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Changes in PINCH and hpTau levels in the CSF of HIV patients correlate with CD4 count

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Abstract

Several studies report associations between the PINCH (particularly interesting new cysteine histidine-rich) protein and HIV-associated CNS disease. PINCH is detected in the CSF of HIV patients and changes in levels during disease may be indicative of changes in disease status over time. PINCH binds hyperphosphorylated Tau (hpTau) in the brain and CSF, but little is known about the relevance of these interactions to HIV CNS disease. In this study, PINCH and hpTau levels were assessed in three separate CSF samples collected longitudinally from 20 HIV+ participants before and after initiating antiretroviral therapy, or before and after a change in the current regimen. The intervals were approximately 1 (T2), and 3-7 (T3) months from the initial visit (baseline, T1). Correlational analyses were conducted for CSF levels of PINCH and hpTau and other variables including blood CD4+ T-cell count, plasma and CSF viral burden, CSF neopterin, white blood cell (WBC) count, and antiretroviral CNS penetration-effectiveness (CPE). Values for PINCH and hpTau were determined for each patient by calculating the fold changes between the second (T2) and third measurements (T3) from the baseline measurement (T1). Statistical analyses showed that the fold-change in CSF PINCH protein from T1 to T2 were significantly higher in participants with CD4 counts >200 cells/mm³ at T2 compared to those with CD4 counts <200 cells/mm³ at T2. This trend persisted irrespective of plasma or CSF viral burden or anti-retroviral therapy CPE scores. The fold-changes in PINCH levels between T1 and T2, and T1 and T3 were highly correlated to the fold changes in hpTau at T2/T1 and T3/T1 (correlation co-efficient = 0.69, p-value < 0.001, correlation co-efficient = 0.83, p-value < 0.0001, respectively). In conclusion, in these HIV participants, changes in CSF levels of PINCH appear to correlate with changes in blood CD4 count and with changes in CSF hpTau levels, but not with plasma or CSF viral burden, neopterin, or WBC, or with anti-retroviral regimen CPE.

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Introduction

Particularly interesting new cysteine histidine rich protein (PINCH), is an adaptor protein involved in cytoskeletal organization, cell attachment and survival [1-3], and is suggested to play an important role in neurodegenerative diseases including human immunodeficiency virus encephalitis (HIVE) and Alzheimer's Disease (AD) [4-6]. PINCH1 protein consists of 5 LIM domains and has no known catalytic activity [1, 2, 7]. The expression of PINCH1 is essential during development for cell proliferation and migration, to maintain neuronal polarity and synaptodendritic connections, and knockout is embryonic lethal [8, 9]. While PINCH is expressed at high levels during development and in disease, in healthy patients, PINCH is nearly undetectable. In the brain, PINCH is upregulated in dystrophic neurons and is also present in brain parenchyma with no apparent association with cellular components [4, 5].

Hyperphosphorylation of Tau results in Tau's dissociation from microtubules and mislocalization to the neuronal soma and dendrites. Accumulation of hyperphosphorylated Tau (hpTau) is a common pathological feature of AD and is reported in HIV, as well [6, 10, 11]. In this context, our recent data show that PINCH binds to hpTau in HIV and AD patients' brains and loses solubility along with hpTau [6]. Moreover, we have shown that the HIV protein Tat and the chemokine TNF- α induce both PINCH expression and hpTau in human primary neurons *in vitro* [5]. However, the significance of the presence of PINCH in the CSF of HIV patients is currently unclear.

The relevance of plasma and CSF viral loads, and CD4 count in HIV-associated neuropathology, prognosis and response to treatment has been extensively studied [12-15]. Numerous studies have addressed potential HIV-associated CSF biomarkers that may be used alone or in combination to either predict CNS disease severity or to predict the progression of HIV CNS disease [16-20]. Similarly, many studies have assessed changes in hpTau levels in CSF as a biomarker in AD, and more recently in HIV [21]. Although results from some reports are conflicting regarding CSF levels of Tau protein in HIV, most agree that elevations in CSF inflammatory factors persist through out disease. For example, a recent review from Price *et al.* describes the value of CSF assessment to improve our understanding of the evolution HIV neuropathology as it relates to changes in immune system function [22]. Additionally, in AD, changes in neurodegenerative markers are reported to precede structural changes in the brain that are detectable by MRI or clinically as alterations in cognition [23]. Therefore, reliable neurodegenerative and neuroinflammatory biomarkers may be useful in predicting changes in disease progression in HIV-associated neurocognitive disorders (HAND), AD and other neurodegenerative diseases and may influence treatment plans for managing patients.

