International Harmonised Approach for Residual Disease Monitoring in CLL

Andy C. Rawstron
on behalf of ERIC consortium

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HMDS
OUTREACH

Timeline for development of assays to detect B-CLL

- 1970s CLL demonstrated to have aberrant phenotype (T-cell antisera, mouse RBC rosetting, polyclonal anti-Ig)
  10-20% response to Chlorambucil
- 1980s Immunophenotypic diagnosis including CD20
  20-30% response to COP/CHOP
- 1990s Low specificity MRD assays (3CLR flow, IgH-PCR)
  40-50% response to Fludarabine
- 2000s High sensitivity MRD assays
  Multiple reports MRD^{NEG} = improved PFS / OS
  Development of consensus methods
  >70% response to chemo-immunotherapy.
- 2010s MRD = independent predictor of PFS and OS

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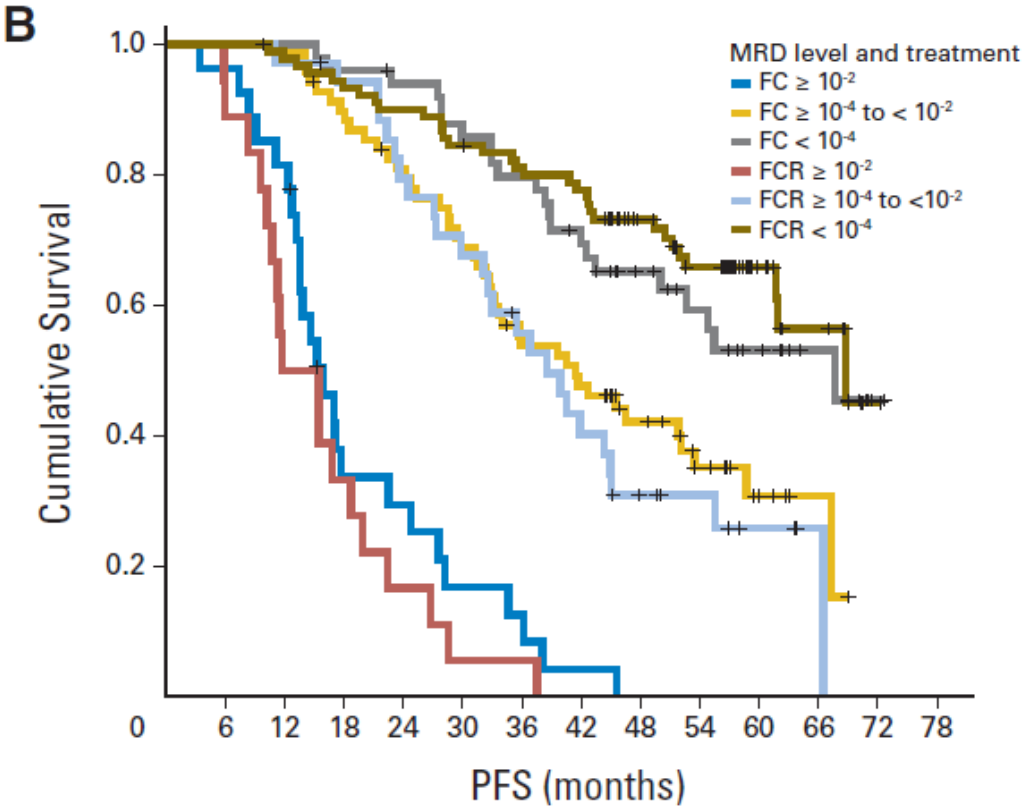
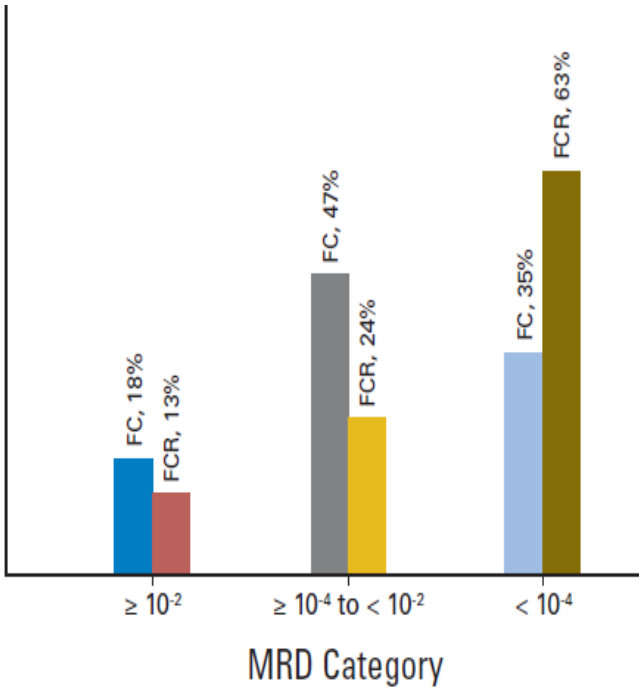
Clonality assessments are not quantitative ➔ not reproducible MRD

CLL cells	Normal B cells	Consensus IgH-PCR	CD19+ κ/λ
0.1% +	0.1%	+	3.4 +
0.1% —	5%	—	1.5 —
0.01% +	0%	+	>10 +
0.01% ?	0.1%	?	1.7 —
0.01% —	5%	—	1.5 —

DCLLSG CLL FC vs. FCR: MRD level not treatment type predicts outcome





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JOURNAL OF CLINICAL ONCOLOGY

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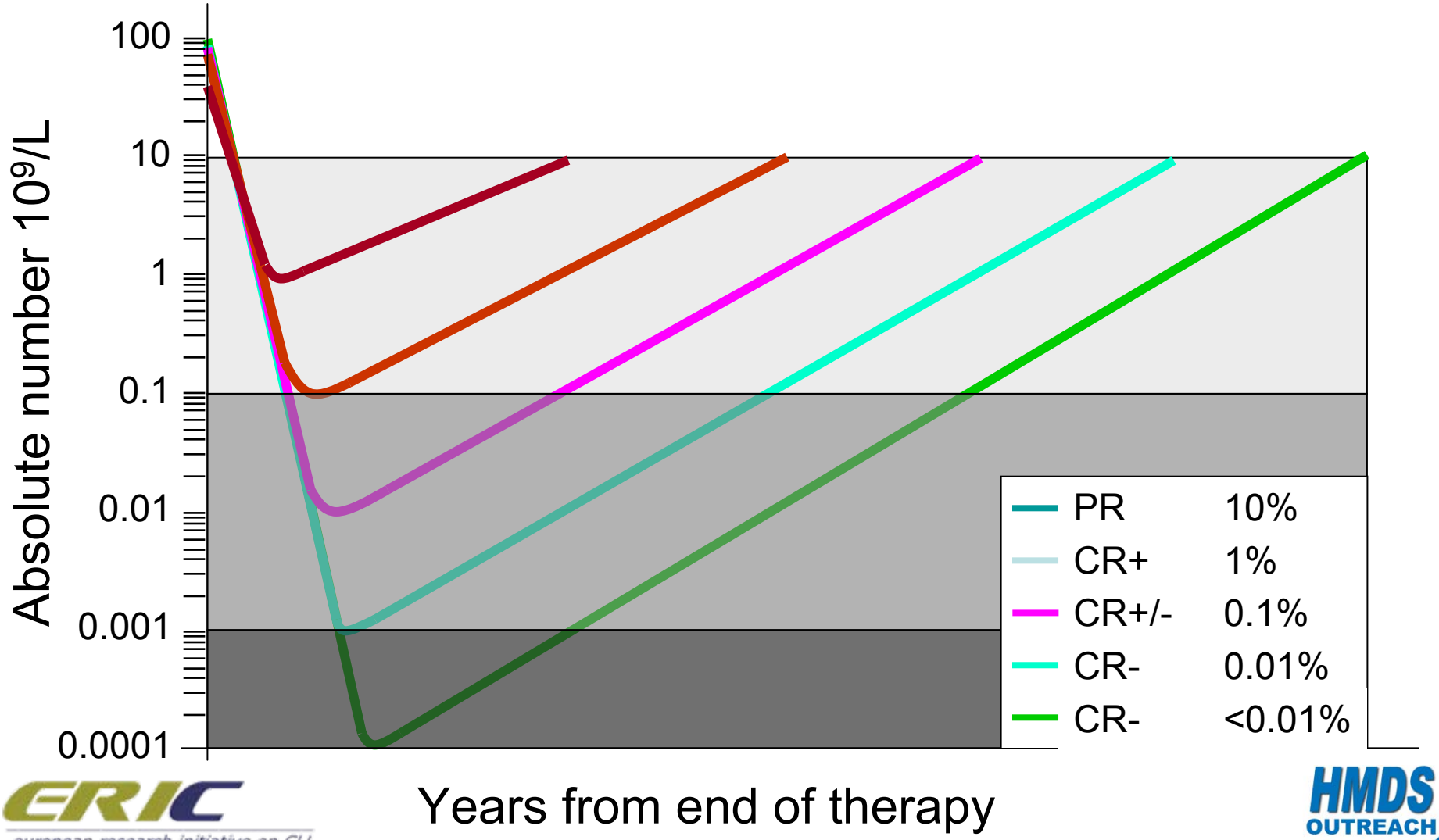
MRD response (<0.01%) is an independent predictor of PFS and OS

Parameter	Progression-free Survival		Overall Survival	
	Univariate (Log-Rank) P Value	Multivariate (Cox) P Value HR (95% CI)	Univariate (Log-Rank) P Value	Multivariate (Cox) P Value HR (95% CI)
Age (60yrs)	0.288		**0.001**	**<0.001 1.08 (1.04-1.13)**
Haemoglobin (110g/L)	0.705		**0.028**	0.479
Platelets (100 x 1^09/L)	**0.002**	0.888	0.058	
Stage (A/B vs. C)	**0.003**	0.902	**0.002**	0.895
Prior treatment (Y/N)	**0.004**	0.072	**0.006**	**0.001 0.36 (0.20-0.64)**
Treatment type	**<0.001**	0.339	**0.017**	0.155
iwCLL Response	**0.008**	0.566	**0.004**	0.731
MRD level (<0.01 / 0.01-0.1 / 0.1-1 / >1%)	**<0.001**	**<0.001 2.18 (1.65-2.87)**	**<0.001**	**0.005 1.40 (1.11-1.76)**
Adverse FISH (del 17p/11q)	**0.009**	**<0.001 0.24 (0.11-0.53)**	**0.038**	**0.033 0.51 (0.28-0.95)**

Kwok et al (ms in prepartion: N=133, age 62 (38-83), 77% male, 41% no prior Rx. Follow-up 5.2yrs (0.5-16.4). Rx: 63 FC/FCR/FCM, 26 Alemtuzumab, 17 Flud, 7 AutoSCT, 20 Clr/other.

CLL with 6 month doubling time ➔ 20 months PFS per log depletion



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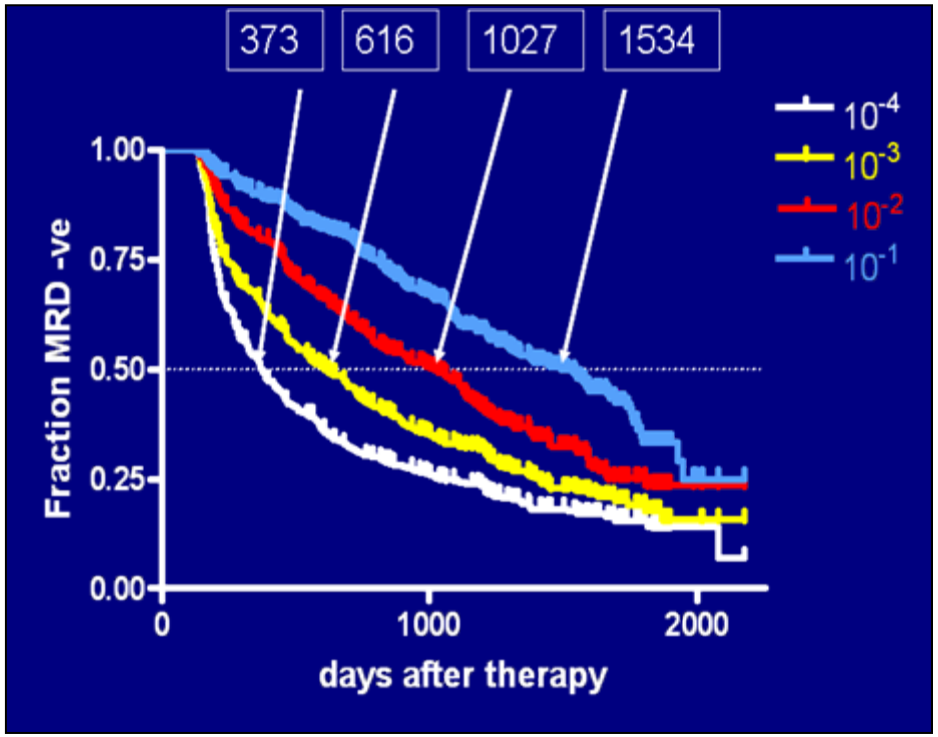
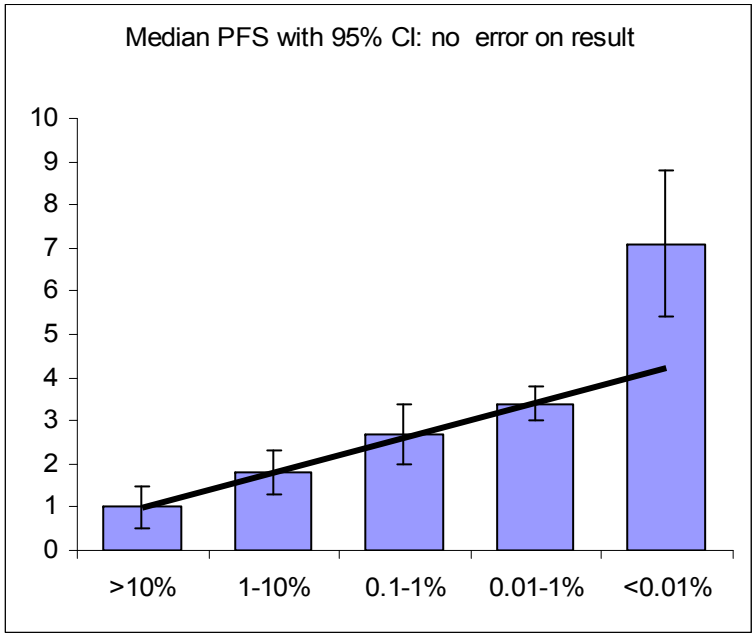
Approximately 8-12 months improvement in PFS per log tumour depletion



