Update on the International Standardized Approach for Flow Cytometric Residual Disease Monitoring in Chronic Lymphocytic Leukaemia

Andy C. Rawstron
on behalf of ERIC consortium
MRD international harmonised approach 2007

- CD19/CD5/Kappa/Lambda
- CD19/CD5/CD3/CD45, with CD19+CD3+ control for limit of detection
  - B/T-cell doublets have characteristics similar to CLL, not so other contaminants
- CD19/CD5/CD20/CD38
- CD19/CD5/CD81/CD22
- CD19/CD5/CD43/CD79b
  - MRD markers chosen from 50 potential combinations for reproducibility of detection

CD19+CD3+ events => similar characteristics to CLL cells
Other “noise” is easy to exclude

International standardised approach for residual disease monitoring in CLL: Leukemia 2007, 21(5): 956-64
Design of the 6CLR panel: combinations, conjugates, contamination and cocktails

<table>
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<tr>
<th>Antibody</th>
<th>FITC</th>
<th>PE</th>
<th>PerC5.5</th>
<th>PE-Cy7</th>
<th>APC</th>
<th>APC-H7</th>
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<tbody>
<tr>
<td>CD3</td>
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<td>CD38</td>
<td>CD5</td>
<td>CD19</td>
<td>CD79b</td>
<td>CD20</td>
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<td>CD81</td>
<td>CD22</td>
<td>CD5</td>
<td>CD19</td>
<td>CD43</td>
<td>CD20</td>
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4-CLR and 6-CLR versions of the CLL MRD harmonised assay

- **Basic Clonality assessment:**
  - CD19/CD5/ Kappa/ Lambda

- **Calculate B-cells as a percentage of leucocytes, contamination control and determine limit of detection:**
  - CD19/CD5/ CD3/ CD45

- **Determine CLL cells as a percentage of B-cells**
  - CD19/CD5/ CD20/ CD38
  - CD19/CD5/ CD81/ CD22
  - CD19/CD5/ CD43/ CD79b

- **Basic clonality assessment, and calculate B-cells as a percentage of leucocytes:**
  - CD19/CD5/ Kappa/ Lambda/ CD20/ CD45

- **Determine CLL cells as a percentage of B-cells, contamination control and determine limit of detection**
  - CD19/CD5/ CD20/ CD3/ CD38/ CD79b
  - CD19/CD5/ CD20/ CD81/ CD22/ CD43
Can any useful information be derived from CD19/CD5/Kappa/Lambda?

- 784 cases after treatment from Barcelona, Kiel and Leeds
  - CD19/5/λ/κ
  - MRD assessment (4CLR flow, ASO-PCR)
CD19/5/κ/λ parameter thresholds to obtain 100% PPV for presence of MRD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Training set: optimal threshold for specificity vs. sensitivity</th>
<th>Training set: threshold for 100% positive predictive value</th>
<th>Validation set: positive predictive value*</th>
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<tbody>
<tr>
<td>CD19 % Leucs</td>
<td>&gt;5.5 (n=165)</td>
<td>&gt;8.9 (n=134)</td>
<td>99.2% (n=132)</td>
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<tr>
<td>CD19+ κ:λ ratio</td>
<td>&lt;0.5: 1 or &gt;8.2:1 (n=198)</td>
<td>&lt;0.04:1 or &gt;61:1 (n=91)</td>
<td>98.8% (n=85)</td>
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<tr>
<td>CD19 % CD5+</td>
<td>&gt;70% (n=185)</td>
<td>&gt;82% (n=160)</td>
<td>100% (n=144)</td>
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<tr>
<td>CD19+5+ κ:λ</td>
<td>&lt;0.6:1 or &gt;5.7:1 (n=218)</td>
<td>&lt;0.05:1 or &gt;32:1 (n=146)</td>
<td>100% (n=128)</td>
</tr>
<tr>
<td>CD19+5+ % sIg-</td>
<td>&gt;21% (n=123)</td>
<td>&gt;54% (n=43)</td>
<td>100% (n=40)</td>
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</tbody>
</table>

Rawstron et al Leukemia, accepted pending minor revisions
Comparison of 4-CLR and 6-CLR assays on samples from patients with CLL after treatment

