

*Invited Review***CROI 2016: Neurologic Complications of HIV Infection****Serena S. Spudich, MD; Beau M. Ances, MD, PhD**

The brain remains a major target for HIV infection and a site of potential complications for HIV-infected individuals. Emerging data presented at the 2016 Conference on Retroviruses and Opportunistic Infections suggest that during the early stages of infection, activated CD4+ cells may traffic the virus into the central nervous system (CNS). HIV is detectable in cells and tissues of the CNS in some individuals despite suppressive antiretroviral treatment. A potential source of cerebrospinal fluid HIV escape may be compartmentalized HIV replication within macrophage lineage cells. Virally infected cells can traffic out of the CNS and may have the potential to reseed the systemic compartment. Additional modifiers of HIV-associated neurocognitive disorder (HAND) were identified, including female sex and hepatic dysfunction. Large epidemiologic studies reported an elevated risk of stroke among HIV-infected individuals, related to traditional vascular risk factors, history of recreational drug use, and HIV measures (lower CD4+ cell nadir and higher viral load). Brain imaging may provide a noninvasive means for detecting early changes in the brain associated with HIV infection and may assist in prognosis of HAND. Some potential adjunctive therapies to standard antiretroviral therapy for HIV-infected individuals were considered.

Keywords: CROI, 2016, HIV, central nervous system, HIV-associated neurocognitive disorder, neuroimaging, neuro-pathogenesis, HIV reservoirs, stroke, cerebrospinal fluid

The status of the central nervous system (CNS) in HIV-infected persons was a central theme in several sessions at the 2016 Conference on Retroviruses and Opportunistic Infections (CROI). One symposium session (Session S-2, “A Beautiful Mind: Keeping It”) highlighted some of the most important themes of CNS HIV infection, including cerebrospinal fluid (CSF) and blood biomarkers of CNS HIV involvement (Abstract 60), state-of-the-art tools and applications for neuroimaging (Abstract 61), establishment and maintenance of the CNS as a site of HIV persistence (Abstract 62), and challenges and approaches for treatment of CNS complications (Abstract 63). Further, CNS-related themes emerged at the conference outside of neurologic-specific sessions, including sessions focused on pediatric HIV infection, basic science investigations, vascular complications, and tissue HIV reservoirs. Neurologic presentations focused on themes relevant to persistent CNS dysfunction in the treated HIV-infected individual: the CNS as

a potential reservoir for HIV, including initiation and persistence of HIV infection in the CNS; contributors to clinical manifestations of CNS HIV; cerebrovascular disease in HIV infection; and neuroimaging tools for detection of CNS abnormalities. Finally, assessment of potential treatments for HIV-associated neurocognitive disorder (HAND) yielded several promising approaches and set the stage for further in-depth studies of therapeutic strategies to address persistent abnormalities affecting some individuals despite well-treated HIV infection.

The CNS as a Site of HIV Persistence: A Barrier to Cure?

The potential of HIV cure—either achieving complete viral eradication or effecting long-term HIV remission in the absence of antiretroviral treatment—has stimulated intense interest in whether tissues and cells outside of the systemic compartment and lymph nodes may be meaningful sites of HIV persistence during therapy. In a symposium talk, Swanson (Abstract 62) provided an overview of the concept of the CNS as an HIV reservoir, describing the emergence of CNS compartmentalization of HIV before the initiation of antiretroviral therapy, cases of viral escape with evidence of HIV replication in the CNS despite systemically suppressive therapy, and the evolution of macrophage-tropic HIV Env presumed to facilitate productive infection of resident CNS macrophages and microglial cells.

Numerous talks and posters at the conference focused on specific topics introduced in Swanson’s overview. Stefic and colleagues (Abstract 400) investigated the mechanisms of CNS compartmentalization of HIV among 9 participants with previous exposure to antiretroviral medications, low CD4+ cell counts (median 163/ μ L), and HAND. Using single-genome amplification of env from paired blood and CSF samples, the investigators detected compartmentalization in 55% of participants. Compartmentalized HIV had a greater degree of diversity between compartments, and sequencing revealed CSF-specific amino acid signatures, some previously reported and some newly described, in the env gene across several subtypes of HIV. The sensitivity to autologous neutralizing antibodies of pseudotyped HIV did not differ among samples derived from blood and those derived from CSF. In contrast, sensitivity to purified broadly neutralizing antibodies differed substantially in many cases between blood and CSF. However, patterns of sensitivity to neutralizing antibodies of

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either type did not relate to viral compartmentalization of CSF. These data suggest that CNS compartmentalization of HIV may chiefly derive from factors outside of selective pressures of autologous neutralizing antibodies, and may relate instead to genetic attributes related to cell entry.

Seipone and colleagues (Abstract 398) approached the question of whether a concurrent opportunistic infection in the CNS might impact the extent of HIV compartmentalization in the CNS by assessing HIV replication among HIV-infected individuals with or without documented tuberculous meningitis (TB meningitis). The investigators found statistically significantly higher levels of HIV RNA in the CSF in 15 individuals with TB meningitis as compared to 22 individuals without TB meningitis. However, in an analysis of env sequencing by single-genome amplification that compared degree of compartmentalization between 4 participants with TB meningitis and 4 without, no clear patterns emerged. This study was limited by a small sample size, but the finding of higher HIV RNA levels in CSF in individuals with an opportunistic infection in the CNS warrants further analysis, specifically of the interaction between HIV replication dynamics and concurrent inflammatory disorders in the CNS, including opportunistic infections.

