Central nervous system as a reservoir for HIV: why it happens and what can be done

Sarah Joseph, PhD
University of North Carolina
HIV reservoirs:
HIV-infected cells that persist during ART
Viral reservoirs are large, capable of quickly reigniting viremia and long-lived.

Most rebounding viral populations are genetically diverse (UNC - Bednar et al, 2016)

Viral reactivation from latency occurs every 5-8 days after treatment interruption (Pinkevych et al, 2015)

Resting CD4+ T cells reservoirs have a mean half-life of ~3.6 years (Crooks et al, 2016)
Talk outline

• Establishing HIV-1 populations in the CNS
  • Studies of subjects off therapy
  • Relationship between HIV-1 replication in the CNS and neurocognitive impairment

• Analyses of viral reservoirs in the CNS
  • Quantitative viral outgrowth assays (QVOA)
  • Viral DNA and/or RNA in brain tissue
  • Viral RNA in CSF

• Conclusions
Establishing HIV-1 populations in the CNS
Establishing HIV-1 populations in the CNS

Diverse, compartmentalized sequences indicate sustained viral replication in the CNS

UNC Joseph et al., THINC study
Establishing HIV-1 populations in the CNS

Historic **errors** in the assessment of macrophage-tropism

1. Does it replicate in a transformed T cell line?

   - Yes
   - No

   T cell-tropic
Establishing HIV-1 populations in the CNS

**Historic errors** in the assessment of macrophage-tropism

1. Does it replicate in a transformed T cell line?
   - Yes → T cell-tropic
   - No → Macrophage-tropic

2. A small number of these were tested for replication in monocyte-derived-macrophage (MDM)
Establishing HIV-1 populations in the CNS

Historic errors in the assessment of macrophage-tropism

1. Does it replicate in a transformed T cell line?
   Only expressed CXCR4, no CCR5

   Yes → T cell-tropic = CXCR4

   No → Macrophage-tropic = CCR5

2. A small number of these were tested for replication in monocyte-derived-macrophage (MDM)
Coreceptor Usage Does Not Define Cellular Tropism

CXCR4 $\neq$ T cell-tropic
CCR5 $\neq$ Macrophage-tropic

The vast majority of HIV-1 is R5 T cell-tropic
T Cells Express 26X Higher CD4 Densities Than Macrophages

ABS = Antibody Binding Sites For An α-CD4 Antibody

UNC - Joseph et al, 2014
Rationale behind macrophage tropism assay

Sustained replication in macrophage/microglia selects for HIV-1 variants with an enhanced ability to enter those cells.

→ Macrophage and microglia express much lower CD4 densities than T cells, thus macrophage-tropic viruses are efficient at entering cells expressing low levels of CD4.
Establishing HIV-1 populations in the CNS

Compartmentalized lineages can be observed in the CSF during the first 2 years of infection

CSF pleocytosis = CSF white blood cell count > 10 cells / µl

UNC - Sturdevant et al 2015
Establishing HIV-1 populations in the CNS

Compartmentalized lineages can be observed in the CSF during the first 2 years of infection.

~16% of subjects had evidence of sustained HIV-replication in the CNS based on either CSF compartmentalization or pleocytosis at multiple time-points.

UNC - Sturdevant et al 2015
Establishing HIV-1 populations in the CNS

Pleocytosis declines as compartmentalization emerges

UNC - Sturdevant et al 2015
Establishing HIV-1 populations in the CNS

Compartmentalized viruses were significantly better at entering cells expressing low levels of CD4

Equilibrated
T cell tropism

Compartmentalized
Intermediate macrophage tropism

UNC - Sturdevant et al 2015
40 HIV-positive, treatment-naïve subjects were enrolled in four cohorts

<table>
<thead>
<tr>
<th>Cohorts</th>
<th>N</th>
<th>Sequencing method</th>
<th>Number of neurocognitive domains tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>THINC</td>
<td>15</td>
<td>SGA and Deep</td>
<td>8</td>
</tr>
<tr>
<td>UCSF</td>
<td>11</td>
<td>SGA</td>
<td>4</td>
</tr>
<tr>
<td>NNTC</td>
<td>5</td>
<td>SGA and Deep</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(no gross motor)</td>
</tr>
<tr>
<td>Emory</td>
<td>9</td>
<td>Deep</td>
<td>8</td>
</tr>
</tbody>
</table>
Relationship between HIV-1 replication in the CNS and neurocognitive impairment