Increased life expectancies of HIV patients have lead to a tremendous growth in the population of older individuals living with HIV. While combination anti-retroviral therapy (CART) is one of the major factors contributing to this phenomenon, both HIV infection and CART accelerate the aging process [24-26]. Antiretroviral therapy causes a substantial decrease in immune activation, and an increase in CD4+ cell count, but recovery in these

patients is still characteristic of immune senescence and is dependent on both CD4+ nadir and age at CART initiation [27, 28].

Several studies report the early development of an “elderly immune system profile” in HIV patients resulting in a depleted naïve T-cell population, lymphoid tissue fibrosis and inverted CD4:CD8 ratio (for review, see [29]). Also, shortened telomeres in T cells are reported in both HIV+ patients and in aging HIV negative adults [30-32]. Furthermore, MRI points to immune suppression as a key player in brain volume reduction in clinically stable HIV patients on CART, correlating with lower CD4+ nadir [33]. These studies suggest the significance of the immune system in accelerating aging in HIV patients.

Despite CART adherence, some patients do not have a favorable response as evidenced by persistent virus in plasma and CSF, and low blood CD4 counts. In this context, immunodeficient HIV patients including immunological non-responders, have increased morbidity and mortality compared to HIV patients with more favorable immune responses [34]; whereas, HIV patients with substantial immune recovery and optimal immune system functioning have life expectancies similar to those of HIV negative individuals [34]. Hence, longitudinal studies and clinical trials exploring biomarkers to examine the relationship between immune system and HIV are essential to effectively evaluate age-related disease CNS progression and treatment efficacy in HIV patients. Earlier studies report that although PINCH CSF levels are significantly elevated in all HIV patients, CSF levels of PINCH are significantly higher in HIV patients without encephalitis compared to those with HIVE [35], suggesting that changes in PINCH CSF levels may relate, in part to inflammatory status of the brain.

In this study, we assessed changes in PINCH levels in a cohort of HIV study participants from whom at least three CSF and blood samples were collected over 3-7 months. Our findings show that as CD4+ cell counts increase, PINCH levels in the CSF increase, as determined by increased levels from one time point to the next. Likewise, changes in levels of CSF PINCH correlate with changes in CSF hpTau levels, but do not correlate with plasma or CSF viral burden. Understanding the pathophysiology of HIV infection of the CNS in the presence of CART, changes in viral load and immune system status may improve clinical management and treatment strategies for HIV patients with neurological disorders.

Methods

Study design and participants

Temple University and UCSD Human Subjects IRB approved the study and a written informed consent was obtained from all study participants. Longitudinal CSF and blood samples were collected from 20 ambulatory HIV+ study volunteers between 1997 and 2004 enrolled at the HIV Neurobehavioral Research Center, University of California San Diego, San Diego, CA. Clinical and laboratory data from CSF and blood samples for study participants are presented in Table I. Mean age of participants was 39 years (range, 27-54 years) and included 12 Caucasians, 5 African-Americans, 1 Hispanic and 1 participant of undetermined background. All study participants received nucleoside or nucleotide reverse

transcriptase inhibitors (NRTI), 12 participants received non-nucleoside RTI, and 11 participants received protease inhibitors.

For each participant, at least three samples were collected with the 1st draw representing baseline, T1. The second draw (T2) ranges from 27-50 days from T1, with a median of 33, and 29 days, representing 1st and 36 representing the 3rd quartile range. Likewise, the third draw (T3) ranges from 60-207 days from T1 with a median of 96 days, and 85 days representing the 1st and 123 representing the 3rd quartile range (Table I). Baseline (T1) CSF and blood draws corresponded to either initiation of CART, or change in treatment regimen due to failure as indicated by one or more of the following parameters: increased plasma and/or CSF viral burden, plasma or CSF viral burden going from undetectable to detectable levels or a significant decrease in CD4+ count or a failure of CD4 count to improve over time. Plasma and CSF HIV viral load, blood CD4+ cell counts and CSF white blood cell (WBC) counts were obtained for most of the participants at each visit. Classification of participants as virologic responders (n=17) or non-responders (n=3) was based on one log reduction of the plasma viral load from baseline (T1) at subsequent collections (T2 or T3).

