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

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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)

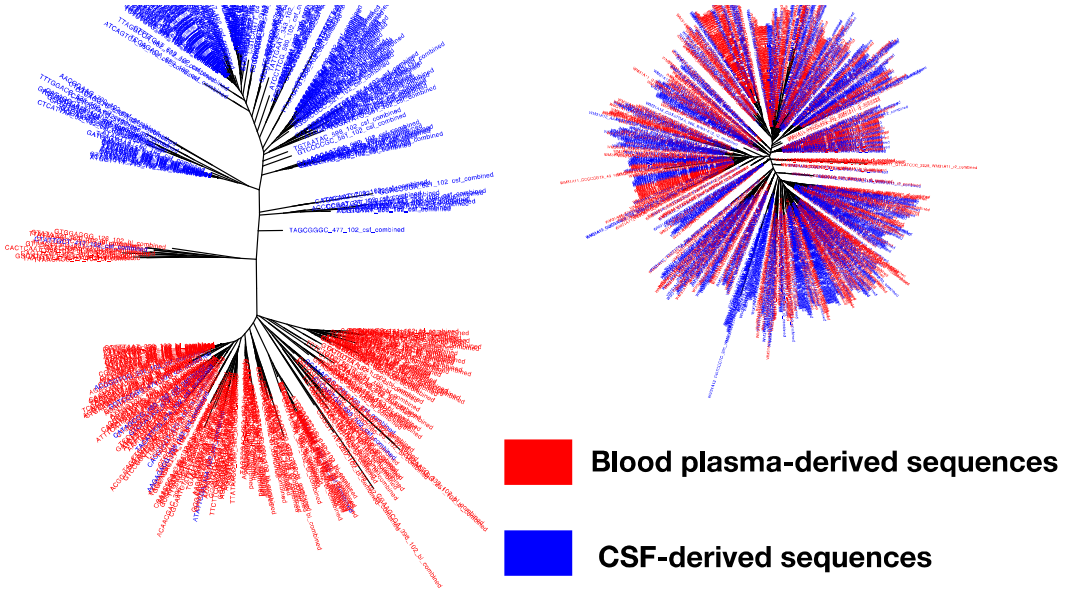
Talk outline

- 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

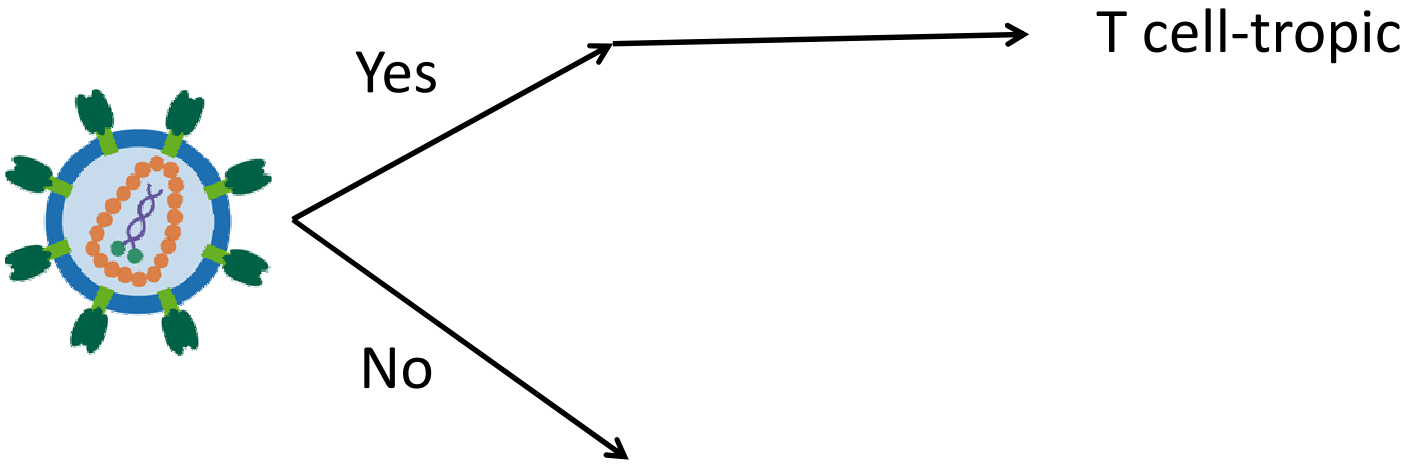


UNC Joseph et al., THINC study

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?



Establishing HIV-1 populations in the CNS

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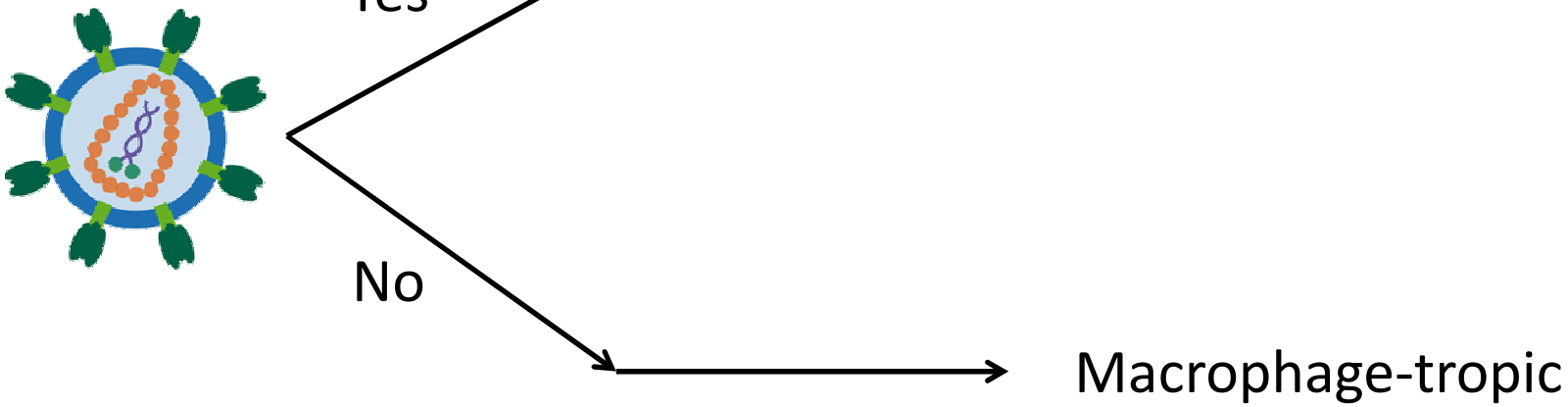


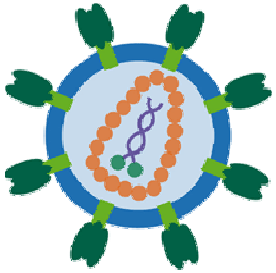
2. A small number of these were tested for replication in monocyte-derived-macrophage (MDM)

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)

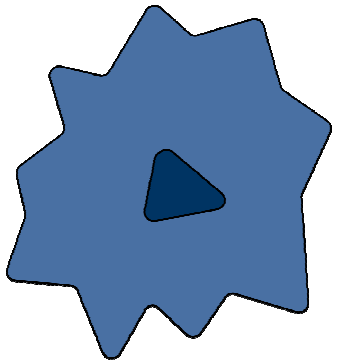
Coreceptor Usage Does Not Define Cellular Tropism

CXCR4 ≠ T cell-tropic

CCR5 ≠ Macrophage-tropic

The vast majority of HIV-1 is R5 T cell-tropic

T Cells Express 26X Higher CD4 Densities Than Macrophages

Monocyte	Monocyte-Derived Macrophage (MDM)	T cell
		
3 CD4 ABS /µm^2	3 CD4 ABS /µm^2	78 CD4 ABS /µm^2

ABS = Antibody Binding Sites For An a-CD4 Antibody

UNC - Joseph et al, 2014

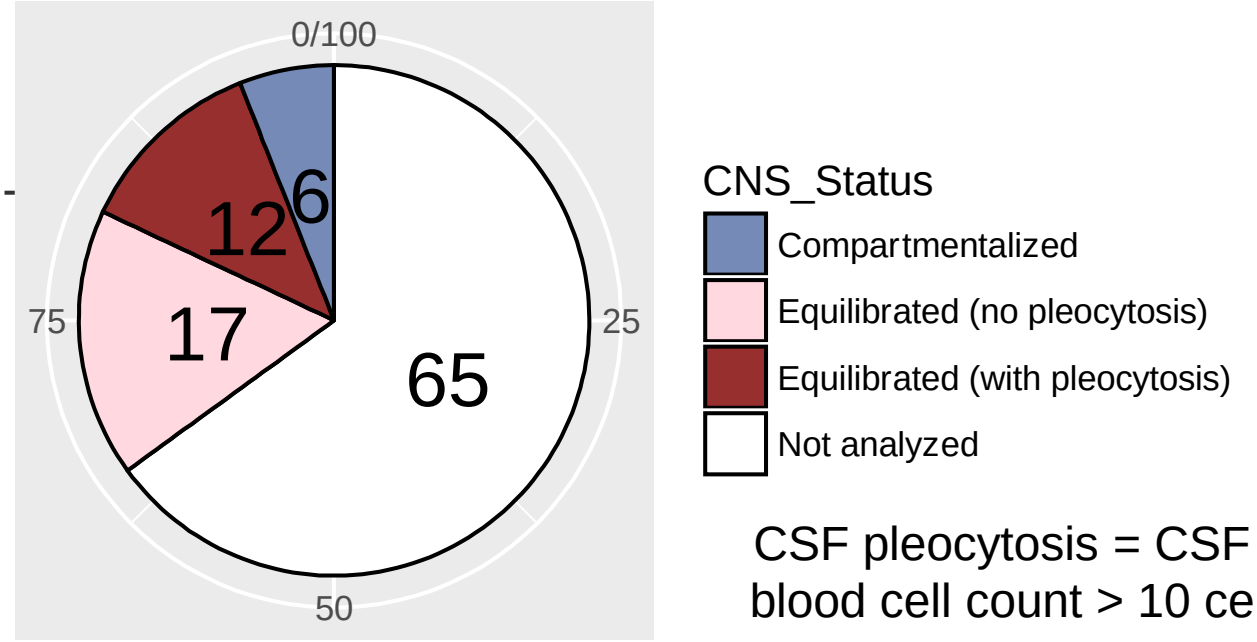
Rationale behind macrophage tropism assay

