

Highlights of the Global HIV-1 CSF Escape Consortium Meeting, 9 June 2016, Bethesda, MD, USA

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Abstract

CSF HIV escape is a recently recognised phenomenon that suggests that despite suppressive treatment, HIV RNA may be detected in the CNS compartment in some individuals. In rare cases this is associated with clinical neurological disease, while in most cases, neurological consequences are not apparent. Attempts at characterising the biological substrates of CSF escape and further investigating the neurological consequences need to be made to better understand the implications of this condition for the HIV cure agenda as well as for clinical outcomes. The Global CSF HIV-1 Escape Consortium meeting, convened by the US National Institute of Mental Health, was a first step to gather investigators from diverse sites to discuss opportunities for future collaborative work on this emerging issue. To better understand CSF HIV escape and allow cross-site data reconciliation, it will be useful to reach a consensus set of definitions of the distinct forms of CSF escape, without which concerted cross-site efforts are difficult.

Introduction

Eradication of HIV-1 from active and latent reservoirs and achieving a functional cure, defined as 'long-term undetectable viraemia for an as-yet-undefined period (probably several years) in the absence of ART' [1], is currently a high priority area for the AIDS research community and the National Institutes of Health (NIH). Successful control of HIV-1 replication in the CNS is one of the key milestones needed to accomplish the goal of a functional cure. Effective antiretroviral therapy (ART) has changed the nature of the epidemic and a majority of patients with HIV-1 are virally suppressed. However, recent findings from several clinical studies have demonstrated that despite stable and successful control of HIV-1 in the periphery, approximately 5–10% of individuals with HIV-1 still have detectable virus in the CSF (CSF escape) [2]. These scientific findings, pertaining to discordance of viral loads between the CSF and periphery in well-controlled patients on ART, present a unique opportunity to study the molecular mechanisms involved in CNS reservoir establishment, compartmentalisation, persistence and resurgence. Studying molecular mechanisms involved in HIV-1 CSF escape and resurgence of CNS-based HIV-1 reservoirs is likely to provide key information needed for designing cure and eradication strategies.

Understanding factors such as genetic make-up of CSF escape variants, influence of host genetics and immune activation on CSF escape, importance of ART regimens and CNS bioavailability of drugs, along with resistance and adherence issues pertaining to ART regimens, will be critical in achieving the goal of HIV-1 cure. Clinical and radiological characterisation of patients exhibiting CSF escape, and pathological assessments of brain autopsies from patients exhibiting CSF escape, are important for us to decipher in order to understand the relationship between CSF escape and neurocognitive impairment. To better comprehend the mechanisms and pathogenesis of CSF escape and achieve the goal of a functional HIV-1 cure it will be important to bring the different investigators pursuing this research together and consolidate the data and samples from these cohorts, especially given the low frequency of occurrence. To this end, the US National Institute of Mental Health (NIMH) held a meeting of investigators that have access to CSF escape cases to establish a 'Global HIV-1 CSF Escape Consortium'. This report summarises the presentations as well as discussions at the meeting and outlines the potential next steps towards formation of a Global HIV-1 CSF Escape Consortium.

Ongoing UCSF HIV-1 CSF escape study

Dr Richard Price, the lead investigator of the UCSF HIV-1 CSF escape study, began the first session of the meeting by describing the rationale for studying HIV-1 CSF escape and provided an overview of the R01 study entitled 'Compartmentalised CSF viral escape and the CNS HIV reservoir'. The key goals of the study are to characterise the evolving molecular genetics of CSF HIV

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isolates and their phenotypic correlates compared to their blood counterparts in the setting of HIV-1 CSF escape in virally suppressed cases. The specific aims also address the neurological implications of both asymptomatic and neurosymptomatic CSF escape as well as those of treatment interruption. Dr Price anticipates a total of 450 samples from the participating clinical sites: Gothenburg University, Sweden; San Raffaele Scientific Institute, Italy; University of California San Francisco (UCSF); University of North Carolina (UNC), Chapel Hill; and Yale University. Further, he presented to the group the architecture and user interface of a dedicated REDCap HIV-1 CSF escape patient database, while alluding to the utility of maintaining such a tool. Following his presentation, there was a brief discussion regarding expansion of studies to include viral reservoirs other than the brain and the need to establish longitudinal cohorts.

Dr Magnus Gisslen, a key collaborator in the above described study presented data pertaining to asymptomatic and secondary HIV-1 CSF escape. He gave insights into the long-standing Gothenburg CSF longitudinal cohort study that began in 1985, involving serial sampling of CSF and blood from both symptomatic and asymptomatic HIV-positive subjects. The key question he is trying to answer is: what is the frequency of observed CSF escape in virally suppressed patients not exhibiting any neurological symptoms? In a sub-study of the Gothenburg cohort involving 75 patients, 23–36% of the patients exhibited CSF blips. However, a proportion of these patients also exhibited blips in the plasma. With more stringent criteria and a larger study population, roughly 5–10% of subjects exhibited a true CSF blip in the absence of a plasma blip. He and others have also made the observation that these blips are associated with increased intra-theal immune activation. Furthermore, Dr Gisslen stressed the importance of understanding secondary CSF escape that can be ‘triggered by a defined or presumed CNS infection or inflammatory process not directly caused by HIV’. Discussion following this presentation touched on the relationship of CSF blips to adherence and the need to differentiate whether active viral replication or inflammation was the underlying reason for the HIV-1 CSF escape.

