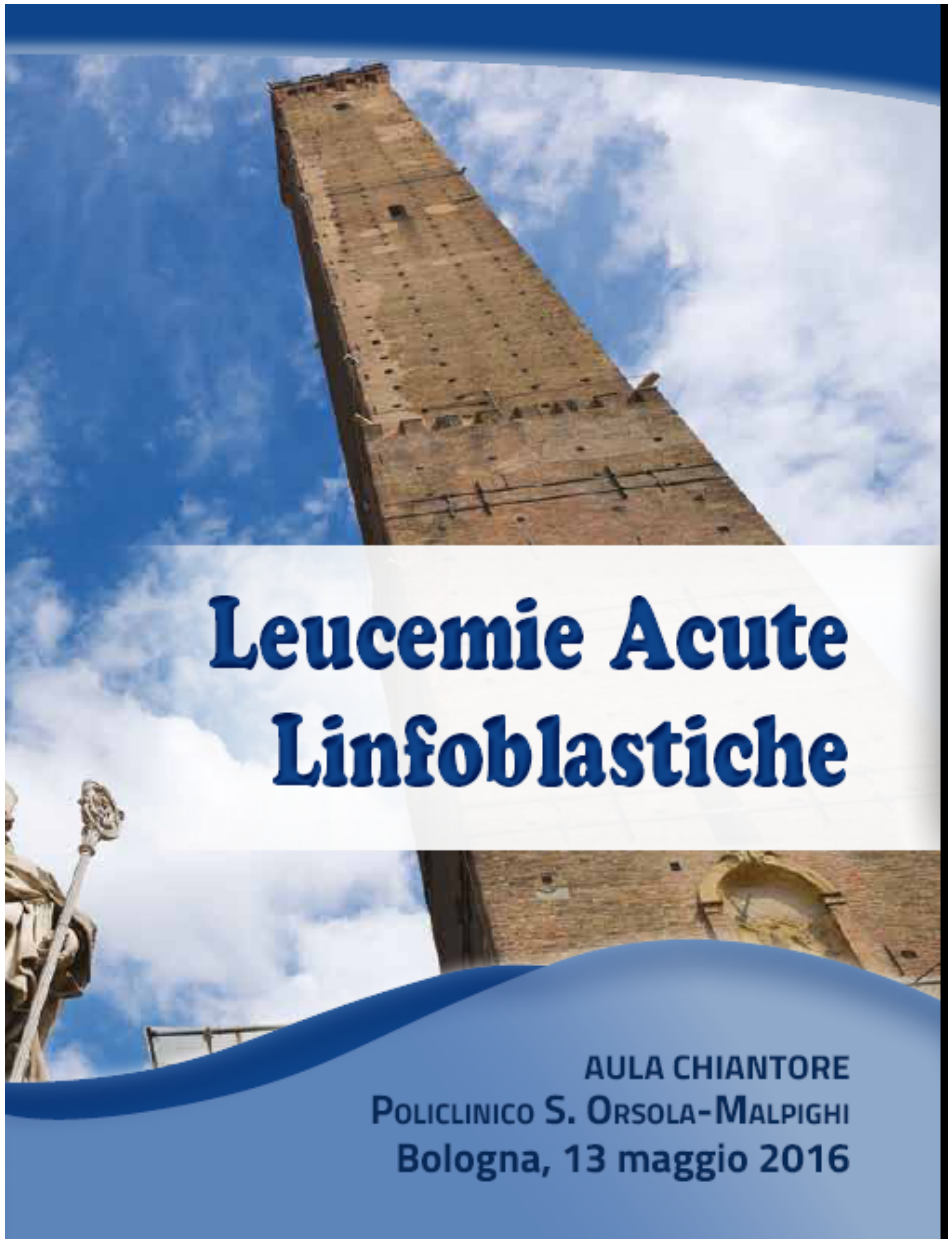


Valutazione MRD: Biologia Molecolare
A.Santoro

Prognostic factor in ALL

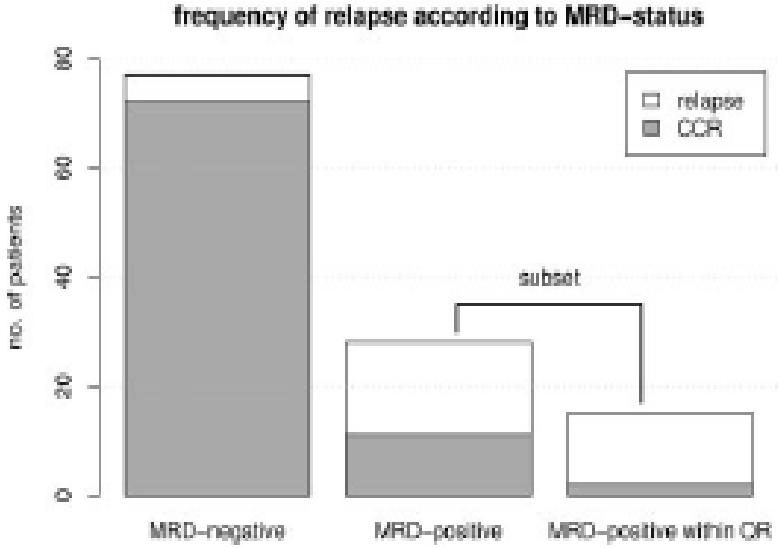
Clinical characteristics	Higher age: > 50 yrs, > 60 yrs High WBC: > 300,00/µl in B-lineage
Immunophenotype	Pro B (B-lin., $CD10^-$) Early T (T-lin., $CD1a^-$, $sCD3^-$) Mature T (T-lin., $CD1a^-$, $sCD3^+$)
Cytogenetics/molecular genetics	t(9;22)/BCR-ABL or t(4;11)/ALL1-AF4
Treatment response	Late achievement of CR: > 3, 4 weeks MRD positivity

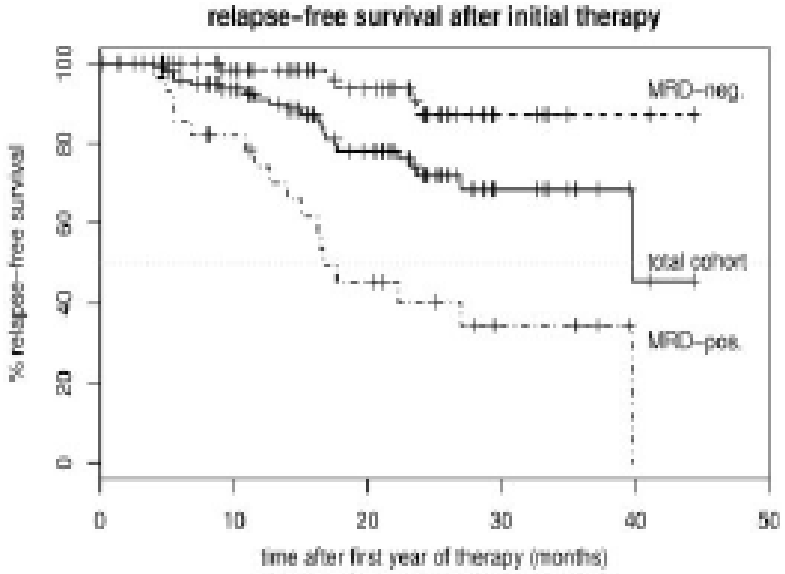
blood

2007 109: 910-915
Prepublished online Oct 5, 2006;
doi:10.1182/blood-2006-07-037093

Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 trials

Thorsten Raff, Nicola Gökbuget, Silke Lüschen, Regina Reutzel, Matthias Ritgen, Sebastian Irmer, Sebastian Böttcher, Heinz-August Horst, Michael Kneba, Dieter Hoelzer, Monika Brüggemann and for the GMALL Study Group





Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL)

Renato Bassan,[1] Orietta Spinelli,[1] Elena Oldani,[1] Tamara Intermesoli,[1] Manuela Tosi,[1] Barbara Peruta,[1] Giuseppe Rossi,[2] Erika Borlenghi,[2] Enrico M. Pogliani,[3] Elisabetta Terruzzi,[3] Pietro Fabris,[4] Vincenzo Cassibba,[4] Giorgio Lambertenghi-Deliliers,[5] Agostino Cortelezzi,[5] Alberto Bosi,[6] Giacomo Gianfaldoni,[6] Fabio Ciceri,[7] Massimo Bernardi,[7] Andrea Gallamini,[8] Daniele Mattei,[8] Eros Di Bona,[9] Claudio Romani,[10] Anna Maria Scattolin,[11] Tiziano Barbui,[1] and Alessandro Rambaldi[1]

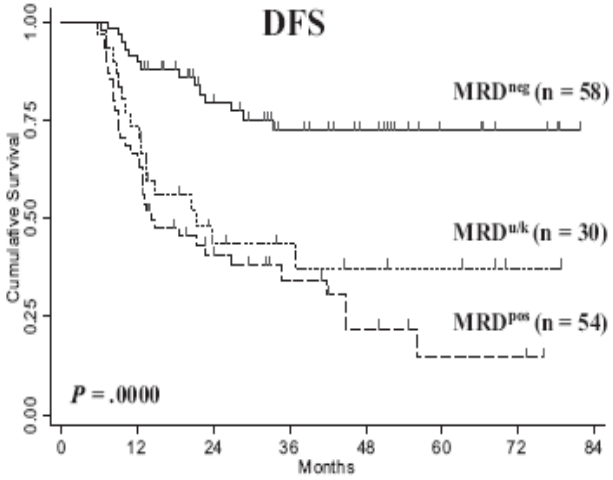


Table 2. Details of 308 molecular probes obtained in 223 patients

Probe type and sensitivity	First probe, 223 patients, no.				Second probe, 85 patients, no.				Total probes, no. (%)
	10^{-5}	10^{-4}	10^{-3}	Total	10^{-5}	10^{-4}	10^{-3}	Total	
IgH/TCR rearrangements									
IgH	25	14	1	40	6	7	3	16	56 (18.2)
Ig—kappa	2	5	1	8	3	5	—	8	16 (5.2)
TCRD	14	15	4	33	5	14	4	23	56 (18.2)
TCRB	5	6	—	11	6	7	—	13	24 (7.8)
TCRG	22	15	7	44	2	10	13	25	69 (22.4)
Fusion genes									
BCR-ABL	61	—	—	61	—	—	—	—	61 (19.8)
MLL-AF4	—	20	—	20	—	—	—	—	20 (6.5)
E2A-PBX1	5	—	—	5	—	—	—	—	5 (1.6)
SIL-TAL1	—	1	—	1	—	—	—	—	1 (0.3)
Total (%)	134 (60)	76 (34)	13 (6)	223 (100)	22 (25.9)	43 (50.6)	20 (23.5)	85 (100)	308 (100)

Randomized study of reduced-intensity chemotherapy combined with imatinib in adults with Ph-positive acute lymphoblastic leukemia