No history of CNS opportunistic infections including cytomegalovirus, toxoplasmosis, or JC virus with progressive multifocal encephalopathy was reported for study participants. Self-reported current and long-term substance abuse and dependence history for alcohol, cocaine and cannabis were obtained at the time of each visit for 9 participants.

CSF and blood measurements

Plasma and CSF HIV RNA levels (log₁₀ copies/ml) were quantified using the Amplicor HIV-1 Monitor test with Ultrasensitive Specimen Preparation (Roche Molecular Systems, Pleasanton, CA). The limit of detection was 50 copies/ml for CSF, 200 copies/ml for plasma and suppression of HIV RNA was defined as a decline in levels from detectable to undetectable [36].

CPE scores for antiretroviral medications

CNS penetration-effectiveness (CPE) ranks were assigned to each component of CART as described by [37]. The CPE rank was derived by dividing the sum of ranks assigned for each antiretroviral drug included in the patient regimen, by the number of medications. Scores ranged from 1 to 4 with a higher score representing more effective penetration.

Sample processing and Western blot analysis

CSF samples were stored at -80° C, thawed on ice and centrifuged briefly at top speed at 4°C to remove cell debris. An equal volume (4ul) of each CSF sample was loaded per well onto 4–12% Bis-Tris pre-cast gels (Invitrogen, Carlsbad, CA, USA). Samples were separated by electrophoresis and transferred onto nitrocellulose membranes (Bio-Rad). Membranes were blocked in 5% nonfat milk or 5% BSA in Tris-buffered saline with 1% Tween-20 (1× TBST) for 30 min before overnight incubation at 4C with primary antibodies. Primary antibodies included PINCH1 (1:1000, BD, Rockville, MD, USA), anti-Tau antibodies, S262 (1:1000, Abcam) and S396 (1:1000, Abcam). Membranes were washed 3× in 1× TBST and incubated in secondary anti-mouse IgG2a or anti-rabbit HRP (1:10,000,

Thermo Scientific, Lafayette, CO) antibody for 1h at room temperature and visualized with enhanced chemiluminescence (ECL) prime (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Densitometry was performed using 3D ImageJ software [38]. To assess the potential correlation of PINCH with hpTau, CD4+ count, CSF and plasma HIV viral burden and CSF WBC count at each time point, fold change of PINCH was calculated as the ratio of the densitometry value for the 2nd time point (T2) and the 1st time point (baseline, T1) represented as T2/T1. Similarly, T3/T1 represents the fold change calculated as the ratio of the densitometry value for the 3rd (T3) time point and the 1st (T1) time point.

CSF Neopterin ELISA

CSF neopterin concentrations were analyzed according to manufacturer's instructions using a commercially available sensitive enzyme-linked immunosorbent assay (Neopterin ELISA, IBL international GMBH, Hamburg, Germany). Cubic spline interpolation with a not-a-knot condition was used to calculate neopterin concentrations from optical density values of duplicate samples (<http://www.akiti.ca/CubicSpline.html>)[39].

Statistical analysis

Median values with interquartile range for plasma HIV RNA log, CSF HIV RNA log, CSF WBC count, CD4+ count for the three time points were computed to describe the distribution of the data. Kruskal-Wallis one-way analysis of variance (ANOVA) test was conducted to assess the difference in mean values for PINCH across the time points and to assess the difference in mean values for PINCH between responders and non-responders. Spearman correlation co-efficient was computed for PINCH and hpTau (S262 and S396) in study participants. Spearman correlation coefficients were also calculated to assess correlation between fold change of PINCH and HIV RNA values in CSF and plasma, as well as between PINCH and CSF WBC count. Similarly, Spearman correlation coefficients were calculated to assess correlation between CD4 + count and fold change of PINCH, and between CD4 + count and S262, and S396. The α value for all the statistical tests was retained at 0.05. Statistical analyses were conducted using Prism 6 (GraphPad software, Inc).

