HIV pathogenesis and persistence in the CNS: The stage is set in primary infection
What is the early natural history of HIV infection and injury in the CNS?

Does early ART impact long term CNS HIV pathogenesis and persistence?*

Does HIV persist in the CNS during ART?

*And how do we test this?
What is the early natural history of HIV infection and injury in the CNS?

Does HIV persist in the CNS during ART?

Does early ART impact long term CNS HIV pathogenesis and persistence?*

*And how do we test this?
Abnormal macrophage/microglial activation detected during ART with plasma viral suppression

Positron emission tomography (PET): Abnormal brain uptake of ligand specific for activated microglial cells (>3 years of ART vs HIV-).

Brain autopsy: Increased CD68+ cells (macrophages/microglia) in > 1.5 years ART vs HIV-.

CSF: Elevated CSF neopterin, marker of macrophage activation, associates with detectable CSF HIV RNA by single copy assay (> 10 years of ART).

SIV & SHIV RNA detected in macaque brain after > 20 weeks of suppressive ART
CNS HIV replication during ART with plasma viral suppression: CSF HIV ‘escape’

Subject 2000

Latest CD4 308 cells/ul; nadir CD4 60 cells/ul

Peluso et al., AIDS, 2012.
CNS HIV replication during ART with plasma viral suppression: CSF HIV ‘escape’

Subject 2000

Plasma HIV RNA (log_{10} cpm)

Years of Follow-up

CSF WBC = 26 cells/ul

Peluso et al., AIDS, 2012.
CNS HIV replication during ART with plasma viral suppression: CSF HIV ‘escape’

<table>
<thead>
<tr>
<th>PI: I13V, K20R, M36I, I54V, L63P, V82A</th>
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<table>
<thead>
<tr>
<th>Drug</th>
<th>Status</th>
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</thead>
<tbody>
<tr>
<td>Zidovudine</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>Didanosine</td>
<td>Resistance</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>Resistance</td>
</tr>
<tr>
<td>Stavudine</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>Abacavir</td>
<td>Resistance</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>Resistance</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>No Evidence of Resistance</td>
</tr>
<tr>
<td>Delavirdine</td>
<td>No Evidence of Resistance</td>
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<tr>
<td>Efavirenz</td>
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<tr>
<td>Saquinavir</td>
<td>Resistance</td>
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<tr>
<td>Indinavir</td>
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<tr>
<td>Ritonavir</td>
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<tr>
<td>Nelfinavir</td>
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<td>Amprenavir</td>
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<tr>
<td>Lopinavir/r</td>
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<tr>
<td>Atazanavir</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>Atazanavir/r</td>
<td>Possible Resistance</td>
</tr>
<tr>
<td>Tipranavir/r</td>
<td>Possible Resistance</td>
</tr>
</tbody>
</table>

Resolution of signs and symptoms

Peluso et al., AIDS, 2012.
CSF HIV escape variants that persist over time suggest a replicating source in the CNS

S. Joseph et al., CID 2018
What is the early natural history of HIV infection and injury in the CNS?

Does early ART impact long term CNS HIV pathogenesis and persistence?*

Does HIV persist in the CNS during ART?

*And how do we test this?
Real-time screening of 285,674 samples in Thailand

Acute HIV infection (n=544 enrolled/681 detected)

Immediate ART (n=539)

Optional procedures (number)
- Lumbar puncture (340)
- Brain MRI /MRS (708)
- Sigmoid biopsy (196)
- Leukapheresis (318)
- Inguinal LN biopsy (156)
- Genital Secretion (1034)

Overall study director: Jintanat Ananworanich

Data as of 31 May 2018
Identification of recent (<12 months) infection by nucleic acid testing or recent negative Ab test

Primary infection enrolled - San Francisco (n=109)

Gothenburg (n=27)
Milan (n=18)
Sydney (n=6)

- Phlebotomy
- Neuropsychological testing
- MRI/MRS
- Lumbar puncture

Follow-up: 6 weeks, each 6 months thereafter (n=136, total visits=711)

Observational study; participants could elect to start ART

ART total visits=379
No ART total visits=332

## Acute and Primary HIV Study Participants

<table>
<thead>
<tr>
<th></th>
<th>RV254/Acute HIV n = 539</th>
<th>PISCES/Primary HIV n = 160</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26 (21 - 36)</td>
<td>37 (29 – 45)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>512 (95%)</td>
<td>150 (94%)</td>
</tr>
<tr>
<td>Estimated duration of infection, days</td>
<td>19 (14 - 25)</td>
<td>88 (52-149)</td>
</tr>
<tr>
<td>Fiebig I/II, n (%)</td>
<td>206 (38%)</td>
<td>NA</td>
</tr>
<tr>
<td>HIV Subtype</td>
<td>81% CRF01_AE</td>
<td>90% presumed B</td>
</tr>
<tr>
<td>Duration of infection prior to ART</td>
<td>21 days</td>
<td>229 days</td>
</tr>
</tbody>
</table>

Values are number (%) or median (IQR)

**Acute HIV Thailand** - Ananworanich, Valcour

**Primary HIV US, Europe, Australia** - Price, Hecht, Gisslen, Hagberg, Cinque, Brew
HIV RNA in CSF parallels plasma in early infection

Spudich et al. JID 2011; Chan et al. JID 2018
Macrophage activation in the CNS is triggered in early infection.

