

Jornadas de Hematología y Medicina Transfusional
Sociedad Chilena de Hematología
6 y 7 de octubre de 2011, Viña del Mar, Chile

Leucemia linfoblástica aguda

Diagnóstico y seguimiento

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ICO-Hospital Universitari Germans
Trias i Pujol. Badalona

Grupo PETHEMA

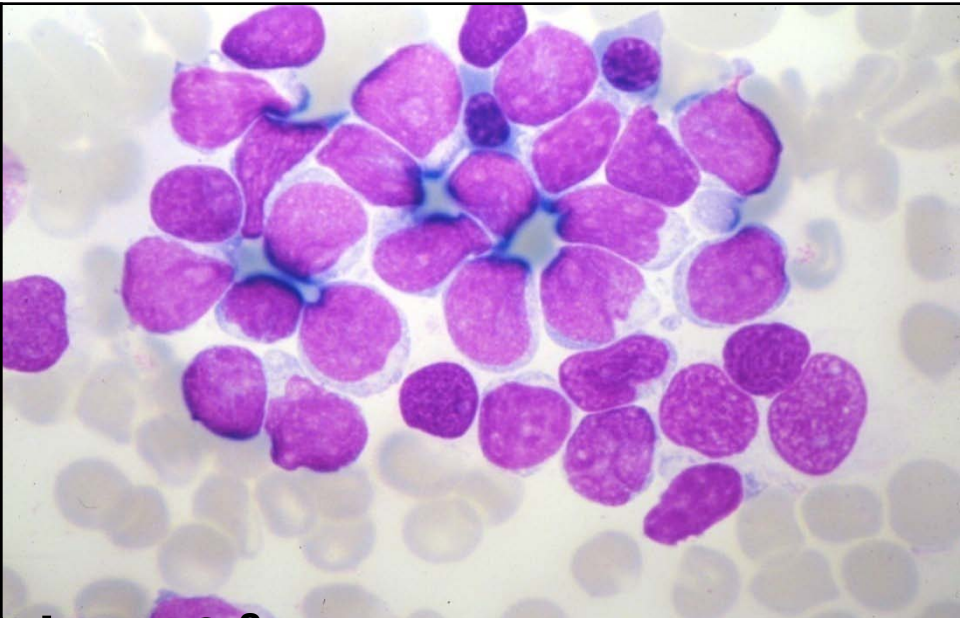
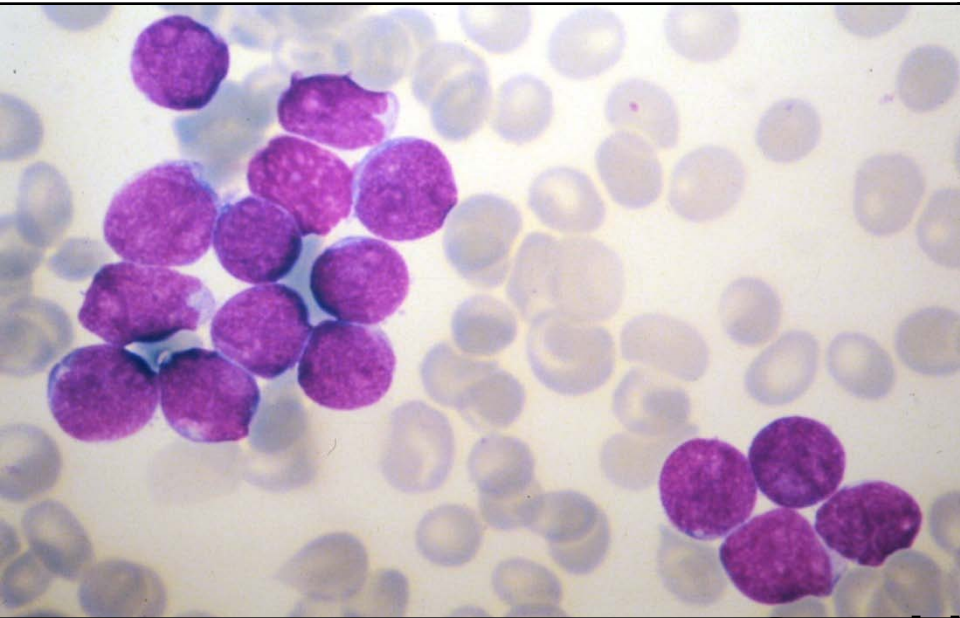
Diagnostic work-up in ALL

- 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.

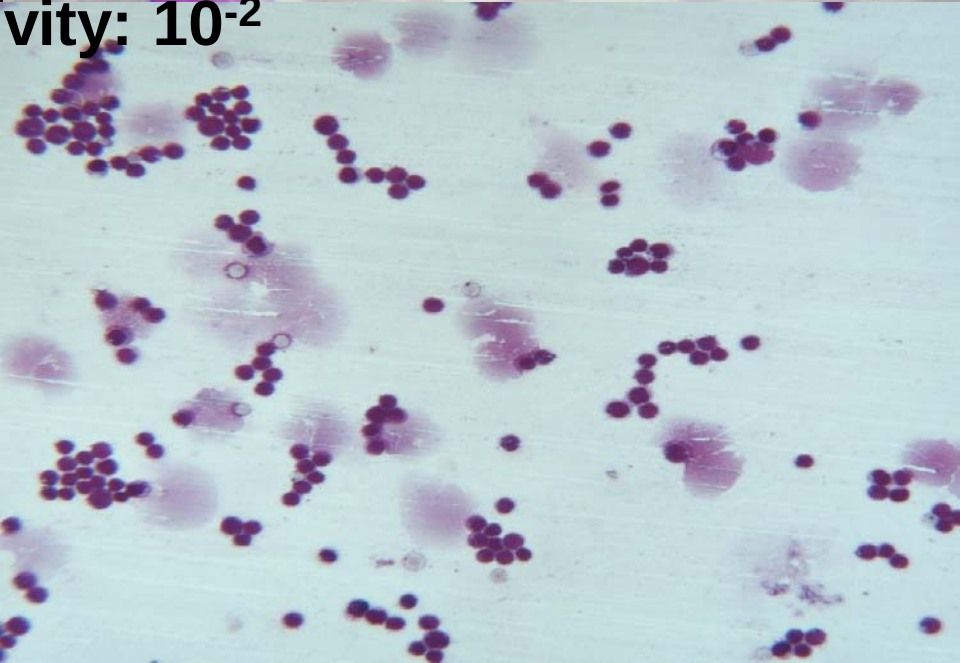
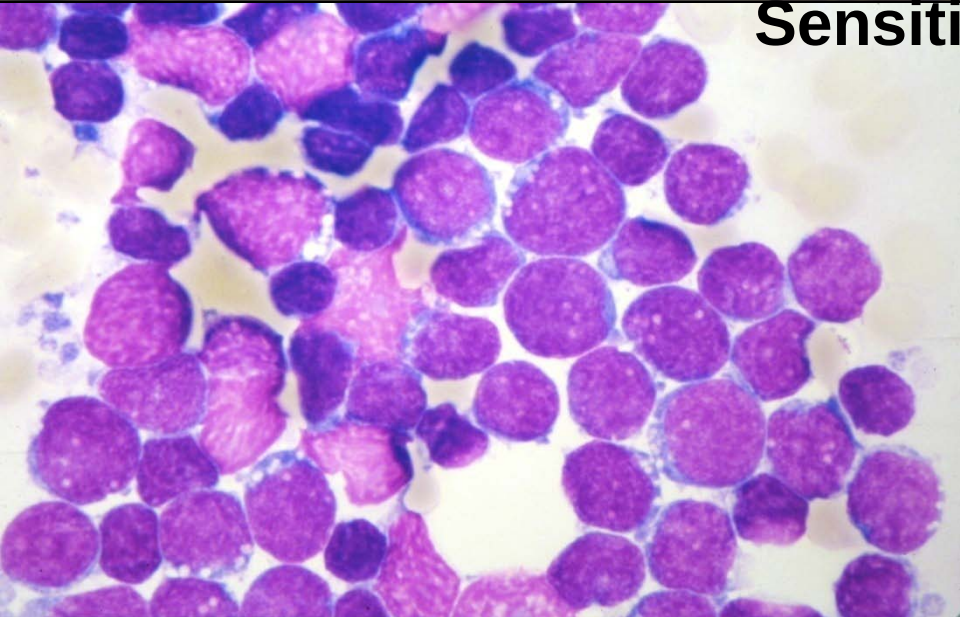
ALL. WHO Classification, 2008

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

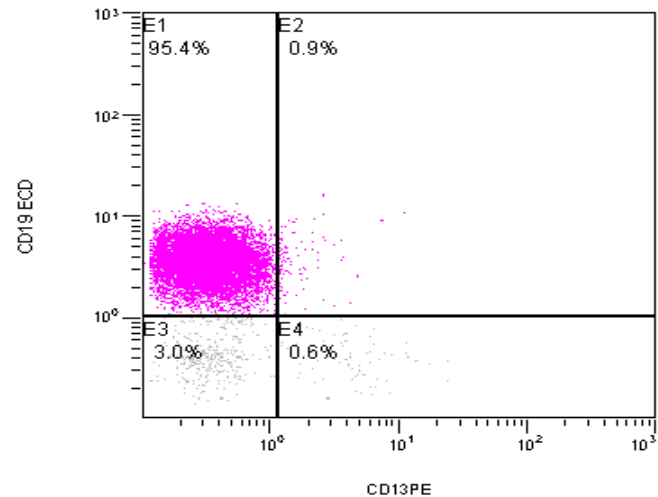
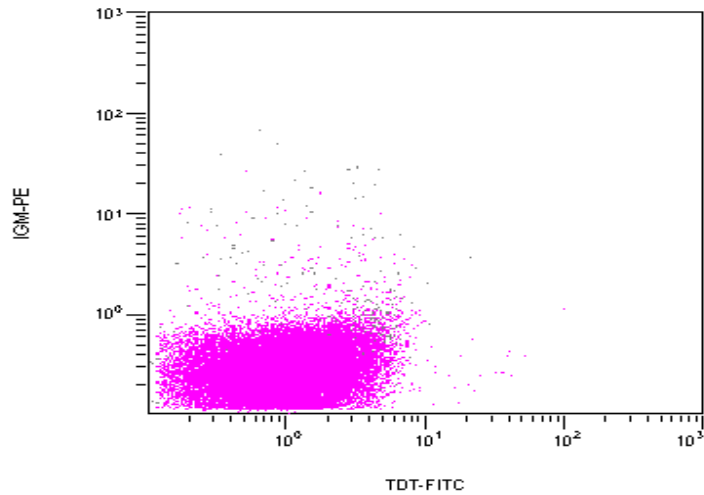
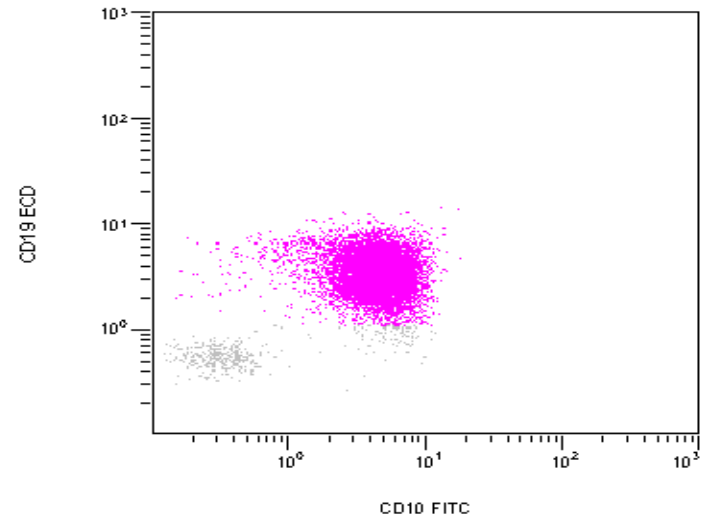
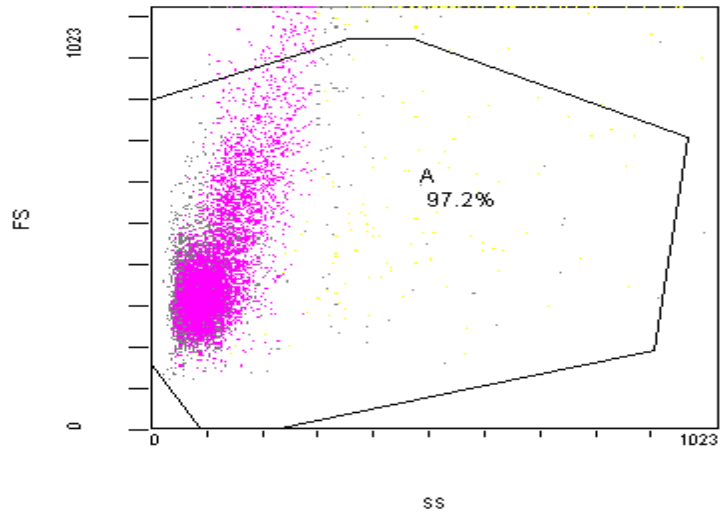
LAL. Morphology



Sensitivity: 10^{-2}



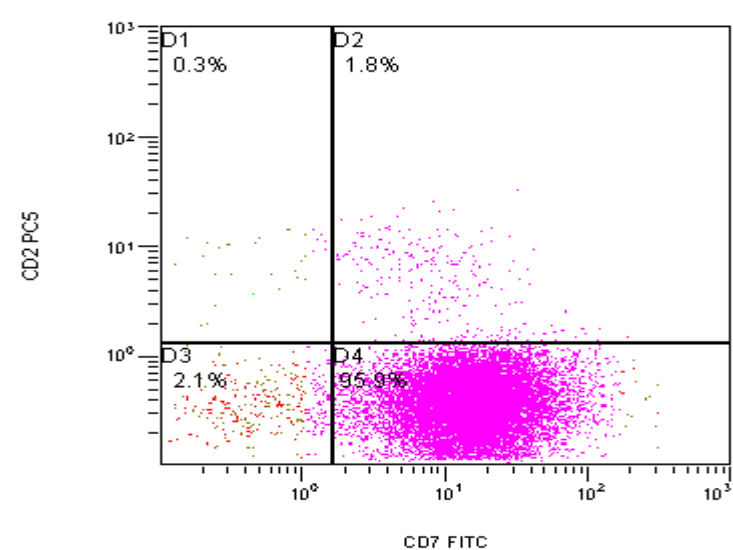
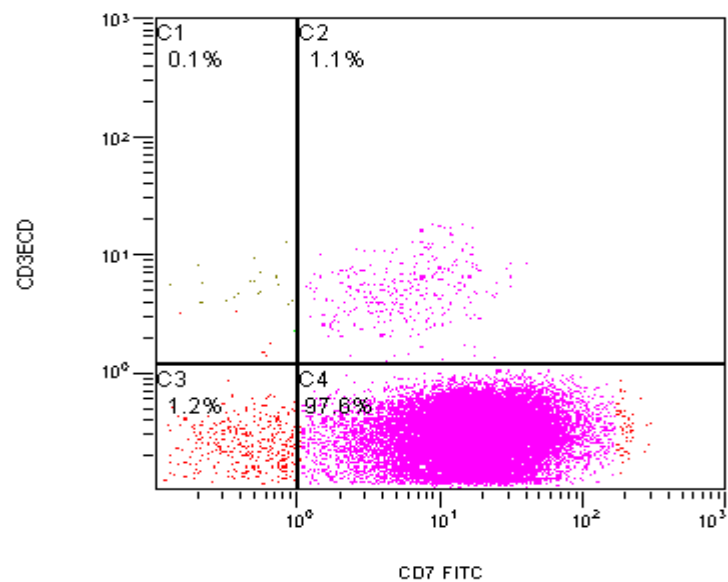
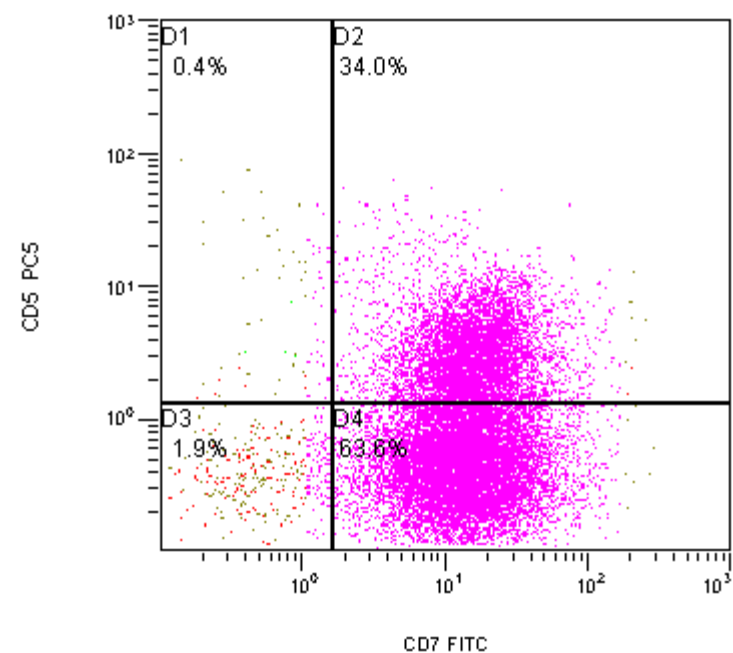
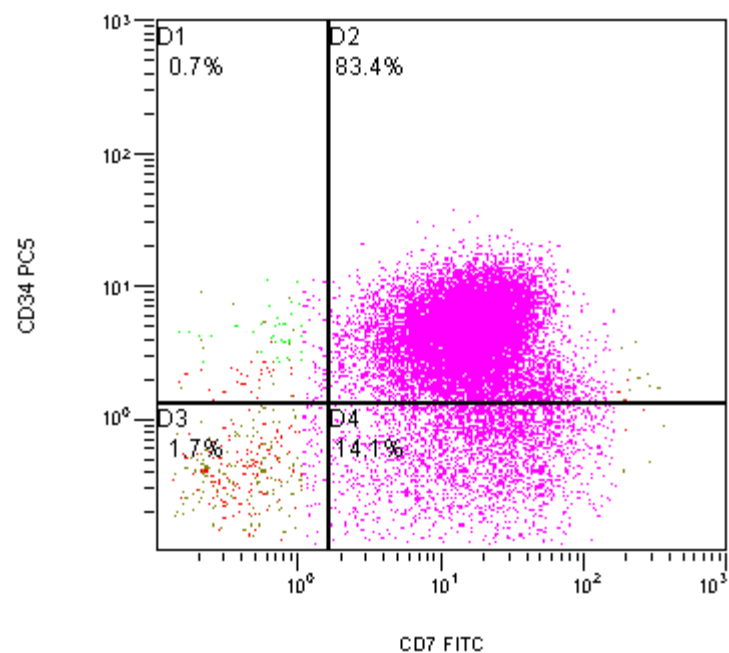
Phenotypic study