Results

Changes in PINCH levels in HIV participants' CSF over time

To assess CSF levels of PINCH after the initiation of CART or after a change in the CART regimen, samples from three different time points per patient were assessed by Western analysis. Distinct PINCH bands were detected at approximately 55 and 71 kDa (Figure 1A), as previously reported [4, 6]. A third PINCH immunoreactive band was detected at approximately 84 kDa in some cases, reminiscent of the approximate size detected by immunoprecipitation of PINCH and hpTau [6]. Detection of multiple PINCH immunoreactive bands has been previously reported, but currently the significance of the different bands is unknown. To determine if PINCH levels within a given participant differed over time, we compared levels of PINCH from the 3 different time points for each participant as shown by a representative blot for 1 virologic non-responder and 1 responder patient (Figure 1A). When we compared the changes at T2 from baseline for all participants,

in 10 of the 17-virologic responder participants and 1 non-responder participant, PINCH levels increased above baseline or remained unchanged at T2 (Figure 1B, blue and black circles, respectively). In the remaining 7-responder participants (yellow circles) and 2 non-responder participants (pink circles), PINCH levels were below baseline at T2 (Figure 1B). This general trend was also observed for T3/T1, but were more variable.

Changes hpTau levels in HIV patients' CSF followed a similar trend as PINCH levels

Changes in hyperphosphorylated Tau levels in the CSF of HIV+ participants were assessed by Western analyses for each of the three collection time points. A representative Western blot from 1 virologic responder (R) and 1 non-responder (NR) shows the typical banding pattern for these participants (Figure 2A). Changes in hpTau levels in approximately 50% of responders (n = 9, blue squares) and 1 non-responder (black squares) increased or remained unchanged at the 2nd time point (T2) (Figure 2B). Likewise, hpTau levels decreased in 8 virologic responders (yellow squares) and 2 non-responders (pink squares) (Figure 2B). Fold changes in hpTau at the 3rd time point (T3) followed a similar trend with 8 responders' levels (blue squares) and 1 non-responder's (black square) level at T2 increasing or remaining unchanged (Figure 2B).

PINCH levels correlate with hpTau levels in CSF of HIV+ patients

To assess if changes in levels of PINCH and hpTau correlate with one another, fold-changes in PINCH and hpTau were determined by Western analyses by calculating the ratio of the densitometry value for the 2nd time point (T2) to baseline levels, and the 3rd time point (T3) to baseline. Spearman correlation coefficient analyses indicated that the fold-change in PINCH levels between 1st and 2nd time points were highly correlated to the hpTau variable (correlation co-efficient = 0.70, p-value = 0.001) (Figure 3A). Fold-changes in PINCH levels between the 1st and 3rd time points correlated with hpTau, as well (correlation co-efficient = 0.83, p-value = <0.0001) (Figure 3B). There was no correlation between the 2nd and 3rd PINCH ratios with hpTau (data not shown). Although the trend persisted, analyses for the correlation between PINCH and hpTau at S396 showed no statistical significance. These findings suggest a relationship between the fold-changes in PINCH and hpTau in HIV participants on CART.

Changes in PINCH CSF levels correlate with CD4+ count in HIV Patients

To assess if changes in CSF PINCH levels correlate with other clinical parameters for this group of study participants, PINCH fold-changes were compared to CD4+ count, plasma and CSF viral RNA, CSF WBC counts and neopterin levels. Spearman correlation coefficients showed no correlation between PINCH and HIV RNA, between PINCH and WBC or between PINCH and CPE scores (not shown). As previously reported by Price *et al*, analyses from a subset of these participants confirmed that CSF neopterin levels and CSF viral load correlated. However, neopterin levels did not correlate with CSF PINCH (data not shown).