In a complementary longitudinal study, Bowman and colleagues (Abstract 401) confirmed a relationship between HIV compartmentalization in the CNS and neurocognitive response to antiretroviral therapy. Using single-genome amplification or deep sequencing of HIV env in CSF and blood, the investigators detected HIV compartmentalization in the CNS in 35% of 28 study participants before the initiation of antiretroviral therapy at CD4+ cell counts below 400 copies/ μ L. The effect of HIV compartmentalization was examined with respect to performance on a detailed neuropsychologic testing battery at baseline and at 6 months and 12 months after starting treatment. At the baseline visit, no laboratory parameters differed between the compartmentalized and noncompartmentalized groups, and neurocognitive impairment was not statistically significantly higher in the group with compartmentalization than in the group without. However, at 6 months and 12 months after initiation of antiretroviral treatment, the overall global deficit score, a measure of neurocognitive impairment, was statistically significantly lower in the noncompartmentalized than in the compartmentalized group. An interpretation of this finding may be that measurable HIV compartmentalization in the CNS before the initiation of antiretroviral therapy reflects a robust site of HIV replication in the CNS that is less responsive to therapy due to reduced antiretroviral exposure in CNS tissues or cells of replication. Additionally, or alternately, compartmentalized HIV replication in the CNS before antiretroviral treatment may result in more severe inflammatory and neural injuries that are irreversible or slower to reverse with therapy.

In a related analysis, Evering and colleagues (Abstract 406) used single-genome amplification to assess the relationship between drug resistance mutations in blood and CSF and the presence of HAND in 12 participants with virologic failure during antiretroviral therapy in the CHARTER (CNS HIV

Antiretroviral Therapy Effects Research) study. Five participants had normal neurocognitive function and 7 had HAND. The presence of drug resistance mutations in CSF and blood was statistically significantly higher among individuals with HAND, and compartmentalization of HIV-1 pol in the CNS was more frequent among individuals with HAND. Moreover, 43% of the participants with HAND had drug resistance mutations detected in CSF that were not detected in the blood. These findings may contribute to an understanding of the pathogenesis of HAND and, particularly, the development of viral escape in CSF among individuals without prolonged effective plasma viral suppression.

To better define the virologic basis of CSF viral escape, Joseph and colleagues (Abstract 402) described the virologic features of HIV env derived from individuals with asymptomatic CSF viral escape identified in a cohort study of 96 individuals receiving more than 1 year of antiretroviral therapy with plasma viral suppression. Six of these individuals were identified as having asymptomatic CSF viral escape. Of these 6 individuals, 2 had longitudinal sampling that revealed resolution of viral escape at 9 months, with 1 participant having continued persistence of viral escape (HIV RNA level 356 copies/mL in CSF, with undetectable plasma HIV RNA) at 8 months. Single-genome amplification sequencing of HIV env from CSF-derived samples in the participant with transient viral escape revealed a purely T-cell tropic, clonally expanded population. Similar examination of samples with persistent CSF viral escape revealed a genetically diverse HIV population with enhanced ability to infect cells with low CD4 receptor density, suggesting adaptation to a macrophage-tropic virus. These data suggest that HIV detected in CSF in individuals receiving systemically suppressive antiretroviral treatment can in some cases reflect low-level viral replication within macrophages or microglial cells.

Although macrophages and microglial cells are the principal sites of HIV replication within the CNS in HIV encephalitis, the possibility that HIV may infect other cell types within the CNS remains a focus of intense investigation. Astrocytes in particular have been controversial as a potential target for HIV, as they lack CD4 surface receptors. Li and colleagues (Abstract 393) investigated possible mechanisms of astrocyte HIV infection in humans, finding that infection by mature HIV virions could be facilitated *ex vivo* by transfecting astrocytes with plasmid encoding the CD4 receptor, by a use of a lysosomotropic agent, or by exposure to Tat peptide. Additionally, these investigators exposed astrocytes to newly produced HIV particles produced by infected CD4+ lymphocytes using a transwell culture pore system and found that after 3 weeks to 4 weeks, the HIV p24 antigen could be measured from culture media around the astrocytes. Anti-CXC chemokine receptor 4 antibodies but not anti-CD4 antibodies inhibited infection. These data that distinguish between mechanisms of infection of astrocytes by mature and newly produced HIV set the stage for future important investigation of the possible conditions for restricted HIV infection of this cell type.

