

The Identification of Intact HIV Proviral DNA from Human Cerebrospinal Fluid

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Background

The central nervous system (CNS) is an HIV reservoir based on a growing body of evidence. More research is needed to better understand this reservoir, of which the cerebrospinal fluid (CSF) is a part. The intact proviral DNA assay (IPDA) has emerged as a technique to identify integrated HIV DNA including non-defective virus that may be the source of virologic rebound.

Methods

We studied CSF from people with HIV (PWH) using IPDA protocols established for blood. IDPA results (3' defective HIV DNA, 5' defective HIV DNA, and intact HIV DNA) were analyzed in conjunction with results from peripheral blood mononuclear cells (PBMC). Results were also analyzed in relation to performance on a battery of nine neuropsychological (NP) tests.

Results

CSF samples from 11 PWH were evaluated with eight matching PBMC samples. Total CSF HIV DNA was detectable in all participants and was significantly higher than in matched PBMC. Intact CSF HIV DNA was detected in 7/11 participants. Intact CSF levels correlated closely with intact blood levels but tended to be higher in CSF than in PBMC. CSF HIV DNA did not correlate with NP performance except for a trend between higher defective HIV DNA level and worse verbal fluency.

ID	Age (years)	Sex	Race	CD4+	CSF WBC	CSF RBC	Plasma HIV log10	CSFHIV log10	NPT-9
124	54	Female	African-American	411	2	120	<1.60	<1.60	41.89
127	47	Male	African-American	1434	5	1	<1.60	<1.60	42.00
130	58	Transgender male to female	African-American	364	1	2	<1.60	<1.60	57.67
132	56	Male	African-American	194	0	0	<1.60	<1.60	55.89
138	47	Male	White	730	0	0	<1.60	<1.60	50.22
139	68	Male	White	245	2	2	<1.60	<1.60	42.33
142	40	Male	African-American	325	0	13	4.68	3.21	56.11
711	36	Male	African-American	51	0	0	4.64	1.61	51.77
717	53	Female	African-American	89	0	0	5.68	4.22	36.99
733	55	Male	White	153	0	0	3.68	3.13	47.44
746	50	Male	African-American	260	0	0	<1.60	<1.60	62.70

Table 1: Demographic/disease characteristics of participants CD4+, WBC, and RBC are reported in cells/microliter

