Update on the International Harmonised Approach for Residual Disease Monitoring in Chronic Lymphocytic Leukaemia: 8-CLR flow cytometry

Andy C. Rawstron

on behalf of ERIC consortium

ERIC european research initiative on CLL

HMDS OUTREACH

Timeline for development of MRD assays in CLL

- 1970s CLL demonstrated to have aberrant CD5 / sIg.
  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
  Development of consensus method
  >70% response to chemo-immunotherapy.

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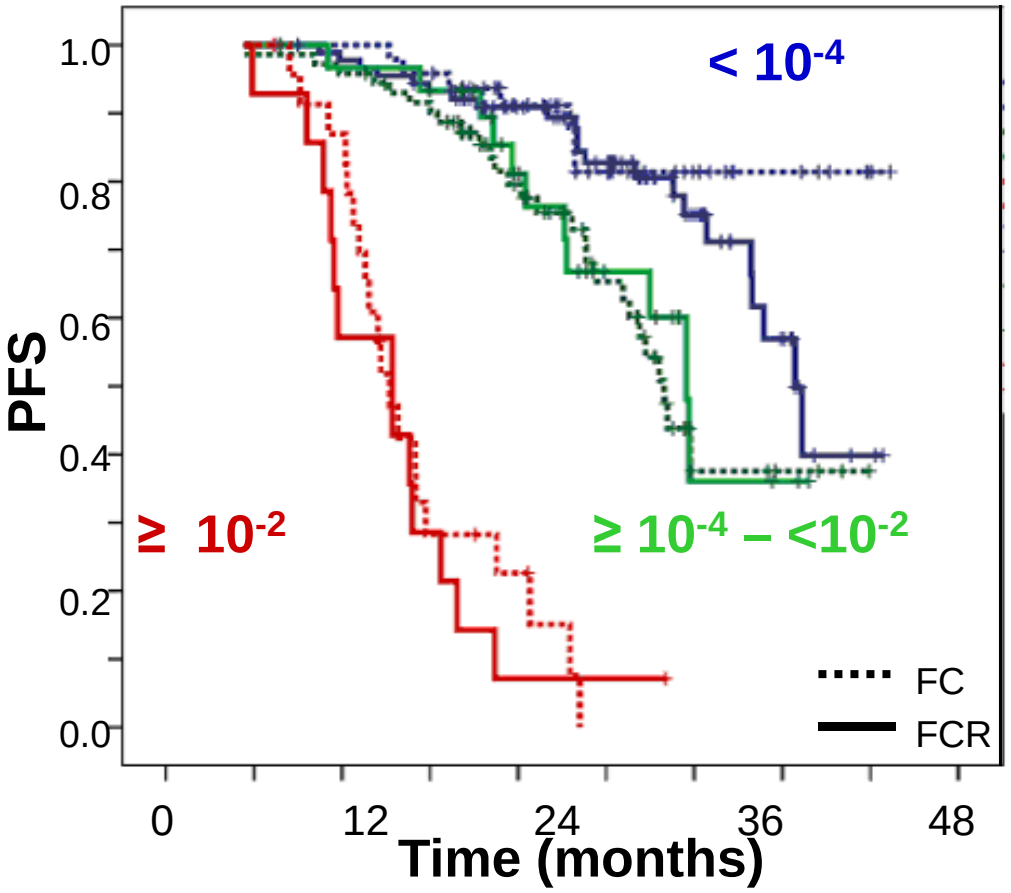
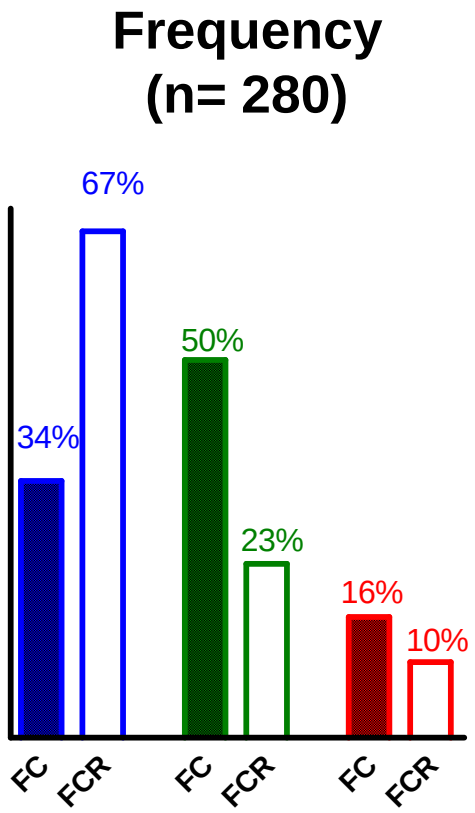
DCLLSG CLL FC vs. FCR: MRD level not treatment type predicts outcome





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Courtesy of Seb Böttcher

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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	Univariate (Log-Rank) P Value	Multivariate (Cox) P Value
Age pre-Rx	0.122		**0.001**	**0.002**
Hemoglobin pre-Rx	0.435		**0.028**	0.921
Leukocytes pre-Rx	0.235		0.092	
Platelets pre-Rx	**0.002**	0.432	**0.028**	0.465
Prior treatment (Y/N)	**0.004**	**0.014**	**<0.001**	**0.035**
Prior fludarabine (Y/N)	0.611		**0.042**	0.078
Type of treatment	**0.017**	0.209	0.214	
iwCLL Response	**<0.001**	**0.023**	**0.015**	0.624
MRD Response	**<0.001**	**<0.001**	**0.002**	**0.012**

Kwok et al ASH 2009: N=137, age 62 (38-83), 77% male, 41% no prior Rx. Follow-up 3.1yrs (0.2-12.7). Rx: 64 FC/FCR/FCM, 29 Alemtuzumab, 17 Flud, 7 AutoSCT, 20 Clr/other.

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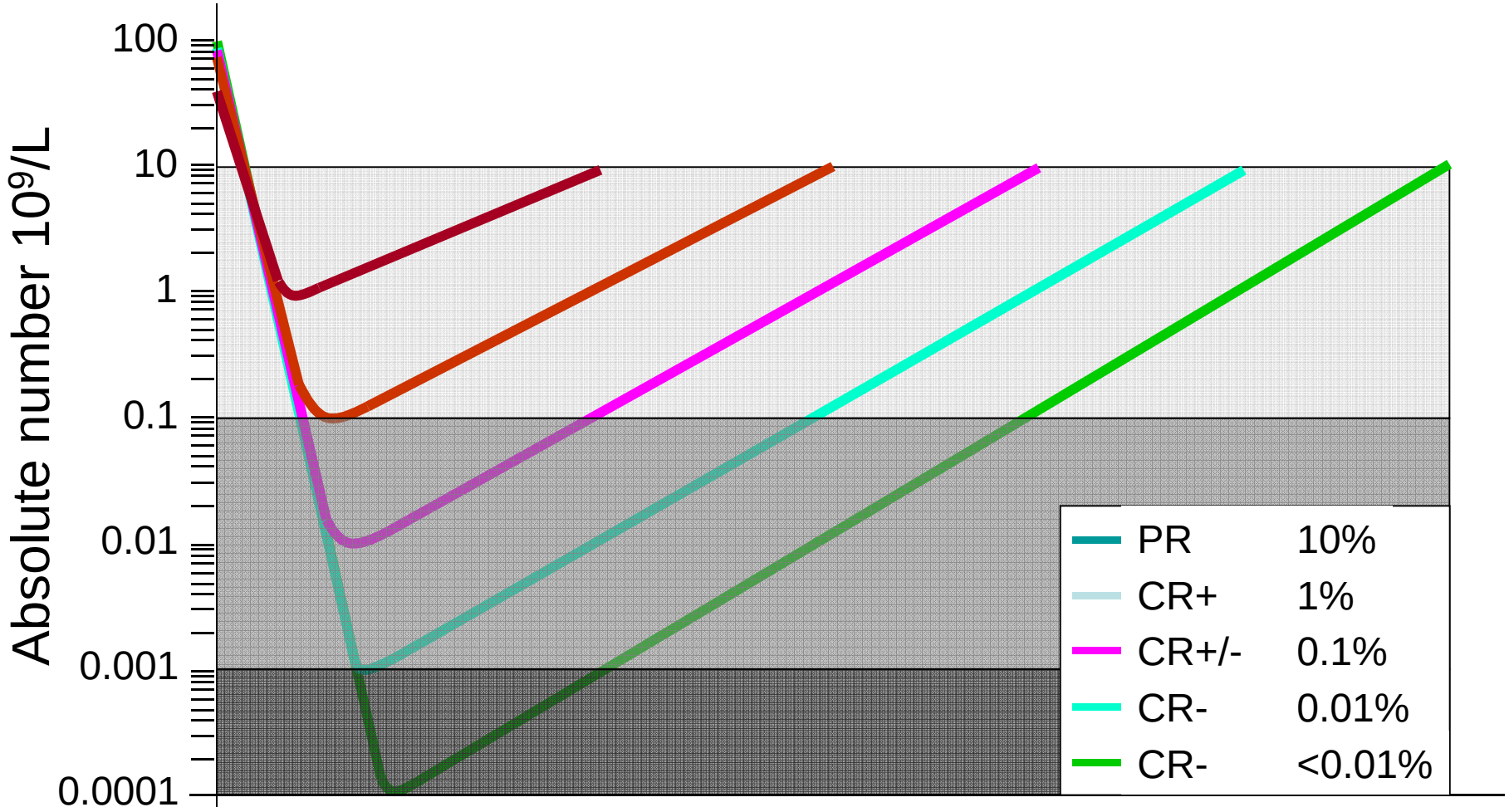
6 month doubling time ➔ 20 months extra progression-free survival per log depletion