Dr Paola Cinque focused her talk on neurosymptomatic CSF escape and gave an in-depth overview of 19 CSF escape cases in Milan. The cases were categorised as: encephalitis ($n=16$); meningoencephalitis ($n=2$) and myelitis ($n=1$). Of the 19 cases, two longitudinal case studies highlighting the links between CSF escape, encephalitis and drug resistance were presented, which led to a discussion of whether CSF escape is the cause or a consequence of drug resistance. Dr Cinque concluded the talk by suggesting that the possible risk factors for HIV-1 CSF escape include the presence and size of the brain HIV-1 reservoir, ARV drug resistance, inadequate ART adherence, ART simplification and inadequate efficacy of individual drugs/regimens.

Dr Serena Spudich focused her talk on how HIV-1 ‘escaping’ from suppression in the CNS after ART interruption can inform the field about CNS reservoirs. She provided examples of cases of HIV-1 rebound and inflammation resulting in neuronal injury within days to weeks following ART interruption. She further presented the selection criteria and recruitment plan for the ART interruption aspect of the planned study, and also data pertaining to the molecular dissection of CSF and plasma HIV-1 variants before and after treatment interruption. In addition, Dr Spudich presented a case study of a participant who voluntarily stopped treatment in a longitudinal observational study. In this participant, an HIV-1 variant detected only in the CSF prior to ART was found to be the predominant population present in the blood after treatment interruption, suggesting the possibility that a CNS-derived virus might have repopulated the periphery after viral rebound. Dr

Spudich suggested that deep sequencing patient samples pre- and post-treatment interruption could help us further understand compartmentalised viral rebound in the plasma and CSF. She closed with a few key questions that need further discussion, such as the cell types that support viral replication in the CNS, the source of rebounding CNS virus, the possibility of the CNS-derived virus reseeding the periphery, and the occurrence of deleterious effects in the CNS in the context of treatment interruption.

Dr Sarah Joseph presented data from the THINC cohort at University of North Carolina, Chapel Hill. Detailed analysis of the available paired plasma/CSF samples revealed that 6% of the HIV-infected subjects in the cohort, on ART for at least a year, had asymptomatic CSF escape, and around 2% had persistent CSF escape due to the presence of an active CNS viral reservoir. For four of the subjects, the study team performed detailed genetic and phenotypic analyses to better comprehend the nature of the active CNS reservoir. Sequencing results for two of the cases were presented, one showing a transient, homogeneous R5 T cell-tropic HIV-1 viral population in the CNS and another with a persistent, heterogeneous HIV-1 viral population with elevated macrophage tropism. Also, data relating to the association of pleocytosis with HIV-1 CSF escape were presented. Dr Joseph gave an overview of a proposed model of HIV-1 persistence in the CNS that suggests active, persistent CNS reservoirs are associated with pre-therapy CNS compartmentalisation of macrophage-tropic variants resulting in infection of long-lived CNS resident cells (macrophage/microglia) and low pleocytosis post-therapy. Discussion following the talk focused on the utility of deep sequencing to observe compartmentalised HIV-1 viral evolution.

International site studies

Dr Alan Winston presented data from the St Mary’s Hospital cohort, UK PARTITION cohort study, POPPY and the Age-HIV Cohort study. His presentation began with a broad overview of the AIDS epidemic in the UK showing that over 90% of HIV-positive subjects attending clinics in the UK have undetectable viral loads. The prevalence data of HIV-1 CSF escape in the various UK cohorts were presented. In the St Mary’s Hospital cohort, 21% of the infected patients on ART had a demonstrably higher CSF viral load than plasma, with 9% of these patients having plasma viral loads below the limit of detection. However, this is a historic cohort with data captured between 2008 and 2010. In the UK PARTITION cohort study, around 18% of the patients, specifically the patient population with consistent low-level viraemia, had a discordance in CSF/plasma viral loads. In a combined study of the POPPY and Age-HIV cohorts (COBRA), of the 134 HIV-positive patients, only one patient had a demonstrable viral load in the CSF. Inclusion criteria to the COBRA study included undetectable plasma HIV-1 RNA, which may partly account for the low numbers with CSF escape observed in this cohort. Further analysis of the demographics of patients enrolled in the POPPY cohort suggested age-related disparities are prevalent, with regards to comorbidities, cognitive function and cognitive impairment. Lastly, Dr Winston shared an exciting new technological breakthrough involving cerebral TSPO PET imaging in acute and chronic HIV-infected cohorts.