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

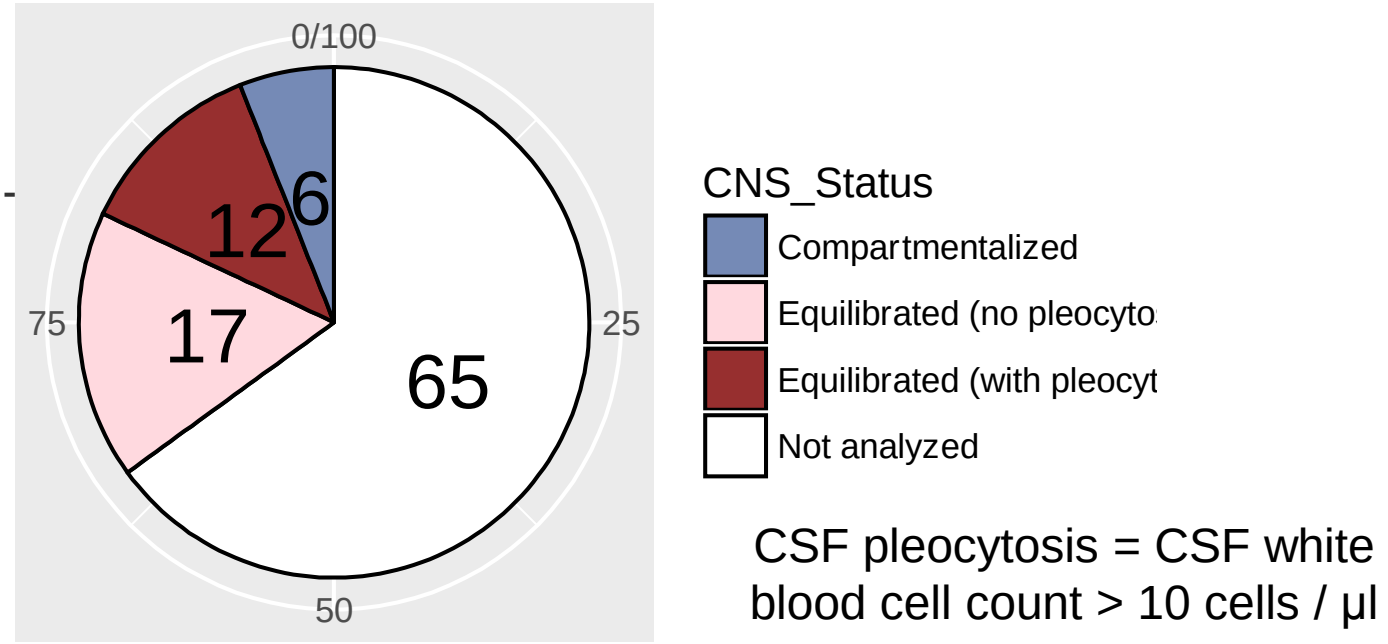


CSF pleocytosis = CSF white blood cell count > 10 cells / µl

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



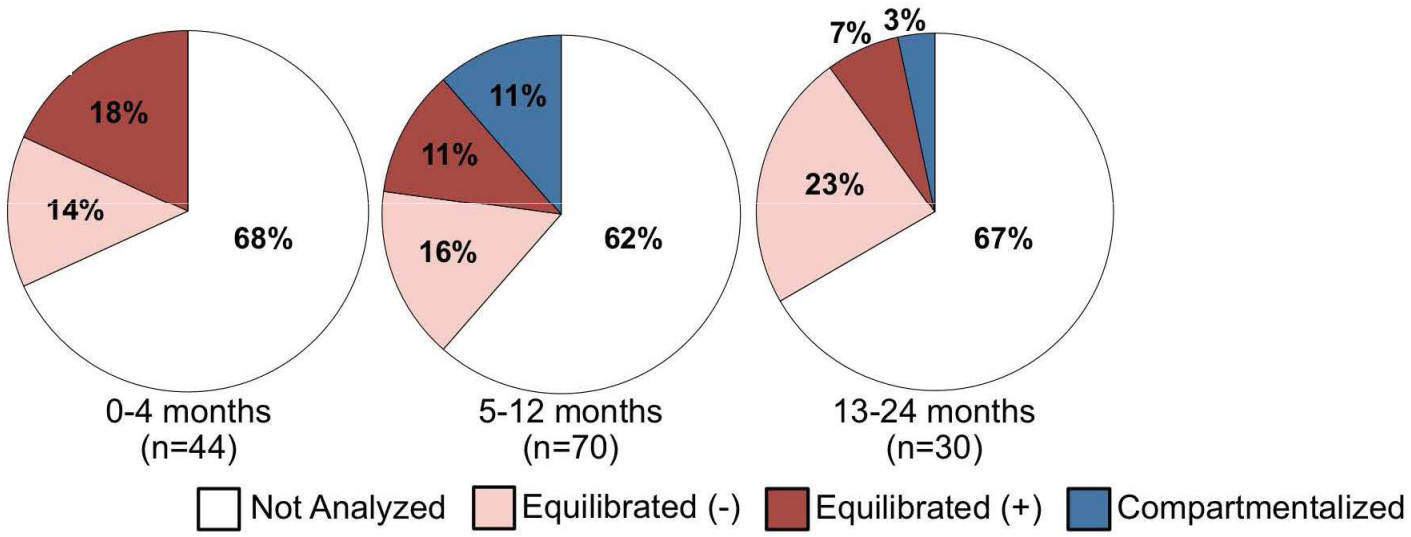
CSF pleocytosis = CSF white blood cell count > 10 cells / µl

~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

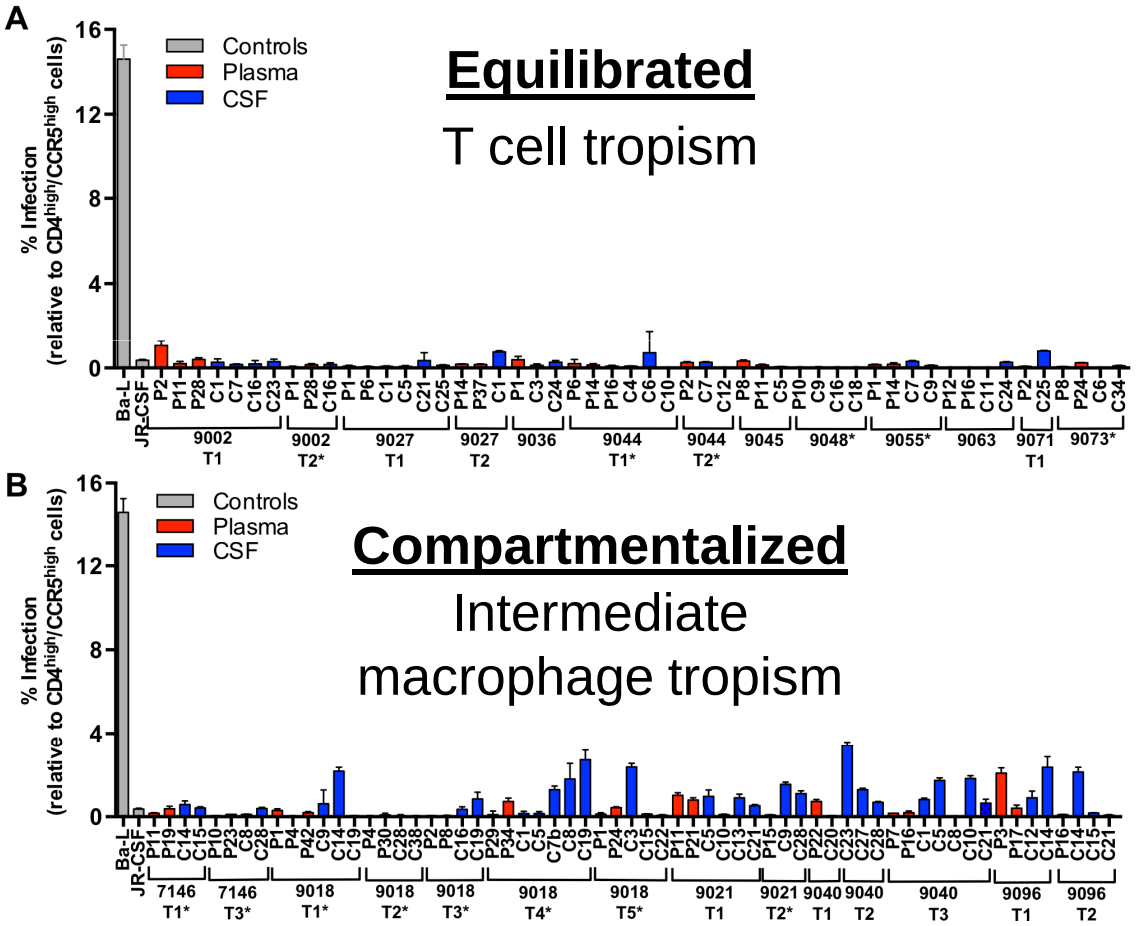
Pleocytosis declines as compartmentalization emerges



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



UNC - Sturdevant et al 2015

Relationship between HIV-1 replication in the CNS and neurocognitive impairment

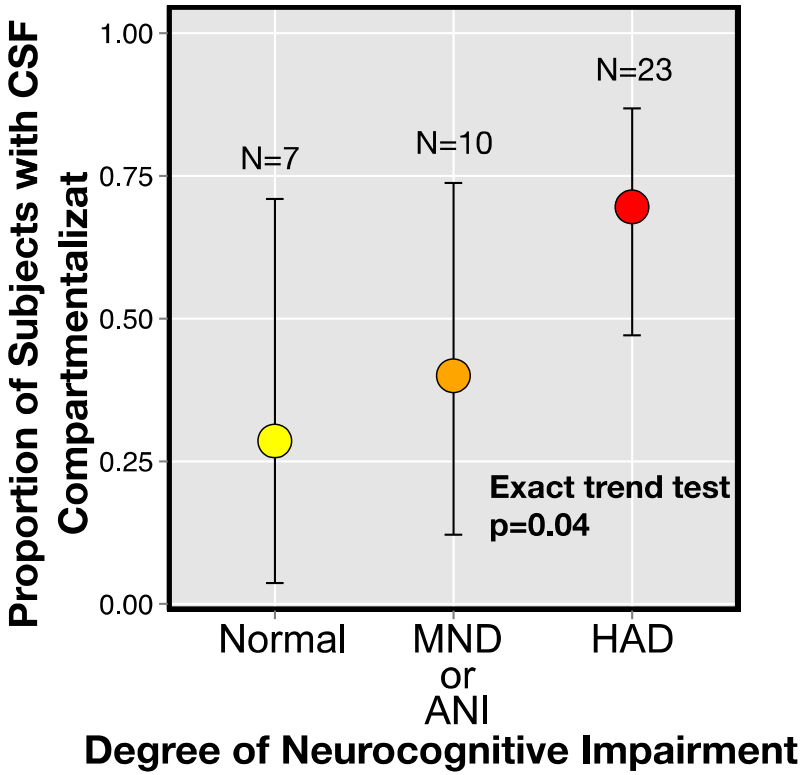
40 HIV-positive, treatment-naïve subjects were enrolled in four cohorts

Cohorts	N	Sequencing method	Number of neurocognitive domains tested
THINC	15	SGA and Deep	8
UCSF	11	SGA	4
NNTC	5	SGA and Deep	7 (no gross motor)
Emory	9	Deep	8

UNC - Joseph et al, CROI 2015

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

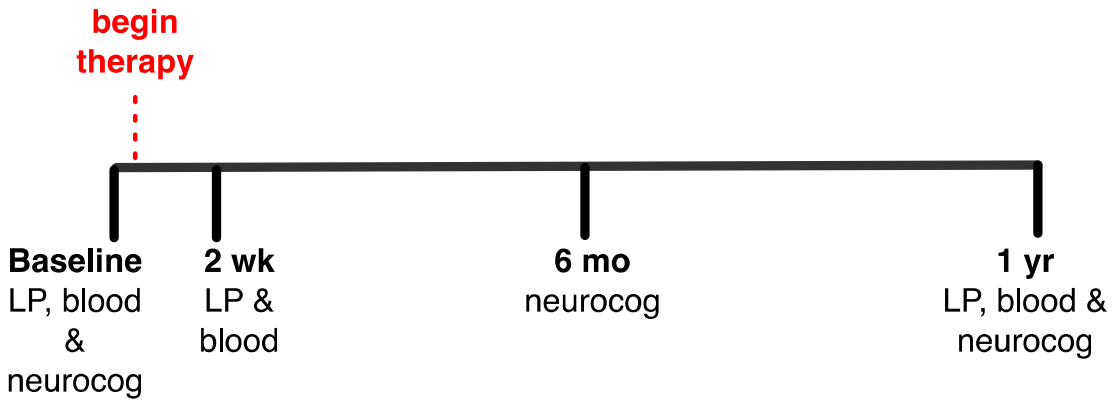
Relationship between HIV-1 replication in the CNS and neurocognitive impairment

THINC study: treatment naive cohort

HIV Tropism, Persistence, Inflammation and Neurocognition in Therapy Initiation

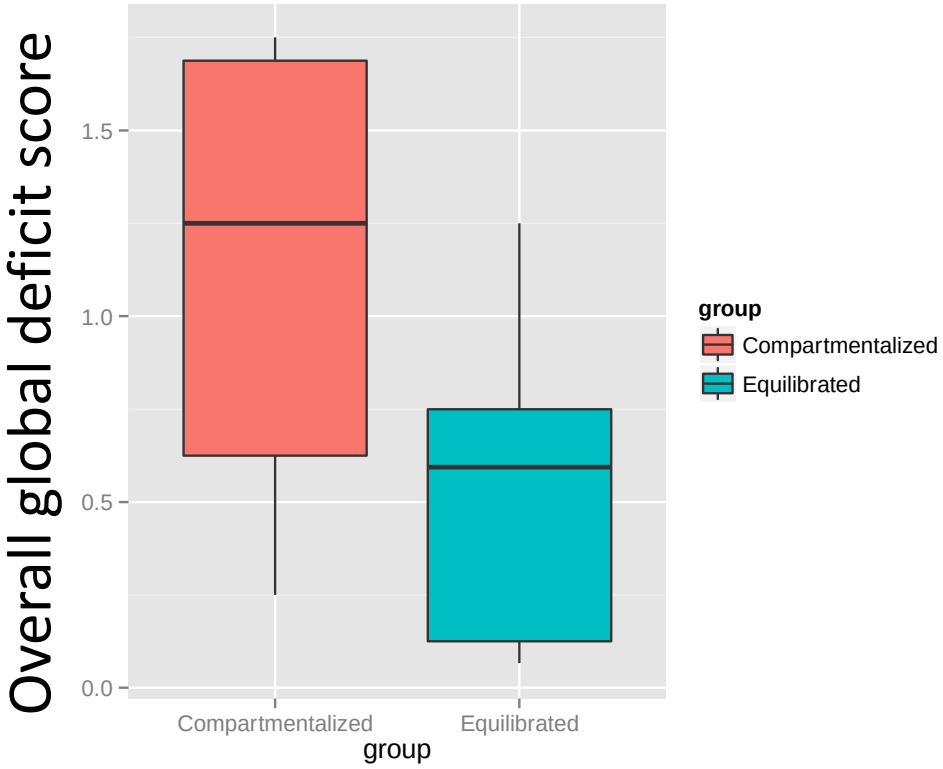
Inclusion criteria:

- 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



Relationship between HIV-1 replication in the CNS and neurocognitive impairment

Neurocognitive impairment due to HIV replication in the CNS does not resolve within a year on ART



UNC - Bowman et al, CROI 2015

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.

