Multiomic dynamics of the cellular HIV reservoir after rebound during ATI

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2022.12.14
The HIV reservoir

Question: Does the phenotype of HIV+ cells change after analytical ART interruption (ATI) + the resumption of ART?
<table>
<thead>
<tr>
<th>Is cell infected?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>scATACseq</strong></td>
</tr>
<tr>
<td>genome wide accessible chromatin</td>
</tr>
<tr>
<td>can also include provirus</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>What is the cell?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>scATACseq</strong></td>
</tr>
<tr>
<td>+ surface protein staining</td>
</tr>
<tr>
<td>accessible chromatin + surface antigen markers</td>
</tr>
<tr>
<td>high resolution identity</td>
</tr>
</tbody>
</table>

**viral scASAPseq** enables direct *ex vivo* identification and analysis of HIV+ cells in their **native and unmanipulated** state

Wu et al., *Nature Immunology* 2022 (in press)

**scATACseq**
(singel-cell Assay for Transposase-Accessible Chromatin) + **scSAPseq**
(singel-cell Select Antigen Profiling)

Mimitou et al., *Nature Biotechnology* 2021
Identification of infected cells from ART-treated PLWH pre and post ATI

**Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption**


**Table**

<table>
<thead>
<tr>
<th>Individual</th>
<th>Total cells</th>
<th>HIV+ cells (% of total cells)</th>
<th>Total HIV DNA copies per 1e6 CD4+ T cells (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A01</td>
<td>14021 (pre-ATI)</td>
<td>9 (0.06%; pre-ATI)</td>
<td>185 (0.019% pre-ATI)</td>
</tr>
<tr>
<td></td>
<td>27065 (post-ATI)</td>
<td>6 (0.02%; post-ATI)</td>
<td>293.8 (0.029% post-ATI)</td>
</tr>
<tr>
<td>A08</td>
<td>18427 (pre-ATI)</td>
<td>46 (0.25%; pre-ATI)</td>
<td>1791.2 (0.18% pre-ATI)</td>
</tr>
<tr>
<td></td>
<td>17461 (post-ATI)</td>
<td>36 (0.22%; post-ATI)</td>
<td>1564.5 (0.16% post-ATI)</td>
</tr>
<tr>
<td>A09</td>
<td>44331 (pre-ATI)</td>
<td>67 (0.15%; pre-ATI)</td>
<td>1297.3 (0.13% pre-ATI)</td>
</tr>
<tr>
<td></td>
<td>32998 (post-ATI)</td>
<td>36 (0.11%; post-ATI)</td>
<td>1221.8 (0.12% post-ATI)</td>
</tr>
</tbody>
</table>
Identification of infected cells from ART-treated PLWH

HIV+ cells are found across many different CD4+ T-cell subsets

Wu et al. Nature Immunology 2022 (in press)

* from Salantes et al., JCI 2018
In(stability) of the phenotype of infected cells after ATI, potentially correlating with extent of viral rebound AUC

(left): Wu et al. *Nature Immunology* 2022 (in press)
(right): modified from Bar et al., *NEJM* 2016
Differential surface protein expression on HIV+ cells before and after ATI

Raw data from Wu et al. *Nature Immunology* 2022 (in press)

Wilcox method in Seurat’s FindMarkers() with Bonferroni correction
Showing only HIV+ CD4+ cells & markers with adj p < 0.05
CD74 is known to be a target by HIV-1 Nef (and Vpu) and may be correlated with immune activation

Left: Stumptner-Cuvelette et al. *PNAS* 2001
Right: Ghiglione et al. *Virus Research* 2011
Differential surface protein expression on HIV+ cells is heterogeneous between individuals

**A08 (higher viral rebound AUC)**

- **Pre-ATI (n = 46)**
- **Post-ATI (n = 37)**

**A09 (lower viral rebound AUC)**

- **Pre-ATI (n = 63)**
- **Post-ATI (n = 35)**

No markers with adjusted $p$ value < 0.05

Raw data from Wu et al. *Nature Immunology* 2022 (in press)

Wilcoxon method in Seurat’s FindMarkers() with Bonferroni correction

Showing only HIV+ CD4+ cells & markers with $p < 0.05$
Community Summary

Key question
• Does the phenotype of HIV+ cells change after ATI + the resumption of ART?

What was accomplished?
• Used our single-cell strategy to phenotypically profile HIV-infected cells at basal (resting) state before and after ATI with VRC01 immunotherapy.

Why is this important?
• Provides the highest resolution understanding of the HIV reservoir during ATI-associated perturbations.
• Understanding HIV reservoir dynamics will help with cure efforts.

Future directions
• Validation of various markers as a biomarker of ATI on HIV+ cells.
• Determine if these findings are specific to ATI + VRC01 or if ATI alone is sufficient for the observed differences.
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  • Felicity Mampe*
  • Katie Bar, MD

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