HIV Persistence and the Central Nervous System as a Sanctuary

David M. Margolis, MD
Professor of Medicine, Microbiology & Immunology, Epidemiology
Persistent HIV Infection despite ART: Is the CNS different?

Rare, persistent proviral genomes

Pierson et al 2001
Residual viral expression (without failure of ART)

a) Pharmacologic and anatomic barriers to drug distribution and activity
   - e.g. CNS penetration
   - Incomplete suppression in the tissues (eg. GALT)

b) Persistent expression in long-lived cells
   - Macrophages, brain microglia

c) Intermittent activation and release of virus from latently infected cells
   - CD4+ cells
   - Recent renewal of the claim that marrow stem cells may be a long-term stable viral reservoir

The clinical detection of this event: **Low-level viremia**

- detected in ca. 75% ART-suppressed patients
- Level correlates with peak viremia, hypothesized to be “slow leak” from resting CD4+ cells by some, or from non-CD4 cells by others
Persistent HIV Production despite ART

Figure 1. No decline in viremia setpoint over 60-110 weeks of therapy

Low-level viremia is unaffected by intensification with PI, NNRTI, RAL, Enf

Raltegravir intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy

Dinoso PNAS 2009

Ghandi PLoS Med 2010
Single Genome sequences from resting CD4 cell outgrowth viruses

Anderson J Virology 2011
HIV Neuroimaging Consortium
Navia et al CROI 2011

• Prospective study of ~300 HIV-infected subjects across 7 centers
  – Brain bank centers at UCLA, UCSD and Harbor-UCLA
  – ACTG and primary HIV clinics Colorado, Pittsburgh, Harbor, Stanford
• Inclusion criteria:
  – Nadir CD4 count <200 cells/mL
  – ART for at least 12 weeks
• Exclusion criteria:
  – Confounding neurological, psychiatric and medical disorders (hepatic, renal, diabetes)
  – Active drug use
Conclusions

• Nadir CD4 prior to the initiation of treatment is significantly associated with persistent injury and NCI.

• Progressive brain injury likely due to the effects of several host and disease-related factors (or their interactions)
  – HIV RNA, immune activation, duration of treatment, duration of infection, and to a lesser extent, aging
  – Co-morbid disorders (hep C, cardiovascular risk factors) may also contribute.
Ongoing viral expression in the CNS
(as there is in the periphery)

• Some evidence that HIV-associated brain injury can continue to unfold in the setting of stable HIV disease and treatment.
• Ongoing evidence of markers of inflammation or injury in the CSF in some studies

- Is that different from the periphery?
- Is it ameliorated by earlier therapy?
- If patients are not treated until later disease, will adjunctive therapy (other than ART) be needed?
# CNS Penetration-Effectiveness Ranks 2010

<table>
<thead>
<tr>
<th>Rank</th>
<th>NRTIs</th>
<th>NNRTIs</th>
<th>PI</th>
<th>Entry/Fusion Inhibitors</th>
<th>Integrase Inhibitors</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>Zidovudine</td>
<td>Nevirapine</td>
<td>Indinavir/RTV</td>
<td>Maraviroc</td>
<td>Raltegravir</td>
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<tr>
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<td>Delavirdine</td>
<td>Darunavir/RTV</td>
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<td>Efavirenz</td>
<td>Fosamprenavir/RTV</td>
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<td>Etravirine</td>
<td>Atazanavir</td>
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<td>Lamivudine</td>
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<td>Nelfinavir</td>
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<td>Tenofovir</td>
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<td>Ritonavir</td>
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<td></td>
<td>Zalcitabine</td>
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<td>Saquinavir</td>
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Improved Delivery of Standard ART

Macrophage Delivery of Nanoformulated Antiretroviral Drug to the Brain in a Murine Model of NeuroAIDS

Huanyu Dou,* Cassi B. Grotepas,* JoEllyn M. McMillan,* Christopher J. Destache,‡
Mahesh Chaubal,§ Jane Werling,§ James Kipp,§ Barrett Rabinow,§
and Howard E. Gendelman²*†

The Journal of Immunology, 2009, 183, 661-669
• Gold nanoparticle preparations with 1 to 170 inhibitor moieties
• Covalently linked to 4 nm gold particle by thiol linker

Bowman JACS 2008
HBMEC monolayer

β-catenin (red) highlights HBMEC tight junctions

After FITC

After FITC-dextran

after FITC-nanocrystals

After FITC/glucose-nanocrystals

confocal image across central cell axis, illustrating intracellular entry of NC

cconfocal image overlaid with phase contrast
Persistent HIV Infection despite ART