HIV-1 replication in the CNS is associated with elevated neurocognitive impairment

UNC - Joseph et al, CROI 2015
Relationship between HIV-1 replication in the CNS and neurocognitive impairment

**THINC study: treatment naive cohort**

HIV Tropism, Persistence, Inflammation and Neurocognition in Therapy Initiation

Inclusion criteria:
- HIV+
- ≥ 18 years of age
- Treatment naïve or off therapy for ≥ 1 yr
- < 400 CD4+ T cells/μl
- Plasma viral load > 10,000 copies of HIV-1 RNA/ml

*Note the timeline for baseline, 2 weeks, 6 months, and 1 year with specified evaluations.*
Neurocognitive impairment due to HIV replication in the CNS does not resolve within a year on ART.
Conclusions – Part 1

1. HIV-1 populations can be established in the CNS within the first 2 years of infection.

2. Between 16 and 70% of untreated subjects have evidence of sustained HIV replication in the CNS.

3. CNS replication is associated with neurocognitive impairment.

4. The effects of HIV-replication on neurocognitive impairment may not be corrected after one year of therapy.
Relationship between HIV-1 replication in the CNS and neurocognitive impairment

CNS biology may hinder the elimination of infected cells from the CNS

1. CD8+ T cells are at a low concentration in the CNS.

1. CD8+ T cells may inefficient at killing infected macrophage.
   Walker-Sperling et al, 2014
   Vojnov et al, 2012

1. Drug concentrations in the CNS are lower than that of the periphery and may be insufficient to completely suppress viral replication in the CNS.

2. Some HIV-1 target cells in the CNS (macrophage and microglia) are long-lived.
Analyses of viral reservoirs in the CNS
HIV reservoirs:
HIV-infected cells that persist during ART
Latent reservoirs:
HIV-infected cells that persist during ART and do not express HIV

Active reservoirs:
HIV-infected cells that persist during ART and express HIV
Most minimally- or non-invasive techniques cannot be used to survey the CNS for viral reservoirs

Whole-body PET scan using an anti-gp120 probe reveals sites of HIV production.
- Probe doesn’t enter the CNS

Santangelo et al 2015

Human follicular dendritic cells (FDCs) isolated from HIV positive subjects on ART treatment retain HIV in endosomal compartments.
- Viable CNS cells can only be collected at autopsy, very soon after death.

Heesters et al 2015
1. Quantitative viral outgrowth assay (QVOA) from CNS-derived cells

Standard quantitative viral outgrowth assay (QVOA)

UNC - Archin et al 2015
1. Quantitative viral outgrowth assay (QVOA) from CNS-derived cells

Proof of concept: МΦ-QVOA can be used to analyze the frequency of infected myeloid cells in untreated, SIV-infected macaques

\[\text{Sacrifice} \rightarrow \text{Isolate brain parenchymal macrophages and Microglia dilute and culture} \rightarrow \text{Measure SIV RNA concentration per well} \rightarrow \text{Calculate infectious units per million (IUPM)}\]

Avalos et al, 2016

МΦ-QVOA has not yet been performed using brain cells from animals/subjects on therapy
2. Analyses of proviral DNA and/or RNA isolated from the brains of subjects on therapy

4149

- Occipital Lobe
  - 1 RNA

- Frontal Cortex
  - 2 DNA

- Basal Ganglia

- Cerebellum

CSF VL < 40 cp/ml
Blood VL < 40 cp/ml

5095

- Dura
  - 2 DNA

- Frontal Cortex
  - ~17 DNA

- Occipital Lobe
  - ~20 DNA

- Temporal Lobe
  - 6 DNA

- Cerebellum
  - 34 DNA
  - 29 RNA

CSF VL < 400 cp/ml
Blood VL < 40 cp/ml

Lamers, CROI 2016
2. Analyses of proviral DNA and/or RNA isolated from the brains of subjects on therapy

Able to detect HIV DNA and RNA in brain samples.
Brain HIV RNA is associated with neuropsychological impairment on ART
Gelman et al, 2013

HIV DNA persists in the brain despite ART
Smith et al, 2014

ART eliminates viral RNA from brain samples collected from SIV-infected macaques, but does not alter the amount of SIV DNA in the brain
Clements et al, 2011
3. Analyses of viral RNA isolated from the CSF of subjects on therapy
3. Analyses of viral RNA isolated from the CSF of subjects on therapy

