Flow Cytometric Analysis of Gut Biopsies

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Overview

- GALT flow cytometry
- GALT flow data from completed studies
  - MTN studies
  - Non-MTN studies
- Lessons learned and questions for the future
GALT Flow Cytometry
GALT Flow Cytometry

- GALT flow cytometry increasingly used to characterize mucosal cell populations in:
  - HIV pathogenesis studies
  - Evaluation of HIV vaccine responses
  - PreP studies
  - Non-HIV related fields such as inflammatory bowel disease research

- Primary approach is to collect biopsies and to mechanically/enzymatically disassociate into single cells
GALT Flow Data
GALT Flow Data (1)

- **HPTN-056**
  - No intervention

- **RMP-01**
  - UC781 gel (Phase 1)

- **RMP-02 / MTN-006**
  - TFV gel & oral (Phase 1)

- **MTN-007**
  - TFV gel (Phase 1)

- **CHARM-01**
  - TFV gel (Phase 1)

- **HVTN-MIG study**
  - No intervention

- **MTN-017**
  - TFV gel/oral (Phase 2)

- **CHARM-03**
  - Maraviroc gel & oral (Phase 1)

- **HPTN-069**
  - Oral TFV, MVC, FTC (Phase 2)
GALT Flow Data (2)

- **ACTG 5330 (Multi-center)**
  - The effect of isotretinoin on immune activation among HIV-1-infected subjects with incomplete CD4+ T cell recovery

- **Dipyridamole (DP) study (Single center)**
  - The effect of DP on HIV-associated immune activation and inflammation

- **ACTG 5341s (Multi-center)**
  - Size and decay of HIV-1 reservoirs in tissues
HPTN-056

- **Population**
  - HIV-negative (N=8)
  - HIV-positive (N=8)

- **Center(s)**
  - Single

- **Sampling**
  - Colon
  - 10 cm and 30 cm
  - BL, +2/52, +4/52

- **Panel**
  - CD3, CD4, CD8, CD45,
  - HLA-DR, CD38,
  - CXCR4, CCR5, DC-SIGN
  - CD19
  - CD16, CD56

McGowan et al. JAIDS 2007
HPTN-056 ICC Scores

McGowan et al. JAIDS 2007
RMP-01

- Population
  - HIV-negative (N=36)

- Center(s)
  - Single

- Sampling
  - Colon
  - 10 cm and 30 cm
  - BL, post single dose, and post seven doses

- Products (1:1:1)
  - UC781 gel (0.1%)
  - UC781 gel (0.25%)
  - HEC placebo

- Panel
  - CD4, CD8, CD45, HLA-DR, CD38
  - CXCR4, CCR5

RMP-01

- Single dose
  - UC781 (0.1%) vs HEC (p >0.05)
  - UC781 (0.25%) vs HEC (p >0.05)

- 7 day exposure
  - UC781 (0.1%) vs HEC (p >0.05)
  - UC781 (0.25%) vs HEC
    - CCR5 RFI on CD4 (p = 0.025)
    - CCR5/CXCR4 on CD4 (p = 0.020)

RMP-02 / MTN-006

- Population
  - HIV-negative (N=18)

- Center(s)
  - 2 sites
  - Samples shipped to UCLA for analysis

- Sampling
  - Colon (15 cm)
  - BL, post single dose, and post seven doses

- Products (2:1)
  - TFV gel (1%)
  - HEC placebo

- Panel
  - CD4, CD8, CD45,
  - HLA-DR, CD38
  - CXCR4, CCR5

Anton PA et al. AIDS Res Hum Retroviruses 2012
### RMP-02 / MTN-006

<table>
<thead>
<tr>
<th></th>
<th>Oral</th>
<th>Single</th>
<th>7 day</th>
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<tbody>
<tr>
<td><strong>P values</strong></td>
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<tr>
<td>CD3+ on CD45+</td>
<td>0.20</td>
<td>0.90</td>
<td>0.32</td>
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<tr>
<td>CD4+ on CD45+</td>
<td>0.46</td>
<td>0.75</td>
<td>0.20</td>
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<tr>
<td>CD8+ on CD45+</td>
<td>0.08</td>
<td>0.44</td>
<td>0.71</td>
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<tr>
<td>CD38+ on CD4+</td>
<td>0.72</td>
<td>0.37</td>
<td>0.86</td>
</tr>
<tr>
<td>CD38 RFI on CD4+</td>
<td>0.76</td>
<td>0.42</td>
<td>0.81</td>
</tr>
<tr>
<td>HLA- DR+ on CD4+</td>
<td>0.53</td>
<td>0.92</td>
<td>0.84</td>
</tr>
<tr>
<td>HLA- DR+ CD38+ on CD4+</td>
<td>0.26</td>
<td>0.93</td>
<td>0.97</td>
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<tr>
<td>CCR5+ on CD4+</td>
<td>0.15</td>
<td>0.29</td>
<td>0.94</td>
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<tr>
<td>CCR5 RFI on CD4+</td>
<td>0.13</td>
<td>0.45</td>
<td>0.83</td>
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</tbody>
</table>

Anton PA et al. AIDS Res Hum Retroviruses 2012
MTN-007

- **Population**
  - HIV-negative (N=60)

- **Center(s)**
  - 3 centers
  - Samples shipped to Pittsburgh

- **Sampling**
  - Anoscopic
  - Flex sig (15 cm)
  - BL, post SD, and post 7D

- **Products (1:1:1:1)**
  - TFV gel (1.0%)
  - N9 gel (2.0%)
  - HEC placebo
  - No Rx