DCLLSG 8: FC or FCR
Courtesy of Seb Böttcher



Leeds Teaching Hospitals

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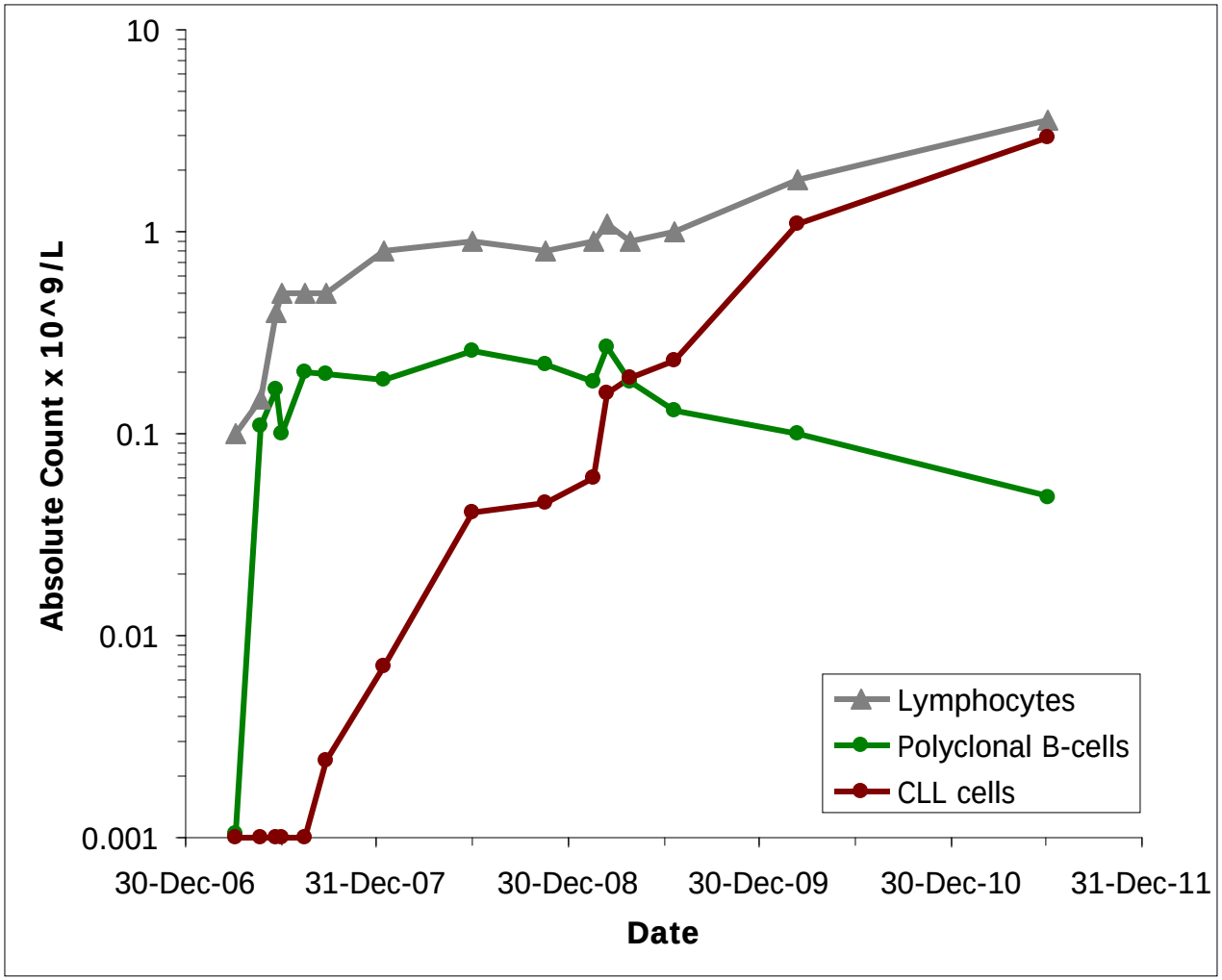
HMDS OUTREACH

Kinetics of relapse: typical pattern



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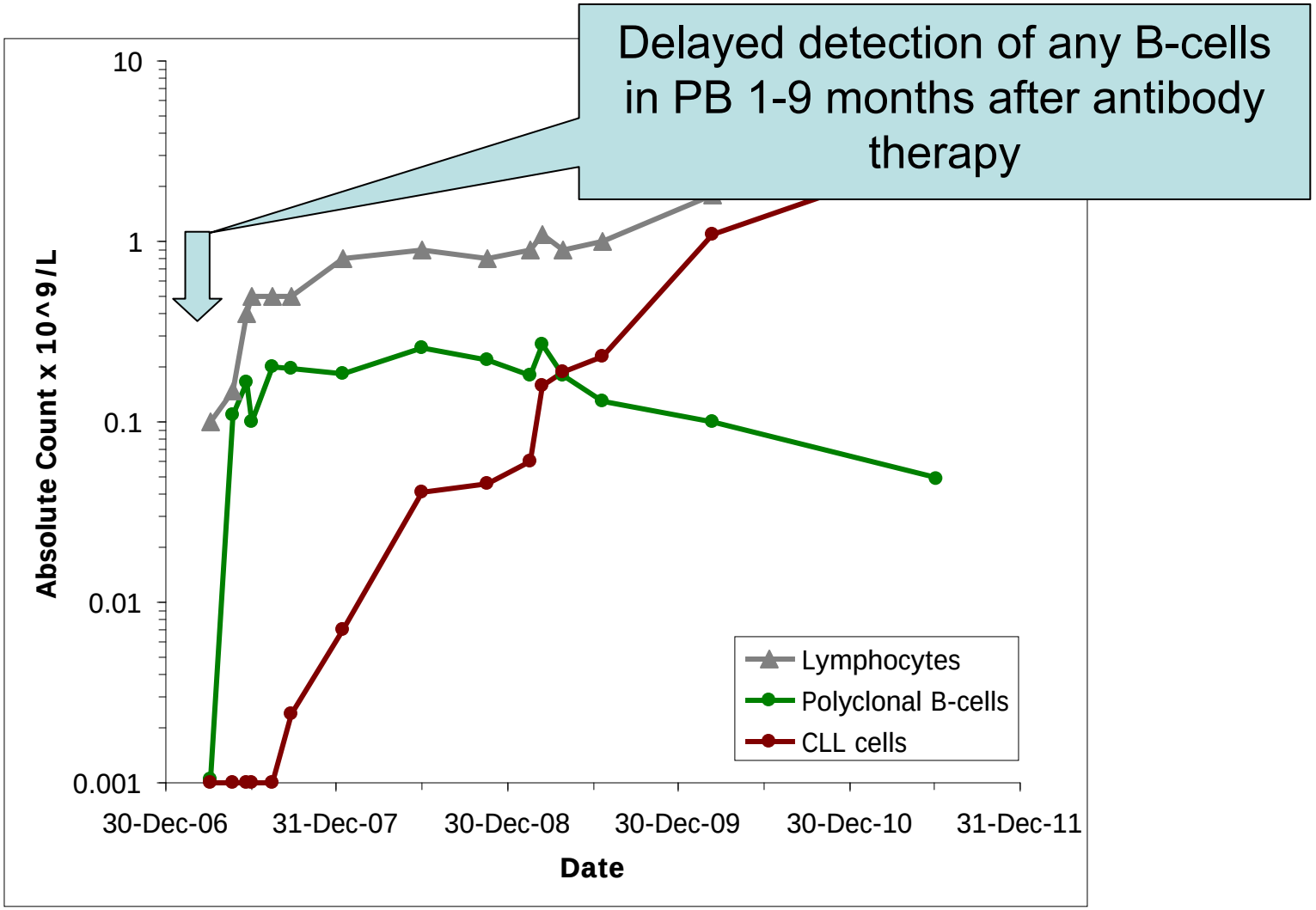
Kinetics of relapse: typical pattern



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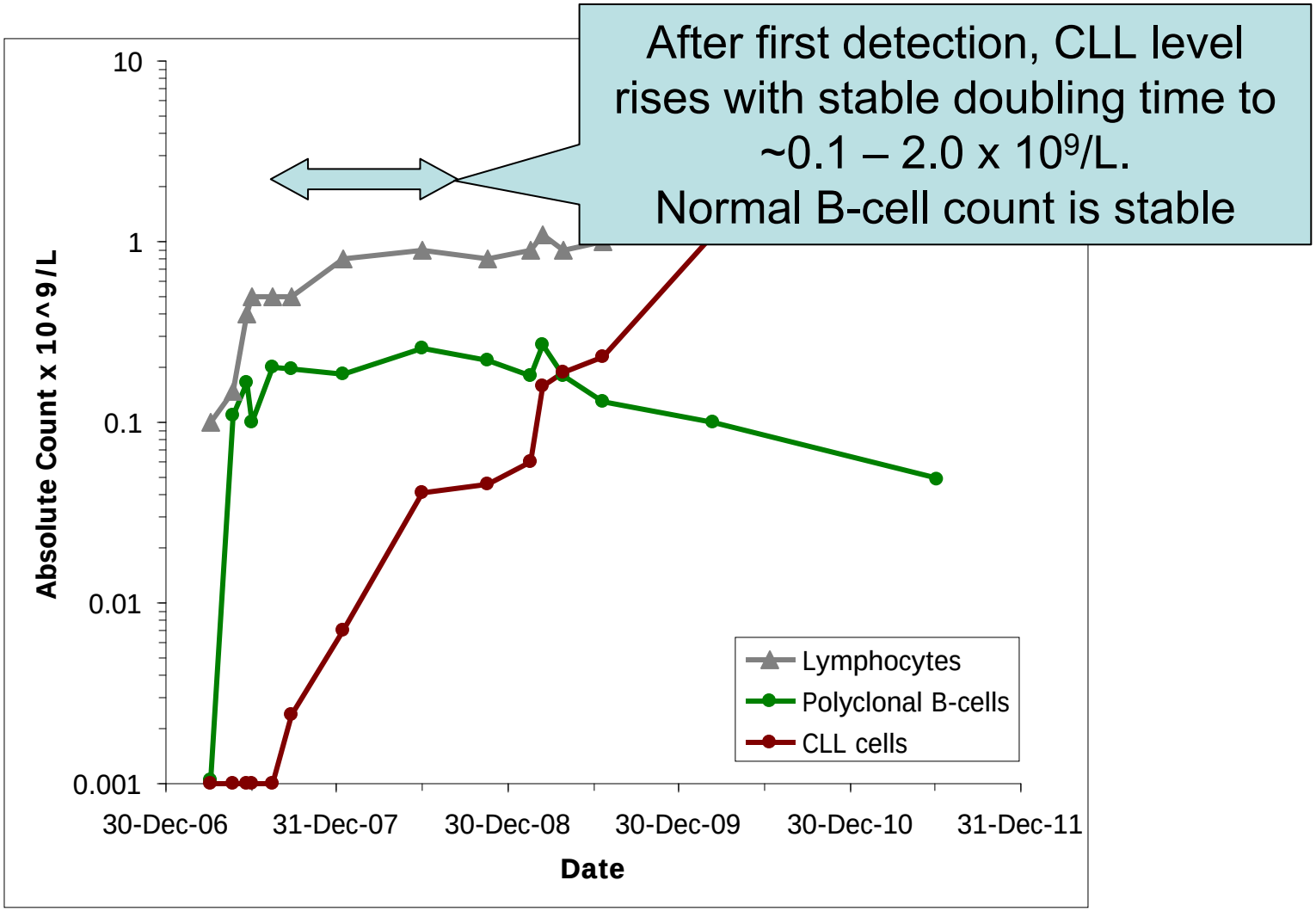
HMDS OUTREACH

Kinetics of relapse: typical pattern



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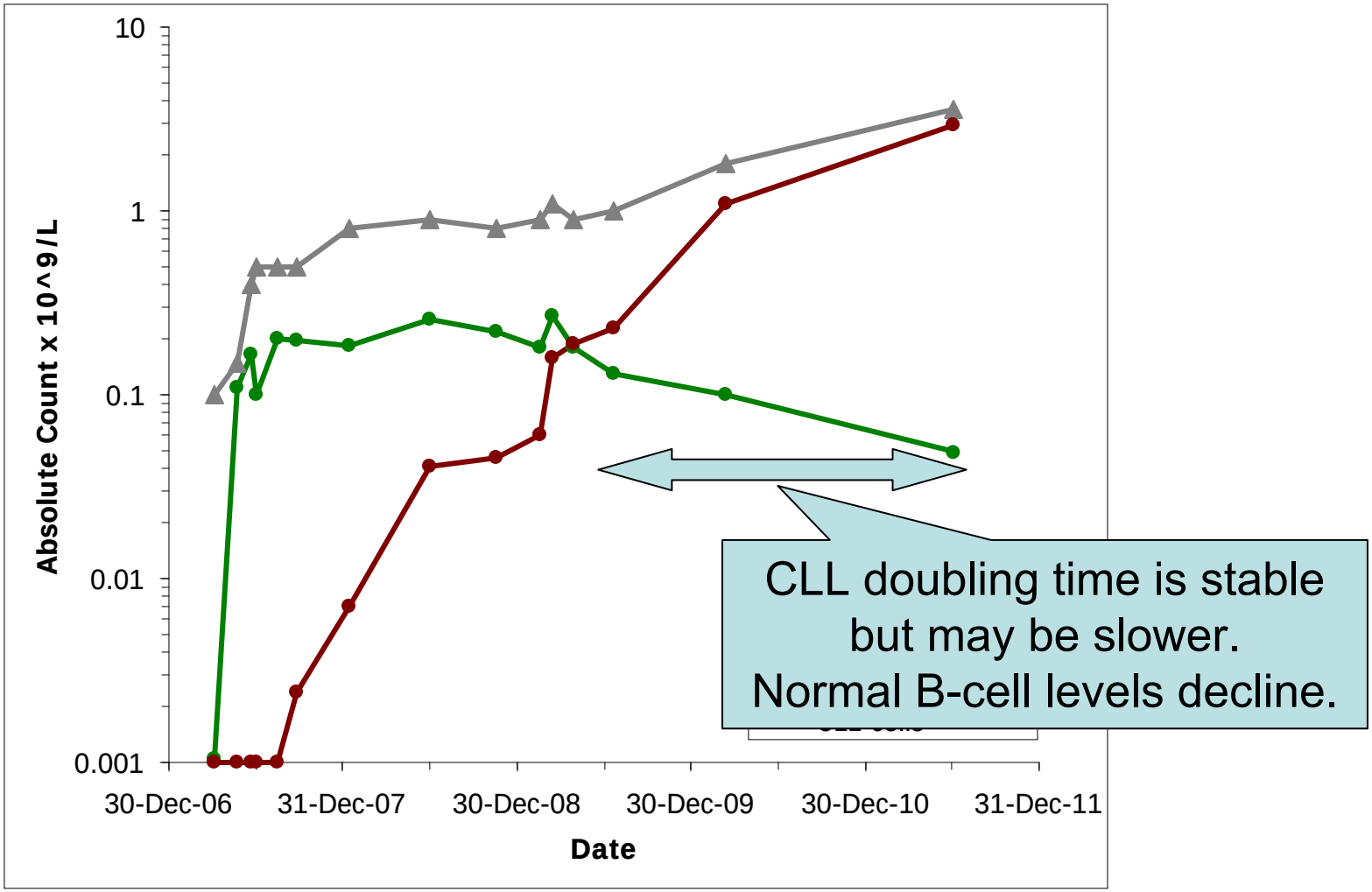
Kinetics of relapse: typical pattern



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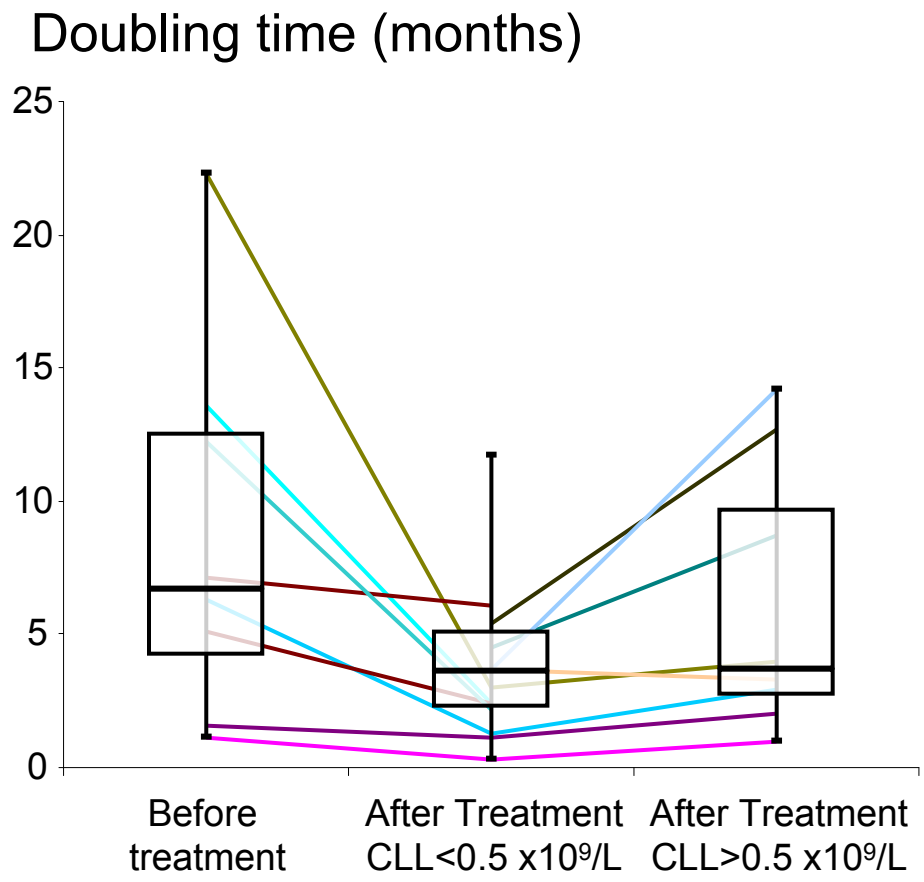
CLL doubling time changes after treatment but is largely stable from first MRD^{POS} time-point

