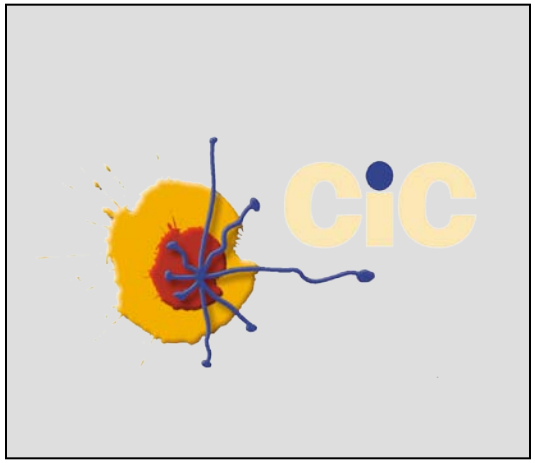
ENFERMEDAD MINIMA RESIDUAL: DECISIONES TERAPEUTICAS



CENTRO DE INVESTIGACIÓN DEL CÁNCER, UNIVERSIDAD y HOSPITAL UNIVERSITARIO de SALAMANCA (ESPAÑA)

XVIII Congreso de la Sociedad Chilena de Hematología
La Serena (Chile), 4 de octubre de 2012

Factors involved in treatment effectiveness in haematological malignancies

Factors	Relative contribution	Solutions
Treatment compliance e.g - duration of Rx - side effects (e.g. allergy)	40%??	- Psychosocial care - Safer drugs
In vivo drug distribution e.g - gastrointestinal absorption - distribution in body (e.g. CNS) - drug metabolism (e.g. polymorphisms in enzymes) - liver excretion - kidney excretion	20%??	Adaptation of drug dosage (based on measurement of therapeutic levels?)
Characteristics of tumor cells e.g - prednison response - *in vitro* drug sensitivity - gene expression profile	40%??	Adaptation of drugs? New drugs?

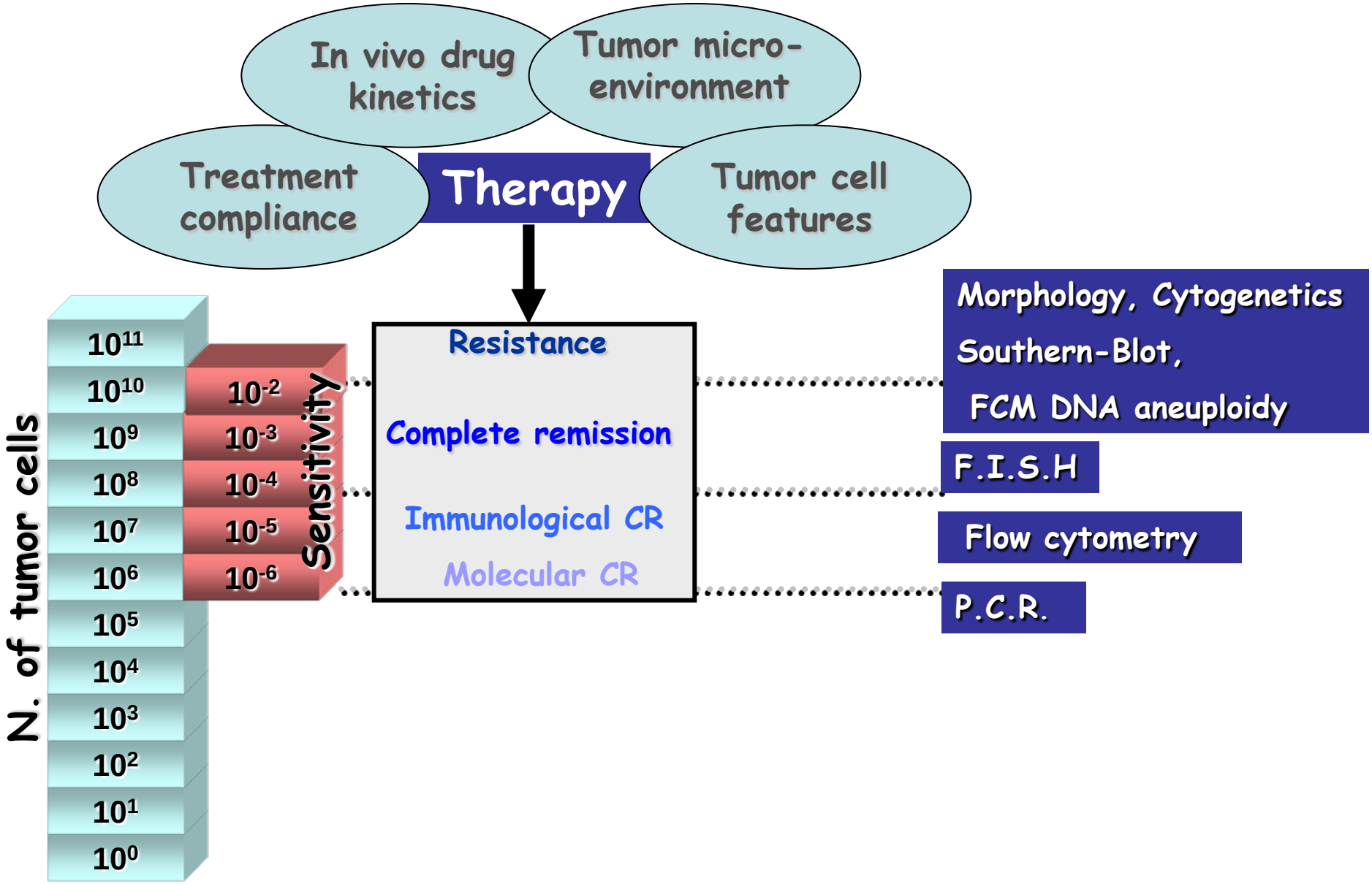
Evaluation of overall treatment effectiveness by MRD diagnostics

↓

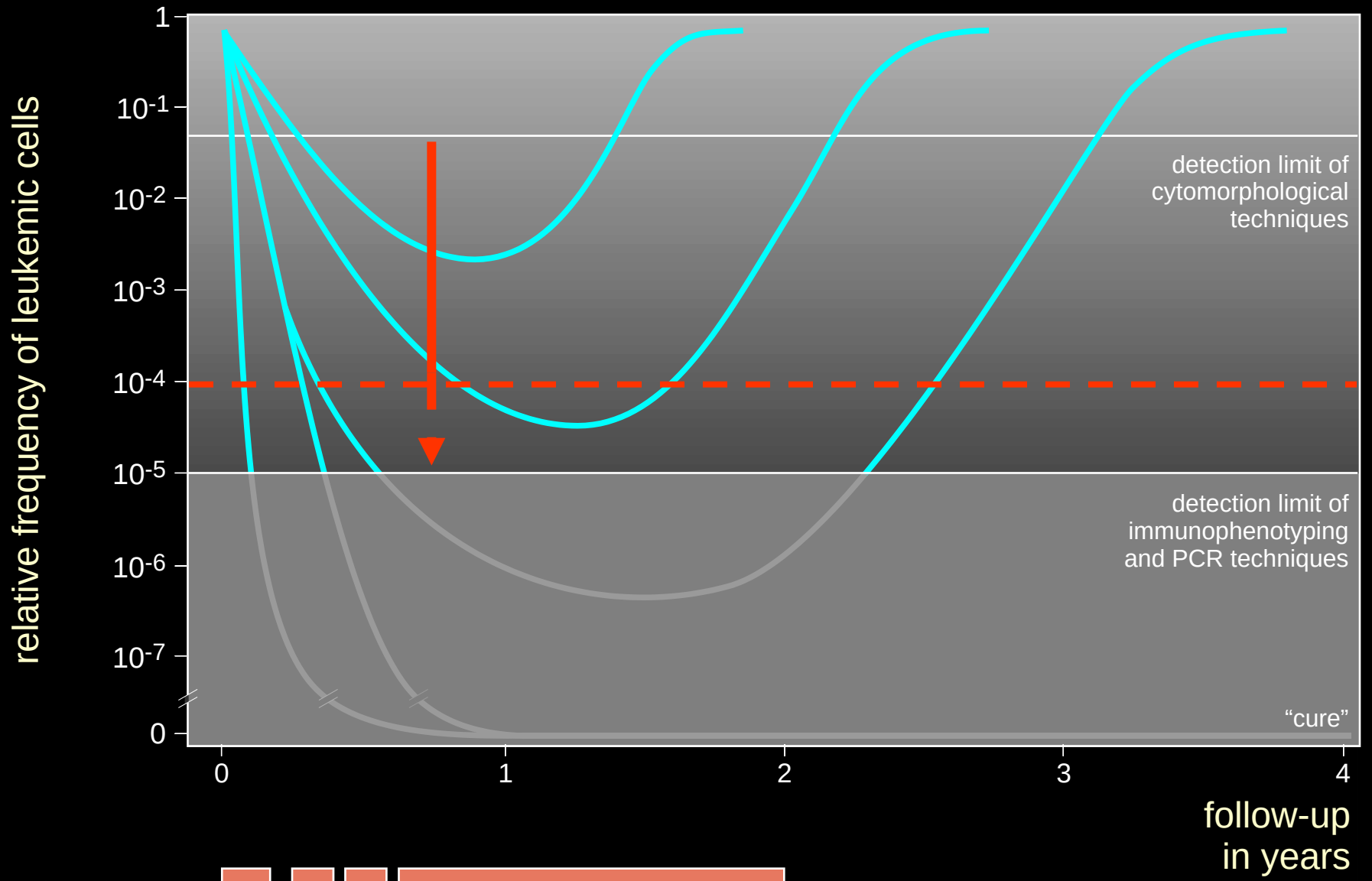
Different treatment arms

MRD MONITORING IN HAEMATOLOGICAL MALIGNANCIES

EuroFlow



Detection of minimal residual disease (MRD)



DCLSG ALL-8 | I | C | II | maintenance Rx

Prepared by J.J.M. van Dongen

FLOW MRD IN HAEMATOLOGICAL MALIGNANCIES

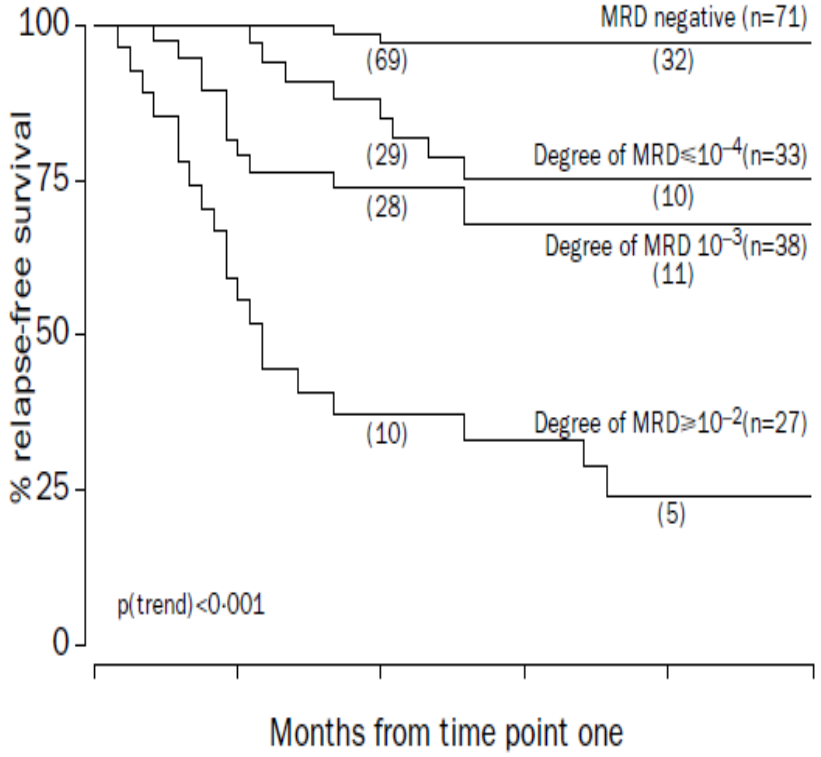
- Does response to therapy impact on long-term patient outcome?

- Does MRD improve prognostic stratification of patients with hematological malignancies?

CLINICAL UTILITY OF MRD IN ALL
(Semiquantitative ASO-PCR: Igs y TCR)

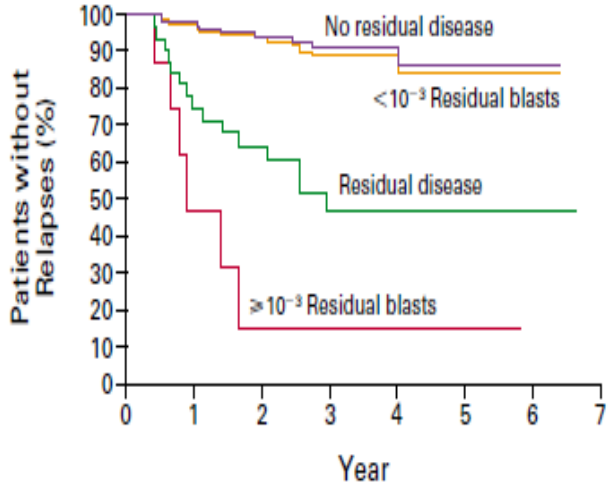
Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood

Van Dongen et al, Lancet 1998; 352:1731-38



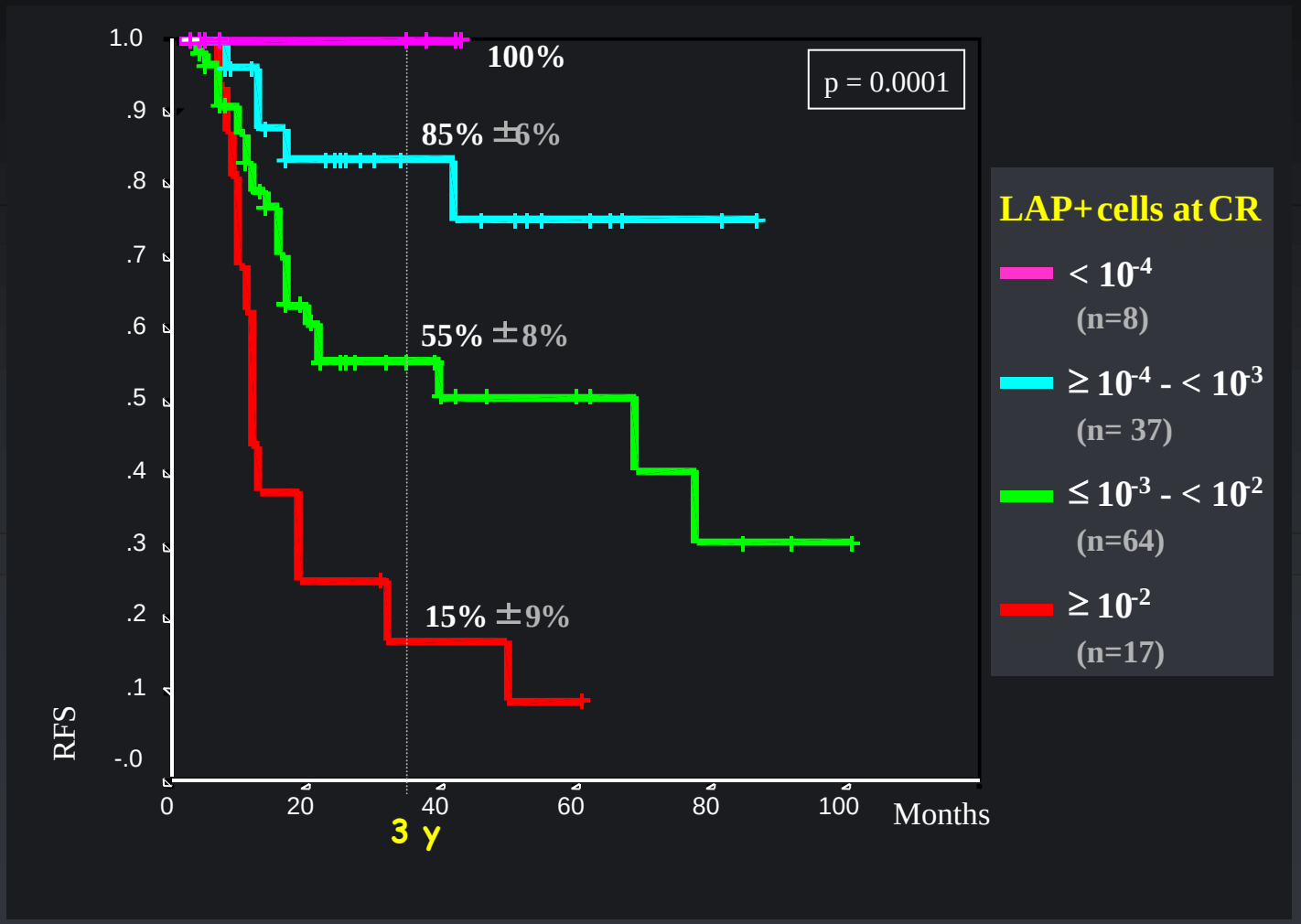
CLINICAL SIGNIFICANCE OF MINIMAL RESIDUAL DISEASE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Cave et al, N Engl J Med 1998; 339:591-598



	No. of Relapses	No. of Patients at Risk						
No residual disease	8	95	92	76	46	20	3	2
Residual disease	15	32	23	17	10	3	3	1
<10⁻³ Residual blasts	11	110	106	88	54	21	4	2
≥10⁻³ Residual blasts	6	8	3	1	1	1	1	0