Dr Luminita Ene started her presentation with an overview of the HIV-1 epidemic in Romania and the cascade of care at the Victor Babes tertiary infectious diseases hospital. The patient population in her study is composed primarily of patients from a former paediatric HIV-1-infected cohort alongside patients infected heterosexually and as intravenous drug users. Currently, 2500 patients are being followed and around 65% of these patients have viral loads below 50 copies/mL. In this cohort, 385 paired

CSF–plasma samples were analysed. Of these, from 50 patients with undetectable plasma HIV-1 loads, 14% (7) had detectable CSF HIV-1 RNA, and from 42 patients with low-level viraemia in the plasma, 25% (11) had detectable CSF HIV-1 viral loads. She then delved into the details pertaining to three distinct types of CSF viral escape (secondary, neurosymptomatic and asymptomatic) observed in the patient population. Dr Ene's data demonstrated that in a clinical setting, CSF/plasma discordances could be due to slower decay of CSF HIV-1 RNA in patients with previous CNS opportunistic infections, secondary to CNS neurological conditions (2%) or asymptomatic (2%). The data presented also suggest that symptomatic CSF escape may present as cognitive decline, as this was seen in two previously stable patients where changes to their ART regimens helped improve the outcome.

Dr Ignacio Pérez Valero presented data from the HIV Unit in La Paz Hospital, Madrid, Spain. Of the 4200 patients treated at this facility, 96% are on suppressive ART. Paired CSF and plasma samples from 125 patients were analysed. Analysis of data from these patients revealed four (3.2%) had low to moderate levels of CSF escape. Dr Perez described the case histories and clinical presentations of these four patients. Three of the four displayed moderate to severe forms of neurological dysfunction that were addressed by changes to their ART regimens. One of these patients had persistent CSF escape associated with blips in plasma despite having no known drug resistance mutations. Dr Pérez Valero concluded by stating that further research on the cases with CSF escape is needed, including analysis of neurological biomarkers, levels of CNS inflammation markers, temporal deep sequencing and an evaluation of ART drug levels in the CSF.

Dr Ned Sacktor presented the details of the Rakai Community-based Cohort Study involving HIV-1 research, PEPFAR care and antiretroviral treatment in 50 communities in the Rakai District of Uganda where HIV-1 prevalence is close to 14%. This study is a collaboration between Uganda Virus Research Institute, Makerere University, and Johns Hopkins University in Baltimore, Maryland. The Rakai-NeuroHIV study team has enrolled 400 HIV-infected, ART-naïve participants and 400 uninfected participants to assess the impact of neurocognitive comorbidities. Thus far, nine out of 91 ART-treated participants with an undetectable plasma viral load have detectable CSF viral load, or CSF escape. Using a battery of neurocognitive assessments, neurocognitive dysfunction was diagnosed in four of the nine participants with CSF escape. The current goals of the Rakai-NeuroHIV Study are to assess whether the trajectory of neurocognitive morbidity over 2 years of follow-up differs by HIV-1 subtype as well as to define and evaluate the association of CSF viral escape cases with neurocognitive dysfunction with respect to the HIV-1 subtype.

Dr Edwina Wright presented an analysis of CSF escape in HIV-positive patients during 2009–2015 in the Alfred Health system. The criterion for selection for the analysis was undetectable plasma HIV-1 viral load in HIV-positive participants within 6 months prior to a detectable CSF HIV-1 viral load test. Eighty-eight of 167 participants had detectable virus in their CSF sample, though only six met the criterion for true CSF escape. The case histories of all six participants, including brain imaging, were presented. Four among the six cases had a diagnosis of neuropsychological dysfunction and two presented with neurological comorbidities (progressive multifocal leukoencephalopathy or amyotrophic lateral sclerosis). One of the six CSF escape cases was deceased and had documented evidence of HIV-associated dementia (HAD). Dr Wright proposed that future research into CSF escape cases should include additional imaging assessments in conjunction with analysis of the paired CSF/plasma samples as well as evaluations of

biomarkers, quantitation of the CNS HIV-1 reservoir and discovery research to identify the source of HIV-1 in the CSF.

Dr Ameet Dravid presented findings from the Pune NeuroAIDS Study conducted in three private hospitals in Pune, India, including Ruby Hall Clinic, Poona Hospital and Noble Hospital, over a 7-year duration (2009–2016). The goal of this prospective observational cohort study was to understand the spectrum of neurological diseases seen in HIV-infected Indian patients on suppressive ART (plasma viral load on ART <1000 copies/mL) and determine the incidence of CSF HIV-1 escape that has been increasingly reported as a cause of severe neurological dysfunction. Of the 1427 virologically suppressed patients in his cohort, 62 developed severe neurological symptoms and 56 (4%) had an incident neurological disease [3]. Neurosymptomatic CSF escape was determined to have occurred in 17/62 (27%) patients based on the combined data from plasma and CSF viral load assays (CSF viral load 1 log higher than plasma viral load). Neurodeterioration was significantly associated with use of ART regimens with low CPE scores (CPE score <6). Change of ART based on CSF genotypic resistance testing and inclusion of drugs with better CNS penetration, like zidovudine, led to resolution of CSF escape in 16 patients. One patient died despite aggressive interventions. HIV encephalopathy due to CSF escape and stroke were found to be the most common infectious and non-infectious causes of incident neurological deterioration in the Pune NeuroAIDS Study cohort.