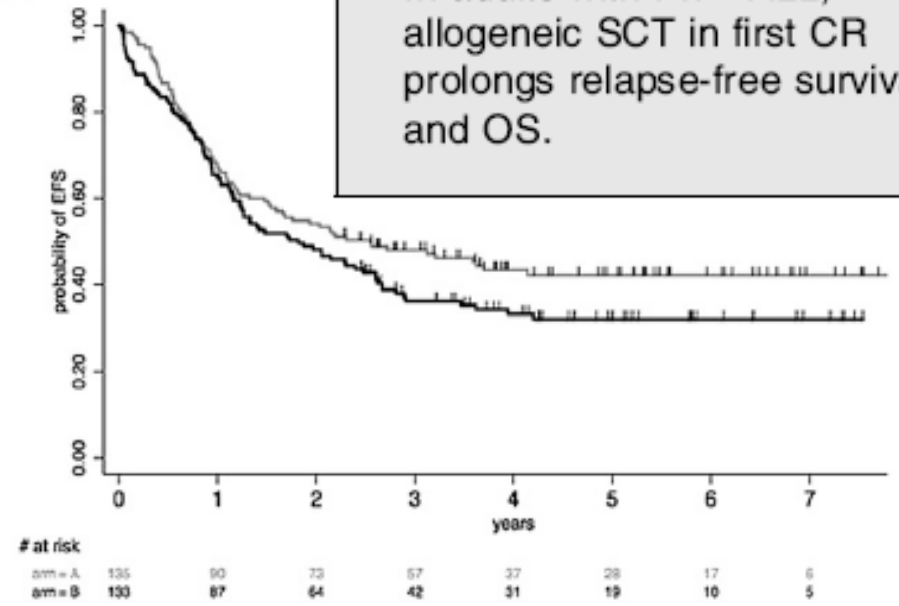
Yves Chalandon,[1,2] Xavier Thomas,[3] Sandrine Hayette,[3] Jean-Michel Cayuela,[4] Claire Abbal,[5] Françoise Huguet,[6] Emmanuel Raffoux,[4] Thibaut Leguay,[7] Philippe Rousselot,[8] Stéphane Lepretre,[9] Martine Escoffre-Barbe,[10] Sébastien Maury,[11] Céline Berthon,[12] Emmanuelle Tavernier,[13] Jean-François Lambert,[2,5] Marina Lafage-Pochitaloff,[14] Véronique Lhéritier,[15] Sylvie Chevret,[16] Norbert Ifrah,[17] and Hervé Dombret,[4] for the Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL)

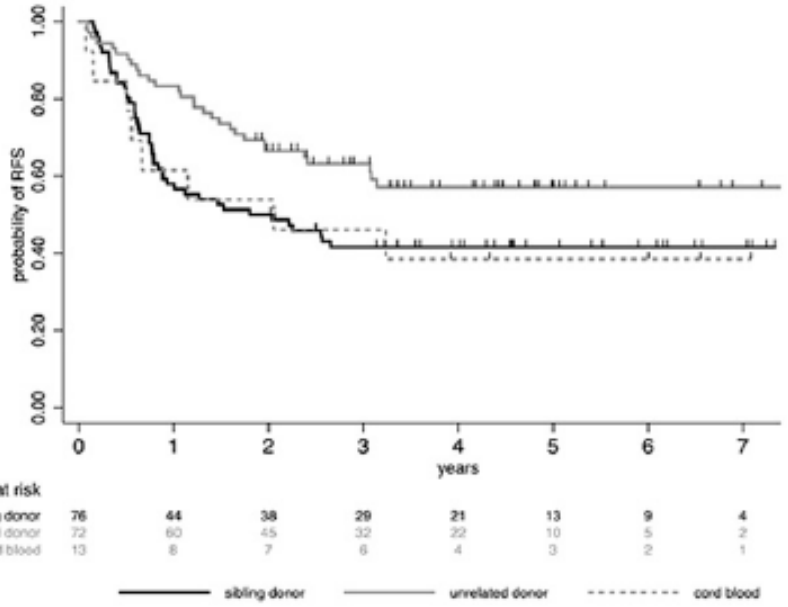
BLOOD, 11 JUNE 2015 • VOLUME 125, NUMBER 24

Key Points

- Initial imatinib-based therapy of Ph+ adult ALL is associated with lower early mortality and higher CR rate.
- In adults with Ph+ ALL, allogeneic SCT in first CR prolongs relapse-free survival and OS.

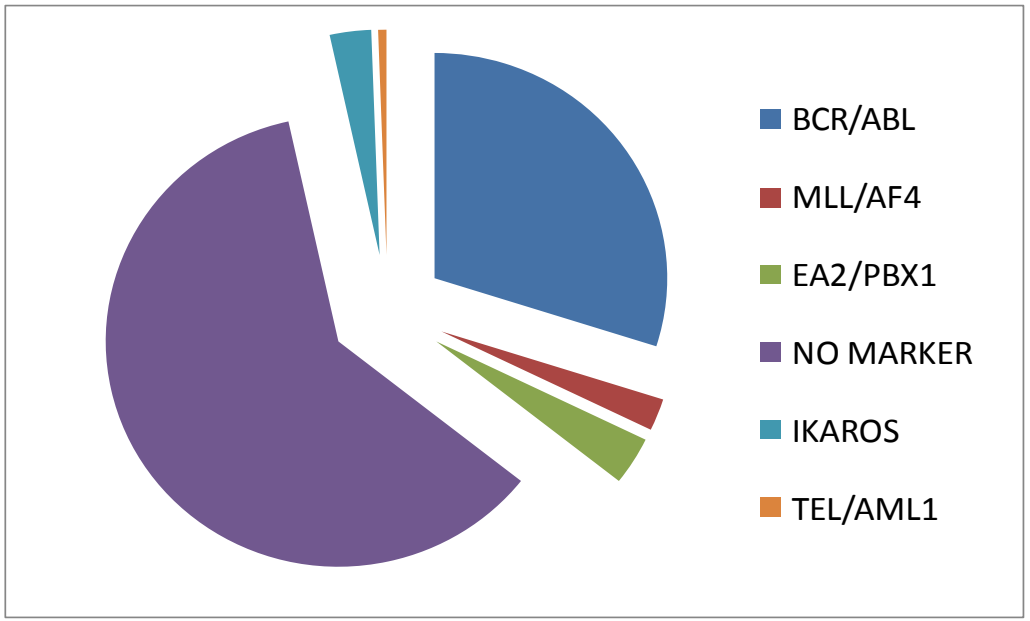
A





ALL Rete siciliana 2011-2015 (170 pts)

Aberration	Target (mRNA or DNA)	Frequency of Applicability (%)[b] Children	Adults
Precursor-B-ALL			
t(9;22)(q34;q11)	*BCR-ABL* (mRNA)	5-8	30-35
t(1;19)(q23;p13)	*E2A-PBX1* (mRNA)	5-8	3-4
t(4;11)(q21;q23)	*MLL-AF4* (mRNA)	3-5[c]	3-4
11q23 aberrations	aberrant *MLL* (mRNA)	5-6[c]	< 5
t(12;21)(p13;q22)	*TEL-AML1* (mRNA)	~30	1-3
TOTAL		**40-45**	**40-45**
T-ALL			
TAL1 deletion	*SIL-TAL1* (DNA/mRNA)	10-25	5-10
t(8;14)(q24;q11)	*c-MYC-TCRA/D* (DNA)	5-10	5-10
t(11;14)(p15;q11)	*LMO1-TCRD* (DNA)		
t(11;14)(p13;q11)	*LMO2-TCRD* (DNA)		
t(1;14)(p34;q11)	*TAL1-TCRD* (DNA)		
t(10;14)(q24;q11)	*HOX11-TCRD* (DNA)		
TOTAL		**25-30**	**10-15**



FUSION GENE TRANSCRIPTS
MINIMAL RESIDUAL DISEASE

Leukemia (2003) 17, 2318–2357

www.nature.com/leu

LEADING ARTICLE

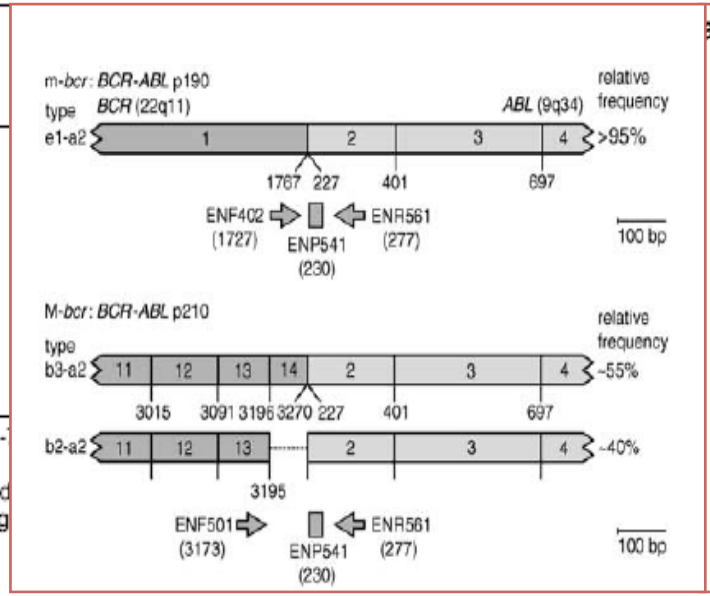
Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – A Europe Against Cancer Program

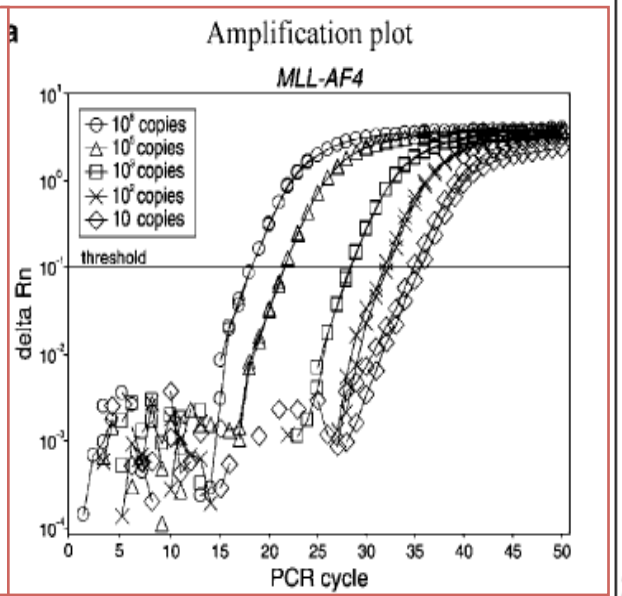
J Gabert[1,17,18,19], E Beillard[1,17], VHJ van der Velden[2], W Bi[3], D Grimwade[4], N Pallisgaard[5], G Barbany[6,20], G Cazzaniga[7], JM Cayuela[8], H Cavé[9], F Pane[10], JLE Aerts[11], D De Micheli[12], X Thirion[13], V Pradel[13], M González[14], S Viehmann[15], M Malec[16], G Saglio[12] and JJM van Dongen[2]

Table 1 Organization of EAC Concerted Action via nine networks for the optimization of the detection of the main fusion gene transcripts in leukemias plus one network focusing on control genes

Chromosome aberration	*RQ-PCR target*
t(1;19)(q23;p13)	*E2A-PBX1*
t(4;11)(q21;q23)	*MLL-AF4*
t(12;21)(p13;q22)	*TEL-AML1*
t(9;22)(q34;q11)	*BCR-ABL m-bcr*
t(9;22)(q34;q11)	*BCR-ABL M-bcr*
del(1)(p32p32)	*SIL-TAL1*
t(15;17)(q22;q21)	*PML-RARA*
inv(16)(p13q22)	*CBFB-MYH11*
t(8;21)(q22;q22)	*AML1-ETO*
—	Control genes