AML:
RFS according to LAP+ cells in 1st BM in mCR after Induction Therapy



LAP: leukemia associated phenotype

San Miguel et al, Blood, 1997 &
San Miguel et al, Blood, 2001

RFS IN AML: Multivariate analysis

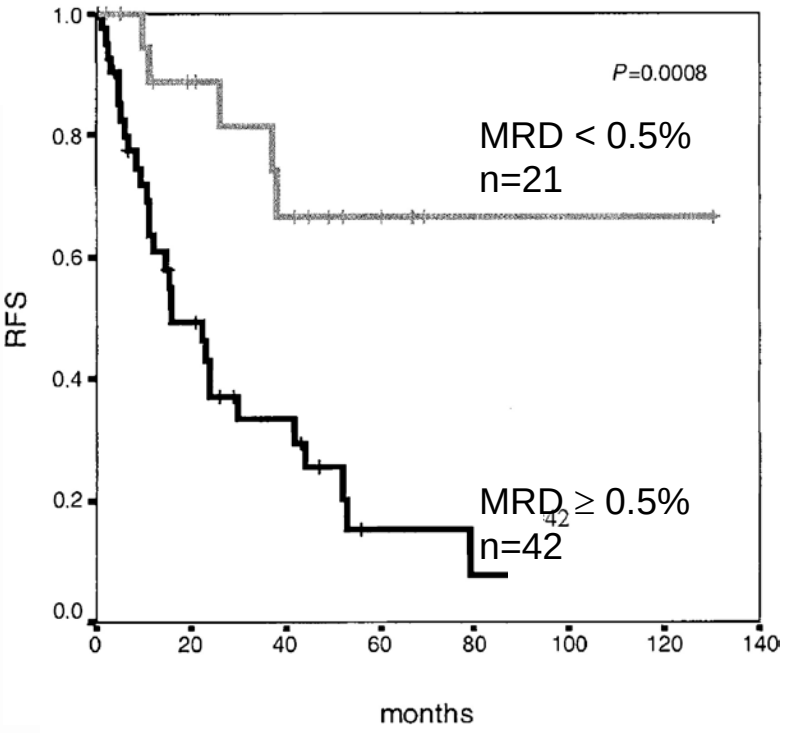
MRD at mRC

- Including cytogenetics (n=96)
  - Cytogenetics p=0.004
  - MRD levels p=0.04
- Excluding cytogenetics (n=126)
  - MRD levels p=0.02

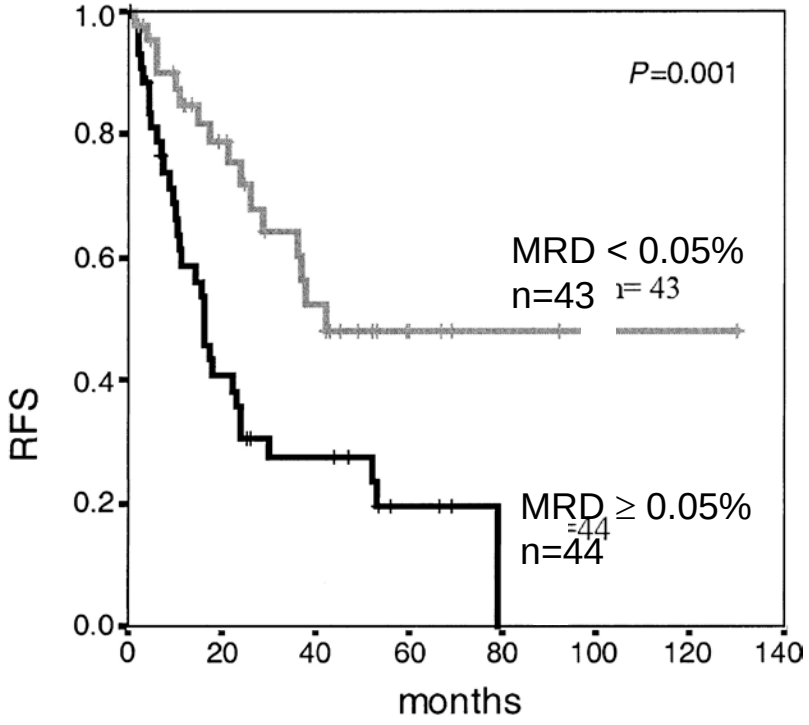
Adult ALL : Prognostic value of MRD detection by flow cytometry

MRD analysis using 3-color flow in adolescents/adults with ALL

day+14



day+35

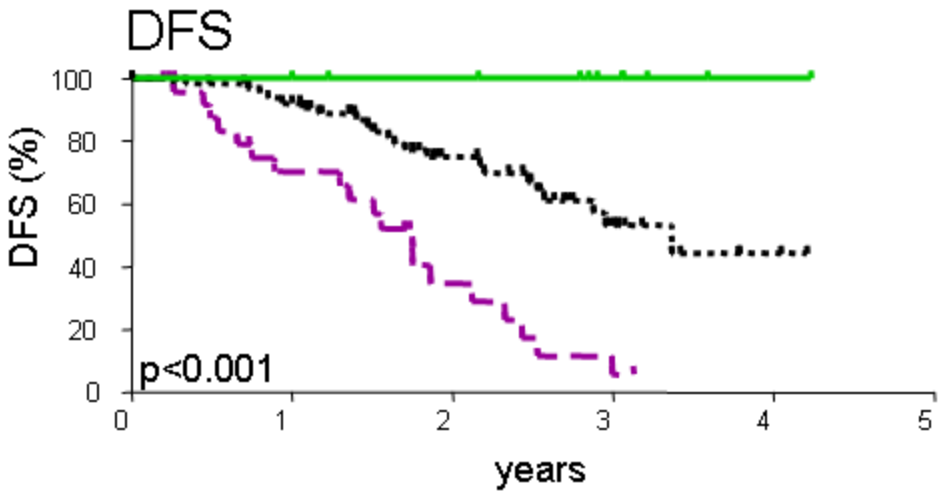


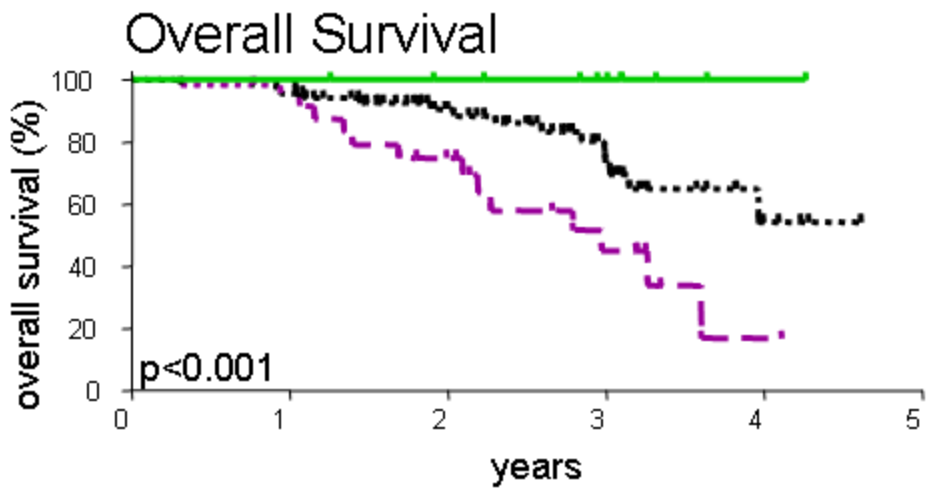
blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

Vidriales, M.-B. et al. Blood 2003;101:4695-4700

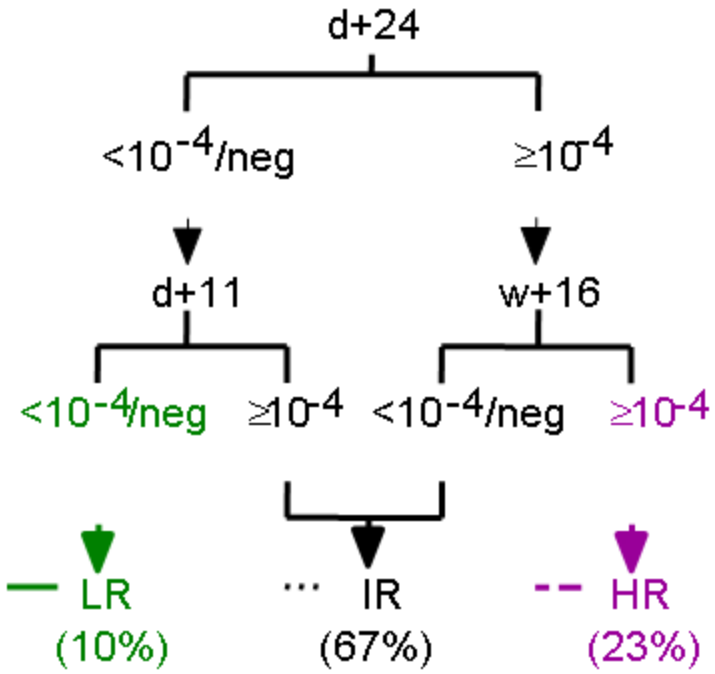
Prognostic significance of MRD in adult ALL

- Standard risk ALL: MRD based risk stratification -





(n: 105)



M Brüggemann et al. Blood 2006; 107: 1116

Prognostic significance of MRD in adults with Ph^{neg} ALL Clinical trials

Author	Year	Group	Method	N	Prognostic Model	EFS
Brüggemann	2006	GMALL	PCR	105 SR	$<10^{-4}$ d11 + $<10^{-4}$ d24	100%
					$>10^{-4}$ d11 + $>10^{-4}$ d24	6%
					All others	53%
Holowiecki	2008	PALG	Flow	115	$< 10^{-3}$ (4 wks)	61%
					$> 10^{-3}$ (4 wks)	17%
Bassan	2009	NILG	PCR	142 SR & HR	$<10^{-4}$ wk16, $<10^{-4}$ neg wk22	72%
					All others	14%

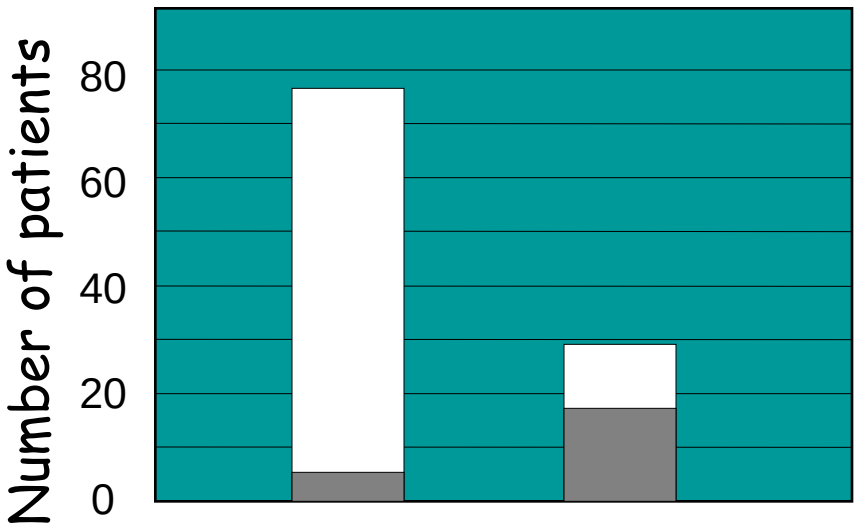
Prognostic significance of MRD in adults with Ph^{neg} ALL Clinical trials

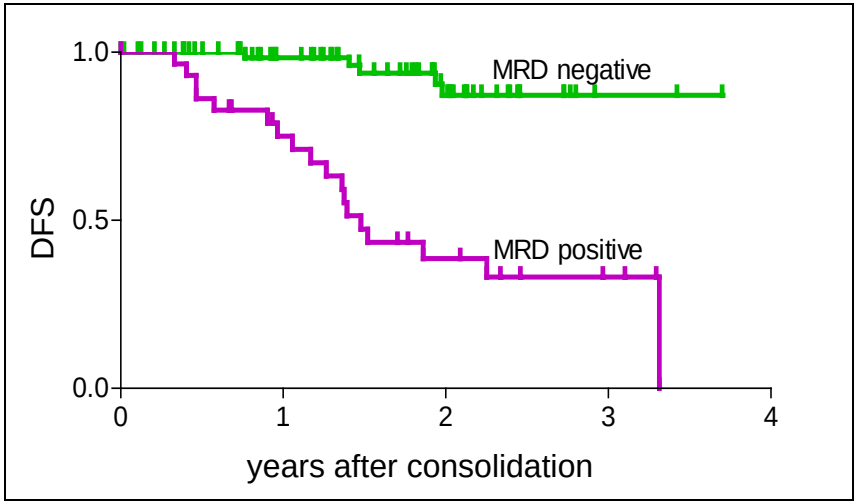
Author	Year	Group	Method	N	Prognostic Model	EFS
Ribera	2009	PETHEMA	Flow	202 HR	$<10^{-3}$(wk5) & $<5x10^{-4}$ (wk16)	54%
					$\geq 10^{-3}$(wk5) & $\geq 5x10^{-4}$ (wk16)	31%
Beldjord	2009	GRAALL	PCR	212	Neg or $<10^{-4}$ wk6	80%
					$>10^{-4}$ wk6	40%
Patel	2010	UKALL	PCR	161 B-lin SR HR	Neg or $<10^{-4}$ wk10*	71%
					$>10^{-4}$ wk 10*	15%

* Prognostic significance for SR patients, or patients randomised to autologous SCT, but not for those allocated to allogeneic SCT. Prognostic significance also seen in other time-points (wk 5, wk 17, 6-9 mo.)

MRD Assessment in ALL Trials:

-Early detection of relapse-





	MRD negative	MRD positive	
CCR	72	11	83
REL	5	17	22
	77	28	n=105

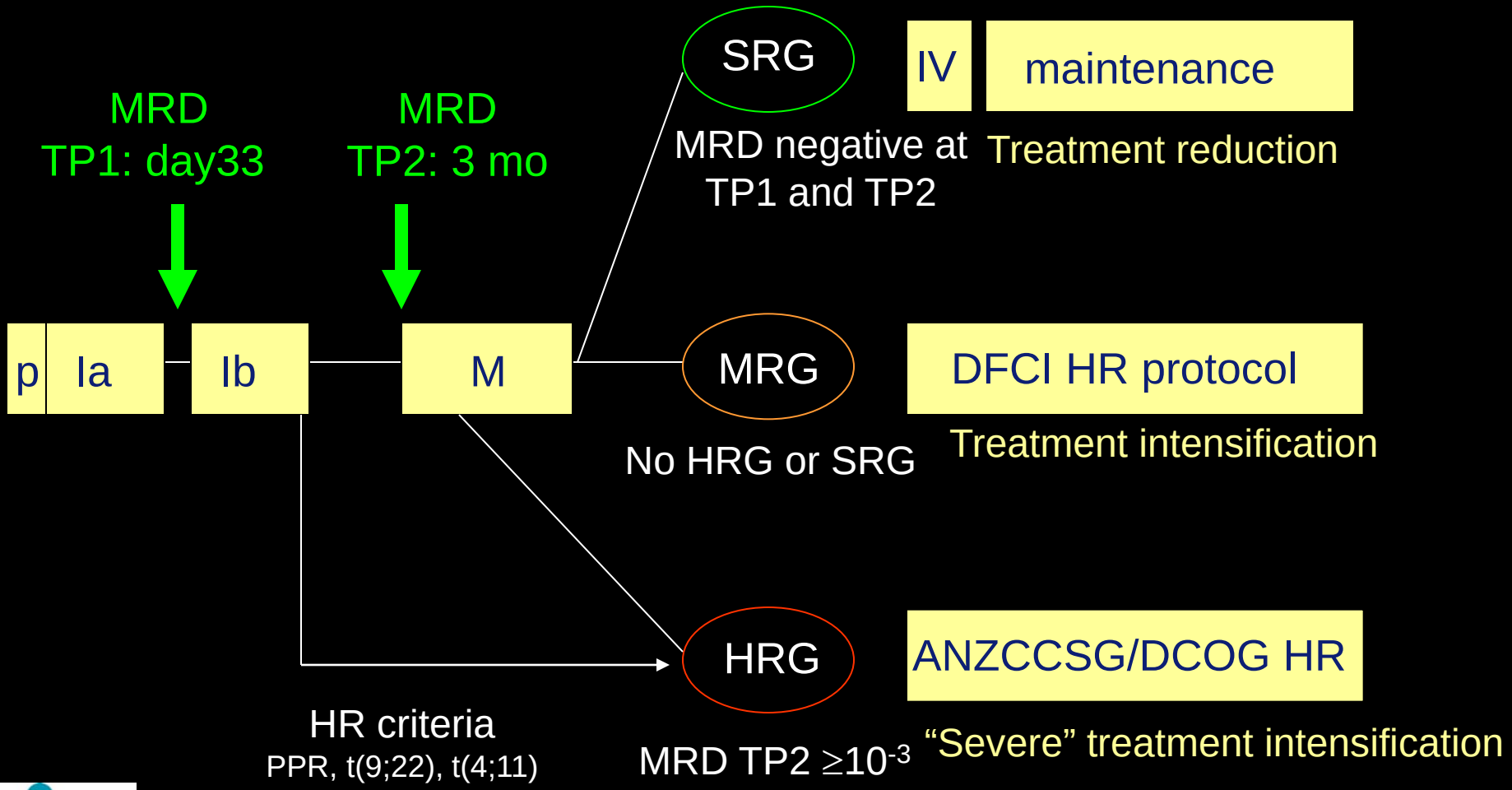
T. Raff et al. Blood 2007

MRD Based Risk Stratification Approaches in Current European Trials for Adult ALL

www.leukemia-net-org

	Time point	Cut-off	Consequence
GMALL *Germany*	Ind+1con 12 mo	10^{-4}	SCT in MRD^+ Stop Tx in MRD^-
GRAALL *France*	Ind + 3 cons	10^{-2}	SCT in MRD^+
NILG *Italy*	Ind + 7 cons	10^{-3}	SCT in MRD^+
PETHEMA *Spain*	Ind+ Cons	10^{-3} $5x10^{-4}$	SCT in MRD^+

MRD-based therapy: ALL10 protocol Started per November 2004



DCOG, The Hague, 2004

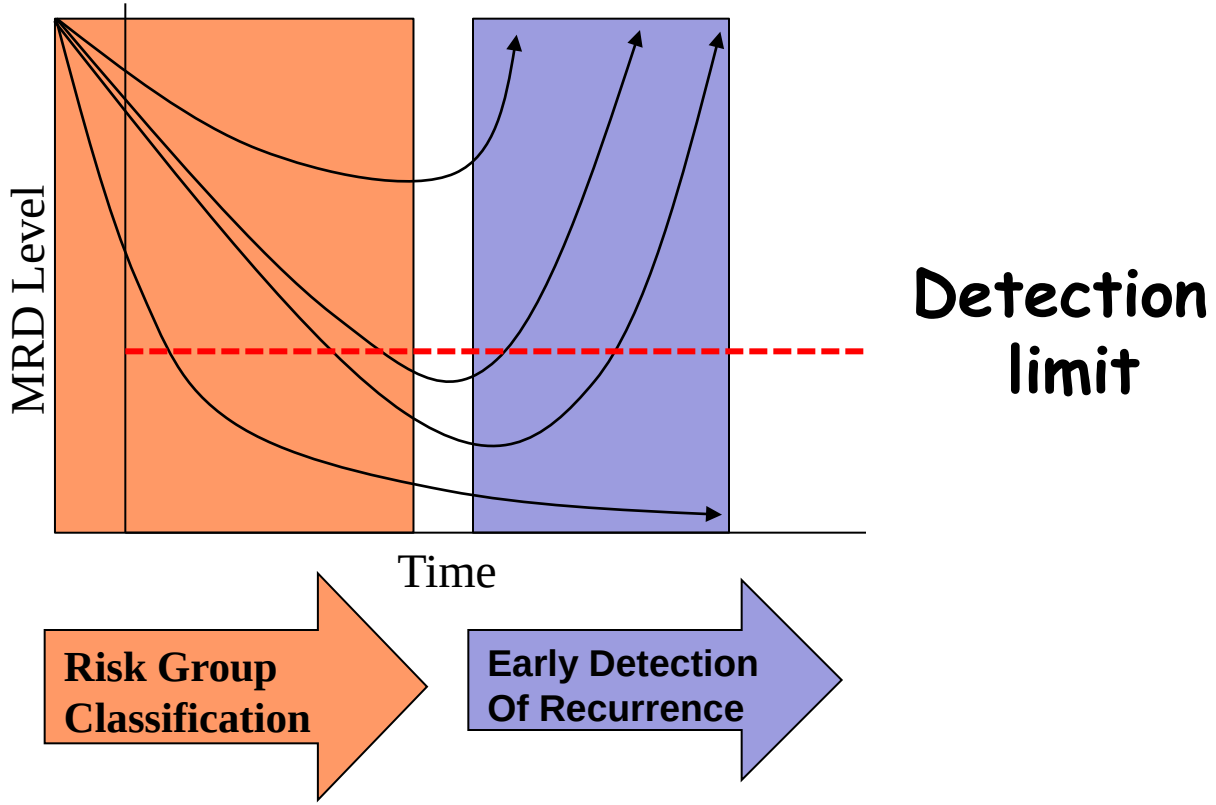
Prepared by JJM van Dongen

POST-TRANSPLANT MRD ANALYSIS WITHIN EUROPEAN TRIALS ON Ph-POSITIVE ALL

	Time point	Cut-off	Consequence
GMALL *Germany*	Day +42	10^{-4}	Intervention withTKI
GRAAPH *France*	Day +100	10^{-2}	Autografting
NILG *Italy*	Every 3 mo	10^{-3}	Intervention with TKI
PETHEMA *Spain*	Day +100	10^{-4}	Intervention with TKI

Modified from Brüggemann et al, Leukemia, 2010; 24: 521-535

Clinical utility of MRD in ALL



Should MRD monitoring be mandatory in ALL?
Which technique should then be applied?