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

Phenotypic classification. Precursor B-ALL

	cCD22	CD19	CD79a	CD34	CD10	TdT	sCD22	CD20	CD38	CD45	Cμ	Slg
Pro-B	+	±	+	+	-	+	±	-	+++	±	-	-
Common	+	+	+	±	+++	+	+	±	+	±	-	-
Pre-B	+	+	+	-	+	+	+	+	±	+	+	-/±
Mature B	+	±	+	-	±	±	±	+	±	+	-	+

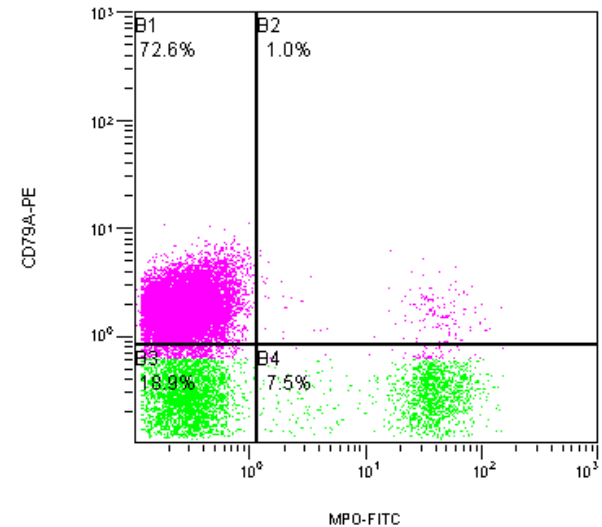
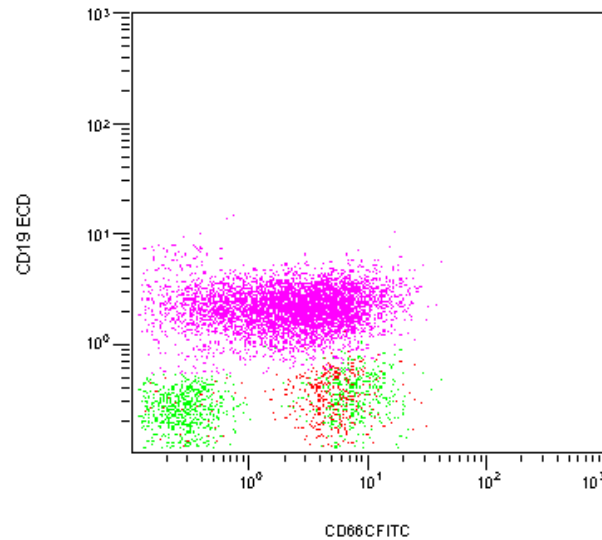
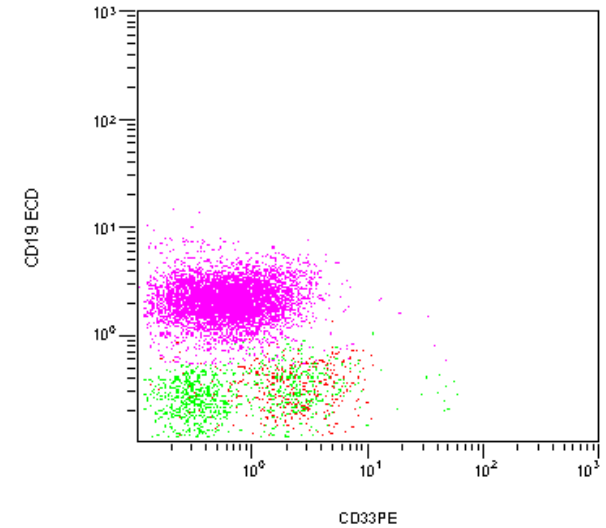
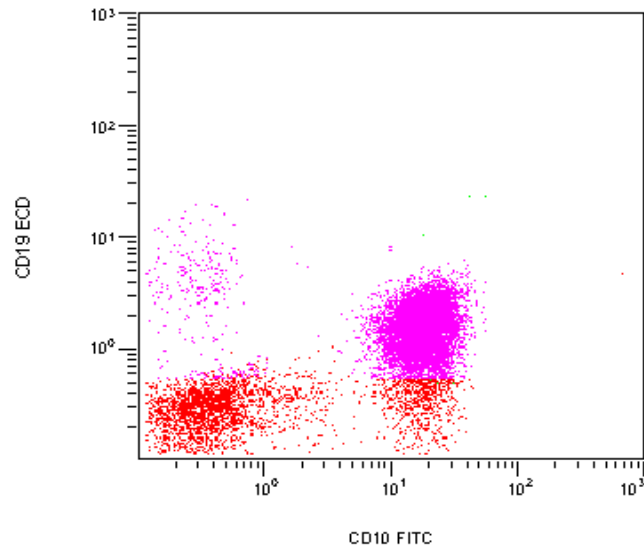


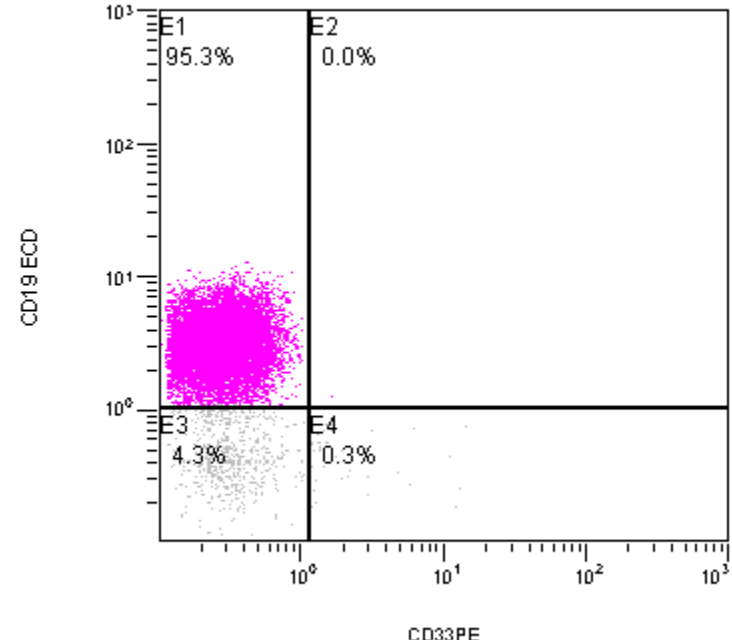
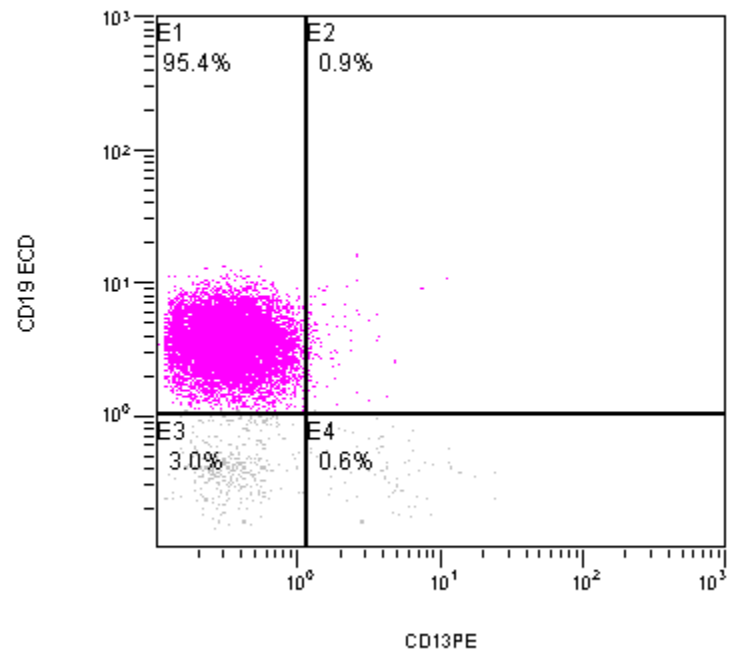
T-ALL

	cyCD3	SCD3	CD7	CD1a	TdT	CD2	CD5	CD4/CD8
Pro-T	+	-	+	-	+/ <u>±</u>	-	-	-/-
Pre-T	+	<u>±</u>	+	-	+/ <u>±</u>	+	+	-/- or +/+
Cortical	+	+	+	+	<u>±</u>	+	+	<u>±</u> / <u>±</u>
Mature	+	+	+	-	<u>±</u> /-	+	+	+/- or -/+

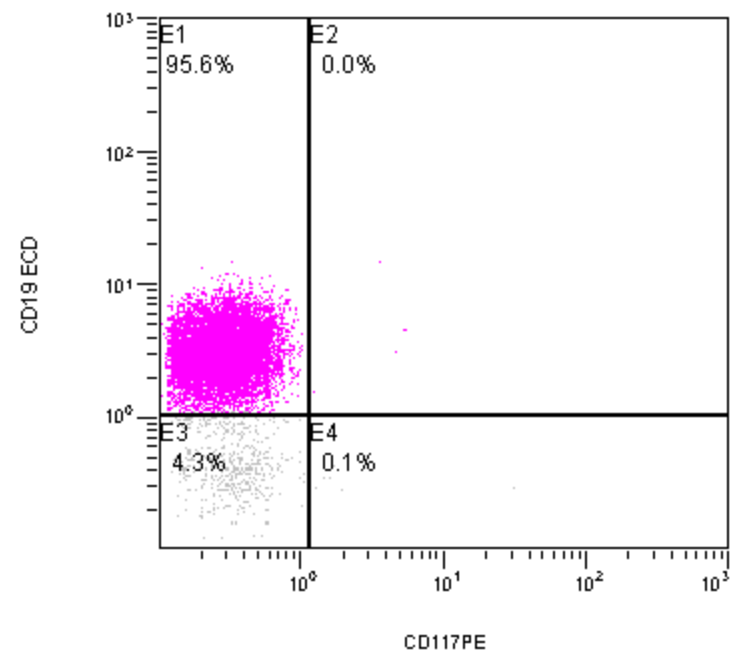
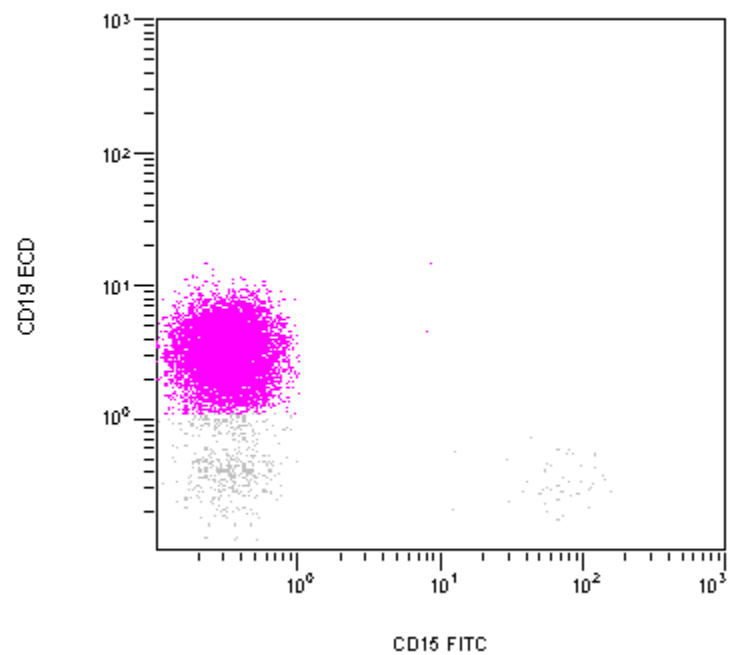
Flow Cytometry

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





ALL My -



Biphenotypic leukemias

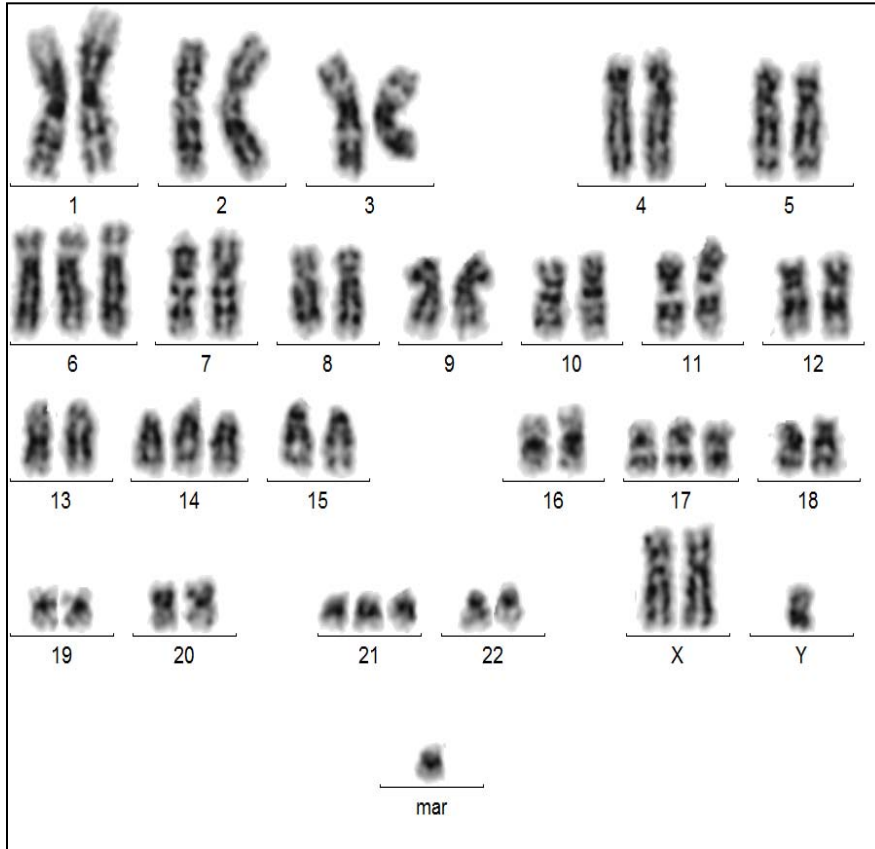
Score	B lin	T lin	Myeloid lin
2	CD79a	CytCD3/CD3s	Anti-MPO
	Cyt IgM	anti-TcR α/β	
	Cyt CD22	anti TcR γ/δ	
1	CD19	CD2	CD117
	CD10	CD5	CD13
	CD20	CD8	CD33
		CD10	CD65
0,5	TdT	TdT	CD14
	CD24	CD7	CD15
		CD1a	CD64

Score ≥ 2 for lineage assignement (myeloid, B or T)