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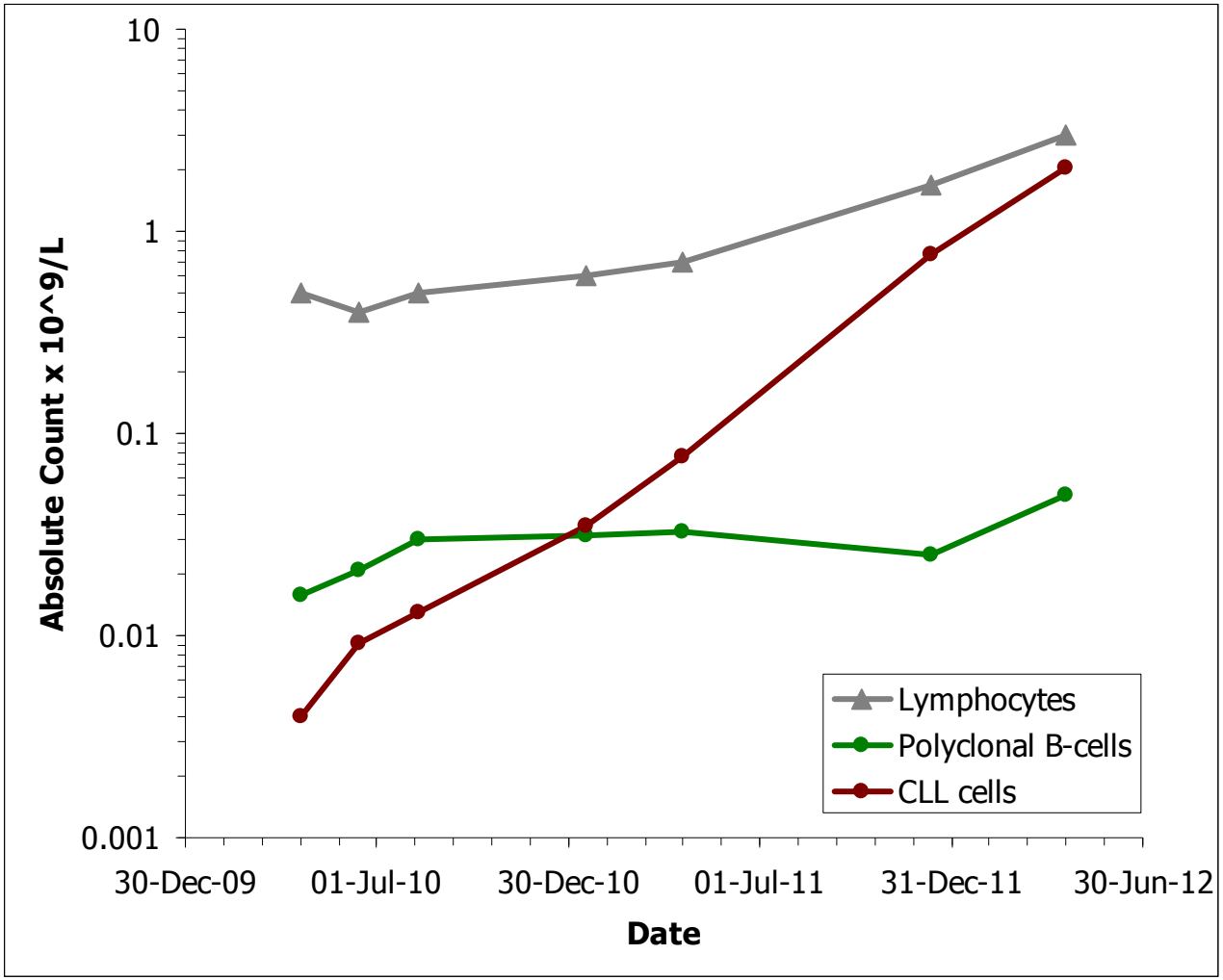
Kinetics of relapse (1)



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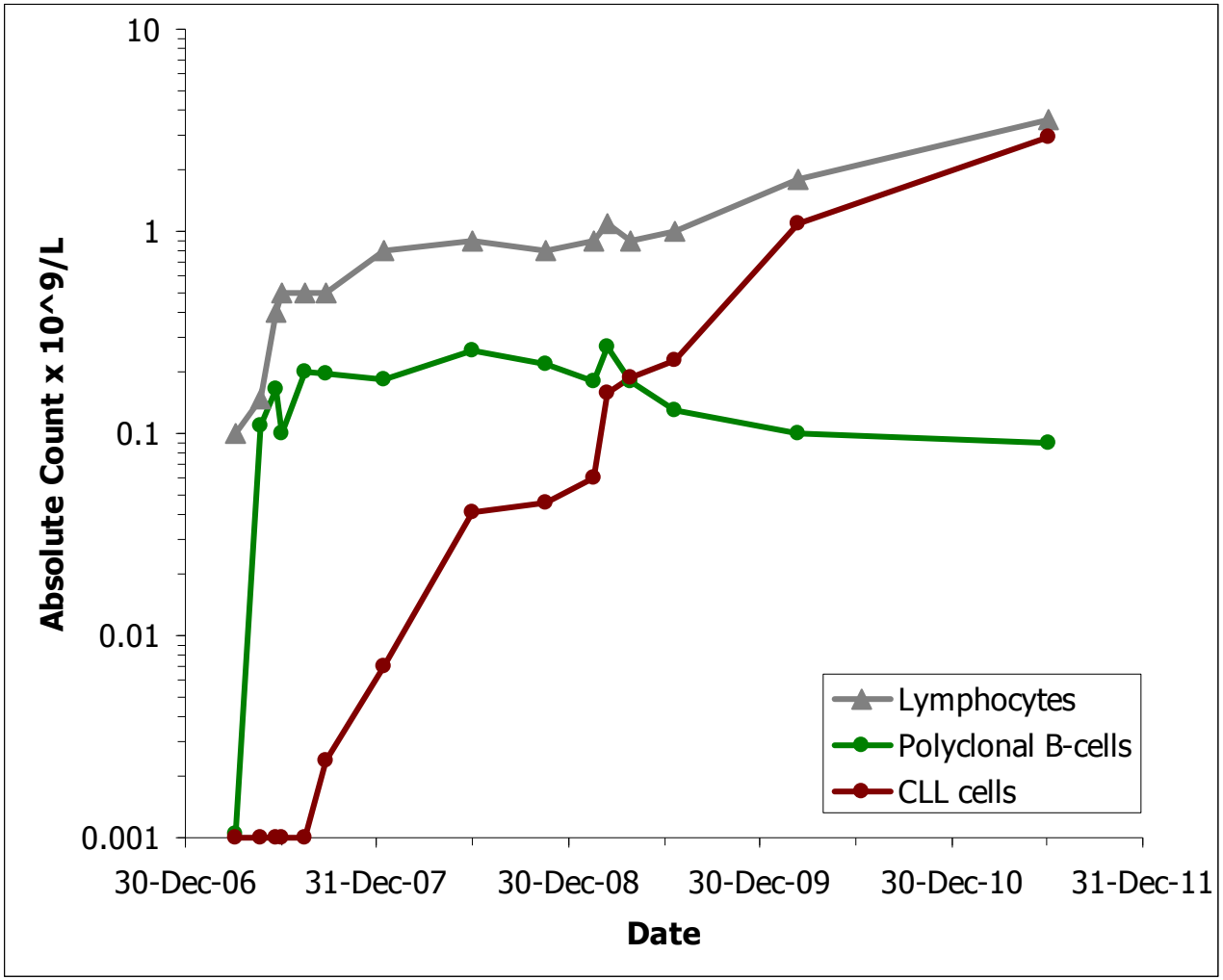
Kinetics of relapse (2)