Prognostic value and clinical applicability of MRD detection in acute leukemias

Disease category	Relative frequency	Type of MRD application			
		Early response to frontline treatment	Continuous monitoring for therapy titration	MRD assessment pre-HSCT	MRD assessment post-HSCT
ALL	~4%	+++	++	+++	++
APL	~2%	-	+++	++	+++
AML (excl. APL)	~10%	++	++	++	+

+++, value of MRD detection in large prospective studies
++, potentially clinically relevant (e.g. in a subset of patients) but not yet proven by large prospective studies
+, MRD results are statistically significant but their clinical implication is not yet established
*, only relevant for patients treated with more aggressive protocols, e.g. including antibody treatment
–, MRD detection has no additional value as compared with conventional cytomorphological techniques

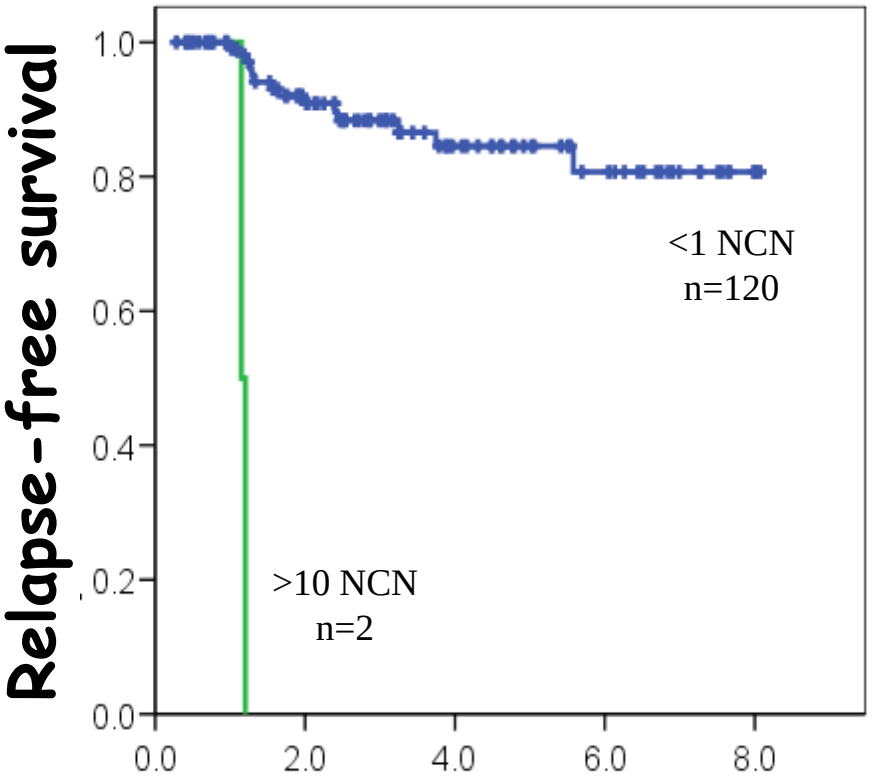
MRD DETECTION BY RQ-PCR IN ACUTE PROMYELOCYTIC LEUKEMIA

Post-Induction

Relapse-free survival

Years

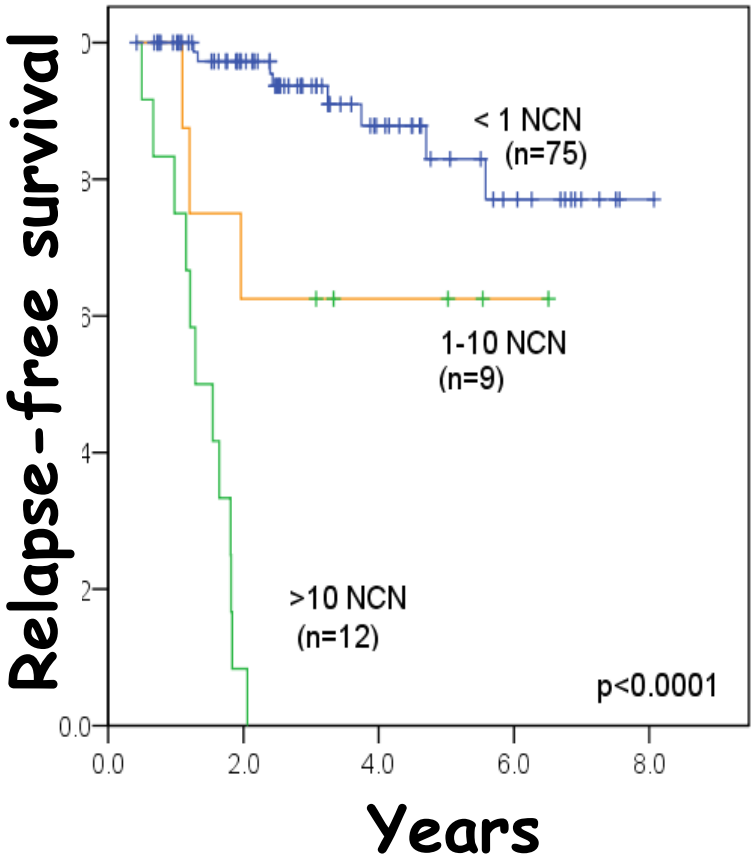
Post-Consolidation



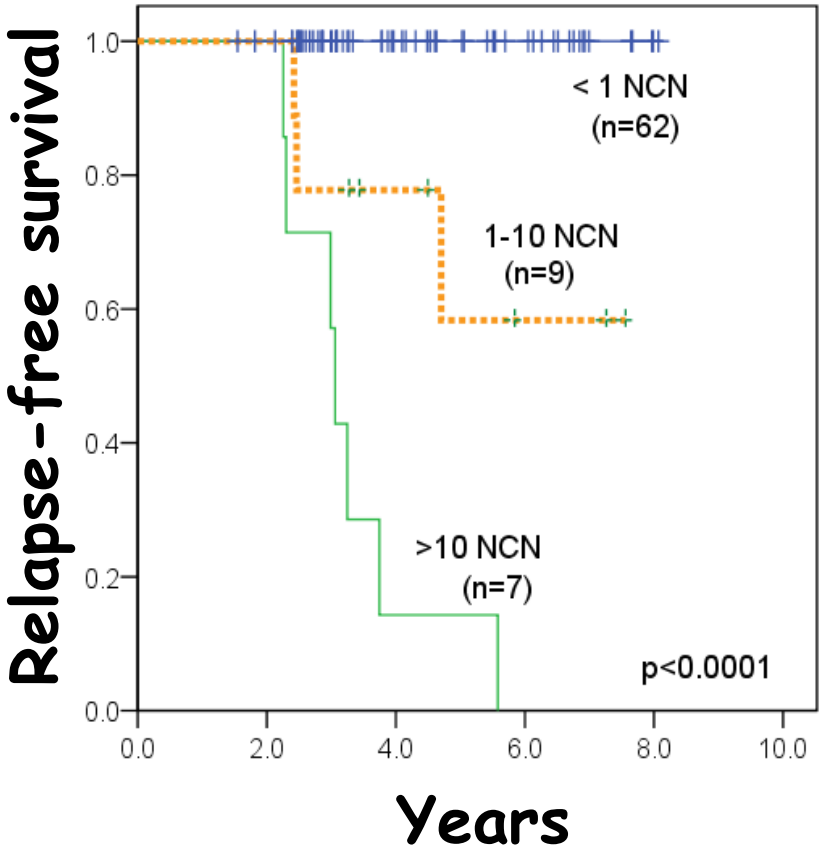
Haematologica, 2007; 92:315-22 .

MRD DETECTION BY RQ-PCR IN ACUTE PROMYELOCYTIC LEUKEMIA

Maintenance



Out of therapy



Haematologica, 2007; 92:315-22 .

PROGNOSTIC IMPACT OF MRD IN CHRONIC LYMPHOCYTIC LEUKEMIA: Trials with deviation from current iwCLL standard

	Reference	N	Therapy	Technique	MRD threshold	PFS by MRD group	p value
1	Robertson 1992	66	Fludarabine + prednisone	2-color flow	Not reported	18 mos vs. NR (TTF)	< 0.001
2	Rawstron 2001	25	auto SCT or alemtuzumab	4-color MRD flow	5×10^{-4}	19 mos vs nr (EFS)	0.0001
3	Moreton 2005	91	Alemtuzumab +/- auto/allo SCT	4-color MRD flow	10^{-5}	20 mos vs nr (TFS)	< 0.0001
4	Wierda 2005	82	FCR	Qualitative *IGHV*PCR	10^{-5}	27 mos vs 44 mos (TTP)	ns
5	Hillmen 2007	36	alemtuzumab vs. chlorambucil	CD19/CD5/κ/λ	not reported	MRD+ CR < MRD- CR	not reported
6	Kay 2007	52	PCR	2-color flow	10^{-2}	18 mos vs 35 mos	< 0.001
7	Tam 2008	266	FCR	3-color flow	10^{-2}	49 mos vs 85 mos	< 0.001
8	Lamanna 2009	23	F→C→R	nested ASO *IGH*PCR	10^{-5}	35 mos vs nr (CR)	0.007
9	Maloum 2009	33	FC	4-color MRD flow	10^{-4}	28 mos vs nr	0.0009
10	Ysebaert 2010	30	FCR	4-color MRD flow	10^{-2}	24 mos vs. nr	< 0.01
11	Parikh 2011	57	CFAR	3-color flow	5×10^{-2}	15 mos vs. nr	< 0.001

Prepared by Sebastian Bötcher

PROGNOSTIC IMPACT OF MRD IN CLL: Trials following current iwCLL standard

	Reference	N	Therapy	Technique	MRD threshold	PFS by MRD group	p value
1	Moreno 2006	17	auto SCT	ASO RQ-PCR	10^{-5}	19 mos vs nr	0.02
		22		3/4-color MRD flow	10^{-4}	16 mos vs 75 mos	< 0.001
2	Bosch 2008	44	FCM	4-color MRD flow	10^{-4}	MRD+ CR < MRD- CR (RD)	0.2
3	Kwok 2009	137	various	4-color MRD flow	10^{-4}	24 mos vs 91 mos	< 0.001
4	Böttcher 2012	290	FC/ FCR	4-color MRD flow	10^{-4} and 10^{-2}	$\geq 10^{-2}$ 15 mos $\geq 10^{-4}$ to $< 10^{-2}$ 41 mos $< 10^{-4}$ 69 mos	< 0.001
5	Fischer 2012	45	BR	4-color MRD flow	10^{-4} and 10^{-2}	$\geq 10^{-2}$ 12 mos $\geq 10^{-4}$ to $< 10^{-2}$ 32 mos $< 10^{-4}$ NR	< 0.001
6	Pettitt 2012	29	Cam-HDMP	4-color MRD flow	10^{-4}	10 mos vs. 24 mos	0.009

Prepared by Sebastian Bötcher

CLL: IMPACT OF MRD MEASUREMENTS IN CLINICAL PRACTICE

Review article

This is a new version, updated and corrected, as of December 8, 2008.

Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute–Working Group 1996 guidelines

Michael Hallek,[1] Bruce D. Cheson,[2] Daniel Catovsky,[3] Federico Caligaris-Cappio,[4] Guillaume Dighiero,[5] Hartmut Döhner,[6] Peter Hillmen,[7] Michael J. Keating,[8] Emili Montserrat,[9] Kanti R. Rai,[10] and Thomas J. Kipps[11]

5.9. Minimal residual disease

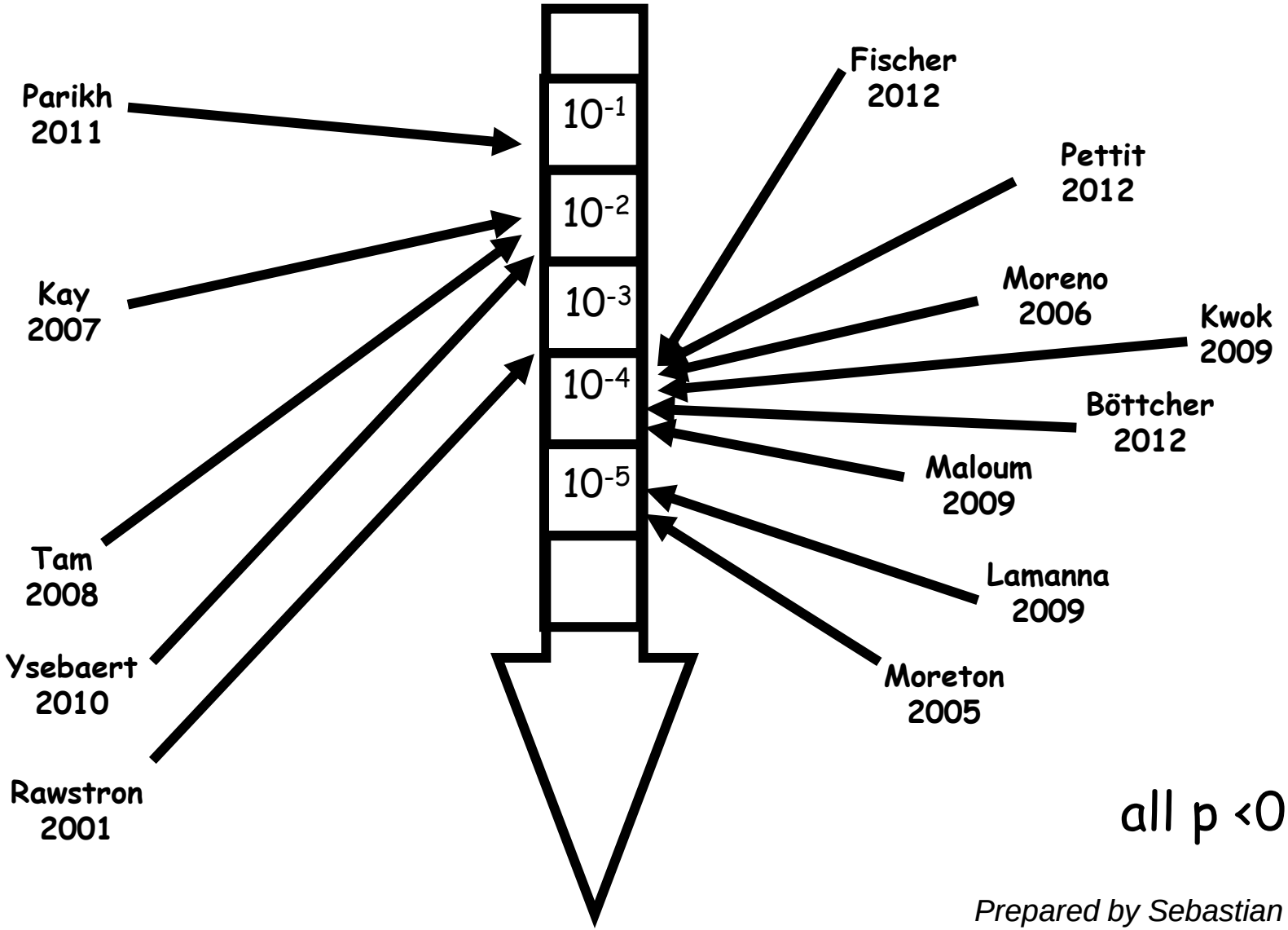
The complete eradication of the leukemia is an obvious desired endpoint. New detection technologies, such as multicolor flow cytometry and real-time quantitative PCR, have determined that many patients who achieved a CR by the 1996 NCI-WG guidelines have detectable MRD. Although eradication of MRD may improve prognosis, prospective clinical trials are needed to define whether additional treatment intended solely to eradicate MRD provides a significant benefit to clinical outcome. The techniques for assessing MRD have undergone a critical evaluation and have become fairly standard.[60] Either 4-color flow cytometry (MRD flow) or allele-specific oligonucleotide PCR is reliably sensitive down to a level of approximately one CLL cell in 10 000 leukocytes. As such, patients will be defined as having a clinical remission in the absence of MRD when they have blood or marrow with less than one CLL cell per 10 000 leukocytes. The blood generally can be used for making this assessment except during the period within 3 months of completing therapy, particularly for patients treated with alemtuzumab, rituximab, and other antibodies targeting CLL. In such