- 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%

Rawstron et al Leukemia, accepted pending minor revisions
Different approaches to improve MRD monitoring

- RQ-ASO IGH-PCR requires patient-specific assay development
  - next generation sequencing is not yet cost-effective or tested in multi-centre setting
- Euroflow approach to standardise the assay and potentially automate analysis
  - Some laboratories require a custom approach
- 10-colour panels incorporate everything into a single tube
  - 10-colour clinical cytometers are not widely available
- Novel markers that allow MRD assessment with fewer markers
  - Not tested in multi-centre settings
- ERIC-initiated project
  - Harmonise 4 / 6 / 8 / (10) CLR assays to provide reproducible monitoring in a variety of laboratories that can be directly related to published clinical trials
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: aim to obtain 100 samples with at least 8-CLR and either 4/6-CLR or PCR, and as many as possible with data on all three approaches.

• **Identify inter-laboratory variation in analysis and evaluate a data analysis QC pilot:** aim to compare all the analyses with at least two other labs + central lab, and to compare the analysis by all laboratories in at least ten samples

• **Assess the potential of an internal data quality check on signal:noise and compensation.**
Internal QC

Correct
CD5- Non-CLL B-cell
vs. CD3+CD19- T-cells
Signal : Noise = 169
T-cell CD20 APC-H7 MFI = 27

Incorrect
CD5- Non-CLL B-cell
vs. CD3+CD19- T-cells
Signal : Noise = 3.9
T-cell CD20 APC-H7 MFI = 1220
ERIC international collaboration with BD Biosciences

- Antibody panel: all the 4/6-CLR reagents in one tube, less CD38 (used to discriminate progenitors from CLL cells and not required when CD81/CD43 present)
- With BD: tested fluorochrome conjugates and custom reagents which were already CE/IVD or could be fast-tracked
- Collaborative agreement with no financial component between BD and ERIC: BD provides reagents in return for access to 8-CLR FCS files

$35K reagents for 50 tests at 12 centres => 600 million cells
## Participants

<table>
<thead>
<tr>
<th>Region</th>
<th>Institute</th>
<th>Contacts</th>
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<tr>
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<td>Jingy Chen / Noel Warner</td>
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ERIC international 8CLR data

- Optimised reagents for whole blood or separated leucocytes (1-3 million cells in 0.1-0.3mL)
- Prepared 4 dilution series to provide example files and for assessing intra-laboratory variation in analysis
- Distributed antibodies to initial group of participating laboratories
- Developed single page gating strategy and protocol in Leeds/Milan, currently under review with other participants

CD5 (L17F12) V450 5µL
CD81 (JS-81) FITC 20µL
CD22 (SHCL1) PerCP-Cy5.5 5µL
CD43 (1G10) APC 5µL

CD3 (SK7) V500-C 5µL
CD79b (SN8) PE 20µL
CD19 (SJ25C1) PE-Cy7 5µL
CD20 (L27) APC-H7 5µL
Example of a case with 0.0025% CLL
Example of a case with 0.0025% CLL
Actions required and timelines

- Participants: please review SOP and undertake cytometer set-up and acquisition of cases
- Participants: attempt analysis of example files and review SOP so that it can be shared with a wider audience
- ACR/PG: prepare ethics / IRB submission
  - No problem to acquire data, and also to share data if suitable local approvals in place
- ACR/PG/MD-SP: RQ-ASO IGHV-PCR analysis
- ACR: prepare samples with varying [Ab] to identify optimal signal:noise for component reagents
- Aim to have all the data acquired before ASH
- ? Comment on NGS approach
High-throughput VDJ sequencing for quantification of minimal residual disease in chronic lymphocytic leukemia and immune reconstitution assessment

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![Graphs A and B](image-url)
Flow cytometry issues:

Median 500K events (150-500K)

CLL phenotype
CD19+CD45+CD3-CD56-CD14-CD5+CD23+
Acknowledgements

Many thanks to Frans Nauwelaers, Lucia Testolin, Jingyi Chen and Noel Warner. BD Biosciences provided custom conjugates and cocktails for parallel testing.

Thanks to Seb Bottcher, Remi Letestu and Peter Hillmen for slides,