Basophil Activation Test: Validation of a Flow Cytometry-Based Ex Vivo Pharmacodynamic Marker

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Introduction

Bruton tyrosine kinase (Btk) activity inhibitors such as PRN1008 and Dasatinib have potent immunosuppressive and anti-inflammatory effects. These properties make them ideal candidates for the therapies of immunologic and inflammatory disorders. Activation of basophils by cross-linking of IgE and FcεRI is Btk-dependent. Thus, ex vivo basophil activation is a useful PD marker for the dose-dependent mechanism of action of Btk inhibitors. However, validation and implementation of a basophil ex vivo activation assay present tremendous challenges due to low basophil counts and the limited target cell stability in the peripheral blood. A flow cytometry-based ex vivo basophils activation assay was validated for its application as a PD biomarker in clinical trials. Basophils were identified in whole blood samples by the co-expression of HLA-DR and CD123 and the up-regulation of a basophil activation marker, CD63, was assessed after stimulation with polyclonal anti-IgE. The fMLP peptide served as positive control for the CD63 up-regulation. Assay performance for total and activated basophils achieved a precision of <12 %CV. The lower limits of quantitation (LLOQ) for the total basophils and Activated Basophils were 0.006% and 5%. Sample stability for all the reportables was established up to 48 hours when maintained at 2-8°C.

Flow Cytometry Panel Configuration and Reportables

Basophil Activation Panel Configuration

<table>
<thead>
<tr>
<th>Detector</th>
<th>Pre-incubation</th>
<th>FL1</th>
<th>FL2</th>
<th>FL3</th>
<th>FL4</th>
<th>FL5</th>
<th>FL6</th>
<th>FL7</th>
<th>FL8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tube 1 (Negative Control)</td>
<td>Wash Buffer/Reagent-A</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CD63</td>
<td>CD123, HLA-DR</td>
</tr>
<tr>
<td>Tube 2 (Positive Control)</td>
<td>fMLP peptide/Reagent E</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CD63</td>
<td>CD123, HLA-DR</td>
</tr>
<tr>
<td>Tube 3 (anti-IgE)</td>
<td>Anti-IgE</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>CD63</td>
<td>CD123, HLA-DR</td>
</tr>
</tbody>
</table>

Reportable Result

- Total Basophils: CD123+, HLA-DR
- Activated Basophils: CD123+, HLA-DR, CD63

Results: Key Assay Performance Parameters

**Intra-Assay Precision:** Four whole blood samples assayed in triplicate. For each sample and each reportable result, the mean, SD, and %CV was calculated. For each reportable result, the Grand Mean %CV was calculated across all samples.

**Inter-Assay Precision:** Four whole blood samples and 2 QC samples (CD-Chex Plus Low and CD-Chex Plus) were assayed in triplicate. Each sample was analyzed in two analytical runs on each two instruments by two technologists for a total of four analytical runs. For each sample and each reportable result, the mean of the replicates was calculated for each analytical run. The Grand Mean (mean of daily means), SD (of daily means) and %CV (of all four runs) was calculated.

Sample Stability: Six samples were assayed as single determinations on the day of collection (baseline) and thereafter, at 24, 26, 32 and 48 hours. The percent change from baseline and the %CV between time points was calculated.

Summary

- The Basophil Activation Panel-2 employs the BD FastImmune™ antibody cocktail comprising of CD123, HLA-DR, and CD63.
- This assay utilizes CD63 positivity on CD123+, HLA-DR basophil population for the measurement of basophil activation.
- The validation was performed as per the guidelines recommended by the ICSH (International Council for Standardization in Hematology) and ICCS (International Clinical Cytometry Society).

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