Standard reporting
$\rightarrow 10^{-4}$

Additional level
$\rightarrow 10^{-2}$

Prepared by Sebastian Bötcher

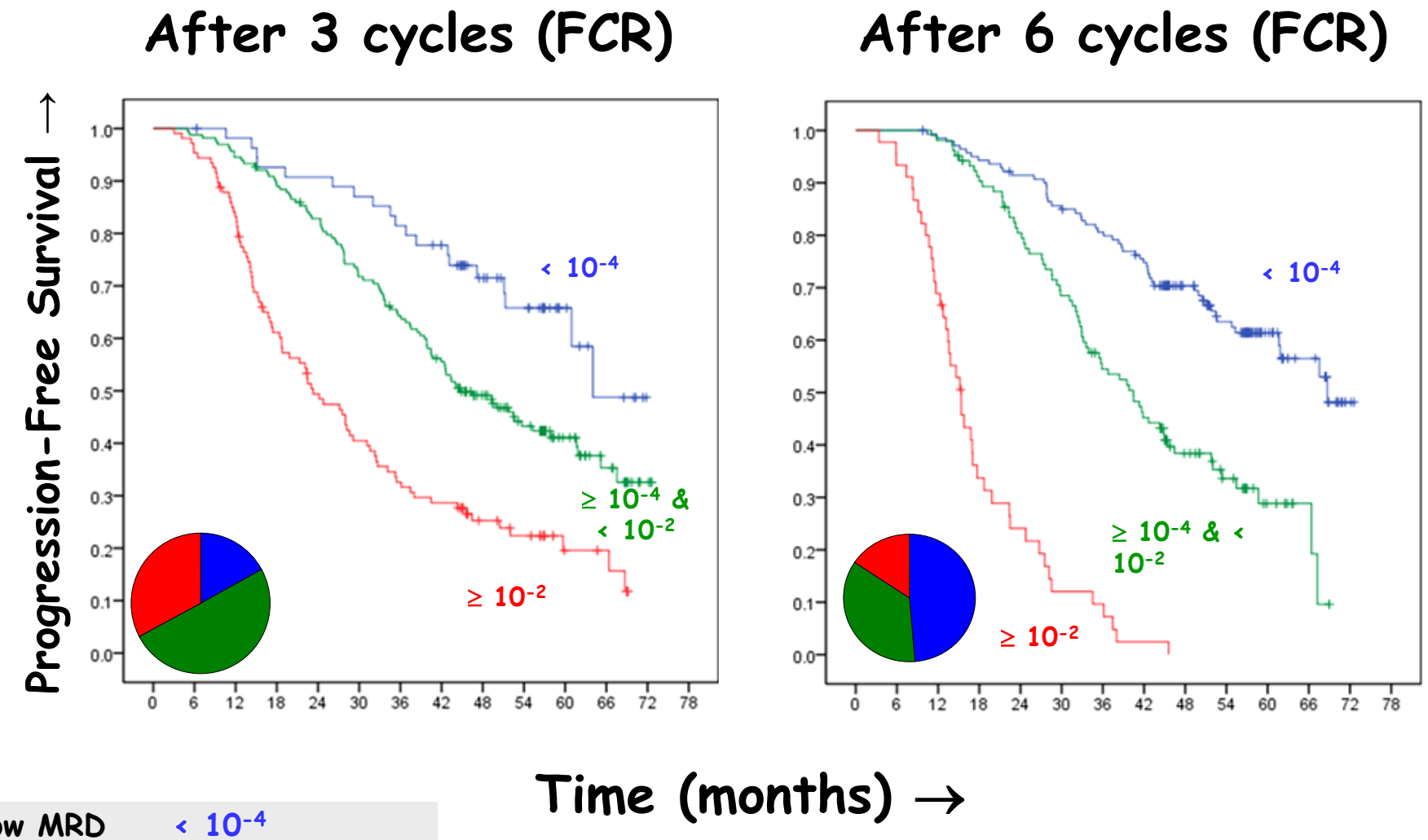
PROGNOSTIC IMPACT OF MRD LEVELS IN CLL:
In the literature any level is prognostic



all p <0.01

Prepared by Sebastian Bötcher

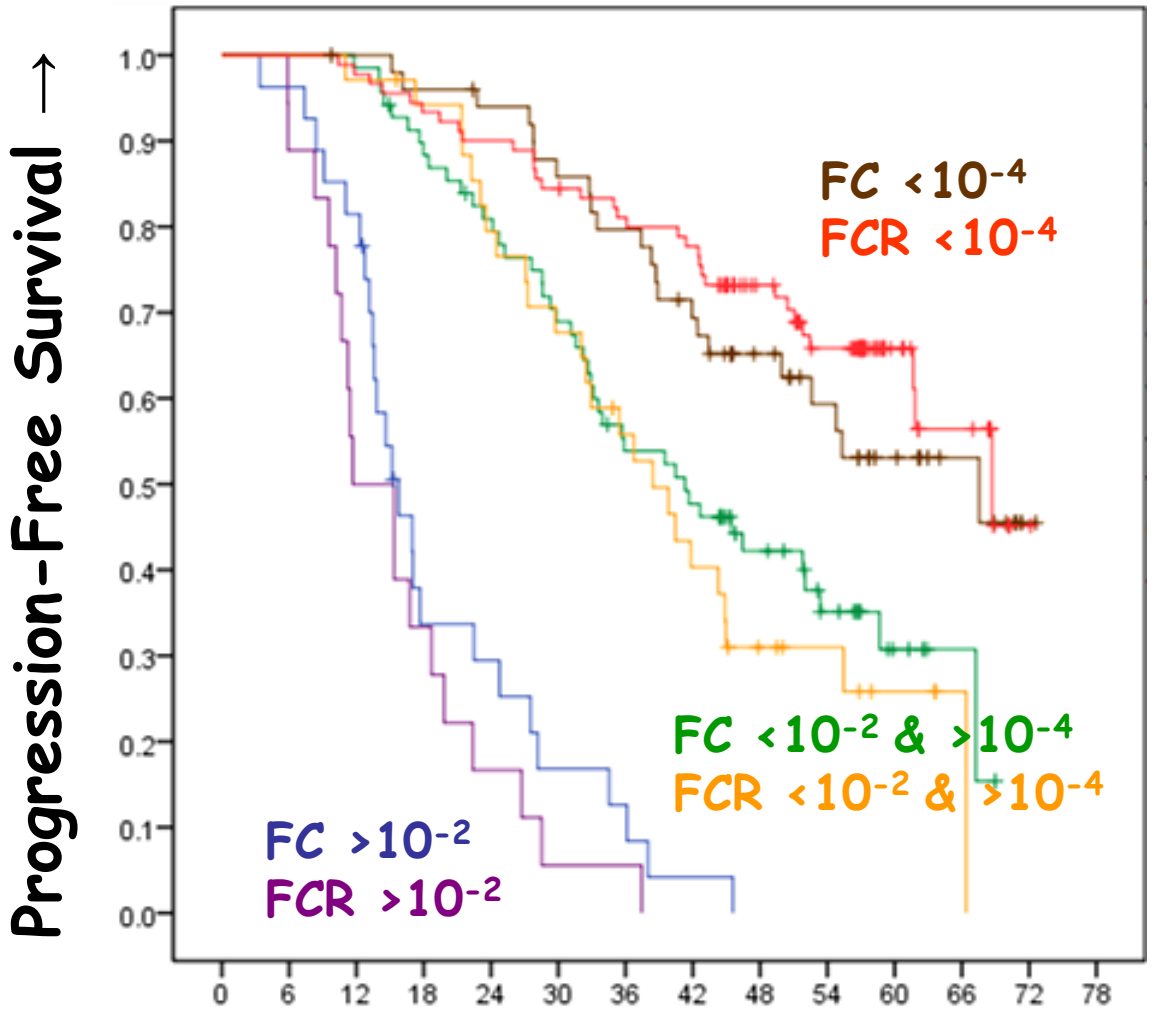
CLL8 DCLLSG TRIAL: MRD at interim and final restaging

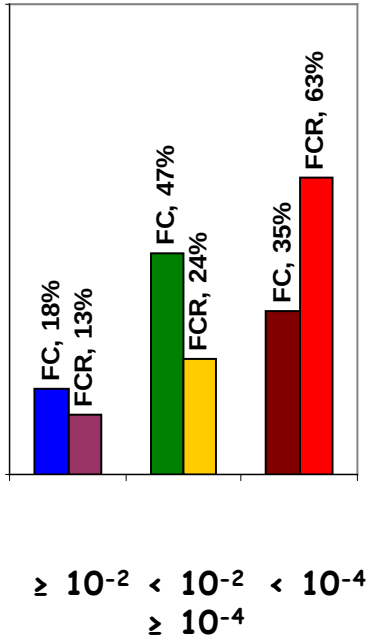


Low MRD < 10^{-4}
Intermediate ≥ 10^{-4} & < 10^{-2}
High MRD ≥ 10^{-2}

Böttcher S et al. J Clin Oncol 2012;30:980-988

CLL8 DCLLSG trial: PFS prediction from Final Restaging (PB) according to treatment arm





Böttcher et al., JCO, 2012

CLL: PFS and OS prediction at Final Restaging (PB) - Multivariate analysis -

		HR PFS		HR OS	
MRD	Overall		p< .0001		p< .0001
	≥ 10^{-4} to <10^{-2} vs. < 10^{-4}	2.8	p< .0001		
	≥ 10^{-2} vs. < 10^{-4}	21.8	p< .0001	7.1	p< .0001
Clinical status	Overall		p= .002		
	PR vs. CR				
	NR vs. CR	4.0	p= .0007		
ECOG	0 vs. > 0			1.8	p= .040
Del(17p)	present	3.6	p= .0005	3.1	p= .004
IGHV	unmutated	2.2	p= .0001		

Böttcher et al., J Clin Oncol, 2012

CLL: UTILITY OF MRD IN CLINICAL PRACTICE

- **After induction therapy predicts PFS and OS:**
  - Independent from treatment
  - Independent from pre-therapeutic risk features (*IGHV*, cytogenetics, stage etc.)
  - Independent from PR/CR status after therapy
  - Preliminary data in unselected first-line patients:
    - Level < 10^{-4} → median PFS ~ 6 years
    - Level ≥ 10^{-4} → median PFS ~ 2.5 years (to be further subdivided at 10^{-2})
- **Predicts GvL after allogeneic stem cell transplantation**
  - (kinetics during tapering of immunosupression)

Prepared by Sebastian Bötcher

CLL: UTILITY OF MRD IN CLINICAL PRACTICE

End-point marker for the identification of:

- superior arm in a randomized clinical trial
- poor-risk patients after induction therapy → candidates for consolidation / maintenance
- good responders after half of induction therapy → candidates for de-escalation
- G v L failures after allogeneic SCT → candidates for donor lymphocyte infusions

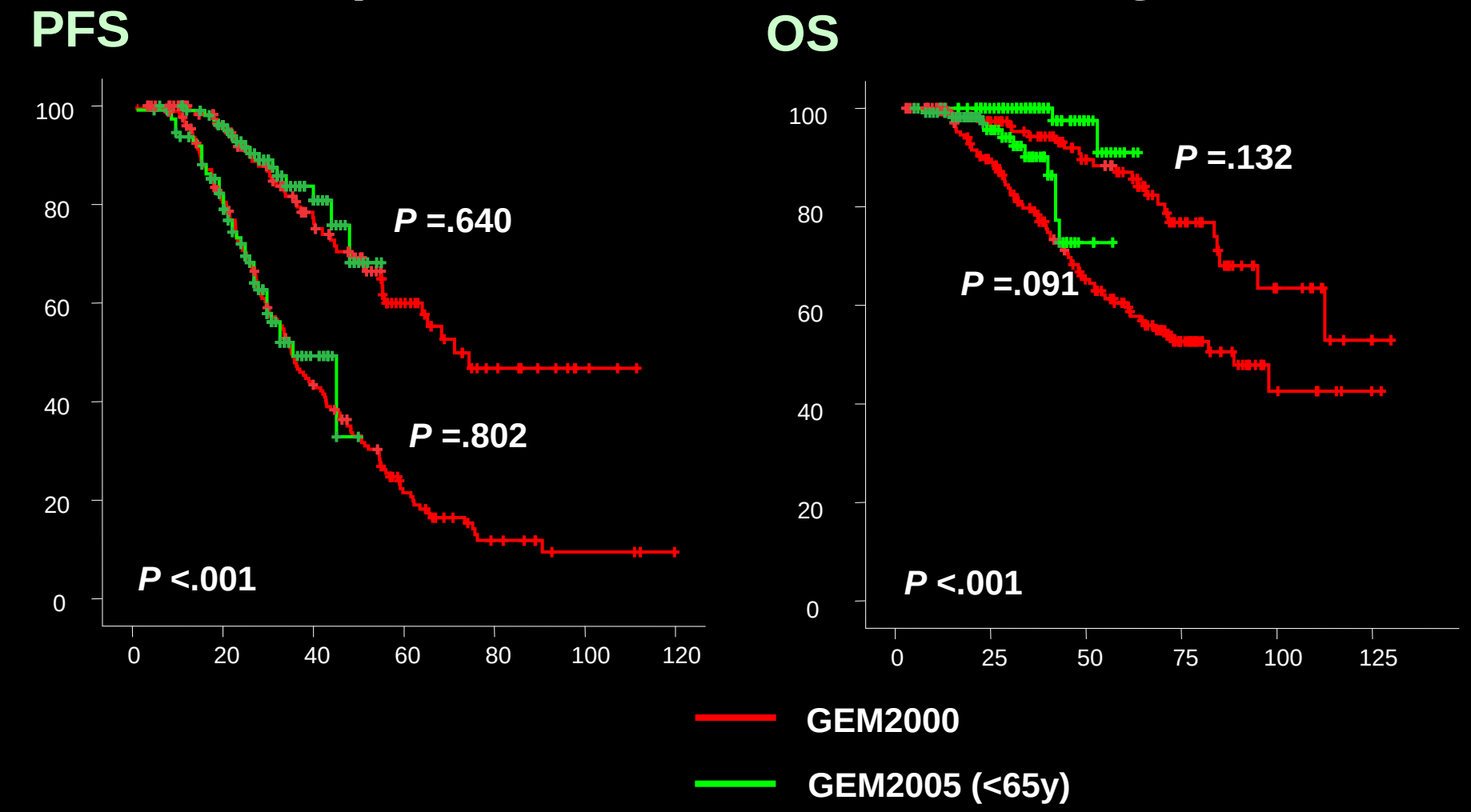
→ DO NOT BASE TREATMENT DECISIONS ON MRD OUTSIDE CLINICAL TRIALS

→ USE STANDARDIZED TECHNOLOGY

Prepared by Sebastian Bötcher

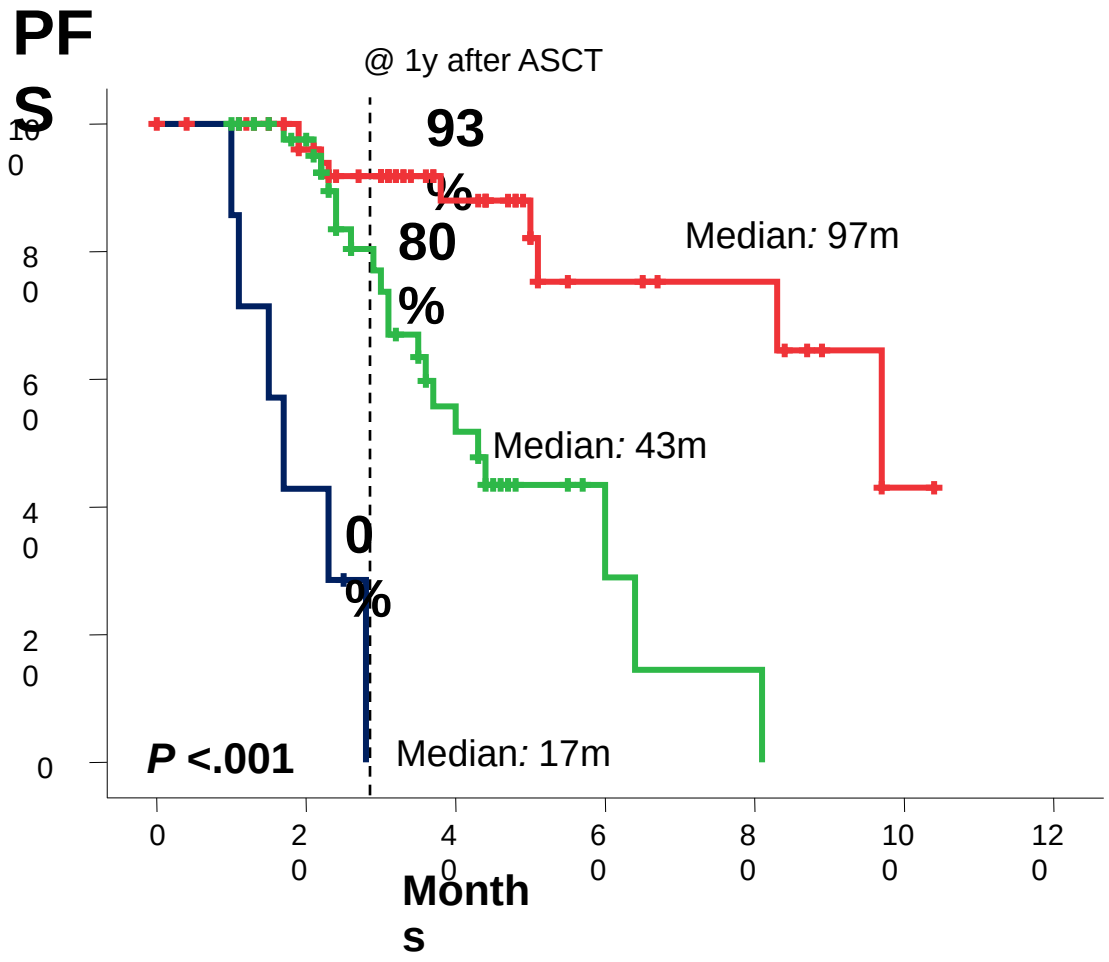
GEM2000 & GEM2005: Impact on survival of achieving an Immunophenotypic CR after HDT/ASCT

