MRD in CLL

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Improving Outcomes in CLL

Adapted from Montserrat et al. Blood 2005;106:2226
The value of response assessment in CLL

- Currently high CR rate (>70%) and long survival (>5yrs)
- Many new agents but the pace of demonstrating clinical benefit has slowed

Hallek et al Lancet 2010; 376: 1164–74
Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial

The Lancet Volume 376, Issue 9747 2010 1164 - 1174
http://dx.doi.org/10.1016/S0140-6736(10)61381-5
### CLL trials assessing MRD as a predictor of survival

<table>
<thead>
<tr>
<th>Reference</th>
<th>Treatment</th>
<th>Initial Status</th>
<th>Response Rate</th>
<th>Complete Remission</th>
<th>MRD Method</th>
<th>MRD Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robak et al. [8]</td>
<td>Cladribine + C</td>
<td>Untreated</td>
<td>82</td>
<td>75</td>
<td>CD19/CD5</td>
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<tr>
<td>Robertson et al. [4]</td>
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<td>159</td>
<td>81</td>
<td>CD19/CD5</td>
<td>&gt;30 vs 19‡</td>
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<tr>
<td>Keating et al. [2]</td>
<td>FCR</td>
<td>Untreated</td>
<td>224</td>
<td>78</td>
<td>CD19/CD5</td>
<td>&gt;48 vs 39‡</td>
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<tr>
<td>Montillo et al. [9]</td>
<td>F/F→A</td>
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<td>34</td>
<td>56</td>
<td>IgH PCR</td>
<td>NA</td>
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<tr>
<td>Del Pesta et al. [10]</td>
<td>F→R</td>
<td>Untreated</td>
<td>60</td>
<td>93</td>
<td>3-color flow</td>
<td>&gt;30 vs 24</td>
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<tr>
<td>Bosch et al. [5]</td>
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<td>60</td>
<td>50</td>
<td>4-color PCR</td>
<td>21 vs 17</td>
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<tr>
<td>Moreton et al. [6*]</td>
<td>A†</td>
<td>Relapsed/refractory</td>
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<td>54</td>
<td>4-color flow</td>
<td>&gt;36 vs 20</td>
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<td>O’Brien et al. [12]</td>
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<td>41</td>
<td>36</td>
<td>ASO-PCR</td>
<td>&gt;24 vs 15</td>
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<td>36</td>
<td>31</td>
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<td>Wierda et al. [3]</td>
<td>FCR</td>
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<td>25</td>
<td>ASO-PCR</td>
<td>44 vs 27</td>
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<td>Milligan et al. [14]</td>
<td>F*→auto</td>
<td>Untreated</td>
<td>115</td>
<td>42</td>
<td>IgH PCR</td>
<td>&gt;33 vs 38</td>
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<tr>
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<td>27</td>
<td>85</td>
<td>4-color flow</td>
<td>73 vs 16</td>
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<td>4-color flow</td>
<td>75 vs 16</td>
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<td>Relapsed/refractory</td>
<td>27</td>
<td>85</td>
<td>ASO-PCR</td>
<td>&gt;60 vs 19</td>
</tr>
<tr>
<td>Sorror et al. [18]</td>
<td>Allo</td>
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<td>64</td>
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<td>IgH PCR</td>
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<tr>
<td>Caballero et al. [19]</td>
<td>Allo</td>
<td>Relapsed/refractory</td>
<td>30</td>
<td>87</td>
<td>4-color flow</td>
<td>NA</td>
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</tbody>
</table>
MRD analysis improves prediction of progression-free survival

- >20 trials with some form of MRD analysis
- All show improved PFS for MRD$^{\text{NEG}}$ response
- Approximately 1-2 years improvement in PFS for CR$^{\text{NEG}}$ vs CR$^{\text{POS}}$
- Wide variety of assays (consensus PCR, ASO-PCR, flow clonality, CD19/CD5, disease-specific MRD flow)
DCLLSG CLL FC vs. FCR: MRD level predicts outcome independent of treatment arm

Böttcher, JCO 2012
Minimal Residual Disease Threshold in CLL – iwCLL Guidelines 2008

• Both 4-colour flow cytometry (MRD flow) and allele-specific oligonucleotide PCR are 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 fewer than one CLL cell per 10,000 leukocytes

• The blood can generally be used to make this assessment except within 3 months of completing therapy, particularly for patients treated with alemtuzumab, rituximab, and other antibodies targeting CLL

iwCLL, International Workshop on CLL; PCR, polymerase chain reaction.