On the other hand, Spearman correlation coefficients for CD4+ count and PINCH indicated significant correlation (correlation coefficient = 0.72, p-value = 0.0067) (Figure 4A, circles), and CD4+ count and hpTau at S262 (squares) yielding a correlation coefficient = 0.59 and

p-value = 0.0381 (Figure 4A). As shown in Figure 4A, PINCH levels increased or remained unchanged in all participants with CD4+ counts over 200 cells/mm³, whereas; all participants whose CD4+ count dropped below 200 cells/mm³ had less PINCH in the CSF (yellow and pink circles). With regard to hpTau levels, this trend was observed for all but 1 participant (Figure 4A, yellow square). In earlier studies, we reported that PINCH levels were significantly increased in HIV participants' brains and CSF.

Taken together, these results show that changes in PINCH CSF levels correlate with changes in CD4+ count rather than viral burden (Figure 4B). Moreover, our data show that increased CSF levels of PINCH correspond with a more favorable immune response as defined by improved CD4 count. Previous data reported that increased PINCH is observed in the brains and CSF of HIV patients, but it was also shown that patients with HIVE had more PINCH in their brains compared to CSF [4]. Likewise, HIV patients with no CNS alterations had less PINCH in the brain and more in the CSF. These findings are supported by our data showing that as CD4+ count increases, PINCH levels in the CSF increase as well.

Discussion

In the current study, we addressed the potential significance of PINCH in the CSF of HIV+ individuals. Clinical and laboratory information including age, blood CD4+ count, CSF and plasma viral load, CSF WBC count, and CPE scores of antiretroviral therapy were compared with fold changes in CSF PINCH and hpTau levels. Our results suggest that changes in CSF levels of PINCH correlate with CSF hpTau levels in this cohort of HIV participants. Previous studies reported that PINCH and hpTau bind one another both *in vitro*, *in vivo* and in the brains and CSF of HIV patients [6]. Our earlier studies also reported higher levels of PINCH in brain and CSF in both HIV with no CNS alterations and HIVE, compared to HIV negative adults. However, PINCH levels in CSF in HIV patients with no CNS alterations were substantially greater than that in HIVE. Similarly, alterations in the solubility of PINCH were also associated with solubility changes in hpTau. These studies support previous findings and suggest a correlation between PINCH and hpTau levels in HIV-associated CNS disease. Although the mechanisms underlying this association are unknown, there are several points of intersection between PINCH and Tau pathways that may contribute to their interaction.

One possible contributing mechanism is through PINCH's binding partners that are involved in hyperphosphorylation of Tau and rely on interacting with PINCH to maintain their catalytic activity. For example, the most well characterized binding partner of PINCH is integrin-linked kinase (ILK). ILK is an ankyrin-repeat containing serine-threonine kinase that phosphorylates multiple kinases such as GSK3 β and AKT involved in aberrant hyperphosphorylation of Tau [40]. In the absence of PINCH, ILK activity is diminished, thus alterations in levels of PINCH may contribute to changes in formation of hpTau. Likewise, PINCH binds to and inactivates protein phosphatase 1 α (PP1 α) that is involved in the dephosphorylation of Tau residues essential for normal Tau-microtubule interactions [41]. The heat shock response represents a third potential pathway that may contribute to PINCH/hpTau interactions, as we have shown that PINCH binds to both hpTau and to the E3 ubiquitin ligase (CHIP) [6]. In this context, knockdown of PINCH in neurons where

hpTau was induced resulted in less hpTau detection, which could be a result of less formation of hpTau, more efficient clearance, or both. The potential interaction(s) among these pathways and PINCH and Tau levels in the CSF are currently unknown, however, changes in binding dynamics and release from the brain into the CSF is an area warranting more investigation.

A second significant and unexpected finding was that the fold-changes in PINCH levels over baseline correlated with blood CD4+ count. Interestingly, all participants with a CD4+ count above 200 cells/mm³ at T2 showed increased or no change in PINCH CSF levels and participants whose CD4+ count decreased below 200 had less PINCH in the CSF. Blood CD4+ count is used as one of the clinical measures of HIV infection, in opportunistic infections in HIV, and as a predictor of response to CART. A CD4+ count less than 200 cells/mm³ is one of the diagnostic markers for acquired immunodeficiency syndrome. The results from our current study might represent an important correlation between PINCH CSF levels and systemic immune response in HIV. The connection between improved immune response and greater levels of PINCH in the CSF is unclear. But as pointed out in earlier studies, HIV patients with less severe CNS alterations may in fact clear both PINCH and hpTau from the brain more efficiently resulting in the detection of more PINCH/hpTau in the CSF relative to patients with increased hpTau accumulation in the brain and by virtue of association, more PINCH in the brain as well.