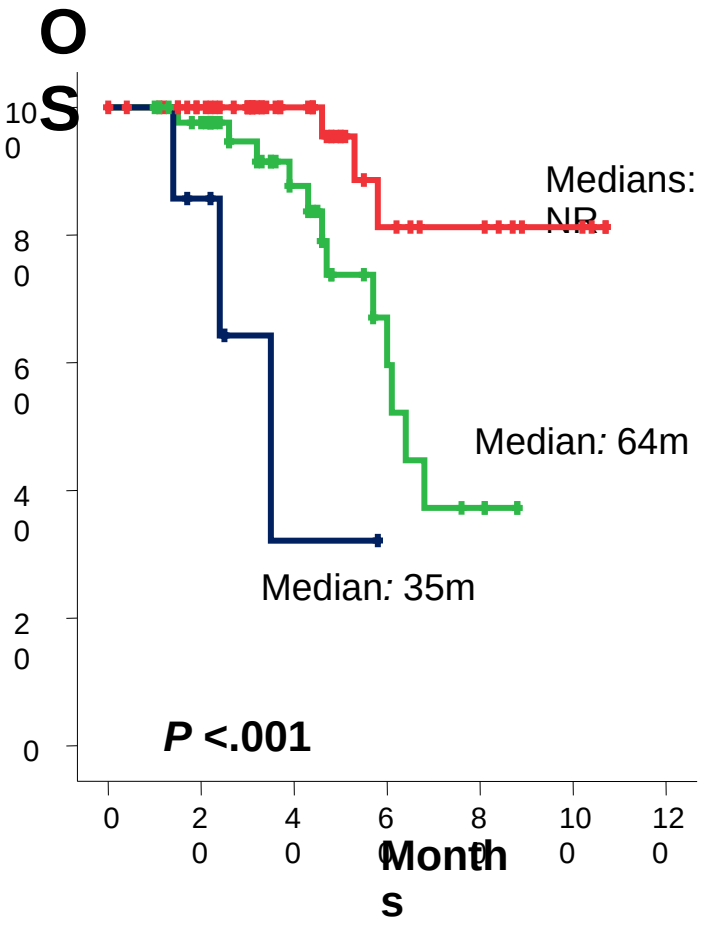
independent of the induction regimen



Paiva et al. Blood 2012

GEM 2000+2005: Immunophenotypic response & FISH for the prediction of early relapse in CR patients after HDT/ASCT (n=241)





MRD negative + Standard risk FISH (n=58)
MRD positive OR High-risk FISH (n=45)
MRD positive + High-risk FISH (n=7)

Paiva et al, Blood 2012

FLOW MRD IN HAEMATOLOGICAL MALIGNANCIES

- Does response to therapy impact on long-term patient outcome?

- Does MRD improve prognostic stratification of patients with hematological malignancies?

- Are MRD techniques well suited for MRD assessment in hematological malignancies?

- Can MRD techniques be used in routine diagnostic labs?

MRD TECHNIQUES FOR HEMATOLOGIC MALIGNANCIES

(Updated from *Szczepanski, Orfao et al, Lancet Oncol, 2001; 2: 409-417*

Disease category	FCM immunophenotyping (sensitivity)		PCR/RT-PCR analyses (sensitivity)	
	LAIP (10^{-3}-10^{-4})	sIgκ/sIgλ or TCRVβ (10^{-2}-10^{-3})	Junctional Reg Ig/TCR genes (10^{-3}-10^{-6})	Chromosomal aberrations (10^{-4}-10^{-6})
Precursor B-ALL				
Children	>90%	NA	95%	40-50%
Adults	>95%	NA	90%	35-45%
T-ALL				
Children	>95%	30-35%	>95%	10-25%
Adults	>95%	?	90%	5-10%
Chronic B-cell leukemias	>95%	>95%	>95%	10-25%
Chronic T-cell leukemias	70-80%	60-65%	95%	<5%
B-cell lymphomas	90%	>95%	70-80%	25-30%
T-cell lymphomas	75-90%	50-60%	95%	10-15%
Multiple myeloma	>90%	>90%	70-80%	NT
AML	70-90%	NA	10%	30-40%*
CML	NA	NA	NA	>95%

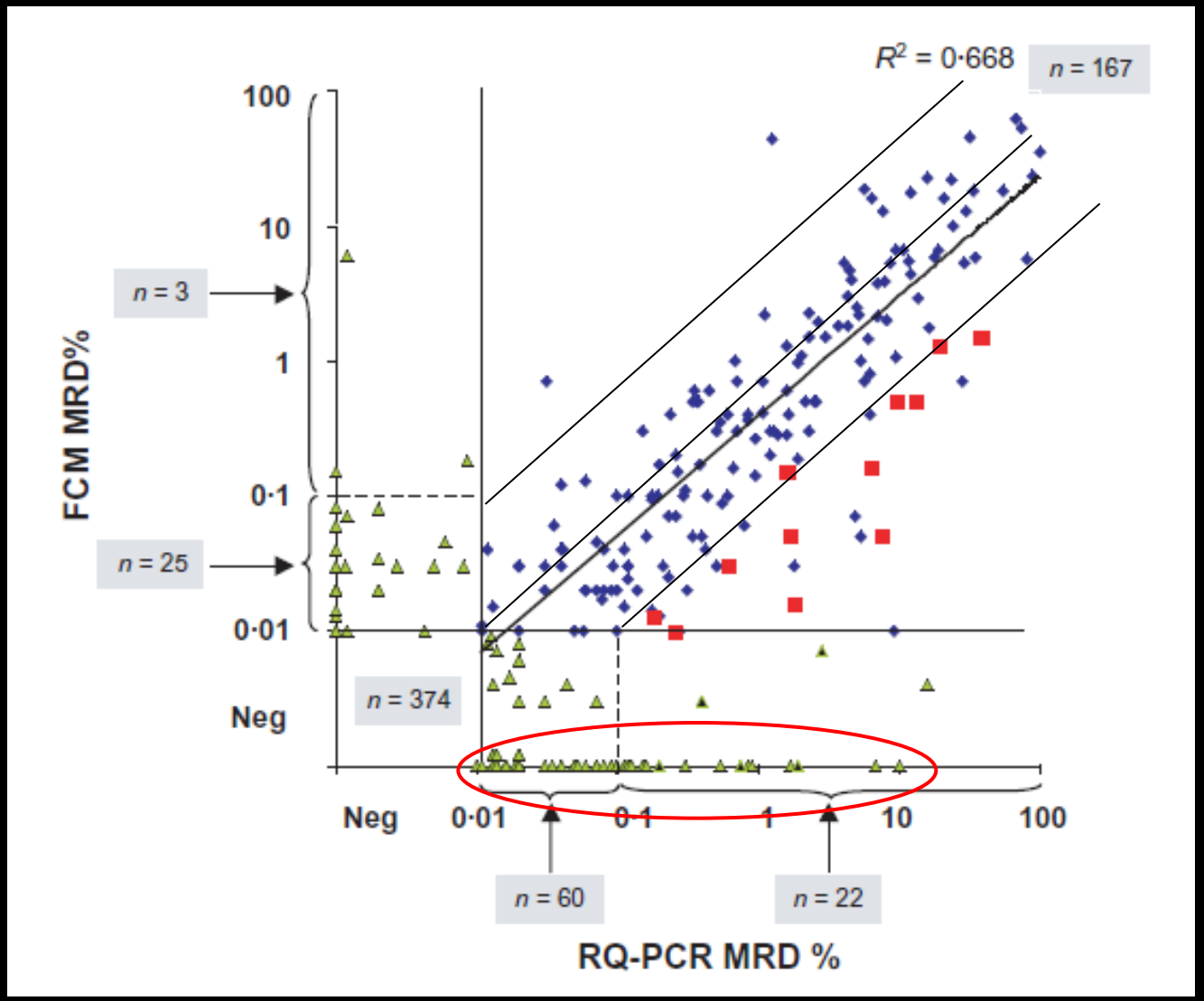
** Increased through the usage of additional molecular markers (e.g.: WT1, NMP1 & FLT3 mutations*

Advantages and disadvantages of the three main MRD techniques

MRD technique	Estimated sensitivity	Advantages	Disadvantages
Flow cytometry -4 colors -6-8 colors	 10^{-3} – 10^{-4} 10^{-4} – 10^{-5} ??	- Fast - Analysis at cell population level or single cell level - Easy storage of data	- Variable sensitivity, because of similarities between normal (regenerating) cells and malignant cells - Limited standardization, no QC rounds
RQ-PCR of Ig/TCR genes	10^{-4} – 10^{-5}	- Applicable in virtually all lymphoid malignancies - Sensitive - Standardized + QC rounds	- Time consuming - Expensive - Requires extensive experience and knowledge
RQ PCR of fusion transcripts and other aberrancies	10^{-4} – 10^{-6}	- Relatively easy - Sensitive - Excellent for specific leukemia subgroups, such as BCR-ABL or PML-RARA	- Limited standardization (harmonization) - Limited QC rounds (with conversion factors) - Limited applicability in broad patient groups (absence of targets)

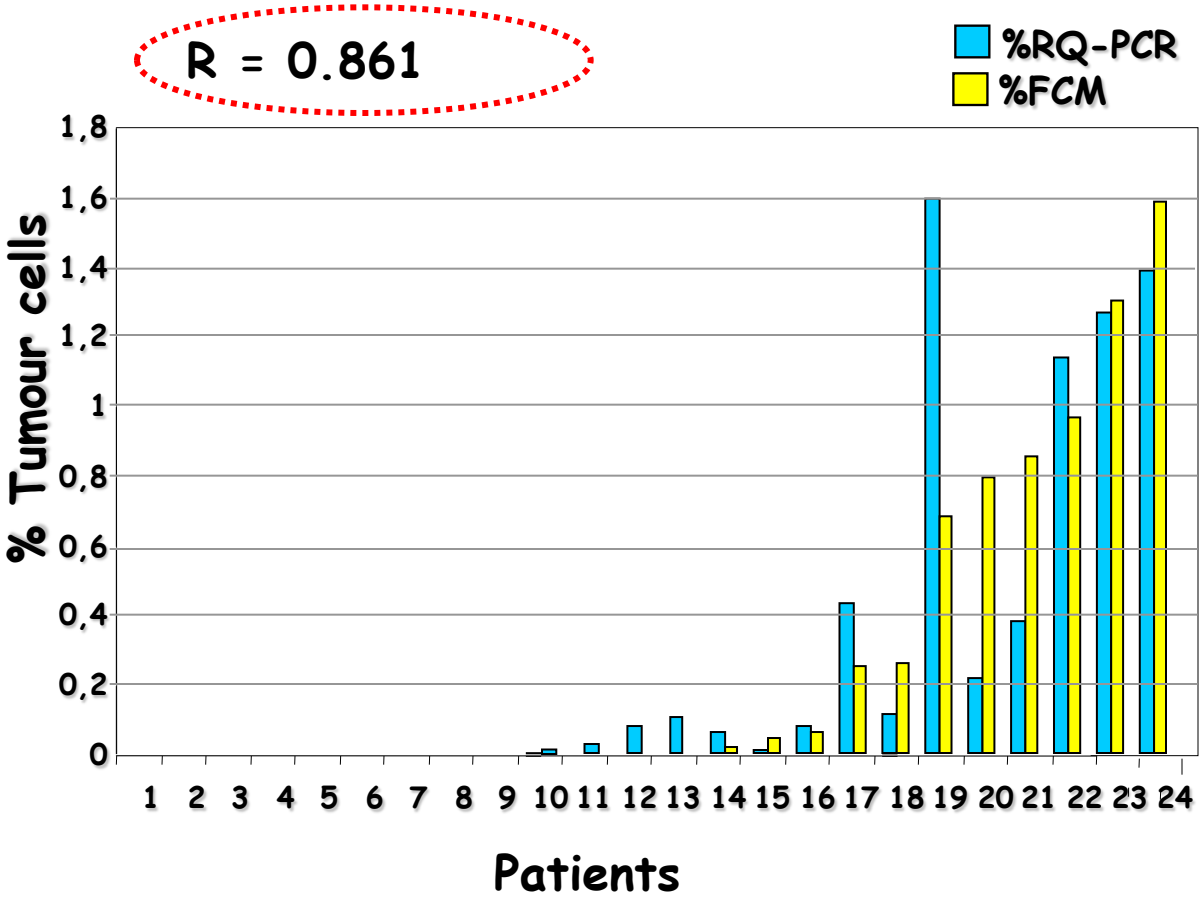
Prepared by JJM van Dongen

RQ-PCR and flowcytometric MRD in childhood ALL



Thörn, I. et al. MRD assessment in childhood ALL: a Swedish multi-centre study comparing real-time polymerase chain reaction and multicolour flow cytometry. Br J Haematol 2011 152: 743-753

MRD IN MM: CORRELATION BETWEEN RQ-PCR & FCM



Cases	%RQ-PCR	%FCM	
1	0	0	
2	0	0	
3	0	0	
4	0	0	
5	0	0	
6	0	0	
7	0	0	
8	0.0009	0	✓
9	0,002	0	X
10	0,003	0	✓
11	0,024	0	✓
12	0,082	0	X
13	0,106	0	X
14	0,062	0,014	
15	0,01	0,047	X
16	0,077	0,065	
17	0,435	0,25	
18	0,113	0,26	
19	1.61	0,686	
20	0,219	0,8	
21	0,382	0,86	
22	1,144	0,97	
23	1,279	1,31	
24	1,399	1,6	

Sarrasquete et al, Haematologica, 2005

MRD IN MULTIPLE MYELOMA: APPLICABILITY OF FCM VS RQ-PCR

Patients in CR (N=53)

Cases with aberrant phenotypes (N=48)

APPLICABILITY OF FCM: 90%

Problems associated with the sample (N=16)

- Very low infiltration (N=9)
- Degraded DNA (N=4)
- Insufficient DNA (N=3)

Problems associated with the method (RQ-PCR) (N= 8)

- No clonal rearrangement detected (N=3)
- Very short N-region (N=3)
- Cases with mutations in the target sequence (N=2)

APPLICABILITY OF RQ-PCR: 75%

Sarrasquete et al, Haematologica, 2005

Open

Leukemia (2012), 1–68

www.nature.com/leu

ORIGINAL ARTICLE

EuroFlow antibody panels for standardized *n*-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes

JJM van Dongen[1], L Lhermitte[2], S Böttcher[3], J Almeida[4], VHJ van der Velden[1], J Flores-Montero[4], A Rawstron[5], V Asnafi[2], Q Lécrevisse[4], P Lucio[6], E Mejstrikova[7], T Szczepański[8], T Kalina[7], R de Tute[5], M Brüggemann[3], L Sedek[8], M Cullen[5], AW Langerak[1], A Mendonça[6], E Macintyre[2], M Martin-Ayuso[9], O Hrusak[7], MB Vidriales[10] and A Orfao[4] on behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708)

Most consensus leukemia & lymphoma antibody panels consist of lists of markers based on expert opinions, but they have not been validated. Here we present the validated EuroFlow 8-color antibody panels for immunophenotyping of hematological malignancies. The single-tube screening panels and multi-tube classification panels fit into the EuroFlow diagnostic algorithm with entries defined by clinical and laboratory parameters. The panels were constructed in 2–7 sequential design–evaluation–redesign rounds, using novel Infinicyt software tools for multivariate data analysis. Two groups of markers are combined in each 8-color tube: (i) backbone markers to identify distinct cell populations in a sample, and (ii) markers for characterization of specific cell populations. In multi-tube panels, the backbone markers were optimally placed at the same fluorochrome position in every tube, to provide identical multidimensional localization of the target cell population(s). The characterization markers were positioned according to the diagnostic utility of the combined markers. Each proposed antibody combination was tested against reference databases of normal and malignant cells from healthy subjects and WHO-based disease entities, respectively. The EuroFlow studies resulted in validated and flexible 8-color antibody panels for multidimensional identification and characterization of normal and aberrant cells, optimally suited for immunophenotypic screening and classification of hematological malignancies.