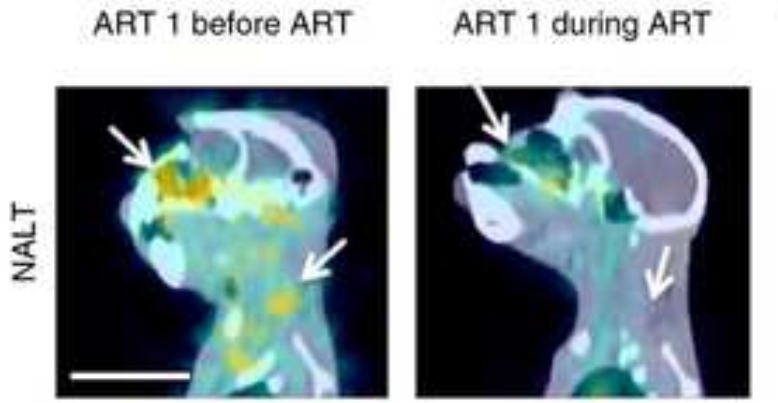
Analyses of viral reservoirs in the CNS

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

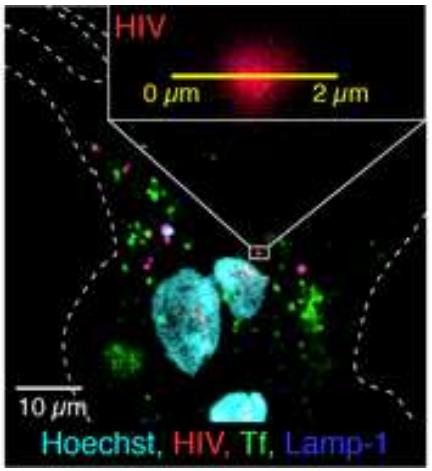
Most minimally- or non-invasive techniques cannot be used to survey the CNS for viral reservoirs



Whole-body PET scan using an anti-gp120 probe reveals sites of HIV production.

- Probe doesn't enter the CNS

Santangelo et al 2015



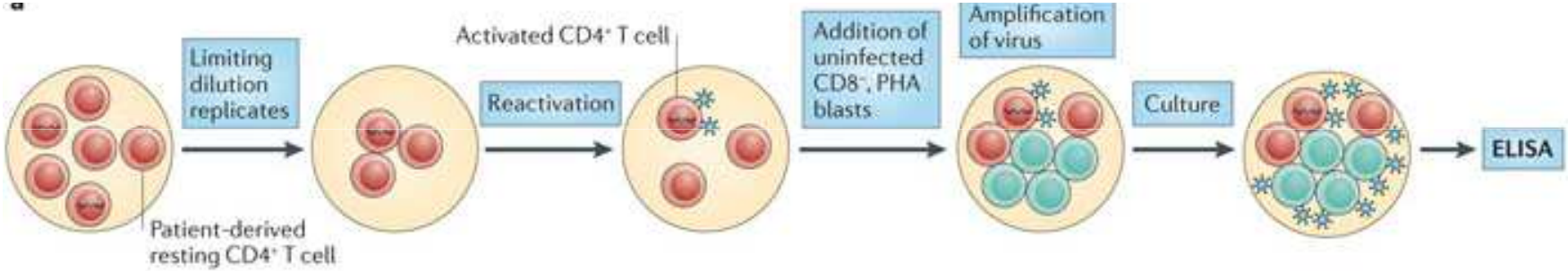
Human follicular dendritic cells (FDCs) isolated from HIV positive subjects on ART treatment retain HIV in endosomal compartments.

- 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

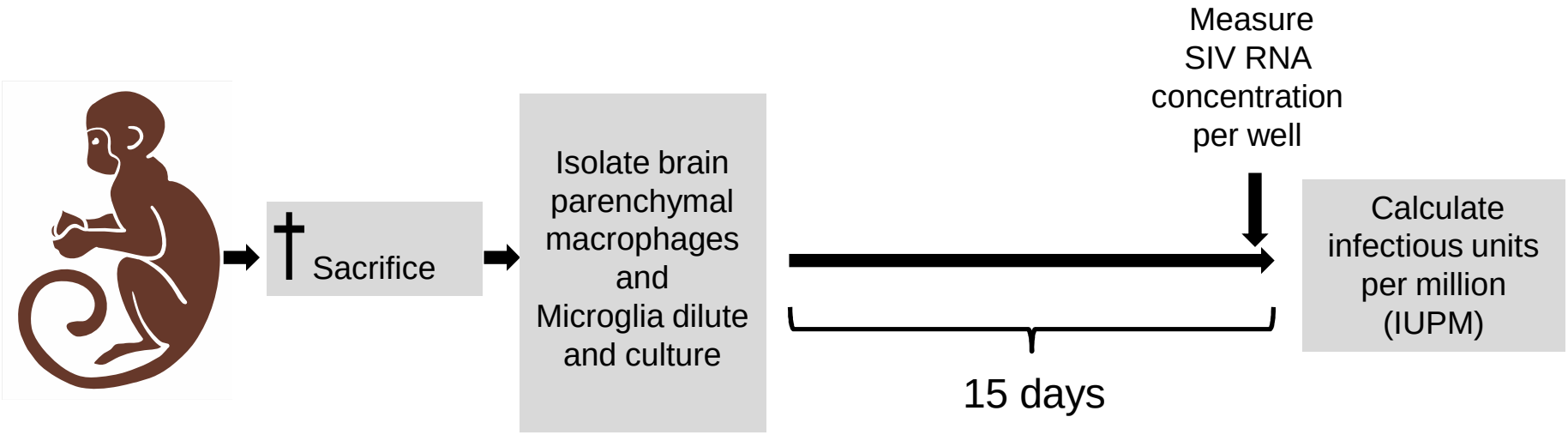
Standard quantitative viral outgrowth assay (QVOA)



UNC - Archin et al 2015

1. Quantitative viral outgrowth assay (QVOA) from CNS-derived cells

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



Avalos et al, 2016

MΦ-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

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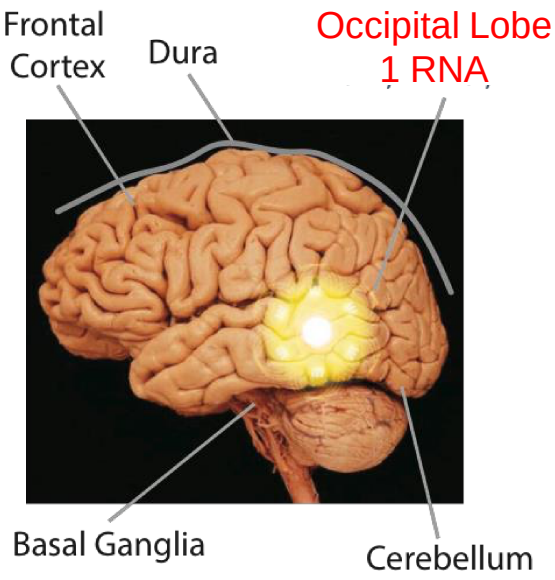
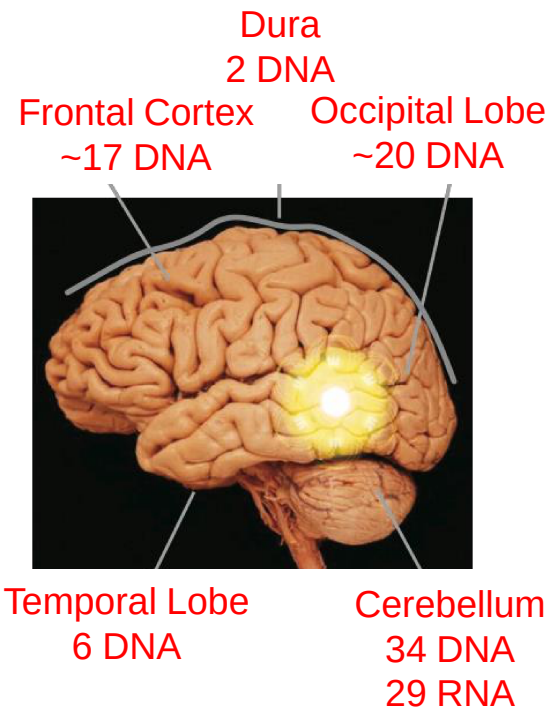


CSF VL < 40 cp/ml
Blood VL < 40 cp/ml

5095



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

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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:

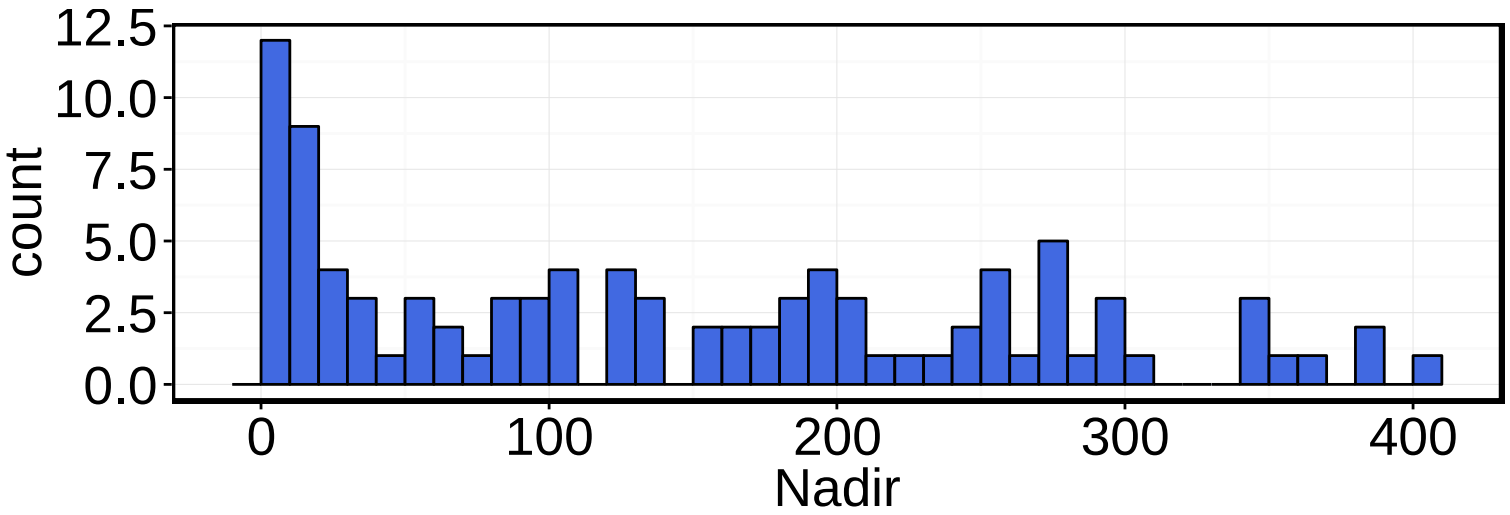
- Examine CSF and blood plasma viral loads.
- Determine whether CSF escape persists across multiple time-points.
- Examine viral diversity.
- Examine macrophage tropism.