Domestic (US) site studies

Dr Scott Letendre presented results from two longitudinal, observational cohorts of HIV-positive adults on ART for more than 6 months: (1) the CNS HIV Antiretroviral Therapy Effects Research (CHARTER) Study; and (2) the HIV Neurobehavioral Research Program (HNRP). In these cohorts, CSF was collected every 6 (CHARTER) to 12 (HNRP) months. CSF escape was defined as CSF HIV-1 RNA levels above the lower limit of detection when blood levels are undetectable, and was observed in 60 (7%) of the 849 participants enrolled in the study. Dr Letendre described three patterns of CSF escape based on longitudinal analysis of paired blood and CSF samples: (1) CSF Blip: one CSF sample from the same individual over time met the criterion for CSF viral escape (37 of 60 participants); (2) CSF Slow Suppression: at least two consecutive CSF samples met the CSF viral escape criterion over time but eventually suppressed and occurred in the context of a more rapid decline in plasma HIV-1 RNA that suppressed prior to CSF suppression (17 of 60 participants); and (3) Persistent CSF Viral Escape: at least two consecutive CSF samples met the CSF viral escape criterion over time with HIV-1 RNA suppression in all paired plasma specimens (six of 60 participants). A surprising outcome of this longitudinal study was the fact that CSF viral escape was not associated with neurocognitive performance over time even though it was associated with risk factors like inflammation, advanced immune suppression and longer duration of disease. One of the likely reasons discussed for the above concerned the results from a sub-analysis that showed persistent CSF viral escape was associated with higher levels of inflammatory cytokines in CSF.

Dr Avindra Nath (Clinical Director of the National Institute of Neurological Disease and Stroke [NINDS], NIH) presented results from the NIH Clinical NeuroHIV Study 'All Hands', with over 160 HIV-positive participants. Among the HIV-positive individuals who agreed to undergo lumbar puncture, 11 (20%) of 56 CSF samples collected displayed HIV-1 levels of greater than 20 copies per mL (CSF range 23–516 copies/mL) while the plasma levels were consistently less than 40 copies/mL for over a year. A key question that the field of HIV-1 reservoir research has been grappling with

is whether replication-competent virus is present in the CNS and whether it could be isolated from the CNS. Dr Nath attempted to address this important question by isolating HIV-1 present within the cells harvested from CSF and performing a viral outgrowth assay. After several days of co-culturing cells isolated from the CSF with PBMCs, HIV-1 p24 expression became apparent, suggesting that the CNS is one of the key reservoirs that needs to be focused upon to achieve the goal of HIV-1 cure. HIV-1 sequencing is ongoing from cells harvested from the CSF and matched PBMCs from the blood in order to characterise the phylogenetic evolution of HIV-1 in CSF escape.

Dr Dana Gabuzda presented a study on CSF escape cases from HIV-infected individuals at four hospitals in Boston, MA and the four National NeuroAIDS Tissue Consortium (NNTC) clinical sites. The study evaluated consecutive plasma and CSF samples from the same individuals over the past decade providing a unique temporal perspective. CSF escape was defined based on paired plasma and CSF viral loads as follows: (1) CSF viral load greater than 50 copies/mL with paired plasma HIV-1 viral load less than 50 copies/mL in virally suppressed subjects on a stable ART regimen for at least 6 months; or (2) CSF viral load 0.5 log higher than plasma viral load in subjects on a stable ART regimen with or without complete viral suppression for at least 6 months. By this definition, 40 CSF escape cases were documented at the Boston hospitals and NNTC clinical sites, with an estimated prevalence of close to 6%. The study further distinguished between cases with incomplete plasma suppression preceded by low or high levels of viraemia, and those with durable plasma suppression and CSF blips or CSF escape. Genotypic analysis of HIV-1 identified a subset of drug resistance mutations that were more prevalent in CSF than plasma, suggesting an effect of compartmentalisation. Clinical risk factors associated with CSF escape in Dr Gabuzda's analysis included HIV-1 diagnosis prior to 1996, plasma/CSF drug resistance mutations and the presence of neurological symptoms.

Dr Valerie Wojna presented the characteristics of the NeuroAIDS Clinical Hispanic Longitudinal Women only Cohort in Puerto Rico with CSF escape. During 2001–2014, 236 HIV-seropositive women at risk of developing cognitive impairment (low CD4 cell count <500 cells/mm³) were evaluated longitudinally including neuropsychology tests and plasma viral loads every 6 months, and CSF samples were collected once per year. Ten of the 380 CSF/plasma paired samples collected from this cohort had CSF HIV-1 RNA levels greater than in plasma (a prevalence of 2.6%); and three of these samples had detectable CSF HIV-1 RNA while plasma levels were below the limit of detection. Nine of the ten women were using combined antiretroviral treatment (ART), including the use of protease inhibitors (7/9), and presented a median CNS penetration effectivity score (CPE) of 7. Among those using ART, they were more likely to be older (median age of 43 years), without cognitive impairment (NPZ median z-score of 0.1) and have a low nadir CD4 and CD4 cell count (median cell count of 57.5 and 398 cells/mm³, respectively). A positive correlation (Spearman's correlation $P=0.047$) was observed between CSF/plasma HIV-1 RNA log ratio and the Beck Depression Index. No correlations were observed between CSF/plasma HIV-1 RNA ratio and age, CPE, CD4 cell counts and cognitive impairment. Dr Wojna is interested in further analysis of these cases of CSF escape and will correlate the findings with prior studies conducted in the cohort.