Leukemia accepted article preview 3 May 2012; doi:10.1038/leu.2012.120

Keywords: euroFlow; antibody panel; lymphoma; flow cytometry; 8-color immunostaining; standardization

Aberrant phenotypes in multiple myeloma: 97%

Leukemia (1999) 13, 110–118

http://www.stockton-press.co.uk/leu

EuroMRD

BIO-TECHNICAL METHODS SECTION (BTS)

Primers and protocols for standardized detection of minimal residual disease in acute lymphoblastic leukemia using immunoglobulin and T cell receptor gene rearrangements and *TAL1* deletions as PCR targets

Report of the BIOMED-1 CONCERTED ACTION: Investigation of minimal residual disease in acute leukemia

MJ Pongers-Willemse[1], T Seriu[2], F Stolz[3], E d'Aniello[4], P Gameiro[5], P Pisa[6], M Gonzalez[7], CR Bartram[2], ER Panzer-Grümayer[3], A Biondi[4], JF San Miguel[7] and JJM van Dongen[1]

Leukemia (2007) 21, 604–611

www.nature.com/leu

LEADING ARTICLE

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)

[1]Department of Immunology, Erasmus MC, Rotterdam, the Netherlands; [2]Centro Ricerca Tettamanti, Universitá Milano-Bicocca, Monza, Italy; [3]Department of Pediatrics, University Hospital Schleswig-Holstein, Campus Kiel, Germany; [4]Department of Cellular and Molecular Medicine, University of Bristol, Bristol, UK; [5]Klinikum der Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany; [6]Children's Cancer Research Institute and St Anna Kinderspital, Vienna, Austria; [7]Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany; [8]Children's Cancer Institute Australia for Medical Research, University of NSW, Sydney, Australia; [9]Laboratoire de Biochimie Génétique, Hopital Robert Debré, Paris, France; [10]Department of Clinical

EuroClonality

Leukemia (2003) 17, 2257–2317

www.nature.com/leu

LEADING ARTICLE

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]

Open

Leukemia (2012), 1–13

www.nature.com/leu

REVIEW

EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations

AW Langerak[1], PJTA Groenen[2], M Brüggemann[3], K Beldjord[4], C Bellan[5], L Bonello[6], E Boone[7], GI Carter[8], M Catherwood[9], F Davi[10], M-H Delfau-Larue[11], T Diss[12], PAS Evans[13], P Gameiro[14], R Garcia Sanz[15], D Gonzalez[16], D Grand[17], Å Håkansson[18], M Hummel[19], H Liu[20], L Lombardia[21], EA Macintyre[22], BJ Milner[23], S Montes-Moreno[24], E Schuuring[25], M Spaargaren[26], E Hodges[27] and JJM van Dongen[1]

MRD DIAGNOSTICS IN CHRONIC MYELOID LEUKEMIA

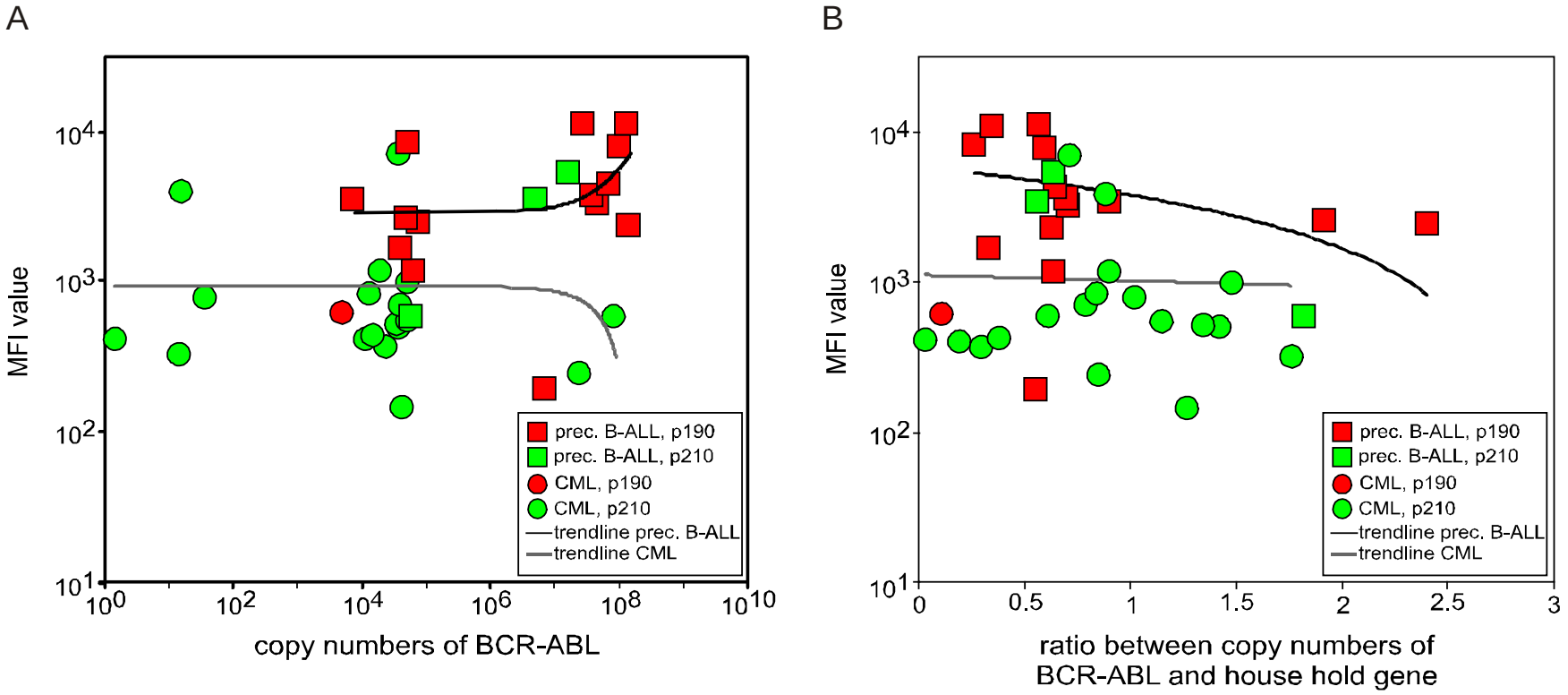
Since the introduction of TKI therapy, CML has become the model disease for MRD-based treatment direction

TECHNICAL APPROACH:

- BCR-ABL transcripts measured by RQ-PCR, but the BCR-ABL transcript levels vary significantly among patients.
- Most CML laboratories have a laboratory-specific conversion factor (CF) that enables to convert local results to an International Scale (IS) for better comparability.
- Unfortunately, it is not clear why CFs are needed.
- The CF system it is not fully validated and not accessible for all CML-MRD labs.

CONSEQUENCE: Highly variable ways by which laboratories perform MRD diagnostics in CML and they interpret individual samples

No straightforward relationship found between BCR-ABL protein levels and transcript levels.



* Relation between BCR-ABL fusion protein levels and *BCR-ABL* transcript levels, expressed as copy numbers of *BCR-ABL* transcripts (A) or as ratio between *BCR-ABL* and control gene transcripts (B).

MRD DIAGNOSTICS IN CHRONIC MYELOID LEUKEMIA

Since the introduction of TKI therapy, CML has become the model disease for MRD-based treatment direction

Clinical response:

- Treatment goal: achievement of Major Molecular Response (MMR), defined as BCR-ABL transcript levels of ≤ 0.1% (Baccarani 2006).
- Complete molecular response (CMR) was initially defined as 2 serial samples with undetectable BCR-ABL transcripts (Baccarani 2006)
- However, with the usage of second-line TKI different definitions of CMR have been proposed, the basic definition is 4.5 log reduction from pretreatment BCR-ABL transcript levels.

CONCLUSION: Decisions made on the basis of MRD results but there is still an urgent need for standardized BCR-ABL MRD diagnostics

MULTIPARAMETER FLOW CYTOMETRY MONITORING OF MRD: problems we are facing

- Many reagents: costly and complex diagnostic panels
- Need for expertise on normal (reference) cell populations
- Technical limitations
- Many ("my" suboptimal) patient-based strategies
- Not standardized: reproducibly harmonized?
- Phenotypic switches (induced or not by therapy)
- Partial and limited clinical utility (and applicability)

EuroFlow

Identification of leukemia-associated immunophenotypes

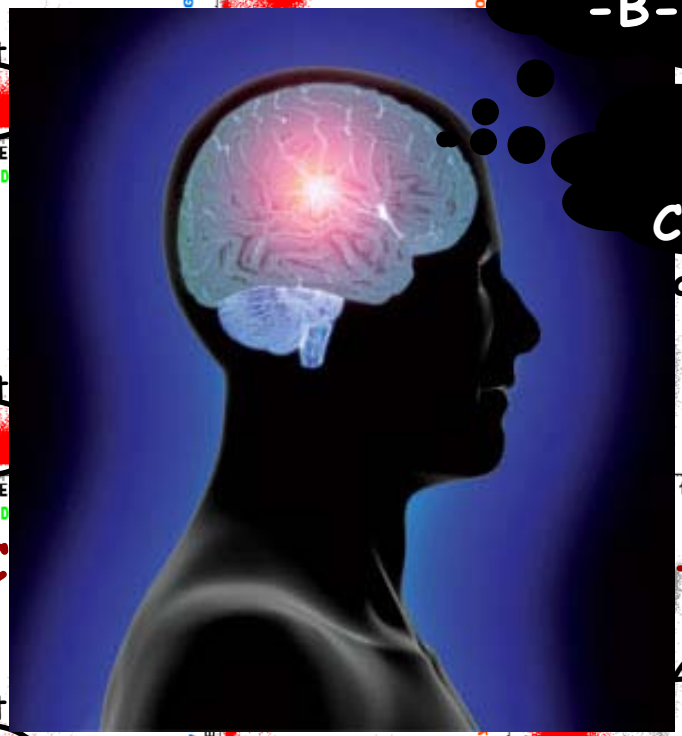
EuroFlow

TdT+ / CD19+ / CD38+

Tube

Tube

Tube



Precursor -B-ALL

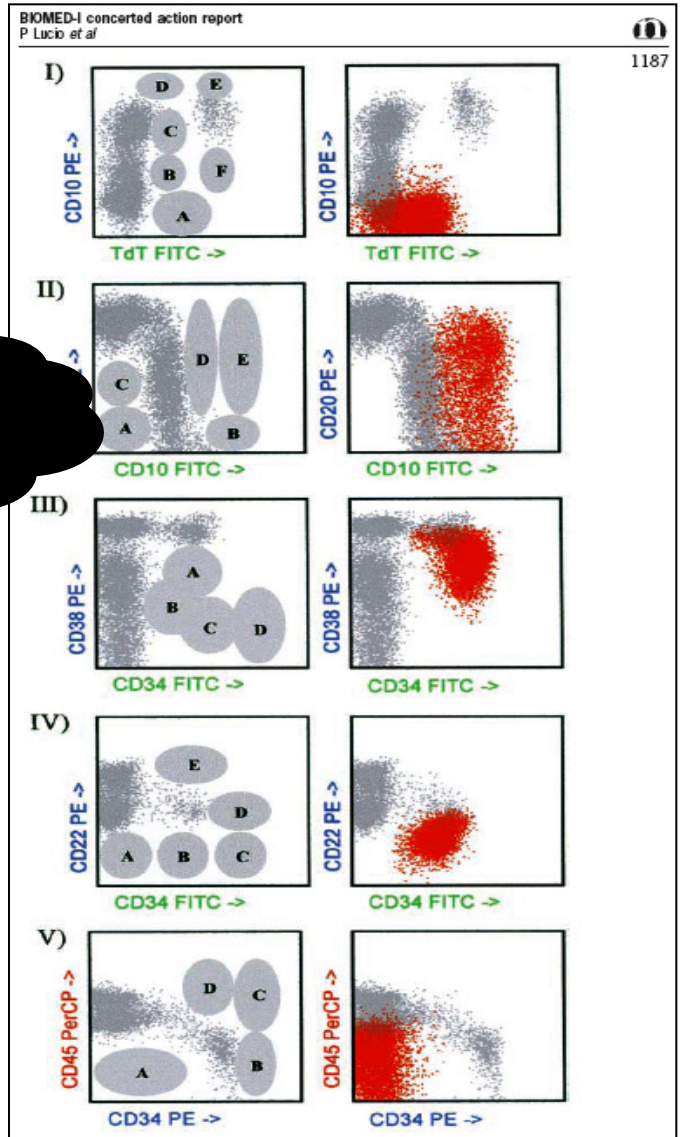
LAIP:

CD45- Tdtlo

Analyse (2D)

Analyse (2D)

JJM van Dongen Department of Immunology, Erasmus MC



Lucio et al, Leukemia, 1999

HOW TO SIMPLIFY, IMPROVE & STANDARDIZE FCM MRD PHENOTYPING?

EuroFlow

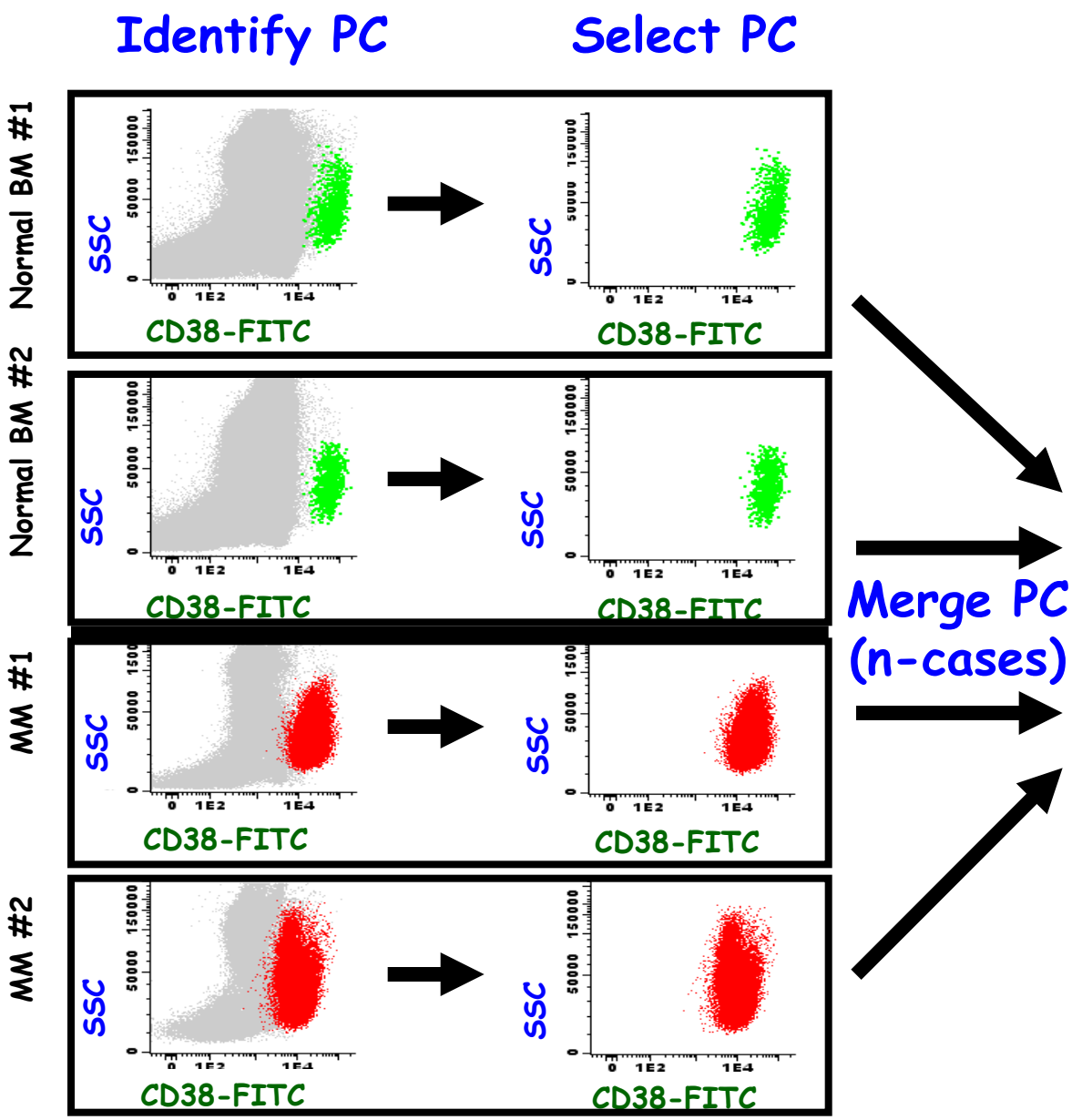
- Improve the design of antibody panels for a greater efficiency and higher reproducibility.

-Construct reference data files for normal and pathologic cell populations (e.g.: per disease category)

- Prospective evaluation of antibody panels and the new data analysis and interpretation tools.

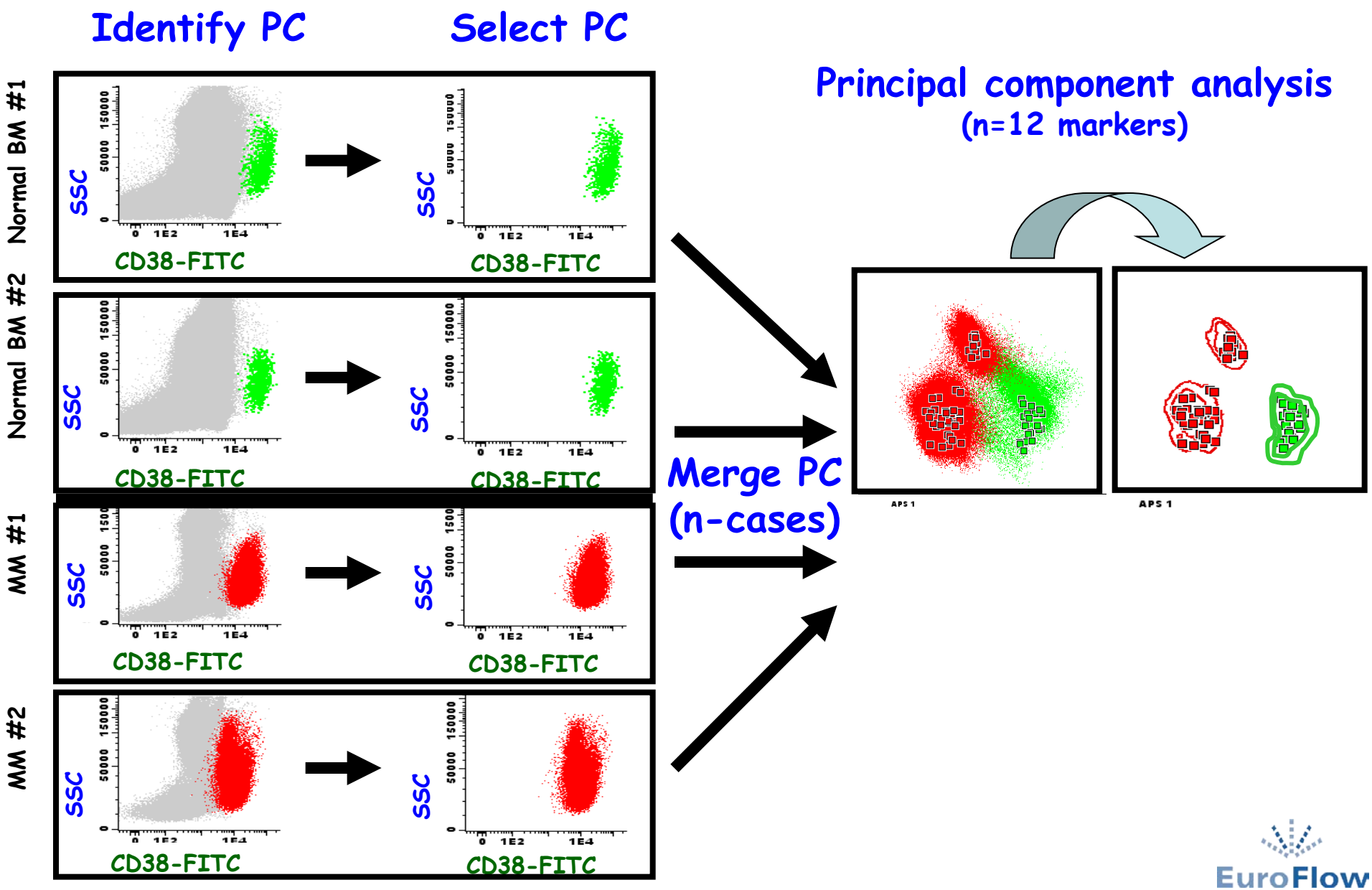
- Development of new tools for data visualization, data analysis and interpretation is required