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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

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4-CLR EU/US harmonisation

Leukemia (2007), 1–9
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ORIGINAL ARTICLE

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

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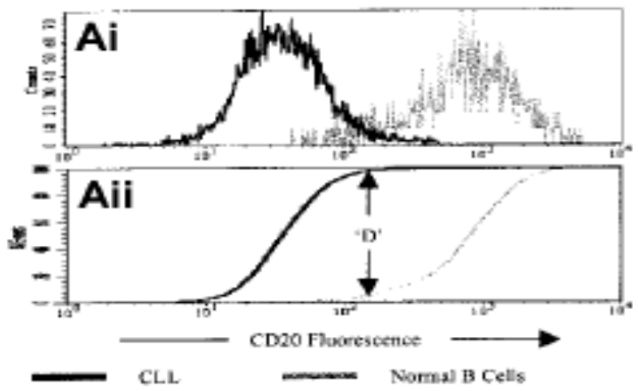
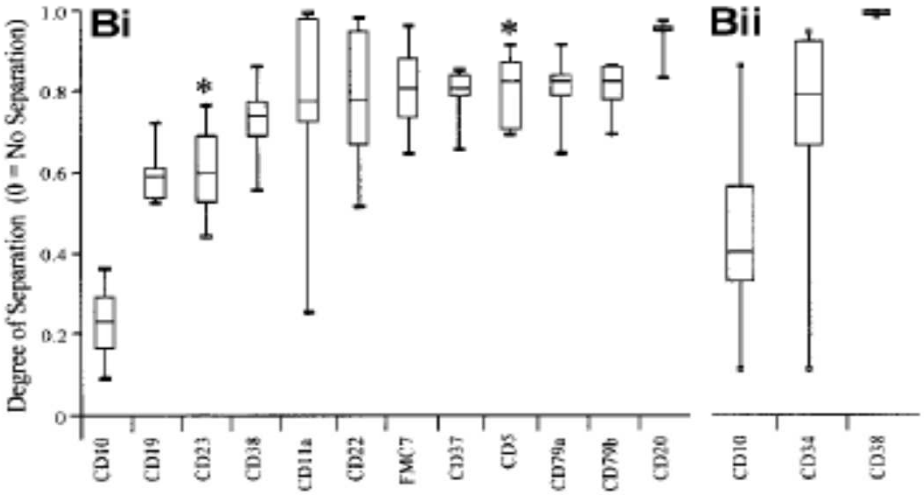
4CLR harmonisation (1): Identification of markers that reproducibly separate CLL





- 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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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.

- Core markers
  - CD5 / CD20 / CD79b
  - CD43 / CD22 / CD81
  - CD38 to exclude progenitors
- Most have been used in CLL diagnostics more several decades

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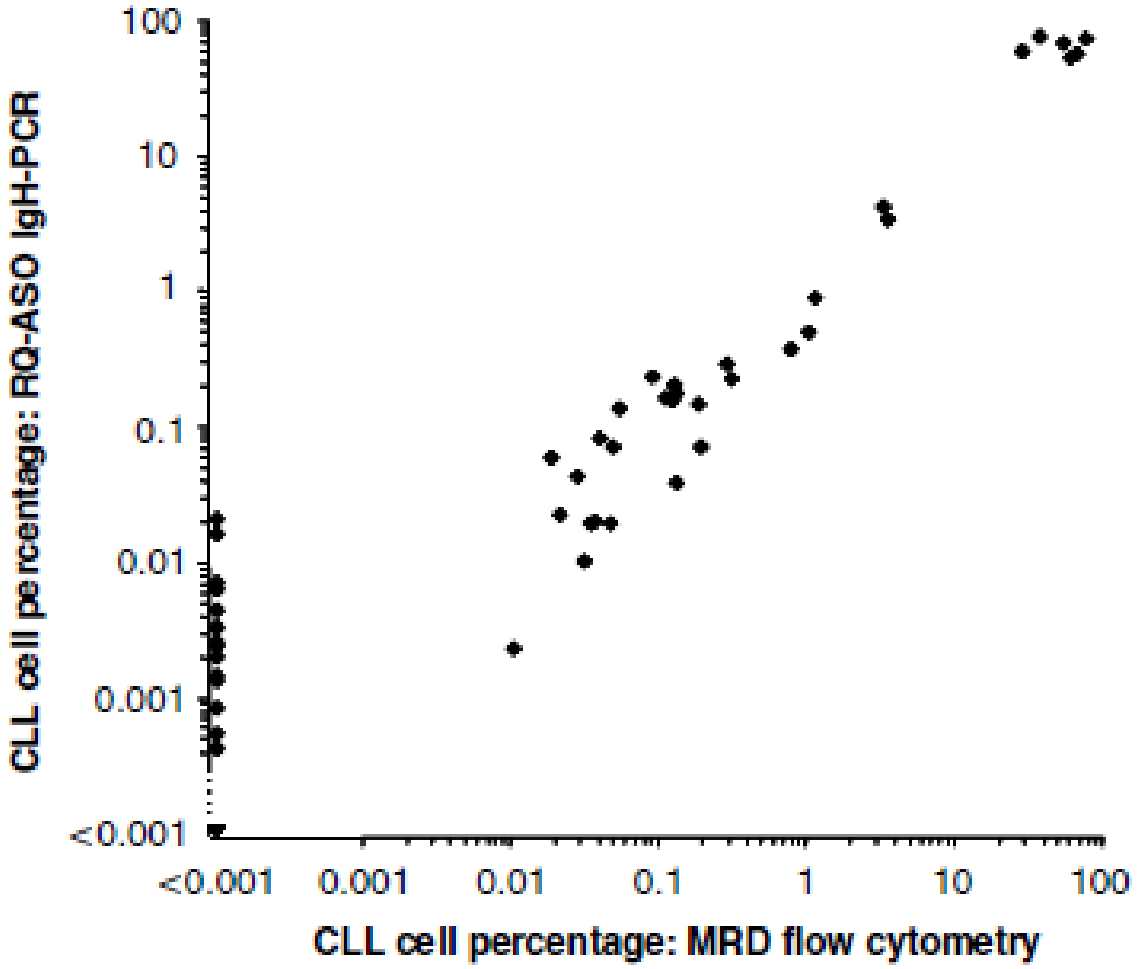
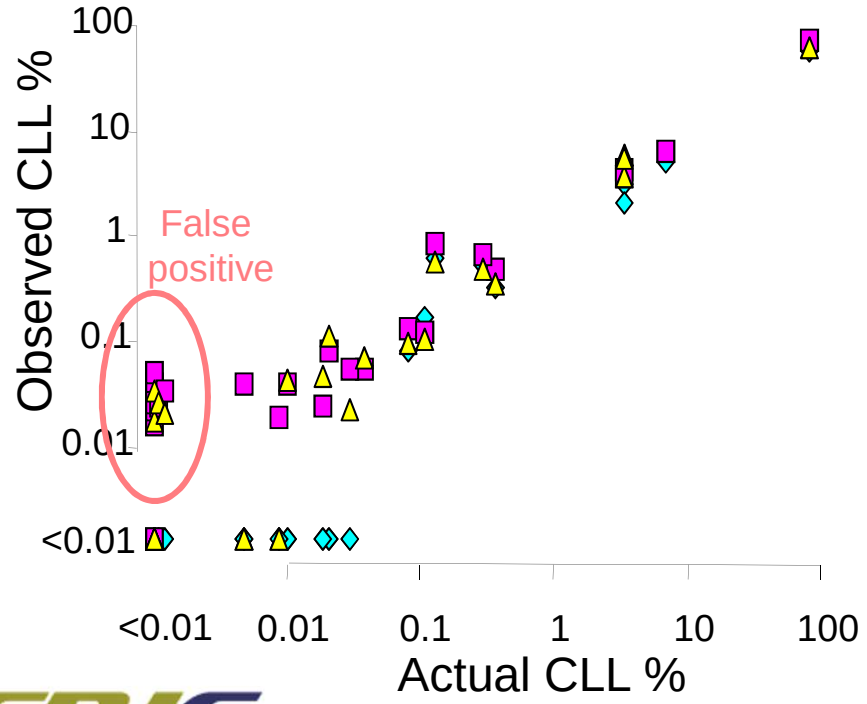
4CLR harmonisation (2): Quantitative approaches equivalent to 0.01% (10E-4)