- Data from Leeds Hospital
- 18 patients with evaluable doubling time
  - Median 6 MRD-positive time-points (range 4-19)
- CLL doubling time relatively stable at MRD levels
  - Median Pearson r = 0.981 (range 0.903 – 0.998)
    - may decrease when CLL count exceeds normal B-cell
    - Significant change (<6M to >12M) in 3/8 evaluable



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MRD analysis in CLL

- Qualitative (clonality) assays are unsuitable because the results vary according to the polyclonal background
- Kinetics of relapse: exponential even at the lowest evaluable levels of disease
- Approximately 8-12 months improvement in progression-free survival per log depletion

ERIC international harmonisation experience in the detection of MRD in CLL

- 4 CLR
  - Markers for separation of CLL from normal B-cells
  - Comparison with RQ-PCR
  - Accuracy and precision with protocol
- 6 CLR
  - Value of clonality assessment
  - Simplify MRD analysis
- 8 CLR
  - Aim to validate to 0.001% (10e-5)
  - Check inter-laboratory variation
  - Internal quality check

4-CLR EU (ERIC) / US (CRC) harmonisation

Leukemia (2007), 1–9
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www.nature.com/leu

ORIGINAL ARTICLE

International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia

AC Rawstron[1], N Villamor[2,3], M Ritgen[4], S Böttcher[4], P Ghia[5], JL Zehnder[6], G Lozanski[7], D Colomer[2,3], C Moreno[2,3], M Geuna[8], PAS Evans[1], Y Natkunam[6], SE Coutre[6], ED Avery[9], LZ Rassenti[9], TJ Kipps[9], F Caligaris-Cappio[5], M Kneba[4], JC Byrd[7], MJ Hallek[10], E Montserrat[2,3] and P Hillmen[1]

[1]HMDS, Leeds Teaching Hospitals, Leeds, UK; [2]Unitat d'Hematopatologia, Departments of Hematology and Pathology, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [3]Unitat d'Hematopathologia, Departments of Hematology and Pathology, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain; [4]Second Department of Medicine, University of Schleswig-Holstein, Campus Kiel, Germany; [5]Department of Oncology, Università Vita-Salute San Raffaele and Istituto Scientifico San Raffaele, Milano, Italy; [6]Department of Medicine, Division of Hematology, Stanford University School of Medicine, Stanford, CA, USA; [7]Division of Hematology and Oncology, The Arthur James Comprehensive Cancer Center, The Ohio State University, Columbus, OH, USA; [8]Laboratory of Cytometry, Institute for Cancer Research and Treatment (IRCC), Candiolo, Italy; [9]Department of Medicine, Division of Hematology/Oncology, University of California, San Diego, UK and [10]Klinik I für Innere Medizin, Universität zu Köln, Köln, Germany

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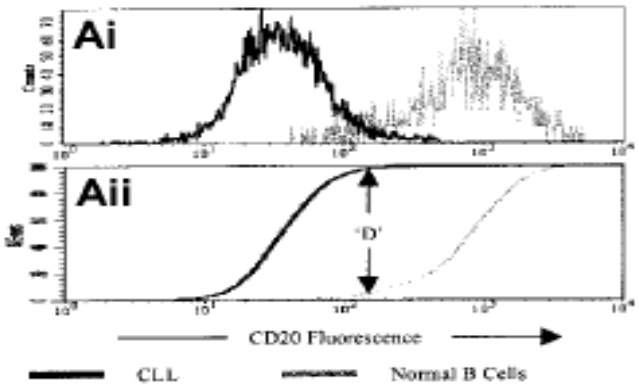
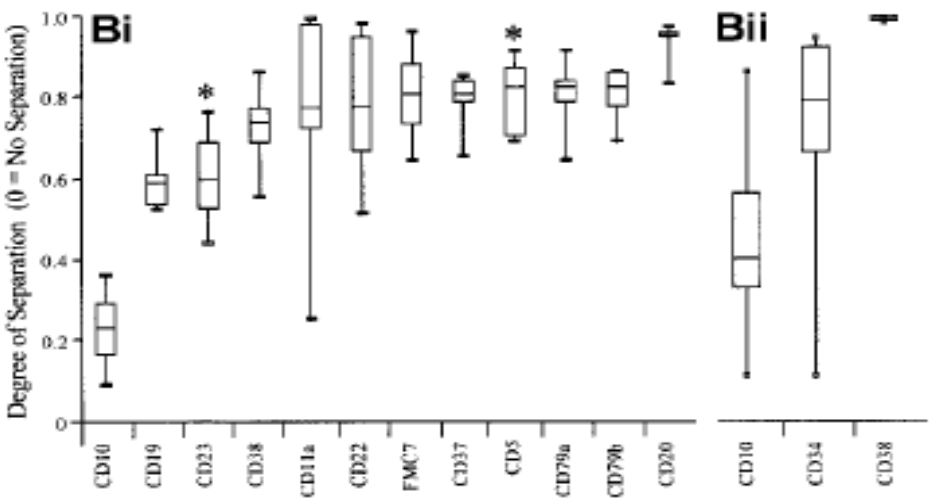
HMDS OUTREACH

4CLR harmonisation (1): Identification of markers that reproducibly separate CLL





- CD10/CD24/CD43, CD10/CD5/CD43, CD10/integrinß7/CD43, CD10/integrinß7/CD5, CD11a/integrinß7/CD5, CD20/CD38/CD5, CD20/CD43/CD5, CD20/integrinß7/CD5, CD21/CD48/CD43, CD21/CD48/CD5, CD21/CXCR5/CD5, CD21/integrinß7/CD5, CD24/CCR6/CD43, CD24/CD27/CD38, CD24/CD40/CD5, CD24/CD48/CD43, CD24/CD48/CD5, CD24/CXCR5/CD43, CD24/CXCR5/CD5, CD24/integrinß7/CD43, CD24/integrinß7/CD5, CD31/CXCR5/CD5, CD37/CCR6/CD43, CD37/CD5/CD43, CD37/CD79b/CD43, CD37/CXCR5/CD5, CD37/integrinß7/CD43, CD40/CD48/CD43, CD40/CD48/CD5, CD40/CXCR5/CD5, CD40/integrinß7/CD5, CD43/CD23/CD5, CD43/CD81/CD38, CD43/CD81/CD5, CD44/integrinß7/CD43, CD44/integrinß7/CD5, CD48/CXCR5/CD5, CD48/integrinß7/CD5, CD48/LAIR-1/CD5, CD48/MPC-1/CD5, CD5/CCR6/CD43, CD5/CXCR5/CD43, CD70/integrinß7/CD5, CD79b/CD21/CD43, CD79b/CD24/CD43, CD79b/CD38/CD43, CD79b/CD39/CD43, CD79b/CD40/CD43, CD79b/CD48/CD43, CD79b/CD5/CD43, CD79b/CD81/CD43, CD79b/CXCR5/CD43, CD79b/CXCR5/CD5, CD79b/integrinß7/CD5, CD81/CD22/CD5

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HMDS OUTREACH

4CLR harmonisation (1): Identification of markers that reproducibly separate CLL

Table 3 Inter-operator variability and false-positive rate for nine selected antibody combinations in comparison to kappa/lambda/CD19/CD5 analysis

Antibody combination	*Inter-operator variability*	*False-positive rate*
CD43/CD79b/CD19/CD5	9.2 (3.9–22.7)	0.8 (0.2–3.1)
CD81/CD22/CD19/CD5	12.6 (6.2–23.7)	0.5 (0.2–1.3)
CD20/CD38/CD19/CD5	13 (8–18.6)	0.3 (0–0.7)
CD20/Integrinβ7/CD19/CD5	14.7 (5.6–28.5)	1.5 (0.6–5.4)
CD43/CD23/CD19/CD5	22 (7.3–40.2)	4.3 (0–25)
CD20/CD5/CD19/CD43	22.8 (5–83.7)	2.4 (0.2–10.5)
Kappa/Lambda/CD19/CD5	29.5 (7.2–76.1)	5.3 (0.3–13.5)
CD37/CD79b/CD19/CD43	31.8 (13.1–53.6)	4.8 (2.3–9.6)
CD38/CD5/CD19/CD43	32.2 (8.3–58.2)	1.1 (0.2–4.1)
CD24/CXCR5/CD19/CD43	48.2 (26.9–64.2)	4.5 (3–6.9)

Combinations were selected according to their efficacy in separating CLL cells from normal B-cells. The inter-operator variability was reported for the percentage of B-cells classified as having a CLL-phenotype by the operators. The false-positive rate was the percentage of normal B-cells that were classified as having a CLL-phenotype.

- CD19/CD5 backbone
  - CD20 / CD38*
  - CD22 / CD81
  - CD43 / CD79b
  - *CD38 to exclude progenitors
- Most of the markers have been used in CLL diagnostics for several decades

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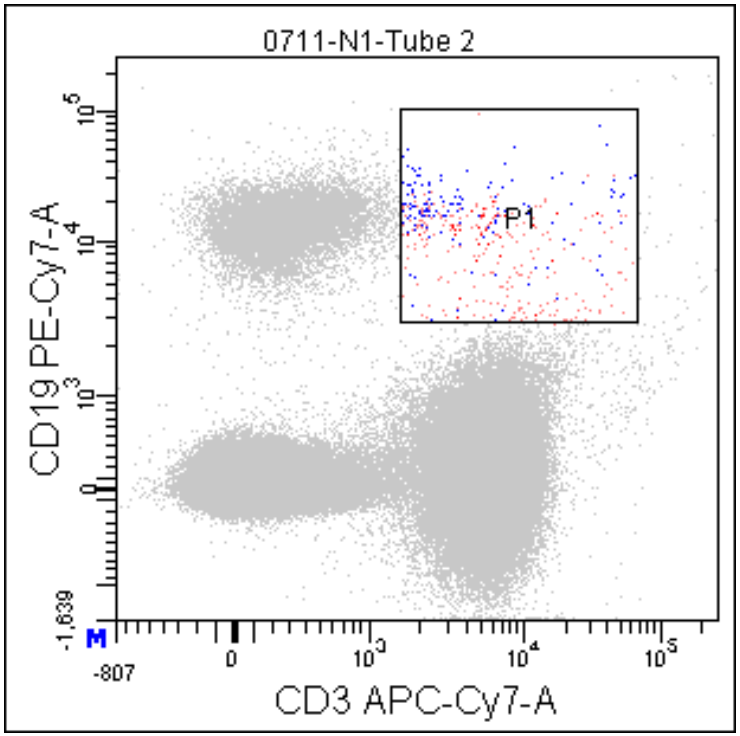
4CLR harmonisation (2): elimination of false-positive contaminants



Correct for $CD19^+CD3^+$ ➔ limit of detection is $\leq 10^{-4}$ (0.01%)

Normal Levels of Peripheral $CD19^+CD5^+$ CLL-Like Cells: Toward a Defined Threshold for CLL Follow-Up—A GEIL-GOELAMS Study

Françoise Durrieu,[1,2] Franck Geneviève,[3,4] Christine Arnoulet,[5] Caren Brumpt,[6] Jean-Claude Capiod,[7] Michel Degenne,[8] Jean Feuillard,[9] Richard Garand,[10] Amina Kara-Terki,[11] Emilienne Kulhein,[12] Marc Maynadié,[13] Maria-Elena Ochoa-Noguera,[14] Adriana Plesa,[15] Mikael Roussel,[16] Houchingue Eghbali,[1,2] Matgorzata Truchan-Graczyk,[3,4] Marcelo de Carvalho Bittencourt,[17] Pierre Feugier,[17] and Marie C. Béné[17*]

[1]Department of Biology, Institut Bergonié, Bordeaux, France
[2]Department of Clinical Hematology, Institut Bergonié, Bordeaux, France
[3]Hematology Laboratory, CHU Larrey, Angers, France
[4]Clinical Hematology Laboratory, CHU Larrey, Angers, France
[5]Biopathology, Institut Paoli-Calmettes, Marseilles, France
[6]Hematology Laboratory, Hôpital Beaujon, Clichy, France
[7]Hematology Laboratory, CHU Nord, Amiens, France
[8]Hematology Laboratory, CHU Bretonneau, Tours, France
[9]Hematology Laboratory, CHU Dupuytren, Limoges, France
[10]Hematology Laboratory, Hôtel Dieu, Nantes, France
[11]Hematology Laboratory, Hôpital Paul Brousse, Villejuif, France
[12]Hematology Laboratory, CHU Purpan, Toulouse, France
[13]Hematology Laboratory, CHU Bocage, Dijon, France
[14]Hematology Laboratory, Hôpital Saint-Louis, Paris, France
[15]Hematology Laboratory, Hôpital Edouard Herriot, Lyon, France
[16]Hematology Laboratory, CHU Pontchaillou, Rennes, France
[17]Immunology Laboratory and Clinical Hematology, CHU Brabois, EA RHEM Nancy Université, Nancy, France

No correction ➔ limit of detection is 1-4 $\times 10^{-4}$ (0.01 – 0.04%)

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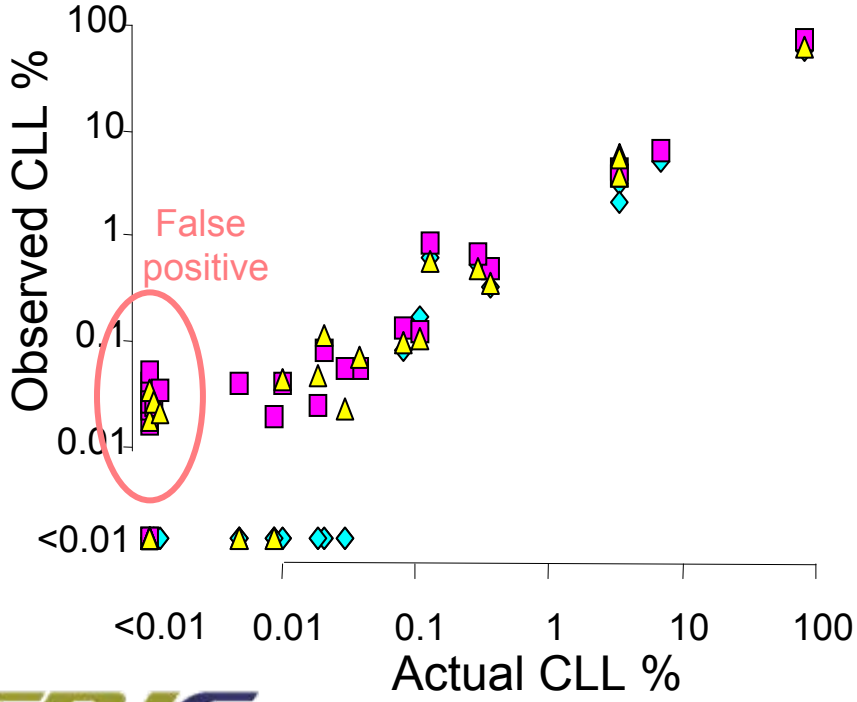
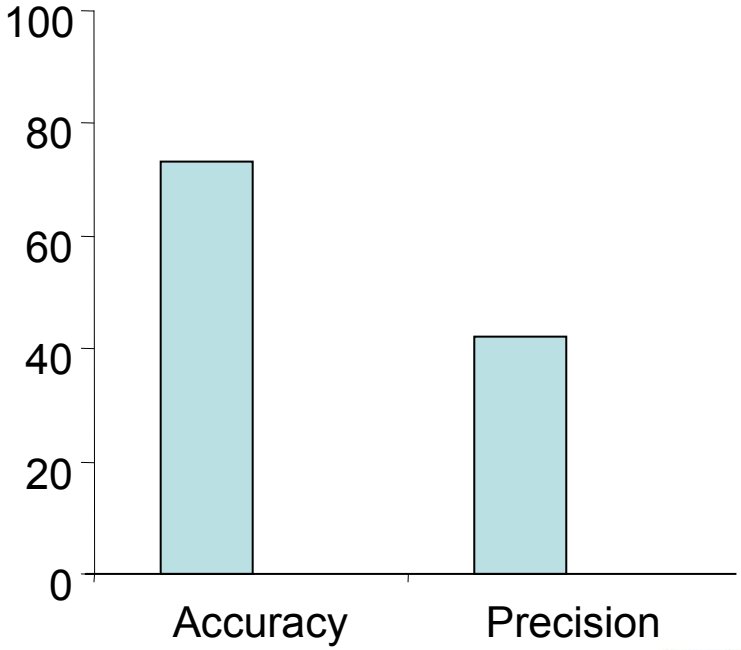
4CLR harmonisation (3): improvement in precision and accuracy using a protocol

Experienced in flow but not MRD analysis: no protocol
Poor accuracy and precision for samples with 0.01% - 0.1% CLL cells (n=145)