Result I: Intact HIV DNA was detected in 7 of 11 CSF samples

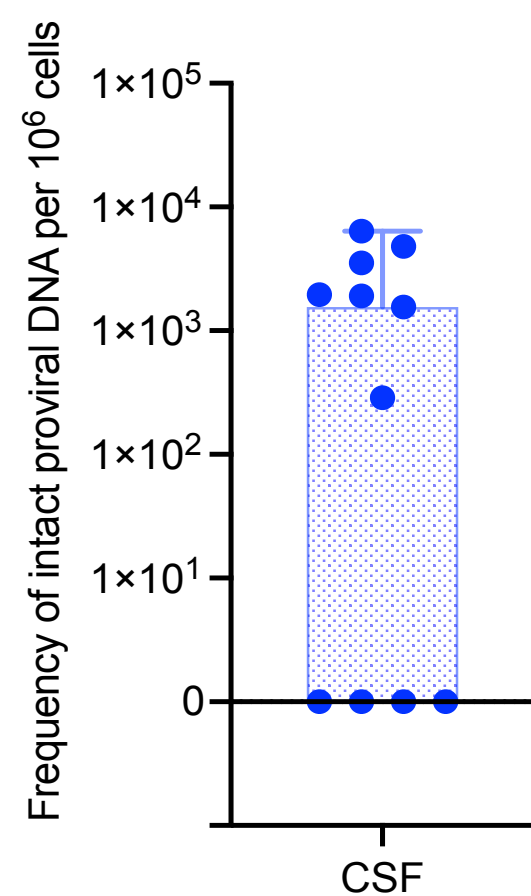


Figure 1: Intact Proviral DNA results from CSF

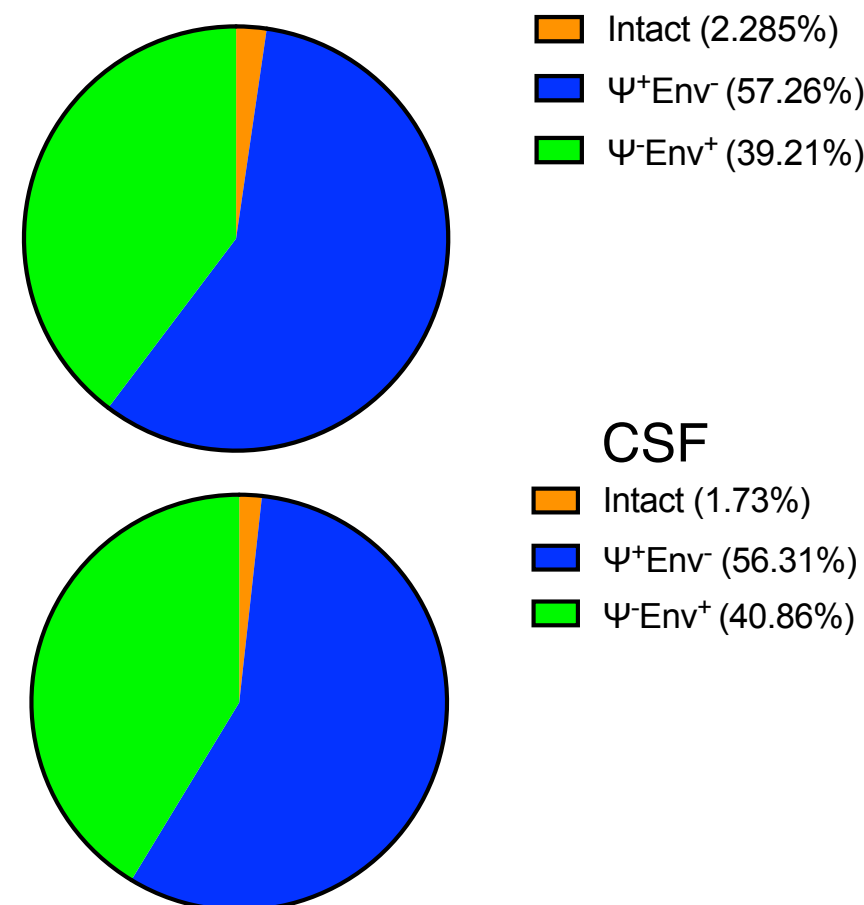