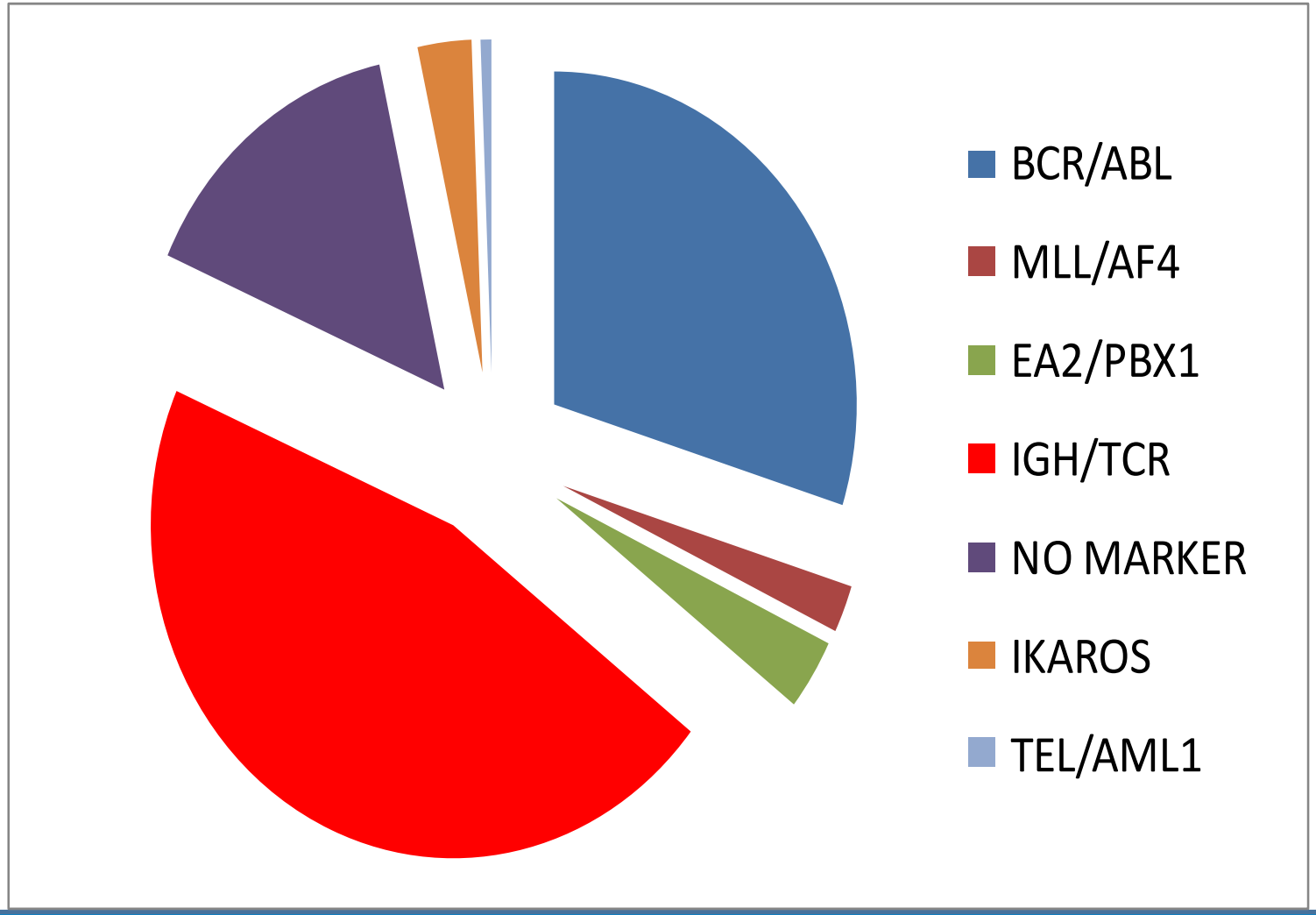
[a]The cell lines used during phases I–IVa are listed; for *SIL-*
[b]Analyzed diagnostic samples during phase IVb.
[c]For *PML-RARA* bcr2, bcr3 and *CBFB-MYH11* type D and
[d]During phases I, II, IIIa and IVa, including the coordinating





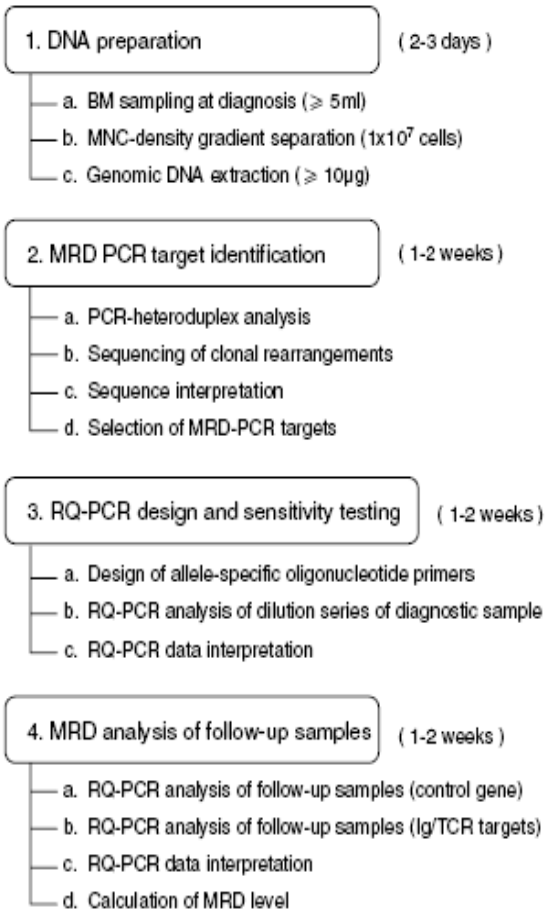
IGH/TCR JUNCTIONAL REGION MINIMAL RESIDUAL DISEASE

ALL Rete siciliana 2011-2015 (170 pts)



	Flow Cytometric Immunophenotyping	PCR Analysis of Chromosome Aberrations (mainly detection of fusion gene transcripts)	PCR Analysis of Ig/TCR Genes (junctional region specific approach)
Sensitivity	10^{-3}–10^{-4}	10^{-4}–10^{-6}	10^{-4}–10^{-5}
Applicability			
Precursor-B-ALL	60-98%	40-45%*	90-95%
T-ALL	90-95%	15-35%**	90-95%
Advantages	• applicable for most patients • relatively cheap • rapid: 1-2 days	• relatively easy and cheap • sensitive and leukemia-specific • stable target during disease course • rapid: 2-3 days • suitable for monitoring of uniform patient groups (e.g., Ph^+ ALL)	• applicable for virtually all patients, if *IGH*, *IGK*-Kde, *TCRG*, and *TCRD* gene rearrangements are used as targets • sensitive and patient-specific • rapid during follow-up: 2-3 days (if junctional region is identified and if RQ-PCR is used)
Disadvantages	• limited sensitivity • need for preferably two aberrant immunophenotypes per patient, because of chance of immuno-phenotypic shifts	• useful in only a minority of patients • cross-contamination of PCR products leading to false-positive results (even at diagnosis)	• time-consuming at diagnosis: identification of the junctional regions and sensitivity testing • relatively expensive • need for preferably two PCR targets per patient, because of chance of clonal evolution

MRD Detection in ALL Patients



Detection and selection of clonal Ig/TCR gene rearrangements at diagnosis:

Ig/TCR PCR

Sequencing of clonal rearrangements

Sequence interpretation

Selection of MRD-PCR targets

Guidelines BIOMED

↓

RQ-PCR sensitivity testing:

