Neuroinflammation associated with intact and 5’ defective proviral DNA persists in the brain of virally suppressed people with HIV

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Neurocognitive disorders in ART-treated PWH

- Despite therapy, people with HIV have an elevated risk of non-AIDS related inflammatory co-morbidities.
- HIV-associated neurocognitive issues occur in ~20% of virologically suppressed people with HIV, ranging from mild to an increased risk of severe impairment
- Associated with impaired cognitive and motor function, ↓ brain volume, and reduced life expectancy.
- Changes in cognitive decline are subtle but sustained over time relative to people without HIV

Impact on daily life:

- Social withdrawal
- Loss of independence
- Full-time care
- Poor ART medication adherence
- Viral recrudescence

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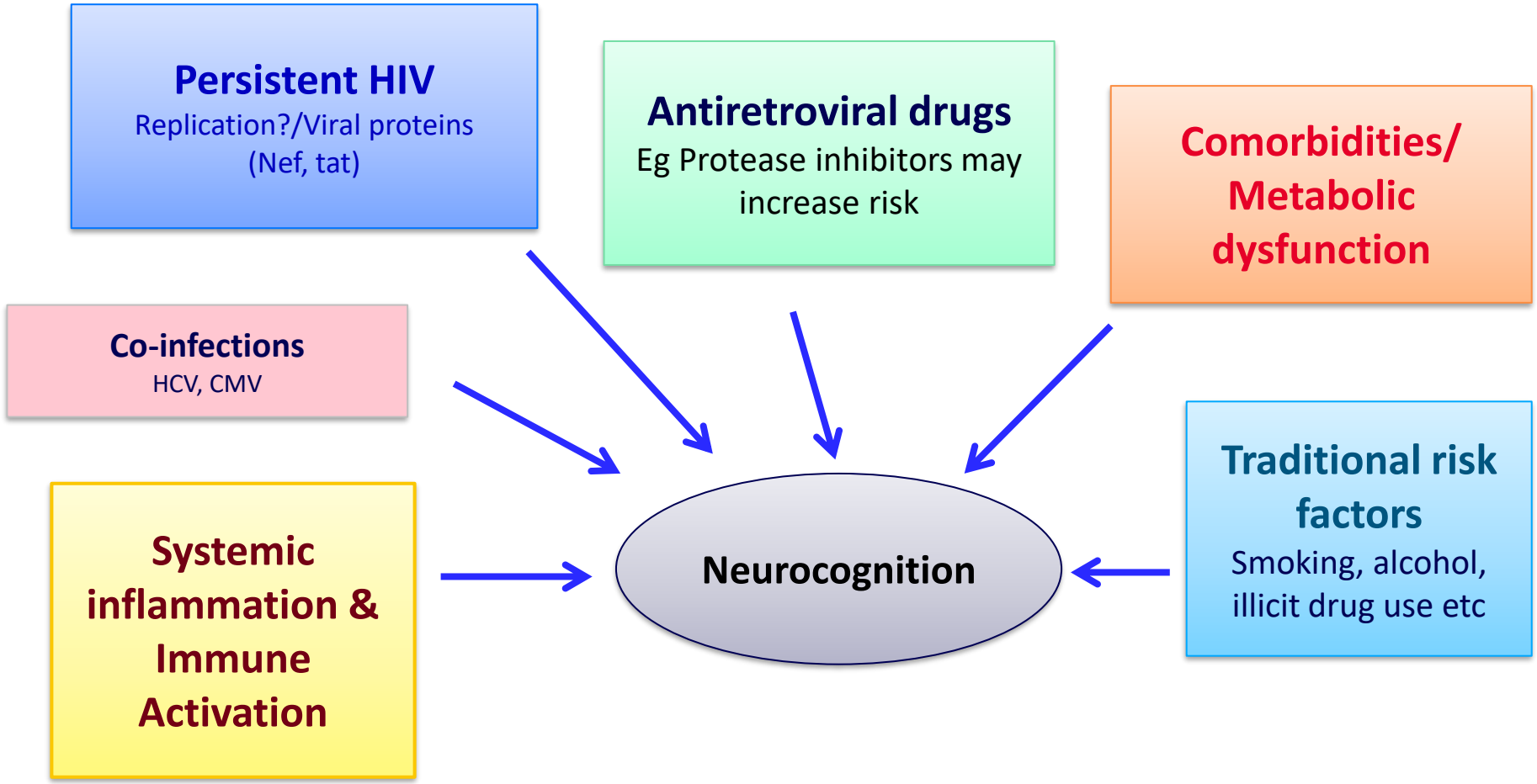
Heaton et al Clin Infect Dis 2015, Heaton et al Brain 2023; Nir et al JAMA 2021; Aung et al eClinical Med 2023

What is driving neurocognitive issues in ART-suppressed PWH?