Dr Deborah Persaud discussed specifics of CNS disease in perinatal HIV-1 infection. The prevalence of perinatal HIV-1 CNS disease in resource-rich settings is 13% to 23% with suppressive ART. Findings in these cases include clinically detectable delays in

cognitive, motor and language development (with gross motor development most adversely affected), and HIV-1 encephalopathy. In ART-naïve children, analysis of the relationships between clinical characteristics and viral genotypic analysis revealed the occurrence of specific viral populations within the CSF which were absent from paired blood samples in half the children by 3 years of age. Dr Persaud also presented data of possible secondary CNS Escape in a very early treated, prenatally HIV-infected infant. The Children with HIV Early Antiretroviral Therapy (CHER) trial that has shown neurodevelopment of infants given early therapy improved significantly compared to infants on deferred ART. The International Maternal Pediatric AIDS Clinical Trials Network, (IMPAACT), is currently beginning studies of the prevalence of CNS escape in paediatric and adolescent age groups. The goal of these studies is to begin systematic evaluation of the CNS in paediatric HIV-1 remission and cure studies.

Dr Howard Fox described the database of the National NeuroAIDS Tissue Consortium (NNTC) housed in the Data Coordinating Center (DCC) at the University of Nebraska Medical Center [4]. He then outlined how the NNTC database could serve as a potential resource for sharing and accessing data associated with the Global HIV-1 CSF Escape Consortium. Further, Dr Fox presented the concept of bio-curators, the biologists who ensure that data entered into large biological databases are standardised and annotated to enable other researchers to understand and work with the data [5]. The meeting participants were asked to explore the possibility of developing and populating a common database to share samples and clinical data within the context of the NNTC.

Discussion

Understanding HIV-1 CSF escape to address key gaps in knowledge in NeuroHIV

Does HIV-1 persist in the CNS in the setting of suppressive ART? If so, can HIV-1 replicate in the CNS on ART? Does CNS HIV-1 replication have the potential to impact the state of systemic HIV-1 infection in the setting of suppressive ART or systemic viral cure? Finally, does low-level HIV-1 replication in the CNS or HIV-1 release from CNS cells contribute to neurological disease in patients on ART? These are the central questions facing HIV-1 investigators and HIV-infected individuals as we look to a known future of treating patients with long-term ART and with a new frontier of research focused on the hope of HIV-1 remission or eradication. Because HIV-1 CSF escape demonstrates that HIV-1 can be released from or replicate in cells of the CNS during cART, the study of CSF escape is critical for the systemic HIV-1 eradication/cure agenda (for discussion, see [6]). Studies of CSF escape have the potential to reveal the CNS cell types producing HIV-1 during ART, factors stimulating CNS HIV-1 release during ART, forces shaping viral evolution in the CNS compartment over time, and whether the possibility exists of re-seeding systemic circulation with CNS variants in the setting of ART or systemic HIV-1 eradication. Additionally, an understanding of the syndrome of CSF escape is important for understanding sources of persistent CNS abnormalities in humans on ART. What is the relationship of escape to neurological and persistent cognitive dysfunction that is observed in patients on ART? Might low-level CSF release from cells or viral production relegated to the CNS compartment drive a component of the immune activation and persistent dysfunction observed in some treated individuals?

Major challenges to development of comprehensive studies of CNS escape relate to its relatively low frequency and the need for a lumbar puncture to determine its presence. Studies to date have

Table 1. Summary of CSF escape cohorts or cases presented at the Global HIV-1 CSF Escape Consortium meeting

Speakers	Study site	Total number of cases	Number of cases of HIV-1 CSF escape	Neurosymptomatic	Asymptomatic	Criteria for determining CSF escape	Estimated prevalence ¹
Price, Gisslen, Cinque, Spudich, Joseph S	Multiple ² (San Francisco, New Haven, Chapel Hill, USA; Sweden; Italy)	N/A	81	42	39	Symptomatic: PVL<50 & CVL>100 or PVL 50–100 & CVL 2 × PVL; or Asymptomatic: PVL<50 & CVL>50	N/A
Joseph S	THINC Study Sites (Chapel Hill, San Francisco, New Haven, USA)	97	6	N/A	6	PVL<40 & CVL>40 or CVL>PVL	6%
Winston (UK)	UK	142	30	3	27	PVL<50 & CVL>200 or log ₁₀ CVL>1.5 × log ₁₀ PVL	21%
Winston (Europe)	EU	134	1	1	N/A	CVL>PVL	0.7%
Ene	Romania/Adult	91	4	2	2	CVL>0.5 log of PVL	4.4%
Perez	Spain	125	4	4	N/A	PVL: not detectable; CVL: detectable	3.2%
Sacktor	Uganda	91	9	4	5	PVL: not detectable; CVL: detectable	10%
Wright	Australia	167	6	3	3	PVL: 6 months not detectable; CVL: detectable	3.5%
Dravid	India	62	17	17	0	CVL: detectable with PVL: not detectable; CVL>1 log of PVL	27.4%
Letendre	CHARTER/HNRC sites	849	60	23	37	CVL>PVL with PVL: not detectable; CVL>1 log of PVL	7%
Nath	Washington DC	56	11	7	4	PVL<40; CVL>20	20%
Gabuzda	Boston, MA/NNTC (four sites)	200/426 (626)	11/29 (40)	11/17	0/12	PVL<50, CVL>50; CVL>0.5 log of PVL	6.4%
Wojna	Puerto Rico**	380	10	3/9	6/9	CVL>PVL	2.6%