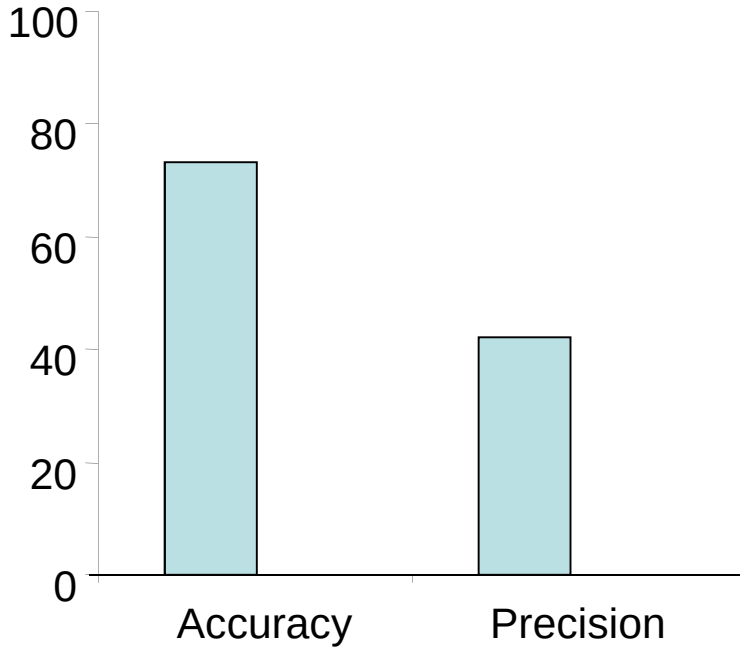


International standardised approach for residual disease monitoring in CLL: Leukemia 2007, 21(5): 956-64

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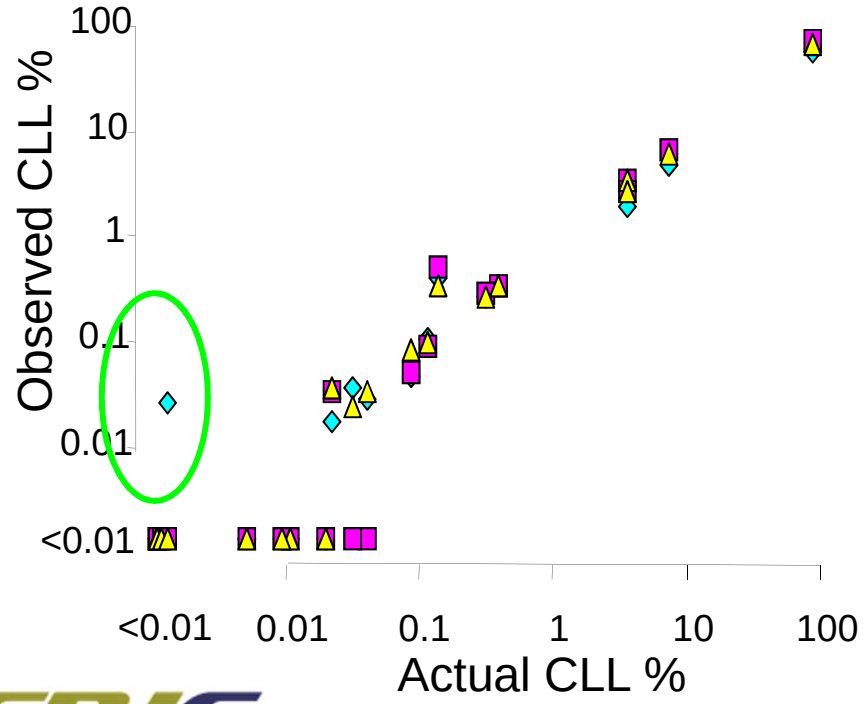
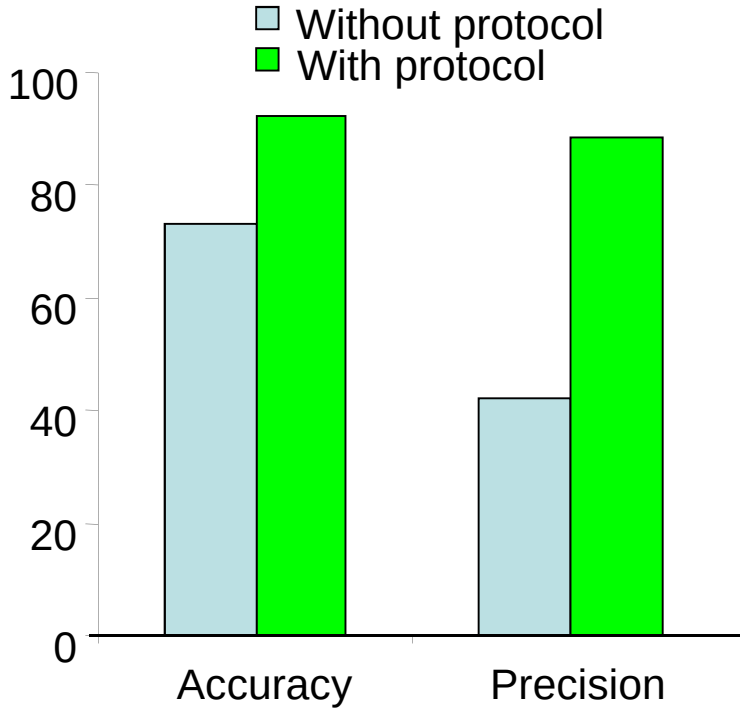
4CLR harmonisation (3): improvement in precision and accuracy using a protocol

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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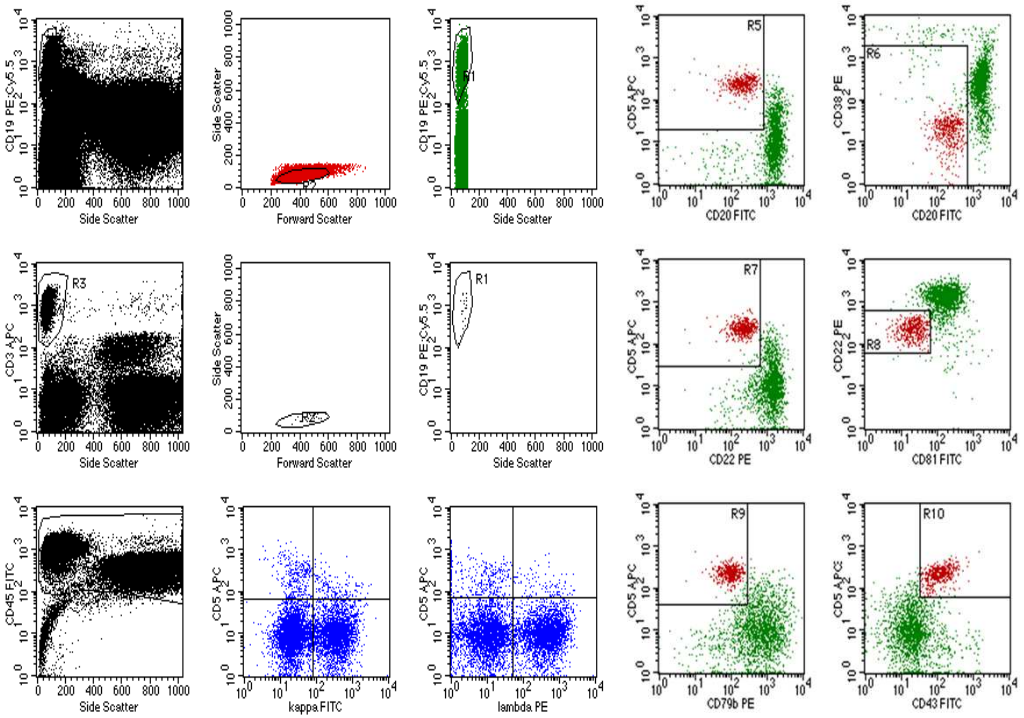
Issues with harmonised 4-CLR assay

