Background and Study Rationale

Inflammation and immune activation play a central role in HIV pathogenesis. An exuberant inflammatory response, termed Immune Reconstitution Inflammatory Syndrome (IRIS), occurs in a subset of patients shortly after starting antiretroviral therapy (ART), and is more common in those with severe lymphopenia and undergoing opportunistic infections. If the IRIS response is due to a previously undiagnosed pathogen, it is termed unmasking IRIS. Exacerbation of symptoms from a previously known or treated disease is called paradoxical IRIS. The pathogenesis and etiology of IRIS remain unclear. Pro-inflammatory biomarkers, such as IL-1, IL-6, IL-18 and CRP, have been shown to be differentially expressed in HIV+ patients who develop IRIS. Furthermore, immunoregulatory markers, such as IL-10, have also been shown to be increased in IRIS patients. The underlying molecular pathways involved in this differential expression are not fully understood.

Gene expression analysis by microarray of patient samples prior to ART initiation could allow the unbiased study of genes and molecular signatures in patients before they develop clinical manifestations of IRIS; helping us understand the molecular pathways triggering the syndrome.

Study Design & Methods

- **Gene expression profiles measured by microarray analysis of peripheral blood mononuclear cells (PBMC) obtained pre-ART from 140 HIV+ patients.** Data analysis was performed using Ingenuity Pathway Analysis (IPA).
- **To detect which molecular pathways might be over-represented in patients that would later develop IRIS we focused on biological function terms as well as upstream regulators that showed statistical significance in IPA.** A heat map of genes associated with those pathways showed statistical significance in IPA. Genes with an asterisk highlighted were found to have a significantly higher signal in IRIS using Wilcoxon analysis. The Bonferroni method was used to adjust for multiple comparisons.
- **Additionally, our group looked at genes known to be associated with IRIS in literature.** We compared those genes in each group using Wilcoxon analysis, and the Bonferroni method was used to adjust for multiple comparisons.

### Results

**Results**

**Table 1.** Characteristics of Study Participants at baseline (pre-ART) | Median values reported with 25th and 75th percentiles.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IRIS</th>
<th>Non-IRIS</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38 (30, 45)</td>
<td>35 (29, 42)</td>
<td>0.25</td>
</tr>
<tr>
<td>Gender (%)</td>
<td>78% Female</td>
<td>77% Male</td>
<td></td>
</tr>
<tr>
<td>CD4 T cells/µL</td>
<td>29 (11, 51)</td>
<td>22 (5, 49)</td>
<td>0.2</td>
</tr>
<tr>
<td>CD4%</td>
<td>3 (2, 7)</td>
<td>3 (1, 6)</td>
<td>0.86</td>
</tr>
<tr>
<td>HIV-RNA (log10 c/mL)</td>
<td>5.08 (4.72, 5.48)</td>
<td>5.29 (4.86, 5.70)</td>
<td>0.02</td>
</tr>
<tr>
<td>Median Days to IRIS</td>
<td>28 (14, 56)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of IRIS</td>
<td>43% Paradoxical, 54% Unmasking</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** A) Heat map of genes found to be of interest using IPA analysis. B) Of those genes, we looked at those known to be associated with the immune system. Genes highlighted in red were found to have a significantly higher and those in blue a significantly lower signal in IRIS using Wilcoxon analysis. Genes with an asterisk remained significant when adjusting for multiple comparisons.

**Figure 2.** Differential gene expression for transcripts canonical signalling pathway: IL-10 Signalling. Literature curated interactions between gene products are portrayed by shapes indicating type of gene product, while colors indicate the magnitude of difference in expression observed in this transcript. Genes in red were more abundant in IRIS patients, in green were more abundant in non-IRIS.

**Table 2.** Review of inflammatory and coagulation biomarkers by IRIS status. Genes in bold were found to be significant when using Wilcoxon analysis. Genes with an asterisk remained significant when p-values were adjusted for multiple testing.

### Conclusion

- This study highlights possible molecular pathways, including IL-1, IL-18, and IL-10, to be involved in IRIS pathology and may have implications for IRIS prevention and treatment.
- Our data suggest a complex interplay between inflammatory and immunoregulatory pathways as well as coagulation factors occurring prior to the IRIS event itself.
- Further analysis is required to gain a better understanding of the connections and associations between the various gene expression and molecular pathways potentially involved in the development of IRIS in HIV+ patients that may also have implications in other settings of reversal of immune suppression.

### References