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)

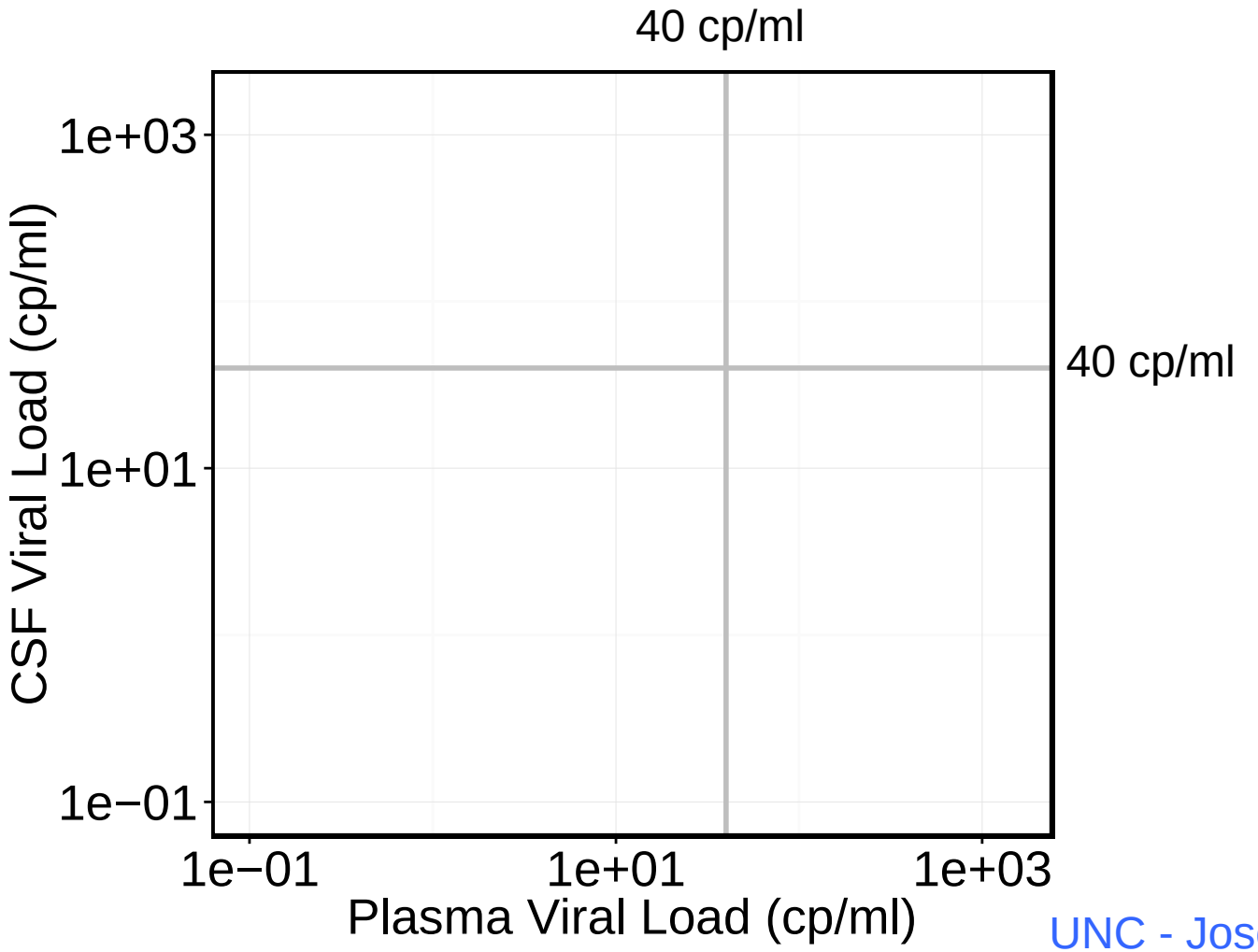
- 114 HIV-infected subjects on ART for > 1 year
- Enrolled at UNC, Yale and UCSF
- 86% Male
- 36% Black, 46% White



UNC Joseph et al., THINC study

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

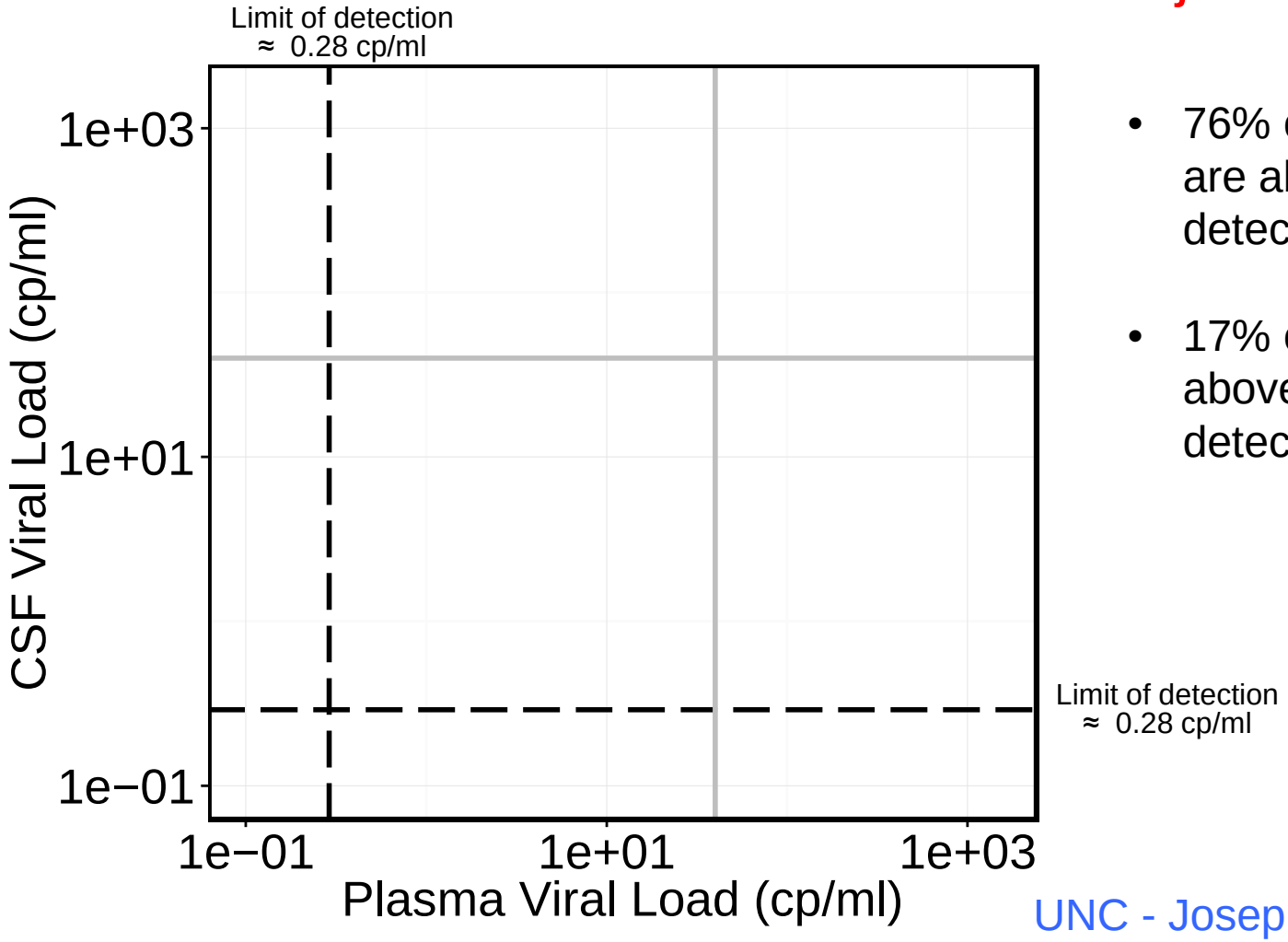
THINC: 7% of subjects (7 of 97) have CSF viral loads > 40 cp/ml



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 HIV in the CSF of most HIV-infected subjects on therapy

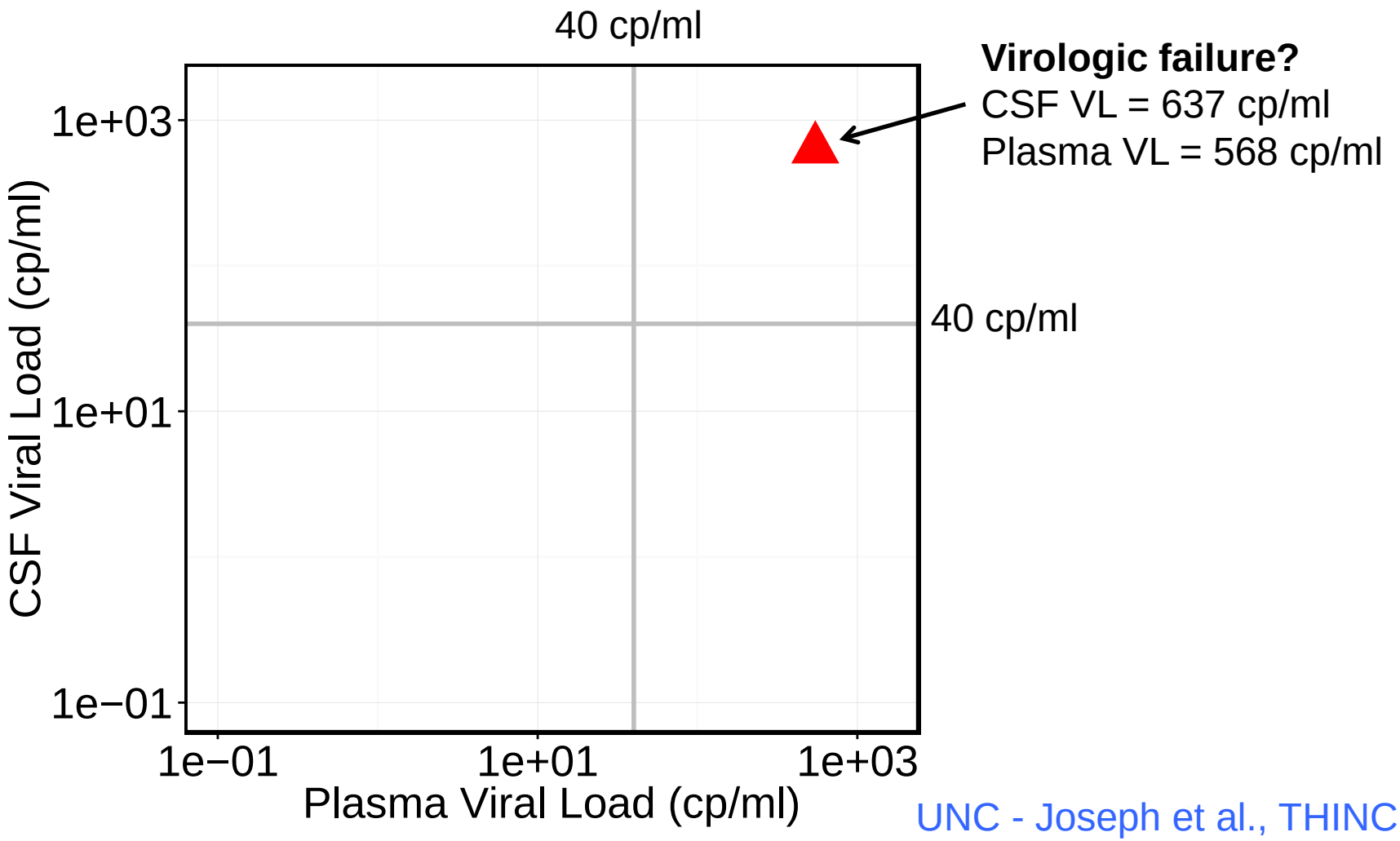


- 76% of plasma samples are above the limit of detection
- 17% of CSF samples are above the limit of detection