Cytogenetics

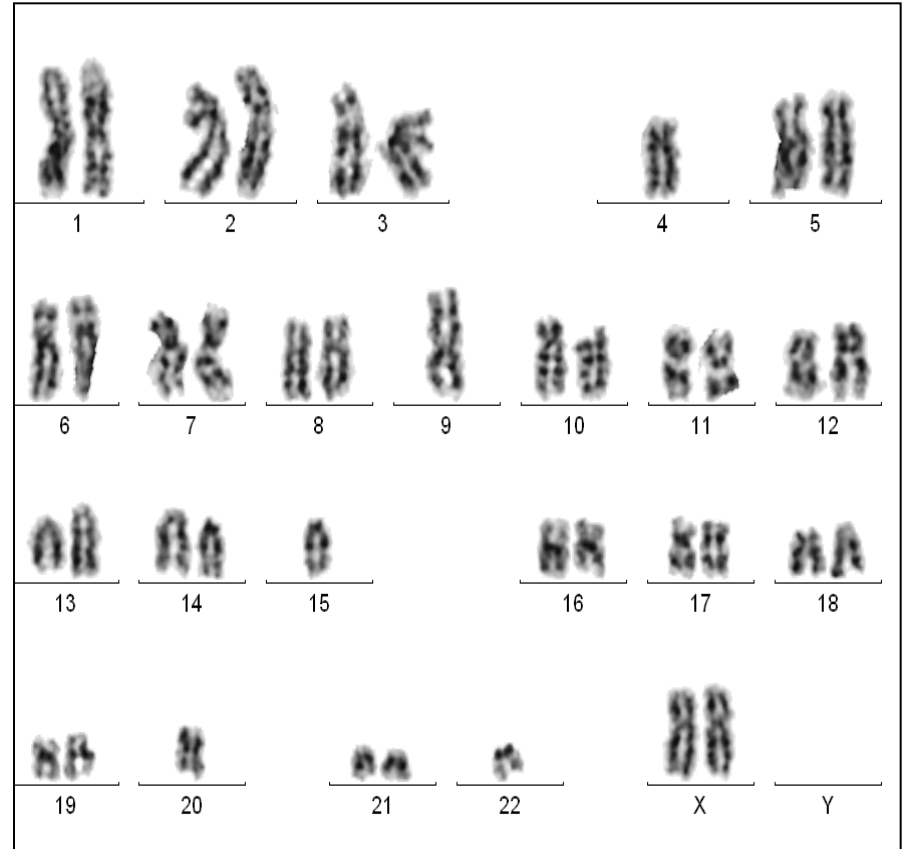
Hiperdiploidy

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



Hipodiploidy

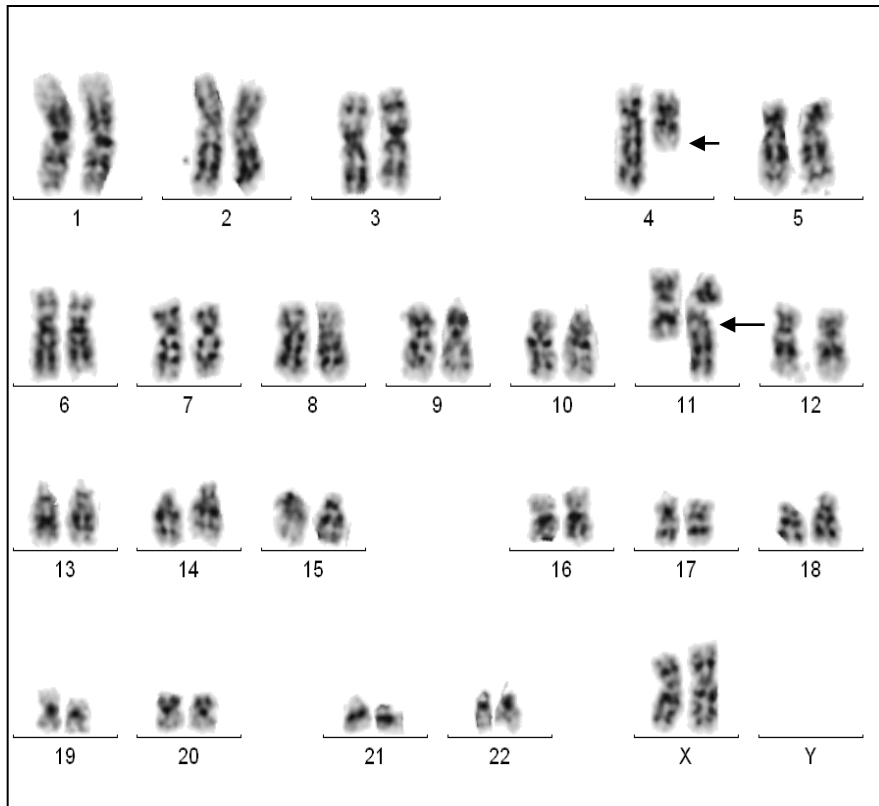
41,XX,-4,-9,add(9)(p21),-15,-20,-22



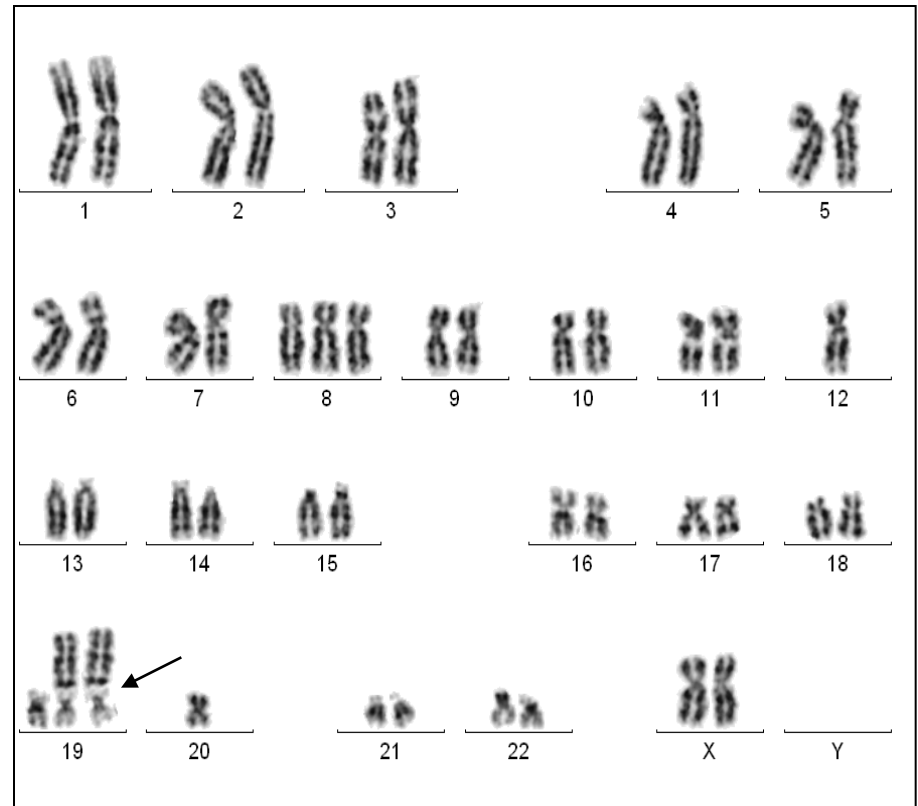
Sensitivity: 10^{-2}

Pseudodiploidy

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



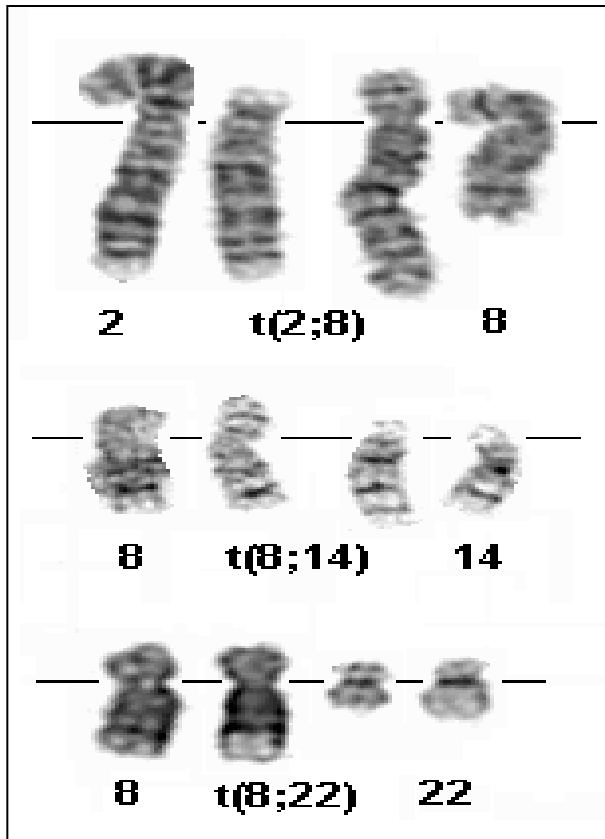
46,XX,+8,-12,der(19)t(1;19)(q23;p13.3),
+der(19)t(1;19)(q23;p13.3),-20



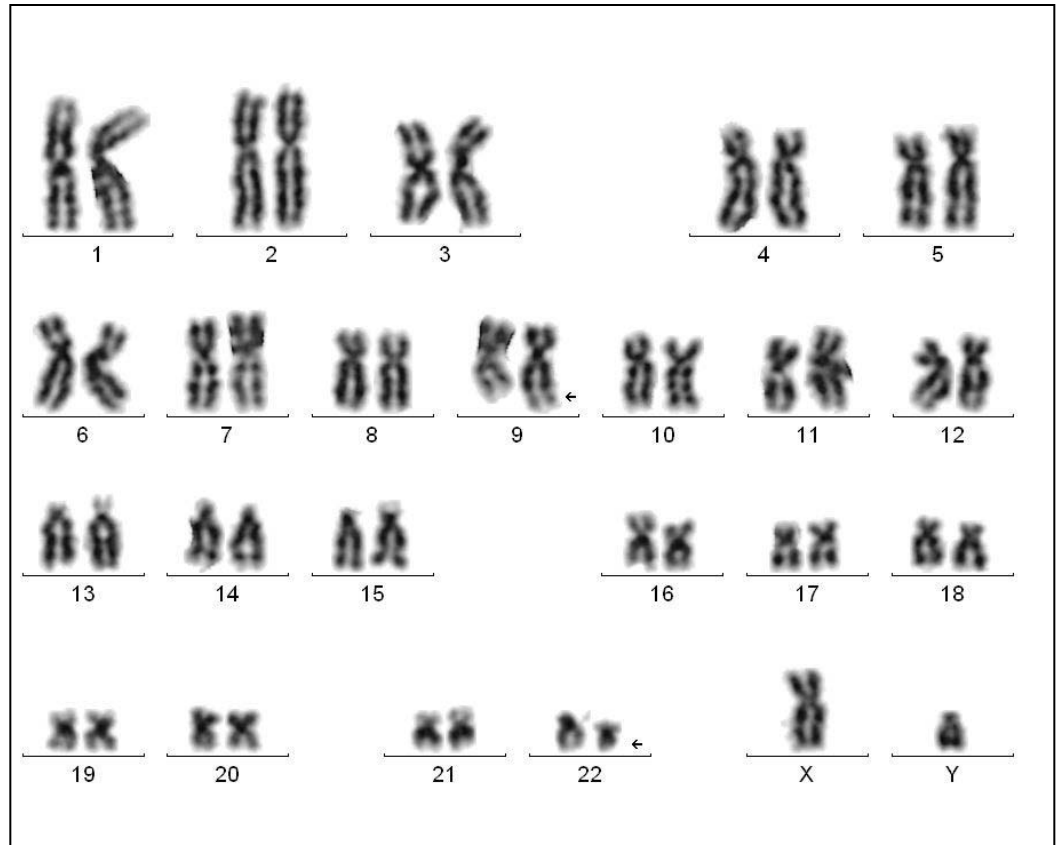
Sensitivity: 10^{-2}

Pseudodiploidy

Burkitt's leukemia

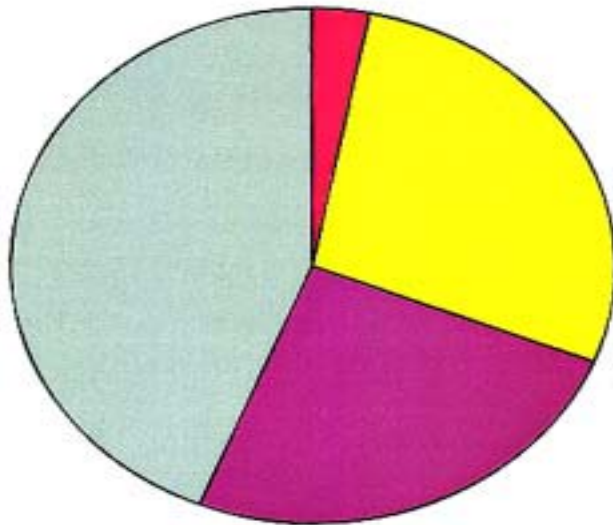


46, XY, t(9;22)(q34.1;q11.2)

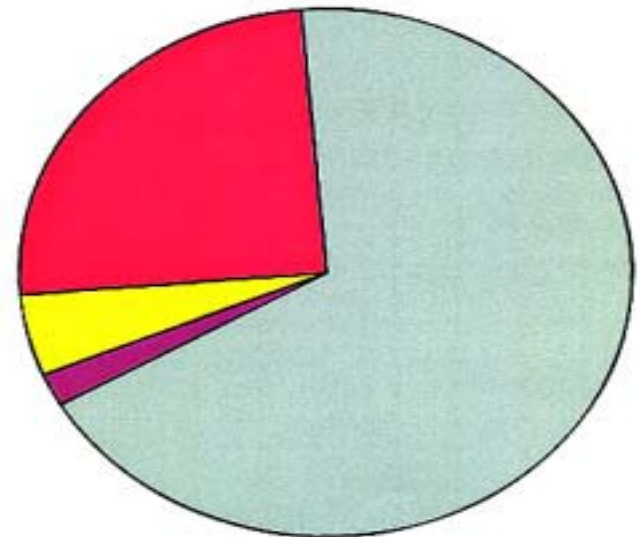


Main cytogenetic differences between ALL in children and adults

Children



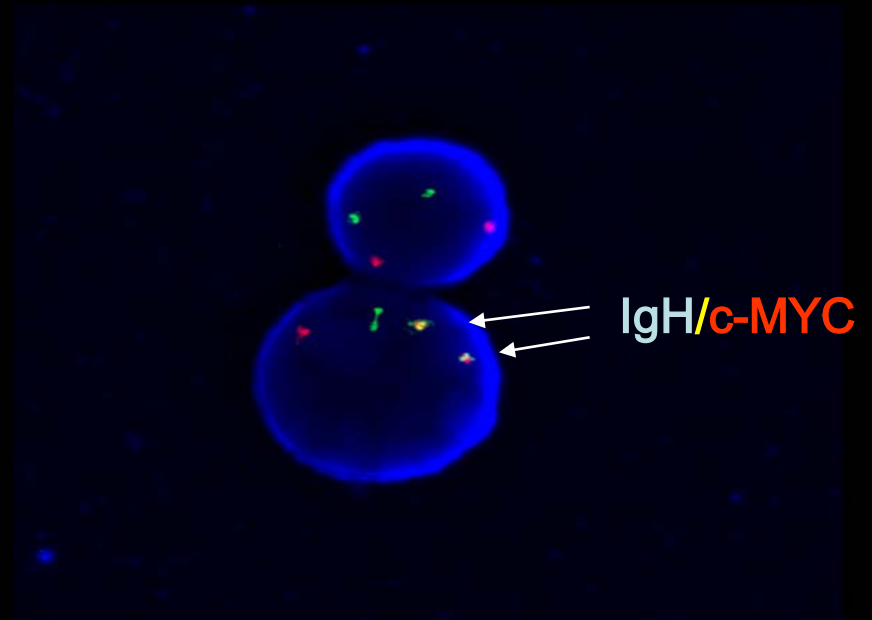
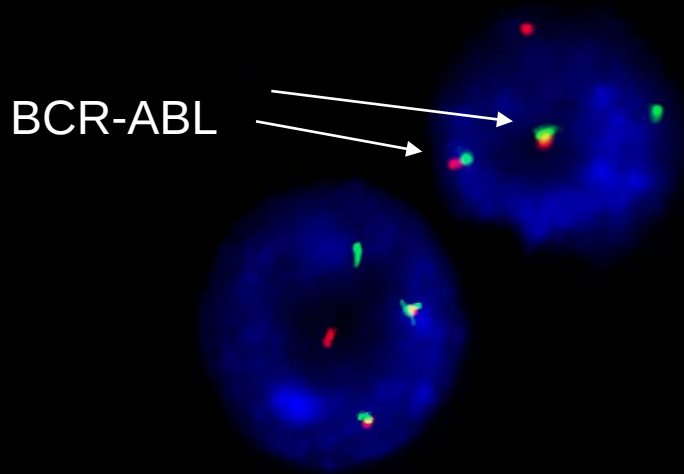
Adults



Incidence (%)		Cure (%)
Children	Adults	

	Philadelphia chromosome	< 5	20-30	< 5 (adults) 25 (children)
	Hyperdiploid	28	< 5	50-80
	TEL-AML1	25	< 5	80-90