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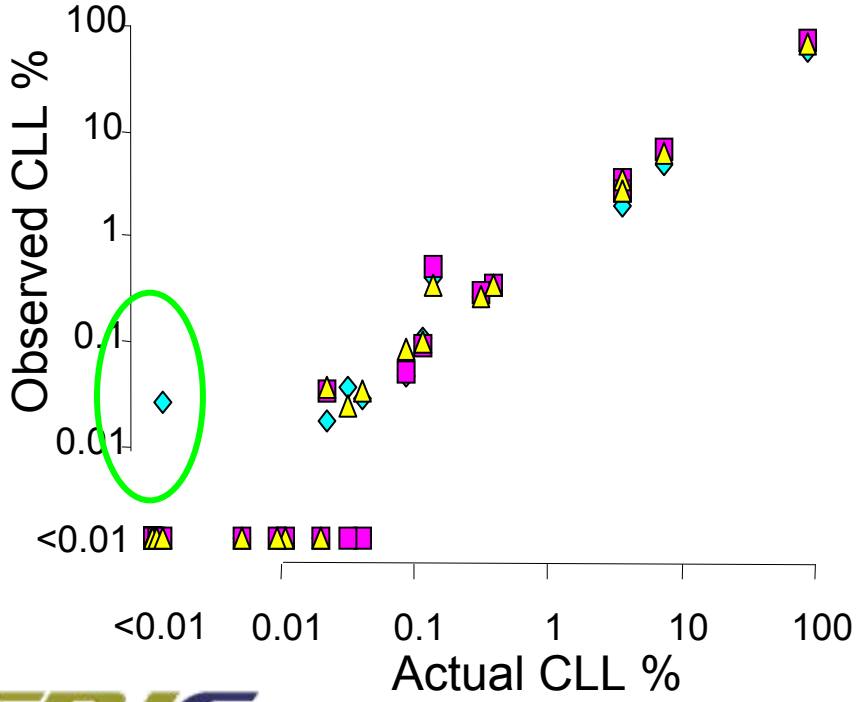
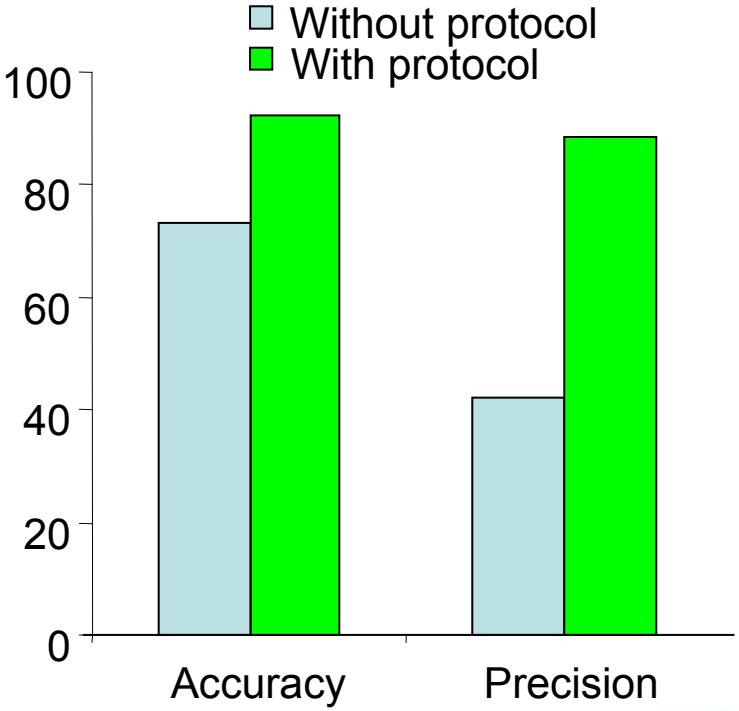
HMDS OUTREACH

4CLR harmonisation (3): improvement in precision and accuracy using a protocol

Experienced in flow but not MRD analysis: with protocol
Can improve accuracy and precision: knowledge of CLL characteristics more important than protocol/template





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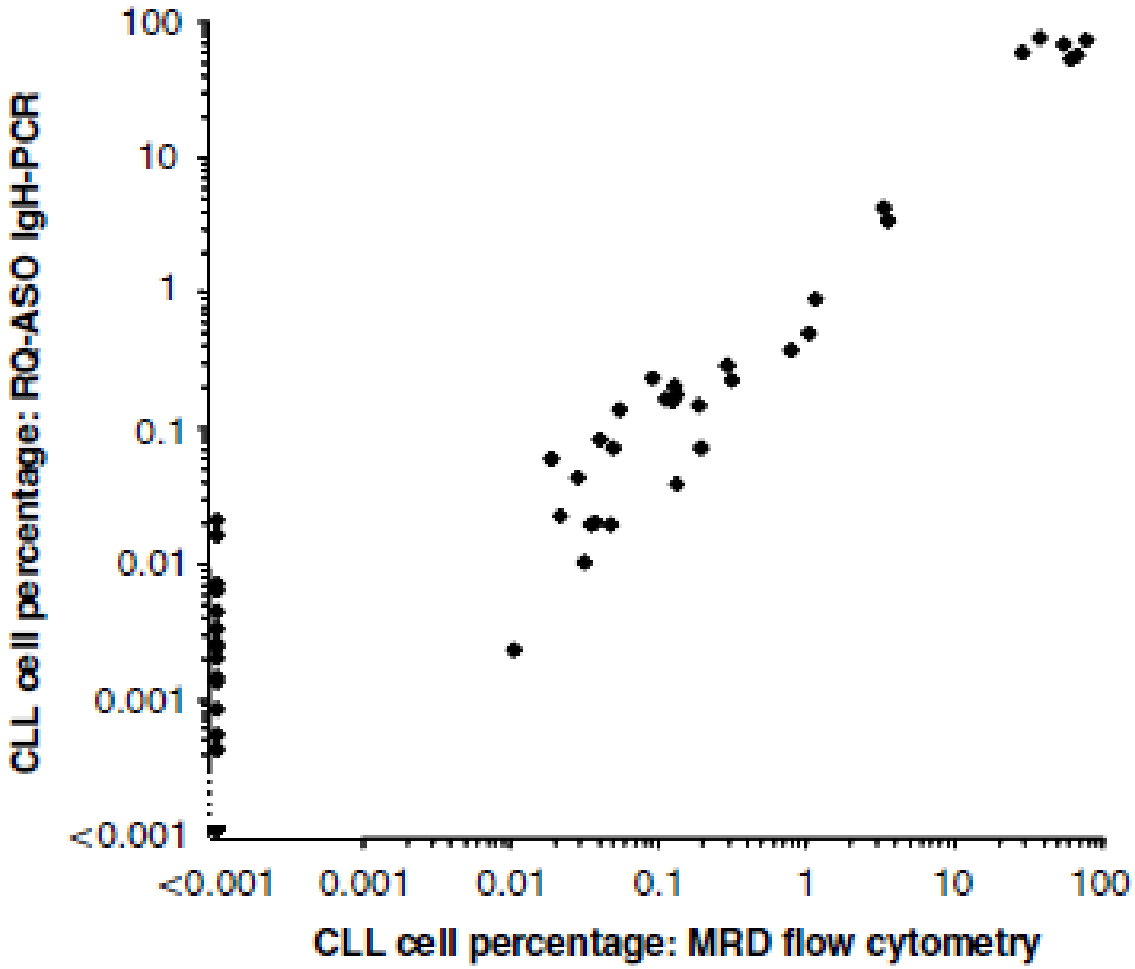
4CLR harmonisation (4): Quantitative approaches equivalent to 0.01% (10E-4)



International standardised approach for residual disease monitoring in CLL: Leukemia 2007, 21(5): 956-64

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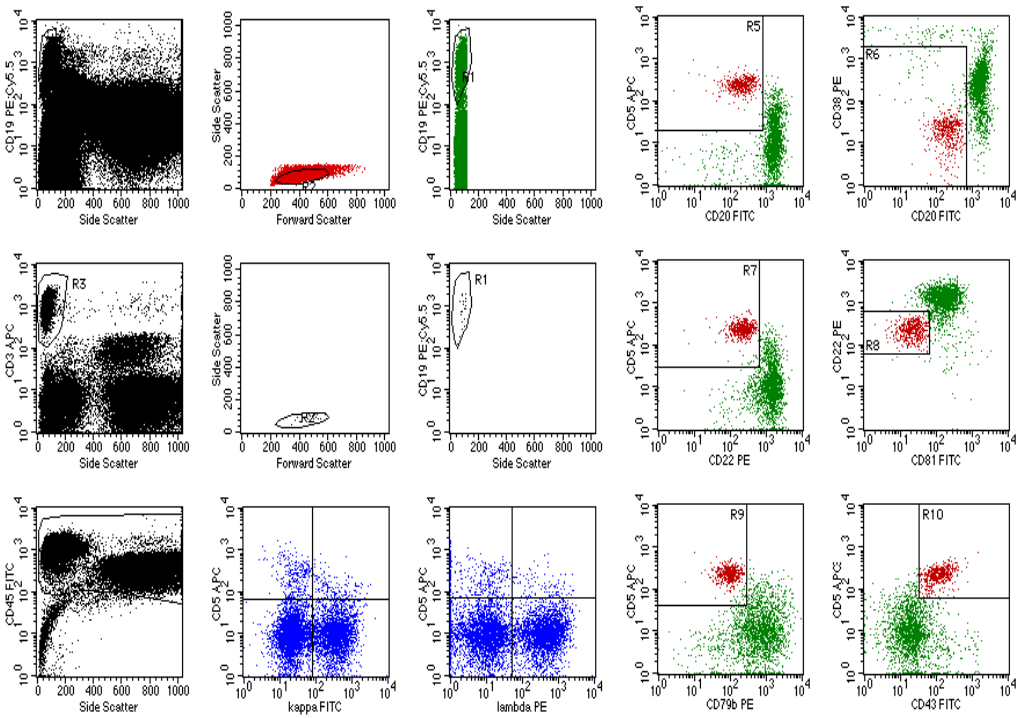
Issues with harmonised 4-CLR assay

- Requires 10 million cells for analysis
  - can be difficult in post-treatment samples
- Slow and difficult to analyse
  - Two pages of analysis, several minutes to update any gate change
- Full panel not required in all cases
  - Clonality assessment still popular but efficacy not known



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6-CLR ERIC harmonisation

ORIGINAL ARTICLE

Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL

AC Rawstron[1,2], S Böttcher[3], R Letestu[4,5], N Villamor[6], C Fazi[7], H Kartsios[1], RM de Tute[1], J Shingles[1], M Ritgen[3], C Moreno[8], K Lin[9], AR Pettitt[9], M Kneba[3], E Montserrat[6], F Cymbalista[4,5], M Hallek[10], P Hillmen[11] and P Ghia[7] on behalf of the European Research Initiative in CLL (ERIC)

(1) Identify situations where a less time-consuming CD19/CD5/k/l analysis would be sufficient for detecting residual CLL

(2) Develop a six-CLR antibody panel that is more efficient for cases requiring full MRD analysis.

Leukemia. 2013 Jan;27(1):142-9. doi: 10.1038/leu.2012.216. Epub 2012 Jul 31.

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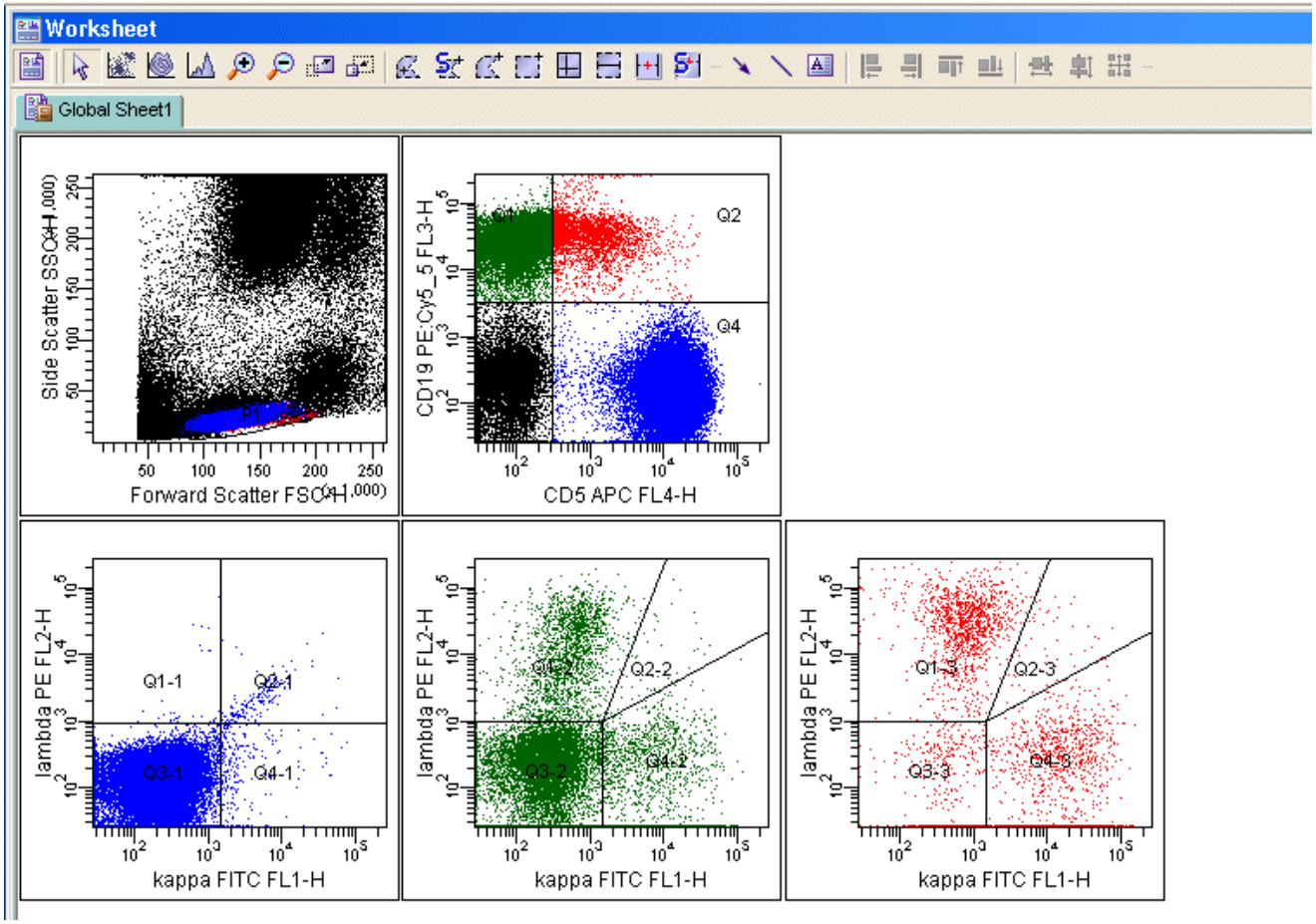
Simple analysis for CD19/CD5/Kappa/Lambda