Figure 2: Median percentages of HIV DNA subsets for blood and CSF

Result III: higher CSF HIV DNA tended to correlate with worse executive function

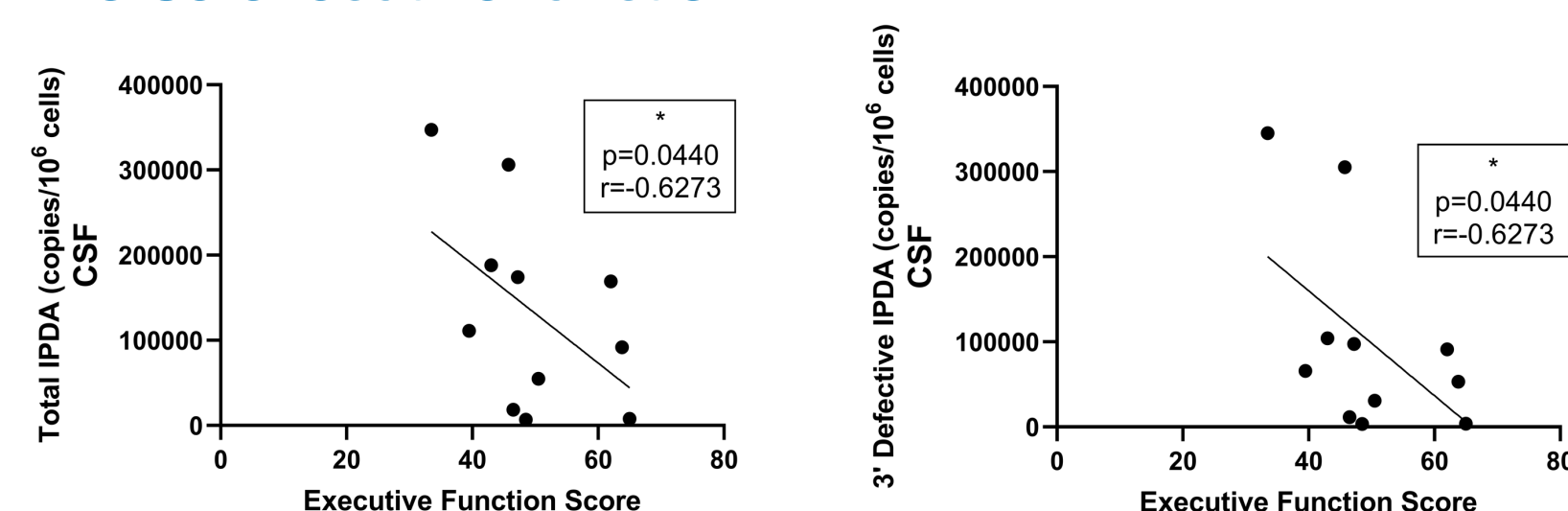
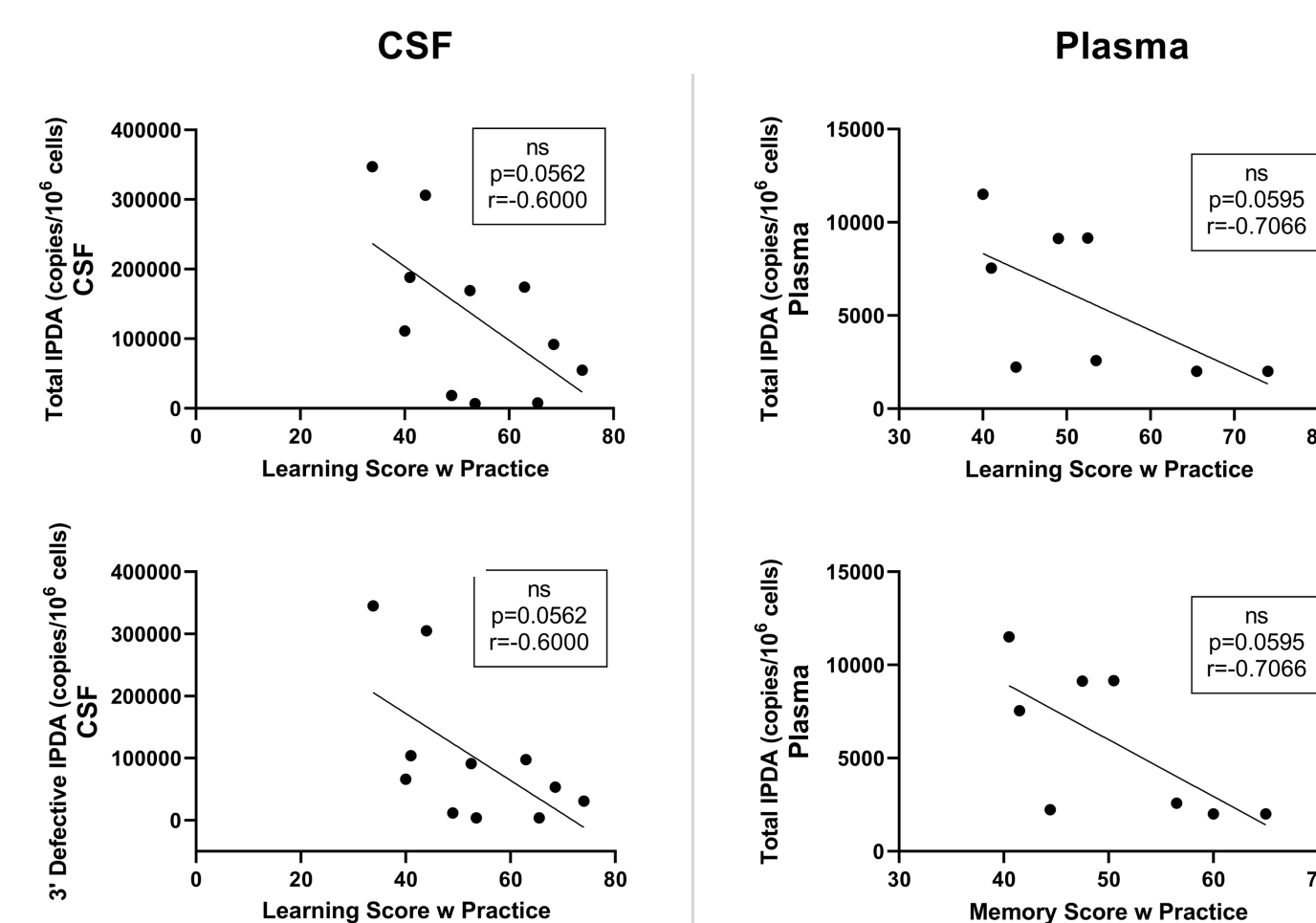