- **Panel**
  - CD3, CD4, CD8, CD45,
  - CD69
  - CXCR4, CCR5

7 doses of TFV gel versus HEC gel associated with a significant increase in CD45+/CD3+ cells isolated from Flex sig biopsies (57% versus 42.8%; p = 0.04)
CHARM-01

- Population
  - HIV-negative (N=14)

- Center(s)
  - 2 centers
  - Samples shipped to Pittsburgh

- Sampling
  - Flex sig (15 cm)
  - BL, and post 7D of each formulation

- Products (crossover)
  - TFV gel (1.0%)
  - RG TFV gel (1.0%)
  - RS TFV gel (1.0%)

- Panel
  - CD3, CD4, CD8, CD45,
  - CD69
  - CXCR4, CCR5

### CHARM-01

<table>
<thead>
<tr>
<th>Flow parameter</th>
<th>Enrollment(n = 14) Mean (SD), Median (25%, 75%)</th>
<th>Mean at 7th Dose N, Mean (SD), Median (25%, 75%)</th>
<th>Change at 7th Dose(n = 13) Mean (SE)</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>% CD3&lt;sup&gt;+&lt;/sup&gt; from CD45&lt;sup&gt;+&lt;/sup&gt;</td>
<td>44.4 (17.4), 47.2 (34.6, 58.9)</td>
<td>11, 53.8 (16.4), 54.8 (46.9, 66.2)</td>
<td>12.23 (5.89)</td>
<td>0.0380</td>
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<tr>
<td>Enrollment vs. RF D7</td>
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<tr>
<td>Enrollment vs. HEC/VF D7</td>
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<tr>
<td>% CXCR4&lt;sup&gt;+&lt;/sup&gt; from CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>71.5 (16.1), 70.3 (57.9, 84.5)</td>
<td>12, 61.1 (23.2), 61.4 (55.0, 79.5)</td>
<td>-10.68 (5.40)</td>
<td>0.0480</td>
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<tr>
<td>Enrollment vs. HEC/VF D7</td>
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<tr>
<td>Change at 7th Dose (RGVF v HEC/VF)</td>
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<td>0.0142</td>
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<tr>
<td>% CD69&lt;sup&gt;+&lt;/sup&gt; from CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>83.4 (6.0), 83.9 (80.4, 87.4)</td>
<td>12, 80.5 (4.9), 82.3 (78.7, 83.5)</td>
<td>-2.27 (0.97)</td>
<td>0.0188</td>
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<td>Enrollment vs. HEC/VF D7</td>
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<tr>
<td>% CXCR4&lt;sup&gt;+&lt;/sup&gt; and CCR5&lt;sup&gt;+&lt;/sup&gt; from CD4&lt;sup&gt;+&lt;/sup&gt;</td>
<td>55.6 (11.9), 55.1 (43.8, 66.2)</td>
<td>12, 45.4 (15.9), 48.7 (41.2, 53.9)</td>
<td>-10.16 (4.20)</td>
<td>0.0157</td>
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<tr>
<td>Enrollment vs. HEC/VF D7</td>
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<tr>
<td>Change at 7th Dose (RGVF v HEC/VF)</td>
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<td>0.0049</td>
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<tr>
<td>% CXCR4&lt;sup&gt;+&lt;/sup&gt; from CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>51.2 (17.1), 47.8 (40.2, 68.1)</td>
<td>12, 39.3 (18.8), 38.3 (33.9, 54.2)</td>
<td>-13.38 (4.58)</td>
<td>0.0035</td>
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<tr>
<td>Enrollment vs. HEC/VF D7</td>
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<tr>
<td>% CD69&lt;sup&gt;+&lt;/sup&gt; from CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>85.7 (6.1), 84.7 (82.1, 90.5)</td>
<td>13, 71.4 (26.6), 80.2 (78.1, 84.3)</td>
<td>-13.54 (6.17)</td>
<td>0.0283</td>
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<tr>
<td>Enrollment vs. RGVF D7</td>
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<tr>
<td>Change at 7th Dose (RF v RGVF)</td>
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<td>0.0336</td>
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<tr>
<td>% CXCR4&lt;sup&gt;+&lt;/sup&gt; and CCR5&lt;sup&gt;+&lt;/sup&gt; from CD8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>43.2 (13.1), 42.5 (35.6, 48.0)</td>
<td>12, 31.5 (13.8), 34.0 (29.1, 37.7)</td>
<td>-12.51 (3.66)</td>
<td>0.0006</td>
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<tr>
<td>Enrollment vs. HEC/VF D7</td>
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</table>
HVTN-MIG

- Feasibility of multi-site flow cytometric processing of GALT samples
- Clinical sites
  - UCLA (N = 18)
  - Pittsburgh (N = 17)
- Samples
  - PBMC, qPBMC, MMC
- Flow analysis
  - Fred Hutchinson CRC

qPBMC Data

MMC Data

MIG Summary

- Ranges for qPBMC CD4/CD8 equivalent at both sites
- Significant differences for the majority of other parameters
- Standardized protocols can reduce but not eliminate variability between sites
- HSV seropositivity influences T cell phenotype
- FMO not routinely required
Lessons Learned
Lessons Learned

- GALT flow cytometry is challenging and variable
- Cryopreservation and centralized analysis may provide more stable data
- Unclear whether flow cytometry routinely required in microbicide studies
- Further data may clarify the situation
  - MTN-017, HPTN-069, CHARM-03
Future Questions
Future Questions

- What studies require flow cytometry?
- What panels should be included?
- Multi-center studies
  - Should sample processing/staining and/or analysis be centralized?
  - Could cryopreservation of tissue samples or isolated MMC lead to more robust data?
Acknowledgements

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