Tested in parallel on 784 post-treatment CLL cases with full MRD data

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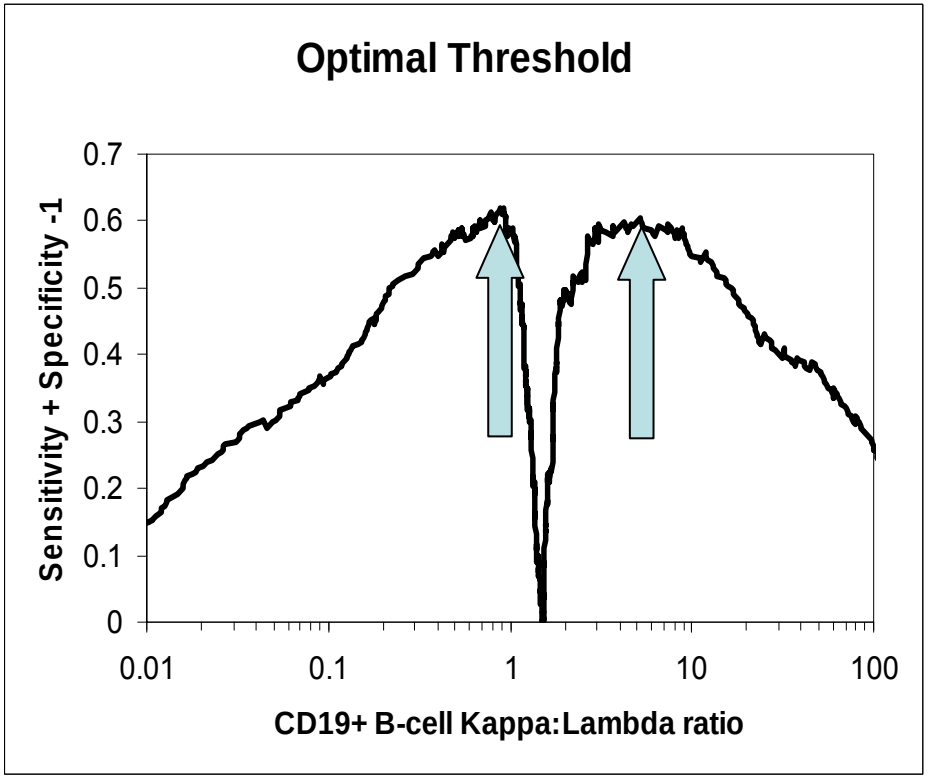
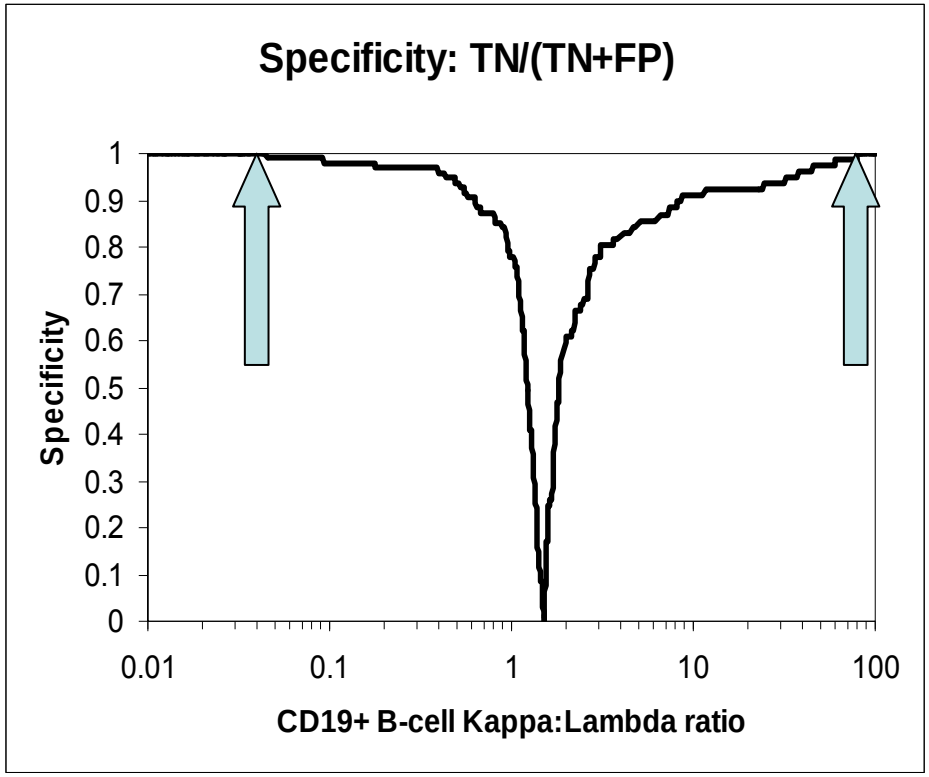
CD19 K:L ratio ROC and specificity





Optimal threshold for presence of MRD: K:L >5.1:1 or <0.91:1
100% specificity for presence of MRD: K:L >80:1 or <0.04:1

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6-CLR harmonisation (1): clonality assessment informative if stringent thresholds are used

	Optimal threshold for specificity vs. sensitivity	Threshold for 100% Specificity
CD19+ K:L	<0.92: 1 or >5.2:1	<0.04:1 or >80:1
CD19+ % CD5+	>61%	>82%
CD19+5+ K:L	<0.64:1 or >3.0:1	<0.05:1 or 32:1
CD19+5+ %sIg-	>21%	>54%

Using just CD19/CD5/Kappa/Lambda it is possible to identify samples which have residual disease with 100% specificity in a large proportion of cases (393/784 in the series assessed)

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Requirement for CD3 contamination control in 6-CLR analysis

Antibody combinations	Paired T-Test for number of events classified as CLL with CD3 data vs. no CD3	Regression Slope for number of events classified as CLL with CD3 data vs. no CD3 data
CD20/CD79b/CD38	0.019	1.13
CD81/CD22/CD43	0.15	1.06
CD81/CD79b/CD43	0.69	1.05

6 files with no CLL (n=2), 20-50 CLL events (n=2) and 50-100 CLL events (n=2)
CD19/CD5/CD3 plus combination above. Files analysed first with CD3 data removed, then reanalysed with CD3 data available

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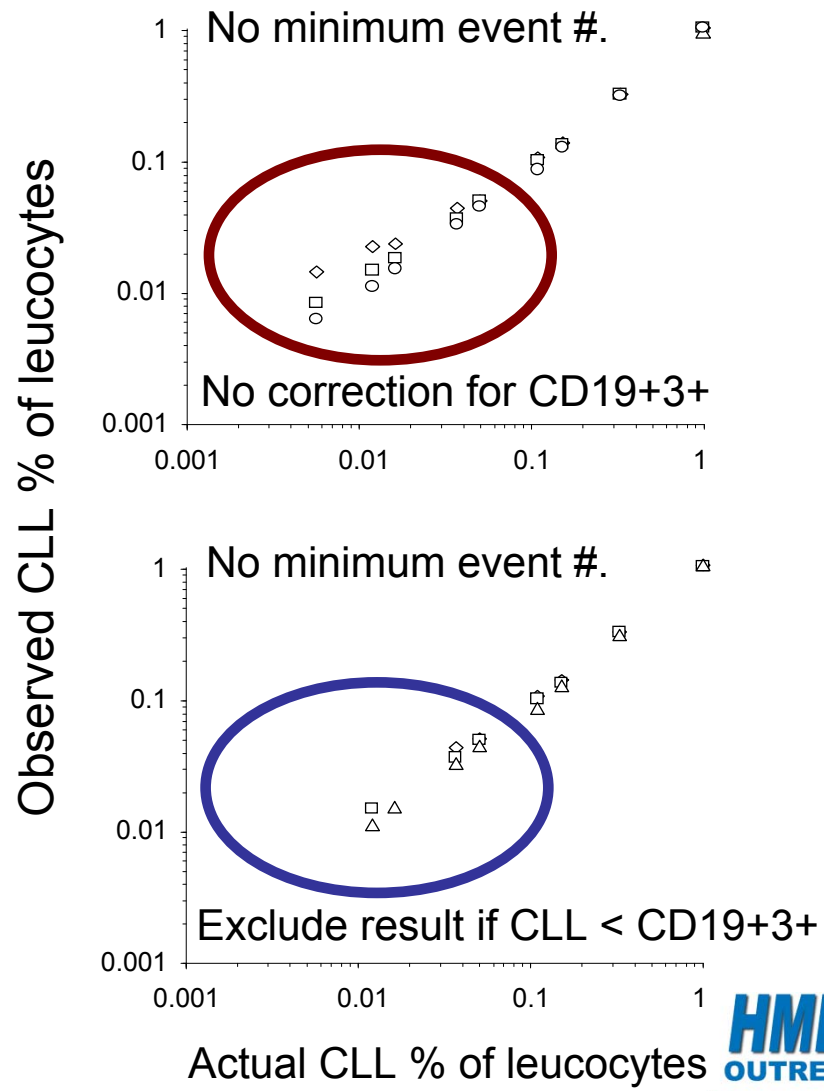
Balance between accuracy/precision and sensitivity for results <0.15%

Minimum events to define a population	Contamination assessment	4 CLR average error (range)	6 CLR average error (range)
50 events	No correction for CD19+3+	13 (2-42)	12 (4-18)
20 events	No correction for CD19+3+	28 (2-111)	15 (4-31)
no minimum	No correction for CD19+3+	20 (2-74)	18 (4-31)
no minimum	Subtract CD19+CD3+	22 (3-38)	38 (3-92)
no minimum	Exclude result if CLL < CD19+3+	7 (2-10)	12 (4-18)



<20 events: no residual disease

>50 events: reproducibly identifiable residual disease

20-50 events: potentially residual disease but poorly reproducible

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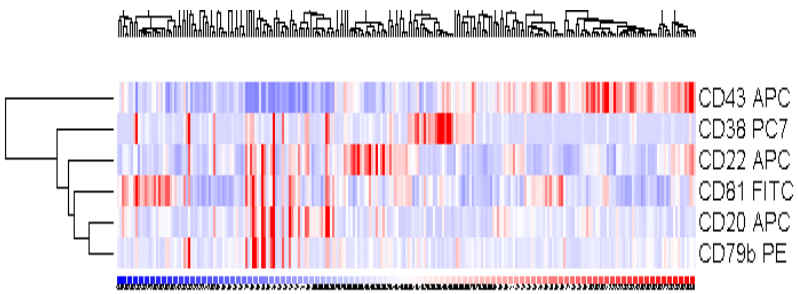
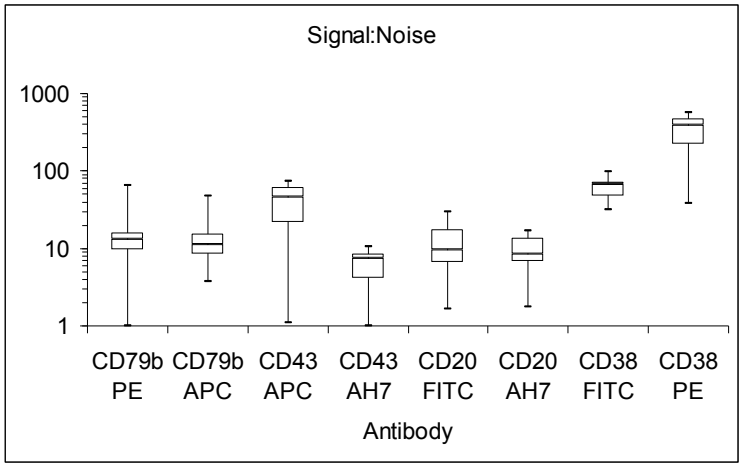
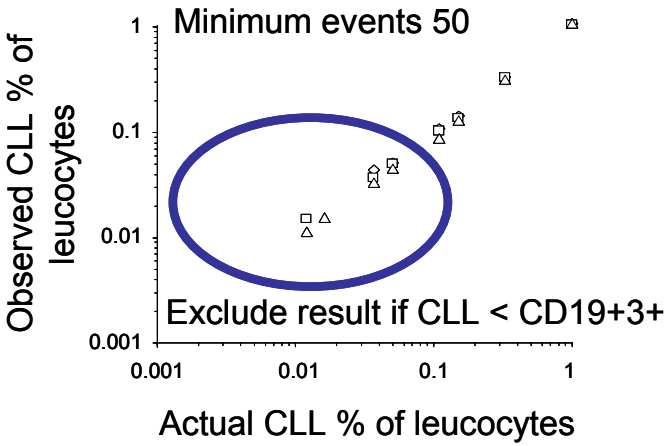
Design of the 6CLR panel: combinations, conjugates, contamination and cocktails



	T-test for # of cells classed as CLL with or without CD3	Regression slope for # of cells classed as CLL +/- CD3
CD20/79b/38	0.019	1.13
CD81/22/43	0.15	1.06
CD81/79b/43	0.69	1.05

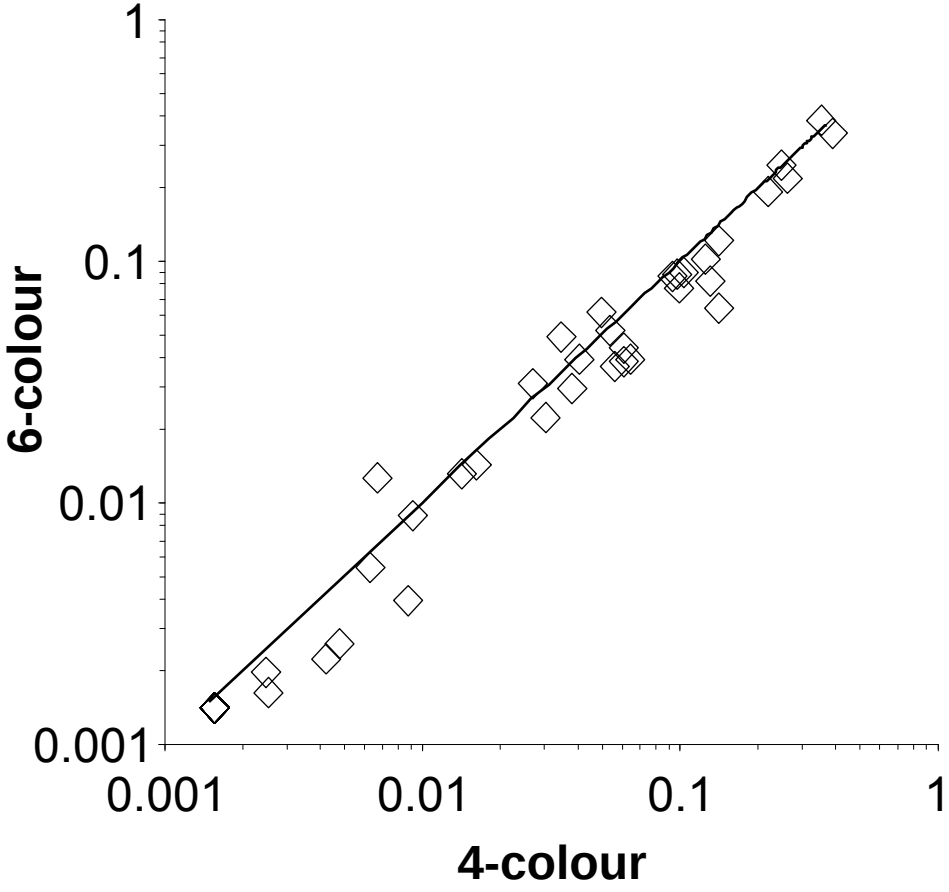




FITC	PE	PerC5.5	PE-Cy7	APC	APC-H7
CD3	CD38	CD5	CD19	CD79b	CD20
CD81	CD22	CD5	CD19	CD43	CD20

6-CLR harmonisation (2): may be possible to achieve detection at 0.001% (10e-5)

- 67 samples with <1% CLL
- Concordance for detection of CLL at 0.01% threshold
  - 98.4% overall
  - 100% if >200,000 events in each tube
- Good linearity to 0.001%



8-CLR MRD: aims of the project

- **Compare 8-CLR with 4/6-CLR and RQ-ASO IGH-PCR to the 0.001% (10-5) level**
- **Identify inter-laboratory variation in analysis and evaluate a data analysis QC pilot**
- **Assess the potential of an internal data quality check on signal:noise and compensation**.