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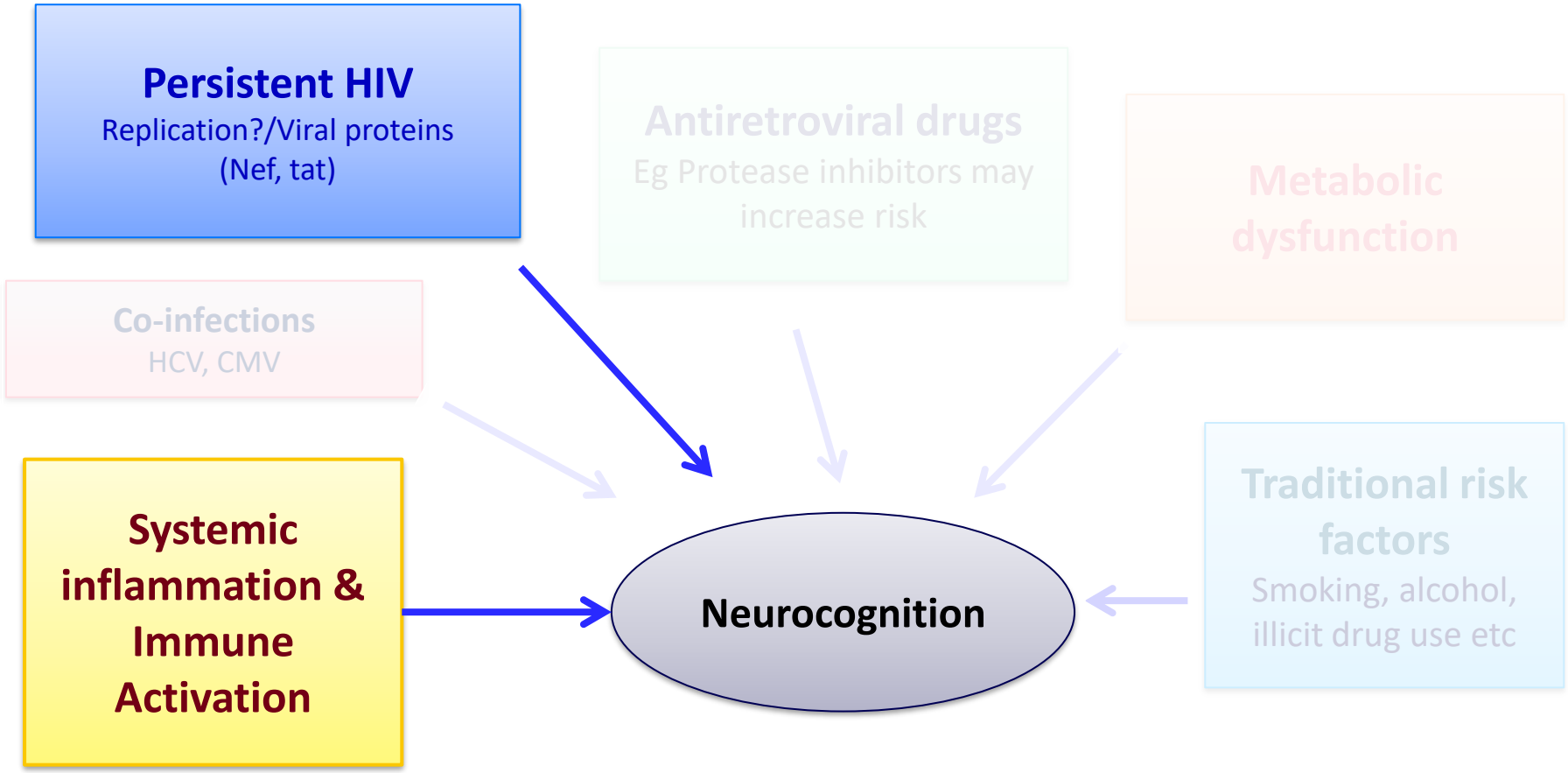
What is driving neurocognitive issues in ART-suppressed PWH?



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Cochrane et al Ann Neurol 2022; Angelovich et al Ann Neurol 2023, Byrnes et al Plos Pathog 2023, Jamal Eddine et al Plos Pathog 2024

What are the viral determinants of neuroinflammation in the brain during long-term viral suppression in PWH?

Overall hypothesis:

Persistent proviral DNA in the brain of virally suppressed PWH contributes to neuroinflammation and may induce neuropathogenesis.

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Cohort

- Well-characterized cohort of human brain tissues (n=41)
- Detailed clinical history and neurocognitive testing data
  - Exclusions: Hepatitis, brain ischemia or focal infarction
- Virally suppressed for a minimum of 18 months

Table 1. Cohort

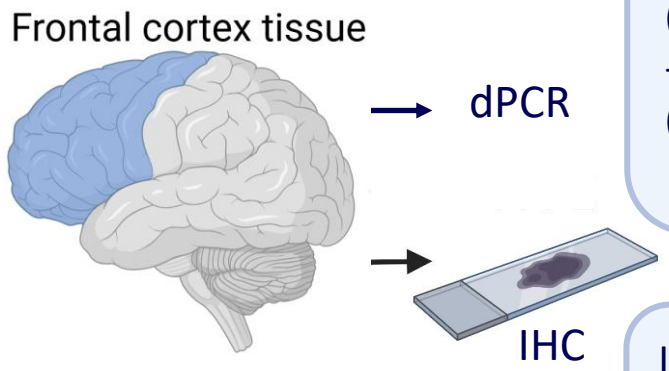
	HIV-seronegative	Non-virally suppressed (nVS)	Virally suppressed (VS)
n	6	17	18
Age	51 (39-61)	47 (40-56)	60 (53-62)
Sex (% Male)	50	52.9	77.5
HIV parameters			
Viral load (plasma)[a]		61223 (17387-367620)	<50
Viral load (CSF)[a]		361 (184-651)	50 (50-55)
CD4 T cells (mm^3)		16 (3-101)	163 (115-376)
Viral suppression (yrs)		-	3.8 (2.49-7.21)