ALL. FISH

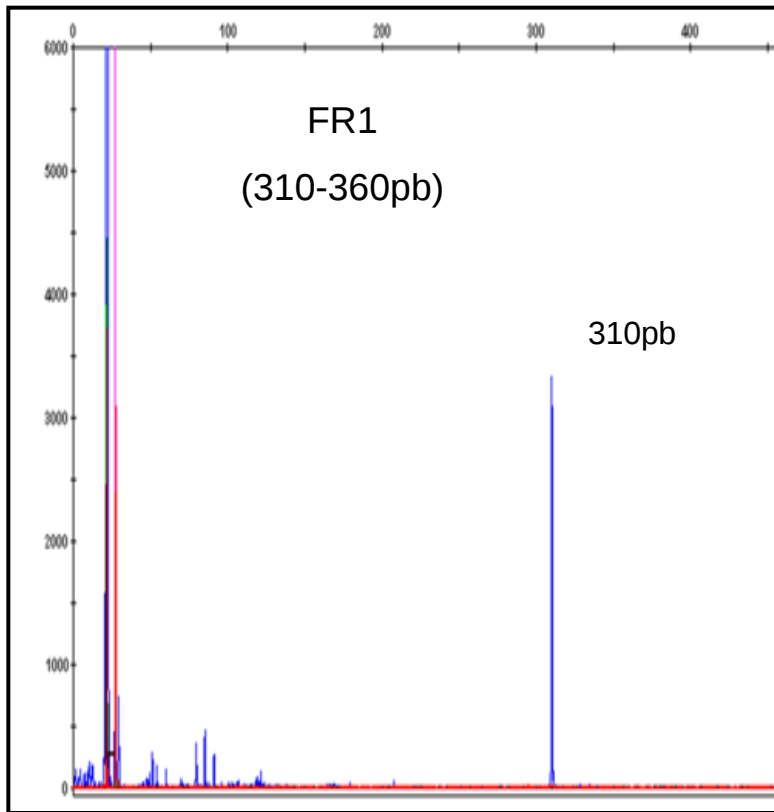


nuc ish(ABL1x3),(BCRx3),(ABL1con BCRx2)[90/100]

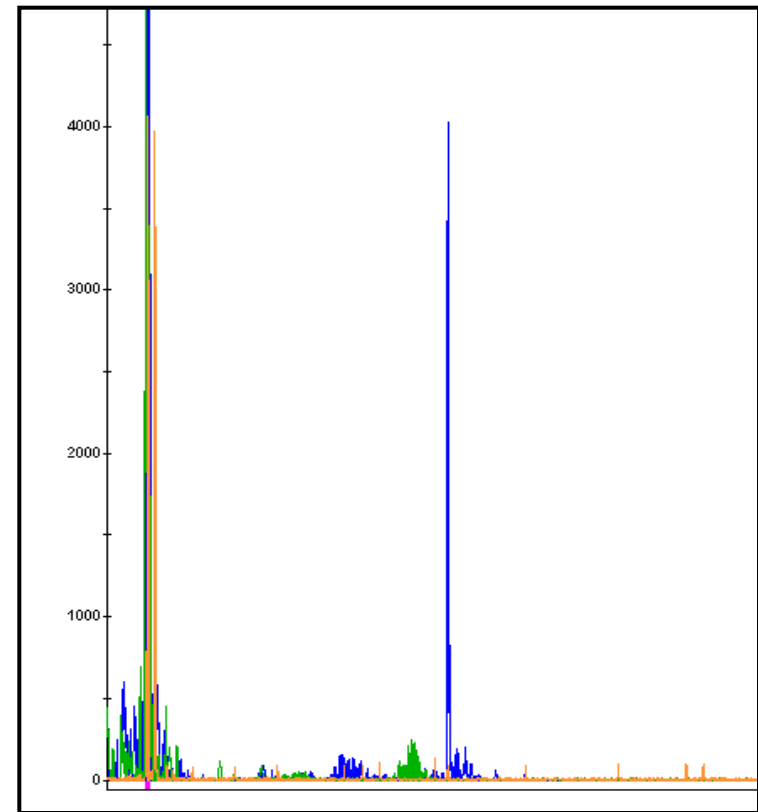
Sensitivity: 5×10^{-2}

Ig & TCR rearrangements

IgH clonal



TCR clonal

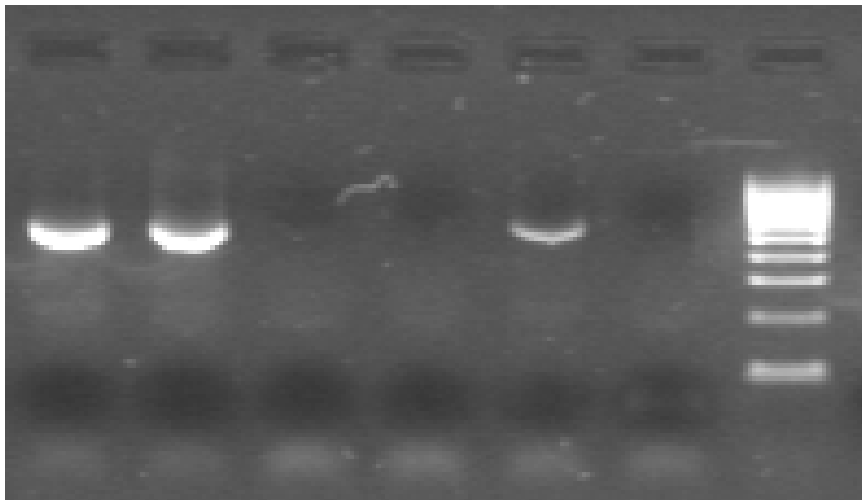


Sensitivity: $10^{-4} - 10^{-5}$ (RQ-PCR)

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)

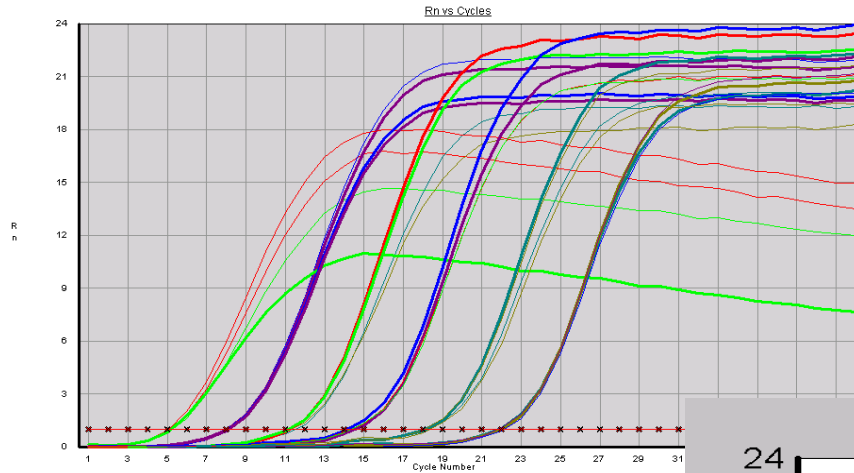
5: Positive control p190

6: Negative control

7: Marker of molecular weight

RQ-PCR

Standard curve



Linear dynamic range (5 Logs)

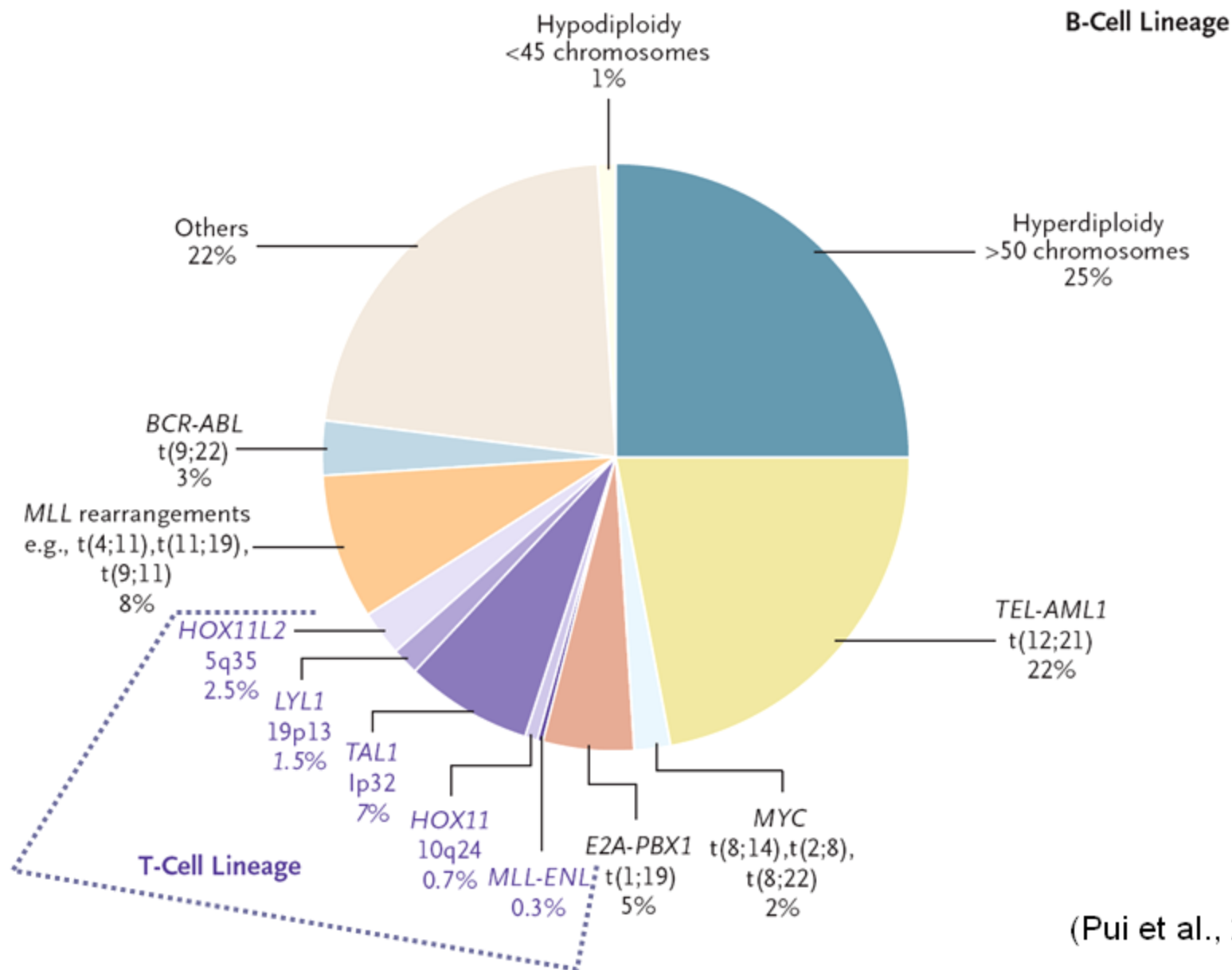
Standard Curve



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

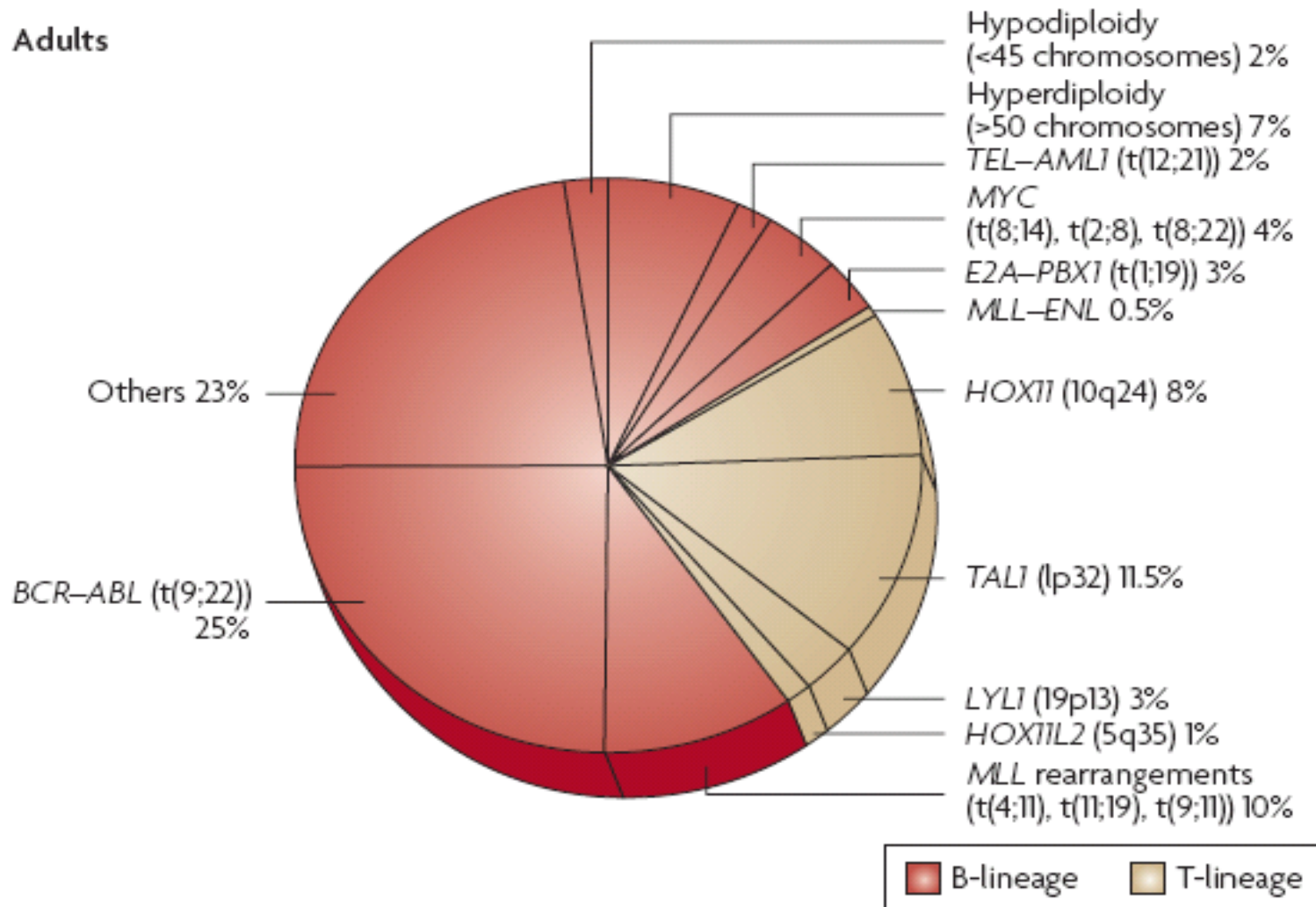
Molecular follow-up (RQ-PCR)