CONSTRUCTION OF MRD EuroFlow PANELS: MM



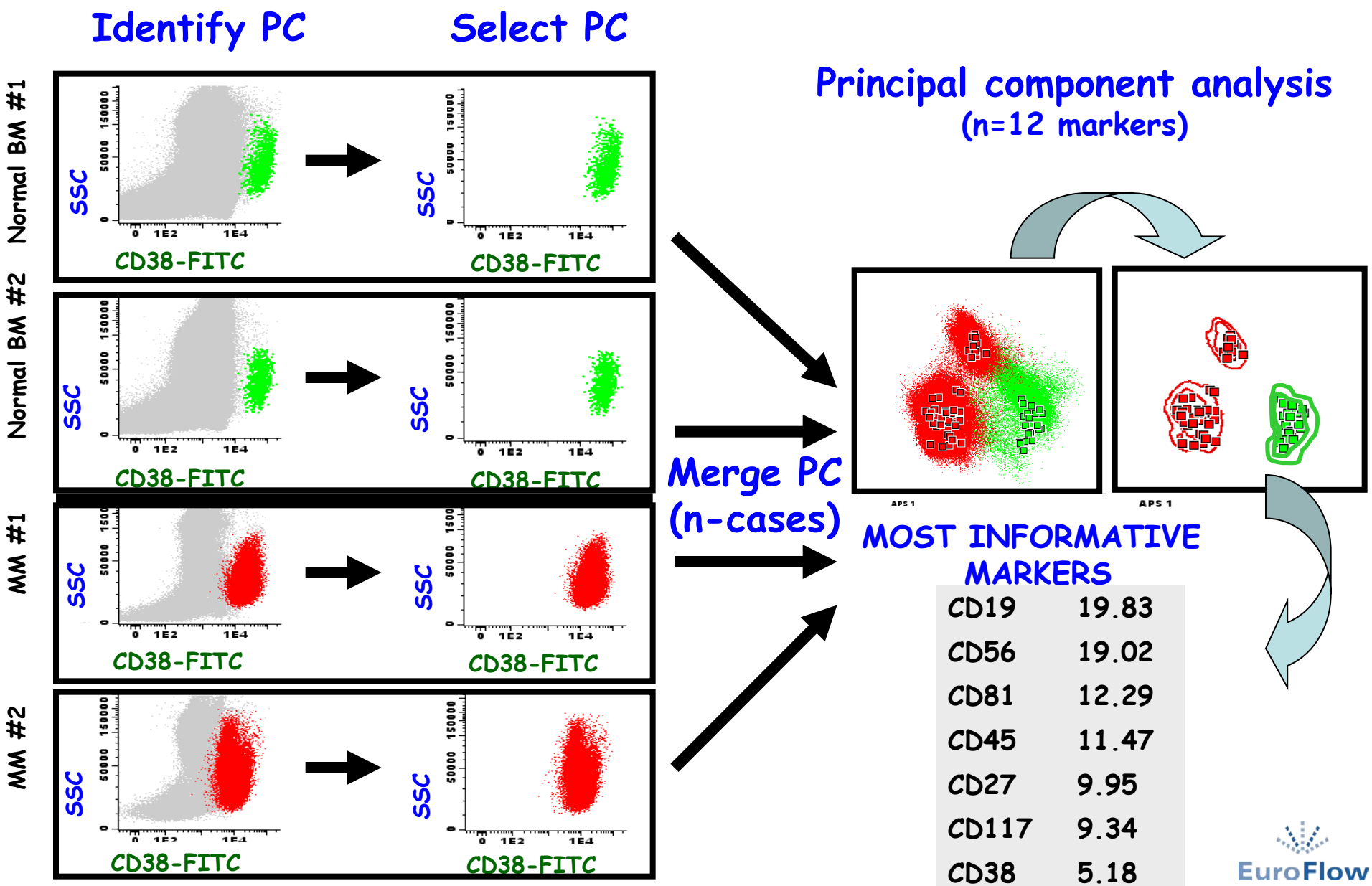
EuroFlow

CONSTRUCTION OF MRD EuroFlow PANELS: MM



EuroFlow

CONSTRUCTION OF EuroFlow MRD PANELS: MM



PCD panel: Backbone markers

	PacB HV450	PacO HV500	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7 APCC750
1	CD138		CD38	CD56	CD45	CD19		
2	CD138		CD38	CD56	CD45	CD19		

	PacB HV450	PacO HV500	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7 APCC750
1	CD138	CD27	CD38	CD56	CD45	CD19	CD117	CD81
2	CD138	CD229	CD38	CD56	CD45	CD19	CyIgk	CyIgλ

Responsible scientist: Juan Flores

EuroFlow

HOW TO SIMPLIFY, IMPROVE & STANDARDIZE FCM MRD PHENOTYPING?

EuroFlow

- Improve the design of antibody panels for a greater efficiency and higher reproducibility.

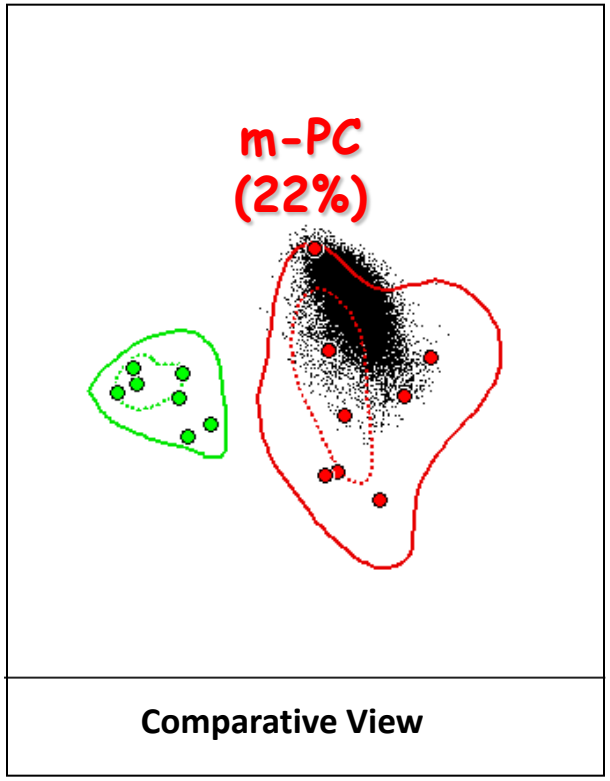
-Construct reference data files for normal and pathologic cell populations (e.g.: per disease category)

- Prospective evaluation of antibody panels and the new data analysis and interpretation tools.

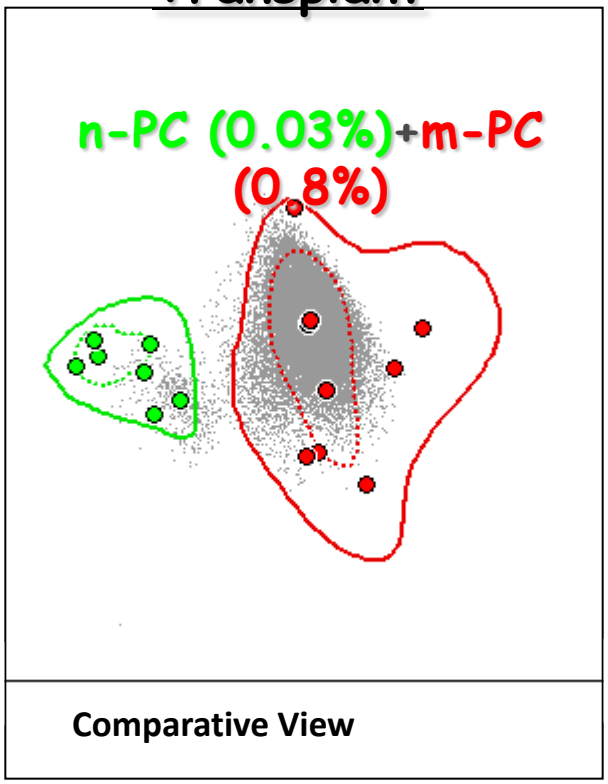
- Development of new tools for data visualization, data analysis and interpretation is required

Automated classification of normal vs aberrant plasma cells in MM

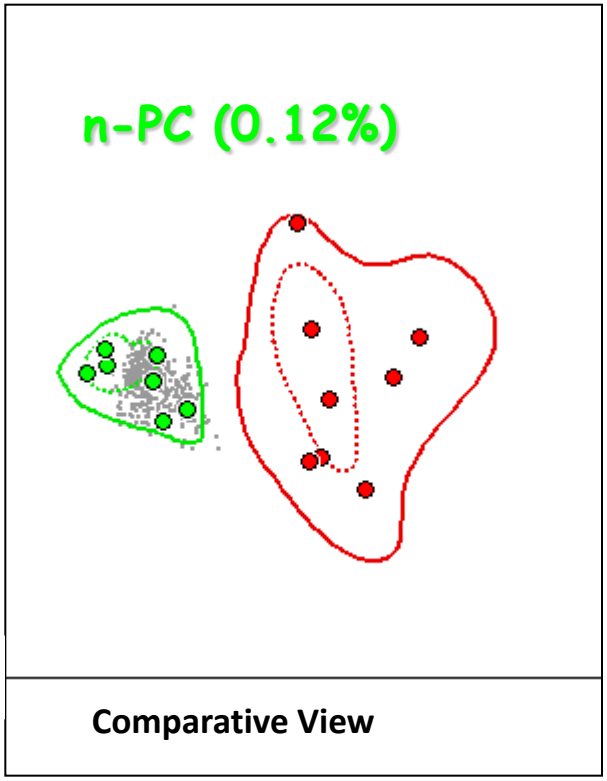
Diagnosis



Pre-Transplant



Post-transplant



HOW TO SIMPLIFY & STANDARDIZE MRD STRATEGIES

EuroFlow

- Improve the design of MRD panels for a greater efficiency and higher reproducibility.

- Construct reference data files for normal and neoplastic cells (e.g.: per disease category)

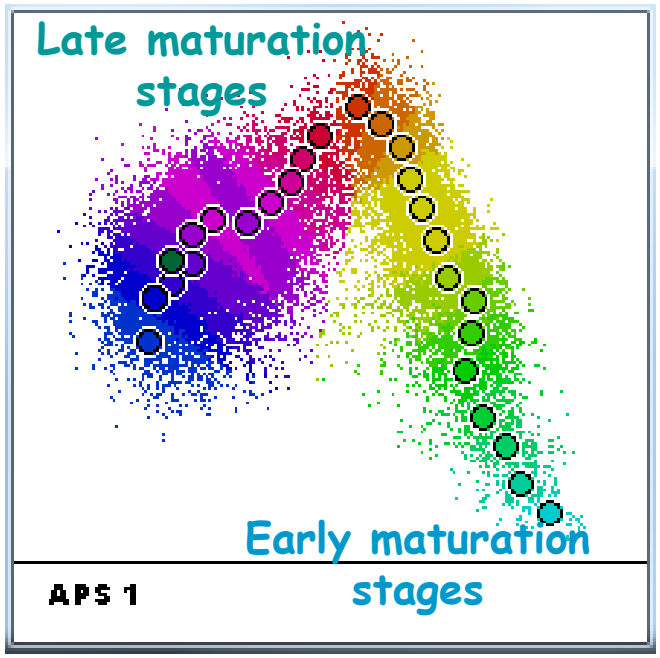
- **Multi-n-dimensional comparison of normal vs neoplastic cell populations (e.g.: at diagnosis and follow-up):**

  - Automated PCA-guided approach for homogeneous cell populations (e.g. lymphoid)
  - **Software tools for heterogeneous cell populations (e.g. maturing myeloid blasts)**

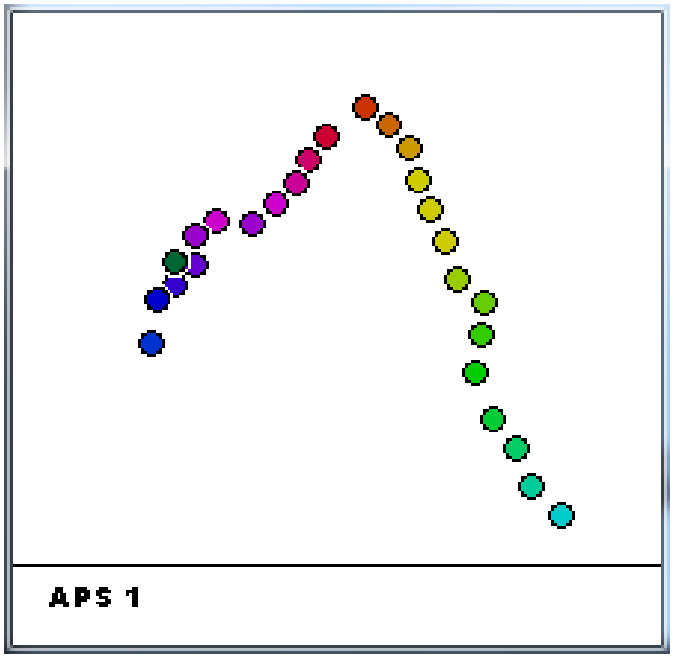
→ **MATURATION EUROFLOW (software) TOOLS**

B-CELL MATURATION IN NORMAL BONE MARROW

Normal BM



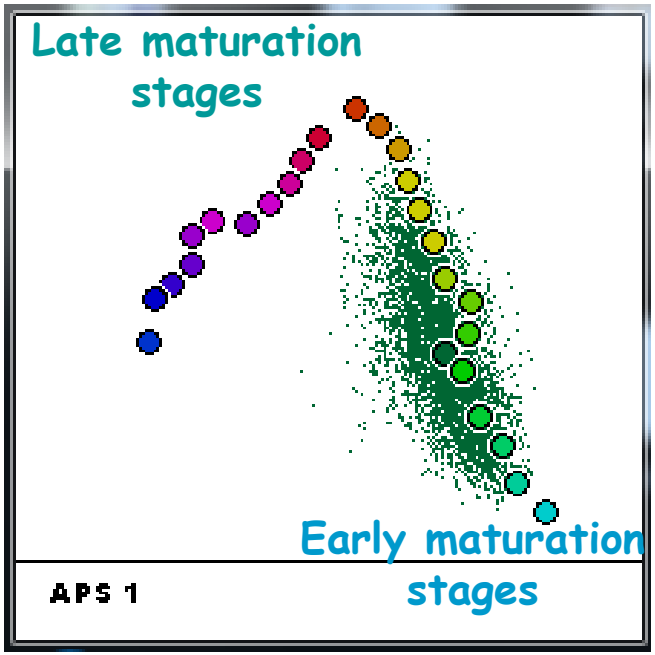
Normal BM

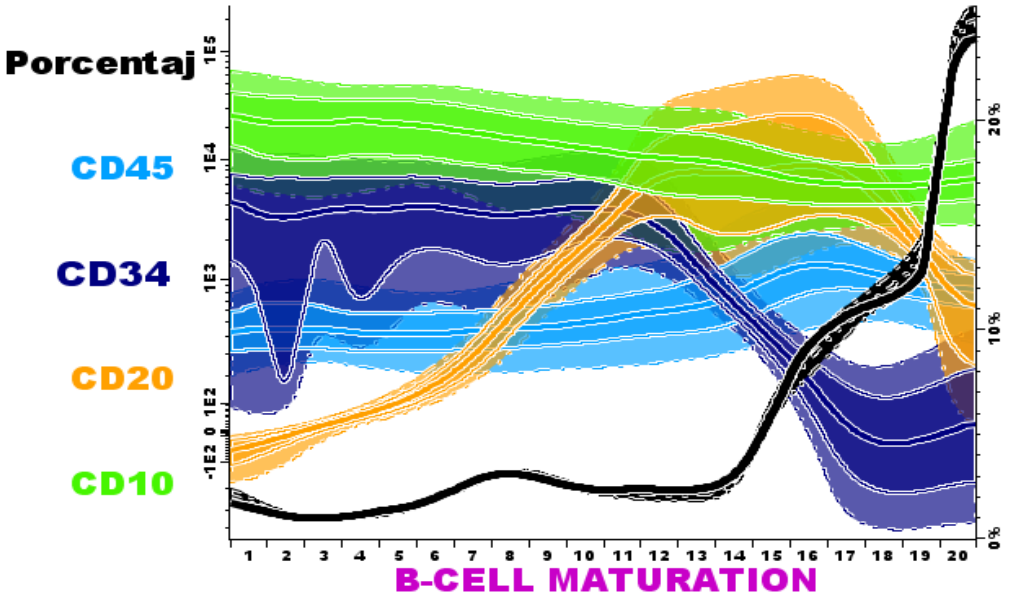


CD19 Gated B-cells (excluding PC)