UNC - Joseph et al., THINC study

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

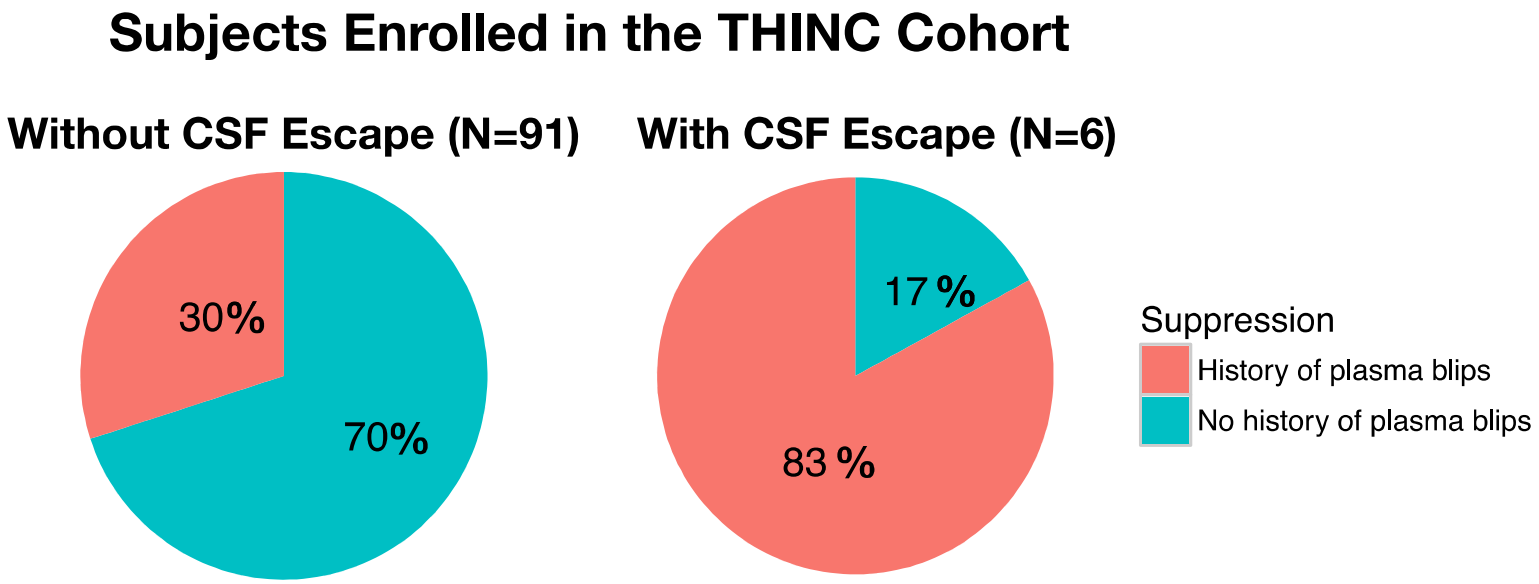
THINC: 6% of subjects (6 of 97) have asymptomatic CSF escape



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

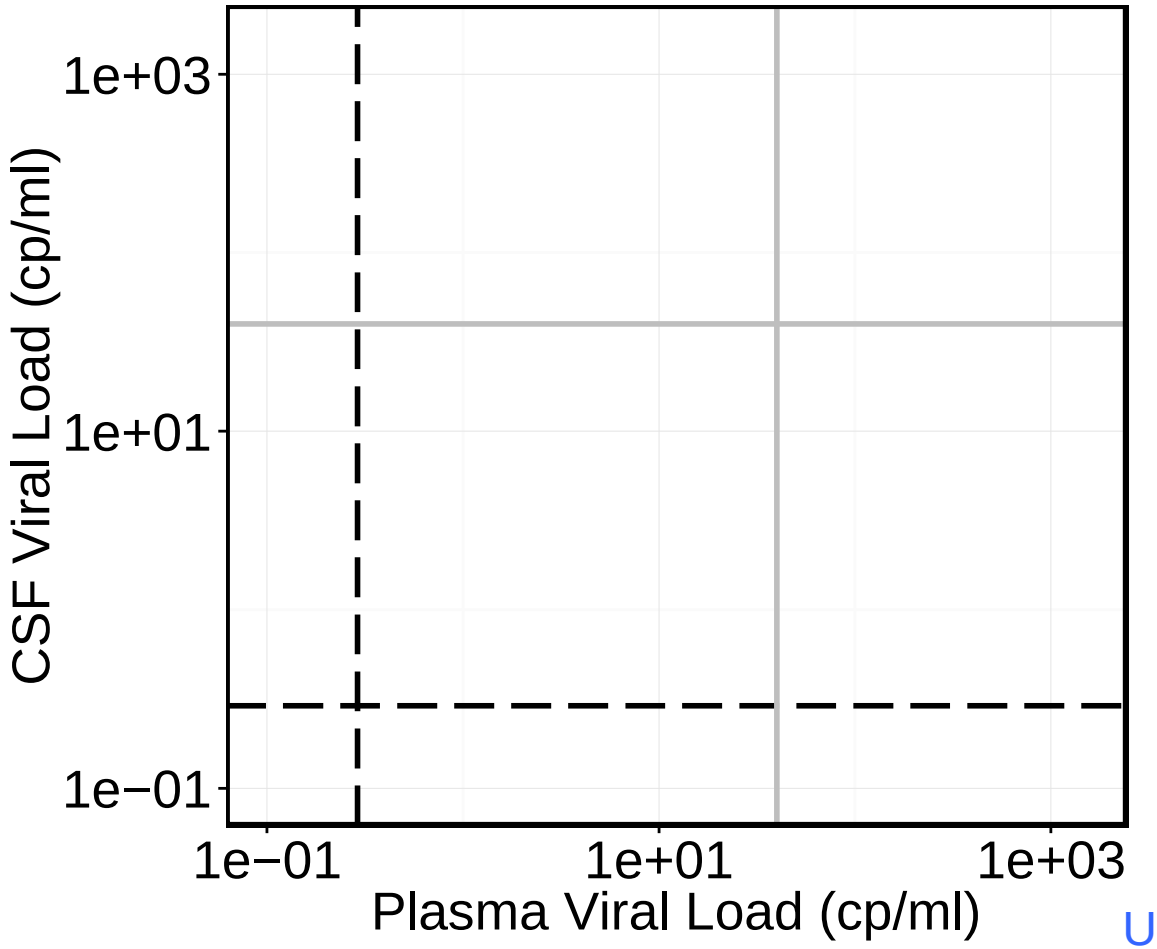


	CSF escape	No CSF escape
History of plasma blip	5	27
No history of plasma blip	1	64

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

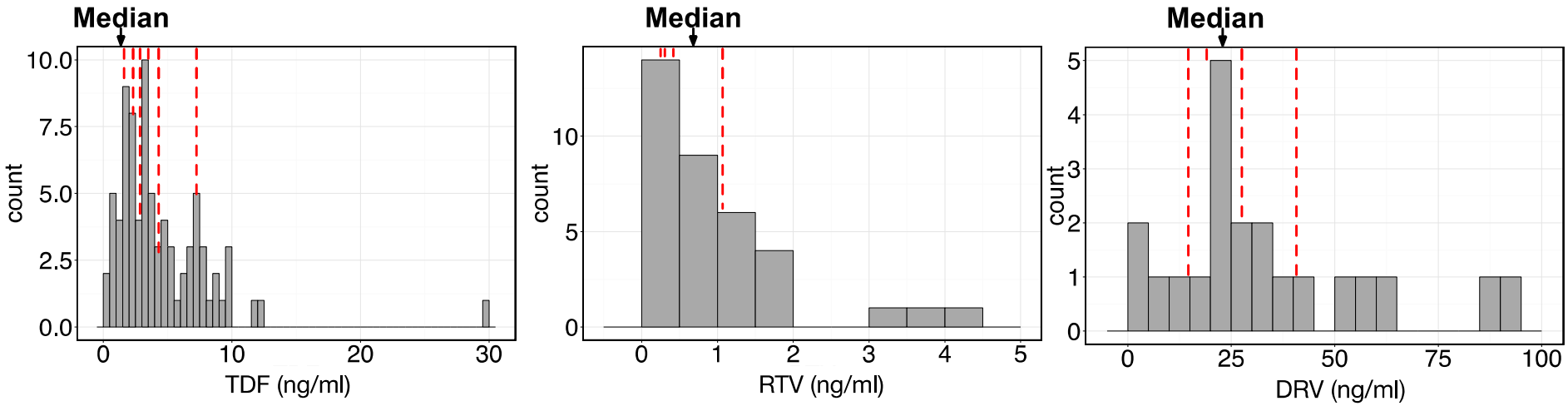


- TDF/FTC/DRV/RTV (3025)
- TDF/FTC/DRV/RTV (3026)
- TDF/FTC/DRV/RTV (340)
- ABC / DRV / DTG / 3TC / RTV / TDF (357)
- EFV/TDF/FTC (1018)
- EFV/TDF/FTC (3017)

UNC - Joseph et al., THINC study

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

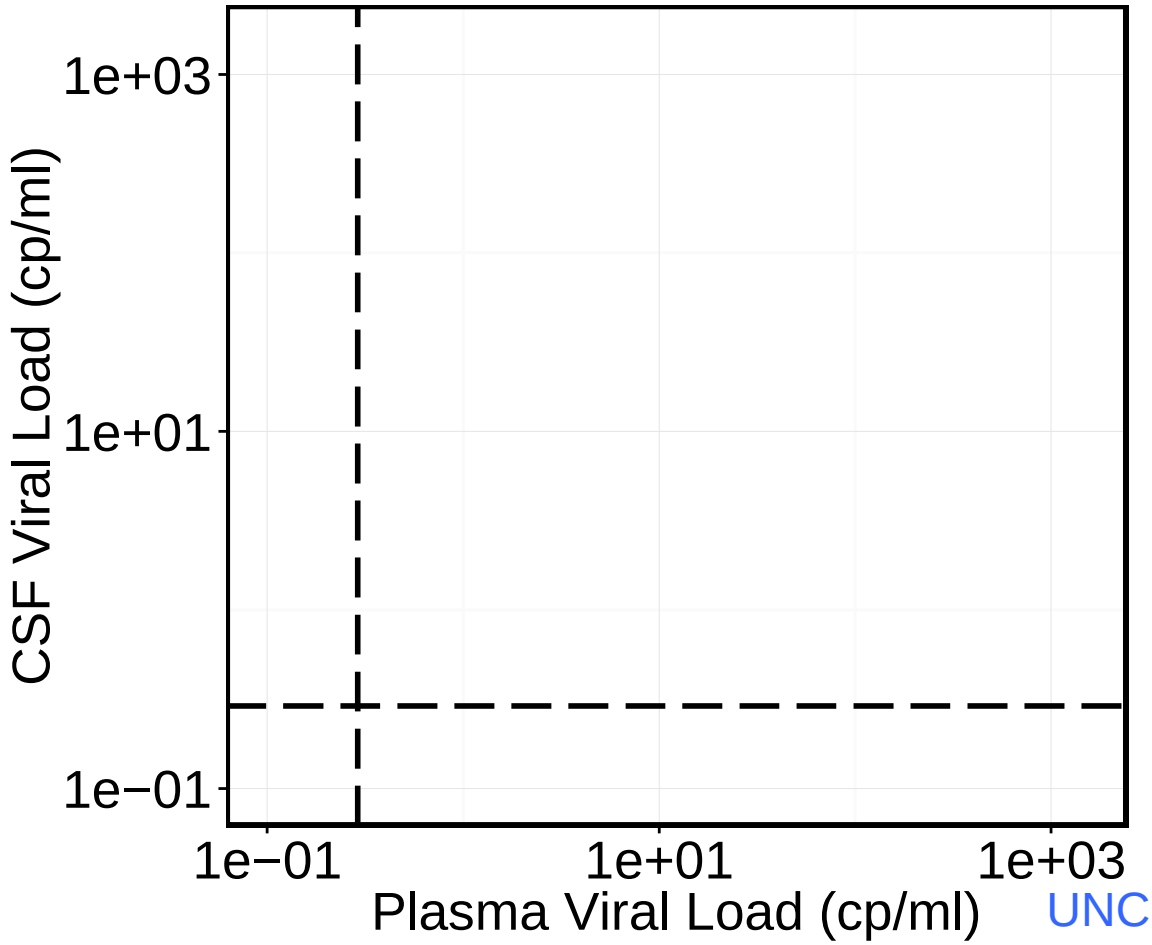
Escape subjects do not have CSF drug concentrations that are consistently lower than cohort median