The existence of a viral reservoir is defined not only by the potential for HIV infection of a given cell or tissue but also by

the persistence of HIV in the setting of apparently effective antiretroviral treatment. de Oliveira and colleagues (Abstract 143) assessed paired blood and CSF samples from 16 participants with suppressed HIV RNA in blood at a median 2 years after initiation of early (before 4 months of estimated infection) or delayed (after 14 months of estimated infection) antiretroviral therapy. Cell-associated HIV DNA in peripheral blood mononuclear cells (PBMCs) and CSF cellular pellets was measured by digital droplet polymerase chain reaction (PCR) testing, followed by next-generation sequencing of partial env from these samples and subsequent phylogenetic analysis. HIV DNA was detected overall in 15 of 16 PBMC samples and 10 of 16 CSF samples. Although CSF interleukin (IL)-6 and tumor necrosis factor- α were lower among those in the early treatment group, there was no difference between HIV DNA detectability in either sample type between the 2 treatment groups. Genetic analysis of env sequences from 8 paired CSF and blood samples revealed that 7 had statistically significant HIV compartmentalization in the CNS, with unique CSF-specific sequences detected despite early treatment and longitudinal persistence of CSF-unique sequences over a 5-month period during treatment. A challenge of these experiments is the low HIV DNA input from the CSF samples, which could bias compartmentalization analysis. Overall, the findings are consistent with recognition of compartmentalization in the CNS early in untreated HIV infection and suggest the potential persistence of compartmentalized HIV in cells of the CNS despite treatment.

Lamers and colleagues (Abstract 345) similarly demonstrated that HIV DNA persisted in the CNS compartment along with other tissue sites among 20 individuals with undetectable HIV RNA (using a lower limit of detection of 40-400 copies/mL) in plasma at autopsy. Fifteen of these individuals had cancer, and the majority were documented as taking antiretroviral treatment at the time of death, with the remainder documented as taking antiretroviral treatment near the time of death. HIV DNA was detected by digital droplet PCR assay in the majority of 87 brain tissue samples analyzed. Nearly all brain tissue samples demonstrated pathology, although only a few showed classic HIV-associated changes of microglial nodule encephalitis or CD68+ infiltrates. An RNA-scope assay detected HIV RNA colocalizing with CD68+ cells, likely macrophages, in brain samples from 2 donors

Emerging data indicate that HIV DNA and RNA may continue to be detectable in cells and tissues of the CNS in some individuals taking suppressive antiretroviral treatment. A potential source of escape of HIV in CSF may be compartmentalized HIV replication within macrophage lineage cells.

with CNS malignancies, and single-genome sequencing revealed clustering of HIV DNA from brain with other anatomic compartments. These data are some of the first to demonstrate the persistence of HIV DNA and even HIV RNA in the brains of infected individuals taking apparently systemically

successful antiretroviral treatment, necessitating further research into potential HIV persistence in the CNS despite suppression of high-level HIV replication.

CNS HIV Entry and Immune Cell Trafficking

A complete understanding of the mechanisms that establish and maintain CNS HIV infection is key to designing interventions to address potential HIV reservoirs in the CNS. HIV enters the CNS in the first weeks after transmission to the host and can be detected throughout the course of untreated HIV infection. However, the means by which HIV is trafficked into the CNS compartment during initial and chronic infection is incompletely understood. A number of studies focused on aspects of trafficking of HIV or immune cells in or out of the CNS during the course of infection.

In an effort to examine determinants of the level of HIV present in the CNS in the earliest stages of infection, Schuetz and colleagues (Abstract 404) examined various sites, including gut mucosa, PBMCs, and CSF in 38 participants in Thailand with antibody-negative acute HIV infection at an estimated duration of infection of 15 days. The investigators found correlations between measures of immune activation (percentage of CD8+ and Ki67+ cells) in the blood and gut and the level of HIV RNA in the CSF, independent of the level of plasma HIV RNA. These data support the hypothesis that peripheral immune activation may facilitate entry of HIV-infected cells into the CNS compartment, mediating the relationship between HIV RNA produced in the periphery and that detected in the CSF.

Two studies examined the relationship between immune cells in blood and CSF during the early stages of HIV infection and the extent of viral trafficking to the CNS. Trautmann and colleagues (Abstract 407) characterized CD8+ cells from the CSF of 28 individuals with acute HIV infection from the same cohort in Thailand. The investigators demonstrated the emergence of high levels of CD8+ cell activation (defined as the percentage of CD38+ and HLA-DR+ cells among all CD8+ cells) in CSF during Fiebig stage II or III HIV infection. The percentage of activated CD8+ cells in CSF correlated with levels of HIV RNA in CSF across Fiebig stages, supporting the concept that immune activation in the CNS is associated with RNA production. A high proportion of CD8+ cells in CSF were HIV-specific cells, and these cells manifested patterns of V beta families distinct from those in blood, suggesting unique T-cell repertoires. These results suggest that even during the initial stages of HIV infection, distinct T-cell responses characterize the CNS and may reflect and facilitate compartmentalization of immune response and perhaps viral infection.

Li and colleagues (Abstract 142) examined CD8+ and CD4+ cells and monocytes in paired CSF and blood samples obtained in a longitudinal study of men recently infected with HIV. Beginning at a median 3.3 months post infection, the percentage of activated CD4+ and CD8+ cells increased at an accelerated rate in the CNS compartment compared with the blood during untreated infection; percentage of activated cells in the CNS compartment did not measurably decline

during 7 months of follow-up during antiretroviral therapy, despite declining in the blood. Moreover, HIV RNA concentrations in the CSF independently correlated with the percentage of activated CD4+ cells but not of CD8+ cells or activated or trafficked monocytes in CSF. These findings suggest that during the early stages of infection, activated CD4+ cells trafficking to the CNS might be a major source of HIV replication, modifying the traditional concept that infected monocytes are the primary “Trojan horse” carrying HIV to the CNS (for review, see Abstract 62).