molecular subgroups in childhood ALL



Genetic Heterogeneity in Adult ALL

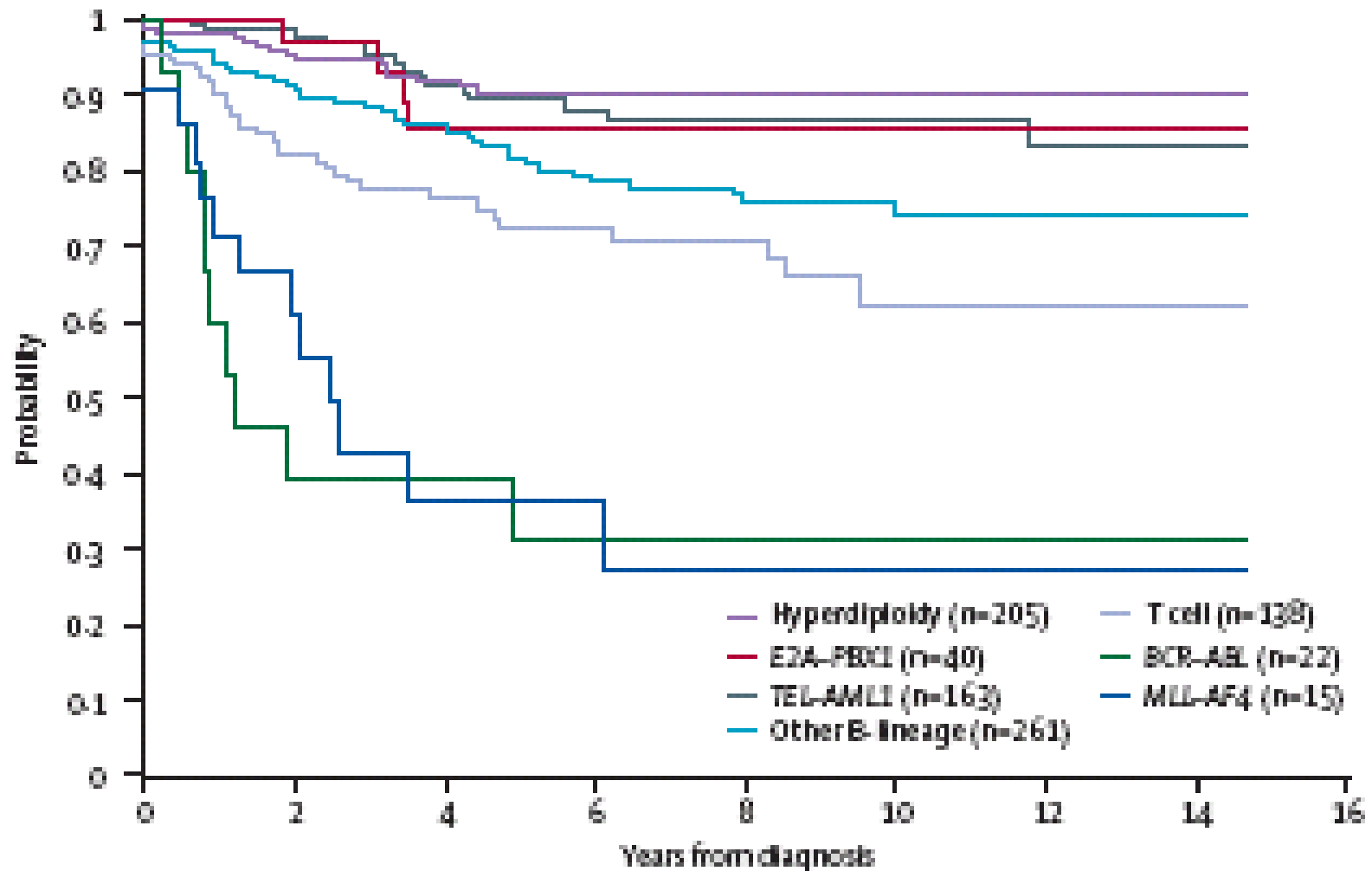
b Adults



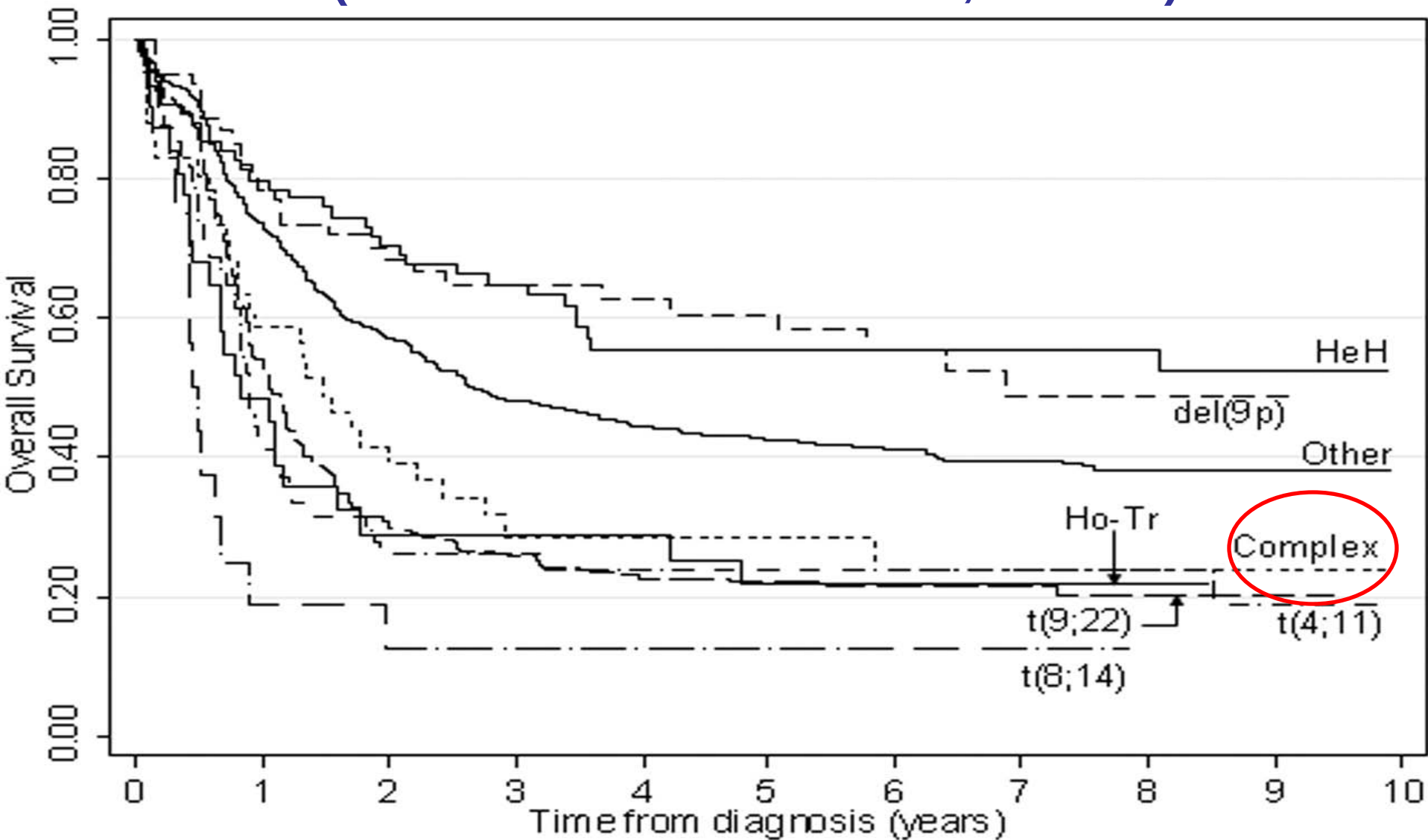
Usefulness of diagnostic work-up

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

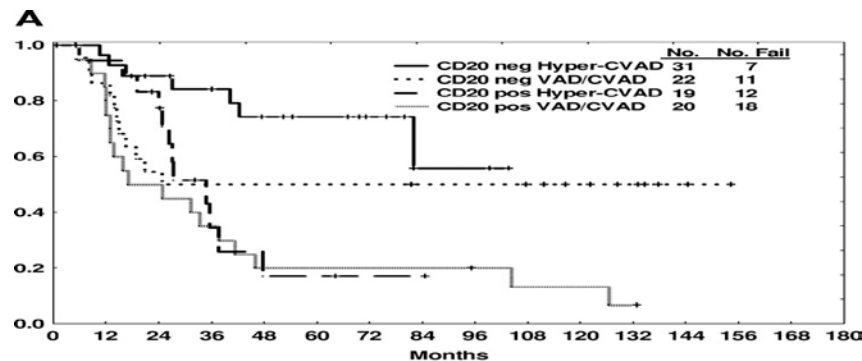
Prognostic impact of genetic and molecular classification of childhood ALL



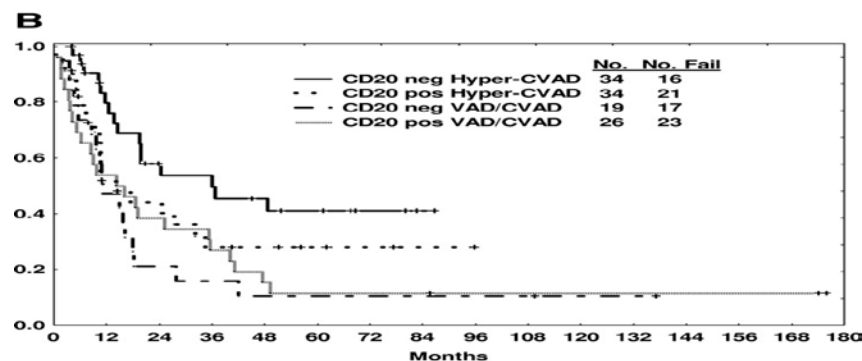
Genetics and prognosis in adult ALL. (MRC UKALLXII/ECOG 2993, n= 1522)



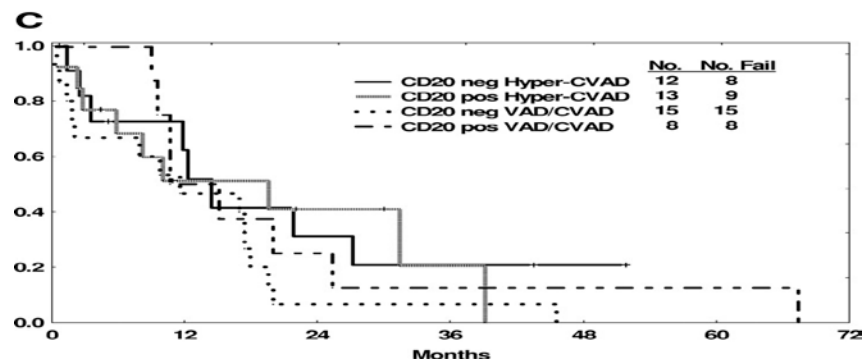
Outcome by CD20 expression and therapy according to age subgroups