CSF: Cerebrospinal fluid. [a] HIV RNA copies/ml

Study design

PWH
Virally Suppressed (VS; n=18)
Non-virally suppressed (nVS; n=17)

HIV seronegative (n=6)

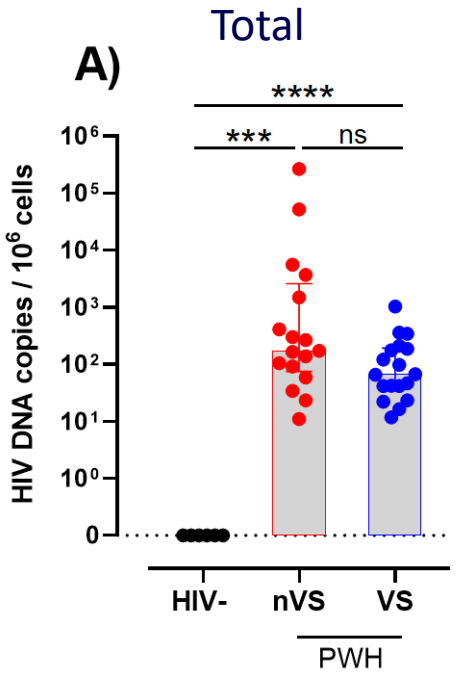


IPDA
- Intact HIV DNA (replication competent)
- Defective HIV DNA (replication incompetent)

Transcription profiling assay
-RNA transcripts
HIV TAR, Long-LTR, Pol, PolyA and Tat/Rev)

IHC
- Cell markers for astrocytes and myeloid cells
- Markers of inflammation and immune regulation (MX1, TNFα, TGFβ)

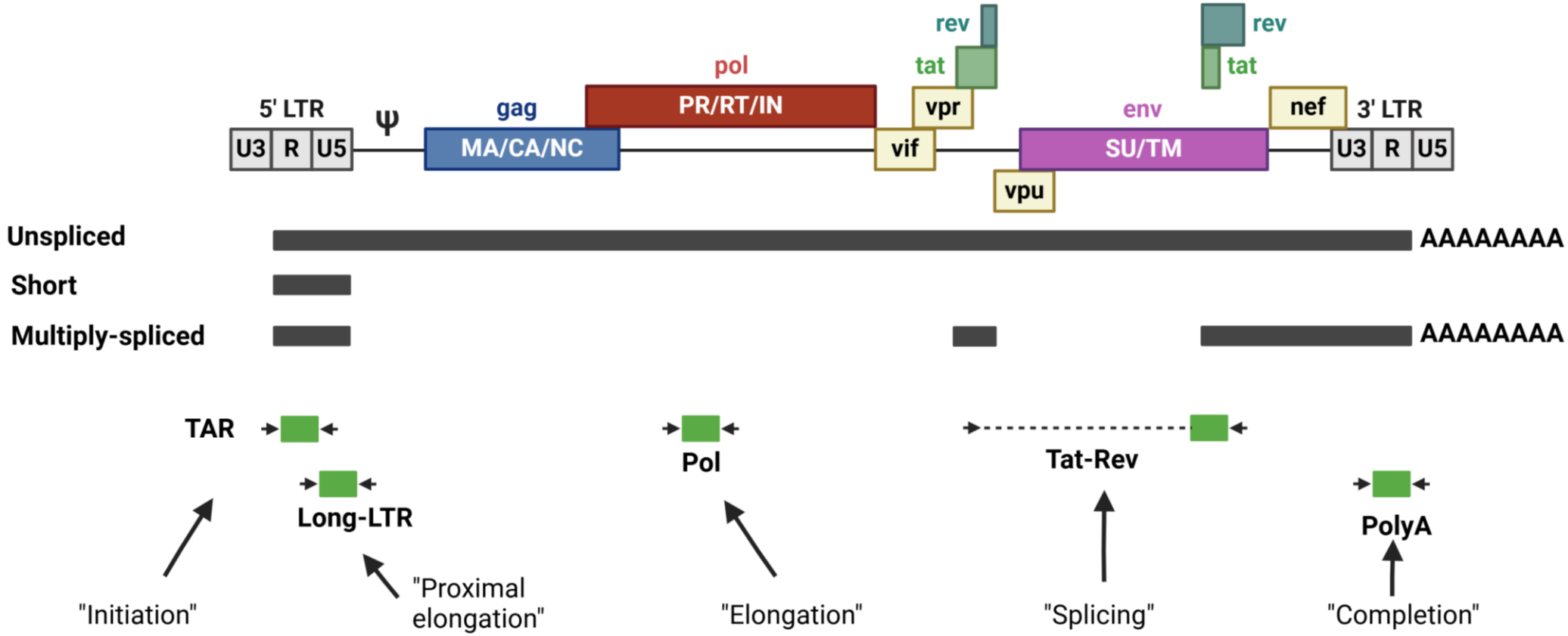
Intact HIV DNA persists in the brain despite suppressive ART



- Total HIV DNA detected in all individuals with HIV
- Intact HIV DNA persists in virally suppressed individuals
- Majority of brain tissue reservoir consists of defective (replication incompetent) proviruses