**Asymptomatic CSF Escape:**
Well controlled plasma viremia but elevated CSF viremia.
No neurologic symptoms.
3. Analyses of viral RNA isolated from the CSF of subjects on therapy

THINC: Asymptomatic CSF escape may reveal information about active CNS reservoirs

**Basic Design:**
- Examine CSF and blood plasma viral loads.
- Determine whether CSF escape persists across multiple time-points.
- Examine viral diversity.
- Examine macrophage tropism.
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC: CSF escape may reveal information about active CNS reservoirs

THINC Cohort (treatment experienced)
- 114 HIV-infected subjects on ART for > 1 year
- Enrolled at UNC, Yale and UCSF
- 86% Male
- 36% Black, 46% White

UNC Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC: 7% of subjects (7 of 97) have CSF viral loads > 40 cp/ml

![Graph showing plasma viral load vs. CSF viral load](UNC - Joseph et al., THINC study)
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

**THINC:** There is overall no evidence of higher levels of HIV in the CSF of most HIV-infected subjects on therapy

- 76% of plasma samples are above the limit of detection
- 17% of CSF samples are above the limit of detection

*UNC - Joseph et al., THINC study*
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC: 6% of subjects (6 of 97) have asymptomatic CSF escape

Virologic failure?
CSF VL = 637 cp/ml
Plasma VL = 568 cp/ml

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

CSF Escape Is Associated With A History of Plasma Blips

Subjects Enrolled in the THINC Cohort

<table>
<thead>
<tr>
<th></th>
<th>Without CSF Escape (N=91)</th>
<th>With CSF Escape (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of plasma blip</td>
<td>30%</td>
<td>30%</td>
</tr>
<tr>
<td>No history of plasma blip</td>
<td>70%</td>
<td>70%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>CSF escape</th>
<th>No CSF escape</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of plasma blip</td>
<td>5</td>
<td>27</td>
</tr>
<tr>
<td>No history of plasma blip</td>
<td>1</td>
<td>64</td>
</tr>
</tbody>
</table>

Fisher’s exact test, p=0.016

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

- TDF/FTC/DRV/RTV (3025)
- TDF/FTC/DRV/RTV (3026)
- TDF/FTC/DRV/RTV (340)
- ABC / DRV / DTG / 3TC / RTV / TDF (357)
- EFV/TDF/FTC (1018)
- EFV/TDF/FTC (3017)

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

Escape subjects do not have CSF drug concentrations that are consistently lower than cohort median

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC: Some subjects with asymptomatic CSF escape have extremely high pleocytosis

Size = CSF pleocytosis

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

Detailed analyses of four subjects can reveal information about active CNS reservoirs

UNC - Joseph et al., THINC study
THINC Subject 1: A transient and homogeneous CSF population is not evidence of a CNS reservoir

3. Analyses of viral RNA isolated from the CNS of subjects on therapy

**THINC Subject 1**: Homogeneous CSF-derived env sequences

**T1**: Blood plasma

**T2**: CSF

Log HIV viral load (RNA copies/ml)

- **Started DRV/r/ TDF/ FTC 7 years after diagnosis**

- Months on HAART

- T1: 20

- T2: 30

- 0.01

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC Subject 1: T cell-tropic based on Affinofile entry assay

UNC - Joseph et al., THINC study
What mechanisms generate clonal viral lineages in the CNS?

A. An infected T cell migrates into CNS

Unlikely because a single infected cell couldn’t produce this much virus
CSF viral load = 1295
1295 X 150 ml of CSF = 194,250 virions

B. An infected T cell migrates into CNS and clonally expands

C. An infected T cell migrates into CNS and produces virus that infects other cells (likely T cells, possibly macrophage)
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC Subject 2: A persistent CSF population

[Graph showing changes in HIV viral load in blood plasma and CSF over time on HAART.]

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

**THINC Subject 2**: A persistent and heterogeneous CSF population is evidence of an active (not latent) CNS reservoir.

UNC - Joseph et al., THINC study
THINC Subject 2: The elevated macrophage tropism of the persistent CSF population is consistent with virus production from myeloid lineage cells

3. Analyses of viral RNA isolated from the CNS of subjects on therapy

![Bar chart showing percent infectivity at low CD4](chart.png)
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC Subject 3: Two clonal lineages. CSF escape observed at two time-points (2 months apart).