Assuming Exponential Growth at the MRD Level → Linear Increase in PFS per Log Tumour Depletion

CR, complete remission; PR, partial remission.
Kinetics of Relapse: Exponential Growth from the Lowest Detectable MRD Level

Serial MRD measurements in a cohort of 32 MRD+ patients in clinical remission with no absolute lymphocytosis after treatment [predominantly FCR] at Leeds.

Total 68 patients monitored, 31 persistent MRD <0.01%, 5 insufficient MRD+ timepoints.
Serial MRD measurements in a cohort of 32 MRD+ patients in clinical remission with no absolute lymphocytosis after treatment [predominantly FCR] at Leeds. Total 68 patients monitored, 31 persistent MRD <0.01%, 5 insufficient MRD+ timepoints.
ADMIRE/ARCTIC Trial (FCR-Based Treatment): Sequential Benefit in PFS per Log Reduction in MRD

**Progression-free Survival**
by bone marrow MRD level at 3 months post treatment

![Graph showing progression-free survival by bone marrow MRD level at 3 months post treatment.](image)

- Log-Rank $\chi^2_4 = 224.125$
- $p<0.0001$

33% (95% CI = 27–38) risk reduction for disease progression per log reduction in MRD level

FCR, fludarabine, cyclophosphamide, rituximab.

MRD in CLL: use as an endpoint in clinical trials

- There are a number of new approaches to treatment but determining the optimal approach will take many years for randomised controlled trials to evaluate progression-free survival (PFS) or overall survival (OS) and using MRD (at 3 months post-treatment) as a trial endpoint could greatly speed the process.


www.fda.gov/Drugs/NewsEvents/ucm340707.htm
MRD Analysis Requires a Quantitative Method That Is Not Influenced by the Polyclonal Background with Prospective Validation

- **Flow cytometry:**
  Multiparameter assessment of CLL phenotype that is not clonality based

- **PCR:** Real-time quantitative ASO-PCR using patient-specific primers, not consensus primers

- **High-throughput sequencing:**
  Amplification of all B-cell sequences and enumeration of CLL-specific immunoglobulin gene

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**Development of ‘MRD’ as a regulatory endpoint:**

1995
- Identify MRD endpoint in clinical trials

2002
- Develop assay

2007
- Standardisation of assay

2012
- Apply standardised assay prospectively

- Apply to regulatory action
Guidelines on the validation of cell-based assays

- **Sensitivity**
  - Either: “lowest signal detectable above background”
  - Or: “true positive / true positive + false negative”

- **Limit of Blank (LOB)** = highest signal in the absence of measurand, calculated as mean (blank) + 1.645 SD (95% of negative values are below this limit)

- **Limit of Detection (LOD)** = level at which 95% of samples with low level of measurand are detected above the limit of blank, calculated as LOB + 1.645 SD

- **Limit of Quantitation (LoQ)** = lowest level of measurand that can be reliably detected and whose total error (bias + Imprecision) meets a desired criterion for accuracy (clinical utility)

Clinical and Laboratory Standards Institute (CLSI) has published the guideline EP17, Protocols for Determination of Limits of Detection and Limits of Quantitation.

UKAS ISO15189 guidelines
Clinically appropriate level of variation:
When BCR-ABL RQ-PCR accepted as a trial end-point, 95% limit of agreement = ± 5-fold

CML: best 95% LOA +/- 2-fold using synthetic standards
CLL: Target: ±3-fold (0.5log), preferably ±2-fold (0.3 log)
Flow Cytometry MRD Detection

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tubes</th>
<th>Detection limit</th>
<th>Cells required for LoD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (4-colour)</td>
<td>4</td>
<td>0.005%</td>
<td>4–20 million</td>
</tr>
<tr>
<td>8 (6-colour)</td>
<td>2</td>
<td>0.001%</td>
<td>2–10 million</td>
</tr>
<tr>
<td>10 (8-colour)</td>
<td>1</td>
<td>0.001%</td>
<td>1–5 million</td>
</tr>
</tbody>
</table>

Mary Sartor Sydney → 10-colour flow assay
Much better reagents became available
Requests to include: CD45, CD38, CD160, CD200, CD305, ROR1

LoD, limit of detection.

