State-of-the-Art Lecture: Molecular basis for HIV

HIV DART & EMERGING VIRUSES
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Well suppressed patients rebound when HAART is stopped
Generation of latently infected cells is probably the result of silencing memory cell differentiation (Siliciano)

CD4+ T-cells

Naive → Activated → Effector to memory transition → Resting Memory Cell

HIV

Latently Infected Resting Memory Cell
QUECEL primary T cell models: Reporter and polarization condition

A

HIV-1 Reporter Virus

CD8α-d2EGFP-IRES-Nef

LTR

5’

Δgag

Env

Rev

Tat

3’

LTR

B

Polarization and infection of effector Th17 T cells

TCR Stimulation & Polarization

Expansion

HIV Infection & Cell Sorting

Induction of Quiescence

Latently infected memory T cell

Naive CD4+ T-cell

Activated CD4+ T-cell

Effector Cell

HIV Infected Effector Cell

Polarization

Infection

Isolate CD8α+ Cells

Quiescent Cells

HIV Reactivation

Day 0

3

6

7

10

21

28

Polarization Cytokines

CD3/CD28 Beads

High IL-2 + IL-23

Low IL-2, IL-23, TGF-β and IL-8

CD3/28 Beads
QUECEL cell models: HIV is silenced as cell enter quiescence
The QUECEL (primary T cell) model is highly reproducible.
RNA Seq demonstrates cell cycle state dominates gene expression profile
Pathway analysis highlights a specific program of gene expression in quiescent cells.
HIV infection induces a gene expression program associated with KLF2 induction of quiescence
Latency reversal is a binary event, dependent upon induction of HIV Tat/P-TEFb.

**Actively transcribed provirus**

- NFAT / NFκB
- LTR → Tat → LTR
- + P-TEFb

**Transcription Blocks**

- NFAT, NFκB, P-TEFb $\downarrow$
- HKMTs $\uparrow$

**Silenced provirus**

- LTR → Tat → LTR
- Chromatin block

**Reinitiation**

- NFAT, NFκB $\uparrow$
- P-TEFb $\uparrow$
NFAT is the dominant transcription initiation factor in primary T-cells

A

**Imagestream Analysis of NFκB P65 and NFATC1 Nuclear Localization**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bright</th>
<th>DAPI</th>
<th>NFκB-P65</th>
<th>Merge</th>
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</thead>
<tbody>
<tr>
<td>Unstim</td>
<td>5.4%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>50.4%</td>
<td></td>
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</tr>
<tr>
<td>SAHA</td>
<td>3.4%</td>
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<tr>
<td>TNF-α</td>
<td>56.3%</td>
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<tr>
<td>IL-15 + IL-7</td>
<td>52.4%</td>
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</table>

B

**Flow cytometry with inhibitors**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Resting</th>
<th>CSFK5 (NFAT)</th>
<th>IKK (NFκB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Nef, pSer175</td>
<td>2.95%</td>
<td>3.96%</td>
<td>2.14%</td>
</tr>
<tr>
<td>HIV Nef, pSer175</td>
<td>0.74%</td>
<td>4.45%</td>
<td>1.45%</td>
</tr>
<tr>
<td>HIV Nef, TCR</td>
<td>6.07%</td>
<td>4.45%</td>
<td>1.45%</td>
</tr>
</tbody>
</table>

C

**HIV expression**

- No Inhibitor
- CsA (NFAT)
- IKK (NFκB)
Reassembly of P-TEFb is essential for HIV proviral reactivation.
CDK9 is cytoplasmic and CycT1 is absent from resting memory T-cells.
Activation of P-TEFb is mediated by Hsp90
Activation of P-TEFb is mediated by phosphorylation of CDK9
pSer175-CDK9 is associated with actively transcribing proviruses
In vivo reactivation of P-TEFb in patients treated with the HDACi Romidepsin (John Mellors)
Epigenetic regulation of genes and HIV

ACTIVE
EUCHROMATIN

INACTIVE
HETEROCHROMATIN

Nucleosome modification

EZH2: H3K27
EHMT2: H3K9
ChIP analysis of latent HIV proviruses in primary cells demonstrates RNAP II pausing and chromatin restrictions.
Both H3K27 (EZH2) and H3K9 (EHMT2) HKMTs are required to maintain latency in primary cells.
KDM6A (UTX-1) and JDMJD3 reverse EZH2 repression

Di/tri-methylation of H3K27
Chromatin compaction

De-methylation of H3K27
Chromatin relaxation

Transcription OFF

Transcription ON
GSK-J4 inhibits proviral reactivation and enhances DNA methylation

A

Assay Design

Isolate Memory Cells from HIV+ donors

Day 0
Treat with GSKJ4

Day 3
Induce with TCR beads

Day 4
Isolate RNA for EDITS Assay

B

HIV-1 reactivation (EDITS)

C

DNA methylation at CpG1

Reactivation (%)

0 20 40 60 80 100

Resting 0 μM 5 μM GSK-J4

TCR beads - + +

Methylation %

0 10 20 30 40

0 μM 5 μM GSK-J4

TCR beads + +
Activation of P-TEFb and Blocking Epigenetic Restrictions Leads to Synergistic Reactivation
IL-15 synergizes with most Latency Reversing Agents
Conclusions: Latency

- From a molecular perspective latency is an integral feature of the HIV life cycle
  - NF-κB/NFAT are only needed to initiate transcription from latent proviruses by reversing epigenetic blocks
  - Non-suppressed patients have latent proviruses
- Transactivation can be thought of as a way to turn on and off transcription in response to changes in the cellular environment
- Rebound is probably going on continuously, but is masked by ART. Rapid rebound may not necessarily be from the fully latent pool but could arise from low level replication and/or poorly suppressed cells.
Conclusions: “Shock, Kill & Contain”

• A wide set of clinically useful “shock” factors is being identified.
  ➢ Synergy between inducers of P-TEFb (PKCa, IL-15) and factors reversing epigenetic silencing (HDACi, HKMTi, ESRi) is biologically plausible and likely to yield the best compound combinations.

• Proviral reactivation strategies should be optimized to be compatible with effector clearance mechanisms and strategies (IL-15 and HDACi).

• Immune enhancements that lead to large scale elimination of the reservoir are likely to be distinct from enhancements needed for long term control. NK cells may be good for acute interventions, while CTLs may be better for long-term containment).
Current Laboratory Members

Curtis Dobrowolski (Th17, EDITS Assay)
Kien Nguyen (Epigenetics)
Uri Mbonye & Fredrick Kizito (P-TEFb)
Mary Ann Checkley & Ben Luttge (NK cells)
David Alvarez (NeuroAIDS)
Saba Valadkhan (Bioinformatics)

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P30 AI036219 (CFAR); D43 TW009780 (Boom)