Design of allele-specific oligonucleotide primers

RQ-PCR analysis of dilution series of diagnostic sample

RQ-PCR data interpretation: quantitative range and sensitivity

Guidelines ESG-MRD-ALL

↓

MRD analysis of follow-up samples

Control gene RQ-PCR analysis

MRD-PCR target RQ-PCR analysis

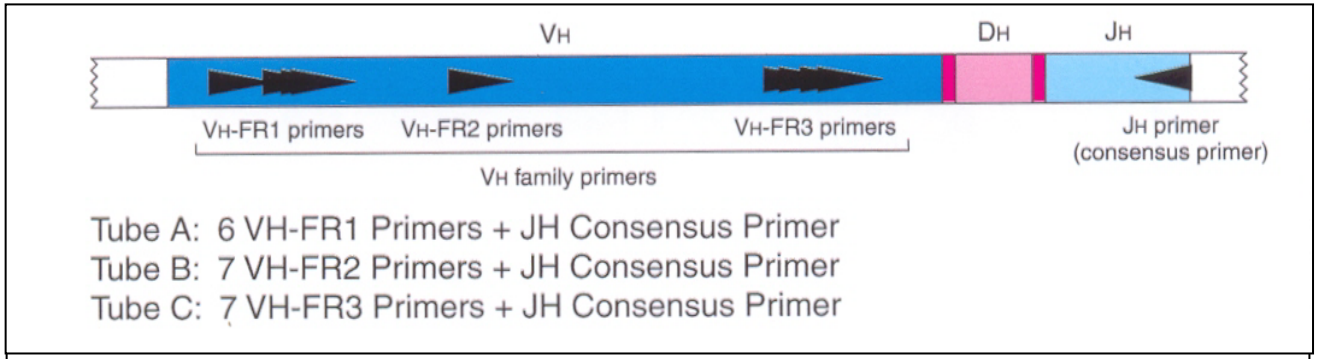
RQ-PCR MRD data interpretation

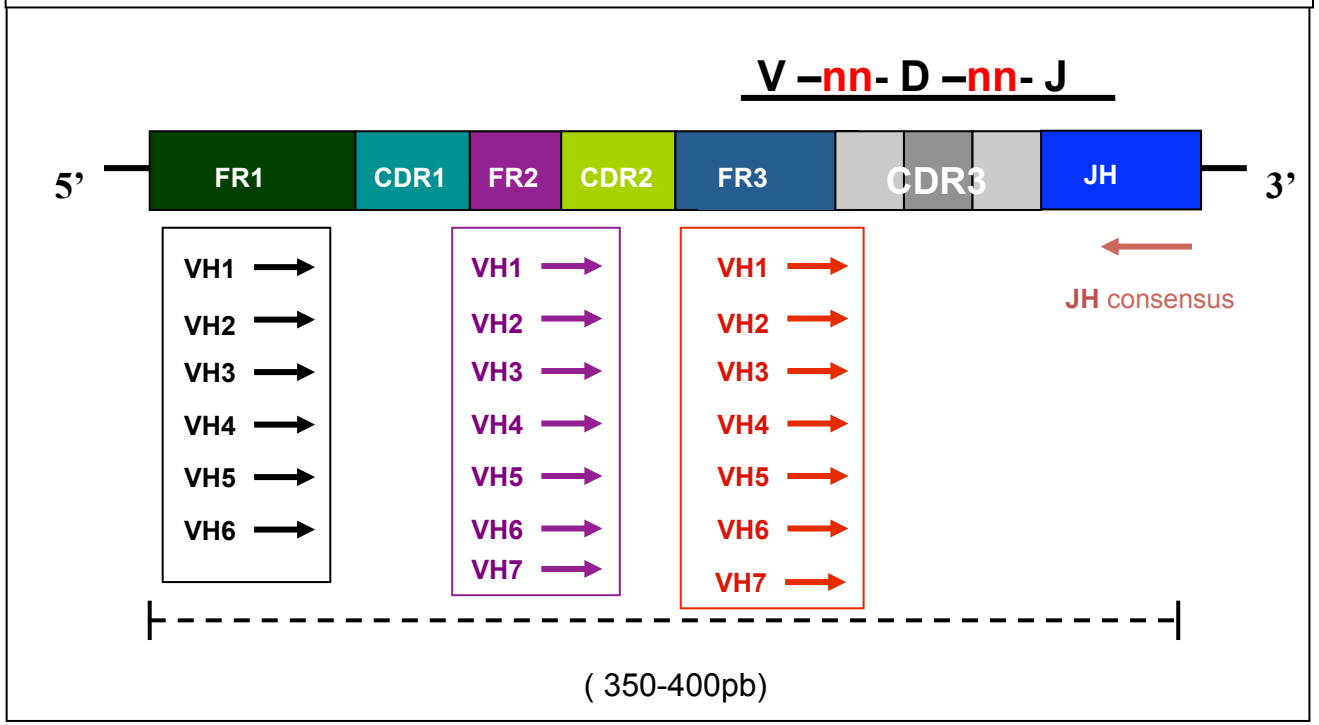
Guidelines ESG-MRD-ALL

Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98-3936

JJM van Dongen[1], AW Langerak[1], M Brüggemann[2], PAS Evans[3], M Hummel[4], FL Lavender[5], E Delabesse[6], F Davi[7], E Schuuring[8,9], R García-Sanz[10], JHJM van Krieken[11], J Droese[2], D González[10], C Bastard[12], HE White[5], M Spaargaren[13], M González[10], A Parreira[14], JL Smith[5], GJ Morgan[3], M Kneba[2] and EA Macintyre[6]

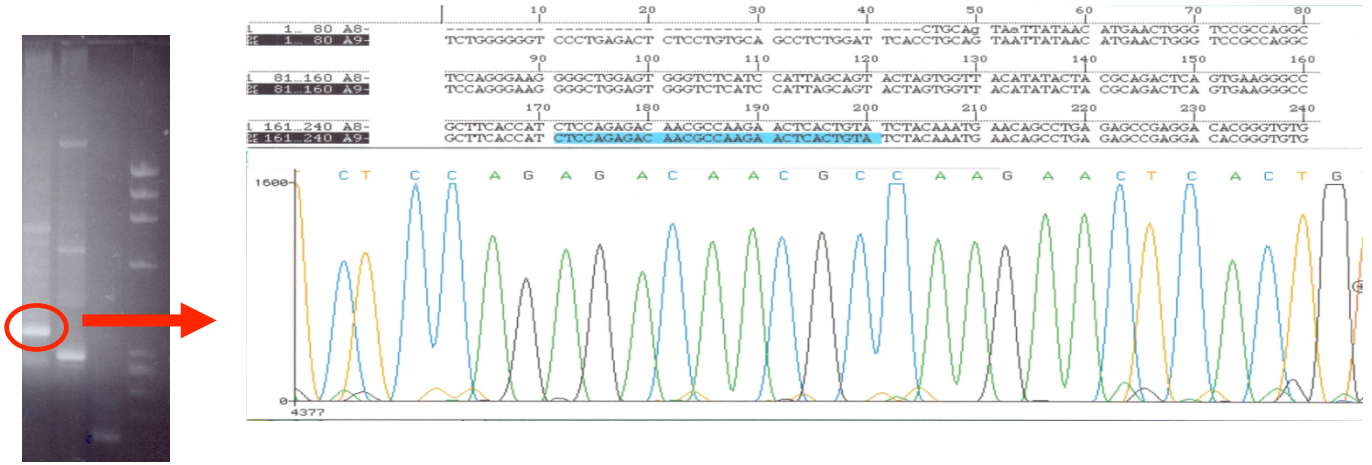
Leukemia (2003) 17, 2257–2317





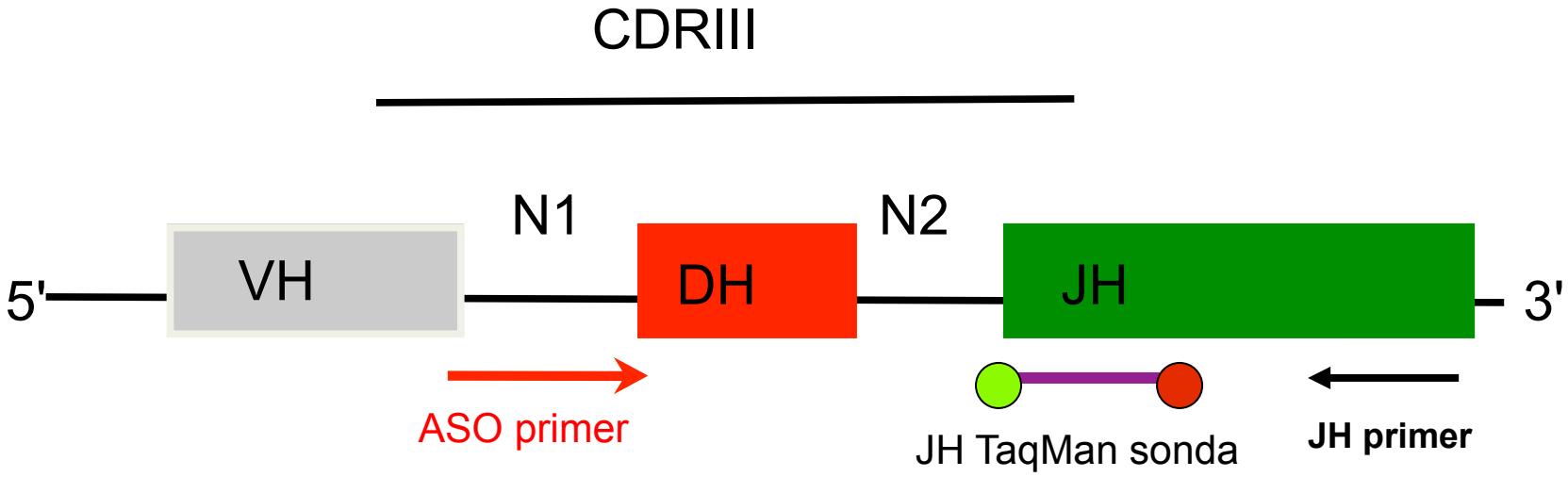
IGH VH-JH
IGH DH-JH
IGK-Kde
TCRB VB-JB
TCRB DB-JB
TCRG
TCRD V-D-J
TCRD V-D
TCRD D-J
TCRD D-D

PCR QUANTITATIVA (Q-PCR) - ASO PRIMER

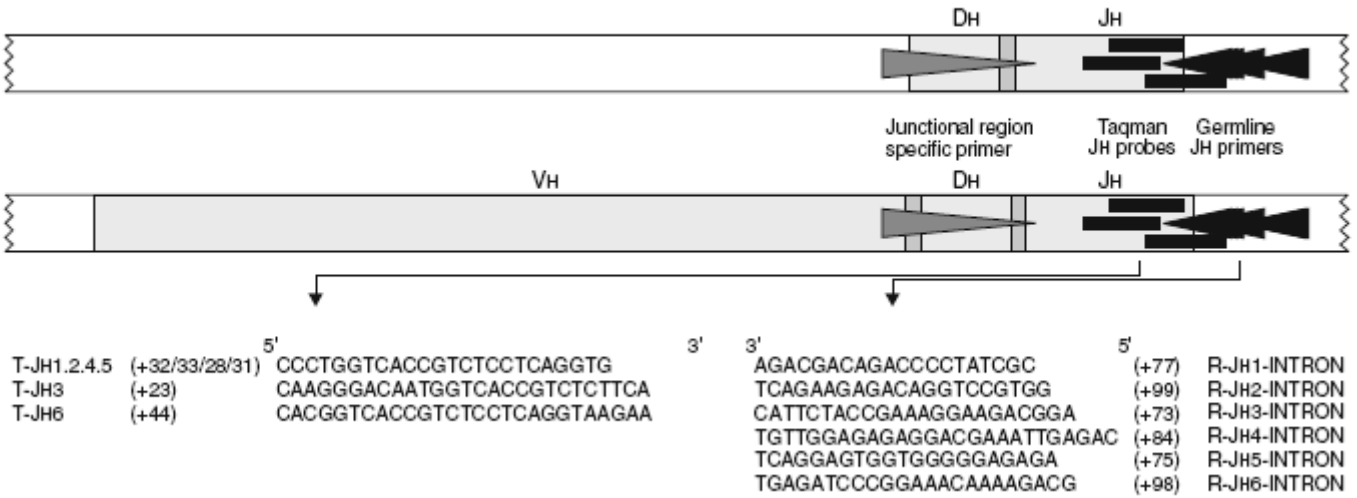


VH2-70*11 D2-2*03 JH6*03 99,6%

CCCCAGGGCAGGGTCACCGACGACCACCTGTCTGGTCCTACGCTGGTGAAACCCACACAGACCCTCACACTGACCTGCACCTTCTCTGGG
TTCTCACTCAGCACTAGTGGAATGTGTGTGAGCTGGATCCGTCAGCCCCCAGGGAAGGCCCTGGAGTGGCTTGCACGCATTGATTGGGATG
ATGATAAATACTACAGCACATCTCTGAAGACCAGGCTCACCATCTCCAAGGACACCTCCAAAAACCAGGTGGTCCTTACAATGACCAACATG
GACCCTGTGGACACAGCCACGTATTACTGTGCACGG**TTCCACTAGGTATATTGTAGTAGTACCAGCTG**CCCCTGGGGTCCCCC**TACTACTAC**
TACTACTACATGGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCCTCAGGTAAGA

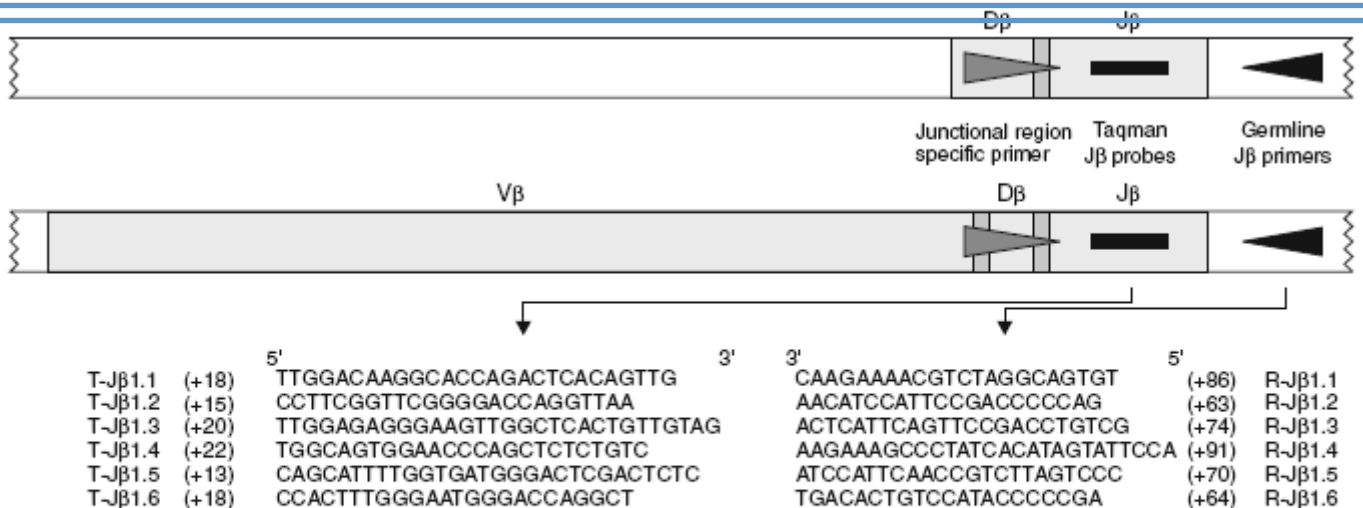


IGH



T-JH1.2.4.5	(+32/33/28/31)	CCCTGGTCACCGTCTCCTCAGGTG	AGACGACAGACCCCTATCGC	(+77)	R-JH1-INTRON
T-JH3	(+23)	CAAGGGACAATGGTCACCGTCTCTTCA	TCAGAAGAGACAGGTCCGTGG	(+99)	R-JH2-INTRON
T-JH6	(+44)	CACGGTCACCGTCTCCTCAGGTAAGAA	CATTCTACCGAAAGGAAGACGGA	(+73)	R-JH3-INTRON
			TGTTGGAGAGAGGACGAAATTGAGAC	(+84)	R-JH4-INTRON
			TCAGGAGTGGTGGGGGAGAGA	(+75)	R-JH5-INTRON
			TGAGATCCCGGAAACAAAAGACG	(+98)	R-JH6-INTRON

TRB

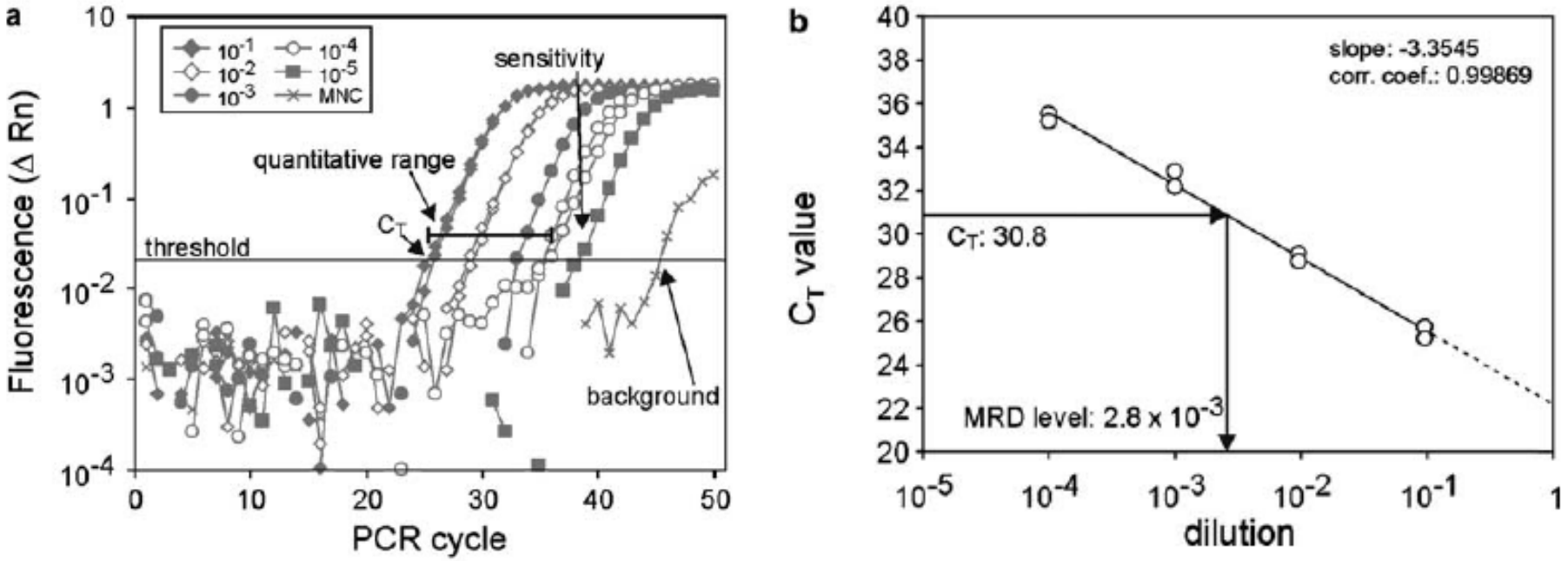


T-Jβ1.1	(+18)	TTGGACAAGGCACCAGACTCACAGTTG	CAAGAAAACGTCTAGGCAGTGT	(+86)	R-Jβ1.1
T-Jβ1.2	(+15)	CCTTCGGTTCGGGGACCAGGTTAA	AACATCCATTCCGACCCCCAG	(+63)	R-Jβ1.2
T-Jβ1.3	(+20)	TTGGAGAGGGAAGTTGGCTCACTGTTGTAG	ACTCATTCAGTTCCGACCTGTCG	(+74)	R-Jβ1.3
T-Jβ1.4	(+22)	TGGCAGTGGAACCCAGCTCTCTGTC	AAGAAAGCCCTATCACATAGTATTCCA	(+91)	R-Jβ1.4
T-Jβ1.5	(+13)	CAGCATTTTGGTGATGGGACTCGACTCTC	ATCCATTCAACCGTCTTAGTCCC	(+70)	R-Jβ1.5
T-Jβ1.6	(+18)	CCACTTTGGGAATGGGACCAGGCT	TGACACTGTCCATACCCCCGA	(+64)	R-Jβ1.6
T-Jβ2.1	(+08)	ATGAGCAGTTCTTCGGGCCAGGGA	AGTGGCACGATCCATTCTTCCC	(+60)	R-Jβ2.1
T-Jβ2.2	(+21)	TTGGAGAAGGCTCTAGGCTGACCGTA	ACCCATTCCTCCGCCAAC	(+66)	R-Jβ2.2
T-Jβ2.3	(+13)	AGTATTTTGGCCCAGGCACCCGG	TGTCACGAGCCATTCGCCC	(+58)	R-Jβ2.3
T-Jβ2.4	(+27)	CGGGACCCGGCTCTCAGTGCT	CATTCGACCCCGGCGG	(+66)	R-Jβ2.4
T-Jβ2.5	(+11)	CAGTACTTCGGGCCAGGCACGC	CGAGGACCACGAGCCACT	(+52)	R-Jβ2.5
T-Jβ2.6	(+10)	AACGTCCTGACTTTCGGGGCCG	CGACTGGCACGACCCACT	(+57)	R-Jβ2.6
T-Jβ2.7	(+18)	CGGGCACCAGGCTCACGGTC	AGAGGGGGGTGGAAGGTCGG	(+79)	R-Jβ2.7

Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data

VHJ van der Velden[1], G Cazzaniga[2], A Schrauder[3], J Hancock[4], P Bader[5], ER Panzer-Grumayer[6], T Flohr[7], R Sutton[8], H Cave[9], HO Madsen[10], JM Cayuela[11], J Trka[12], C Eckert[13], L Foroni[14], U zur Stadt[15], K Beldjord[16], T Raff[17], CE van der Schoot[18] and JJM van Dongen[1], on behalf of the European Study Group on MRD detection in ALL (ESG-MRD-ALL)

Leukemia (2007) 21, 604–611



Quantitative Range: Precisione dei replicati / Separazione dal background

Sensibilità: Separazione dal background

Ricerca e selezione del marcatore all'esordio

Frequency of Ig/TCR gene rearrangements in childhood ALL

Gene	Type of rearrangement	Precursor-B-ALL (%)	T-ALL (%)
IGH	V_H-J_H	80–85	~5
	D_H-J_H	~20	~20
	Total *IGH*	>95	20–25
IGK	Vκ-Kde	45	0
	Intron-Kde	15–25	0
	Vκ-Jκ	30	NT
	Total *IGK*-Kde	60–75	0
IGL	Vλ-Jλ	15–20	NT
TCRB	Vβ-Jβ	25–30	80
	Dβ-Jβ	15	55
	Total *TCRB*	35	90
TCRG	Vγ-Jγ	50–60	95
TCRD	Vδ2-Dδ3 or Dδ2-Dδ3	40	5–10
	Vδ-Jδ1 or Dδ2-Jδ1	<1	50
	Total *TCRD*	40	55
TCRD/A	Vδ2-Jα	40–45	NT

Overall sensitivities of Ig/TCR gene rearrangements in RQ-PCR assays

Rearrangement		Quantitative range of at least 10^{-4} (%)[a]	Sensitivity of at least 10^{-4} (%)[a]
IGH	DJ	50	75
	VDJ	80	95
IGK-Kde		80	90
IGK Vκ-Jκ		45	80
Vλ-Jλ		50	80
TCRD	Incomplete	45	90
	Complete	80	95
Vδ2-Jα		75	90
TCRB	VDJ	70	90
	DJ	55	90
TCRG	precursor-B-ALL	25	45
	T-ALL	70	80

[a]Percentage of rearrangements with quantitative range/sensitivity of at least 10^{-4}

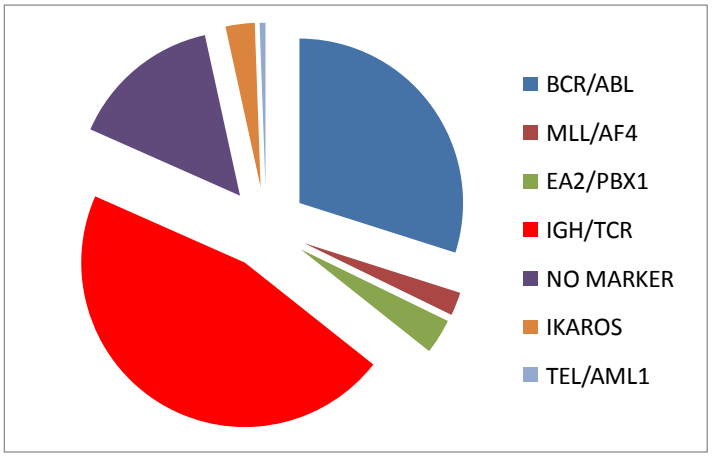
Il doppio marcatore

Stability of Ig/TCR gene rearrangements between diagnosis and relapse in childhood ALL patients

PCR target	Monoclonality (%)	Stability at relapse (%)		
		Monoclonal	Oligoclonal	All
Precursor-B-ALL				
IGH	60–70	86	45	61
IGK-Kde	90	95	<50	90
Vκ-Jκ	60–70	>95	70	89
Vλ-Jλ	Nt	77	Nt	77
TCRB	90	Nt	Nt	73
TCRG	60–65	Nt	Nt	75
TCRD	60	86	26	63
Vδ2-Jα	65	83	44	66
T-ALL				
TCRB	>95	Nt	Nt	80
TCRG	>95	Nt	Nt	86
TCRD	>95	Nt	Nt	100