### Antigen Typical expression (% positive vs control) Control population in normal peripheral blood Minimum relative fluorescence intensity (preferred)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Typical expression (% positive vs control)</th>
<th>Control population in normal peripheral blood</th>
<th>Minimum relative fluorescence intensity (preferred)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD5</td>
<td>Positive (&gt;20%)</td>
<td>CD3+ T-cells</td>
<td>CD19+ B-cells</td>
</tr>
<tr>
<td>CD20</td>
<td>Weak</td>
<td>CD19+ B-cells</td>
<td>CD3+ T-cells</td>
</tr>
<tr>
<td>CD43</td>
<td>Positive (&gt;20%)</td>
<td>CD3+ T-cells</td>
<td>CD20+ B-cells</td>
</tr>
<tr>
<td>CD79b</td>
<td>Weak</td>
<td>CD20+ B-cells</td>
<td>CD3+ T-cells</td>
</tr>
<tr>
<td>CD81</td>
<td>Weak</td>
<td>CD3+ T-cells</td>
<td>Granulocytes</td>
</tr>
</tbody>
</table>

Weak indicates ≤20% reduction in fluorescence intensity relative to the median expression observed with a reference population of polyclonal B-cells using the same antibody. The minimum relative fluorescence intensity would provide separation of CLL cells from normal B-cells in >95% of cases with a preferred relative fluorescence intensity being the level at which 99% of cases have optimal separation of CLL cells from normal B-cells.

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**Requires ≥6 markers to achieve 0.01% – available to most labs**

**The core panel must meet these 6 specifications, but is flexible thereafter**

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**ERIC**

European Research Initiative on CLL

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Examples of MRD analysis in patients treated with non-FCR regimens

PB, peripheral blood.
* Following treatment cessation.
Progression-Free Survival by Bone Marrow MRD Status and iwCLL Response with FCR-Type Therapy

- Requires ≥6 markers to achieve 0.01% – available to most labs
- The core panel must meet these 6 specifications, but is flexible thereafter
- Backwards-compatible and applicable to current treatments
- Validated prospectively in FCR-based treatment

9 MRD-negative PR: 5 splenomegaly, 3 nodes >1.5 cm, none with BM or >1 site involved

Rawstron AC, et al Haematologica 2015; 100 (S1):Abstract S794.
Flow Cytometry and qPCR

- Patient-specific ASO IgH gene PCR
- (Quasi) Quantitative analysis
  - Calibrate to standard curve generated by serial dilution of pre-treatment material into normal DNA

Previous more sensitive than flow cytometry ($10^{-5}$–$10^{-6}$); good correlation to $10^{-4}$

The assay has to be validated for each patient (not because of the primer design requirement)

ERIC harmonised approach

ASO, allele-specific oligonucleotide;
IgH, immunoglobulin heavy;
qPCR, quantitative polymerase chain reaction;
RQ, real-time, quantitative.

The sensitivity of an RQ-PCR assay is dependent on several factors, including the type of rearrangement, the size of the junctional region and the amount of DNA in each reaction.

The standard curve, including the lowest dilution of the ‘quantitative range’ and all previous dilutions:
- must include at least three dilution points
- must have a minimum range of two logs
- must have a slope between -3.1 and -3.9; and
- must have a correlation coefficient \( \geq 0.98 \).

Vincent van der Velden et al, Leukemia (2007) 21, 604-611
The sensitivity of an RQ-PCR assay is dependent on several factors, including the type of rearrangement, the size of the junctional region and the amount of DNA in each reaction. The sensitivity is the lowest dilution that meets all the following criteria:

- specific amplification, as determined by the shape of the amplification curve
- ≥ one positive replicate
- lowest CT value ≥ 1.0 lower than the lowest CT value of the background
- lowest CT value <20 cycles from the undiluted sample or, if this undiluted sample is not included in the standard curve, from the intercept of the standard curve (representing the $10^0$ dilution).

Vincent van der Velden et al, Leukemia (2007) 21, 604-611
Sensitivity → Limit of detection
Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data

- The sensitivity of an RQ-PCR assay is dependent on several factors, including the type of rearrangement, the size of the junctional region and the amount of DNA in each reaction.