The concept that CD4+ T lymphocytes may be a key cell type trafficking to the CNS early in the course of infection was supported in a presentation by Vasan and colleagues (Abstract 405) regarding CNS findings after acute infection in a nonaccelerated simian-human immunodeficiency virus (SHIV) model. This model closely resembles human disease in terms of systemic viral load and immune responses, as well as CSF biomarker patterns. On examination of tissues, CD8+ cellular infiltrates were noted to be surrounding blood vessels in the brain, and CD4+ cells were found clustered in the meninges of SHIV-infected macaques at 12 weeks after infection but not in uninfected animals. These CD4+ cells aggregating in the meninges might be a CNS-specific source of SHIV production in the early stages of infection, contrasting with macrophage and microglial cell sources of viral RNA present in established infection and encephalitis.

In a study focused on a macaque model of late-stage simian immunodeficiency virus (SIV) disease, Mallard and colleagues (Abstract 403) found shared SIV gp120 sequences to be present between monocytes and macrophages detected in the bone marrow and the brain of CD8-depleted animals with SIV encephalitis. Analysis using Bayesian evolutionary analysis sampling trees (BEAST) to determine time to most recent common ancestor revealed recent viral spread between bone marrow and brain, suggesting a bone marrow source of virally infected cells in the CNS during later stages of infection.

Although many presentations centered on ingress of cells and HIV into the CNS, the possibility that cells originating in the CNS may traffic out of this compartment into the periphery is a concern of key importance to HIV eradication efforts. If HIV-infected immune cells or free virus might egress from the CNS, then persistent infection in CNS cells may lead to re-seeding of HIV in the systemic compartment despite successful systemic HIV remission or eradication. Recent evidence that the brain has a dedicated lymphatic system that allows for trafficking of immune cells directly from the CNS to the deep cervical lymph nodes supports this concept,¹ but trafficking of infected cells has not previously been observed.

Alvarez and colleagues (Abstract 141) presented evidence that infected cells traffic from the CNS, employing superparamagnetic iron oxide nanoparticles (SPIONs; foreign particles likely to be ingested by phagocytic cells, including macrophages and microglial cells in the brain). The investigators injected fluorescent SPIONs into the cisterna magna of the brain of SIV-infected or uninfected macaques, monitoring SPION uptake by perivascular cells during the first day after injection. Robust transit of SPION-containing cells from the

CNS to the cervical lymph nodes was observed over the following 7 days in both infected and uninfected animals, and these cells were characterized as CD163+ cells, indicating a macrophage-monocyte lineage. With staining, the investigators confirmed that some of the SPION-containing cells in the lymph nodes of infected animals were SIV infected, demonstrating that in this accelerated macaque model, infected cells were trafficked from the CNS to the cervical lymph node. These data suggest a route of viral infection from the CNS to the periphery, highlighting the need to adequately treat the CNS compartment during standard HIV treatment and to consider this compartment in viral eradication efforts.

The ability to address immune abnormalities that persist in the CNS in the context of suppressive antiretroviral therapy will be essential to optimal treatment of HIV infection. HIV RNA levels, soluble measures of inflammation, and CSF cell characteristics improve in response to systemically suppressive antiretroviral therapy but may not completely normalize despite prolonged treatment.^{2,3} Chung and colleagues (Abstract 411) demonstrated the feasibility of applying both flow cytometry and newly emerging mass spectrometry methods to examine characteristics of CSF and blood cells in HIV-infected individuals well treated with antiretroviral medications, despite having low clinical CSF white blood cell counts. Mass spectrometry, unlike flow cytometry, uses heavy

A detailed understanding of HIV and immune cell trafficking is emerging from studies using human and animal models of acute and chronic HIV infection, suggesting a role of activated T lymphocytes and macrophages that migrate into and out of the CNS.

metals rather than fluorescein dyes to label cell surface receptors, allowing for resolution of up to 40 distinct surface markers simultaneously in a single sample. Among 7 individuals taking antiretroviral treatment, with a median white blood cell count of 2/μL, mass spectrometry generated interpretable data and identified a unique subset of effector memory cells comprising the majority of T cells in CSF but not blood. These methods have potential in future investigations of individuals with treated HIV infection, as a means of identifying unique abnormalities that should be mitigated in the optimal treatment of HIV infection in the CNS.

Contributors to Presentation and Progression of HAND

A key concern for clinicians and persons living with HIV infection is HAND, a condition that can impact individuals despite treatment with antiretroviral therapy. Numerous studies focused on potential factors that may contribute to HAND. Potential differences between HAND in women and in men were explored by Maki and colleagues (Abstract 416), through a comparison of cognitive test performance among women enrolled in the WIHS (Women’s Interagency HIV Study)

study and men enrolled in the MACS (Multicenter AIDS Cohort Study) (429 HIV-infected and 281 uninfected individuals in each group). The investigators compared performance on 4 common neuropsychologic tests utilized in both studies, using a mixed-effects regression analysis that matched participants by demographic and disease variables. HIV-infected women performed statistically significantly worse on Trail Making and Grooved Pegboard tests, tests of executive function and processing speed, than HIV-infected men. Differences between women and men in these measures may reflect biologic or sociodemographic differences, but this study provides rationale to further investigate the sources of these differences and whether distinct interventions may be required to address them.