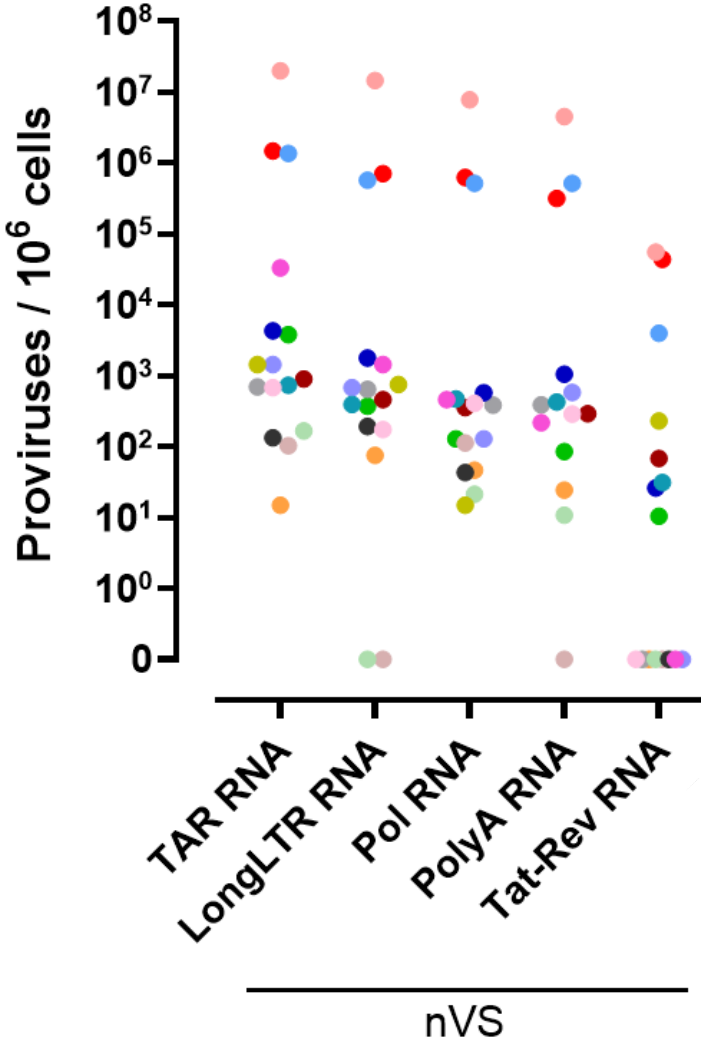
ART does not eradicate the HIV proviral DNA reservoir in the brain of PWH

Are brain reservoirs active *in vivo*?



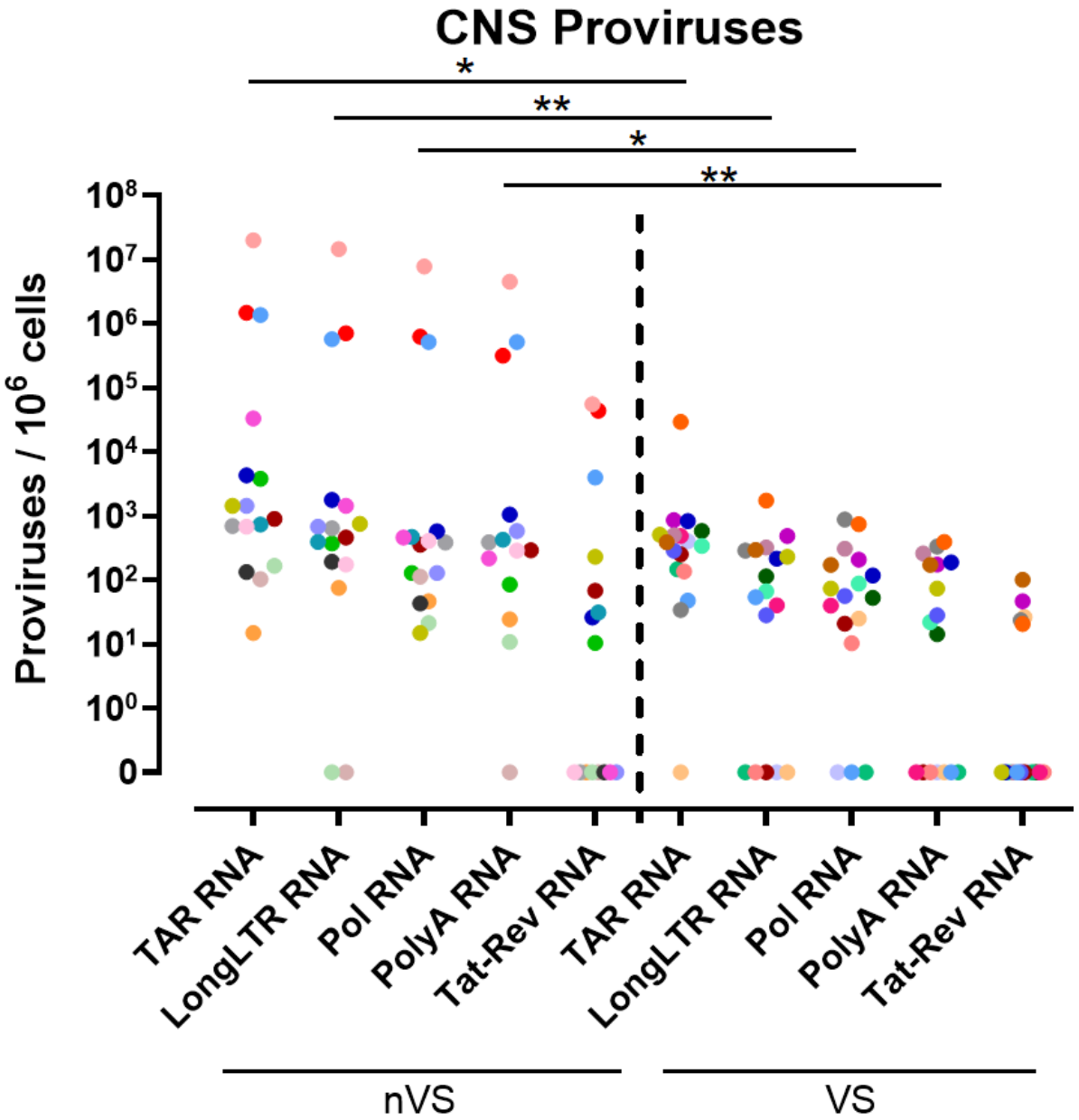
Yukl et al Sci Trans Med 2018; Telwatte et al Plos Pathog 2018