- Centres in the EU / US and Australia participating
- Antibodies generously provided by BD but platform independent study

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ERIC 8CLR MRD Participants

Region	Institute	Contacts
EU	Amsterdam	Arnon Kater / Johan Dobber
EU	Barcelona Sant Pau	Carol Moreno / Josep Nomdedeu
EU	Barcelona University	Neus Villamor / Julio Delgado
EU	Barcelona Vall d'Hebron	Francesc Bosch / Carlos Palacio
EU	Brno	Sarka Pospisilova / Michael Doubek
EU	Dublin	David O'Brien
EU	Leeds	Andy Rawstron / Peter Hillmen
EU	Milano San Raffaele	Paolo Ghia / Claudia Fazi
EU	Paris AP-HOP	Remi Letestu / Florence Cymbalista
EU	Salzburg	Alex Egle
US	Genzyme	Henry Dong
US	Mayo	Curtis Hanson / Tait Shanafelt
US	MD Anderson	Jeff Jorgensen / Bill Wierda
US	NIH	Maryalice Stetler-Stevenson
US	UCSD / Genoptix	Beth Broome / Laura Rassenti / Tom Kipps
US	UPMC Pennsylvania	Fiona Craig
NZ/AUS	Sydney	Stephen Mulligan
NZ/AUS	Sydney	Mary Sartor
OTHER	BD	Jingy Chen / Noel Warner
OTHER	ERIC	Emili Montse / Colm Bradley / Michael Hallek
OTHER	Roche	Michael Wenger / Kathryn Humphrey / Rober Xin

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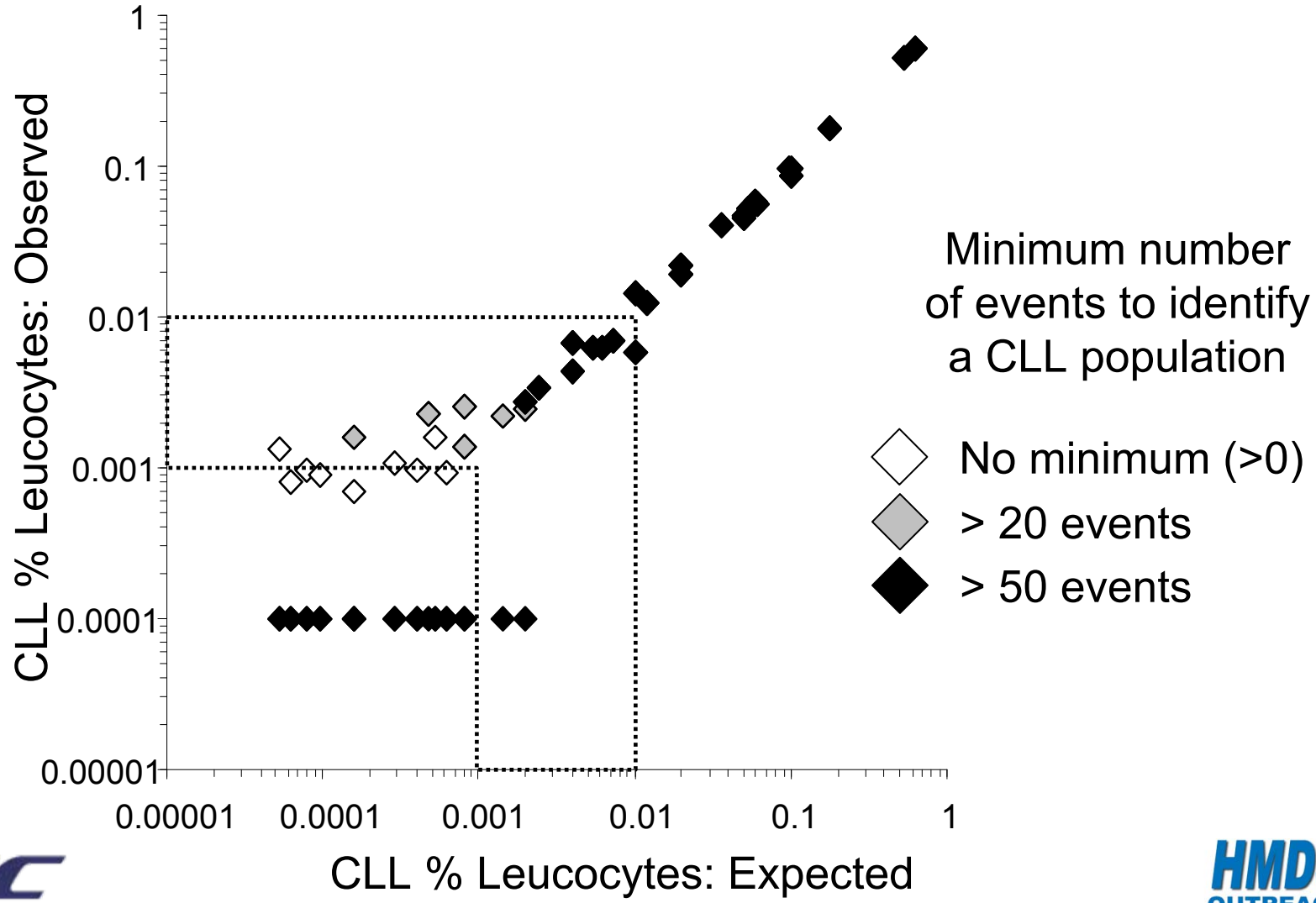
8-CLR harmonisation (1): dilution studies ➔ detection limit 0.001 - 0.003%



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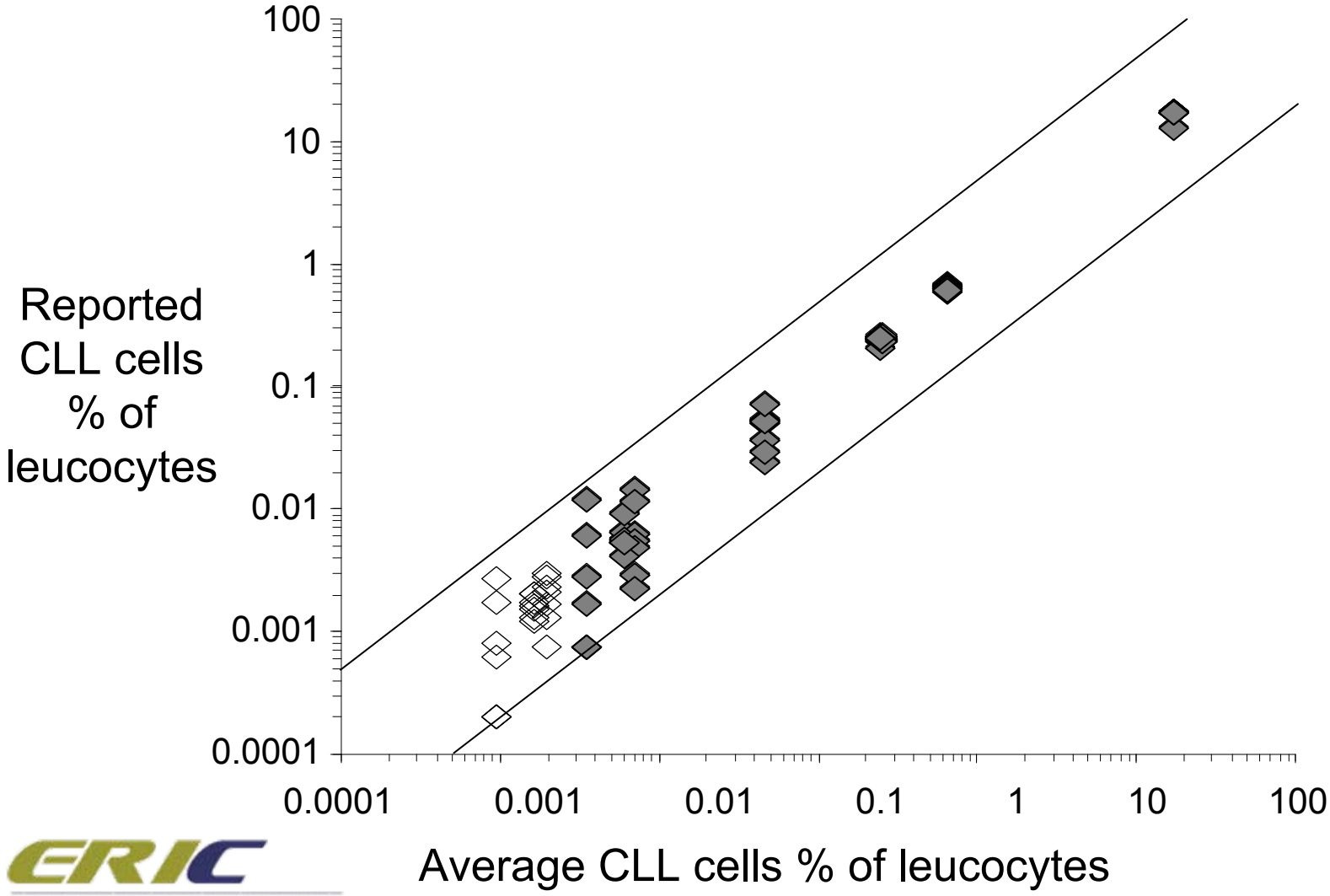
8-CLR harmonisation (2): analytic variation up to 0.5log for samples with <0.01% (10e-4) CLL



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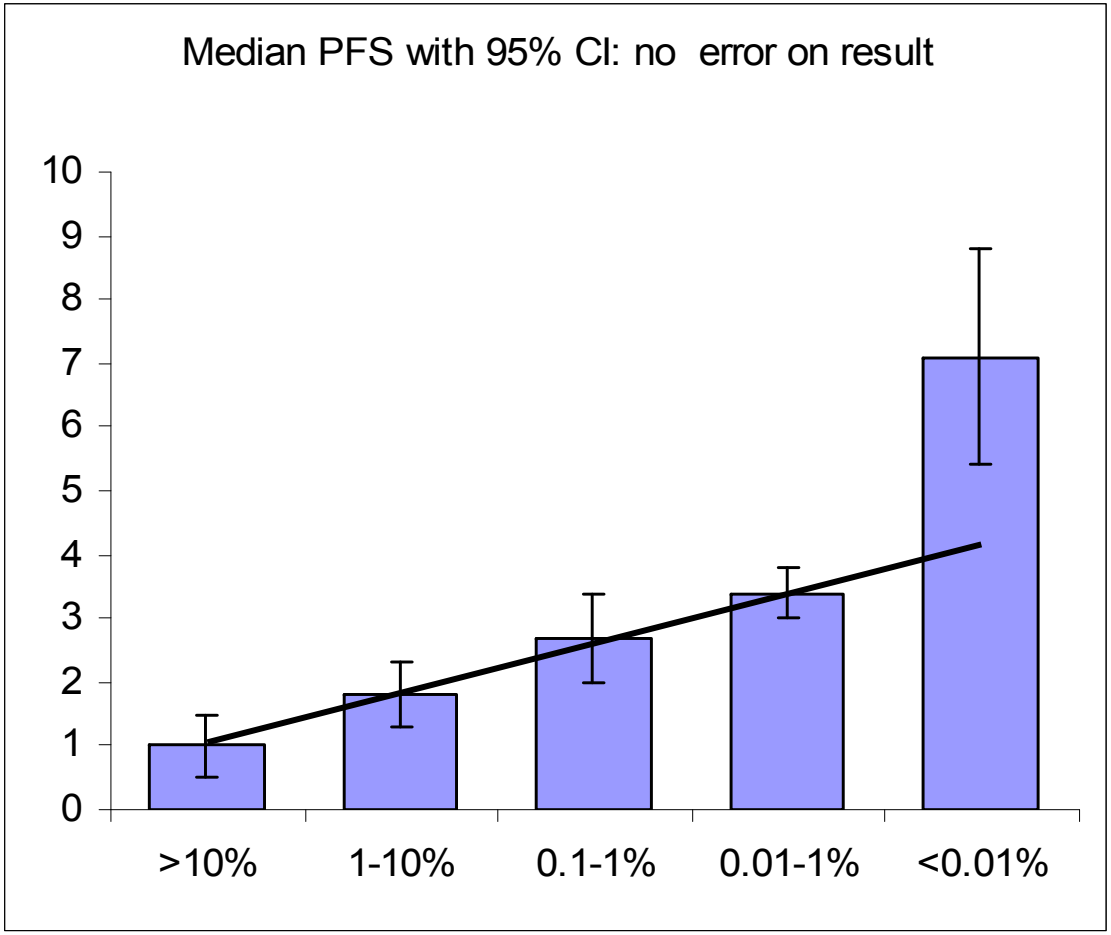
HMDS OUTREACH

Evaluation of Leeds MRD data since 1995: what is an “acceptable” error?



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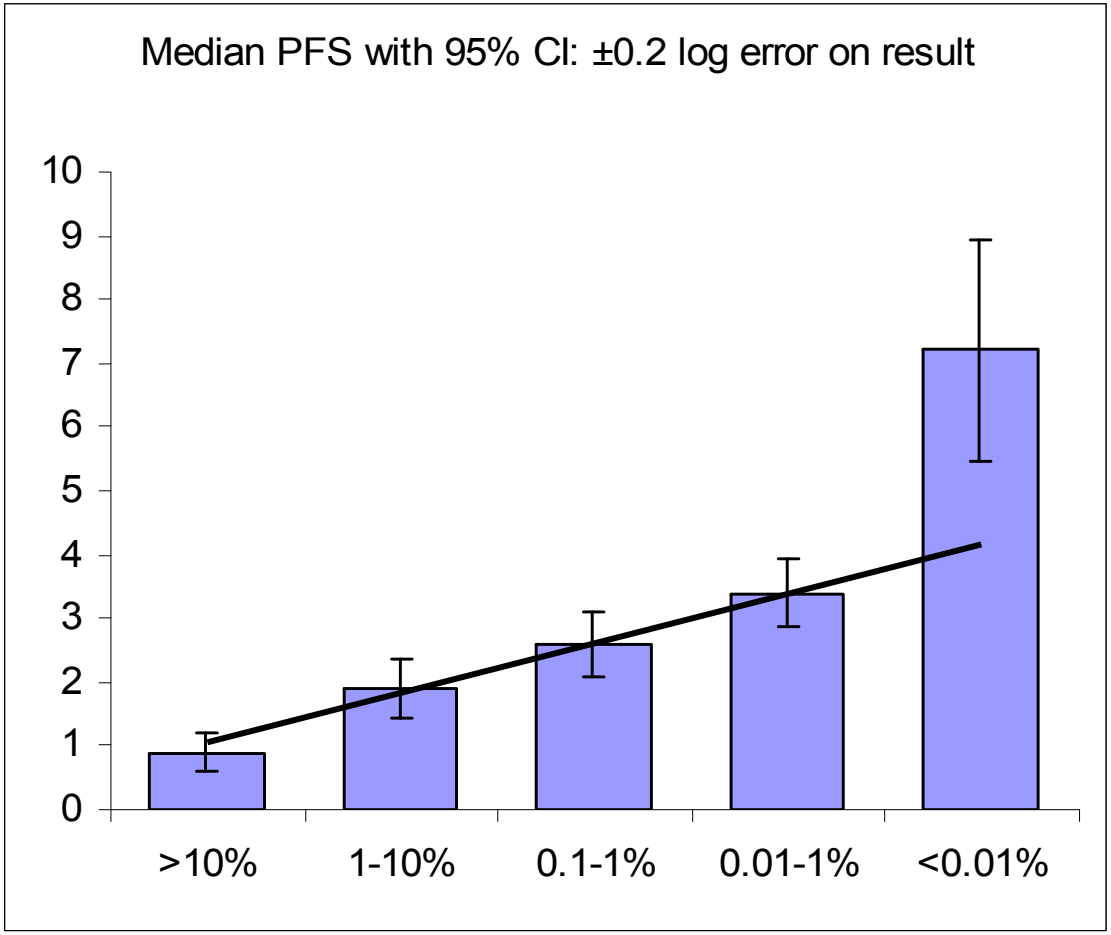
HMDS OUTREACH

Evaluation of Leeds MRD data since 1995: what is an “acceptable” error? ±0.2 log?



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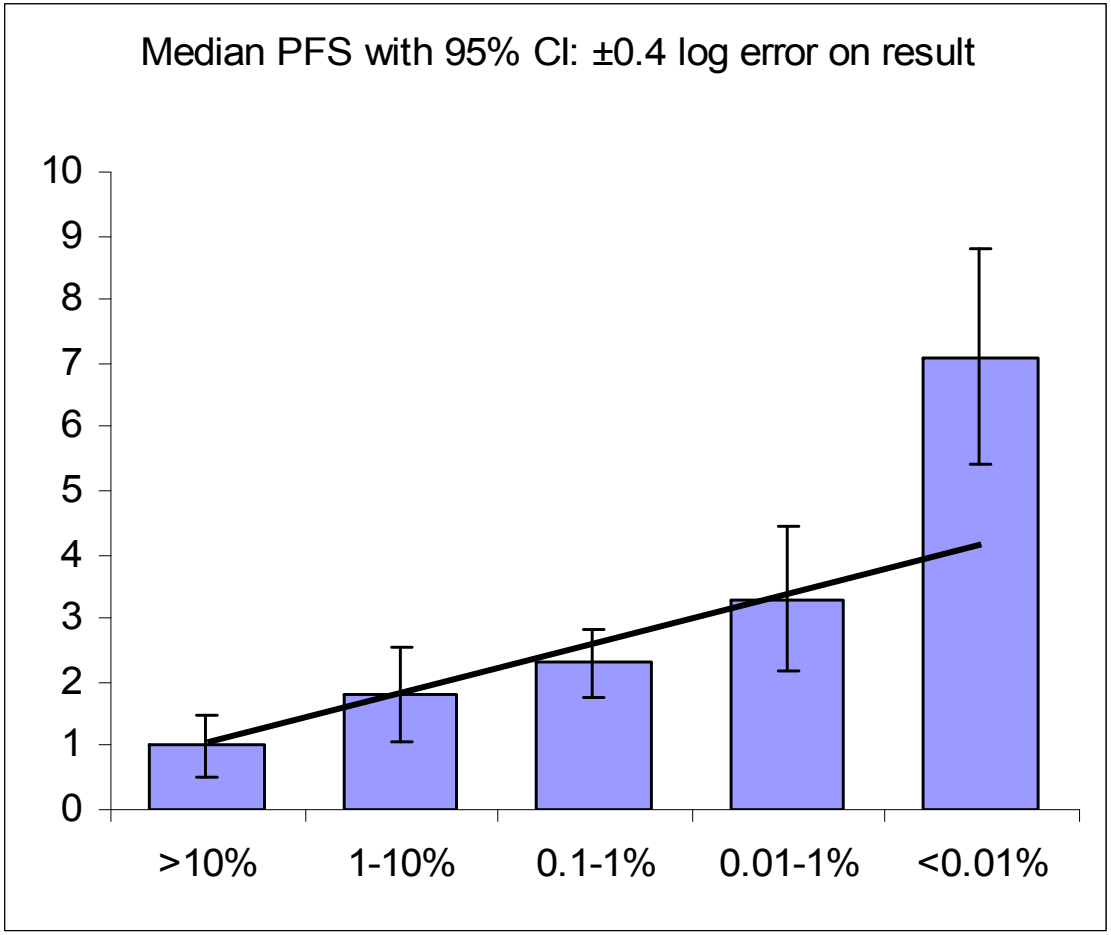
HMDS OUTREACH

Evaluation of Leeds MRD data since 1995: what is an “acceptable” error? ±0.4 log?