Valcour and colleagues (Abstract 422) performed a novel exploration of the potential contribution of minimal hepatic encephalopathy associated with liver fibrosis and HAND among women in the WIHS study. Neuropsychologic testing results among 258 women with liver fibrosis—defined by aspartate aminotransferase (AST)-to-platelet ratio index—were compared with those of 1221 women without liver fibrosis within the WIHS study. After adjustment for hepatitis C virus (HCV) infection status and HIV disease markers, the presence of liver fibrosis was associated with poorer overall cognition and performance on verbal learning, executive function, verbal memory, psychomotor speed, fluency, and fine motor tasks. HCV infection status had no independent association with cognition. These findings suggest that liver fibrosis may be an additional contributor to cognitive dysfunction in HIV-infected women in particular and possibly in persons living with HIV infection more generally. These results suggest possible new specific therapeutic interventions for HAND in individuals who have concomitant hepatic dysfunction.

In another assessment of potential toxic metabolic contributors to HAND, mitochondrial dysfunction and neurocognitive performance were evaluated by Samuels and colleagues (Abstract 144), in a cohort of 1011 participants from the CHARTER study. This study revealed a relationship between mitochondrial DNA content in blood cells and the presence of cognitive impairment. This association was primarily driven by correlations among individuals with only incidental comorbidities, suggesting that mitochondrial DNA content in blood cells may be a contributor to HAND in the absence of other factors. In a subset of 335 study participants, elevated cell-free mitochondrial DNA in the CSF was associated with increased measures of HIV RNA, inflammation, and iron metabolism in the CSF but not with neurocognitive performance.

Hellmuth and colleagues explored the neurologic manifestations (Abstract 415) and psychiatric symptoms (Abstract 414) accompanying acute HIV infection in individuals identified with very early antibody-negative infection in Bangkok, Thailand. Mild neurologic signs, including slowed fine finger movements and neuropathy, and mild symptoms of cognitive difficulties affected the majority of individuals during very early infection. However, in this cohort that received antiretroviral treatment during acute infection, the majority of findings remitted during 3 months of follow-up. Self-reported

mood difficulties, including depression and anxiety, were also highly prevalent at baseline in this cohort and statistically significantly correlated with HIV disease indices, including lower CD4+ cell counts in blood and higher levels of HIV RNA and neopterin (a macrophage activation marker) in blood or CSF. Prevalence of depression and anxiety decreased dramatically after 12 weeks of antiretroviral treatment in this cohort. These findings suggest that although abnormal neurologic and psychologic findings may manifest extremely early in HIV infection, early initiation of treatment may help to ameliorate processes that contribute to HAND in chronic HIV infection.

Perhaps consistent with this hypothesis is a report from Vassallo and colleagues (Abstract 408) who investigated the longitudinal relationship between CD4+/CD8+ cell ratio in blood and neurocognitive dysfunction over approximately 2 years among 96 participants in the Neuradapt study, a prospective study of HAND. The investigators found that in this cohort, in which 73% of individuals had plasma HIV RNA suppressed to less than 200 copies/mL, a decline in CD4+/CD8+ cell ratio was associated with a decline in performance on neurocognitive testing in a multivariable model (odds ratio, 3.70; $P = .007$). As the CD4+/CD8+ cell ratio reflects both immune suppression and excess systemic immune activation, this measure serves as a more complex index of immune dysfunction of persons living with HIV infection than CD4+ cell count or CD4+ cell count nadir alone. Although mechanisms driving the decline or recovery of the CD4+/CD8+ cell ratio are still largely unknown, early initiation of antiretroviral treatment leads to relative preservation of the CD4+/CD8+ cell ratio, potentially protecting the CNS from immunologic factors that contribute to HAND.

Mukerji and colleagues (Abstract 145) examined longitudinal associations between neuropsychologic testing results and both blood lipid parameters and the presence of an apolipoprotein E4 (APOE4) allele in men enrolled in the MACS study. The investigators examined trajectories of neurocognitive performance among 273 men with HIV infection who were taking antiretroviral therapy (aged 50-65 years) and among 516 matched uninfected men (aged 50-65 years). Among the HIV-infected men, but not uninfected men, abnormal lipid profiles (ie, elevated total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels, and reduced high-density lipoprotein level) were associated with accelerated declines in neuropsychologic testing performance in this age span. Similarly, the presence of an APOE4 allele was associated with a pattern of enhanced decline among HIV-infected men compared with uninfected men in the same age range. These findings suggest a possible important role of abnormal lipid metabolism in the development of neurocognitive impairment among middle-aged HIV-infected men taking HIV treatment. As statin use was not controlled for in the analysis, it is possible that individuals with lower-range lipid measurements had disproportionately higher statin use, which might benefit neurocognition in the context of HIV infection through lipid-independent antiinflammatory effects. However, it is also plausible that lipid metabolism impacts HAND by increasing the risk of vascular dysfunction that was

previously tied to the presence of HAND in the MACS and other cohorts,^{4,5} providing a rationale for use of statins or other lipid-lowering agents in persons with HAND.