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

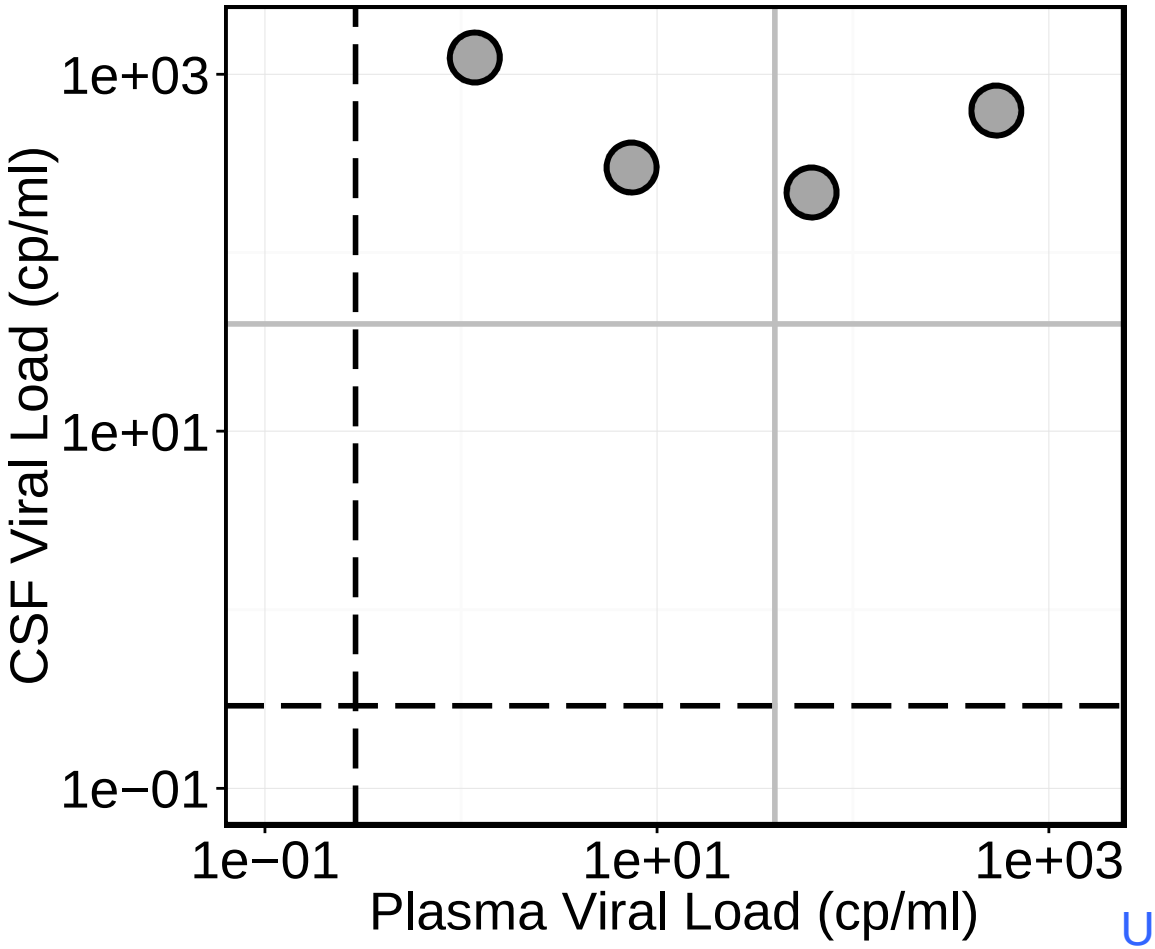


Size = CSF pleocytosis

UNC - Joseph et al., THINC study

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

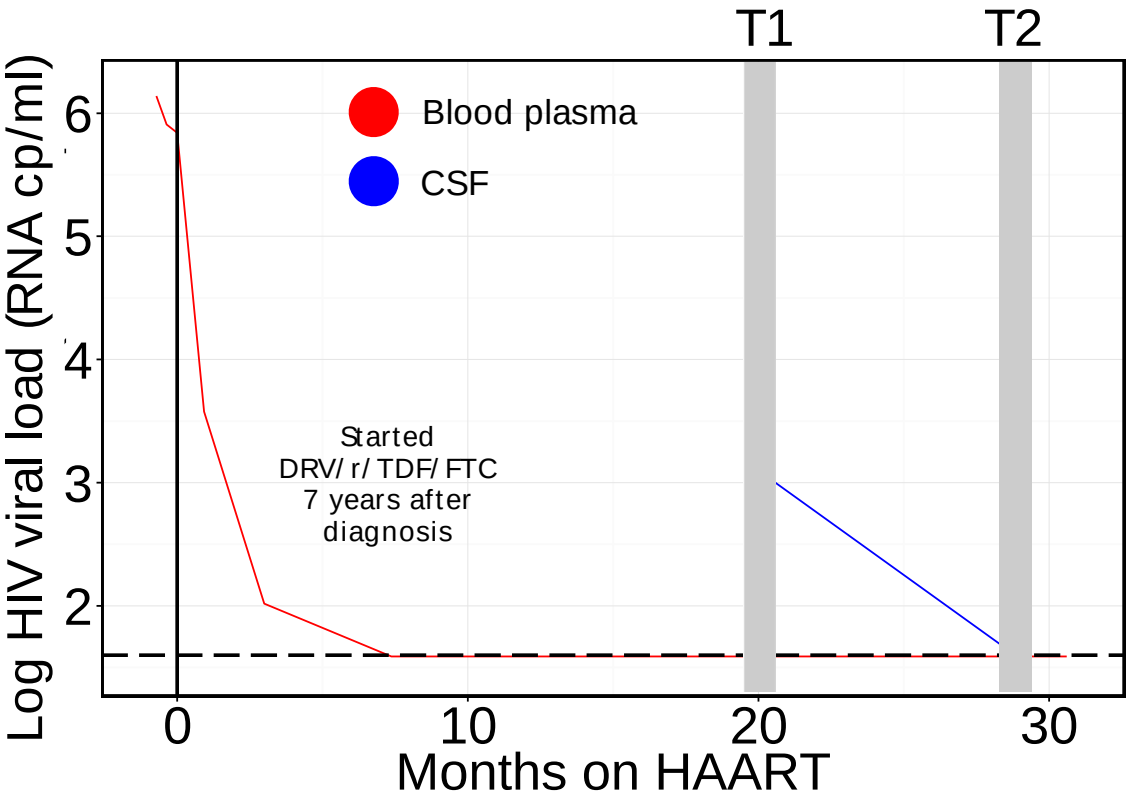
Detailed analyses of four subjects can reveal information about active CNS reservoirs



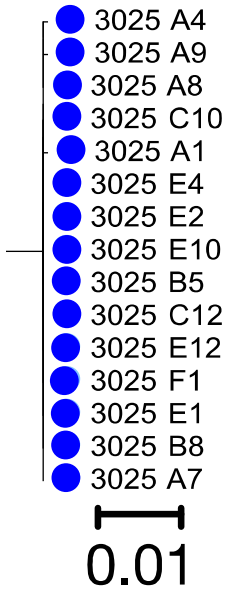
UNC - Joseph et al., THINC study

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

THINC Subject 1: A transient and homogeneous CSF population is not evidence of a CNS reservoir



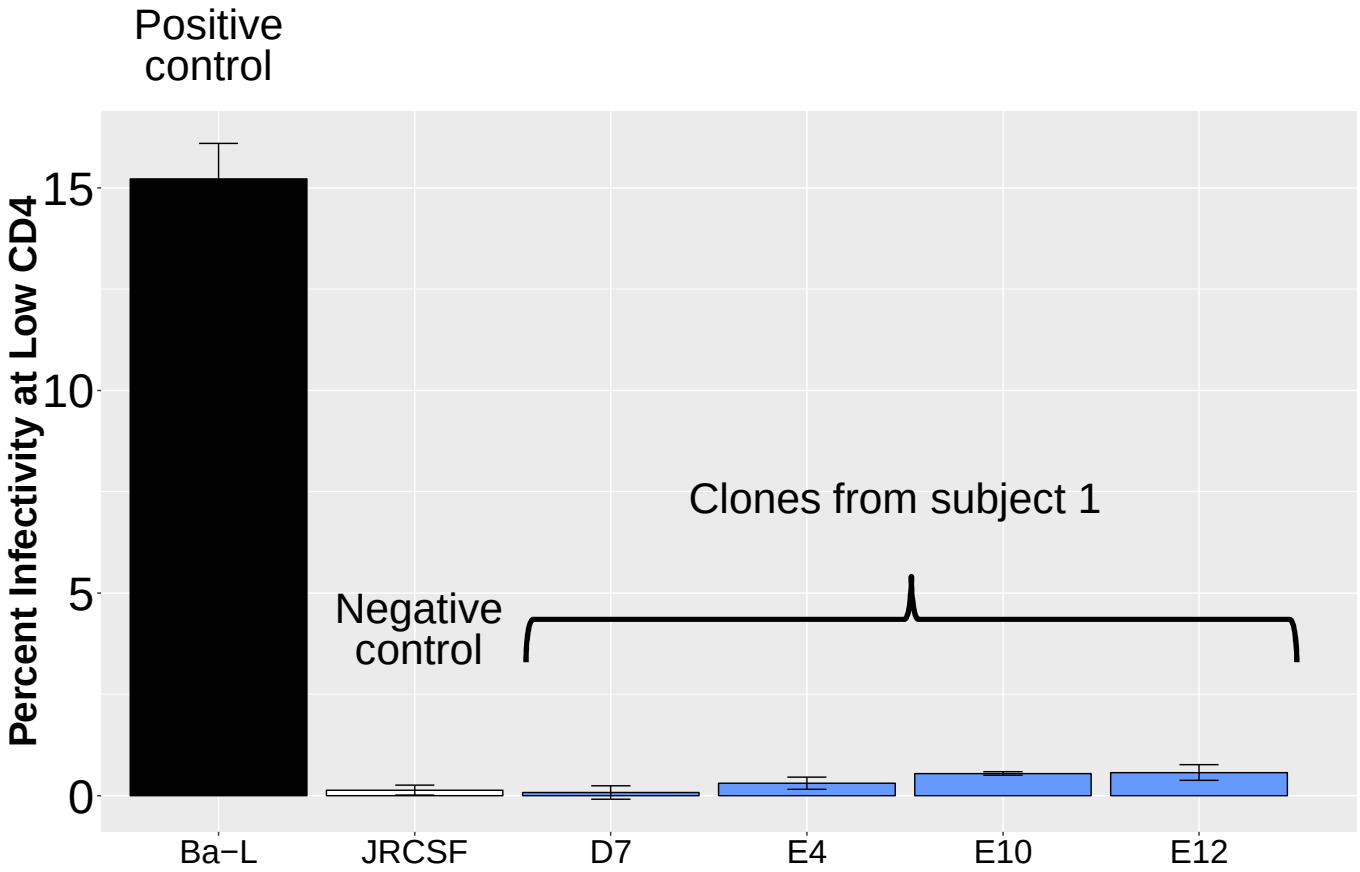
T1: Homogeneous CSF-derived *env* sequences



UNC - Joseph et al., THINC study

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

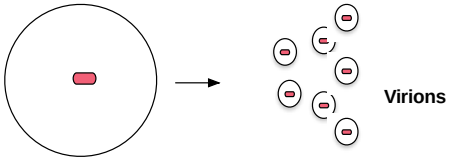
THINC Subject 1: T cell-tropic based on Affinofile entry assay



UNC - Joseph et al., THINC study

What mechanisms generate clonal viral lineages in the CNS?

