The Immune Checkpoints PD-1, LAG-3 and TIGIT are Biomarkers of HIV Infected Cells During ART and Identify Distinct Cellular Reservoirs

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Identifying biomarkers of latently infected cells is of primary importance to specifically target and eliminate the persistent reservoir.

Approach: Combining the “Where” with the “How”

Where: HIV persists in discrete subsets of cells during ART

How: Mechanisms driving the establishment and persistence of the HIV reservoir

Biomarkers of latently infected cells could be:
- surrogate markers of higher susceptibility to HIV infection
- markers of persistence providing selective advantages (latency maintenance, immune escape, replenishment)
Why immune checkpoints (ICs) could be biomarkers for HIV persistence during ART?

- ICs, negative regulators of T cell activation, regulate T cell proliferation and cytokine production.

- Several of these molecules are associated with T cell dysfunction in chronic HIV infection (PD-1, CTLA-4, TIM-3, CD160).

- Immune dysregulations persist during ART (residual immune activation, incomplete CD4 T cell restoration, T cell dysfunction).

Chen L, Nat Rev Imm. 2013


Hatano et al, JID 2012; Kelley et al, CID 2009
By inhibiting T cell activation, negative regulators (Immune Checkpoints, ICs) may actively maintain viral latency and identify reservoir cells during ART.
**Study population:**
48 HIV infected subjects virally suppressed for at least 3 years with CD4>350 c/µL

**Methods:**
- Multiparametric flow cytometry analysis of the expression of 8 ICBs (PD-1, LAG-3, TIGIT, CTLA-4, BTLA, CD160, 2B4, TIM-3) in PBMCs
- Ultrasensitive qPCRs to measure the frequency of CD4 T cells harboring virological markers of HIV persistence

The frequency of CD4 T cells expressing PD-1, LAG-3 and TIGIT are positively correlated with the frequency of CD4 T cells harboring integrated HIV DNA during ART.
PD-1 identifies $T_{CM}$ and $T_{TM}$ CD4 T cells enriched in integrated HIV DNA.

The frequency of cells harboring integrated HIV DNA is significantly higher in PD-1 expressing $T_{CM}$ and $T_{TM}$ when compared to their PD-1 negative counterparts.
LAG-3 identifies $T_{CM}$ and $T_{TM}$ CD4 T cells enriched in integrated HIV DNA.

Differentiation

The frequency of cells harboring integrated HIV DNA is significantly higher in LAG-3 expressing $T_{CM}$ and $T_{TM}$ when compared to their LAG-3 negative counterparts.
TIGIT identifies $T_{EM}$ CD4 T cells enriched in integrated HIV DNA.

The frequency of cells harboring integrated HIV DNA is significantly higher in TIGIT expressing $T_{EM}$ when compared to their TIGIT negative counterparts.
Can we further enrich in the reservoir by combining multiple ICs?

Memory CD4 T cells expressing multiple ICs are highly enriched for integrated HIV DNA.
Is the virus carried by latently infected cells expressing ICs functional?

**A** Principle of “Tat/Rev Induced Limiting Dilution Assay” (TILDA)

Memory CD4 T cells expressing LAG-3 and/or PD-1 and/or TIGIT are highly enriched for inducible HIV latently infected cells.
1. **Biomarkers**

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<th>Biomarkers</th>
<th>CM</th>
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<tr>
<td>PD-1</td>
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<td>TIGIT</td>
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2. 

Altogether, our data suggest that blocking ICs may reactivate HIV from latency and paves the way for the development of novel strategies to cure HIV infection.
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