HIV RNA transcripts persists in brain despite suppressive ART



- TAR RNA detected in all individuals with HIV (initiation)

HIV RNA transcripts persists in brain despite suppressive ART



- TAR RNA detected in all individuals with HIV (initiation)
- While transcription occurs in the brain of both nVS and VS PWH, **ART reduces** the frequency and number of individuals with transcripts (TAR, LongLTR, Pol and PolyA) associated with initiation, elongation and completion of transcription

The brain is an 'active' reservoir of HIV that is impacted by ART

Implications of HIV persistence on cell activation in the brain

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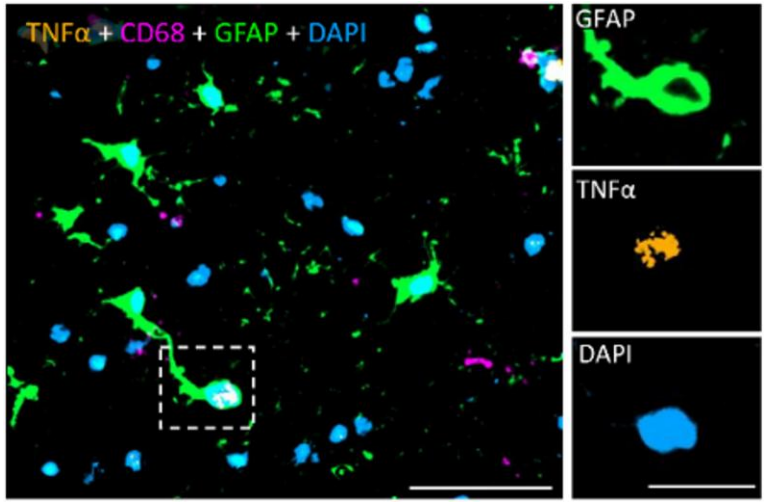
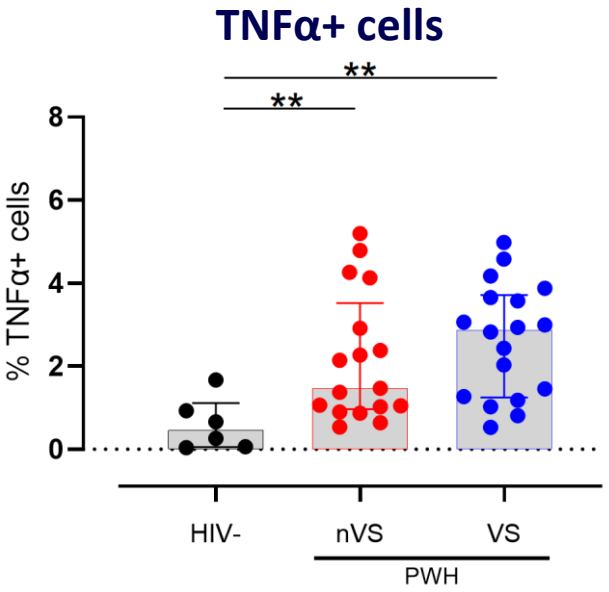
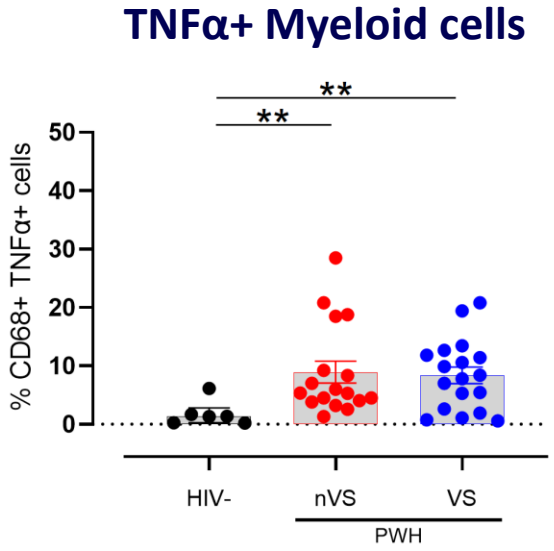
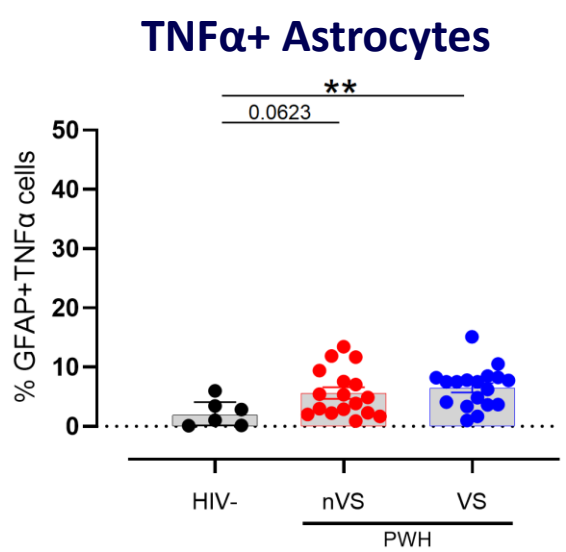
Enhanced immune activation persists in the brain of ART-suppressed PWH: TNFα