- 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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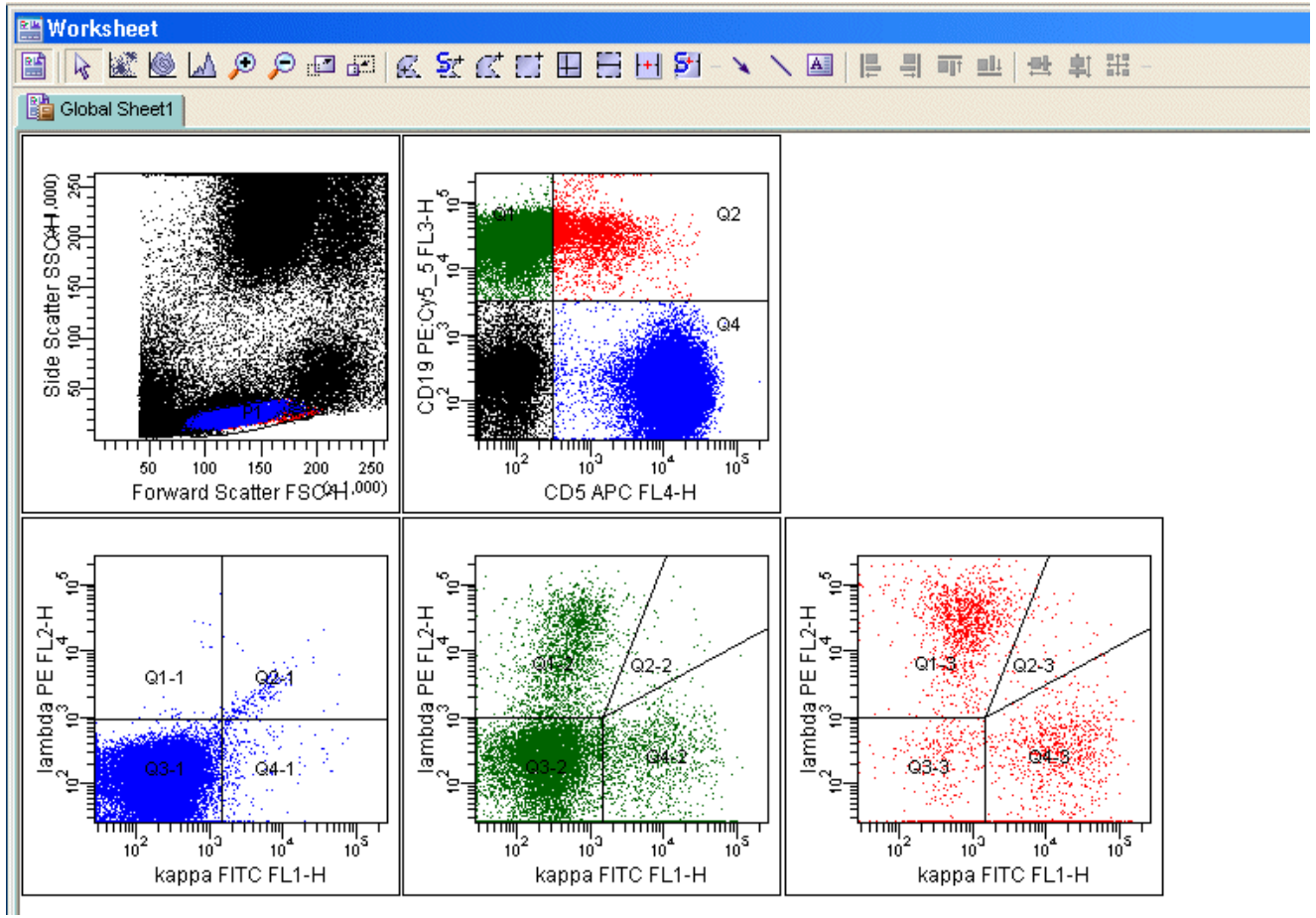
Simple analysis for CD19/CD5/Kappa/Lambda



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

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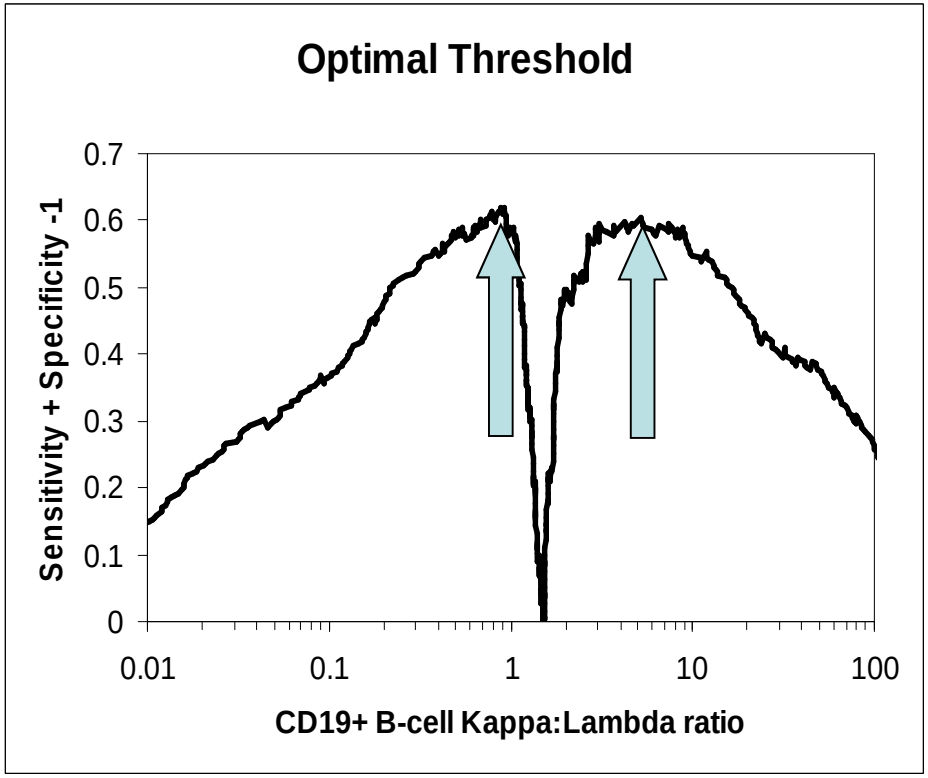
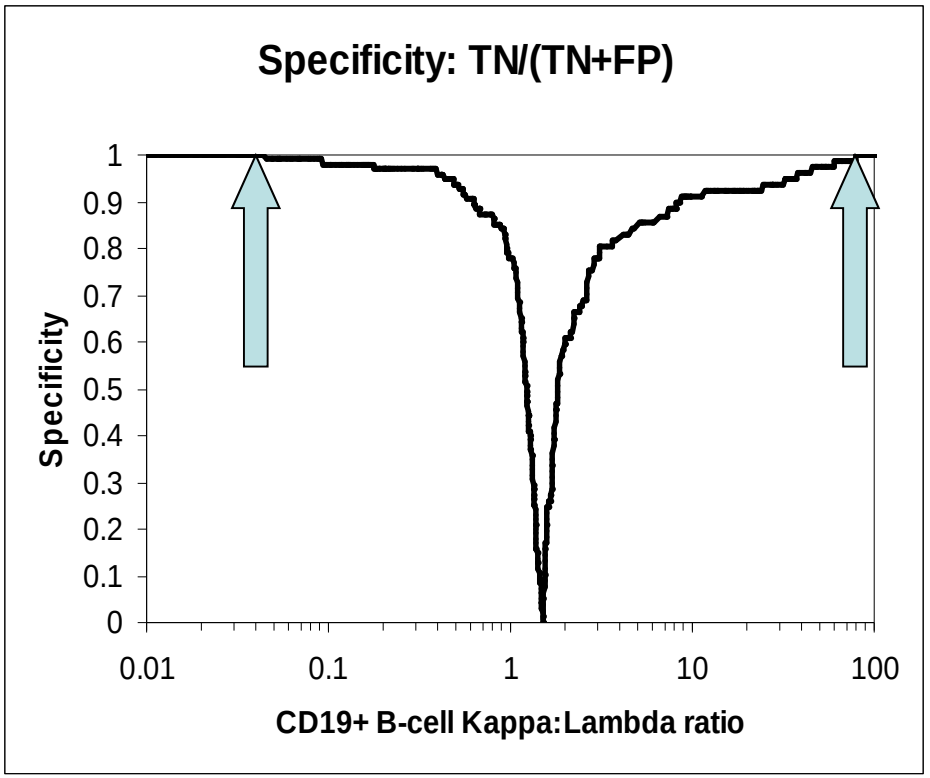
CD19 K:L ratio ROC and specificity