LAL rete Siciliana : MRD data

(80 Patients/ 8 Centers)



500 MRD DETECTION TEST

	1x10E-3	5x10E-4	1x10E-4	1x10E-5
Sensibilità	**100%**	**95%**	**85%**	**60%**
Quantitative Range	**100%**	**85%**	**60%**	**5%**

GIMEMA LAL 1913: MRD data

	Roma Lab (Della Starza)	Palermo Lab (Santoro)	Bergamo Lab (Spinelli/Tosi)	Total
Case no.*	42	37	36	**113**
Center no.	19	15	11	**35**
Cases per center, median (range)	2 (1-7)	1.5 (1-4)	3 (1-7)	**2.8 (1-7)**
MRD probe(s), no. (%)	30 (71.5)	35 (94)	31 (86)	**96 (85)**

*patient-specific samples received for MRD analysis

ESLHO european scientific foundation of laboratory hemato oncology

EuroMRD a division of ESLHO

IKAROS

The NEW ENGLAND JOURNAL of MEDICINE

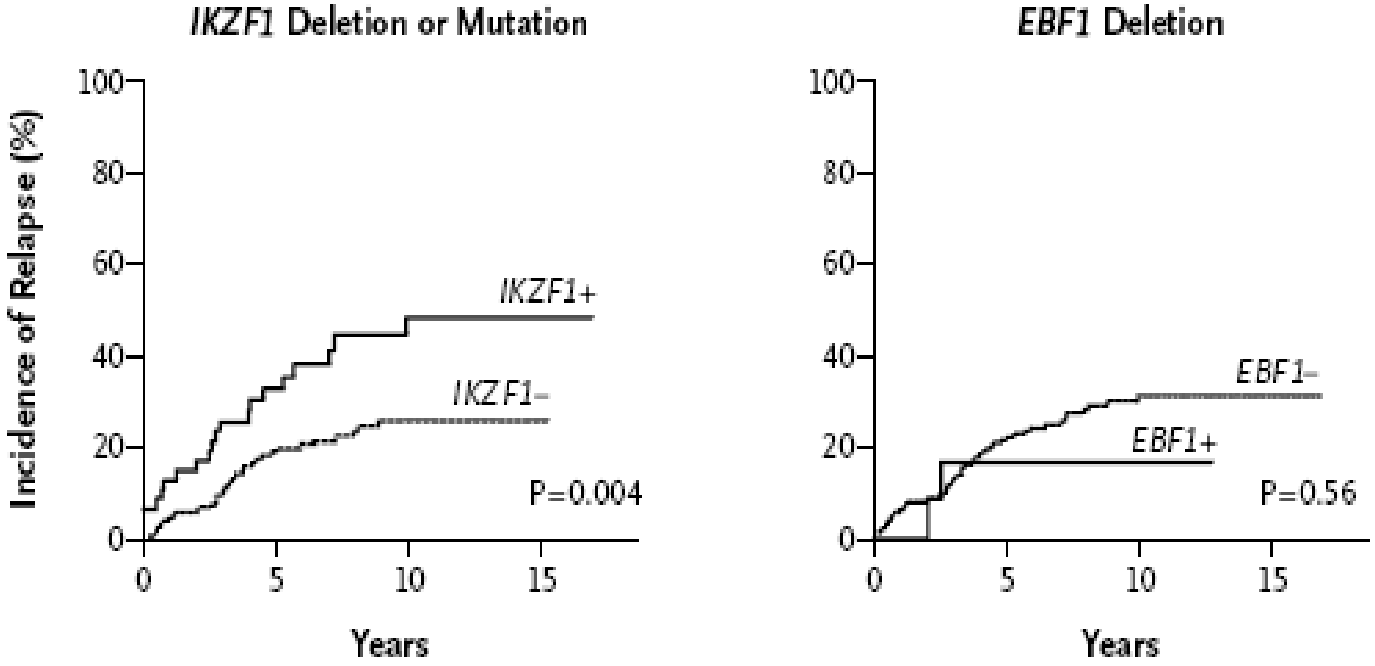
2009

Deletion of *IKZF1* and Prognosis in Acute Lymphoblastic Leukemia

Charles G. Mullighan, M.D., Xiaoping Su, Ph.D., Jinghui Zhang, Ph.D.,

More than 50 recurring copy-number abnormalities were identified, most commonly involving genes that encode regulators of B-cell development (in 66.8% of patients in the original cohort); *PAX5* was involved in 31.7% and *IKZF1* in 28.6% of patients.

B Validation Cohort, All Patients with B-Cell–Progenitor ALL (N=258)



IKZF1 (Ikaros) Deletions in *BCR-ABL1*–Positive Acute Lymphoblastic Leukemia Are Associated With Short Disease-Free Survival and High Rate of Cumulative Incidence of Relapse: A GIMEMA AL WP Report

VOLUME 27 · NUMBER 31 · NOVEMBER 1 2009

JOURNAL OF CLINICAL ONCOLOGY

Giovanni Martinelli, Ilaria Iacobucci, Clelia Tiziana Storlazzi, Marco Vignetti, Francesca Paoloni, Daniela Cilloni, Simona Soverini, Antonella Vitale, Sabina Chiaretti, Giuseppe Cimino, Cristina Papayannidis, Stefania Paolini, Loredana Elia, Paola Fazi, Giovanna Meloni, Sergio Amadori, Giuseppe Saglio, Fabrizio Pane, Michele Baccarani, and Robin Foà

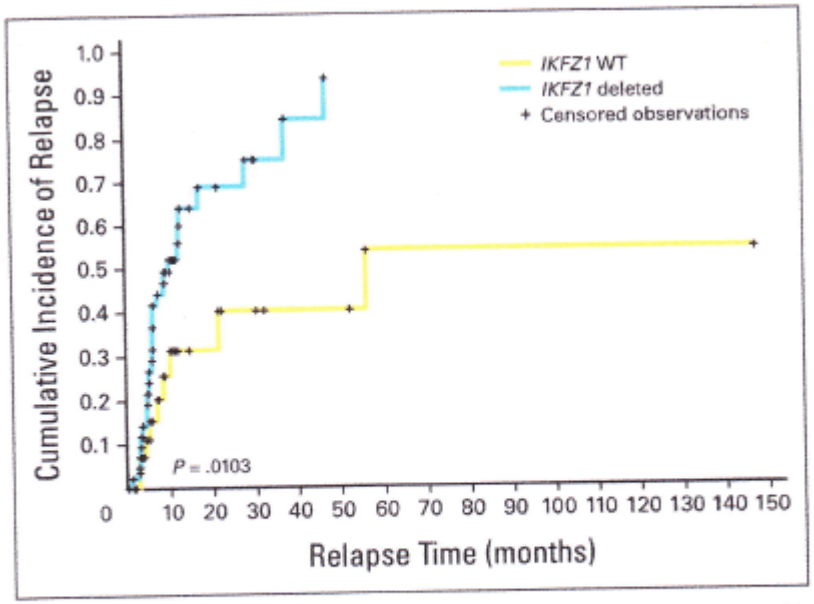


Fig 2. Cumulative incidence of relapse of de novo–treated patients with Philadelphia chromosome–positive acute lymphoblastic leukemia treated with conventional or investigational therapy including tyrosine kinase inhibitor (imatinib or dasatinib) regimens and with *IKZF1* deletion compared with patients treated with the same protocols without *IKZF1* deletion (*IKZF1* wild type [WT]).

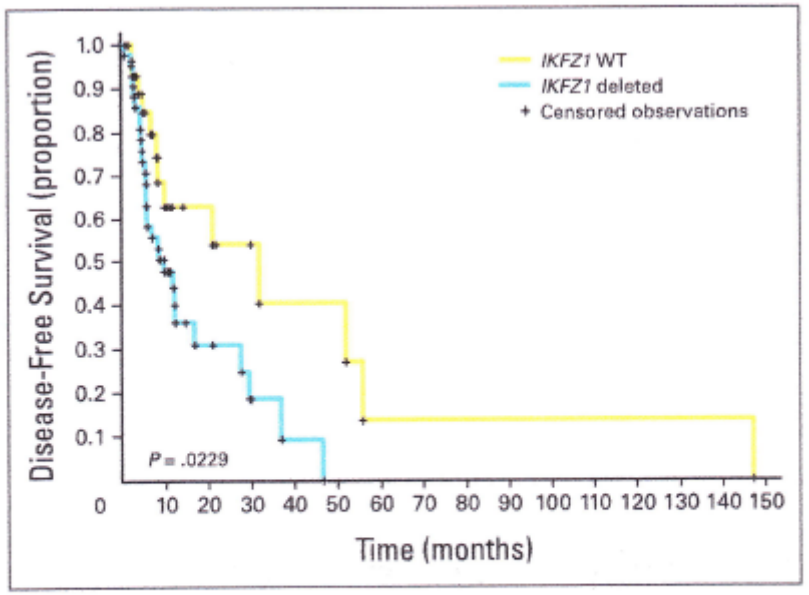
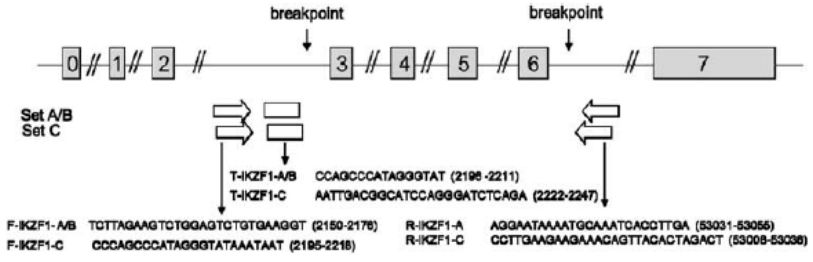


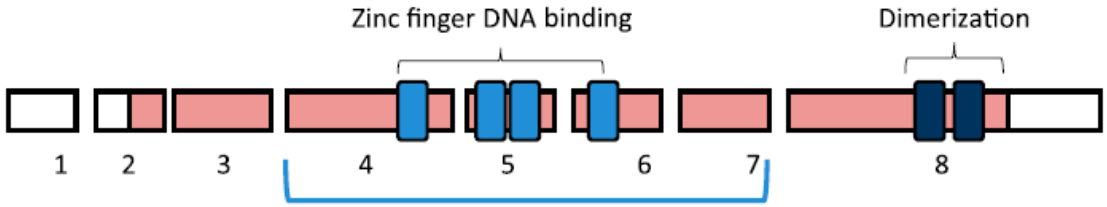
Fig 3. Disease-free survival of de novo–treated patients with Philadelphia chromosome–positive acute lymphoblastic leukemia treated with conventional or investigational therapy including tyrosine kinase inhibitor (imatinib or dasatinib) regimens and with *IKZF1* deletion compared with patients treated with the same protocols without *IKZF1* deletion (*IKZF1* wild type [WT]).