¹ Values shown for estimated prevalence are estimates for the selected populations. Variability in the values reflects differences in study populations, methods, definitions and selection criteria.

² Data represent participants enrolled plus projected for R01 project entitled 'Compartmentalized CSF viral escape and the CNS HIV reservoir' (Price, Principal Investigator).

** Women-only cohort.

PVL: plasma viral load; CVL: CSF viral load; N/A: not applicable due to not determined or study not designed to assess.

either been small series or case reports [7–13] (for the clinically apparent, 'symptomatic' version) or relatively small studies to evaluate prevalence in settings where lumbar punctures are employed in screening of patients or study participants [1,14–16] (for the 'asymptomatic' version). The Global CSF HIV-1 Escape Consortium meeting brought together numerous investigators who have access to cases of individuals with CSF escape across five continents (see Figure 1), with the goals of reviewing the data already collected from these disparate settings, and exploring the possibility of forming a consortium of investigators for future research in this area. Summaries of the cases identified at each site are included above, revealing the potential of more comprehensive studies of CSF escape based on the many cases identified across broad geographic and clinical settings. However, the presentations and extensive discussion between attendees at the meeting highlighted some of the challenges to interpreting data obtained in these different settings. Most critically, if data and/or samples from multiple sites and studies are to be combined to perform larger studies, there is a need for some congruence in definitions of escape and methods of identifying individuals with escape. Table 1 demonstrates the range of definitions and criteria used to determine CSF escape across sites, emphasising the need for agreement on these factors for interpretation of data in a collective manner. The large variance in estimated prevalence of CSF escape cases across sites shown in Table 1, ranging from

0.7% to 27%, reflects differences between study populations, and the different methods, definitions and selection criteria used by each site.

Strategies for collaboration using HIV-1 CSF escape cases

Formation of a consortium to facilitate collaboration across multiple sites is an optimal research approach to the study of CSF HIV-1 escape, and has already successfully been initiated in a small scale (five clinical sites) in the ongoing UCSF HIV-1 CSF escape study. A multisite collaborative structure is appropriate, even necessary, for comprehensive investigation of rare conditions such as CSF HIV-1 escape. Multisite, consortium-type study designs have been successfully applied in HIV-1 research, yielding important understanding of HIV-infected individuals with specialised conditions (including HIV-1 elite controllers and individuals with acute infection). Similarly, coordinated efforts towards studying neurological disorders have been developed through the NINDS supported NeuroNEXT Network for Excellence in Neuroscience Clinical Trials, and successfully applied to rare conditions such as spinal muscular atrophy and myasthenia gravis (see <https://www.neuronext.org>).

A consortium focused on broad studies of CSF HIV-1 escape would require common definitions of 'escape' (see below), a centralised database and willingness of investigators to share data (see Table