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+ %slg-	>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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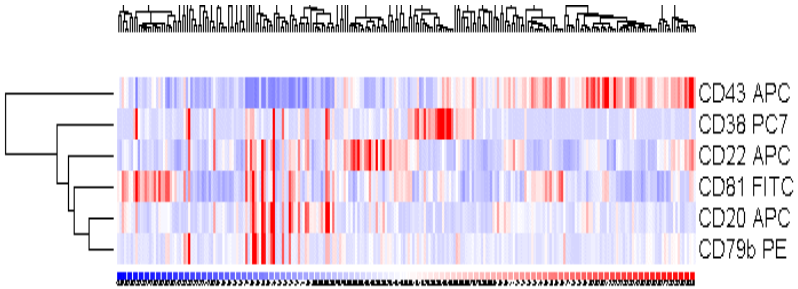
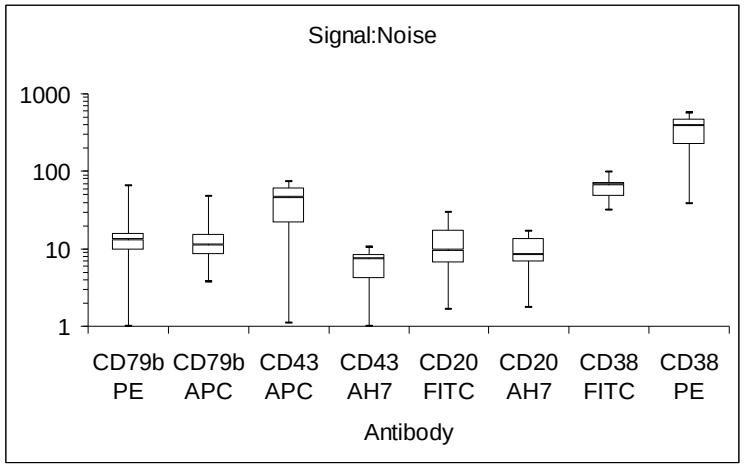
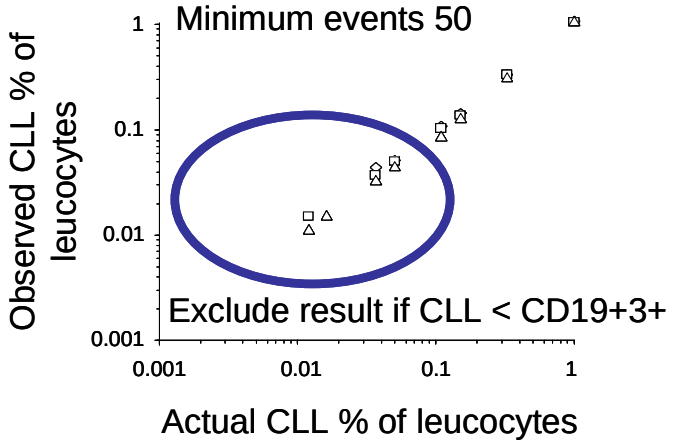
Design of the 6CLR panel: combinations, conjugates, contamination and cocktails





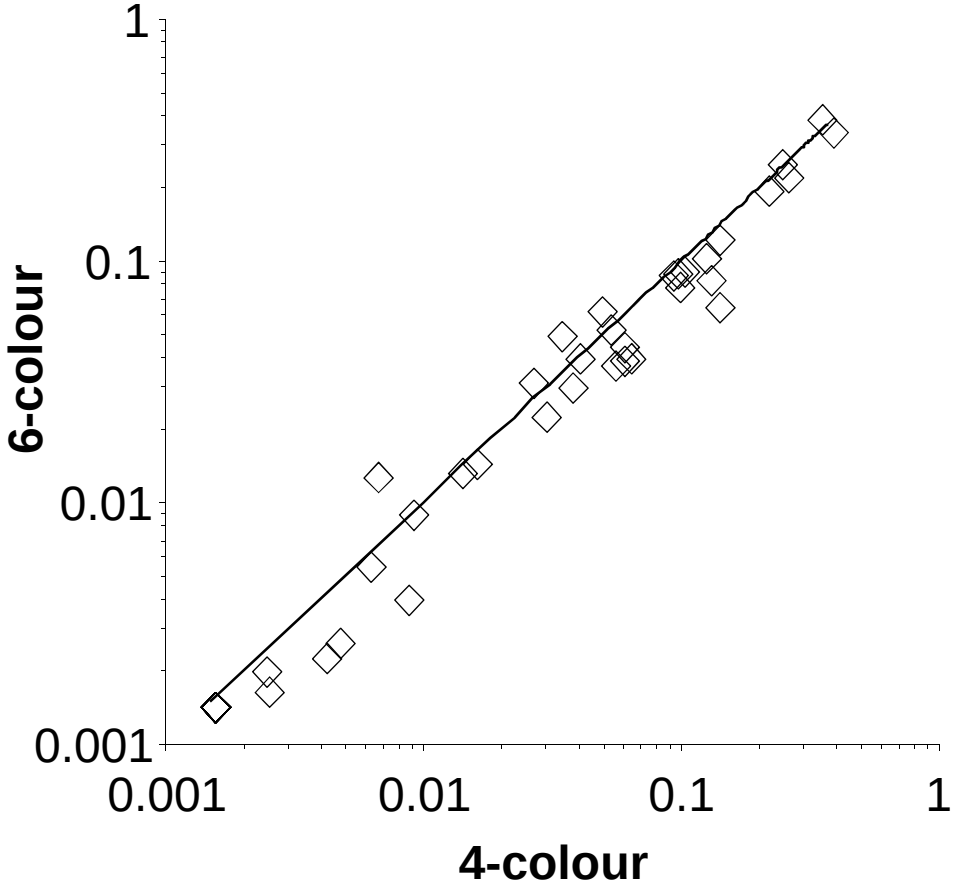
	T-test for # of cells classed as CLL with or without CD3	Regression slope for # of cells classed as CLL +/- CD3
CD20/79b/38	0.019	1.13
CD81/22/43	0.15	1.06
CD81/79b/43	0.69	1.05