A. An infected T cell migrates into CNS

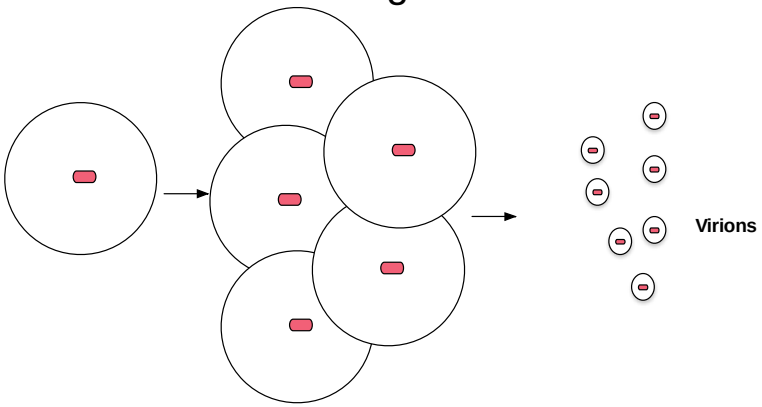


Unlikely because a single infected cell couldn't produce this much virus

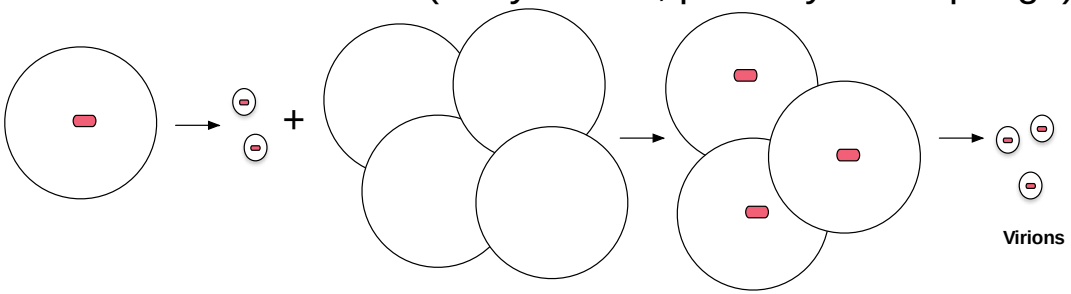
CSF viral load =1295

1295 X 150 ml of CSF = 194,250 virions

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

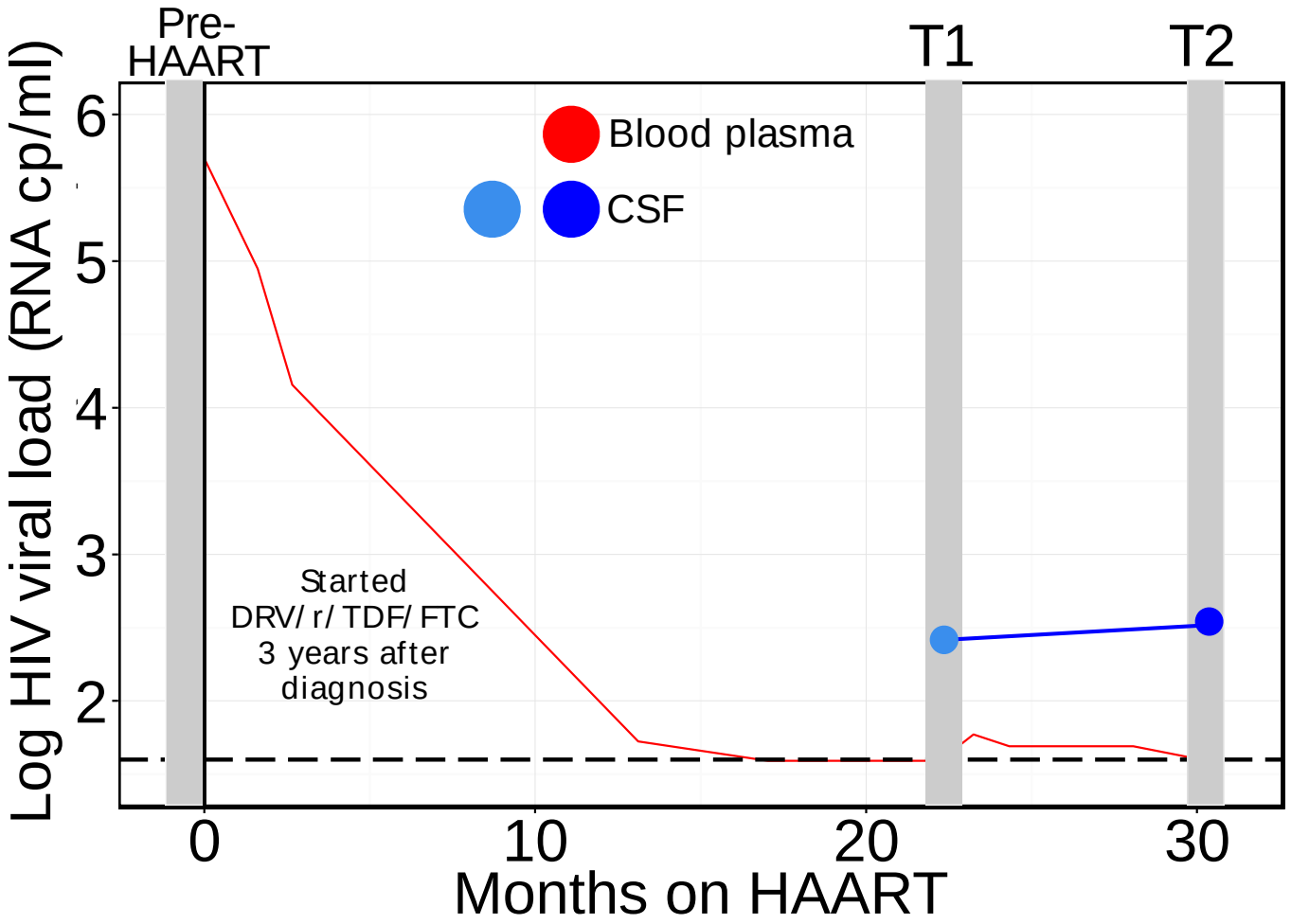


C. An infected T cell migrates into CNS and produces virus that infects other cells (likely T cells, possibly macrophage)



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

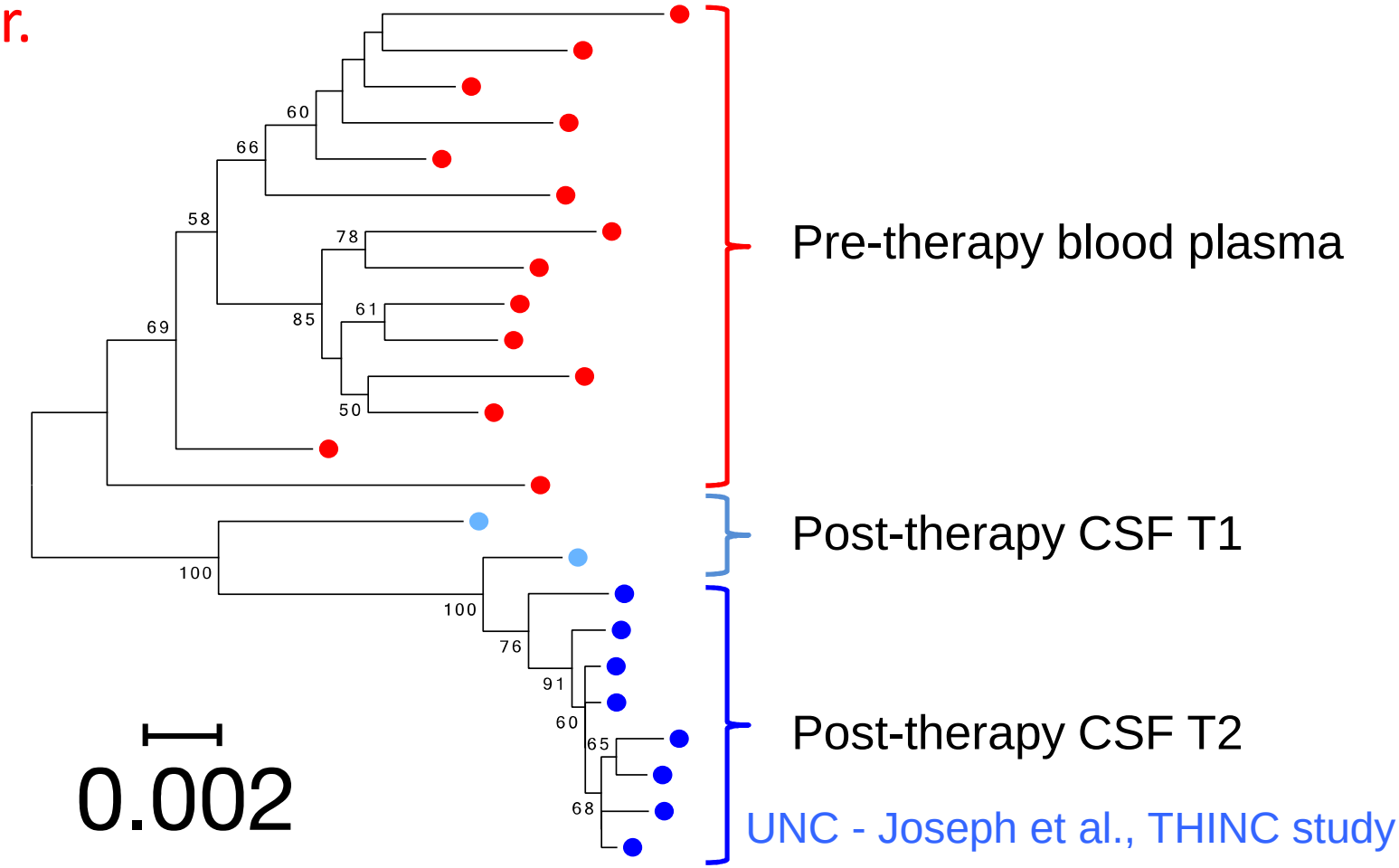
THINC Subject 2: A persistent CSF population



UNC - Joseph et al., THINC study

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

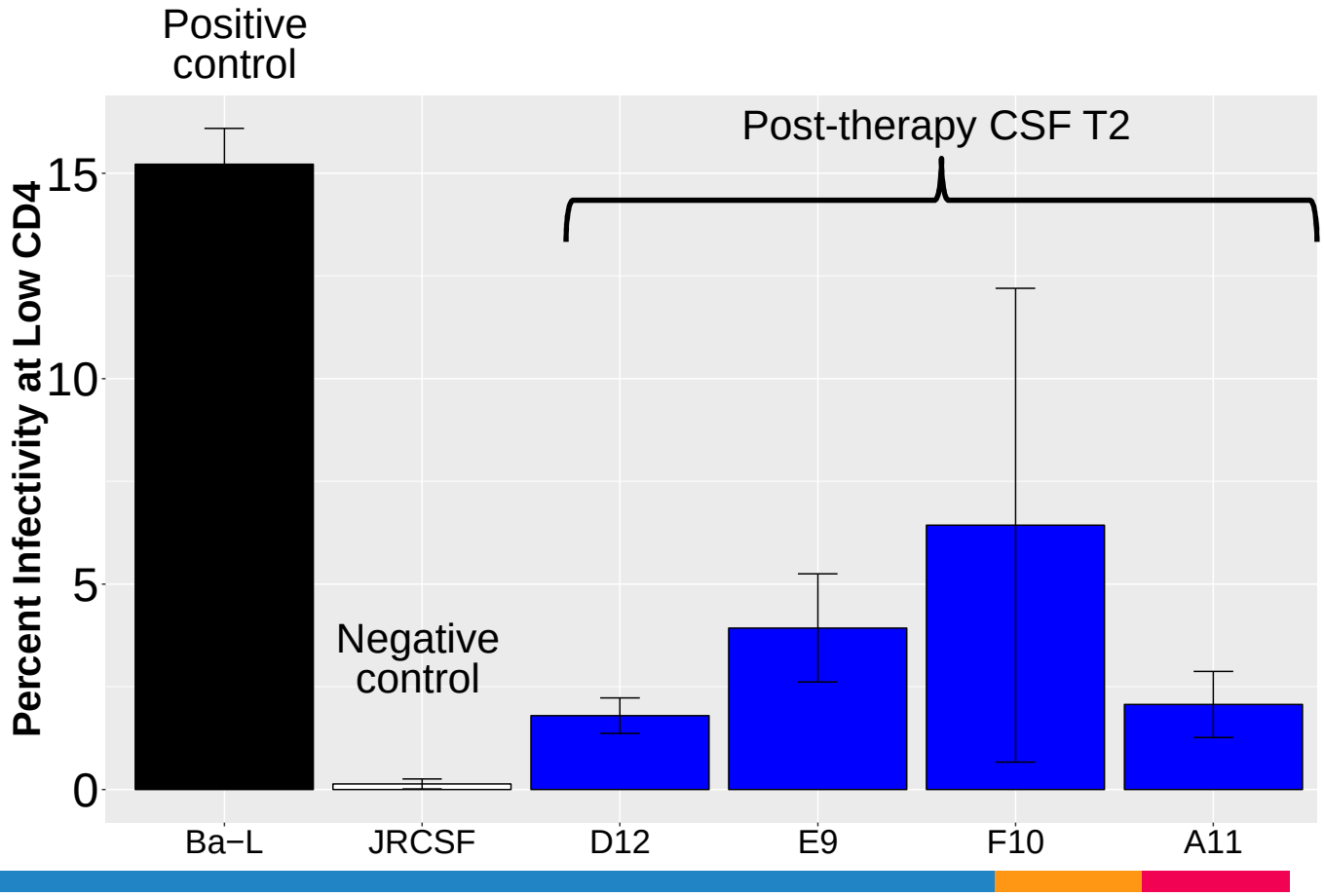
THINC Subject 2: A persistent and heterogeneous CSF population is evidence of an active (not latent) CNS reservoir.