Protocol

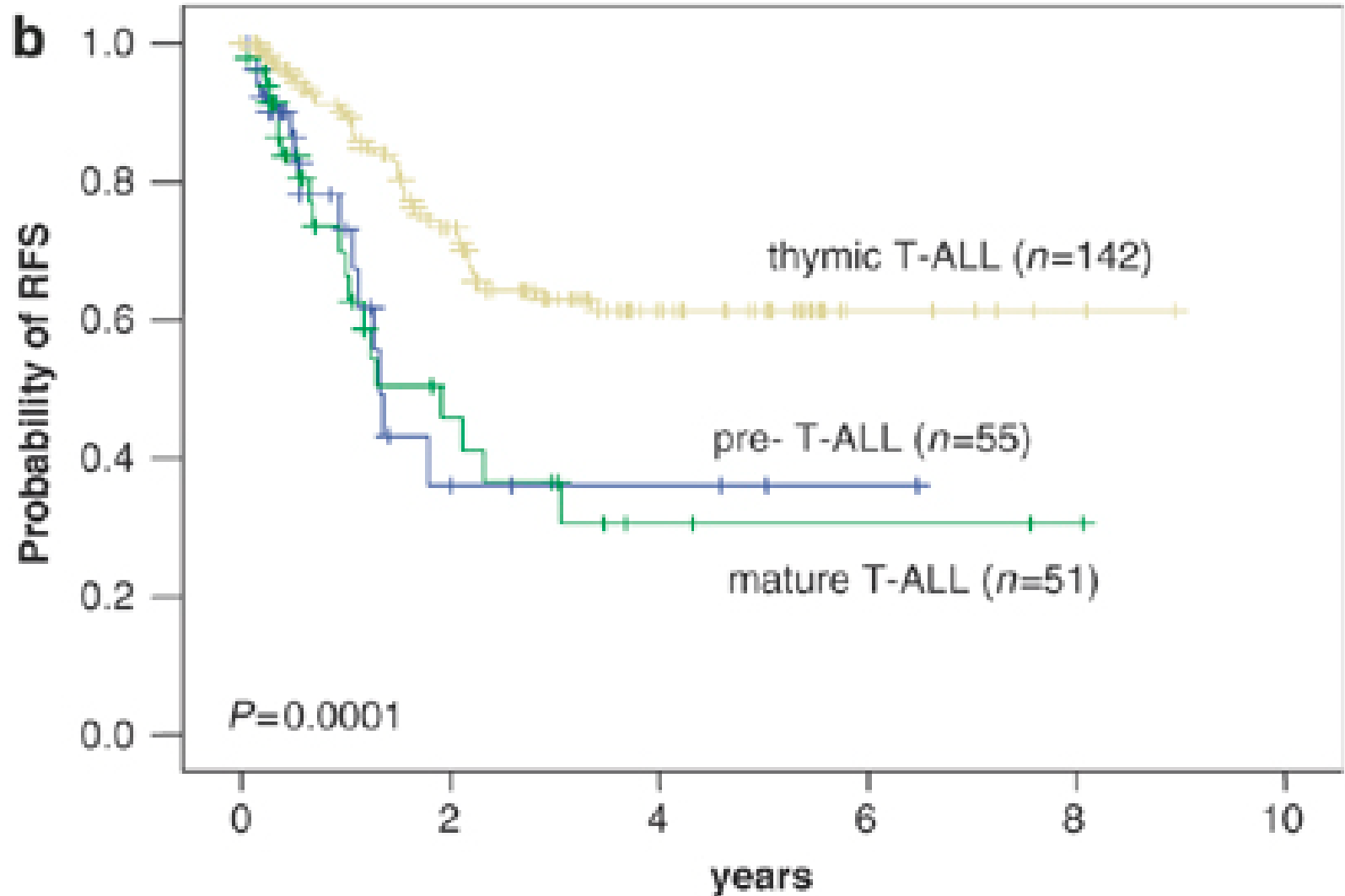


Young

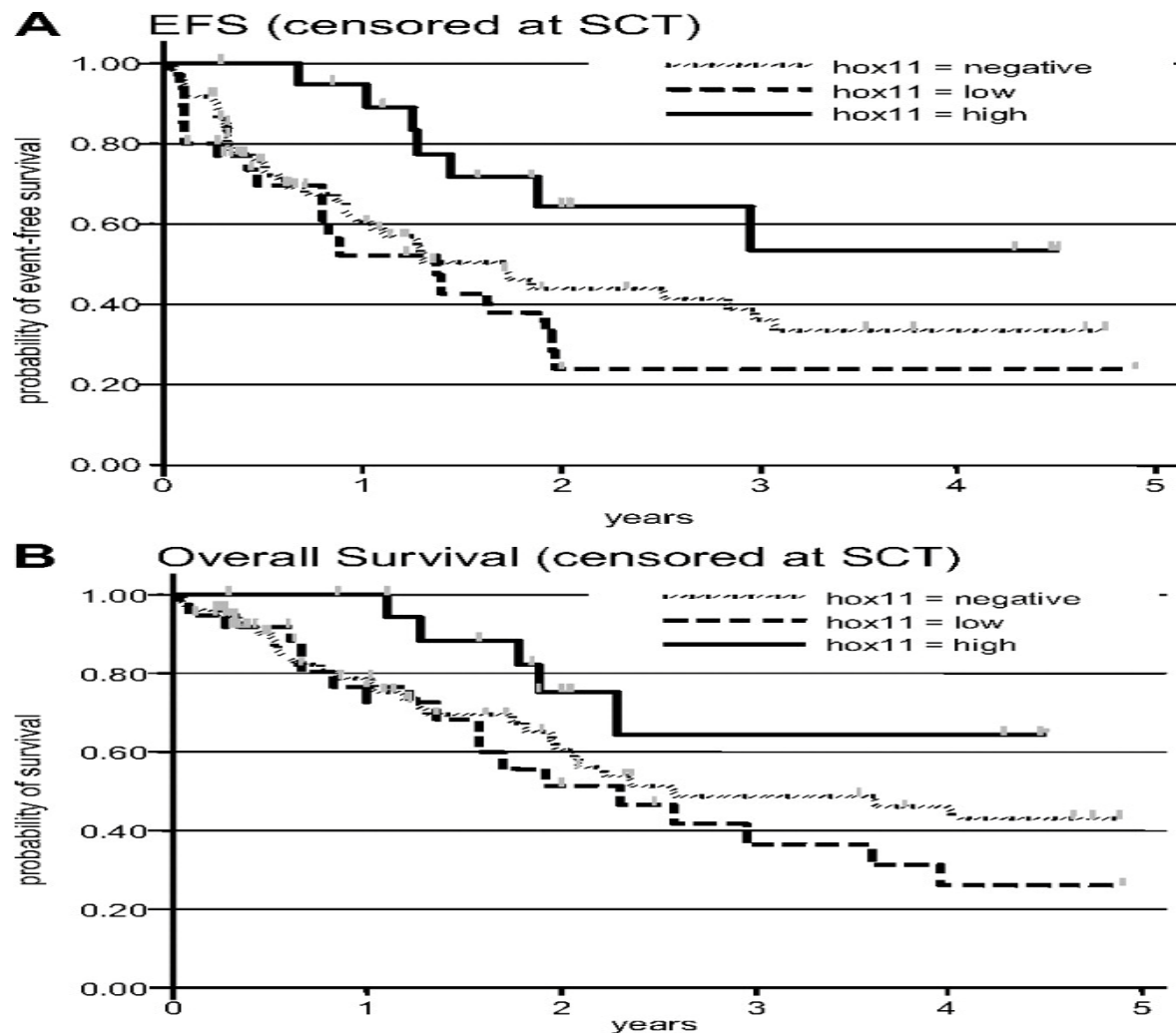


Elderly

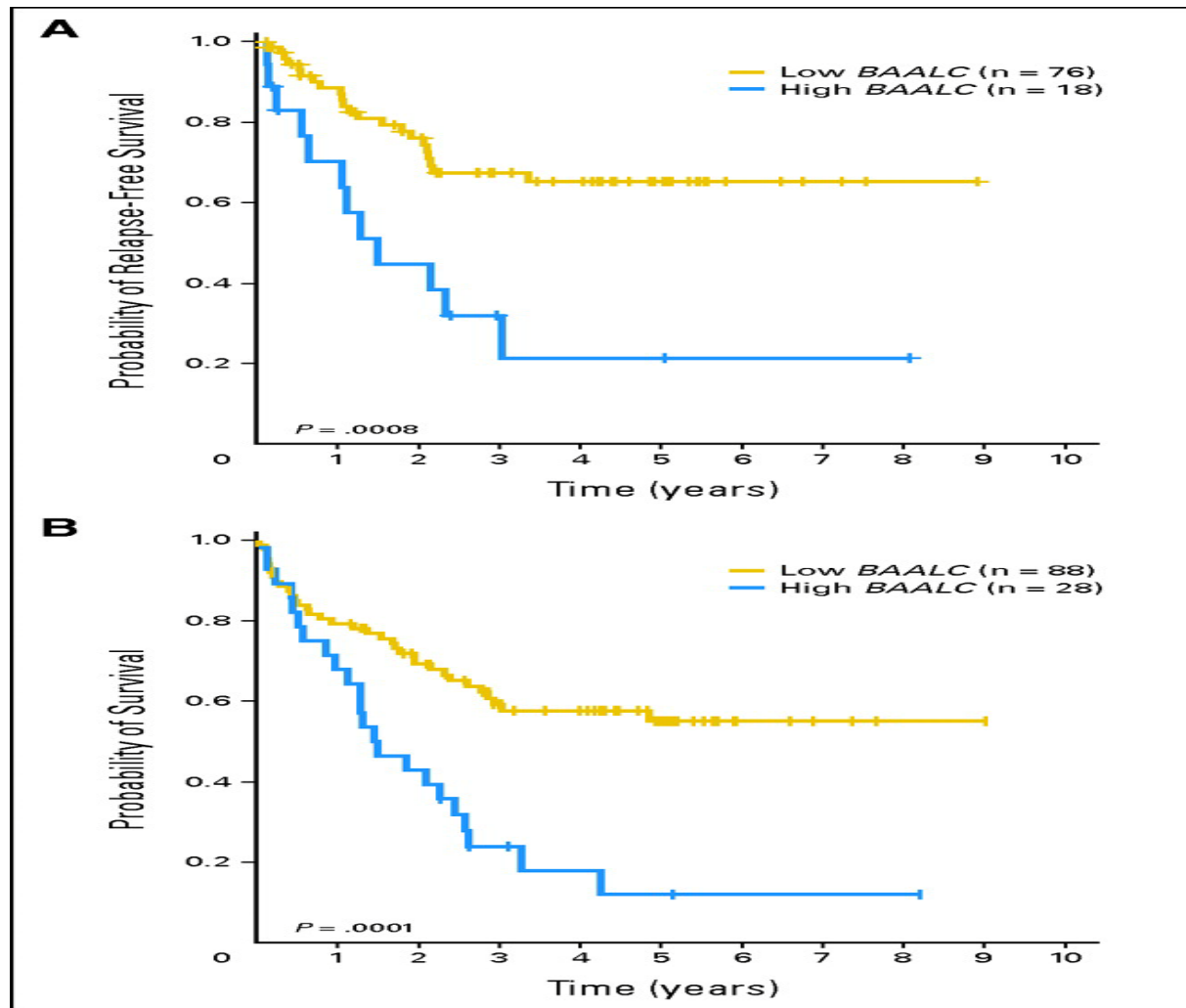
T-ALL: prognostic value of differentiation stage/phenotype



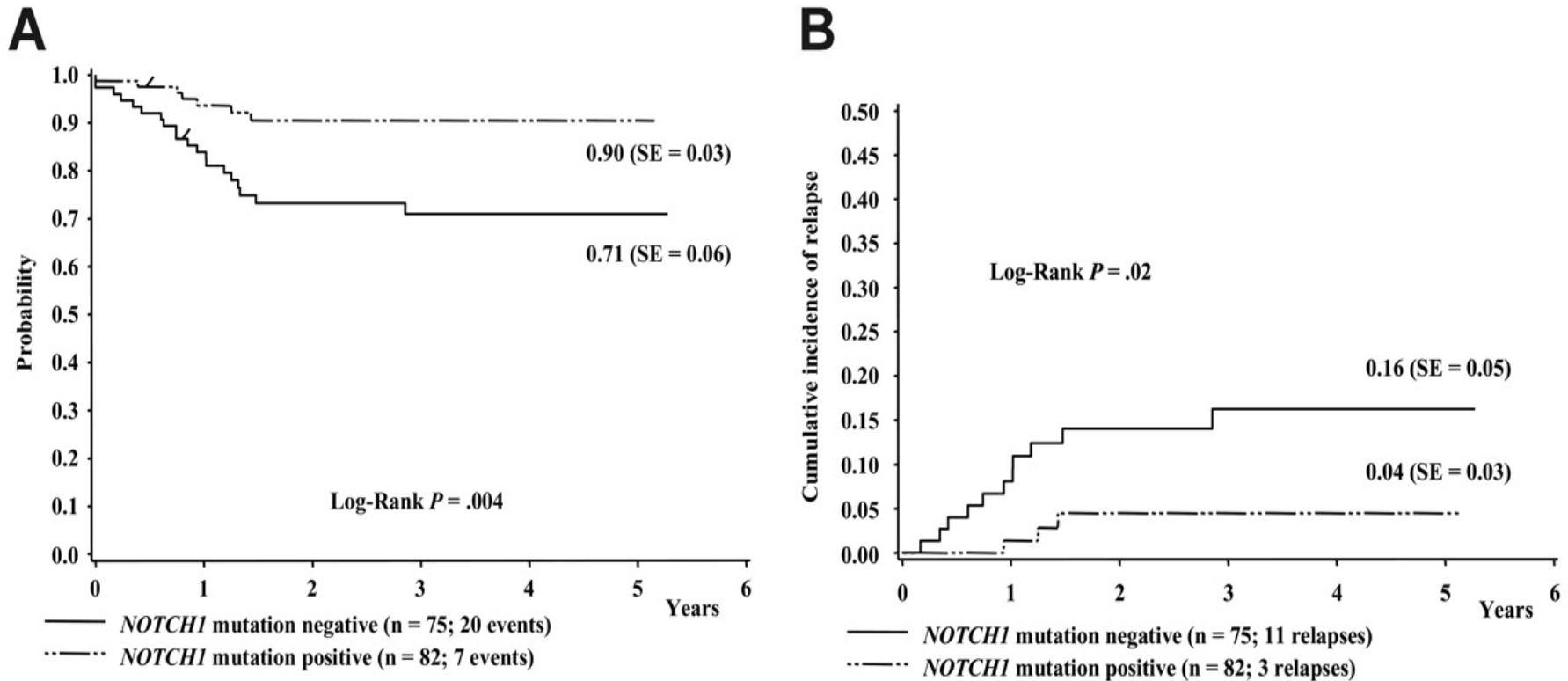
Prognostic impact of HOX1/TLX1 in adult T-ALL



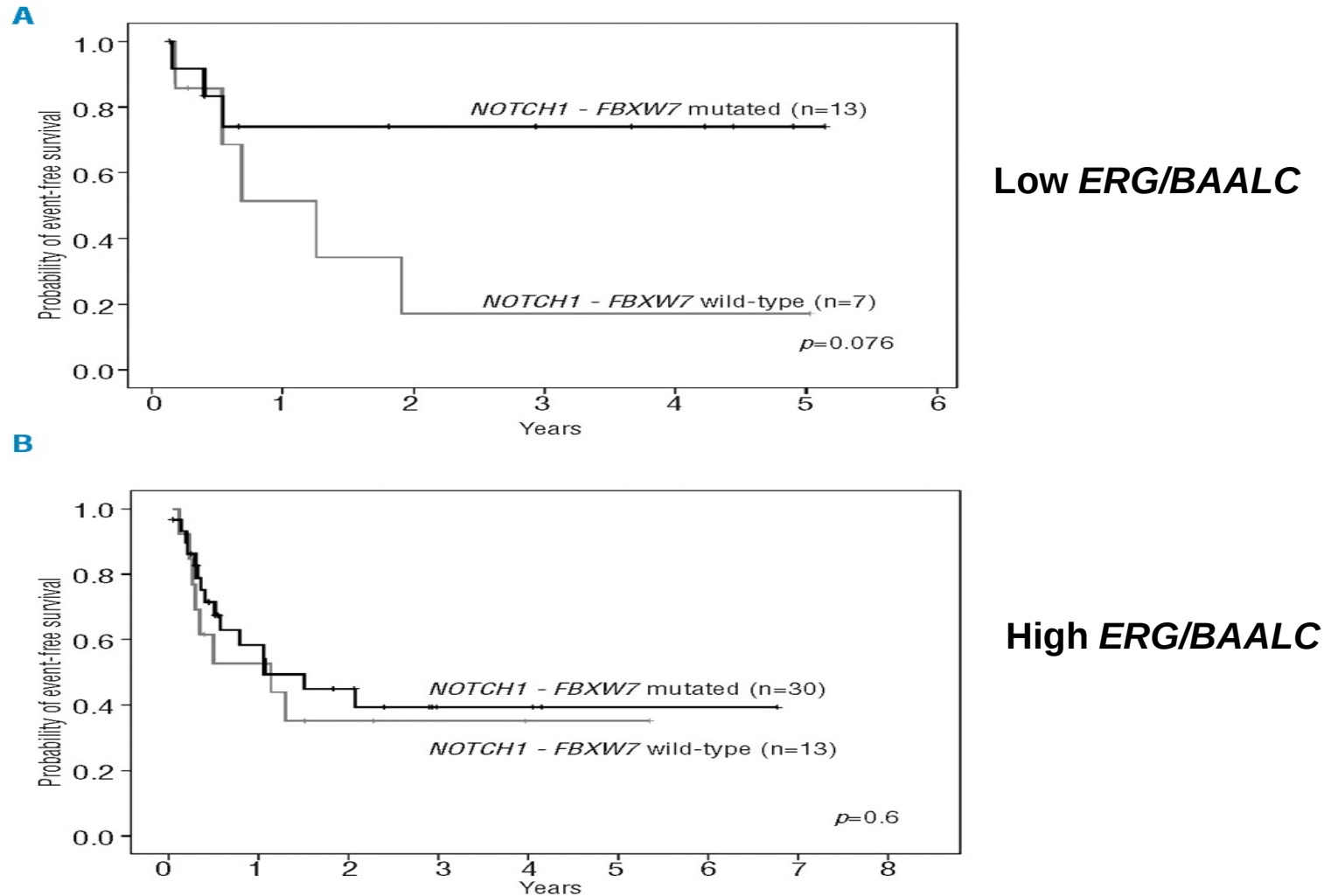
Impact of BAALC expression on survival in adult T-ALL



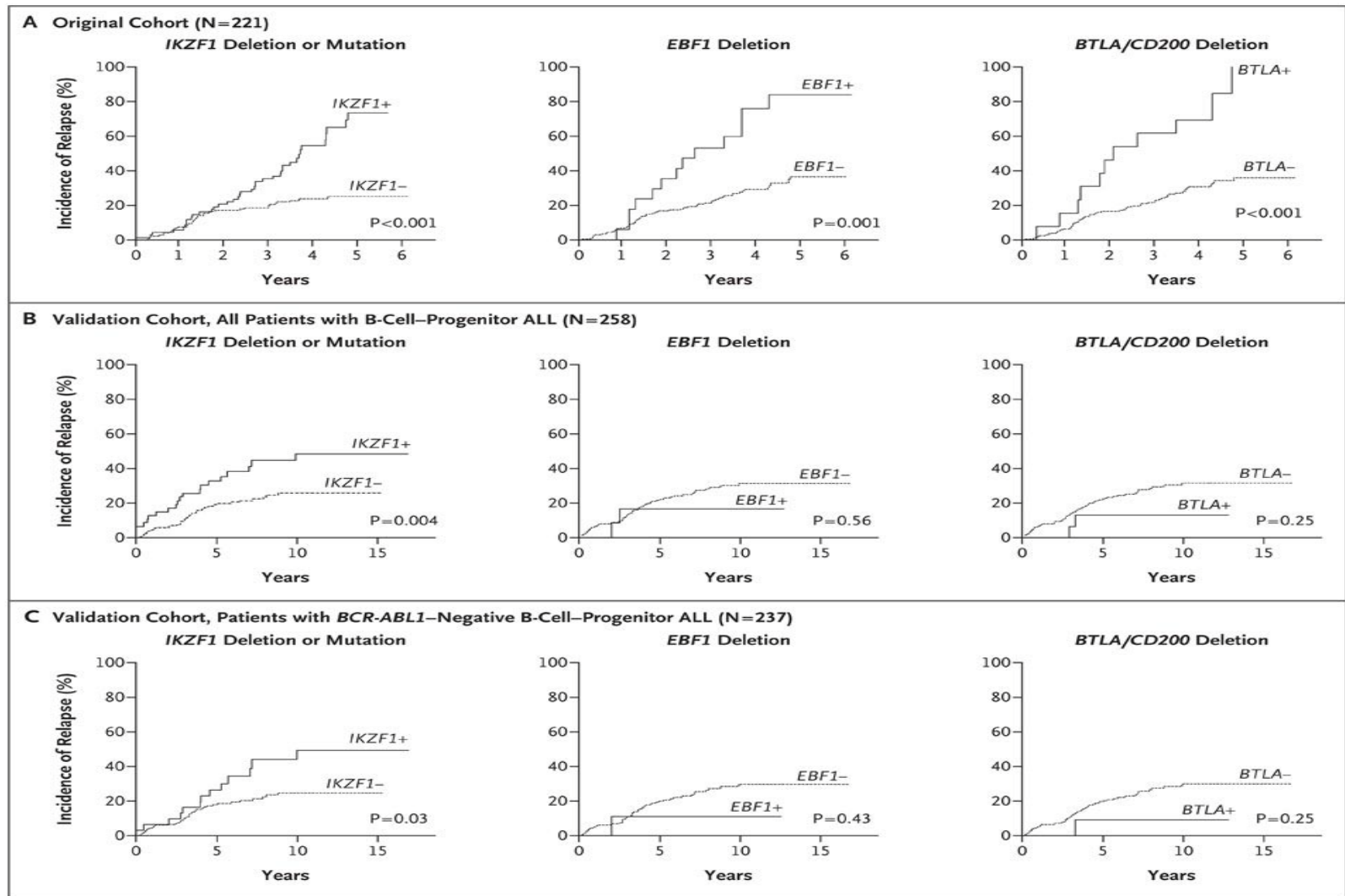
Effect of NOTCH1 mutation status on long-term prognosis in childhood T-ALL



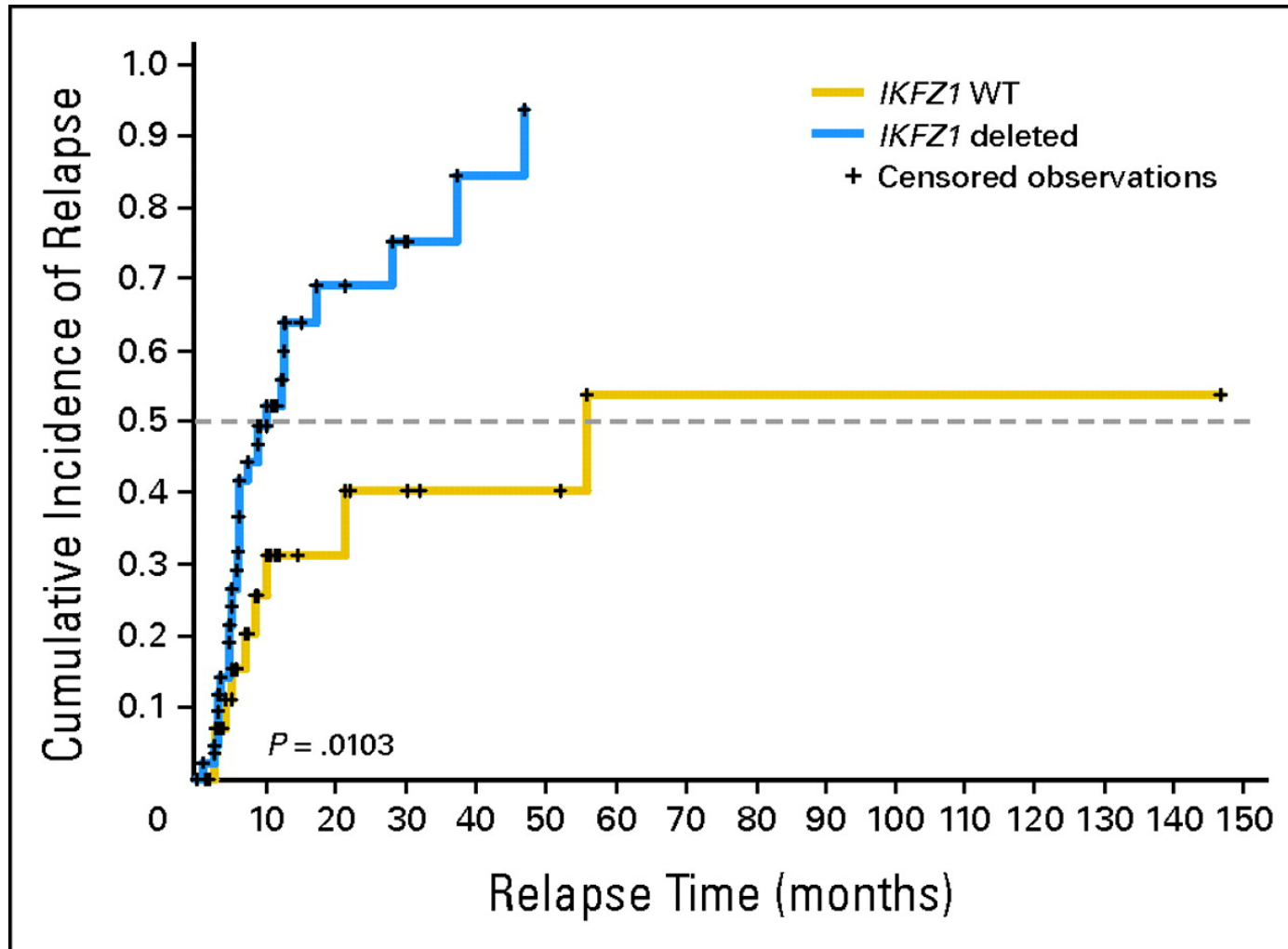
EFS impact of *NOTCH1*-*FBXW7* mutations within *ERG*/*BAALC* expression groups