- Quantitative range
  - Specific amplification (shape of amplification curve)
  - Reproducible amplification (replicate ΔC_T <1.5)
  - All C_T values >3.0 lower than the lowest C_T value of the background
  - Mean C_T value must be within a defined range from the mean C_T value of the previous dilution point:
    - 2.6–4.0 C_T between 10-fold dilutions (e.g. 10^-3 to 10^-4)
    - 0.5–1.5 C_T between two-fold dilutions (e.g. 10^-3 to 5x10^-4)

Vincent van der Velden et al, Leukemia (2007) 21, 604-611
Quantitative range → Limit of quantitation
Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data

- RQ-PCR data should be corrected for the amount and ‘amplifiability’ of the DNA of the diagnosis and the follow-up samples by analyzing a control gene in parallel to the Ig or TCR gene target.

- Quantitation is performed:
  - using the standard curve, excluding dilutions outside the ‘quantitative range’, of the involved Ig/TCR target;
  - using the mean CT of the triplicates of the follow-up sample and
  - correcting the MRD level according to the DNA quality/quantity of the diagnostic sample and the follow-up sample as determined by RQ-PCR of the control gene.

Vincent van der Velden et al, Leukemia (2007) 21, 604-611
High-Throughput Sequencing with Universal Reagents (IGH-VDJ, IGH-DJ, IGK) Acceptable Linearity to 1 in 10^{-6}

Case 1

Case 2

Case 3

Sequence 1 (productive)

Sequence 2 (non-productive)

Average of sequences 1 and 2

CLL % Leukocytes Using Adaptive ClonoSEQ

Expected CLL % Leukocytes

HTS: likely to predict very good outcome for MRD <10^{-6}, reproducible but can have >1log difference from RQ-PCR

Disease-Free Survival (%)

Months after Transplant

0 12 24 36 48 60 72 84 96

MRD <10^{-6}

MRD ≥10^{-6}

p=0.0002

r=0.64
p<0.0001

IGH-HTS CLL Cells per 10^6 Genomes

p=0.0002

r=0.64
p<0.0001

0 10^0 10^1 10^2 10^3 10^4 10^5 10^6

0 10^0 10^1 10^2 10^3 10^4 10^5 10^6

CAP accredited & CLIA approved

Issues to be resolved for quantitative HTS using universal primers

Correction factors
• Calculations based on addition of reference DNA
• Estimation of total leucocytes,
• Amplification bias → probably requires calculation of quantitative range per patient as RQ-PCR
• Replicate amplicons and non-functional rearrangements

False positive results
• ERIC: no “CLL-associated” sequences present in an unrelated sample at the level of 0.010% or greater,
• Four CLL-associated sequences were detected in one or more unrelated cases, representing a median 0.00080% (range 0.00046-0.0019%)

Prospective Validation

Selection of techniques for measuring MRD as a clinical trial endpoint

- Requires a quantitative method that is not influenced by the polyclonal B-cell background
- PFS/OS benefit is detectable with a 1-2 log depletion
  → MRD assay is ideally quantitative to ±0.3 log
- Prospective validation
  - multi-parameter cytometry and RQ-PCR
- Validated assays require “limit of detection” for each result stated on the report
  - Cellular assays: number of events acquired
  - Molecular assays: total DNA, patient-specific IGH effects
- Combination of both ≥6-CLR flow cytometry and RQ-PCR/HTS
BCR-pathway inhibition: different pattern of disease depletion

MRD suitable as an endpoint for therapies aimed at disease eradication, not disease control: evaluate at response assessment after treatment
Monitoring CML response to Imatinib: the white cell count is not informative
Monitoring CML response to Imatinib: inhibitor dependent on molecular response

CLL: Identifying combinations which achieve <0.01% MRD in the majority of patients
Measuring the kinetics of response to BCR-pathway inhibitors

IcIICLLe: https://www.clinicaltrialsregister.eu/ctr-search/trial/2012-003608-11/GB
Measuring the kinetics of response to BCR-pathway inhibitors

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IcICLLe: https://www.clinicaltrialsregister.eu/ctr-search/trial/2012-003608-11/GB
Measuring the kinetics of response to BCR-pathway inhibitors

Optimal response assessment requires MRD analysis (or at least high-sensitivity B-cell enumeration)

?? Similar to CML ??