Highly sensitive MRD tests for ALL based on the IKZF1 Δ3–6 microdeletion

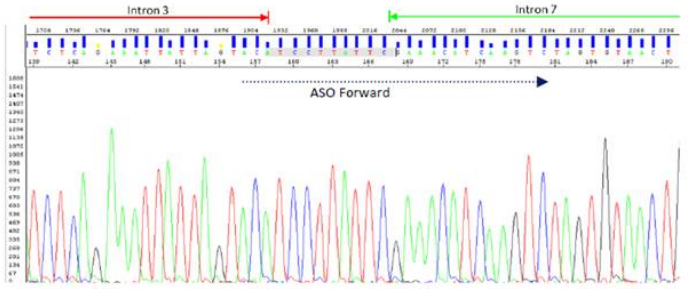
NC Venn[1], VHJ van der Velden[2], M de Bie[2], E Waanders[3], JE Giles[1], T Law[1], RP Kuiper[3], V de Haas[4], CG Mullighan[5], M Haber[1], GM Marshall[1,6], Norris MD[1], JJM van Dongen[2] and R Sutton[1]

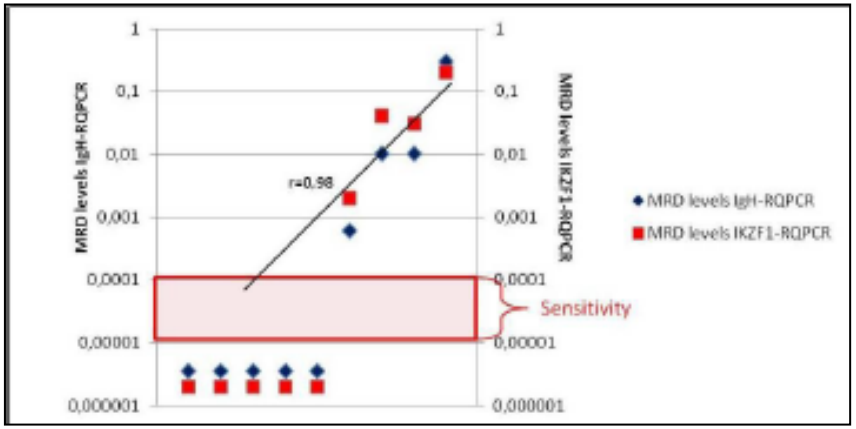
Leukemia (2012) **26,** 1414–1416;





Case 1





Next Generation Sequencing

DIVERSE TECNOLOGIE

- **Ion torrent**

- **Genome analyzer Illumina**

- **454 Roche tecnology**

Approccio "Standard" BIOMED 2-RQPCR

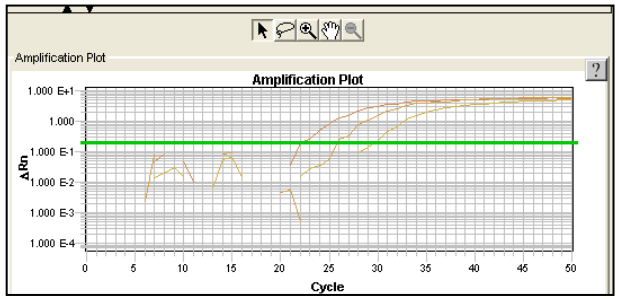
1- Screening

- IGH -DH
- IGK-Kde
- TRB
- TRG
- TRD

2- Disegno e verifica ASO primer

3-MRD Detection

- Amplificazione specifica con metodologia TaqMan



Approccio "NGS"

1-Screening

- IGH -DH
- IGK-Kde
- TRB
- TRG
- TRD

2-MRD Detection

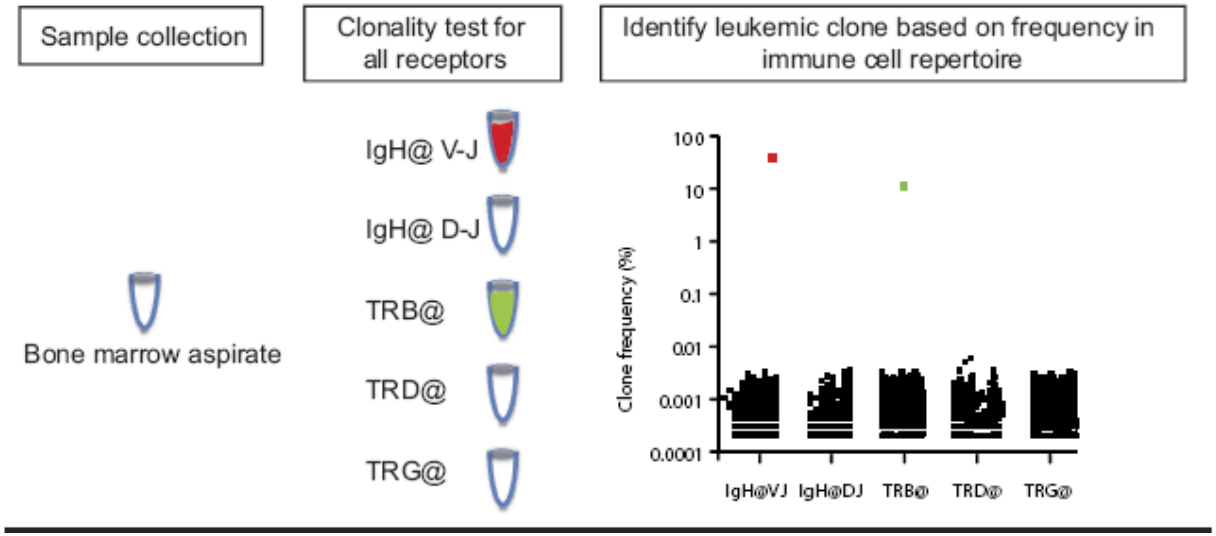
- Marker A
- MarkerB

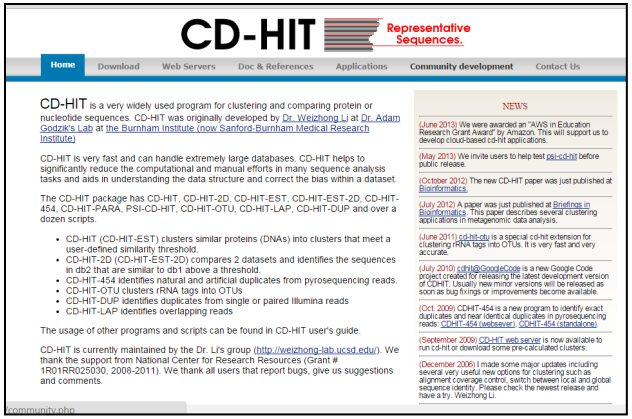
Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia

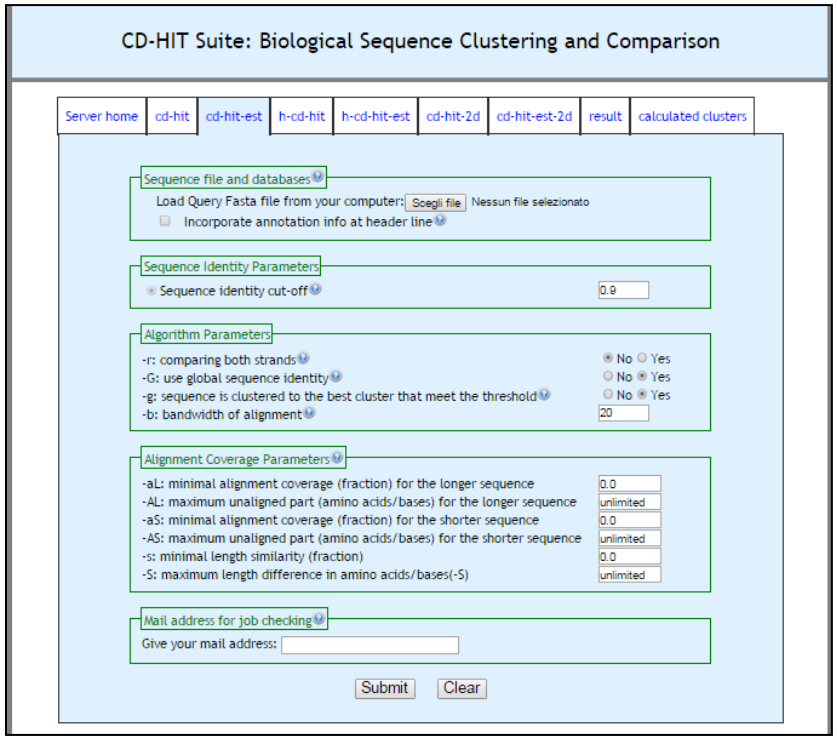
Malek Faham,[1] Jianbiao Zheng,[1] Martin Moorhead,[1] Victoria E. H. Carlton,[1] Patricia Stow,[2] Elaine Coustan-Smith,[3] Ching-Hon Pui,[2] and Dario Campana[3]

[1]Sequenta Inc, South San Francisco, CA; [2]Department of Oncology, St Jude Children's Research Hospital, Memphis, TN; and [3]Department of Pediatrics, National University of Singapore, Singapore

STEP 1: IDENTIFICATION OF LEUKEMIC CLONE IN DIAGNOSTIC SAMPLES







Huang Y at al. Bioinformatics, 2010(26): 680-682

Cluster >5%

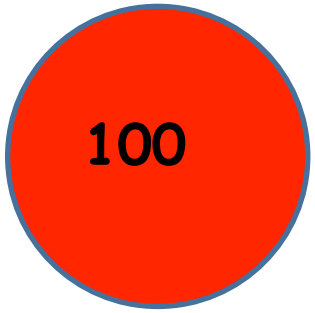
Cluster 0, No. sequences: 17822
Representative: I6JKX5O01CVONS
>I6JKX5O01CVONS length=545
xy=1063_3590

VH4-55*02 DH3-09*01JH6*03
VH4-55*02 DH3-09*01JH6*03
IGKV1-37*01
IGKV1-37*01-Kde
IGKV4-1*01
IGKV4-1*01-Kde
IGKV2-30*01
IGKV2-30*01-Kde

Vk2-30-Kde
Vk2-30-Kde
Vk3D-20
Vk3-20-Kde
DH2-2*01-JH5*01
VH6-1*02DH2-2*02JH5*02
VH3-11*06 DH5-18*01JH4*02
VH3-11*06 DH5-18*01JH4*02

VH2-5*02 DH3-22*01JH4*02
VH2-5*02 DH3-22*01JH4*02
IGKV1D-33*01
IGKV1D-33*01-kde

NGS 70.000- 100.000 SEQUENZE

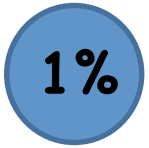


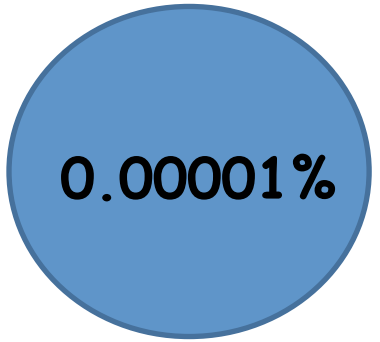
AMPLICONI

1000 Seq X amplicone

100000 Seq X amplicone

SENSIBILITA'





Screening dei cloni

NGS MRD DETECTION

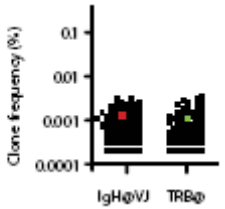
STEP 2: DETECTION OF LEUKEMIC CLONE IN FOLLOW-UP SAMPLES

Serial follow-up sample collection

MRD test for positive receptors

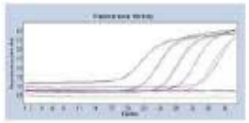
Monitor MRD over time in serial follow-up samples