Stroke and Cerebrovascular Disease in HIV: An Emerging Concern

As HIV becomes more of a chronic disease, additional cofactors (eg, cerebrovascular disease) are becoming increasingly important when treating HIV-infected individuals.⁶ Becker and colleagues (Abstract 388) suggested that cardiovascular disease risk factors may be as important as HIV-associated factors in predicting rate of change in the brain. Although the overall incidence and mortality of stroke among HIV-infected individuals has decreased, Berenguer and colleagues (Abstract 639) demonstrated that the risk of stroke still remains elevated among HIV-infected individuals compared with uninfected individuals, based on large epidemiologic studies. Even after controlling for traditional risk factors, HIV-infected individuals have a substantially higher risk of ischemic stroke than uninfected individuals.⁷ Crane and colleagues (Abstract 636) showed that most strokes seen among HIV-infected individuals are ischemic rather than hemorrhagic in nature.

The overall incidence of ischemic stroke was 1.69% among HIV-infected individuals according to data presented by Chow and colleagues (Abstract 43). Although the risk of ischemic stroke is elevated across the lifespan of an individual, the greatest risk was actually seen in younger HIV-infected individuals. In particular, Chow and colleagues demonstrated that HIV-infected women have a higher risk of stroke even after adjustment for age, race, vascular, and sex-specific risk factors (Abstracts 638 and 43). The greatest risk of stroke among HIV-infected women occurred among those aged 40 years to 49 years. Chow and colleagues (Abstract 43) and Crane and colleagues (Abstract 636) identified traditional risk factors associated with ischemic stroke, including older age, elevated blood pressure, active or past recreational drug use, current smoking, diabetes, and HIV-associated variables (lower CD4+ cell nadir and higher viral load). The increased risk of ischemic stroke associated with unsuppressed virus corresponded to an effect of aging by approximately 15 years.

Hatleberg and colleagues (Abstract 637) identified risk factors for hemorrhagic stroke, including elevated blood pressure and poor renal function. Controversy still remains concerning the impact of HCV infection, as one study demonstrated an increased risk for hemorrhagic stroke among HIV/HCV-coinfected individuals. Although Berenguer and colleagues (Abstract 639) identified an increased risk of stroke among a large cohort of HIV/HCV-coinfected individuals in Spain, Crane and colleagues (Abstract 636) and Chow and colleagues (Abstract 43) did not observe a substantially increased risk among participants with HIV/HCV coinfection compared with those with HIV monoinfection in studies performed in the United States. For all epidemiologic studies, careful analysis and adjudication are needed, as the diagnosis and medical coding of stroke are often complicated and may be over-represented (approximately 45% of strokes coded using the

The risk of stroke remained elevated among HIV-infected individuals compared with uninfected individuals, based on large epidemiologic studies. Traditional vascular and HIV disease-related risk factors could be used to specifically target interventions toward high-risk HIV-infected populations.

International Classification of Diseases [ICD]-9 required additional discussion by clinicians). As noted by Crane and colleagues (Abstract 636) and Chow and colleagues (Abstract 43), additional limitations of these large observational datasets include that information is not collected at regular intervals using standard metrics and that, typically, homogenous convenience samples are utilized. These cohorts often have a higher proportion of white men.

Neuroimaging studies may assist in evaluating the effects of cerebrovascular disease and in visualizing preclinical changes, as noted by Becker and colleagues (Abstract 388). Janjua and colleagues (Abstract 640) noted increases in incidental carotid plaque (calcified and noncalcified) among HIV-infected individuals who were free of known cardiovascular disease compared with matched uninfected controls. In addition, the presence of carotid plaque was associated with an increased incidence of subsequent cerebrovascular events.

Stroke may be underreported as a distinct end point in many clinical trials involving HIV-infected individuals. Certain groups of HIV-infected individuals (eg, women or those of black race) may be at increased risk for stroke. These groups may merit specific targeting for possible interventions. Within the HIV-infected population, traditional (eg, hypertension, hyperlipidemia, diabetes, smoking, etc) and disease-related (eg, detectable HIV RNA or presence of immune activation) risk factors could be specifically targeted for future interventions.

Neuroimaging to Diagnose and Assess the Mechanisms of HAND

There is an expanding interest in using neuroimaging methods to study the effects of HIV infection on brain structure and function. Brain imaging may detect HIV-associated changes soon after initial infection.^{8,9} In a cohort of HIV-infected individuals in Thailand, structural brain volumetric and metabolite measurements were performed by Killianpur and colleagues (Abstract 384) at diagnosis of acute HIV infection (< 1 month after seroconversion) and at 2-year follow-up. A 2% to 3% rate of atrophy was observed primarily in subcortical areas (caudate, putamen, and globus pallidus). Decreases in subcortical brain volumetrics correlated with increases in neuronal loss and inflammation (as measured by magnetic resonance spectroscopy). Slowly evolving, multidimensional changes may continue to progress if HIV-infected individuals remain untreated (Abstract 63). A number of groups, including Guha and colleagues (Abstract 382) and Cysique and colleagues (Abstract 391), demonstrated that continued active

viral replication and inflammation are often associated with subcortical changes. In particular, continued presence of virus was associated with reduced putamen volume in an analysis by Guha and colleagues (Abstract 382). With regard to progressive immunosuppression, Schonfeld and colleagues (Abstract 383) demonstrated that a lower CD4+ cell count nadir was associated with volumetric loss in cortical areas (including frontal, temporal, and parietal lobes).