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%



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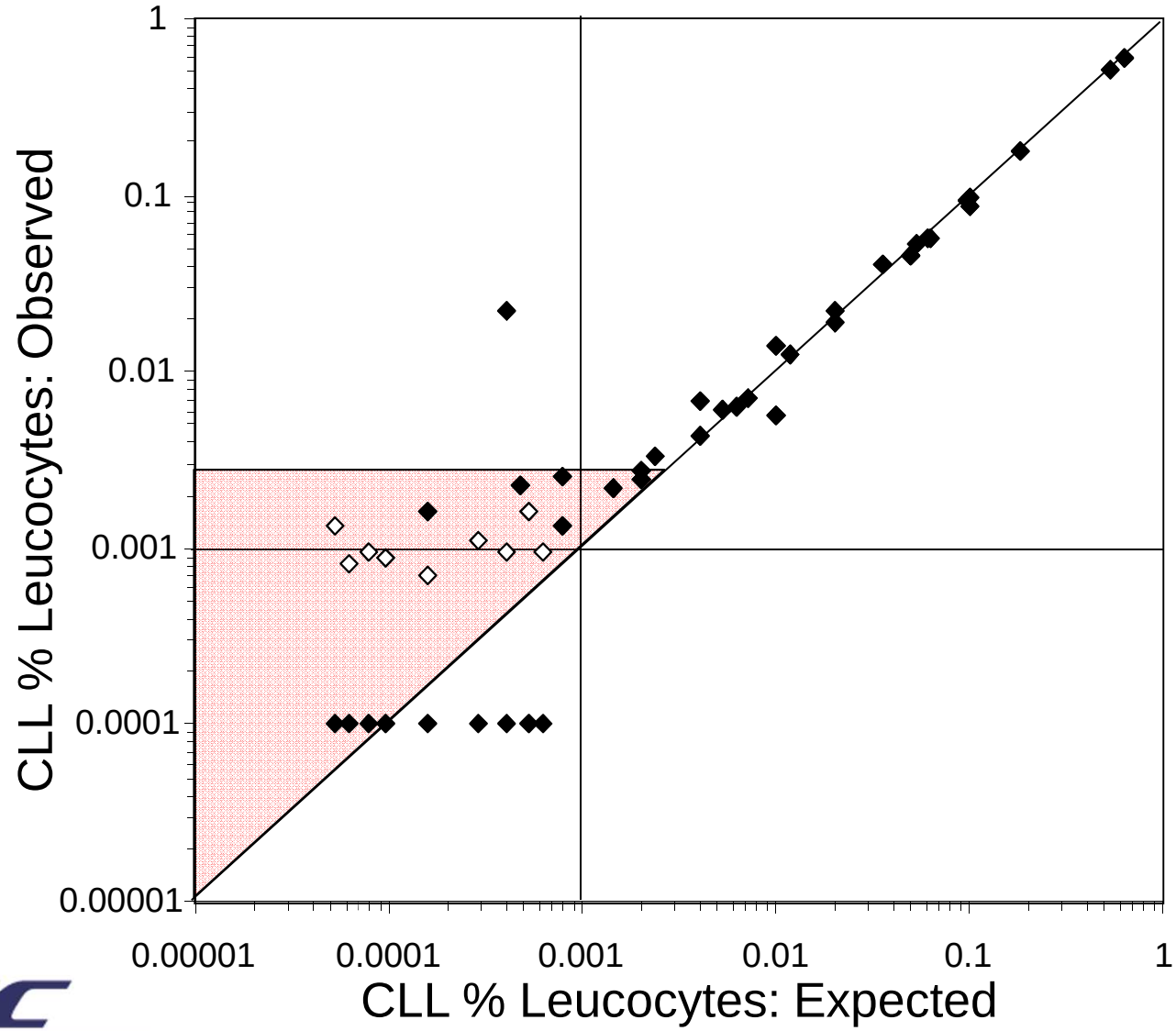
8-CLR harmonisation (1): dilution studies ➔ detection limit 0.003%



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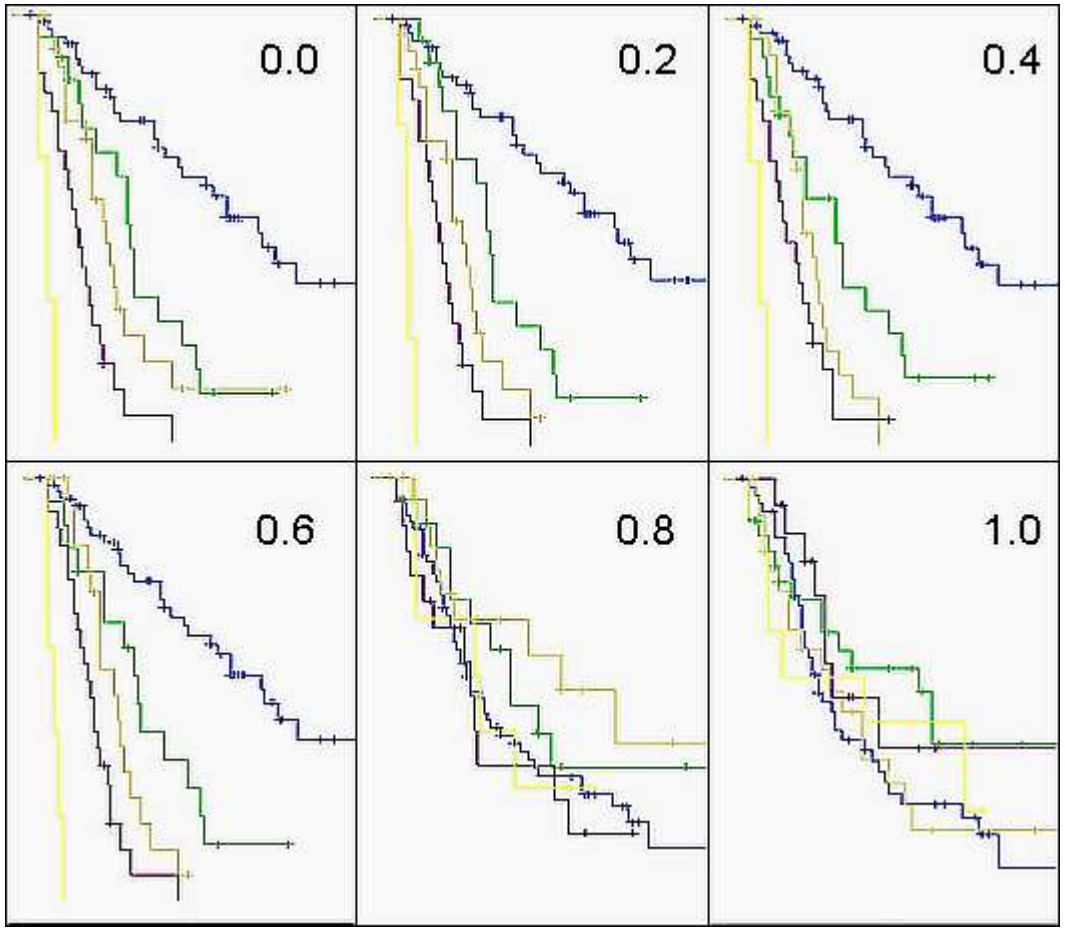
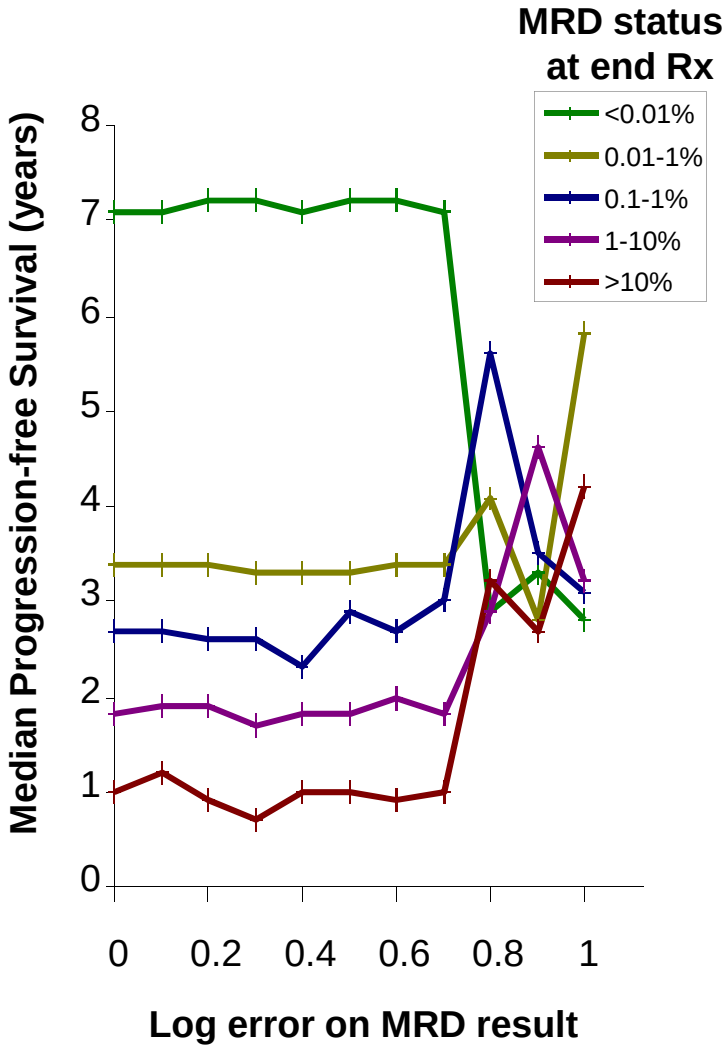
Evaluation of Leeds MRD data since 1995: what is an “acceptable” error?