Bone marrow aspirate
OR
Peripheral blood



NON B CELL LEUKOCYTE
B CELL
LEUKEMIC B CELL

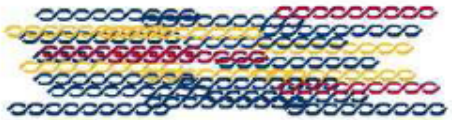
QUANTITATE DNA



D = Amount of DNA K = DNA per Cell

$N_{TOT} = D/K$ = Total Number of Leukocytes

EXTRACT DNA



ADD KNOWN QUANTITY OF REFERENCE IgH

N_R = Number of Reference Molecules

AMPLIFY IgH MOLECULES

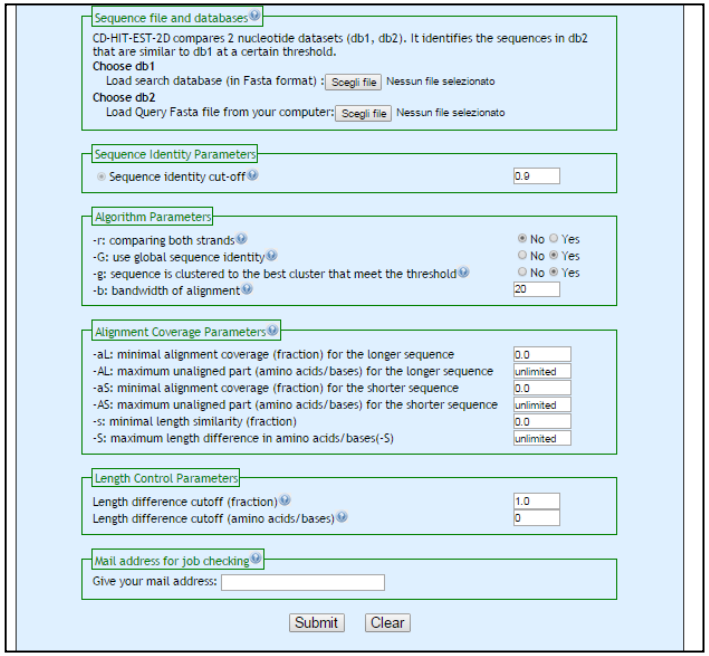
SEQUENCE MOLECULES

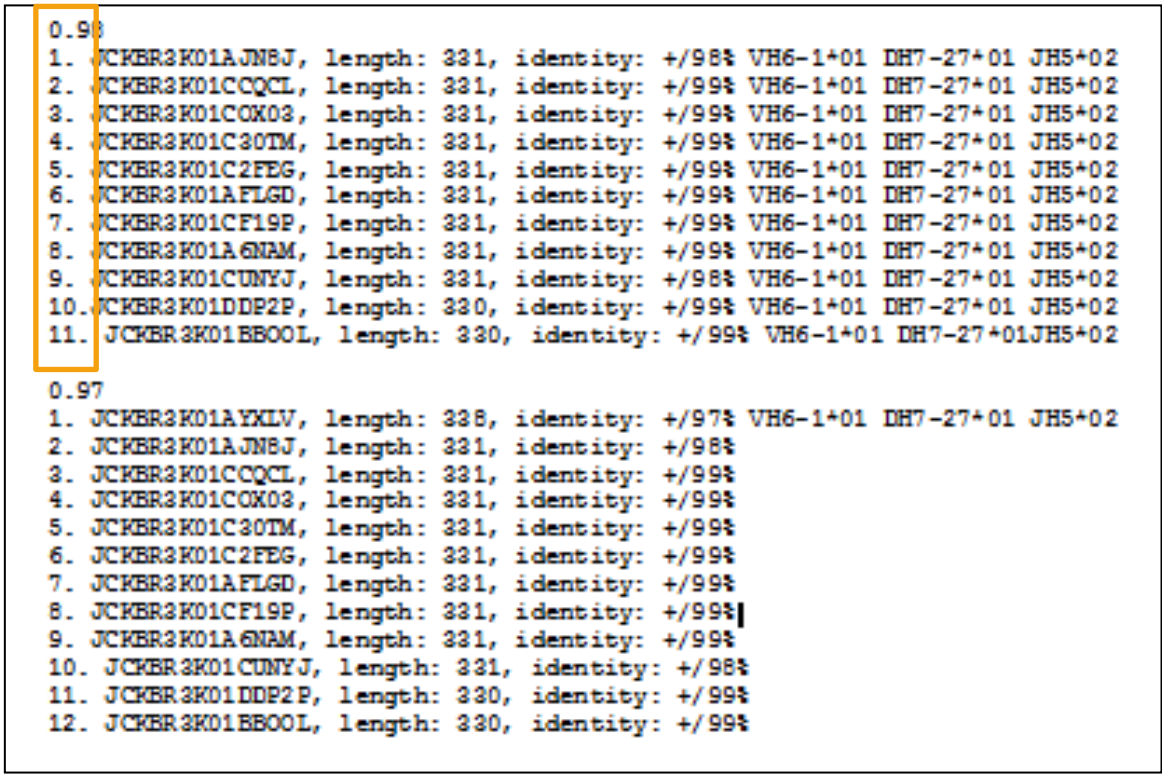
S_B = Number of B Cell Reads

S_L = Number of Leukemia Reads

S_R = Number of Reference Reads

CD-HIT-EST-2D





NGS MRD12/12986
0.9x10E-3

EURO-MRD vs NGS-MRD

	Sensibilità	QR	MRD TP1	MRD TP2
VH6-1*01 EURO-MRD	1x10E-5	1x10E-4	1x10E-3	
VH6-1*01 NGS-MRD	1x10E-4	1x10E-4	0.9x10E-4	
VH3-30-3*01 EURO-MRD	1x10E-5	1x10E-4	5x10E-4	2x10E-4
VH3-30-3*01 NGS-MRD	5x10E-5	5x10E-5	1x10E-4	1x10E-4
TRG V8*01 EURO-MRD	1x10E-5	1x10E-4	5x10E-4	2x10E-4
TRG V8*01 NGS-MRD	5x10E-5	5x10E-5	3x10E-4	3x10E-4

High-throughput VDJ sequencing for quantification of minimal residual disease in chronic lymphocytic leukemia and immune reconstitution assessment

Aaron C. Logan[a], Hong Gao[b], Chunlin Wang[b], Bita Sahaf[a], Carol D. Jones[c], Eleanor L. Marshall[c], Ismael Buño[d], Randall Armstrong[e], Andrew Z. Fire[c], Kenneth I. Weinberg[f], Michael Mindrinos[b], James L. Zehnder[g], Scott D. Boyd[c], Wenzhong Xiao[b,h], Ronald W. Davis[b,1], and David B. Miklos[a,1]

Divisions of [a]Blood and Marrow Transplantation and [g]Hematology, Department of Medicine, [c]Department of Pathology, and [f]Division of Pediatric Stem Cell Transplantation, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305; [b]Stanford Genome Technology Center, Stanford, CA 94304; [d]Department of Hematology, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Spain; [h]Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114; and [e]Stanford Cellular Therapeutics and Transplantation Laboratory, Stanford Hospital and Clinics, Stanford, CA 94305

Contributed by Ronald W. Davis, November 11, 2011 (sent for review September 3, 2011)

TRANSPLANTATION

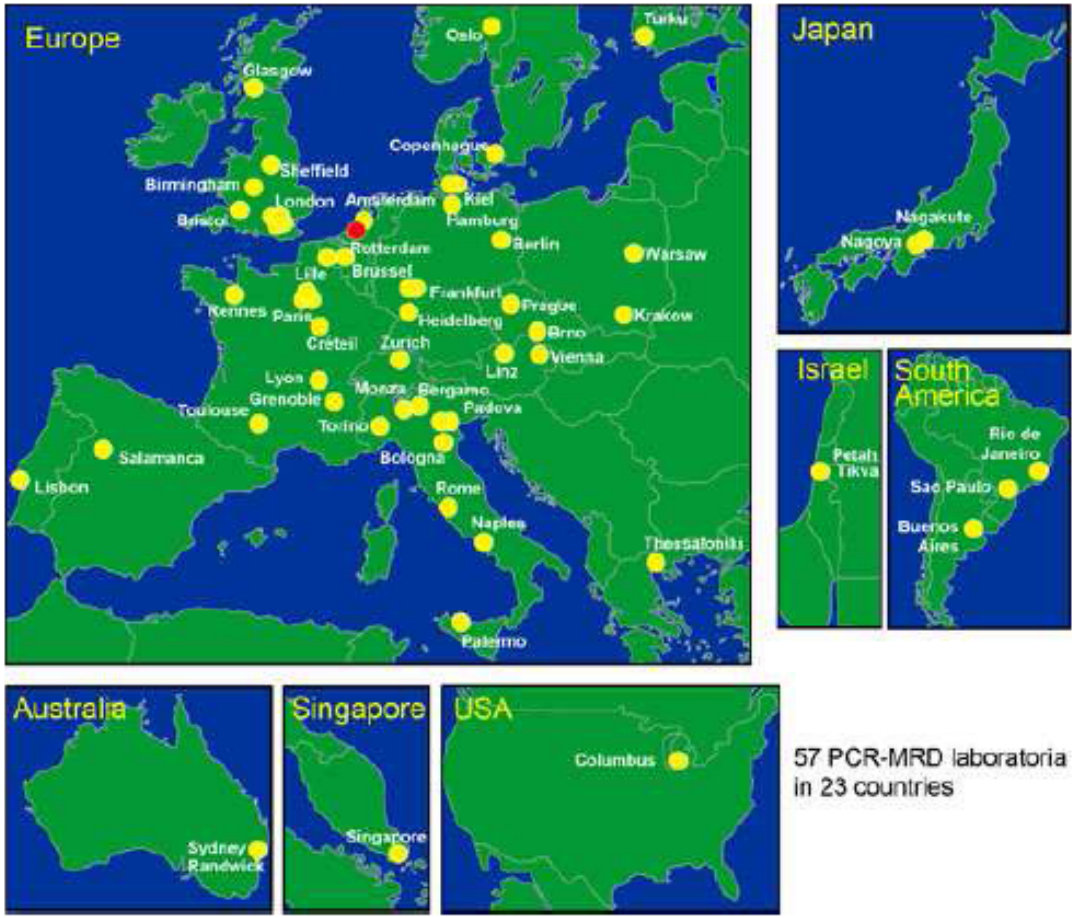
IgH-V(D)J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients

Michael A. Pulsipher,[1] Chris Carlson,[2,3] Bryan Langholz,[4] Donna A. Wall,[5] Kirk R. Schultz,[6] Nancy Bunin,[7] Ilan Kirsch,[3] Julie M. Gastier-Foster,[8-10] Michael Borowitz,[11] Cindy Desmarais,[3] David Williamson,[3] Michael Kalos,[12] and Stephan A. Grupp[7,13]

BLOOD, 28 MAY 2015 • VOLUME 125, NUMBER 22

ESLHO
european scientific foundation
of laboratory hemato oncology

EuroMRD
a division of ESLHO



57 PCR-MRD laboratoria
in 23 countries

NGS-MRD QC29