CORRELATION BETWEEN A 10-COLOUR FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE (MRD) ANALYSIS AND MOLECULAR MRD IN ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL)

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Background: MRD monitoring in ALL is a strong predictive factor and a stratification tool for treatment intensification. The currently accepted standard of molecular monitoring with either immunoglobin heavy or kappa chain (IG) or T-cell receptor (TCR) quantitative PCR (qPCR) in Philadelphia negative (Ph-) ALL offers high sensitivity, but accessibility is limited by expertise, cost and turnaround time. Flow cytometric assays are increasingly utilised and improved sensitivity is seen with multi-parameter flow cytometry at 8 or more colours.

Methods: We developed a 10-colour single tube flow cytometry assay (Figure 1). Samples were subject to bulk ammonium chloride lysis to maximise cell yields with a target of 1 x 10⁶ events. Once normal maturation patterns were established, patient samples were analysed in parallel to standard molecular monitoring with either IG/TCR qPCR in Ph- disease or BCR-ABL qRT-PCR in Ph+ disease. Statistical analysis was performed in Graphpad Prism v7.0.

Results: Flow cytometry was performed on 47 samples from 16 patients. 13 samples were at diagnosis or morphologic relapse. An informative immunophenotype was identifiable in all patients; however a molecular assay could not be developed in one patient.

38 samples were tested for MRD by flow cytometry (Figure 2). In 2 samples, flow cytometric MRD was detected despite blinatumomab (anti-CD19) therapy.

27 samples were tested concurrently for MRD by both molecular and flow cytometric methods (Figure 3A and 3B). There was a strong correlation between molecular and flow cytometric MRD (R²=0.909, p<0.001; Figure 3B). Correlation was strong with both IG/TCR-based (n=16; R²=0.955, p<0.001) and BCR-ABL-based (n=11; R²=0.957, p<0.001) assays. The cost was significantly lower than IG/TCR qPCR (eg. Cost for four time-points per patient approximately $1200 vs $3700).

Conclusion: Our 10-colour flow cytometric MRD assay attained sensitivity of ≤0.01% in 87% of samples, and correlated strongly with molecular MRD. By including CD22 in our panel, we demonstrated that we were also able to detect MRD in patients who had been treated with anti-CD19 therapies such as blinatumomab. This technique offers rapid and affordable testing in B-ALL patients, including cases where a suitable molecular assay cannot be developed.

Figure 1: 10-colour antibody panel

These markers were selected to provide at least two targets for identification of B-lineage cells (including after anti-CD19 therapy such as blinatumomab) and to include the most frequently aberrant markers in precursor B-lineage ALL.

Figure 2: Sensitivity of flow cytometry for MRD detection

<table>
<thead>
<tr>
<th>Lower limit of detection</th>
<th>Median 0.0064%</th>
<th>Range 0.005% to 0.028%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower limit of quantification</td>
<td>Median 0.017%</td>
<td>Range 0.002% to 0.07%</td>
</tr>
</tbody>
</table>

Sensitivity ≤0.01% attained in 33 of 38 samples (87%)

* Sensitivity was reduced in remaining samples due to low event detection.

Figure 3A: Qualitative correlation between flow cytometric and molecular MRD

<table>
<thead>
<tr>
<th>Molecular</th>
<th>Flow cytometry</th>
<th>MRD</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>14</td>
<td>2*</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>13</td>
<td>27</td>
<td></td>
<td></td>
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</tbody>
</table>

*In both samples where MRD was detected only by molecular methods, the quantification was low (unquantifiable level in one, and amplification of only one of three replicates in the second).

Figure 3B: Quantitative correlation between flow cytometric and molecular MRD

R² = 0.909