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



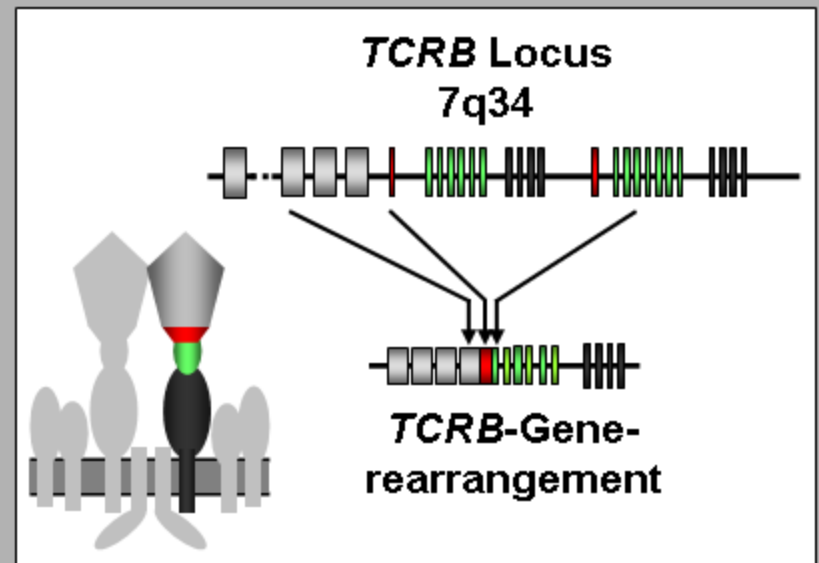
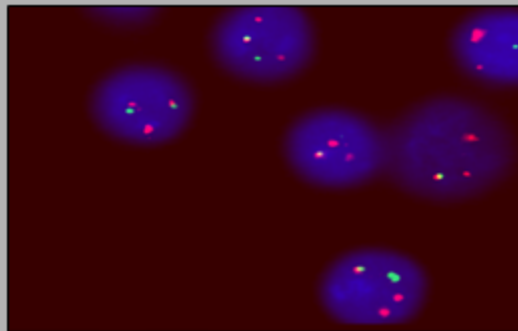
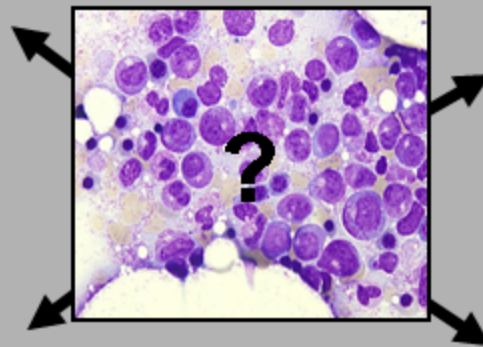
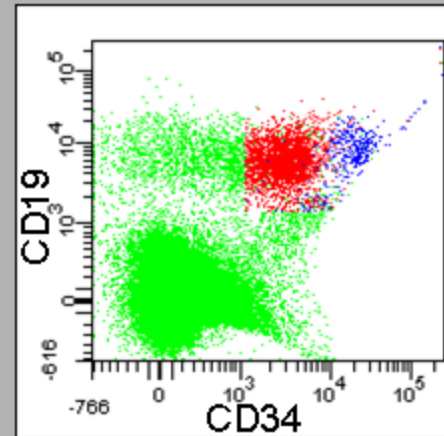
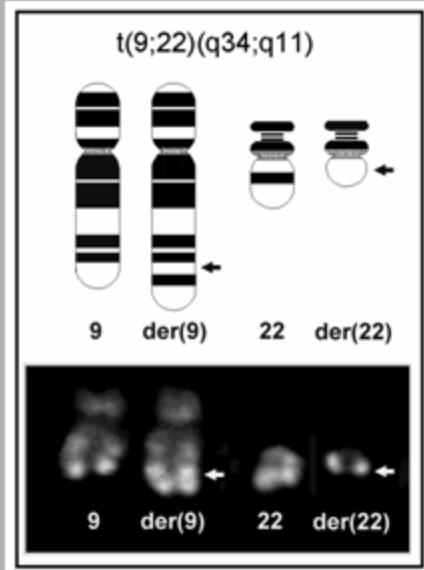
CIR according to *IKZF1* deletion in *BCR-ABL*+ ALL



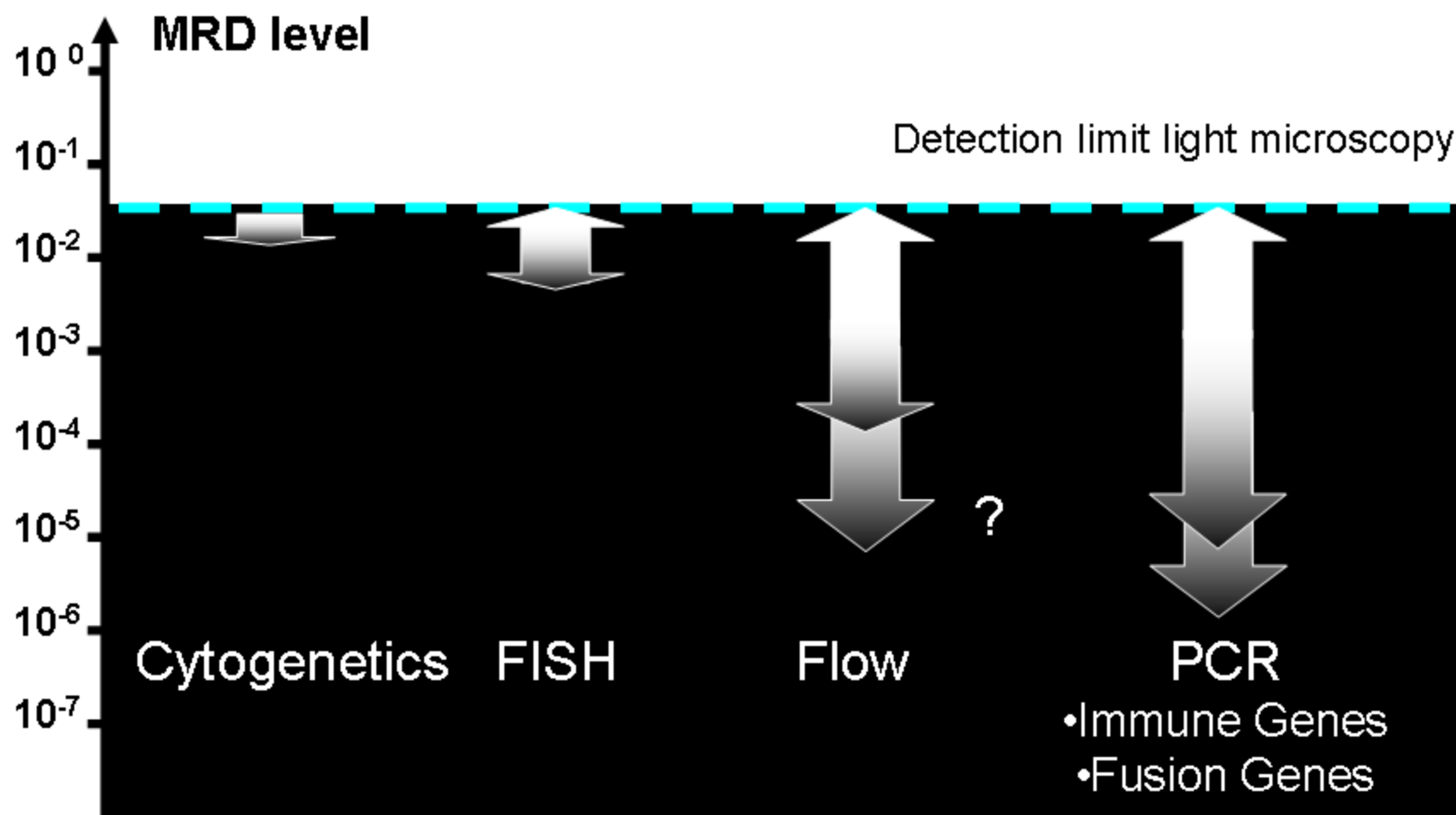
Usefulness of diagnostic work-up

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

MRD analysis in ALL – How?



MRD analysis in ALL – How?



Study of Fusion Transcripts

- Advantages

- High sensitivity: 1×10^{-5} - 10^{-6}
- Leukemia-specific
- Stable target during evolution
- Standardized: useful in cooperative multicenter clinical trials

- Pitfalls

- Not patient-specific
- Useful in a minority of patients
- Risk of contamination
- Poor reproducibility when small numbers of transcripts are present

Comparison of PCR and Flow Cytometry for MRD Study in Ph-negative ALL

	PCR analysis of Ig and TCR gene rearrangements	Multiparameter flow cytometry
Sensitivity	RQ-PCR: $10^{-4} - 10^{-5}$	3- to 4-color: $10^{-3} - 10^{-4}$ 6- to 9-color: $10^{-4} - 10^{-5}$
Applicability	Precursor B-ALL: 90-95% T-ALL: 90-95%	Precursor B-ALL: 80-95% T-ALL: 90-95%
Advantages	- High sensitivity - High degree of standardization - Applicable for almost all cases - DNA stability (multicenter setting)	- Applicable for almost all cases - Rapid - Information on benign cells - Information on malignant cells - Increasing standardization
Disadvantages	- Time consuming - Potential instability of targets (clonal evolution phenomena), two independent targets recommended - Extensive knowledge/experience needed - Relatively expensive	- Immunophenotypic shifts in precursor-B-cells during regeneration - Modulation of antigen expression during induction therapy - Low cellularity during/after induction - Limited sensitivity/applicability using 3- to 4-color flow cytometry - Extensive experience needed in ≥ 6-color flow cytometry

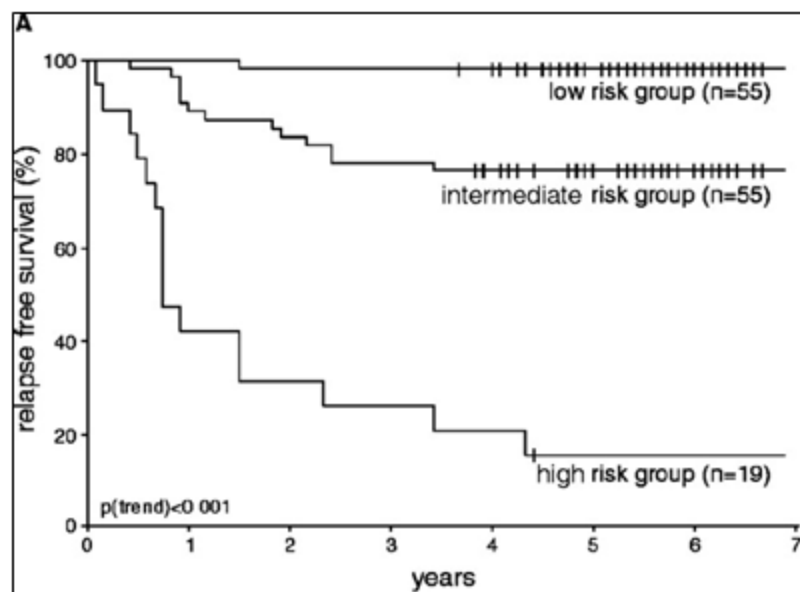
Implications of MRD as a Prognostic Factor

- Assessment of response to therapy
- Assignment of risk group to modulate treatment
 - **Intensity**
 - High-risk group: intensification of therapy, SCT
 - Low-risk group: deintensification (balance toxicities – including TRM- and risk of relapse)
 - **Duration**
 - **Timing of stem cell transplantation**
 - **Treatment post-SCT**
 - **New drugs in low MRD status (i.e.: alemtuzumab (CALGB), blinatumomab (EWALL))**
- Detection of early relapse