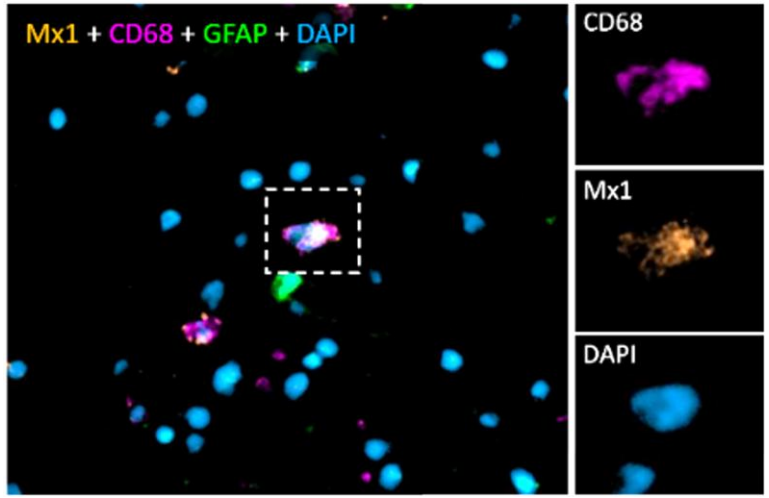


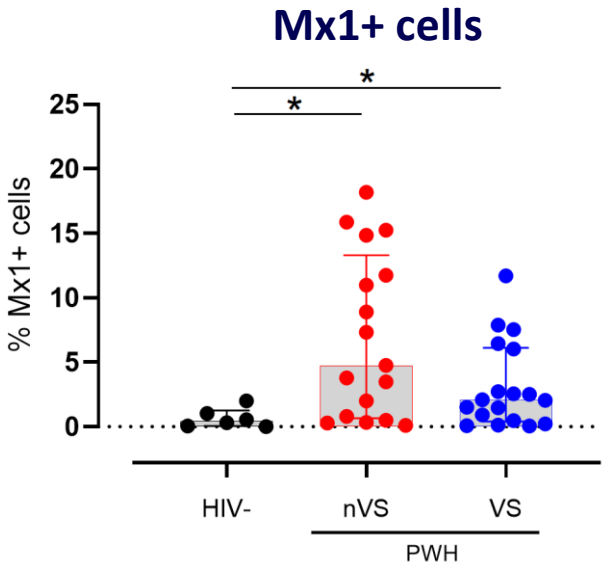
- ↑ TNFα (proinflammatory cytokine) in nVS and VS PWH
- TNFα produced by astrocytes and myeloid cells
  - ➢ No change in immune activation despite viral suppression

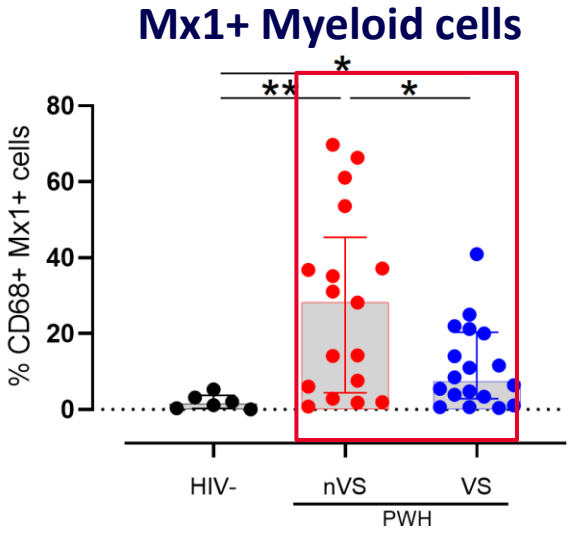
TNFα remains elevated in the CNS of PWH despite viral suppression