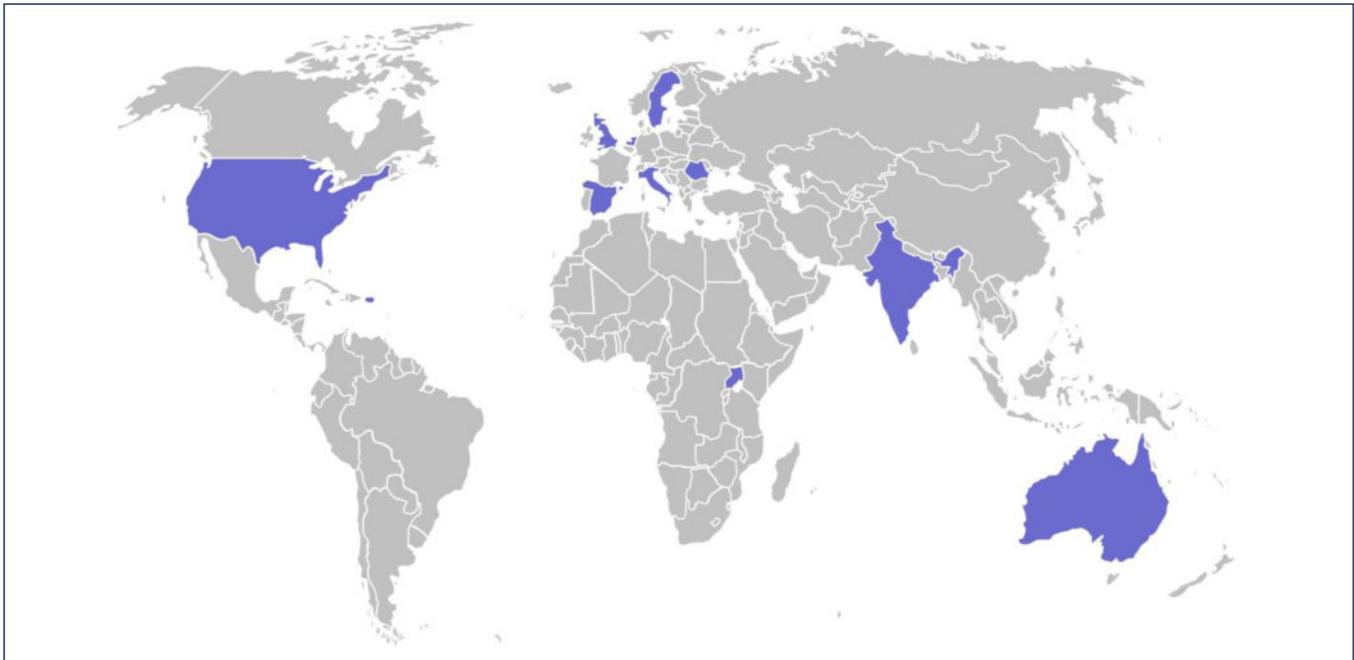


Figure 1. Geographic distribution of cohorts presented at the Global HIV-1 CSF Escape Meeting

Table 2. Challenges to consortium studies of CSF HIV-1 escape

Need for common definitions of CSF escape

- Category of escape with ‘undetectable’ plasma viral load: which assay measurements (assay platform/method, lower limit of detection, cutoff for ‘undetectable’ definition)?
- Category of escape with CSF/plasma HIV discordance in treated patients: what ratio considered ‘discordant,’ what plasma viral load is considered evidence of ‘treatment’?
- Category of ‘symptomatic’ viral escape: which clinical manifestations fulfil criteria for ‘symptomatic’?
- Category of ‘asymptomatic’ viral escape: what evaluation required to define as ‘asymptomatic’?

Determination of ART regimens considered ‘treatment’: include ‘old’ regimens, ‘atypical’ regimens, ‘simplified’ (two-drug) regimens?

Enrolment/recruitment methods

- Include participants referred for LP for clinical reasons?
- Screening in research-only participants, clinical setting?
- Any requirement for screening for concomitant CNS infection/inflammation (to assess for ‘secondary’ CSF escape)?

Data collection, dissemination, interpretation.

- Agreement on common elements of clinical and demographic data to be interpreted across sites, including methods?
- Agreement on neuropsychological test and neuroimaging methods and standardisation across sites?
- Common open database?
- Willingness to share data across sites?

Samples to be collected

- Agreement on sample types (CSF supernatant, plasma, CSF pellets, PBMC, other tissues)?
- Common methods for sample collection, processing, storage?
- Willingness to share samples across sites for specialty assays?

Infrastructure and support

- Funding mechanism for research studies that required collaboration between investigators?
- Organisation of and support for consortium teleconferences and in-person meetings?

2). Furthermore, a consortium for CSF escape should ideally be more than simply a specimen collection system, but instead a scientific think-tank type collaboration with input and distinct contributions (samples, expertise, methods, assays) from different

sites and investigators, working together on problems and issues identified together. This latter point emphasises the need for the possibility of sharing samples for highly specialised assays, which may present challenges for some sites acquiring samples in

particular regions of the world. For those measurements (HIV-1 RNA, basic CSF measures, some immune activation measures, neuroimaging) that may be shared but that are more likely to be performed at the individual sites, congruent or consistent validated assays for shared measurements will need to be identified. Resources of a shared database are already available through the NNTC, as outlined by Dr Fox above, which is a funded database already designed to collect data relevant to neuroAIDS and with support staff and infrastructure available. Finally, to encourage participation in this consortium, future funding sources will need to require collaborative work between sites for grant awards to facilitate analysis plans that include data and samples from multiple institutions and clinical settings, rather than fostering competition between sites.

In order to collect data that can be meaningfully shared between investigators, common criteria for the definition of CSF escape study participants will be key. Questions to consider are whether only those who are 'durably' suppressed in plasma should be included, and how to define this requirement of suppression. Should standardised assays be required? Clearly, a lower limit of 'cutoff' for the detection of escape should be consistent across studies. Alternately, should individuals with some definition of a 'disproportionately elevated' CSF HIV-1 RNA, as compared to well-controlled but not suppressed plasma HIV-1 RNA, be included, and if so, what ratio between these should be considered disproportionate? Should only those with 'modern' ART regimens be included, and should 'simplified' regimens that are in common use in some regions be included? Distinct categorisation based on whether CSF escape is detected on a lumbar puncture performed for a neurological condition (either work up for HIV-1 encephalopathy or other, e.g. LP for syphilis) versus performed for research (the latter better reflects prevalence) is likely necessary. Such issues require consensus definitions between collaborating investigators.