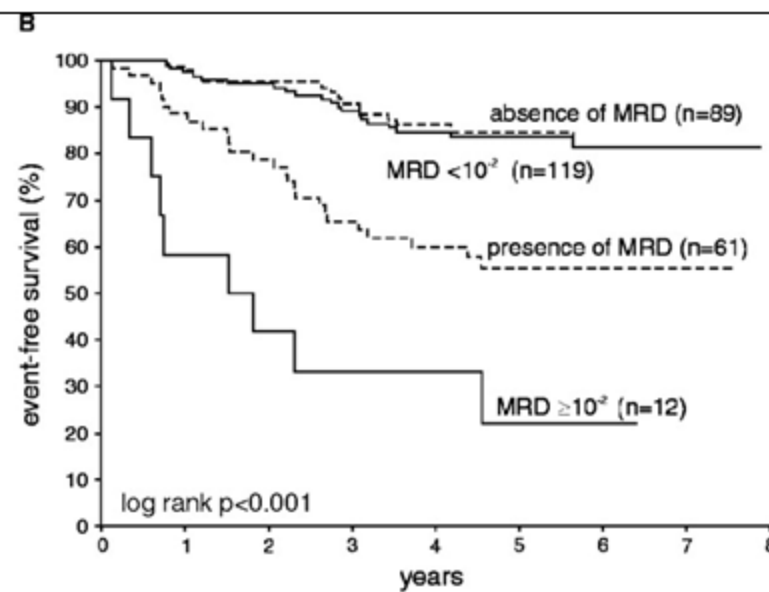
Predictive value of MRD

- Childhood ALL -

„MRD after induction is most important independent prognostic factor“

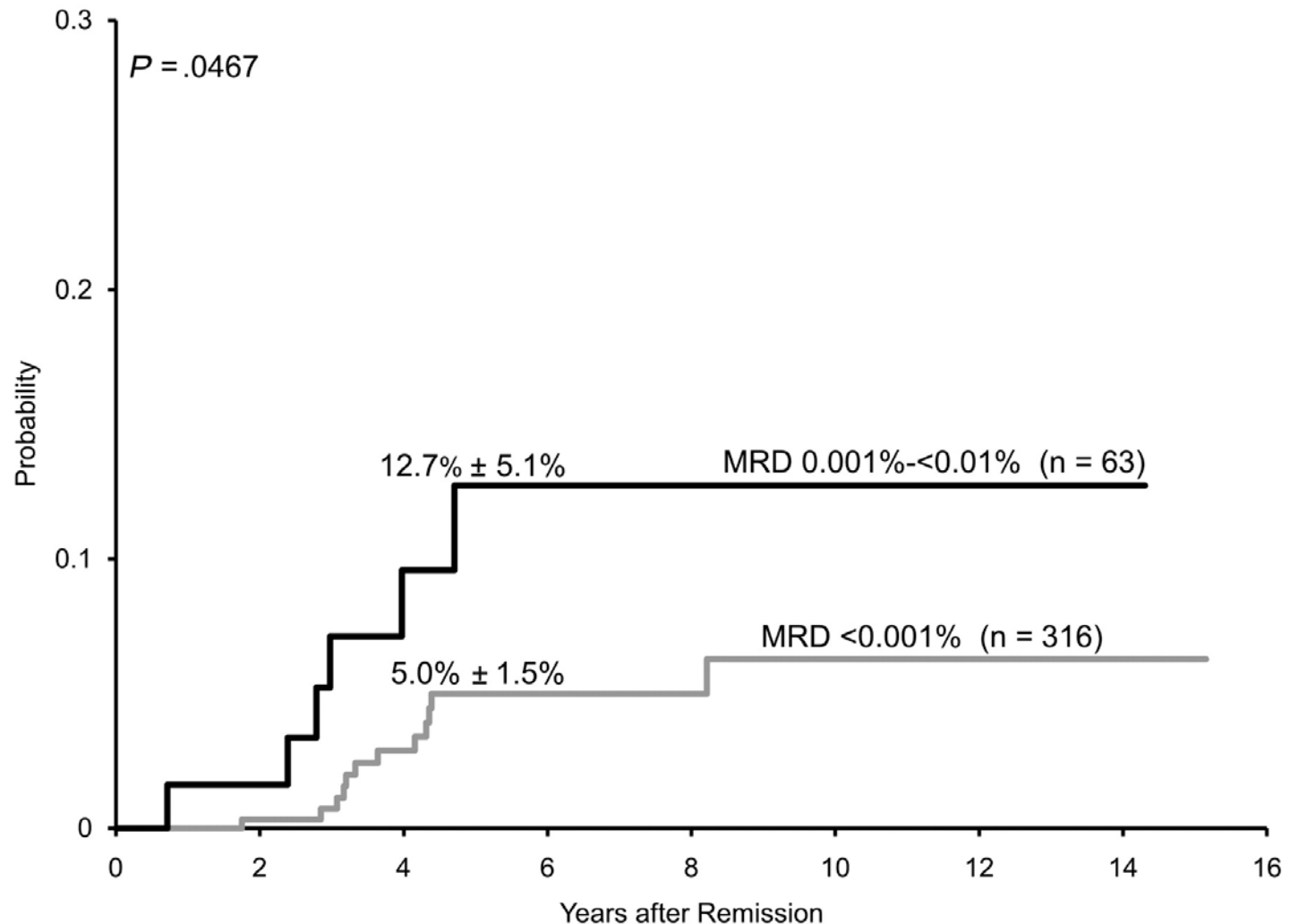


I-BFM-SG



EORTC trial 58881

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



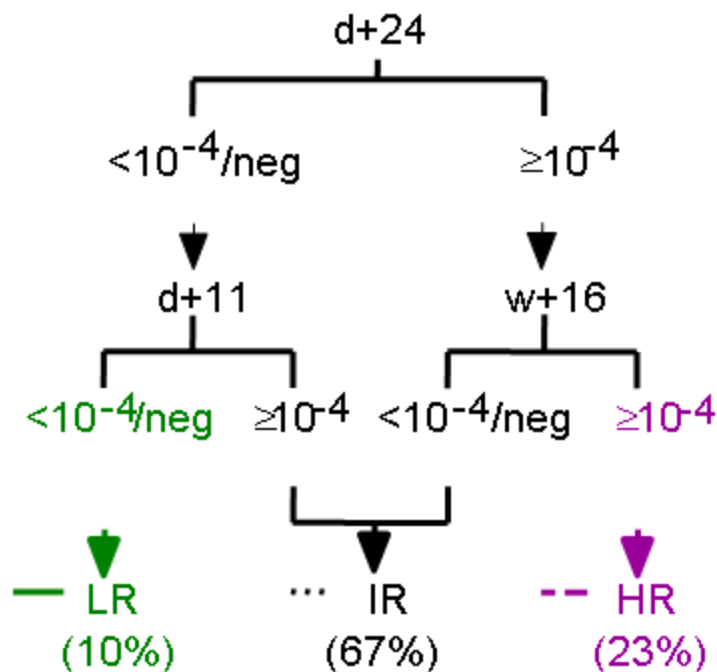
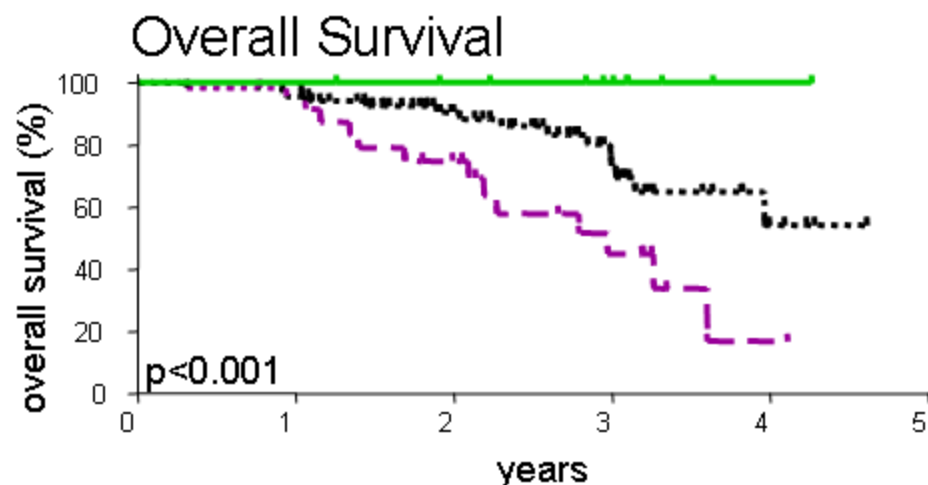
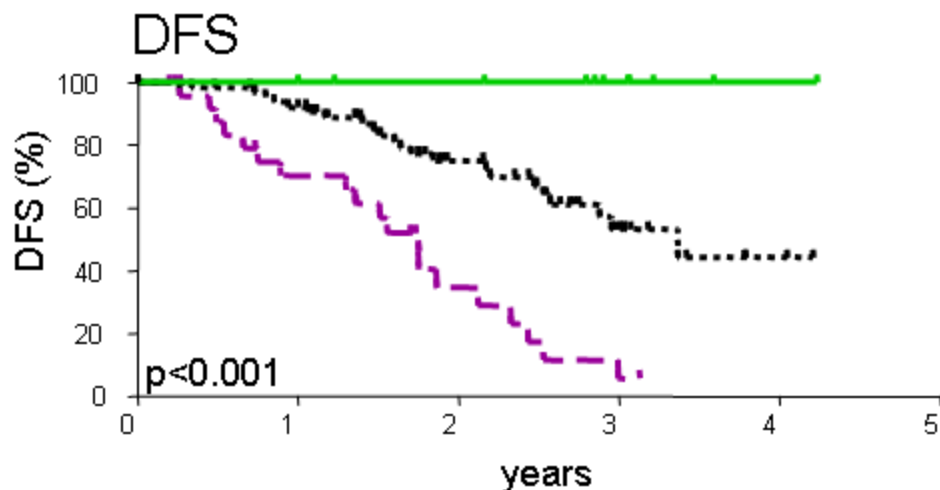
Prognostic Significance of MRD in Adults with Ph-negative ALL

Clinical Trials with Therapeutic Consequences

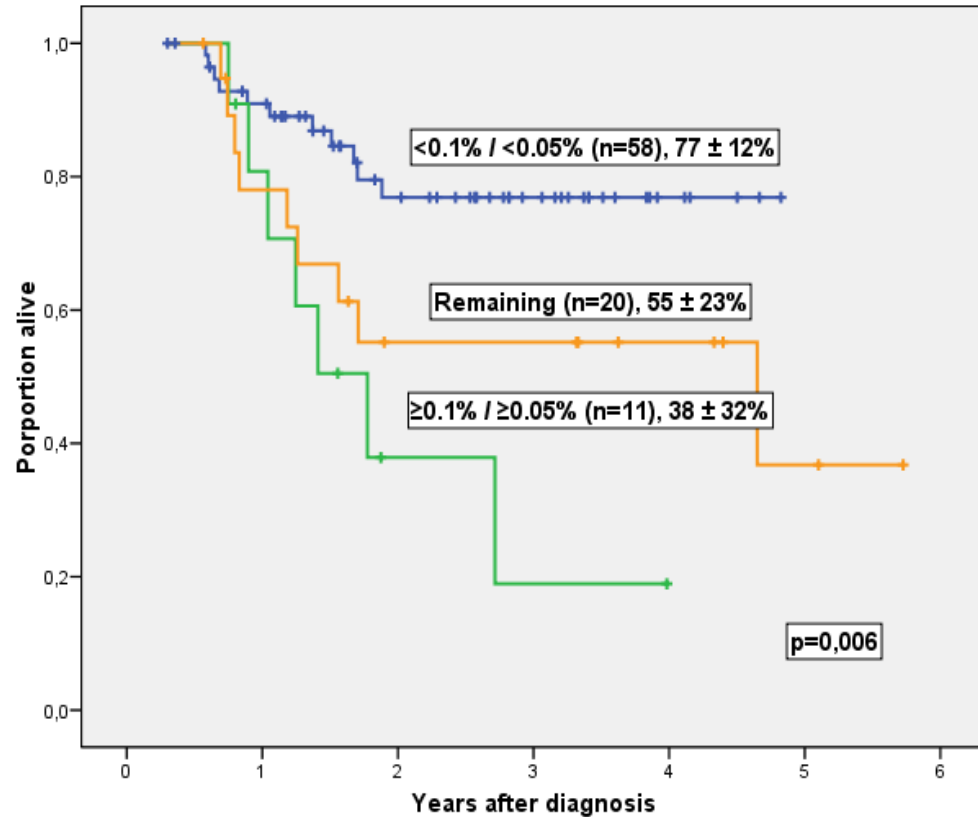
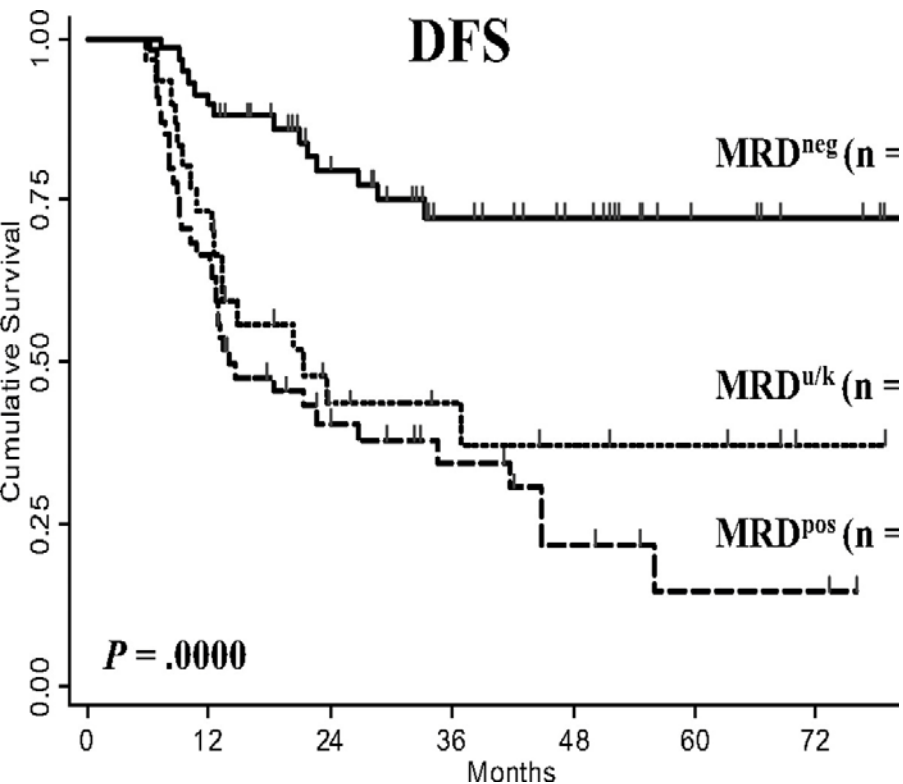
Author	Year	Group	Method	N	Prognostic Model	DFS
Brüggemann	2006	GMALL	PCR	105 SR	$<10^{-4}$ d11 + $<10^{-4}$ d24	100%
					$>10^{-4}$ d24 + $>10^{-4}$ w16	6%
					All others	53%
Bassan	2009	NILG	PCR	142 SR & HR	$<10^{-4}$ wk16, $<10^{-4}$ wk22	72%
					All others	14%
Ribera	2009	PETHEMA	Flow	202 HR	$<10^{-3}$ (wk5) & $<5 \times 10^{-4}$ (wk16)	77%
					$\geq 10^{-3}$ (wk5) & $\geq 5 \times 10^{-4}$ (wk16)	31%

Prognostic significance of MRD in adult ALL

- Standard risk ALL: MRD based risk stratification -



Prognostic significance of MRD in adult ALL



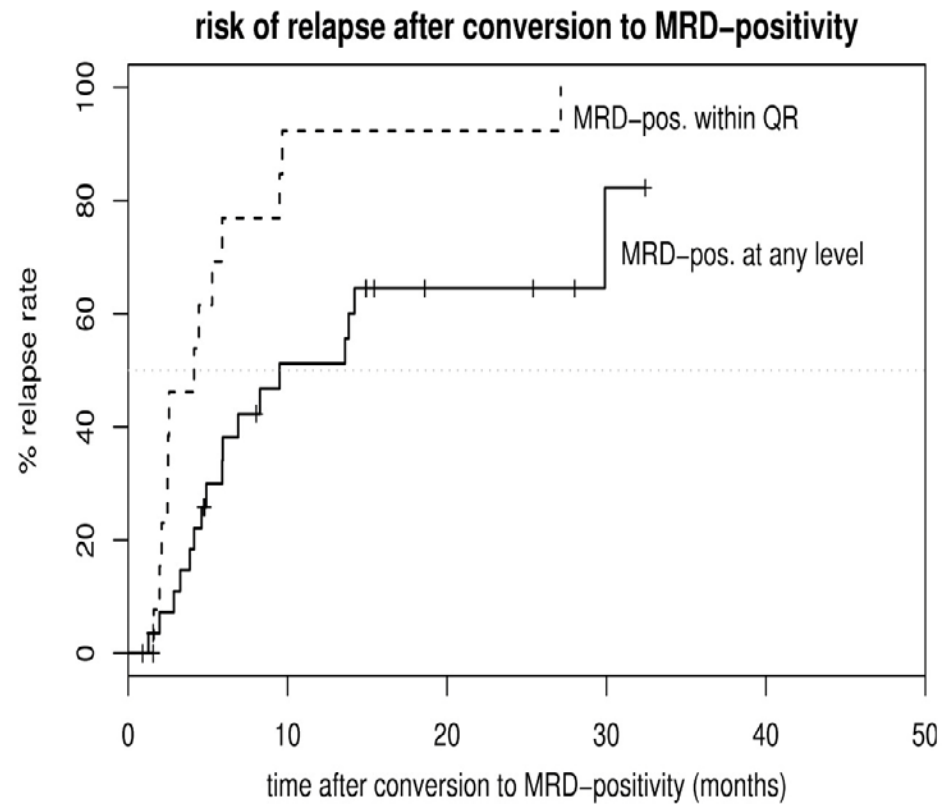
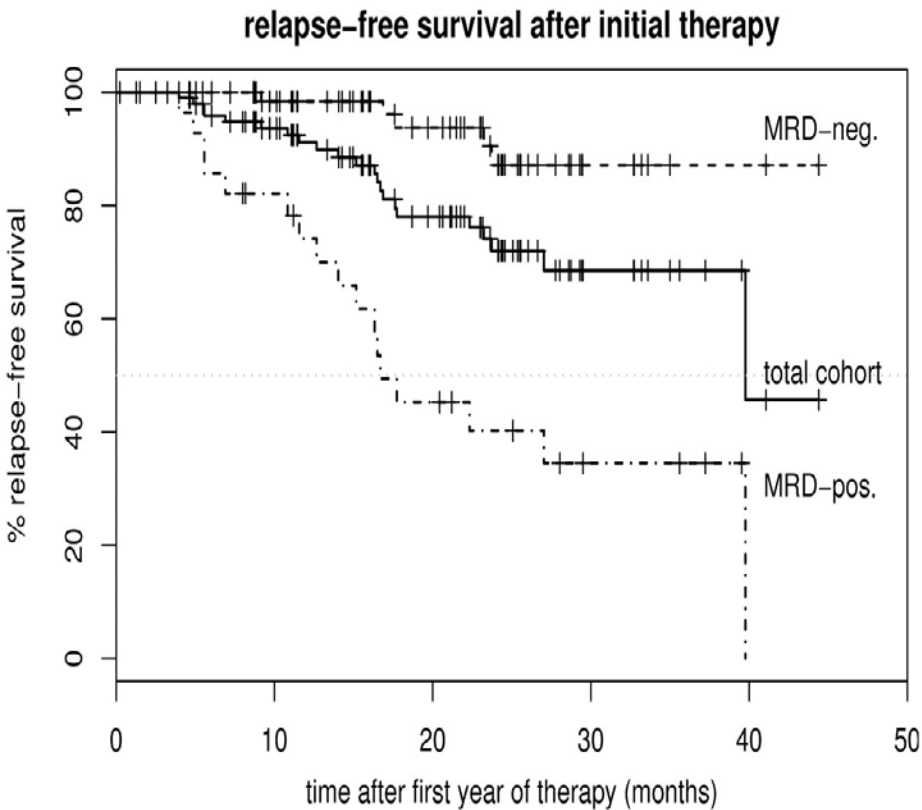
Bassan R, et al. Blood 2009; 113: 4153-4162

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



Prognostic Significance of MRD before SCT

Author	Year	N	Relapse rate (%)	
			MRD-pos	MRD-neg
Patel	2010	25 (A)	75%	23%*
Giebel	2009	123 (A)	57%*	17%*
			62%**	8%**
Bader	2009	91 (P)	57%	13%
Spinelli	2007	37 (A)	46%	0%
Sramkova	2007	25 (P)	75%	6%
Sanchez	2001	40 (A/P)	67%	17%
Van der Velden	2001	17 (P)	67%	20%

* B-lin ALL, ** T-ALL

A microscopic image showing several large, round white blood cells with prominent, textured nuclei and thin rims of cytoplasm, characteristic of lymphoblasts. The cells are stained in shades of orange and yellow against a dark background.

Thank you!

**White blood cells from a patient with acute lymphoblastic
leukaemia**

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