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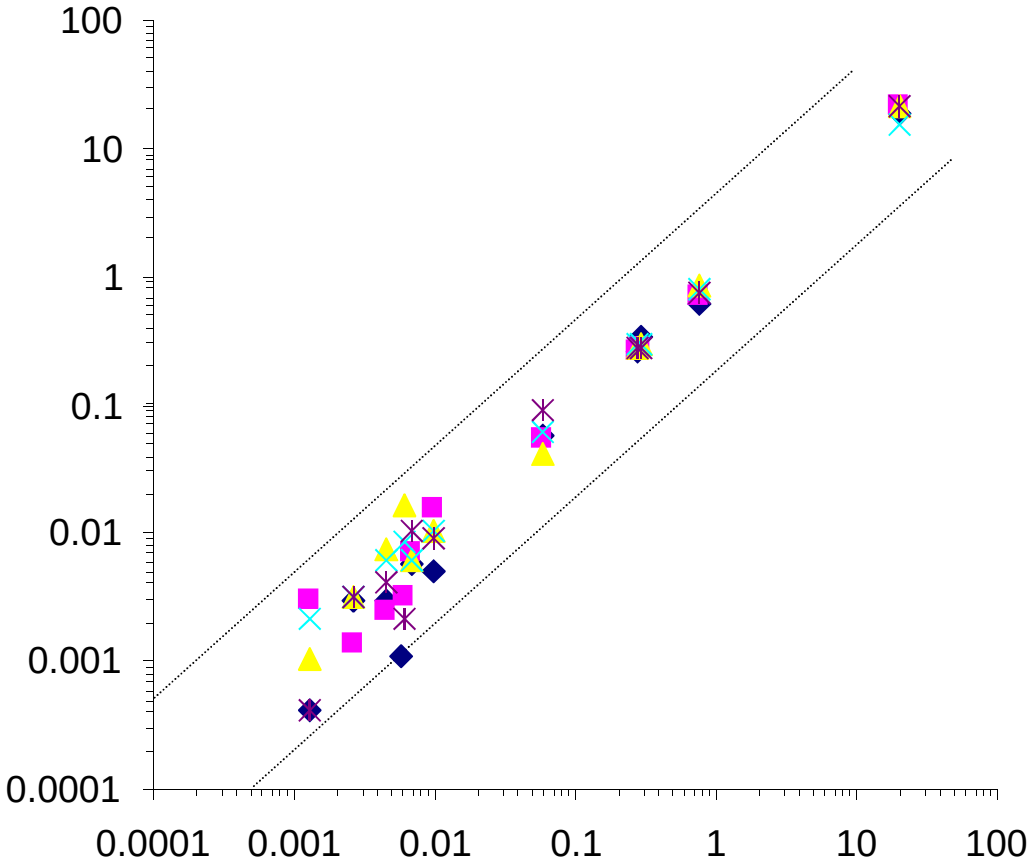
8-CLR harmonisation (2): analytical variation is <0.5log for samples with <0.01% (10e-4) CLL



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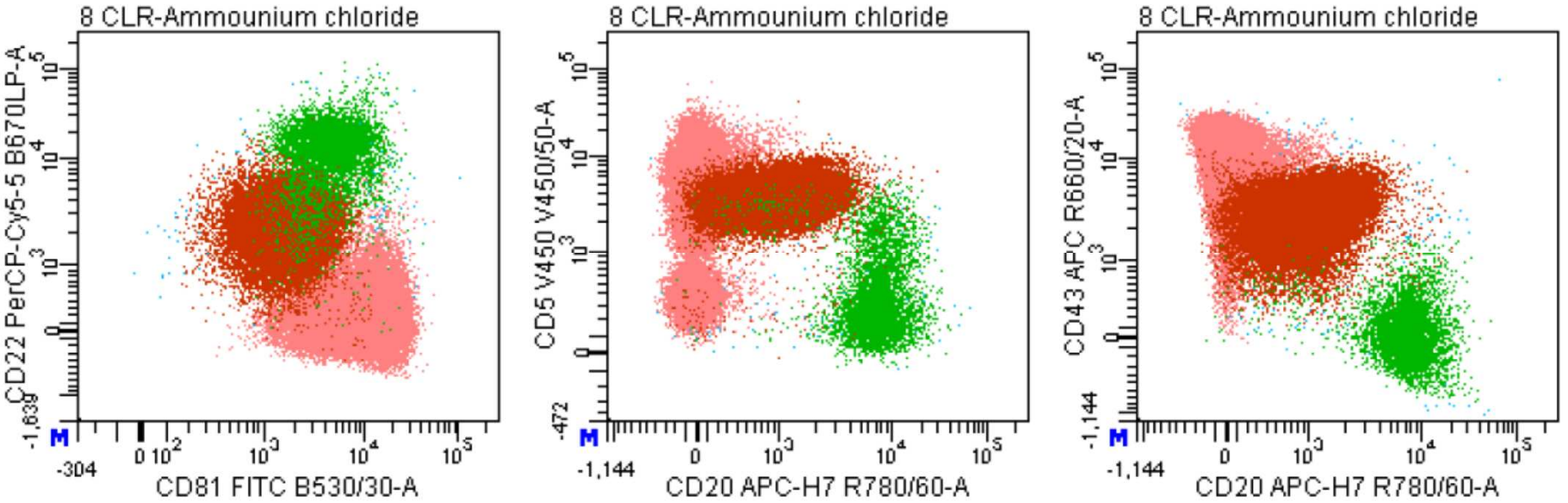
8-CLR harmonisation (3): internal data QC



- 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

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 have been in diagnostic use for decades
- Inter-laboratory errors well 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.

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Participants

Region	Institute	Contacts	Abs	vs 4CLR	vs PCR
EU	Amsterdam	Arnon Kater / Johan Dobber	??	??	??
EU	Barcelona Sant Pau	Carol Moreno / Josep Nomdedeu	Yes	Yes	Store
EU	Barcelona University	Neus Villamor / Julio Delgado	Yes	Yes	Store
EU	Barcelona Vall d'Hebron	Francesc Bosch / Carlos Palacio	Yes	Yes	Store
EU	Brno	Sarka Pospisilova / Michael Doubek	Yes	Yes	Yes
EU	Dublin	David O'Brien	Yes	Pseudo	Store
EU	Leeds	Andy Rawstron / Peter Hillmen	Yes	Yes	Yes
EU	Milano San Raffaele	Paolo Ghia / Claudia Fazi	Yes	Yes	Yes
EU	Paris AP-HOP	Remi Letestu / Florence Cymbalista	Yes	Pseudo	Store
EU	Salzburg	Alex Egle	Yes	Pseudo	Store
US	Genzyme	Henry Dong	??	??	??
US	Mayo	Curtis Hanson / Tait Shanafelt	Yes	??	Store
US	MD Anderson	Jeff Jorgensen / Bill Wierda	Yes	Yes	Store
US	NIH	Maryalice Stetler-Stevenson	Yes	Pseudo	Store
US	UCSD / Genoptix	Beth Broome / Laura Rassenti / Tom Kipps	Yes	Yes	Store
US	UPMC Pennsylvania	Fiona Craig	No	Analysis	No
NZ/AUS	Sydney	Stephen Mulligan	Yes	??	Store
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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