The mechanism by which pathologic spread occurs from subcortical to cortical areas remains unknown, but an interesting study investigated the neurovascular unit. De Alwis and colleagues (Abstract 389) observed that HIV pathology

Neuroimaging may provide a noninvasive means to evaluate progressive changes in the brain caused by HIV infection. Changes may initially occur within subcortical brain regions and spread to cortical areas with more advanced disease.

caused intracranial vessel wall thinning and loss of vascular plasticity. This can cause an expansion of the vessel lumen and poorer regulation of perfusion to subcortical and cortical brain regions. Noninvasive imaging of changes in the arterial wall could potentially serve as an *in vivo* marker for monitoring disease progression and evaluating the potential benefits or deleterious effects of antiretroviral therapy in smaller vessels in HIV-infected individuals.¹⁰

A larger number of neuroimaging studies have also begun to focus on the effects of HIV infection in pediatric populations. Hoare and colleagues (Abstract 821) showed that HIV-infected children performed statistically significantly worse on neuropsychologic performance tests in various domains (ie, processing speed, memory, language, and flexibility) than well-matched uninfected children. These HIV-infected children also had substantial abnormalities in brain structure, especially within the corpus callosum, compared with uninfected children. In addition, HIV-infected children whose initial antiretroviral regimen failed had greater white matter brain dysfunction.

In another series of studies, a cohort of youths with perinatally acquired HIV infection who were taking antiretroviral therapy had both cortical (as demonstrated by Williams and colleagues, Abstract 822) and subcortical (as demonstrated by de los Angeles and colleagues, Abstract 823) structural changes in the brain compared with uninfected youths in a matched cohort. The greatest decreases in cortical and subcortical volumetrics were associated with higher peak plasma viral loads and unsuppressed virus. In particular, de los Angeles and colleagues (Abstract 823) showed that subcortical (putamen, globus pallidus, caudate nucleus, and thalamus) structural changes were correlated with poorer scores on neuropsychologic performance testing. Williams and colleagues (Abstract 822) showed that alcohol and marijuana use were also linked to lower brain volumes, suggesting that not only HIV infection but other factors may influence brain development in HIV-infected youths.

Initiation of antiretroviral therapy leads to improvements in brain function. Schiffito (Abstract 392) reported encouraging results that 12 weeks after initiation of antiretroviral therapy, statistically significant improvements in functional connections were observed between the posterior cingulate cortex and other brain regions among previously treatment-naïve HIV-infected individuals. Ances (Abstract 61) and Calcagno (Abstract 63) suggested that initiation of antiretroviral therapy soon after seroconversion may be most beneficial, although treatment does not lead to a complete normalization. High variability exists in penetration of the CNS. Numerous clinical and demographic factors may also affect concentrations of medications. Calcagno (Abstract 63) showed that HIV-infected individuals with well-controlled virus may still have residual HIV replication or residual systemic and CNS immune activation. Questions still remain concerning the optimal antiretroviral regimen and the best means to assess the effects of treatment (eg, CNS penetration effectiveness or monocyte efficacy score).

Overall, use of antiretroviral therapy has led to a reduction in the incidence but not prevalence of more severe forms of HAND.^{11,12} Findings continue to suggest that despite the introduction of antiretroviral therapy and subsequent virologic control, there appears to be a substantial percentage of HIV-infected individuals who still have evidence of poorer cognitive performance, atrophy of grey and white matter, and abnormalities in white matter (Abstract 61). Underwood and colleagues (Abstract 148) used a k-means cluster method and found that brain and cognitive abnormalities often occurred together in HIV-infected individuals. In particular, increased atrophy of grey matter was associated with lower fractional anisotropy as measured by diffusion tensor imaging. Cysique and colleagues (Abstract 391) showed that HIV-infected individuals with a history of neurocognitive impairment often have loss of neuronal integrity within subcortical and cortical regions. Granziera and colleagues (Abstract 381) showed that volumetric changes in the brain may have longitudinal predictive power to detect subsequent changes in neuropsychologic performance. A combination of methods (neuroimaging, CSF analysis, and neuropsychologic performance testing) may therefore provide a more complete understanding of changes in the brain caused by HIV infection (Abstract 61). As noted by many of these studies, additional

A combination of methods (neuroimaging, cerebrospinal fluid analysis, and neuropsychologic performance testing) may provide a more complete understanding of brain changes caused by HIV infection.

investigations are needed that 1) pool data from various modalities and cohorts (Abstracts 383 and 386); 2) longitudinally assess HIV-infected individuals (especially those with well-controlled virus) (Abstracts 384 and 381); and 3) include appropriate uninfected controls for comparison (Abstracts 61 and 146).