UNC - Joseph et al., THINC study

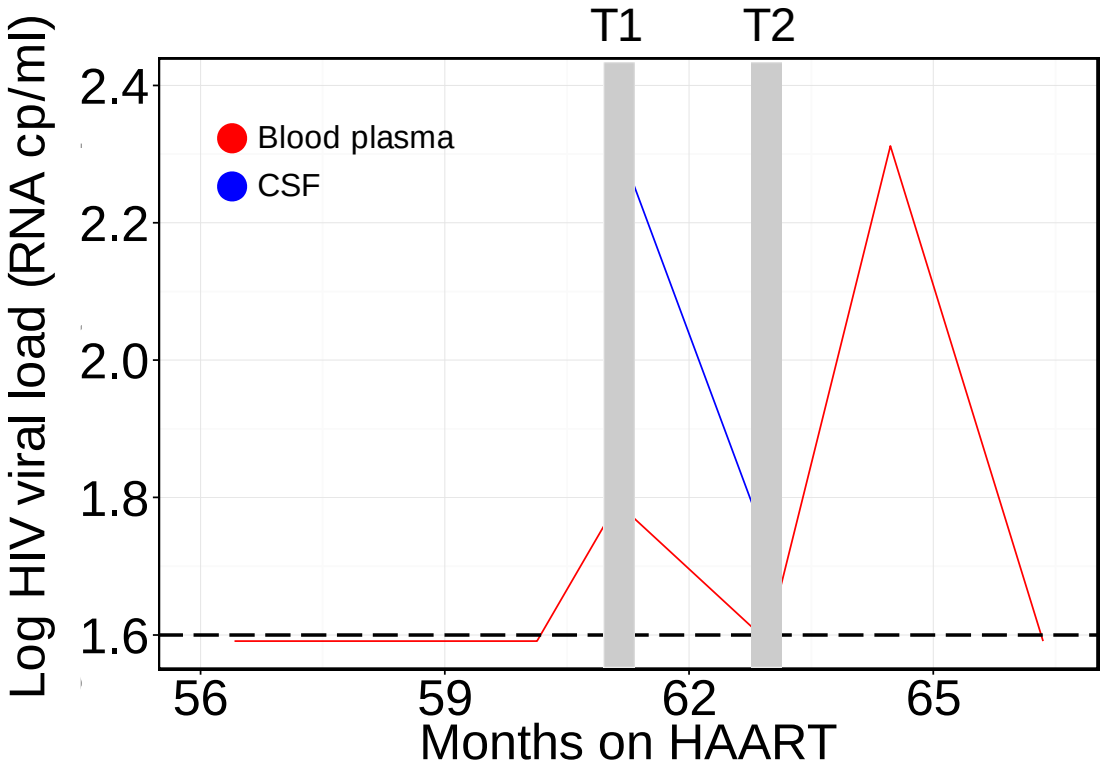
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

THINC Subject 2: The elevated macrophage tropism of the persistent CSF population is consistent with virus production from myeloid lineage cells

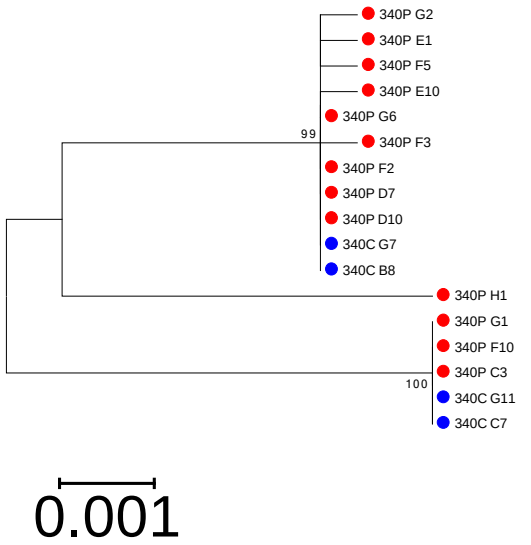


3. Analyses of viral RNA isolated from the CNS of subjects on therapy

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



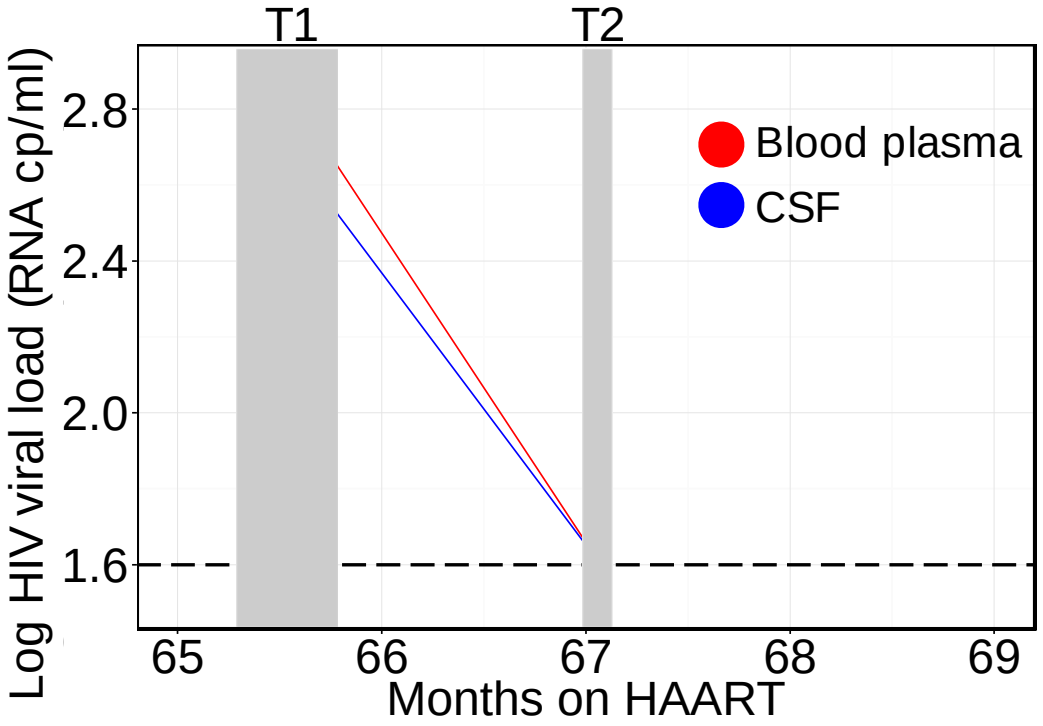
T1: Two clonal lineages of CSF-and blood-derived *env* sequences and an outlier



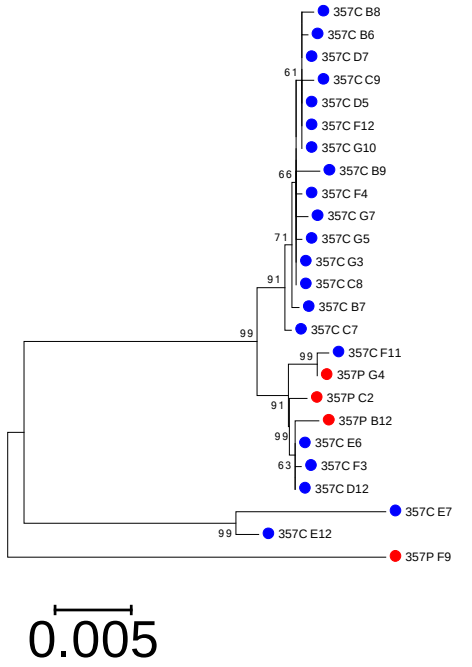
UNC Joseph et al., THINC study

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

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)?



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



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

THINC: HIV-1 can persist in the CNS during therapy, but this may be rare

Subject	Persistent CSF escape?	Diverse CSF population?	Elevated macrophage tropism?	Drug resistant?	Evidence of persistent CNS reservoir?
1	No	No	No	No	No
2	Yes	Yes	Yes	?	Yes
3	Yes	Yes	?	?	Yes
4	No	Yes	?	?	?

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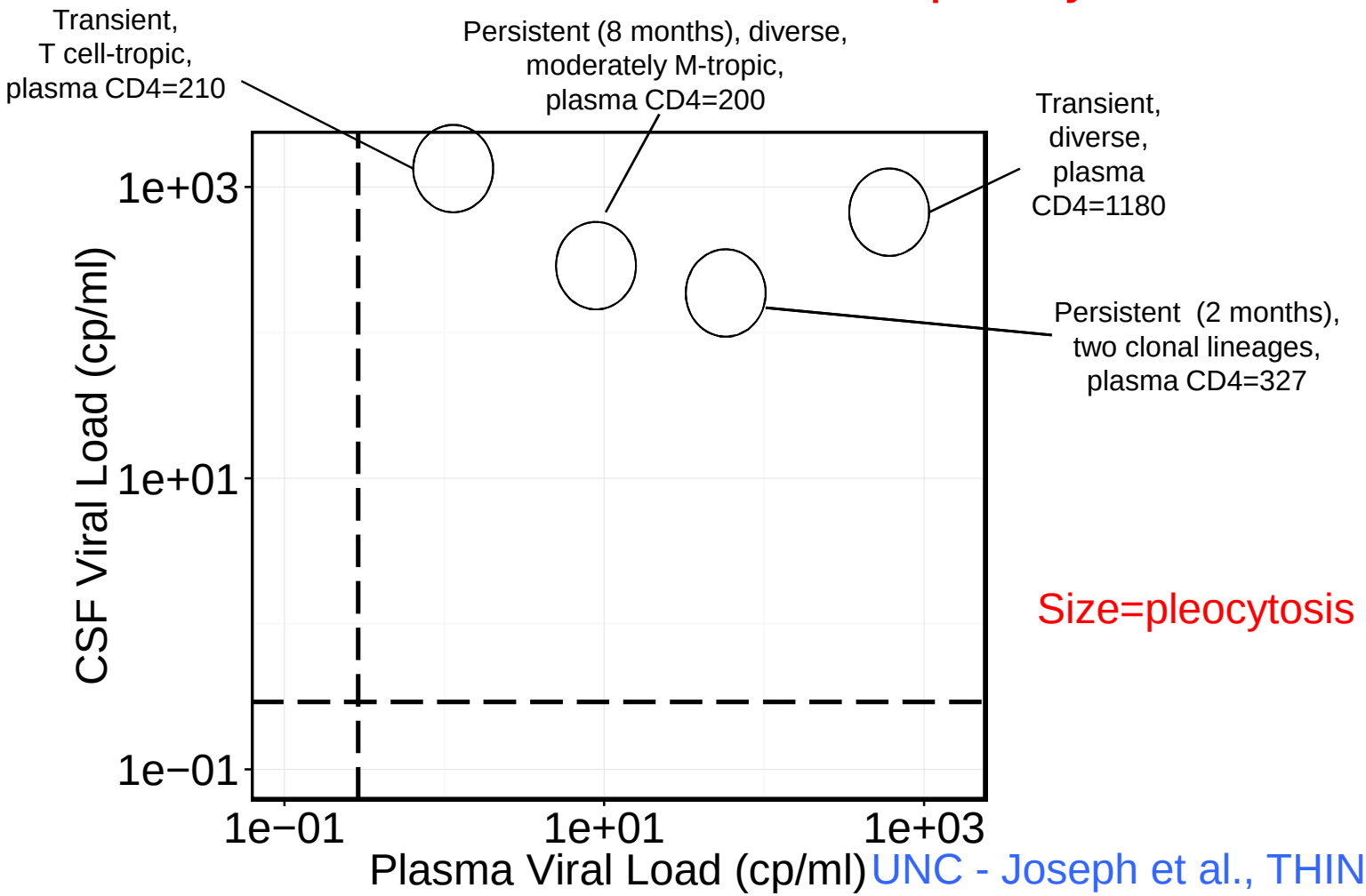
3. Analyses of viral RNA isolated from the CNS of subjects on therapy

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



Size=pleocytosis

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Conclusions – Part 2

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   - What proportion of these cells can produce HIV?

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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:

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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

UCSF

Richard W Price
Jaclyn Javerbaum
Julia Peterson
Alex Russell

Yale

Serena Spudich

Emory

Albert Anderson
David Loring

Swanstrom Lab

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

NNTC

Funding

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