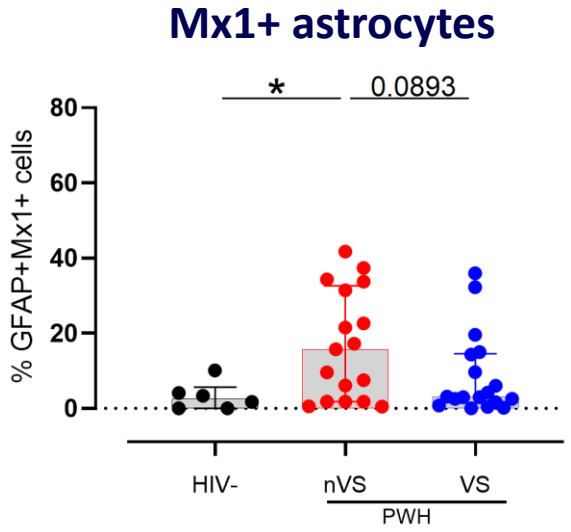
Byrnes *et al* Front Immunol 2025

Antiviral signalling in the brain of ART-suppressed PWH remains elevated: Mx-1







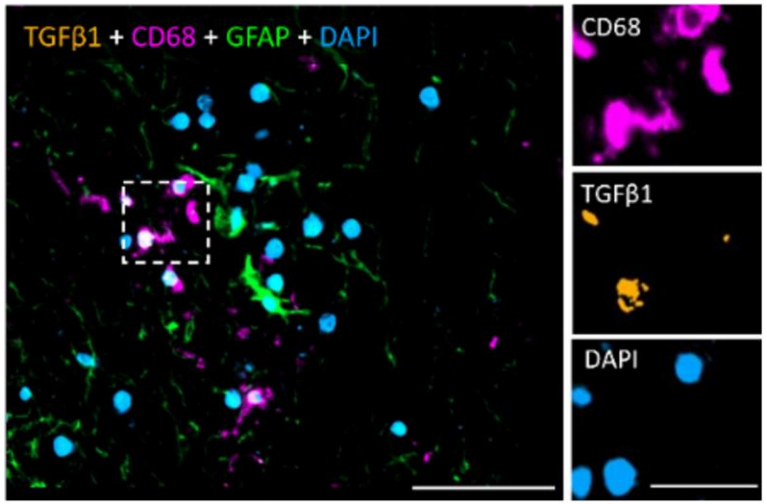


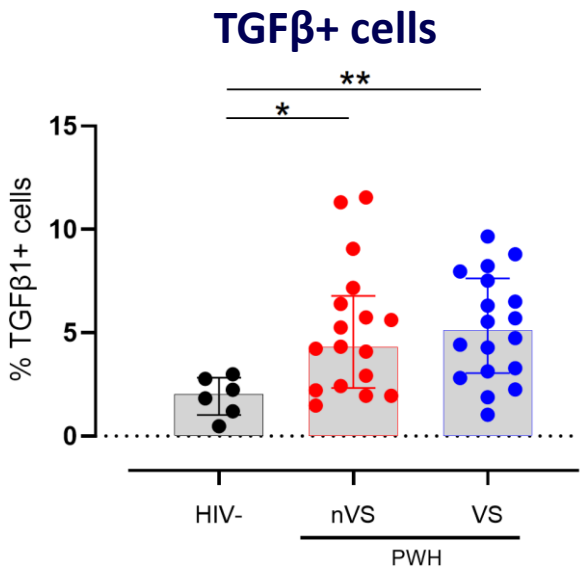
- ↑ Mx1 (type I interferon response) in nVS and VS PWH
- Majority of Mx1 produced by myeloid cells compared to astrocytes
- Mx1 myeloid cells elevated in nVS and VS
  - ➢ reduced in VS PWH compared to nVS PWH

Mx1 remains elevated in the CNS of PWH despite viral suppression

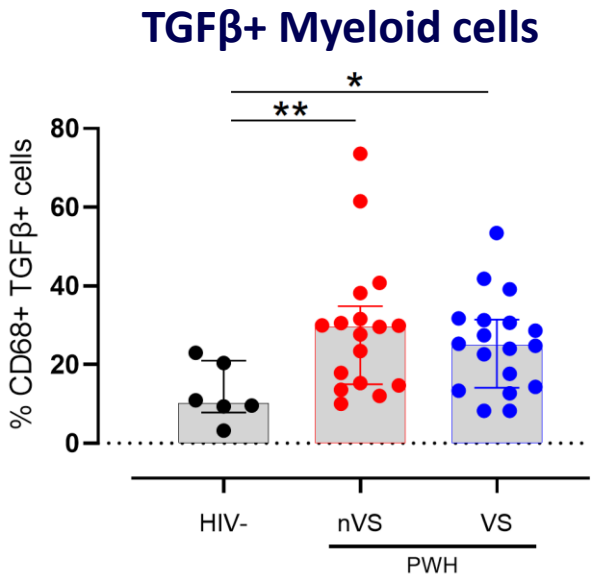
Byrnes *et al* Front Immunol 2025

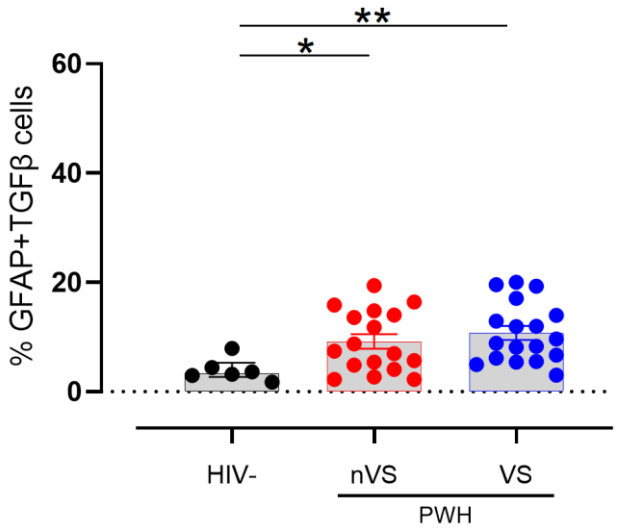
Enhanced immune regulation persist in the brain of ART-suppressed PWH: TGF-β1