![Graph showing viral load over time](image)

**T1:** Two clonal lineages of CSF- and blood-derived env sequences and an outlier

Log HIV viral load (RNA cp/ml)

- Blood plasma
- CSF

Months on HAART

UNC Joseph et al., THINC study
THINC Subject 4: A transient, but heterogeneous CSF and blood population. No evidence of a CNS reservoir. Virologic failure (CSF VL = 637 cp/ml, Plasma VL = 568 cp/ml)?

3. Analyses of viral RNA isolated from the CNS of subjects on therapy

**T1:** Heterogeneous CSF-and blood-derived **env** sequences

UNC Joseph et al., THINC study
THINC: HIV-1 can persist in the CNS during therapy, but this may be rare

<table>
<thead>
<tr>
<th>Subject</th>
<th>Persistent CSF escape?</th>
<th>Diverse CSF population?</th>
<th>Elevated macrophage tropism?</th>
<th>Drug resistant?</th>
<th>Evidence of persistent CNS reservoir?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Yes</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>No</td>
<td>Yes</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

UNC - Joseph et al., THINC study
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC: Is persistent CSF escape associated with an absence of pleocytosis?

- Transient, T cell-tropic, plasma CD4=210
- Persistent (8 months), diverse, moderately M-tropic, plasma CD4=200
- Transient, diverse, plasma CD4=1180
- Persistent (2 months), two clonal lineages, plasma CD4=327

Size=pleocytosis

UNC - Joseph et al., THINC study
Conclusions – Part 2

1. Detection of HIV-1 proviral DNA in brain tissue collected from subjects on ART indicates that HIV-infected cells can persist in the brain during ART.
   • What proportion of these cells can produce HIV?
Conclusions – Part 2

1. Detection of HIV-1 proviral DNA in brain tissue collected from subjects on ART indicates that HIV-infected cells can persist in the brain during ART.
   • What proportion of these cells can produce HIV?

2. HIV-1 RNA is not consistently identified in brain tissue.
   • Is this due to degradation of RNA prior to analysis or suppression?
Conclusions – Part 2

1. Detection of HIV-1 proviral DNA in brain tissue collected from subjects on ART indicates that HIV-infected cells can persist in the brain during ART.
   • What proportion of these cells can produce HIV?

2. HIV-1 RNA is not consistently identified in brain tissue.
   • Is this due to degradation of RNA prior to analysis or suppression?

3. ~6% of HIV-infected subjects on ART have asymptomatic CSF escape.
   • How well does CSF sample escape populations in the brain parenchyma?
Conclusions – Part 2

1. Detection of HIV-1 proviral DNA in brain tissue collected from subjects on ART indicates that HIV-infected cells can persist in the brain during ART.
   • What proportion of these cells can produce HIV?

2. HIV-1 RNA is not consistently identified in brain tissue.
   • Is this due to degradation of RNA prior to analysis or suppression?

3. ~6% of HIV-infected subjects on ART have asymptomatic CSF escape.
   • How well does CSF sample escape populations in the brain parenchyma?

1. ~2% of HIV-infected subjects on ART have evidence of active, persistent CNS reservoirs.
   • Do these active reservoirs generate neurocognitive damage?
   • Is drug resistance, macrophage-tropism and/or pleocytosis associated with persistent CSF escape?
Acknowledgements

THINC members:

**UNC**
- Joe Eron
- Prema Menezes
- Jessica Margolis
- Natalie Bowman
- Chris Lippincott
- Michael Vinikoor
- Laura Kincer
- Kevin R Robertson
- Sarah Yosief
- Charlie Upton
- Paul Camarena
- UNC retrovirology

**Emory**
- Albert Anderson
- David Loring

**Swanstrom Lab**
- Ron Swanstrom
- Kate Arrildt
- Laura Kincer
- Li-Hua Ping, MD
- Gretja Schnell
- Maria Bednar
- Christa Sturdevant

**UCSF**
- Richard W Price
- Jaclyn Javerbaum
- Julia Peterson
- Alex Russell

**Yale**
- Serena Spudich

**NNTC**

**Funding**
- National Institutes of Health (NIH)
- National Institute of Mental Health (NIMH)