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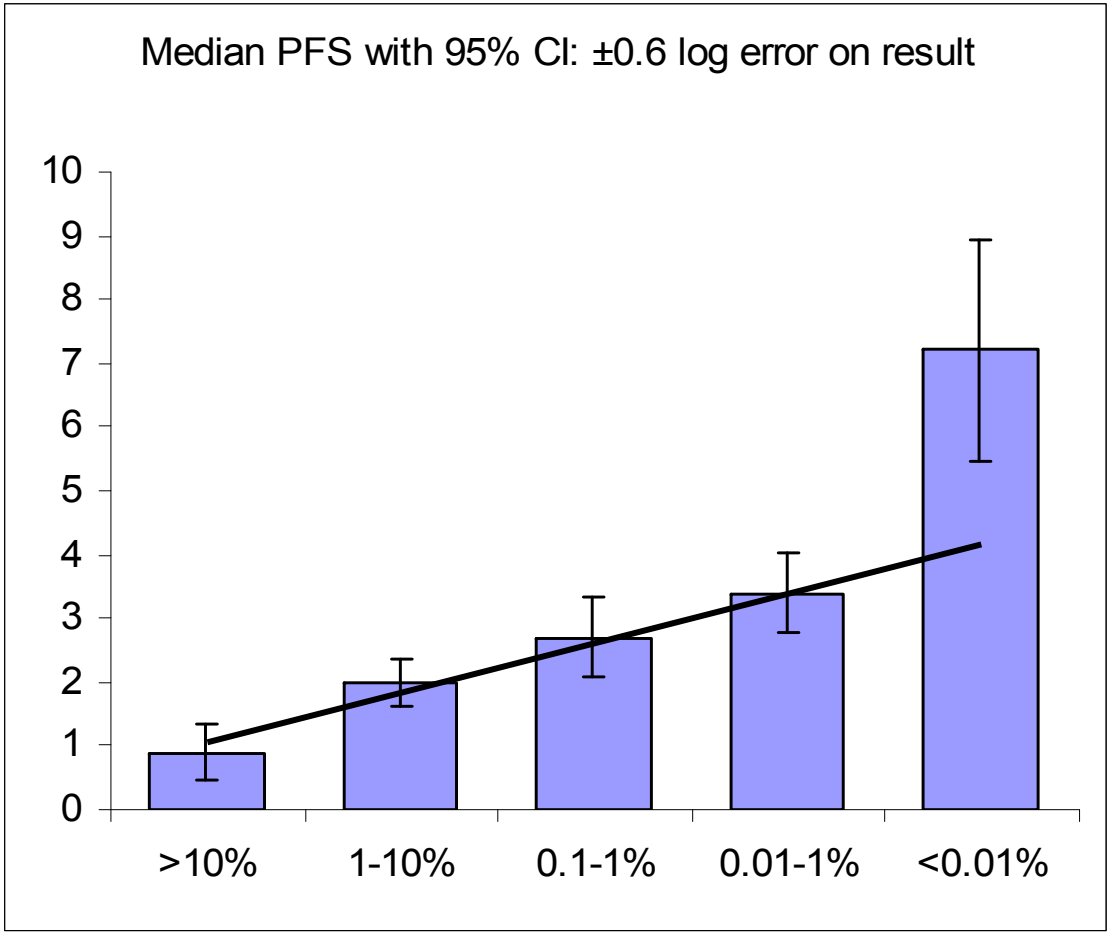
HMDS OUTREACH

Evaluation of Leeds MRD data since 1995: what is an “acceptable” error? ±0.6 log?



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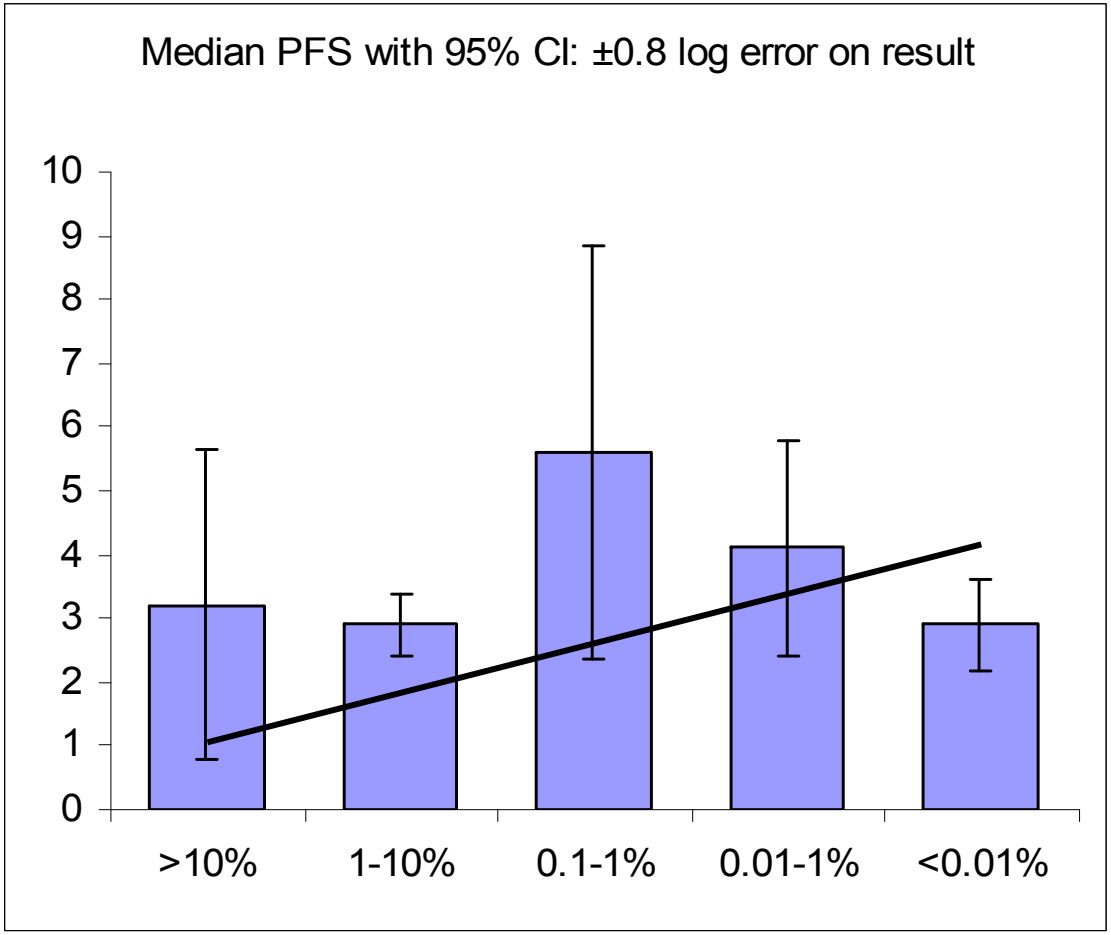
HMDS OUTREACH

Evaluation of Leeds MRD data since 1995: what is an “acceptable” error? ±0.8 log?



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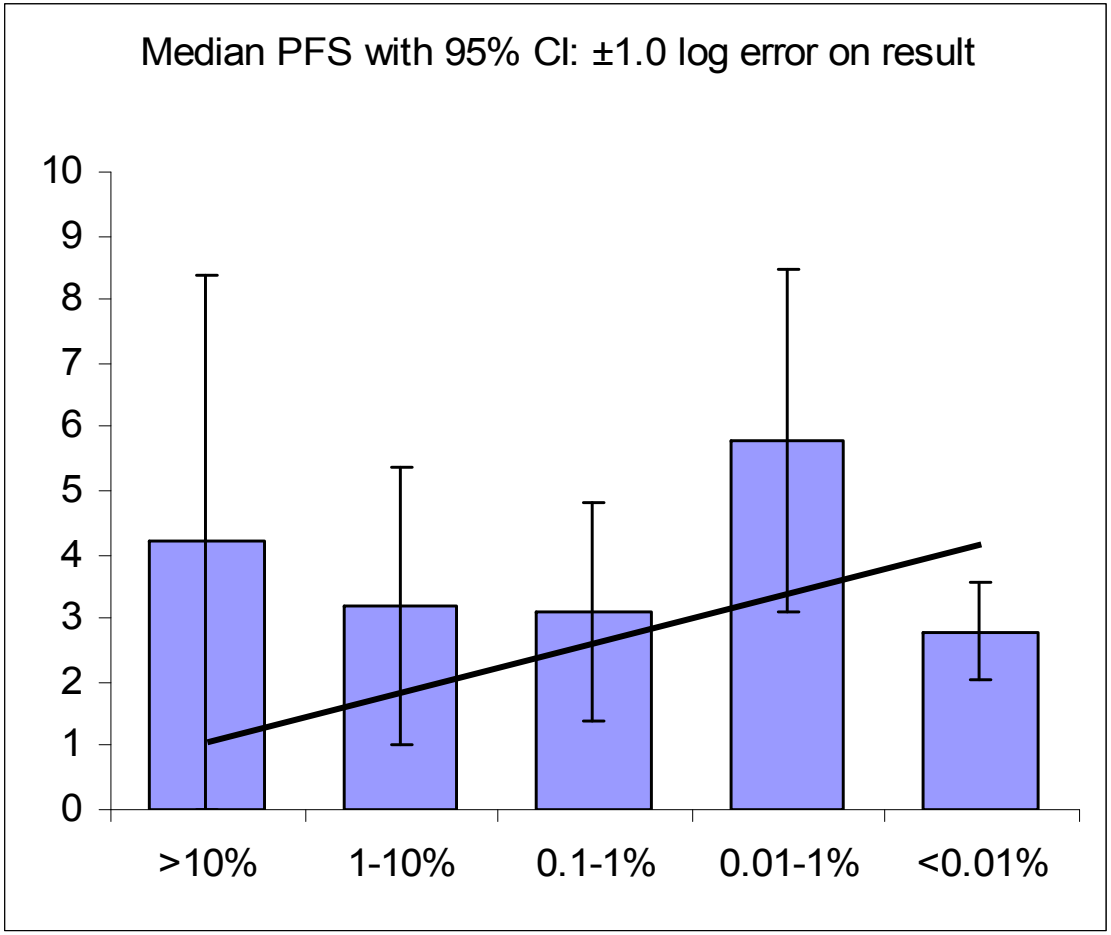
HMDS OUTREACH

Evaluation of Leeds MRD data since 1995: what is an “acceptable” error? ±1 log?



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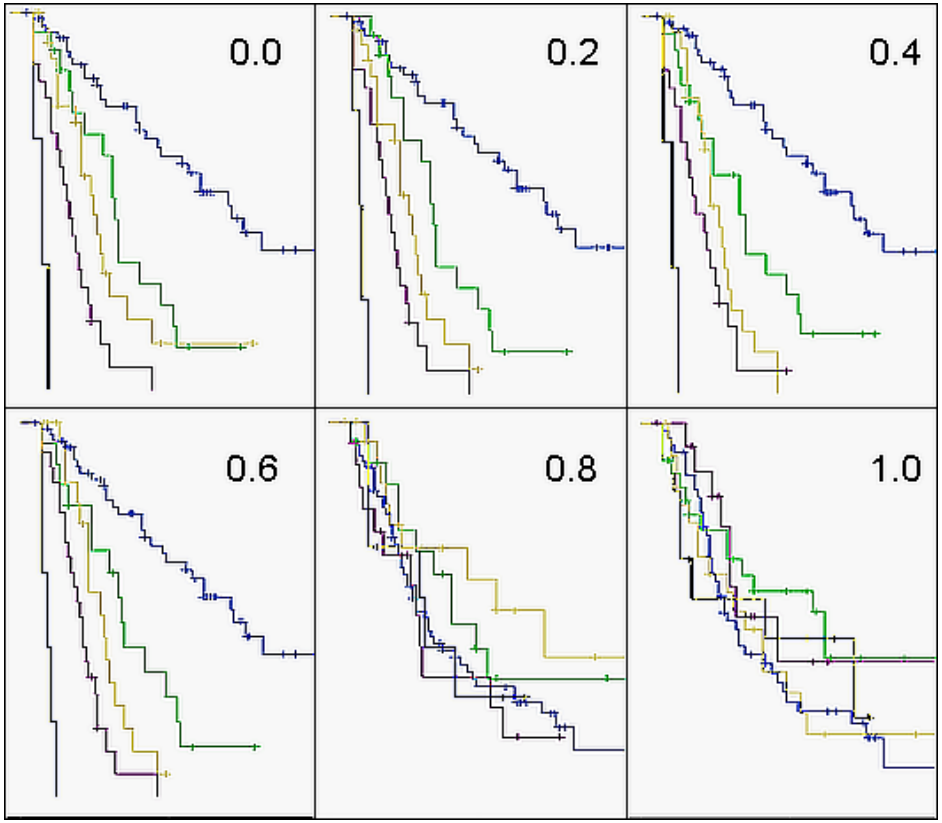
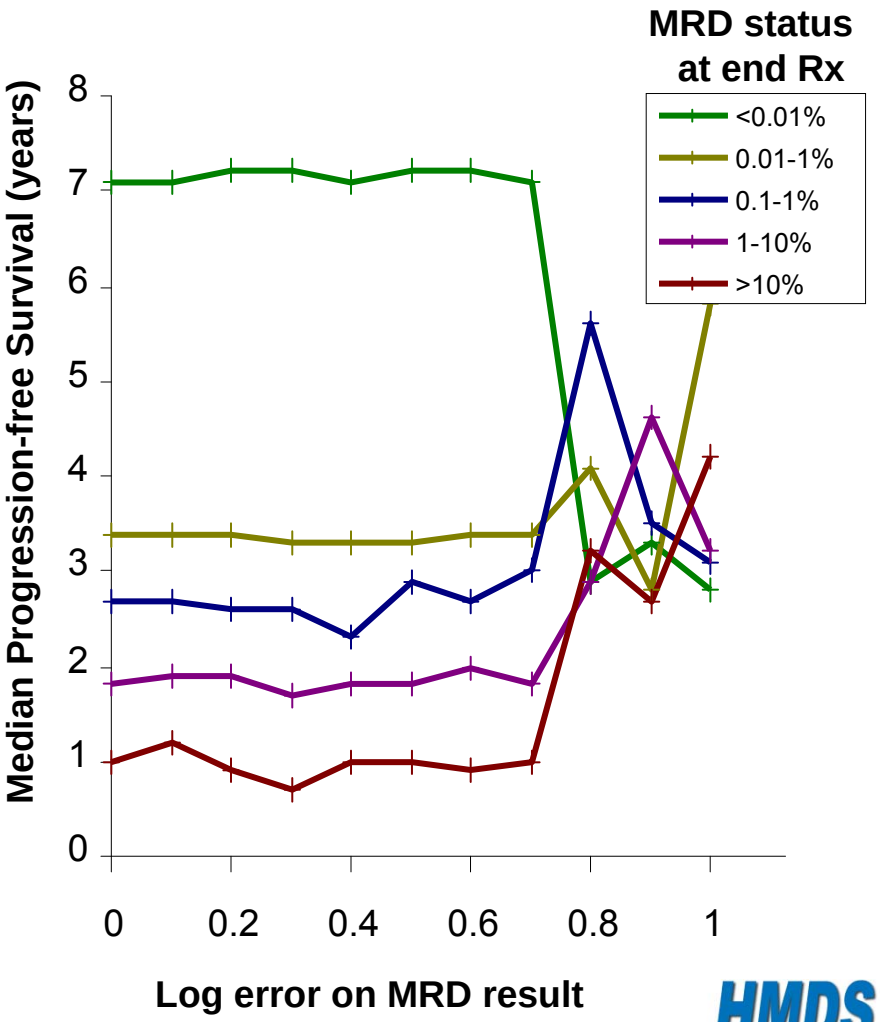
Acceptable error on MRD data may be 0.5 log (e.g. 0.01% = 0.005-0.05% or 1% = 0.05-5%)