Figure 4: Executive function scores with practice in virally suppressed people with HIV negatively correlate to both total and 3' defective HIV proviral DNA copies per 10⁶ cells in CSF (determined via IPDA). Correlations performed via Spearman's rho using Graphpad Prism v9.5.1 (α=0.05).



Supplemental Figure: Learning scores with practice in virally suppressed people with HIV (PWH) trend (p=0.0562) toward negative correlation to both total and 3' defective HIV proviral DNA copies per 10⁶ cells in CSF (determined via IPDA). Learning and memory scores with practice in virally suppressed PWH trend (p=0.0595) toward negative correlation to total HIV proviral DNA copies per 10⁶ PBMC. Correlations performed via Spearman's rho using GraphPad Prism v9.5.1 (α=0.05).

Result II: Intact, 3' defective (Psi+env-) and 5' defective (Psi-env+) HIV DNA were both higher in CSF than blood in matched pairs

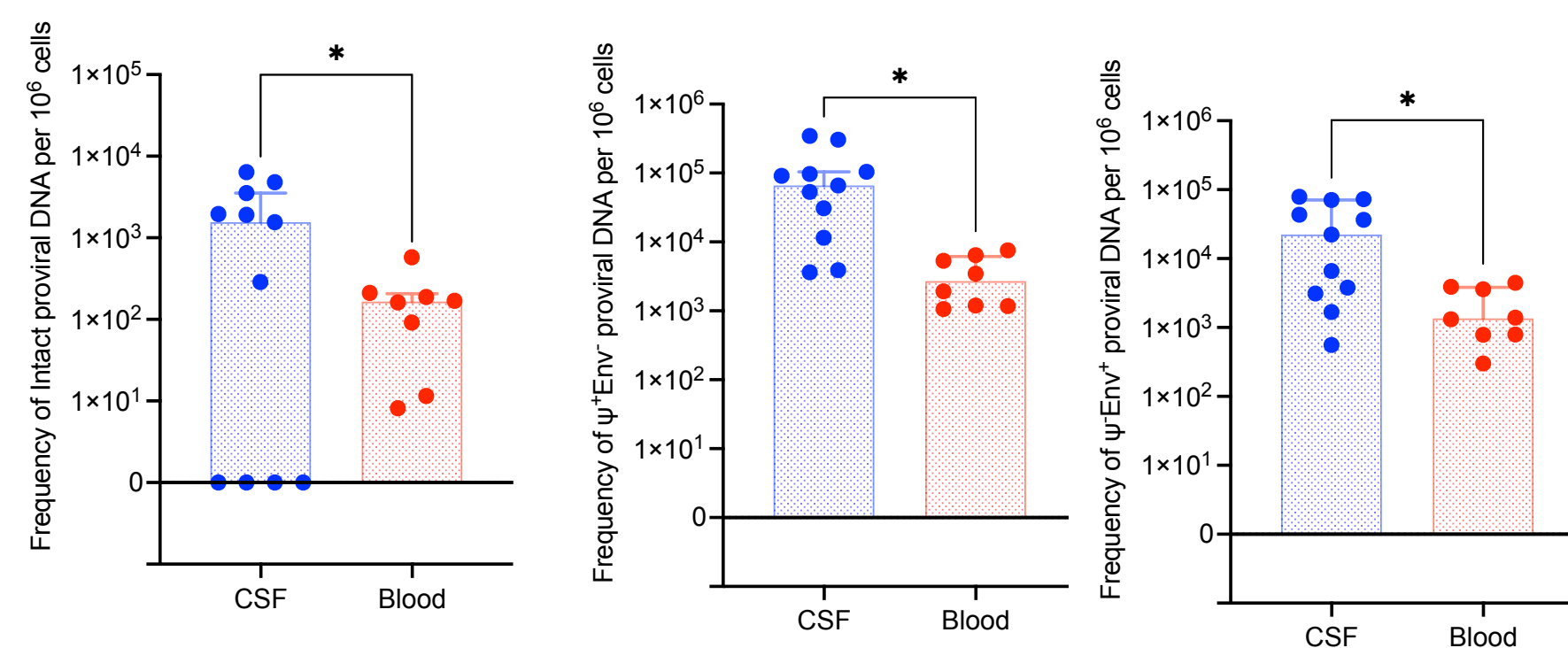
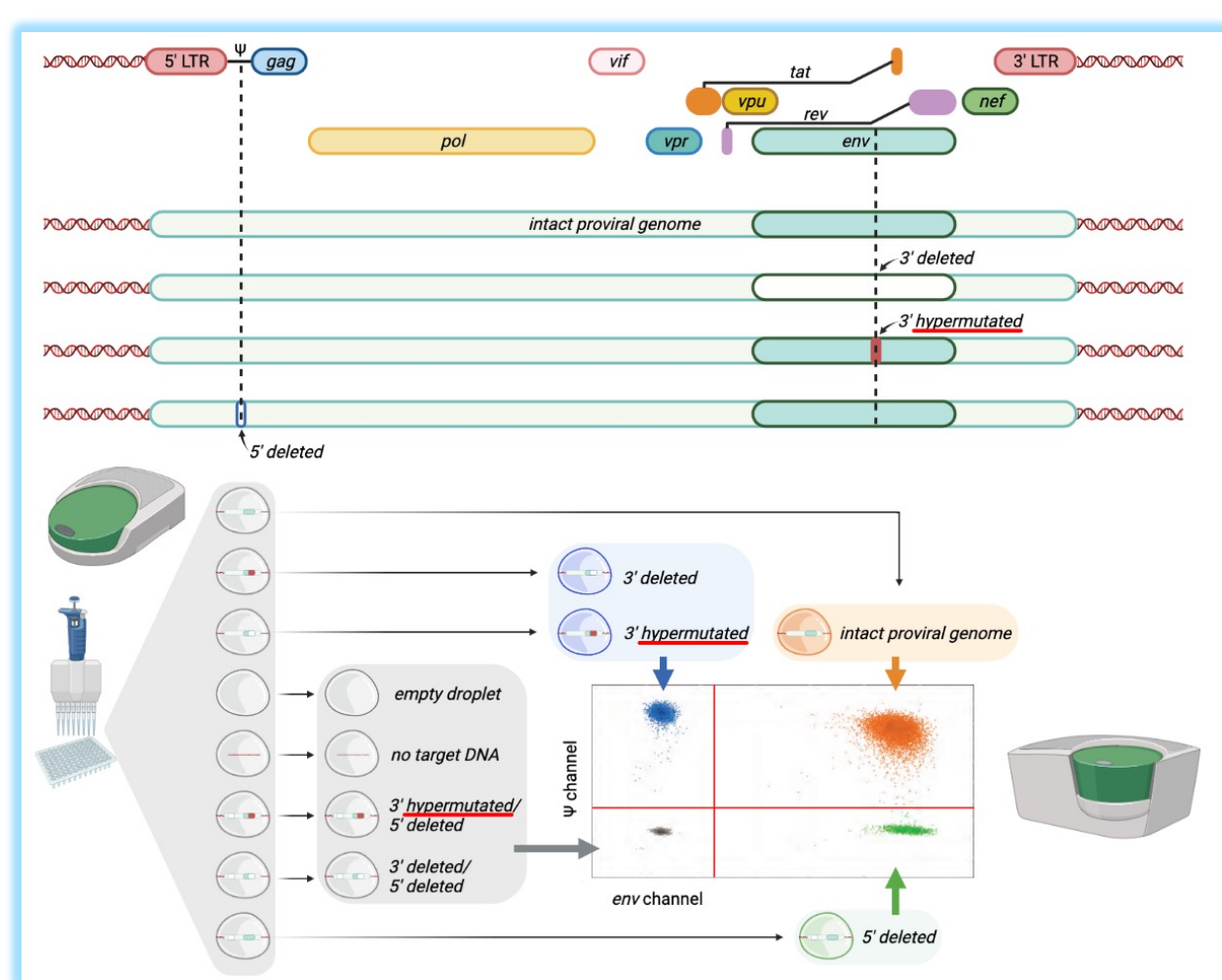
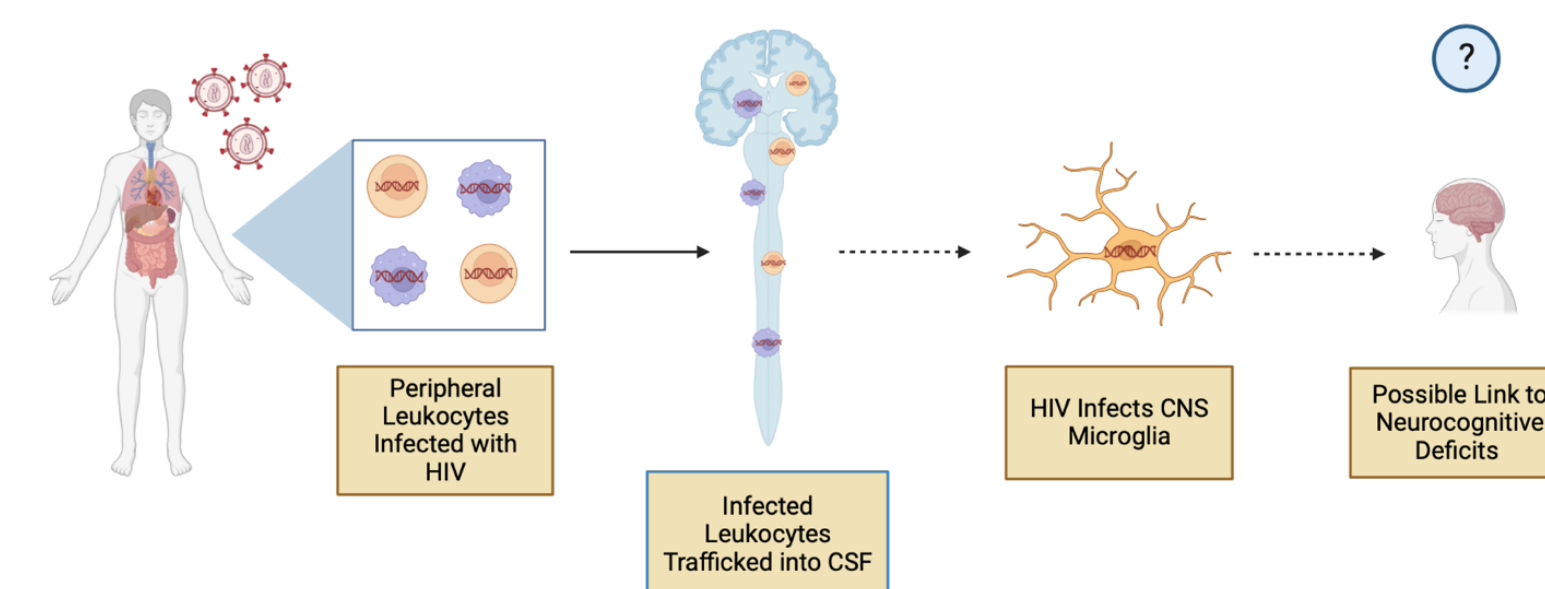


Figure 3: Proviral HIV DNA results from CSF versus Blood From left: Intact, 3' defective, and 5' defective



Schematic overview of Intact proviral DNA assay (IPDA). Top half: Illustration of IPDA-targeted defective regions of Psi and Env. Bottom half: Multiplex PCR-based 2D plot allows to discriminate the intact proviral DNA from the defective ones.



Discussion and Conclusions

Intact HIV DNA is frequently present in the CSF of PWH on and off ART. This further supports the presence of an HIV CNS reservoir and provides a method to study the CNS during HIV cure studies. Larger studies are needed to evaluate relationships with CNS clinical outcomes.

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