Therapeutics for HAND

A major priority for HIV research efforts is the development of effective treatment strategies to improve neurocognitive disorders or to prevent the development of HAND. Several strategies were evaluated as adjunctive therapies to standard antiretroviral therapy. Most interventions, although promising in concept, did not have a measurable impact on clinical or laboratory outcomes. CC chemokine receptor R5 (CCR5) inhibitors have potential antiinflammatory and antileukocyte trafficking properties that may reduce immune activation and infection in the CNS. Winston and colleagues (Abstract 423LB) presented results from a study comparing neurologic outcomes in antiretroviral therapy-naïve participants randomly assigned to initiate a protease inhibitor-based regimen of tenofovir disoproxil fumarate (TDF), emtricitabine, and ritonavir-boosted atazanavir or a protease inhibitor-based regimen of abacavir, lamivudine, ritonavir-boosted darunavir, and the CCR5 inhibitor maraviroc. Thirty participants were randomly assigned to each arm and followed up for 48 weeks; all participants achieved plasma viral suppression at follow-up. Both groups experienced improvement in neurocognitive performance, with no statistical differences detected between the groups.

Data from the large AIDS Clinical Trials Group (ACTG) A5303 study, which focused on the possibility that maraviroc might specifically benefit the CNS, were presented by Robertson and colleagues (Abstract 147). Neurocognitive performance was assessed in this randomized placebo-controlled study that compared neuropsychologic testing at baseline, 24 weeks, and 48 weeks among antiretroviral therapy-naïve individuals initiating treatment with maraviroc with a placebo or TDF with a placebo plus ritonavir-boosted darunavir and emtricitabine. One hundred nineteen participants were randomly assigned to the maraviroc-containing arm, and 111 were assigned to the TDF-containing arm. Consistent with the findings in the smaller study presented by Winston, there were no differences in global deficit score between the 2 arms at baseline, 24 weeks, or 48 weeks. Both groups improved, but there was no difference in change in score from baseline to 48 weeks between study arms. Overall, 50% of individuals with HAND improved to the unimpaired level with treatment at week 48. These findings may suggest that in the context of the potent immune and viral effects of antiretroviral therapy, any relative benefit of one regimen over another may be too subtle to detect. Studies are ongoing to further examine whether treatment intensification with maraviroc for individuals taking suppressive antiretroviral therapy may cause an improvement in neurocognitive function.

Based on promising data from single-arm studies, Decloedt and colleagues (Abstract 419) performed a randomized placebo-controlled trial of lithium as adjunctive therapy to stable suppressive antiretroviral therapy for treatment of HAND (N = 66). This trial demonstrated improvement over time on repeated neuropsychologic testing but no difference in neurocognitive outcomes between the 2 study arms (those who received lithium and those who received a placebo).

Two studies documented that simplification strategies for antiretroviral treatment appeared safe with regard to neurocognitive outcomes after 1 year of follow-up. Ciccarelli and colleagues (Abstract 417) described 151 participants whose antiretroviral regimen was switched to ritonavir-boosted atazanavir and lamivudine (dual therapy) or who maintained their original 3-drug regimen (triple therapy), as part of the Italian ATLAS-M (Atazanavir and Lamivudine for Treatment Simplification–M) study, and had neuropsychologic testing available. There were no differences in any neuropsychologic testing parameters between the 2 arms at 48 weeks of follow-up. Similarly, Perez-Valero and colleagues (Abstract 424LB) presented data from a neurologic substudy (n = 96) of the SALT study that investigated whether a regimen of ritonavir-boosted atazanavir and lamivudine (dual therapy) was non-inferior to a regimen of 2 nucleoside analogue reverse transcriptase inhibitors and ritonavir-boosted atazanavir (triple therapy). At 96 weeks of follow-up, the researchers detected no differences in neurocognitive measures between the 2 groups, although 2 participants in each group developed neurocognitive impairment. These studies suggest that, at least early in follow-up, strategies for antiretroviral treatment simplification may be safe for neurocognitive outcomes in stably treated individuals.

Sacktor and colleagues (Abstract 146) presented results of a double-blind placebo-controlled clinical trial of therapy with paroxetine and fluconazole to address residual inflammation and oxidative stress in the CNS in individuals taking antiretroviral treatment. After screening to identify compounds with potential neuroprotective effects, paroxetine and fluconazole were identified as medications that promoted hippocampal neuron survival in cell culture and protected against neuronal injury in an SHIV model. Individuals with plasma viral suppression were enrolled and underwent neuropsychologic testing at baseline, then were randomly assigned to 1 of 4 treatment arms: fluconazole alone (n = 11), paroxetine alone (n = 11), fluconazole and paroxetine together (n = 12), or a placebo (n = 11). Repeat neuropsychologic evaluation at 24 weeks revealed a benefit in a summarized score of neuropsychologic testing and in a computerized test battery in the paroxetine-containing arms compared with fluconazole alone or a placebo. Depression symptomatology was evaluated at each visit and did not differ between the groups. The group that received fluconazole alone did not exhibit improvement in cognitive testing but did have a greater decrease in CSF ceramide, a measure of oxidative stress, relative to baseline than did the other groups. This is the first adjunctive therapy

One small study demonstrated a benefit of adjunctive therapy with the antidepressant medication paroxetine in individuals taking stable suppressive antiretroviral treatment.

to demonstrate a beneficial impact on neuropsychologic testing performance in well-treated HIV-infected individuals with HAND. Whether this improvement is attributable to the

neuroprotective effects of paroxetine or to an undetected impact on mood, the documented benefit of this treatment in the small number of participants studied provides a rationale for a larger clinical trial to examine the potential effect of paroxetine in ameliorating HAND in individuals taking suppressive antiretroviral therapy. 

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