Rare, persistent proviral genomes

Pierson et al 2001
MULTIPLE HURDLES TO VIRAL ESCAPE FROM LATENCY

- Cellular state
- Integration Site
- Chromatin modification
- Tat deficiency
- Factor deficiency
- Txn INTERFERENCE
HIV lives within chromatin

“Closed” Nucleosome
- Hypo-Acetylated Histone tails
- Stable, Compact Chromatin
- Less accessible to Transcription Factors
- Transcription Repressed

“Open” Histones
- Acetylated Histone tails
- Reduced Higher Order Structure
- Access to Transcription Factors
- Transcription Active

deacetylated

acetylated

30 nm

6-8 nucleosomes per turn

11 nm

nucleosome

linker DNA

histone octamer core

histone H1
Infected units per billion resting CD4+ T cells

Limit of detection

PHA

VPA global HDAC inhibitor

Class II inhibitor

Class I inhibitor

Archin AIDS 2009
Free virions

Productively infected CD4+ T lymphoblasts

Resting CD4+ T cells with unintegrated HIV-1 DNA

Virions + T-DC

Infected macrophages

Resting CD4+ T cells with integrated provirus

Half-life (days)

0 30 60 90 120 ?

Microglia
Astrocytes
Where can HIV eradication approaches be studied?
Simian Immunodeficiency Virus–Infected Macaques Treated with Highly Active Antiretroviral Therapy Have Reduced Central Nervous System Viral Replication and Inflammation but Persistence of Viral DNA. Zink et al.

• Simian immunodeficiency virus in which all pigtail macaques develop AIDS and 90% develop CNS disease by 3 months
• Tenofovir disoproxil fumarate, saquinavir, atazanavir, and an integrase inhibitor starting at 12 days after inoculation and were euthanized at 175 days.
• Plasma and CSF viral loads declined rapidly
• Brain viral RNA was undetectable at necropsy
• Viral DNA levels were not different from untreated macaques.
• CNS inflammation was significantly reduced
Where can HIV eradication approaches be studied?

“Humanized” mice
Generation of Bone Marrow/Liver/Thymus (BLT) Mice

Laboratory group of Victor Garcia-Martinez, UNC
Systemic infection of BLT mice with HIV-1

In situ hybridization analysis for the presence of HIV RNA in tissues from an infected mouse.

Jake Estes
Reconstitution of BLT mice brain with human CD68$^+$ macrophages and MHC II$^+$ cells
Modeling ART to examine persistent CNS HIV infection in a small animal model?
Suppression of plasma viremia in humanized mice

Raltegravir (2 mg)
FTC (3.5 mg)
TDF (5.2 mg)

Denton & Garcia unpublished
Purification of Resting hu-CD4+ T cells from hu-Rag2\(^{-/-}\)γc\(^{-/-}\) Mice and Resting CD4+ Cell Outgrowth Assay

Harvest lymphocytes from Blood, LN, Spleen, BM, FRT, Lung, Liver and Thymus

Rest cells for 2 days in media with HIV RT and Integrase Inhibitors

24 hr maximal activation with PHA, IL-2, allogeneic cells

HIV outgrowth assay in limiting dilution of activated CD4\(^{+}\) T feeder cells

Calculate frequency of resting cell infection by maximum likelihood method

97-99% pure resting CD4\(^{+}\) T cells

0.5-1.0 million cells/mouse

Choudhary, Margolis CROI 2011
Purification of Resting Human CD4+ T Cells from Humanized BLT Mice

A. Before Column Purification

B. After Column Purification

Frequency of human resting CD4+ T cell infection: 7 to < 3 per million

Resting human CD4+ T cells
Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation

Gero Hütter, M.D., Daniel Nowak, M.D., Maximilian Mossner, B.S., Susanne Ganepola, M.D., Arne Müßig, M.D., Kristina Allers, Ph.D., Thomas Schneider, M.D., Ph.D., Jörg Hofmann, Ph.D., Claudia Kücherer, M.D., Olga Blau, M.D., Igor W. Blau, M.D., Wolfr K. Hofmann, M.D., and Eckhard Thiel, M.D.

Monocyte and CNS reservoirs not targeted
How to attack persistent neuro HIV infection?

• New and better measures of persistence in patients
• Studies across all models: cell lines, primary cell models, patient cells, animal models, and patients
• Approaches to persistent viremia
  – Resting CD4+ T cells
  – Are there other persistent cells with HIV
  – Do we need better drugs
• Approaches to persistent provirus
  – In resting CD4+ T cells
  – In other cells?
  – HDAC inhibitors, other epigenetic drugs
  – Combination therapies to induce expression and cell death
  – Paradox of oncologic approach
Special thanks to our study volunteers