Participants and specific designs for cohorts at each site might depend on aims for individual projects, for example incidence or prevalence of escape in well-suppressed patients, association with residual neurological dysfunction, biology of HIV-1 reservoirs, or relationship to plasma/CSF resistance or viral tropism. Certain questions will need to be answered as a consortium, however, to ensure that data obtained at distinct sites could be usefully analysed together. One question relates to study participant information that will need to be documented at each participating study site. This may include current and prior antiretroviral drug regimens, current and/or prior concomitant infections/inflammatory conditions/malignancies (CNS or systemic), plasma HIV-1 RNA history, with assay/platform used and lower limit of detection noted.

Another key question that will need to be answered collectively across sites is what samples and types of assessment should be collected to provide maximal information from an amalgamated study. Large volume CSF collection (25 mL or more) with paired plasma samples, ideally with longitudinal samples in at least some cases, could be valuable. It is likely that further important knowledge would be gained from collection and cryopreservation of CSF cell pellets and peripheral blood mononuclear cells, where available, with procedures between sites standardised. In order to assess the relationship between CSF escape and neurological impairment in HIV-1, neuropsychological evaluation should be collected. Use of standardised, low time-intensive measures (for example the computerised Cogstate, NIH Toolbox, or a brief NPZ battery), is most likely to be successfully obtained across multiple sites. Finally, studies employing neuroimaging across sites would require common or comparable imaging platforms and, in the case

of MRI, the same magnet strength across sites. Additionally, modalities (MRI, MRS, DTI, rs-fMRI, BOLD imaging, PET with specific ligands) would need to be identified for studies at multiple sites and prescriptions for acquiring imaging will need to be standardised.

Underexplored aspects of HIV-1 CSF escape

The main areas of focus of prior and ongoing CSF escape studies have been examination of prevalence of this condition, quantitation of HIV-1 RNA in the CNS versus the blood, clinical manifestations of neurological disease in patients with CSF escape, and assessment of HIV-1 derived from CSF specimens for virological characterisation and resistance genotypes. Numerous additional aspects of this syndrome are likely to be of central importance to unravelling its importance to CNS and systemic disease in HIV-1. In particular, immune activation/dysregulation is a hallmark of clinically symptomatic CSF escape and is detected in research participants noted to have subclinical CSF HIV-1 escape. Moreover, there are likely important overlaps between CSF HIV-1 escape and the syndrome of CD8 + T cell encephalitis [17,18], which has been posited to be an immune consequence of a robust or reconstituted immune system in the setting of possibly low-level HIV-1 antigen. Key questions relate to whether CNS immune activation or overt inflammatory damage is a result or an aetiology of viral escape, which cells or signals are involved in this process, and a possible beneficial role of immune-directed rather than virus-directed therapeutics.

The possibility that co-infection with pathogens besides HIV-1 is important is suggested by the identification of CSF HIV-1 escape in individuals with other CNS infections (see 'secondary CSF escape' defined in the Gisslen summary and noted by other investigators above). Along these lines, some cases of CD8+ T cell encephalitis have been reported to follow or occur concomitantly with systemic or CNS infections besides HIV-1. These observations indicate that further investigations for links between infections as possible triggers for CSF escape may be warranted. Genetic predisposition to neuroinflammation or autoimmune responses might have a role in the aetiology of CSF HIV-1 escape, or specific pharmacogenetics may place certain individuals at risk for poor CNS effectiveness of ART and enhanced risk of escape in the CNS compartment. Additional important avenues of exploration in individuals with CSF escape include potential relation to cellular reservoirs of HIV-1 DNA (in CNS and/or blood) and HIV-1 replication/reservoir burden in other tissues, including lymph node, gut and genital tract.

Neuroimaging has shown promise in revealing inflammatory changes in the brain, both on clinical evaluation in symptomatic CSF escape (see Cinque above and [12]) and in neuroasymptomatic cases in a research setting (Winston, above), but has not been used systematically in any studies of CSF escape. Transient inflammation or neural network disruption might be detected in 'asymptomatic' CSF blips using methods sensitive to dynamic changes such as MRS or rs-fMRI. Specific methods for imaging viral latency or viral replication in the brain might be developed in the future to assess viral reservoirs underlying the occurrence of HIV-1 CSF escape. Several studies have documented genotypic resistance of HIV-1 variants emerging in the CNS during HIV-1 CSF escape, and presumed reduced CNS penetration of drugs in regimens in individuals with this syndrome. However, further careful studies examining ART penetration/macrophage/microglial efficacy and CSF pharmacology are needed to understand their relationship to this syndrome. Finally, the question of whether CSF escape occurs in ART-treated children/adolescents with perinatal or behaviourally acquired HIV-1, and whether there may be

distinctions in its manifestations in children (for example leading to neurodevelopmental delays or behavioural syndromes) versus adults has not been studied to date.

Through achieving consensus on definitions and methods, and initiating collaborations between diverse geographic sites, the Global CSF HIV-1 Escape Consortium seeks to address these and other key questions to better understand this emerging topic in the HIV cure research agenda.

Acknowledgements

The authors would like to acknowledge Leandra Stubbs, for capturing the various discussions during the HIV-1 CSF Escape Consortium Meeting, and Dr Pim Brouwers for his valuable and timely suggestions. The authors would also like to thank Dr Dianne Rausch as well as the Office of AIDS Research, NIH for their support of this meeting.

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