PFS

Yrs from Rx (0-10)



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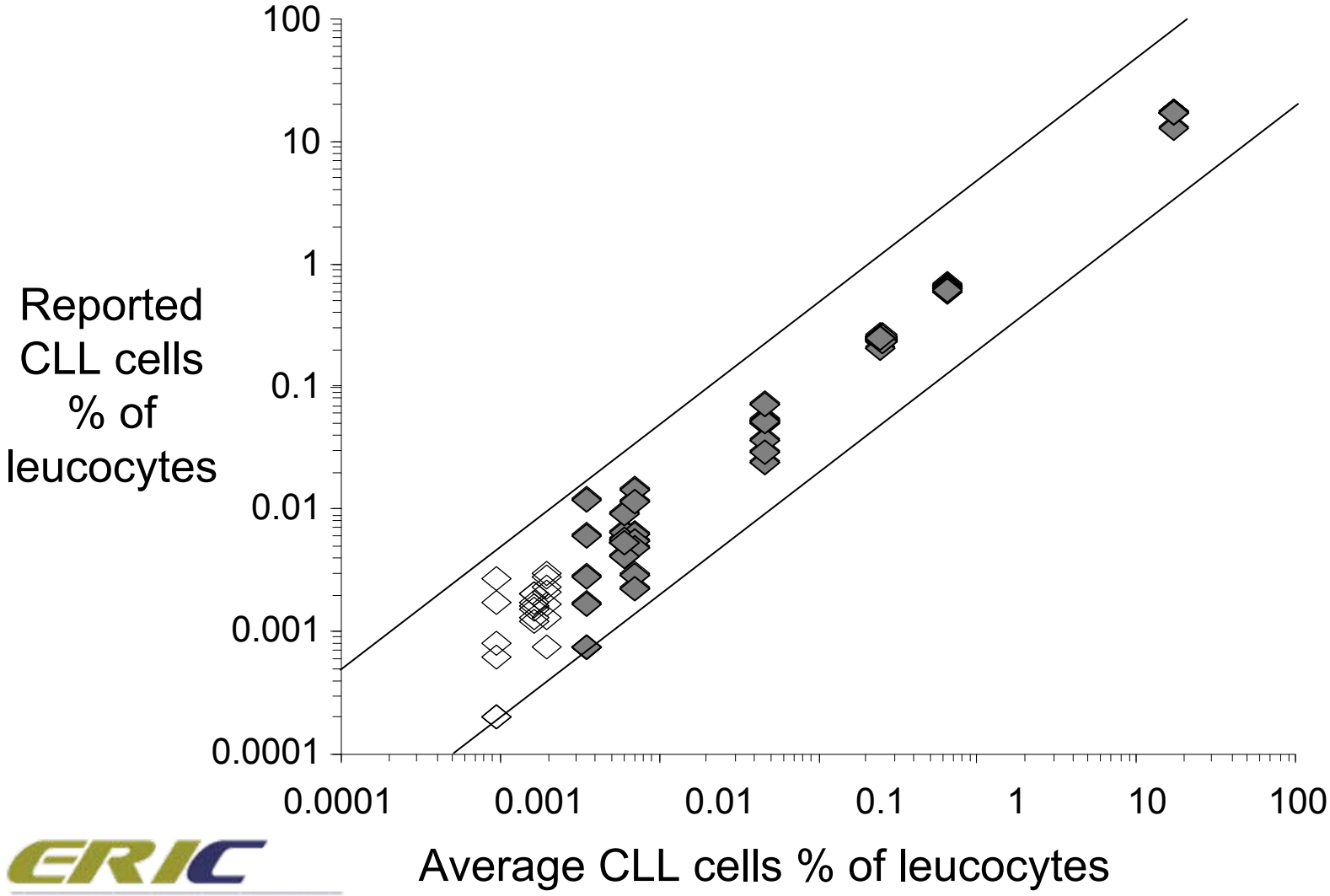
8-CLR harmonisation (2): analytical variation is <0.5log for samples with <0.01% (10e-4) CLL



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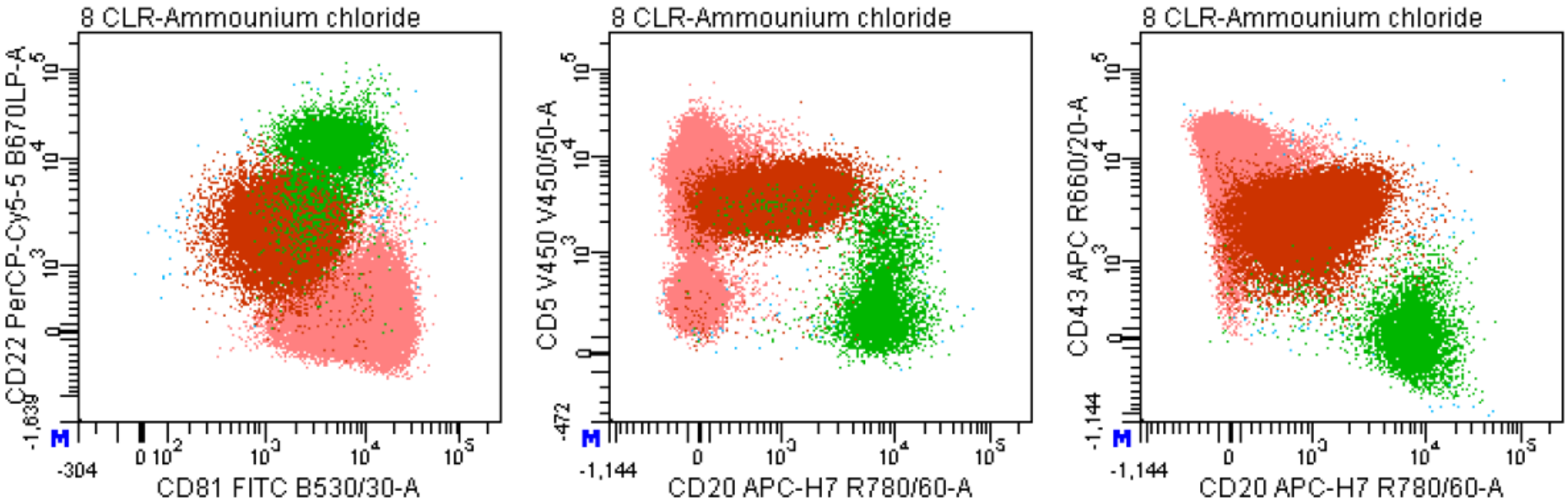
HMDS OUTREACH

8-CLR harmonisation (3): internal data QC



- Internal controls: Normal T-cells, B-cells, granulocytes
- T-cells: CD5+ CD43++ CD22- CD20-
- CLL: CD5+ CD43+ CD22wk CD20wk
- B-cells: CD5+/- CD43- CD22++ CD20++

- Use data from the assay to validate machine set-up, antibody signal:noise, CLL-cell phenotype

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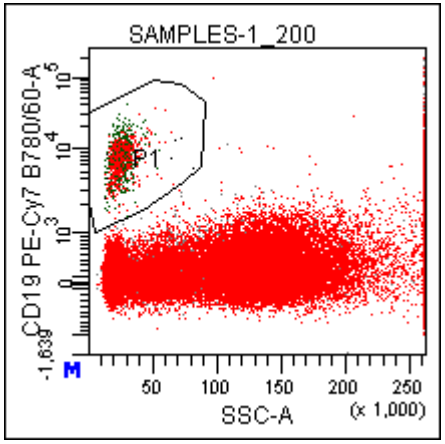
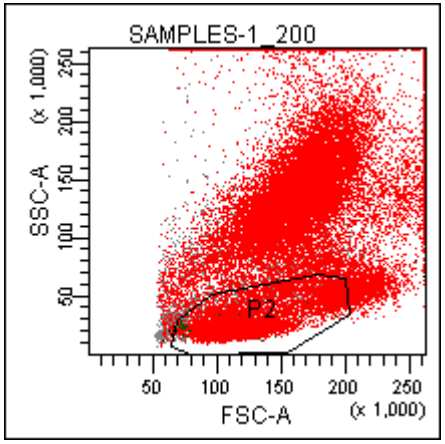
HMDS OUTREACH

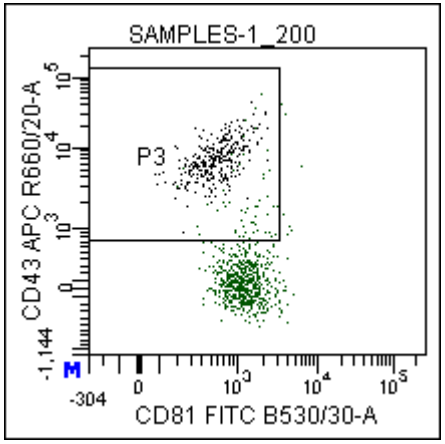
Proficiency testing: first evaluation of UK NEQAS stabilised sample shows good potential

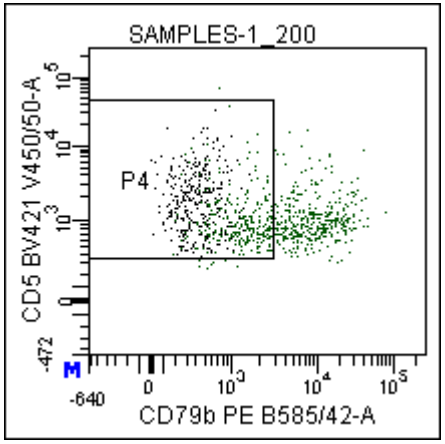


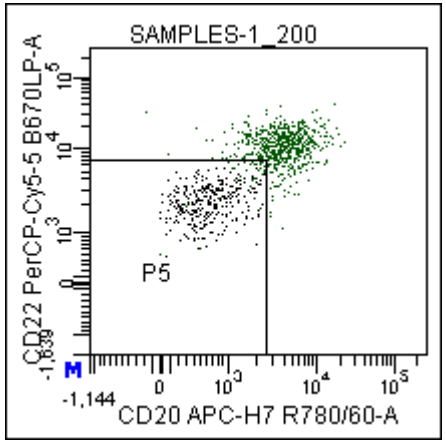


Specimen Name: SAMPLES
Tube Name: 1_200

Population	#Events	%Total
All Events	39,960	100.0000
P1 AND P2	969	2.4249
P3 AND P4 AND P5	303	0.7583
P7	36,366	91.0060







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ERIC international harmonisation experience in the detection of MRD in CLL

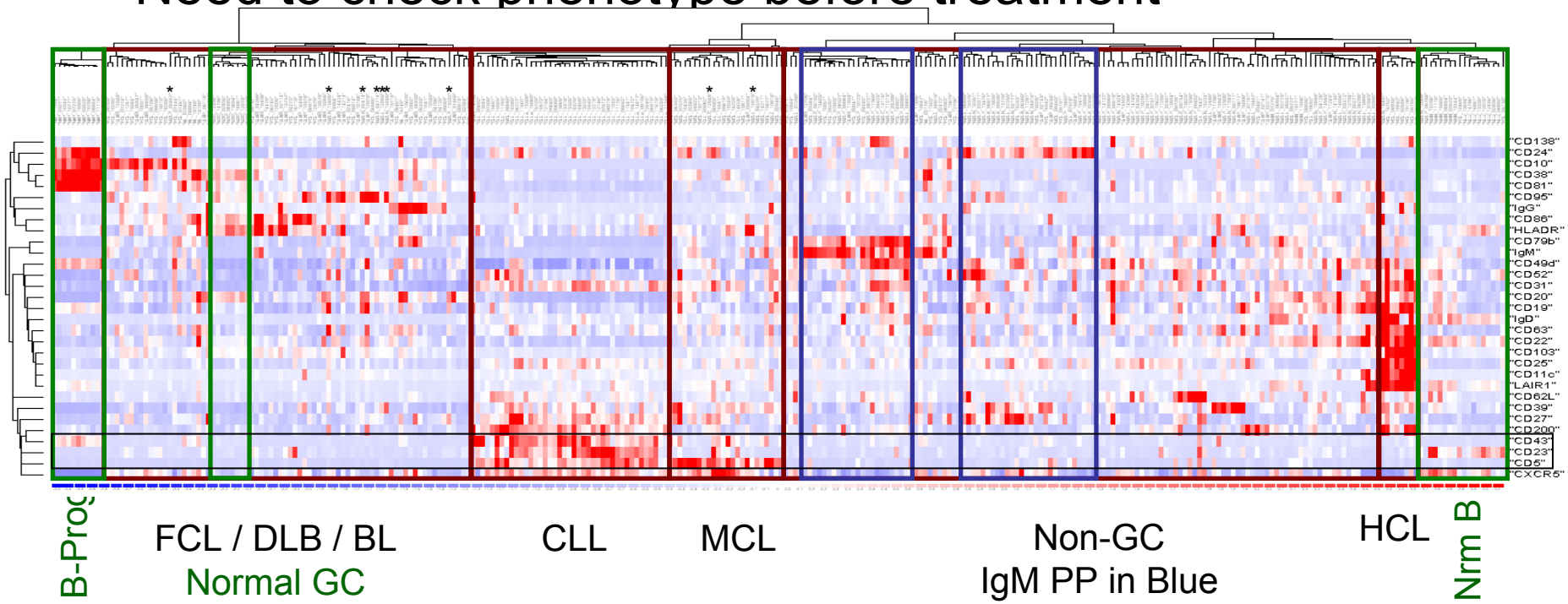
- Multi-parameter flow readily applicable to the quantitative detection of CLL to 0.01% (10e-4)
- 4 / 6 / 8 CLR assays validated, using the same core antibodies, most of which have been in diagnostic use for decades
- Inter-laboratory analytic errors within acceptable range
  - Substantial improvements in survival (e.g. >1 year) require >1 log depletion of CLL, therefore enumeration errors up to 0.5 log (e.g. 0.01% = 0.005 – 0.05%) do not impair prediction of outcome.
- Proficiency testing (EQA) available soon.

Lessons from CLL 207 trial

- Sequential monitoring after treatment, MRD-positive → alemtuzumab
- 102 BM & 411 PB samples, majority of samples were MRD-negative
  - <20 events in 500,000 / <0.004% / <4 x 10^{-5}
- Quality check on laboratory procedures: clonality / B-cell enumeration assay run independently from CLL MRD assay
  - Different person, time, batch and cytometer
- ~2% (11/513) repeat assessments required to check for discrepancy
- 1-2 (0.2-0.4%) discrepant results despite independent checks

Atypical phenotype

- 1-5% referred to CLL trials are not (typical) CLL
- CD5/CD23 less relevant for defining CLL cluster and suitability for MRD monitoring than CD43/CD81
- Need to check phenotype before treatment



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Recommendations for clinical trials

- Quantitative CLL-specific assay, not qualitative clonality-based approach
- Pre-treatment work-up to include suitability for MRD monitoring
- Reproducible limit of detection 0.01%
  - 20-50 events likely to represent a population of CLL but less reproducible than 50 event population minimum
- Contamination control (CD3) for results in the 0.005-0.05% level
- Independent validation of results
  - Partial spillover control in 4/6-CLR multi-tube assays
  - Independent preparation of component tubes
  - Validate with independent assay, RQ-ASO IGH-PCR
- If treatment decision based on MRD result, multiple positive time-points with rising MRD level indicated.

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- Leeds
  - Peter Hillmen, Andrew Jack, Ruth de Tute
- ERIC
  - Emili Montserrat, Paolo Ghia, Michael Hallek
- BD Biosciences
  - Frans Nauwelaers, Lucia Testolini, Mark Herberger, Jingyi Chen, Noel Warner
- UK NEQAS
  - Matt Fletcher, David Barnett

- Thank you

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