About 20% of HIV patients are immunological non-responders with low blood levels of immune cells, increased immune activation, immunosenescence and apoptosis even in the presence of effective antiretroviral therapy and significant viral suppression. Various causes for poor immunological response include older age, ineffective antiretroviral therapy, and low CD4 nadir with a CD4+ count < 200cells/ul. Interestingly, the two virologic responders in our study with a CD4+ count < 200cells/mm³ and a decrease in PINCH ratios were over 45 years of age; whereas, others were younger than 40 years of age, suggesting that age may be a factor in CD4+ count and PINCH levels.

There were several limitations in this study. Although this study was longitudinal in some aspects, the collection dates among participants for each time point were not conducted at the exact same intervals. Rather collection points for T2 and T3 were 33 (29-36) days and 96 (85-123), respectively, which represent the median (interquartile range with 1st and 3rd quartile). This rather wide range may account for the variation observed at T3 in PINCH and hpTau (Figure 1B, 2B). Of note, changes in PINCH levels at 1-1.5 months from baseline showed stronger correlations with CD4+. Another limitation in the study is that we were unable to consider CD4+ nadir in our correlation analyses since these data were not available for all patients. Because CD4+ nadir is a better clinical and diagnostic measure of immune system response to HIV infection and treatment, future studies will consider the CD4+ nadir to increase our understanding of the relationship between CSF PINCH and immune system. Thus, future studies to understand the potential significance of PINCH in brains and CSF of HIV patients should be expanded to include many more patients and consider age, immune response, and response to treatment.

In this context, in our current study we observed a trend of decreased fold change for PINCH in older HIV participants in the responder treatment group with CD4 + count below 200 cells/mm³, suggesting PINCH protein's association with aging in defining disease pathology. Thus, future studies exploring the effects of aging in HIV patients and levels of PINCH in brain and CSF in response to hpTau are needed. Furthermore, CD4+/CD8+ ratios in CSF and blood should also be analyzed for assessing the response to treatment, effect of immunosenescence in HIV and disease pathology in the context of PINCH expression levels. Understanding the role of PINCH in CNS of HIV infection would help better define the function of PINCH in the pathophysiology of aberrant tau formation in HIV, in immune response to infection and therapy, and the significance of its detection in CSF and brain in HIV, AD and other neurodegenerative diseases. In conclusion, PINCH CSF levels appear to correlate with immune responses in HIV participants, suggesting another role for PINCH in CNS disease pathology including in HIV and other neurodegenerative diseases.

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Figure 1. PINCH levels in the CSF of HIV participants

A) Representative Western blot of PINCH in CSF from two different HIV+ participants, 1 non-responder (NR) and 1 responder (R). Western blot of CSF collected at three different time points and reacted with anti-PINCH antibody detects bands at molecular weights of approximately 55, 71 or 84 kDa. T1 (baseline), T2 and T3 correspond to 1st, 2nd and 3rd time points of sample collection, respectively. **B)** Kruskal-Wallis one-way ANOVA of PINCH comparing fold changes to baseline values in each patient yielded statistically non-significant results. T2/T1 and T3/T1 represent fold change from the baseline value (T1). Blue circles represent responder HIV+ participants with increased fold change of PINCH at T2 or T3 compared to T1. Yellow circles represent responder HIV participants with decreased fold change of PINCH at T2 compared to T1. The black circle represents a non-responder HIV+ participant with an increased fold change of PINCH at T2 or T3 compared to T1. Pink circles represent non-responder HIV + participants with decreased fold change of PINCH at T2 or T3 compared to T1.