IclICLLe: https://www.clinicaltrialsregister.eu/ctr-search/trial/2012-003608-11/GB
Guiding Treatment

- **GALACTIC:** GA\textsuperscript{101} (obinutuzumab) monoclonal Antibody as Consolidation Therapy In CLL
  - Phase 2/3, multicentre, RCT testing obinutuzumab versus no consolidation in CLL patients with >0.01% MRD at 3–12 months after 1st–3rd-line therapy

- Stopping treatment: 6 months MRD <0.01% in PB (3 assessments), confirmed in BM → end treatment
  - FLAIR: Ibrutinib + rituximab versus FCR (n=754)
  - IcICLLe: Ibrutinib monotherapy (n=40)
  - CALiBRe: Idelalisib monotherapy (n=40)
  - IcICLLe extension: Ibrutinib + obinutuzumab (n=40)
  - CLARITY: Ibrutinib + venetoclax (n=50)

GALACTIC: https://www.clinicaltrialsregister.eu/ctr-search/trial/2014-000880-42/GB (accessed May 2016);
FLAIR: https://www.clinicaltrialsregister.eu/ctr-search/trial/2013-001944-76/GB (accessed May 2016);
IcICLLe & extension: https://www.clinicaltrialsregister.eu/ctr-search/trial/2012-003608-11/GB (accessed May 2016);
Compartment effect

<table>
<thead>
<tr>
<th>Trial</th>
<th>Treatment</th>
<th>n</th>
<th>MRD assessment</th>
<th>Paired PB &amp; BM samples (n)</th>
<th>N° with MRD BM &gt;0.01% &amp; PB &lt;0.01% (%)</th>
<th>N° with &gt;2 log difference between PB &amp; BM (%)</th>
<th>Median log difference* (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLL 202 CAMFlud, CLL 207</td>
<td>Alemtuzumab +/- Fludarabine</td>
<td>97</td>
<td>End of treatment</td>
<td>95</td>
<td>16 (16.8%)</td>
<td>5 (5.2%)</td>
<td>1.7 (-1.3 to 3.3)</td>
</tr>
<tr>
<td>CLL 201, MO20927, ADMIRE + ARCTIC</td>
<td>Rituximab + FC or FCM</td>
<td>567</td>
<td>3M post end of treatment</td>
<td>333</td>
<td>68 (20.4%)</td>
<td>3 (0.9%)</td>
<td>0.69 (-1.3 to 2.4)</td>
</tr>
<tr>
<td>CALiBRe, IcICLLe</td>
<td>Idelalisib, Ibrutinib</td>
<td>60</td>
<td>1M &amp; 6M</td>
<td>112</td>
<td>0 (0%)</td>
<td>-</td>
<td>0.0 (-0.72 to 1.1)</td>
</tr>
</tbody>
</table>

- Discrepant peripheral blood and bone marrow in all chemoimmunotherapy trials
  - Discrepancy frequency varies from 7% to 57% of samples per trial

The Outcome for Patients with MRD Detectable in BM but Not in PB (PB - / BM +) Differs According to Type of Therapy

FCR-based therapy
~3 yrs median follow-up

PFS for PB-BM+ PFS similar to PB-BM- in the short term

Alemtuzumab therapy

Log-Rank $\chi^2 = 17.1020$
p=0.0002

PFS for PB-BM+ similar to PB+BM+

The Outcome for Patients with MRD Detectable in BM but Not in PB (PB - / BM +) Differs According to Type of Therapy

<table>
<thead>
<tr>
<th>Months after randomisation</th>
<th>PB MRD ≥0.01%</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M post-treatment MRD</td>
<td>12 18 24</td>
</tr>
<tr>
<td>BM &lt;0.01%</td>
<td>2.0% 6.0% 9.7%</td>
</tr>
<tr>
<td>PB &lt;0.01%</td>
<td>3/148 8/133 12/124</td>
</tr>
<tr>
<td>BM &gt;0.01%</td>
<td>33% 62% 81%</td>
</tr>
<tr>
<td>PB &lt;0.01%</td>
<td>13/39 18/29 17/21</td>
</tr>
</tbody>
</table>

PFS for PB-BM+ PFS similar to PB-BM- in the short term

FCR-based therapy ~3 yrs median follow-up

Log-Rank $\chi^2 = 112.565$

Rawstron AC, et al. Haematolgica 2015; 100(S1):Abstract S794 (oral presentation)
Data updated June 2016.
The Outcome for Patients with MRD Detectable in BM but Not in PB (PB - / BM +) Differs According to Type of Therapy