Neopterin, biomarker of macrophage activation, is elevated in the CSF compared to HIV negative (dotted line) throughout early infection.
Neuronal injury begins during primary infection

Neurofilament light chain (NFL) = specific CSF biomarker of axonal injury

Acute HIV = median 19 days post infection
Primary HIV = median 90 days post infection

Thalamus volume declines in early infection prior to ART (red line)

Peluso et al., JID 2013; Sanford et al, CID 2018
When does CNS HIV compartmentalization start during the course of infection?
No CNS compartmentalization detected during acute HIV by single genome amplification of env.

Median duration of infection = 17 days

Phylogenetic tree of plasma & CSF HIV env sequences

* Multiple T/F

Plasma

CSF

CM244

INHC Internal
Enrichment of minor variants in CSF during acute infection detected by deep sequencing

IonTorrent PGM deep sequencing platform, n = 17 acute HIV participants

Frequency of major, minor#1, minor#2 and recombinants in protease (PR) and reverse transcriptase (RT)

Sirijatuphat, et al, CROI 2017; Tovanabutra et al., submitted
When does CNS HIV compartmentalization start during the course of infection?
Variable compartmentalization of *env* sequences between plasma and CSF in primary infection

A. Equilibrated

- **Sub. 9001**
  - 308 d.p.i.

B. Compartmentalized

- **Sub. 9040**
  - 352 d.p.i.

- **Sub. 9096**
  - 348 d.p.i.

Blood

CSF

Not Analyzed

Equilibrated (-)

Equilibrated (+)

Intermediate

Compartmentalized

---

Sturdevant C., *PloS Pathogens* 2015
Compartmentalized evolution of CSF HIV variants over first two years of infection

CSF HIV RNA

TMRCA: 134 days

days p.i. P/C
165
352
644
918

Sturdevant C., *PloS Pathogens* 2015
What is the early natural history of HIV infection and injury in the CNS?

Does early ART impact long term CNS HIV pathogenesis and persistence?*

Does HIV persist in the CNS during ART?

*And how do we test this?
ART initiated in acute HIV effectively reduces CSF HIV RNA

Low rate of CSF escape after treatment in acute HIV (n=1/89, 1% vs ~10% reported after treatment in chronic HIV).

Handoko et al., CROI 2019.
ART initiated in acute HIV normalizes CSF myeloid activation (neopterin) & chemokines

**CSF Neopterin**

- HIV negative
- AHI cART naive
- AHI cART 6m
- AHI cART 24m

*\( p<0.001 \)  *\( p<0.001 \)

**CSF CCL2/MCP-1**

- HIV negative
- AHI cART naive
- AHI cART 6m
- AHI cART 24m

*\( p=0.004 \)  *\( p=0.010 \)  *\( p=0.008 \)

Hellmuth et al, JID, 2019.
ART initiated at a median of 225 days post-infection.

Months on cART=13.3 (5.7, 35.3)

ART was initiated in primary HIV does not reverse blood brain barrier injury

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days following cART initiation (t)</td>
<td>1E-5</td>
<td>0.083</td>
</tr>
</tbody>
</table>

Rahimy et al., JID 2017
ART initiated in primary HIV attenuates but does not reverse accumulated brain inflammation

N = 26
ART initiation WPI = 28 (12, 73)
Follow-up weeks = 121 (38, 171)

Young et al., *Neurology* 2014
Compartmentalized CSF HIV detected by single genome sequencing despite ART in primary infection

Blood  CSF

Grey/black – pre-ART sequences
Colors – on ART sequences: interval 1, 2

Participant 9058
Treated in primary Infection

Tree rooted with pre-ART plasma sequence.

Dahl et al., JID, 2014.
HIV-specific CD8 T cells in CSF after ART in acute HIV

Presence of CSF CD8 T cells pre-ART (V1), and after 6 (V13) and 24 months (V19) of ART.

Subra et al. CROI 2019
CNS monitoring of ART interruption after treatment during Fiebig I acute HIV

No detectable CSF HIV RNA\(^1\) (<80 copies/ml) during post-TI rebound plasma viremia.

No significant change in:
- MRI measures of inflammation and neuronal integrity.
- CSF inflammatory measures (neopterin, MCP1, IP10, and sCD14).
- Neuropsychological test performance over time.

\(^1\)Standard Roche COBAS TaqMan HIV-1 Test V2.0.
HIV DNA detected in CSF cells during long-term ART initiated in chronic HIV

ACTG 5321

Median ART duration = 8.6 years

HIV Persistence Measures in CSF
n = 69 samples

Boxes indicate % positive; bars represent 95% confidence intervals

- Among those with detected CSF CA-HIV DNA, median level is similar to blood: 2.1 (range 0.12 - 7.00) copies/10^3 cells.
- CSF cell-associated HIV RNA = intracellular transcribed virus.

A5321 Study Team, submitted
Single cell RNA seq identifies myeloid cell clusters found predominantly in CSF in chronic suppressed HIV

Myeloid-2 cells – share RNA expression patterns with brain-derived microglial cells

During primary HIV infection:
- HIV enters the CNS and immune activation is established.
- HIV can begin to evolve independently within the CNS.

During chronic HIV infection:
- CNS inflammation and viral persistence can be detected during ART.

ART in early infection:
- Reduces inflammation in the CNS.
- May impact long-term viral persistence.

Key questions:
- How early is early enough for ART to prevent neuropathogenesis and establishment of CNS HIV persistence?
- Is adjunctive therapy with immune modulating agents during early HIV needed?
- What is the optimal approach to assess CNS HIV persistence?
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