B-CELL MATURATION IN (REFERENCE) REGENERATING BONE MARROW

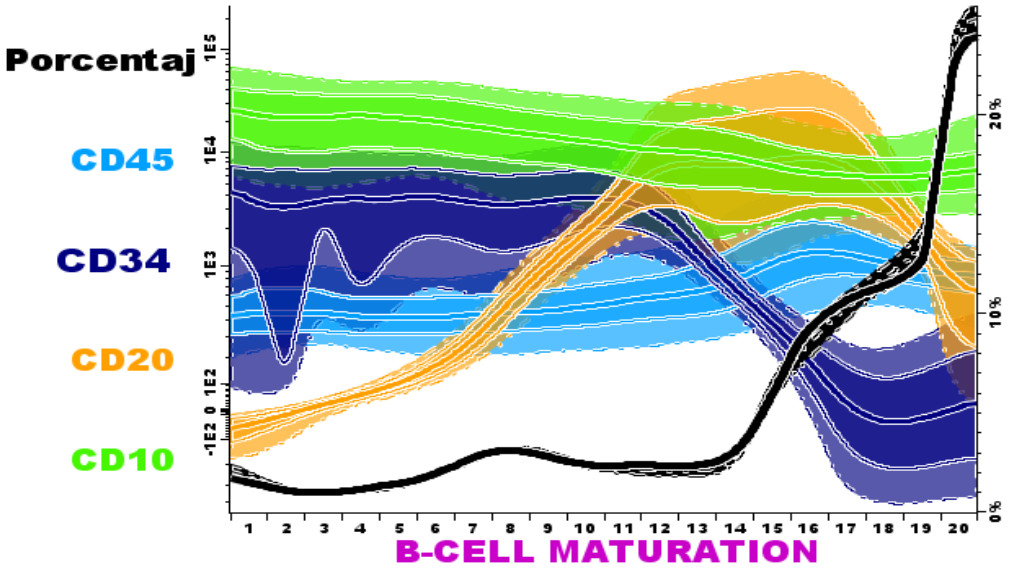
Normal vs Regenerating BM

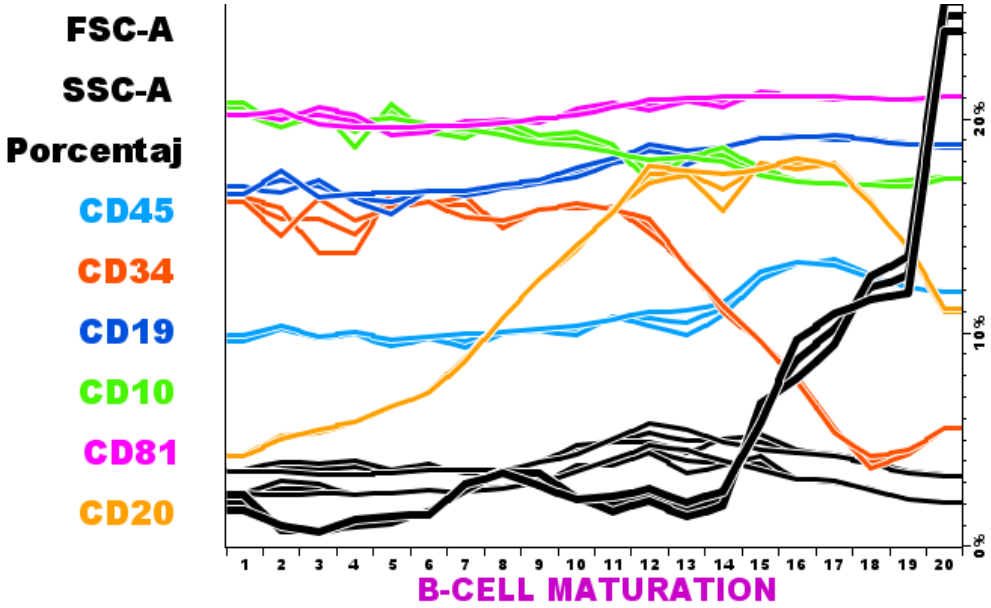




EuroFlow

B-CELL MATURATION IN (REFERENCE) REGENERATING BONE MARROW





REFERENCE DATAFILES FOR BCP-ALL MRD

Normal/Regenerating BM case 1

Normal/Regenerating BM case 2

Normal/Regenerating BM case 3

Merged Reference Data file

Responsible scientist: Esther Mejstrikova & Vincent van der Velden

EuroFlow

REFERENCE DATAFILES FOR BCP-ALL MRD

Normal/Regenerating BM case 1

Normal/Regenerating BM case 2

Normal/Regenerating BM case 3

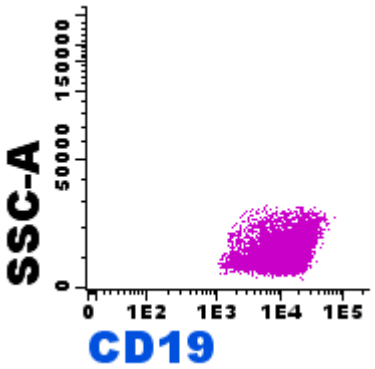
Merged Reference Data file

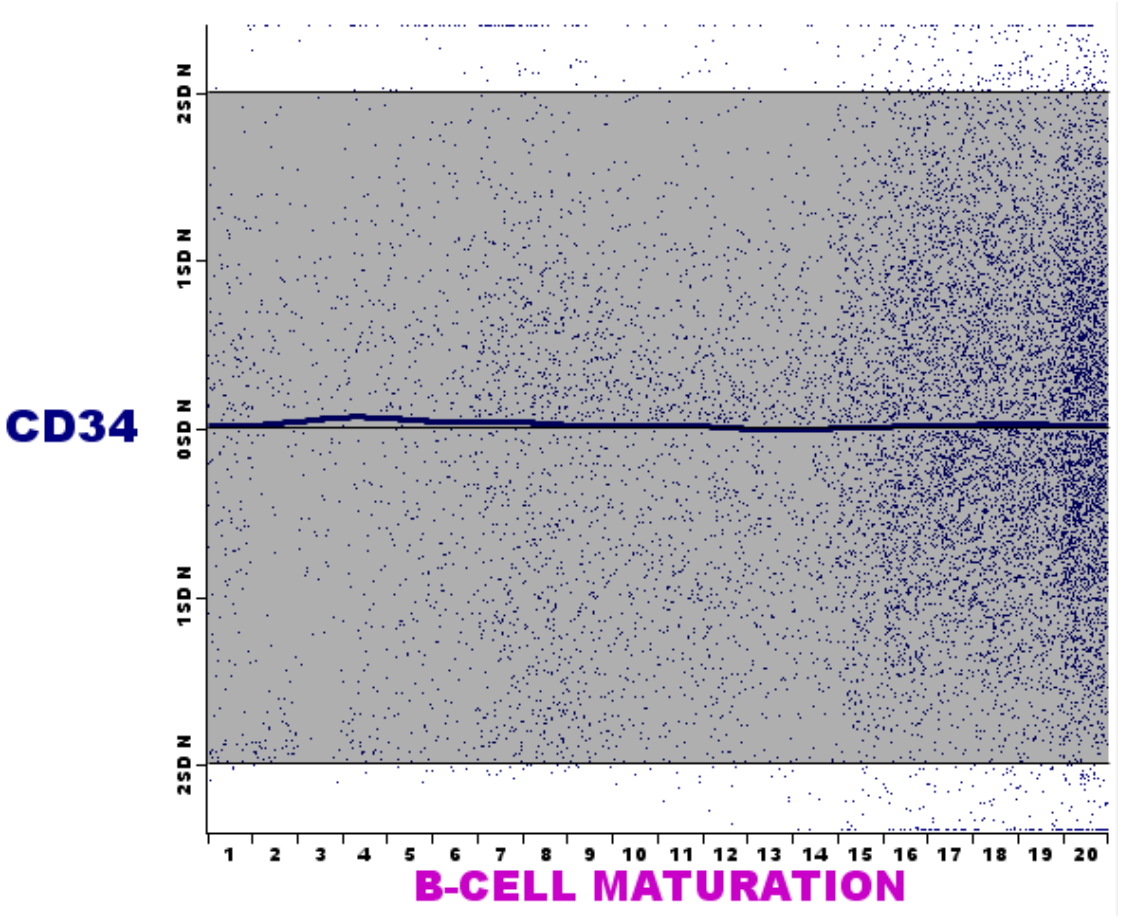
BCP-ALL case 1

Responsible scientist: Esther Mejstrikova & Vincent van der Velden

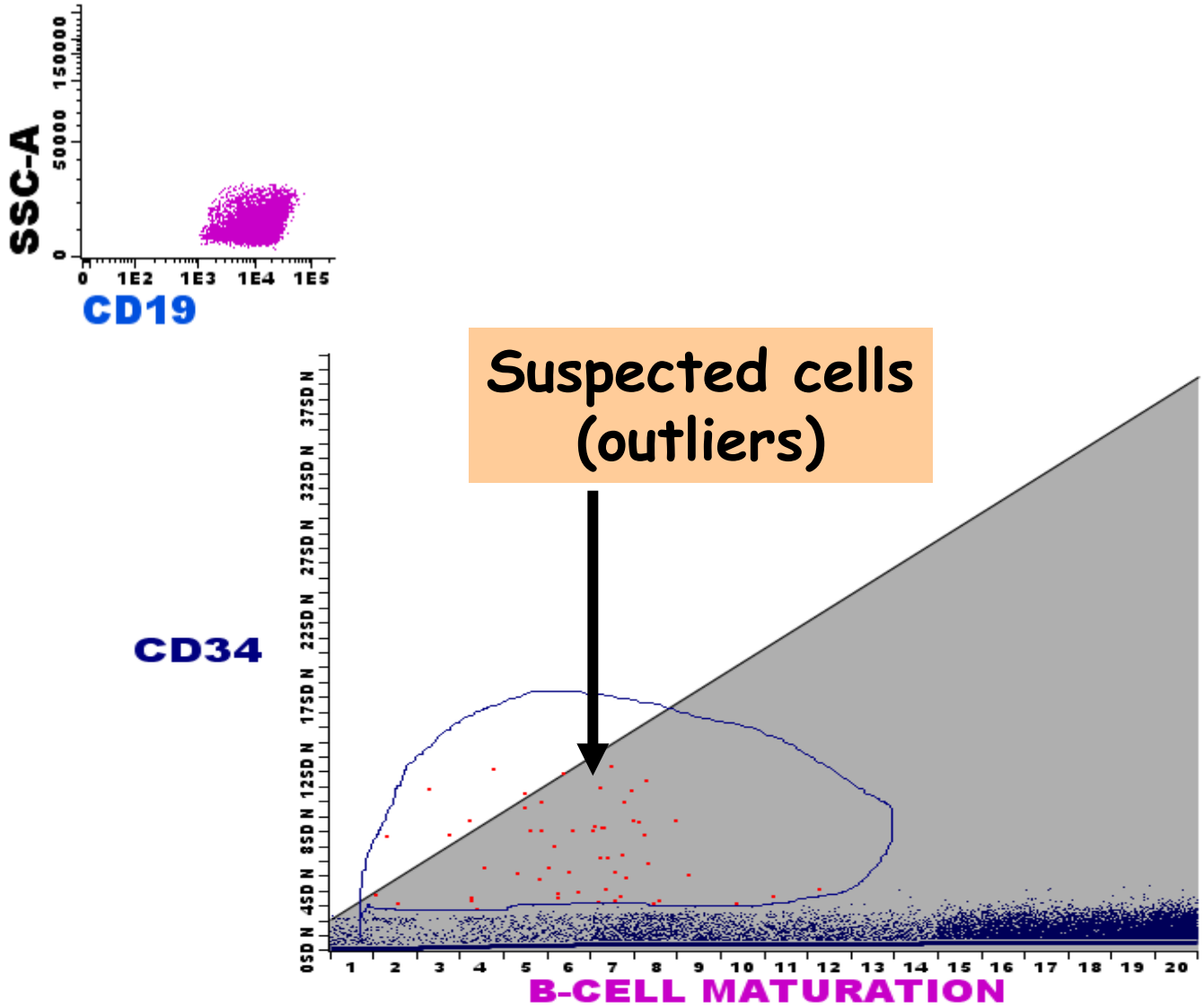
EuroFlow

MRD detection in BCP-ALL by comparison with regenerating BM B-cell maturation

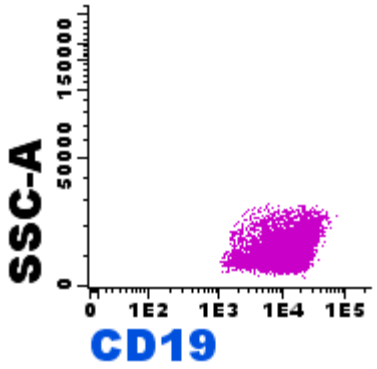




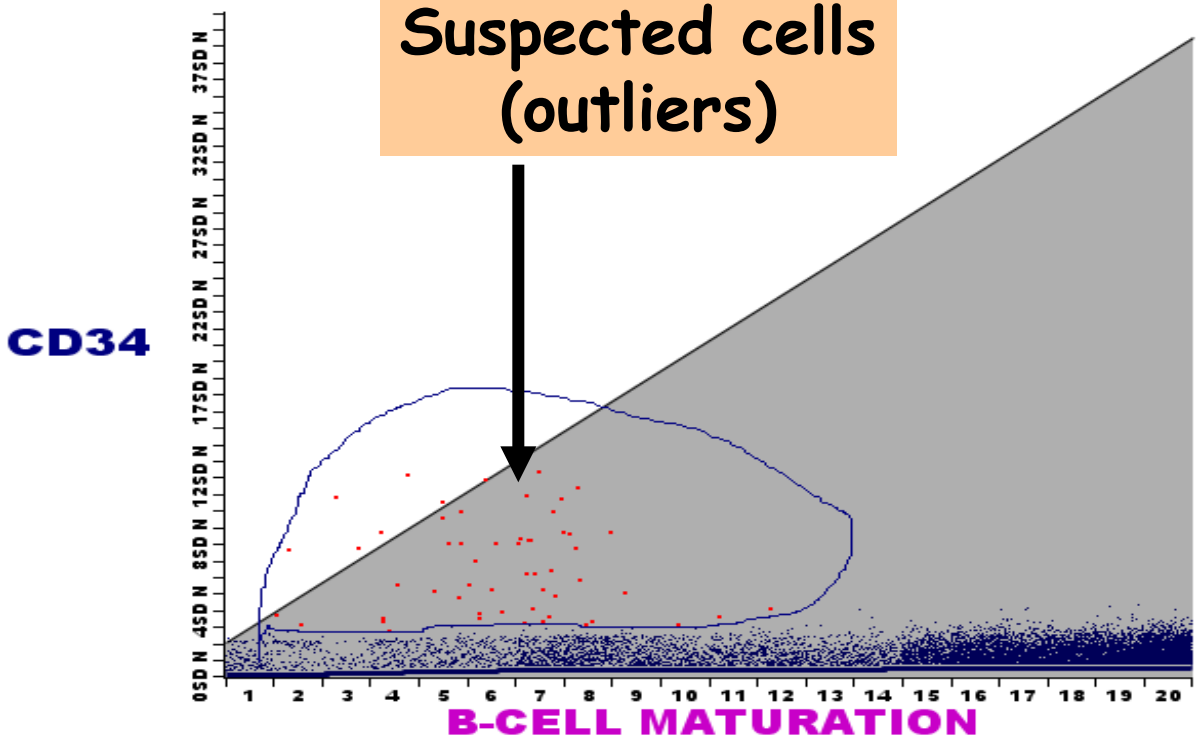
MRD detection in BCP-ALL by comparison with regenerating BM B-cell maturation



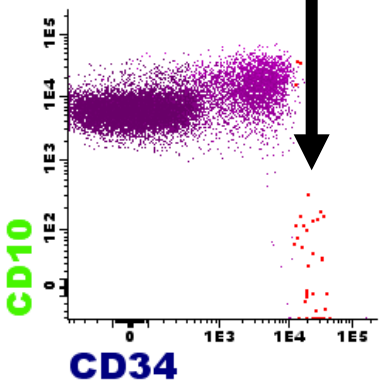
MRD detection in BCP-ALL by comparison with regenerating BM B-cell maturation

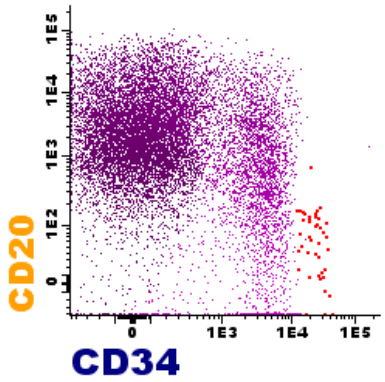


Suspected cells (outliers)



Phenotype of outliers





DEVELOPMENT OF 8-COLOR EUROFLOW MRD ANTIBODY PANELS

EuroFlow

Single-tube antibody EuroFlow MRD protocols under evaluation

1. Acute leukemias (include recognition of normal precursors)

- Acute myeloid leukemia panel (AML-MRD): 1-3 tubes (A. Orfao)
- **B-cell precursor (BCP-ALL-MRD):** 1 tube (V. van der Velden, E. Mejstrikova)
- **T-cell ALL (T-ALL-MRD):** 1 tube (V.Asnafi)

2. Chronic lymphoproliferative disorders

- **Chronic lymphocytic leukemia (CLL-MRD):** 1 tube (L. Lhermitte)
- **Hairy cell leukemia (HCL-MRD):** 1 tube (E. Macintyre)
- Mantle cell lymphoma (MCL-MRD): 1 tube (S. Böttcher)
- Follicular lymphoma (FL-MRD): 1 tube (S. Böttcher)
- Marginal zone lymphoma (MZL-MRD): 1 tube (R. de Tute)
- Lymphoplasmacytic lymphoma (LPL-MRD): 1 tube (R de Tute)
- Diffuse large B-cell lymphoma (DLBCL-MRD): 1 tube (P. Lucio)
- **Burkitt lymphoma (BL):** 1 tube (R. de Tute)
- **T-chronic lymphoproliferative diseases (T-CLPD-MRD):** 1 tube (J. Almeida)
- **Multiple myeloma (MM):** 1 tube (J. Flores)

EuroFlow

EuroFlow consortium aims at innovation in flow cytometry (www.euroflow.org)

www.euroflow.org

EuroFlow participants

EuroFlow

University Institutes / Medical Schools

Erasmus MC, Rotterdam, NL	J.J.M. van Dongen, V.H.J. van der Velden…
USAL, Salamanca, ES	A. Orfao, J. Flores, J. Almeida, Q. Lecrevisse…
IMM, Lisbon, PT	P. Lucio, A. Mendonça, A. Parreira a.o…
UNIKIEL, Kiel, DE	M. Kneba, S. Böttcher, M. Ritgen, M. Brüggemann …
AP-HP, Paris, FR	E. Macintyre, L. Lhermitte, V. Asnafi …
UNIVLEEDS, Leeds, GB	S. Richards, A.C. Rawstron. P. Evans …
DPH/O, Prague, CZ	O. Hrusak, T. Kalina, E. Mesjstrikova …
SAM, Zabrze, PL	T. Szczepanski, L. Sedek …
DCOG, The Hague, NL	E. Sonneveld, A. van der Sluijs-Gelling ...
KUL, Leuven, BE	N. Boeckx …
HGSA, Porto, PT	M. Lima, AH Santos
UFRJ, Rio de Janeiro, BR	C. Pedreira, E.S. Costa

Companies (SME's)

DYNOMICS, Rotterdam, NL	E. Dekking, F. Weerkamp …
CYTOGNOS, Salamanca, ES	M. Martin, J. Bensadon, J. Hernandez, M. Muñoz …

THANK YOU

The MRD Marker Must Be Reliable

- **Specificity**: to discriminate malignant-normal cells
- **Sensitivity**: to detect at least 1 malignant cell in a background of ≥10,000 normal cells.
- **Applicability**: to be applicable in virtually every patient
- **Reproducibility**: standardized methods
- **Clinical utility**: available resources & results obtained in a timely manner