FCR-based therapy
~3 yrs median follow-up

- Log-Rank $\chi^2 = 112.565$

- Proportion Alive and Progression Free

- Months from Randomisation

- PFS for PB-BM+ PFS similar to PB-BM- in the short term

FCR-based therapy
4 yrs median follow-up

- Log-Rank $\chi^2 = 153.742$
p<0.0001

- Proportion Alive and Progression Free

- Months from Randomisation

Rawstron AC, et al. Haematologica 2015; 100(S1):Abstract S794 (oral presentation);
Data updated June 2016.
Compartment effect

- PB MRD <0.01%: patient may have extensive disease during or shortly after antibody therapy
- It is possible to assess the compartment effect per treatment
  - Alemtuzumab: 2-log higher MRD levels in BM than in PB
  - Rituximab: 0.7-log higher MRD levels in BM than in PB
  - Ibrutinib/Idelalisib: no compartment effect
  - Ibrutinib+Obinutuzumab: 0.3-log higher MRD levels in BM > PB
- PB is acceptable for monitoring (during and after therapy) but BM may be required if there is a significant compartment effect
- PB >1% → BM does not provide additional information

MRD in CLL

• Know the limit of detection (background) and limit of quantitation (sensitivity)
  • FLOW: LoD 20 events, LoQ 50 events. Total events 2 million \( \rightarrow \) LoD \( 10^{-5} \)
  • PCR/HTS: 500ng \( \rightarrow 10^5 \) cells

• Factors that may impact individual assay results
  • FLOW: Non-typical phenotype or treatment-associated phenotypic shift/drift
  • PCR/HTS: Amplification bias

• Compartment effect: hemodilute samples
  • FLOW: assess BM-restricted cell populations within the assay
  • PCR/HTS: flow/morphology on paired sample
## Acknowledgements

<table>
<thead>
<tr>
<th>Name and Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johan Dobber, Arnon Kater</td>
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<tr>
<td>Remi Letestu, Florence Cymbalista</td>
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<tr>
<td>Martin Spacek</td>
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<td>Neus Villamor, Julio Delgado</td>
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<td>Josep Nomdedeu, Carol Moreno</td>
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<td>Claudia Fazi, Paolo Ghia</td>
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<td>Andy C. Rawstron, Ruth M. de Tute, Peter Hillmen</td>
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<td>Beth Broome, Thomas J. Kipps</td>
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<td>Frans Nauwelaers, Lucia Testolin, Jingyi Chen and Noel Warner. BD Biosciences provided custom and commercial conjugates for testing</td>
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<td>Michael Wenger for meeting support</td>
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HTS vs. Sanger: Andreas Agathangelidis
Dilution studies: Claudia Fazi

Ruth de Tute, ANDREW JACK and PETER HILLMEN

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Comparison of flow vs. next generation sequencing
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I only want MRD on 700 patients at 20 time points...

HMDS, Leeds

Talha Munir
Ruth de Tute
Cathy Burton

NAP

Abbvie
Napp

Abbvie
Pharmacyclics
Roche

University of Leeds

The Royal Liverpool and Broadgreen University Hospitals

Bloodwise
Flow cytometry for MRD detection
Flow cytometry for MRD detection
Key gating points for CLL MRD detection
Start with “CLL regions” in the centre of the normal B-cell population
Adjust regions: CLL CD81/CD79b/CD20 lower than median of normal B-cells
Adjust regions: CLL CD81/CD79b/CD20 lower than median of normal B-cells
Progenitors and plasmablasts have strong CD43/CD81 and no CD5
Contaminating T-cells have strong CD43/CD81 and (usually) no CD20
Contaminating T-cells usually removed by adjusting B-cell gate
Contaminating T-cells usually removed by adjusting B-cell gate
CLL cells have similar CD19 expression to normal B-cells
Exclude events that are not fully resolved from normal B-cells by ≥1 parameter

Not CLL
Exclude events that are not fully resolved from normal B-cells by ≥1 parameter
Exclude events that are not fully resolved from normal B-cells by ≥1 parameter

CLL
Be very cautious around the limit of detection

CLL events n=51
Be very cautious around the limit of detection

Not CLL events