Figure 2. hpTau levels in CSF of HIV+ participants

A) Representative Western blot of hpTau (S262) in CSF from two different HIV+ participants, 1 non-responder (NR) and 1 responder (R). Western blot of CSF collected at three different time points and reacted with anti-S262 hpTau antibody detects bands at an approximate molecular weight of 55 kDa. T1 (baseline), T2 and T3 correspond to 1st, 2nd and 3rd time points of sample collection. **B)** Kruskal-Wallis one-way ANOVA of S262 comparing fold changes to baseline values in each patient yielded statistically non-significant results. T2/T1 and T3/T1 represent fold change from the baseline value (T1). Blue squares represent responder HIV+ participants with increased fold change of hpTau at T2 or T3 compared to T1. Yellow squares represent responder HIV+ participants with decreased fold change of hpTau at T2 or T3 compared to T1. The black squares represent non-responder HIV+ participants with an increased fold change of hpTau at T2 or T3 compared to T1. Pink squares represent non-responder HIV+ participants with decreased fold change of hpTau at T2 or T3 compared to T1.

Figure 3. Changes in PINCH levels in CSF correlate with changes in hpTau levels

A) Fold change in PINCH (y-axis) compared to hpTau (X-axis) from baseline (T1) to T2, or **B)** from baseline (T1) to T3. Blue circles represent responder HIV+ participants with increased fold change of PINCH at T2 or T3 compared to T1. Yellow circles represent responder HIV+ participants with decreased fold change of PINCH at T2 or T3 compared to T1. The black circle represents a non-responder HIV+ participant with an increased fold change of PINCH at T2 or T3 compared to T1. Pink circles represent non-responder HIV+ participants with decreased fold change of PINCH at T2 or T3 compared to T1. Spearman correlation coefficients for PINCH ratios and pTau (S262) at **A)** T2/T1, p-value = 0.001 and **B)** T3/T1, p-value <0.0001.

Figure 4. Changes in PINCH and hpTau in CSF correlate with blood CD4+ count

A) T1 and T2 correspond to 1st and 2nd time points of sample collection. T2/T1 represents fold change from the baseline value (T1). Blue represents responder HIV+ participants with increased fold change of PINCH (circles) or hpTau (squares) at T2 compared to T1. Yellow represents responder HIV+ participants with decreased fold change of PINCH (circles) or hpTau (squares) at T2 compared to T1. Pink represents non-responder HIV+ participants with decreased fold change of PINCH (circles) or hpTau (squares) at T2 compared to T1. Black represents a non-responder HIV+ participant with an increased fold change of PINCH (circle) and hpTau (square) at T2 compared to T1. Circles represent PINCH, and squares represent pTau (S262). Spearman correlation coefficients fold change of PINCH at T2 compared to T1 and CD4 + count at T2, p-value = 0.0067 and fold change of pTau (S262) at T2 compared to T1 and CD4 + count at T2, p-value = 0.0381. **B)** Venn diagram depicting

the correlations between fold change of PINCH at T2 compared to T1 and CD4 + count at T2 in responders and non-responders. The blue circle represents responders, the pink circle represents non-responders and the intersecting yellow region represents responders with decreased PINCH ratio and CD4 + count < 200 cells/mm³.

Table 1

Summary statistics representing the median and interquartile range for plasma HIV RNA load, blood CD4 + count, and for CSF HIV RNA load, and CSF white blood cell count.

Sample collection	Median (interquartile range)
Plasma HIV RNA (log ₁₀ RNA copies/mL)	
1 st time point (Baseline, T1)	4.92 (4.71-5.54)
2 nd time point (T2)	2.99 (2.54-3.2)
3 rd time point (T3)	2.30 (2.30-2.53)
CSF HIV RNA (log ₁₀ RNAcopies/mL)	
1 st time point (Baseline, T1)	3.54 (2.82-3.86)
2 nd time point (T2)	1.95 (1.73-2.26)
3 rd time point (T3)	1.69 (1.69-1.69)
CSF White Blood Cell count (cells/mm ³)	
1 st time point (Baseline, T1)	3 (1-12)
2 nd time point (T2)	3 (2-4)
3 rd time point (T3)	2 (1-3)
CD4 + count (cells/mm ³)	
1 st time point (Baseline, T1)	139(67-237)
2 nd time point (T2)	285 (183-375)
3 rd time point (T3)	328 (233-362)