- ↑ TGFβ (immune regulation)
- Majority of TGF-β produced by myeloid cells
- Higher frequency of TGFβ+ myeloid cells and astrocytes in nVS and VS PWH



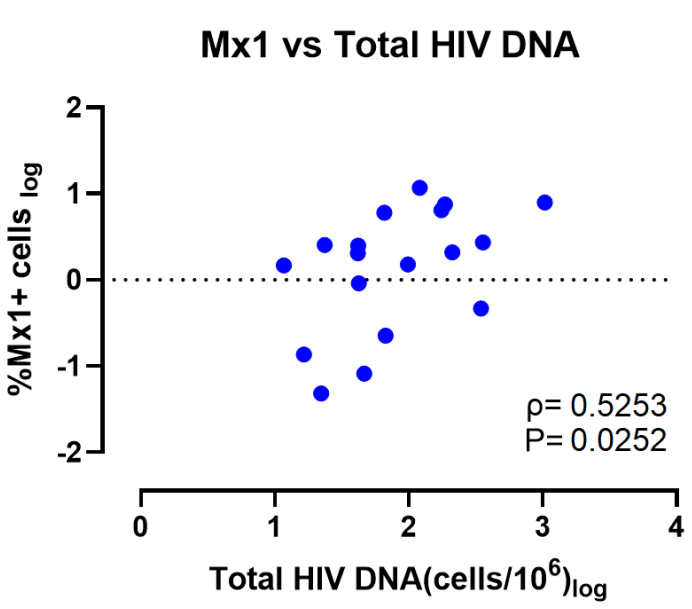


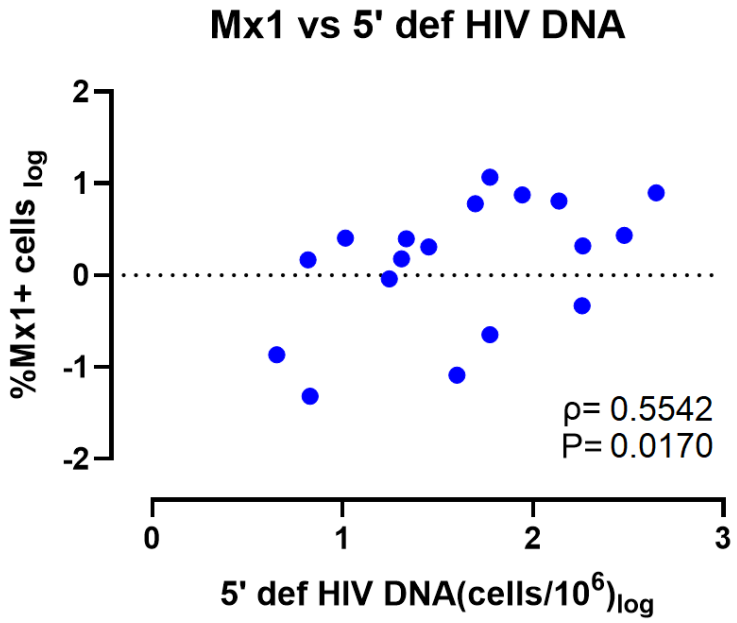
TGF-β remains elevated despite viral suppression

Byrnes *et al* Front Immunol 2025

Mx1 is associated with HIV DNA in the brain of virally suppressed PWH

● VS





- Total HIV DNA correlates with Mx1+ cells in VS PWH
- Importantly, MX1+ cells correlate with 5’ defective HIV DNA, suggesting the ability of defective proviruses to impact neuroinflammation

Intact and defective proviral DNA in the brain may be a driver of neuroinflammation

Byrnes *et al* Front Immunol 2025

Summary and implications

HIV persists in the brain of PWH

- Proviral DNA reservoir not impacted by ART
- 'active' reservoir (RNA) is impacted by ART

Ongoing immune activation is observed in the brain of virally suppressed PWH

- Elevated inflammatory and regulatory immune response in PWH despite ART
  - Mx1+ myeloid cells persist in the brain of both nVS and VS PWH, however, ART reduces the levels of Mx-1+ cells during viral suppression

Neuroinflammation persists in the brain and is caused, in part, by local and potentially peripheral transcriptionally active HIV reservoirs

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Byrnes *et al* PloS Pathog 2023

Community summary

Key question

- Does HIV persist in the brain tissue despite viral suppression?
- Does persistent HIV in the brain drive neuroinflammation?

Key findings

- HIV DNA is not impacted by viral suppression, however, the activity of the HIV reservoir in the brain is reduced
- Elevated inflammation persists in the brain of PWH, however, some markers of inflammation are reduced by ART
- The majority of HIV DNA in the brain is defective

Next steps

- Determine the mechanisms to alleviate ongoing neuroinflammation in the brain of PWH to improve brain health

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OHSU OREGON NATIONAL PRIMATE Research Center

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