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# Biotypes of Central Nervous System Complications in People With Human Immunodeficiency Virus: Virology, Immunology, and Neuropathology

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Despite viral suppression with antiretroviral therapy (ART), people with human immunodeficiency virus (HIV) continue to experience central nervous system (CNS) complications, primarily in the form of mild cognitive impairment and mental health disorders (eg, depression, anxiety, other neuropsychiatric problems). The multifactorial pathogenesis and heterogeneity of mechanisms likely underlying CNS complications must be addressed in the development of preventive interventions and effective treatments. The biotyping approach has previously been useful to define phenotypes of other CNS diseases based on underlying mechanisms and could be translated to the field of neuroHIV. The purpose of the Biotype Workshop series, and the Virology, Immunology and Neuropathology Working Group in particular, is to capitalize on current and new technologies and guide future research efforts using the wealth of available immunological, virologic, and neuropathological data collected from people with HIV on and off ART.

**Keywords.** HIV-associated cognitive disorder; HAND; neuroHIV; biomarkers; inflammation; biotype.

Human immunodeficiency virus type 1 (HIV) can be detected in the central nervous system (CNS) days after infection [1] and is associated with cognitive, neurological, and mental health complications [2]. Antiretroviral therapy (ART) has dramatically reduced CNS morbidity; however, many people with HIV (PWH) continue to experience CNS complications, primarily in the form of mild cognitive impairment and mental health disorders (eg, depression, anxiety, other neuropsychiatric problems) [3–5]. Understanding the complex relationship between HIV infection and neuropathogenesis remains a research priority even though ART that suppresses HIV

replication reduces how HIV directly and indirectly affects the CNS.

Decades-long research efforts have not generated a complete understanding of the mechanisms by which HIV infection generates and maintains CNS complications during ART, and no effective therapies exist to reverse these complications. Inflammation has long been viewed as one of the primary drivers of CNS complications. While an early-stage study aimed at reducing monocyte activation in the CNS showed promise for improving cognition in PWH [6], several clinical trials aimed at reducing neuroinflammation in ART-treated PWH generated minimal or no improvement in cognition [7, 8]. Such findings may be explained by the following, not mutually exclusive, possibilities: (1) neuroinflammation is not the exclusive driver of adverse CNS outcomes; (2) clinical trials included interventions that were unable to sufficiently inhibit the type of inflammation responsible for CNS complications; (3) participants had accumulated irreversible CNS damage prior to the intervention (so-called legacy effect); (4) these trials only examined differences in specific subgroups, which might not be representative of the entire populations of PWH; and/or (5) most studies focused on global cognition and it is possible that certain drugs influence some, but not all, domains. These findings highlight the need for new approaches for studying neuroHIV.

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<sup>b</sup>L. C. N. and S. S. contributed equally to this work (shared last authors).

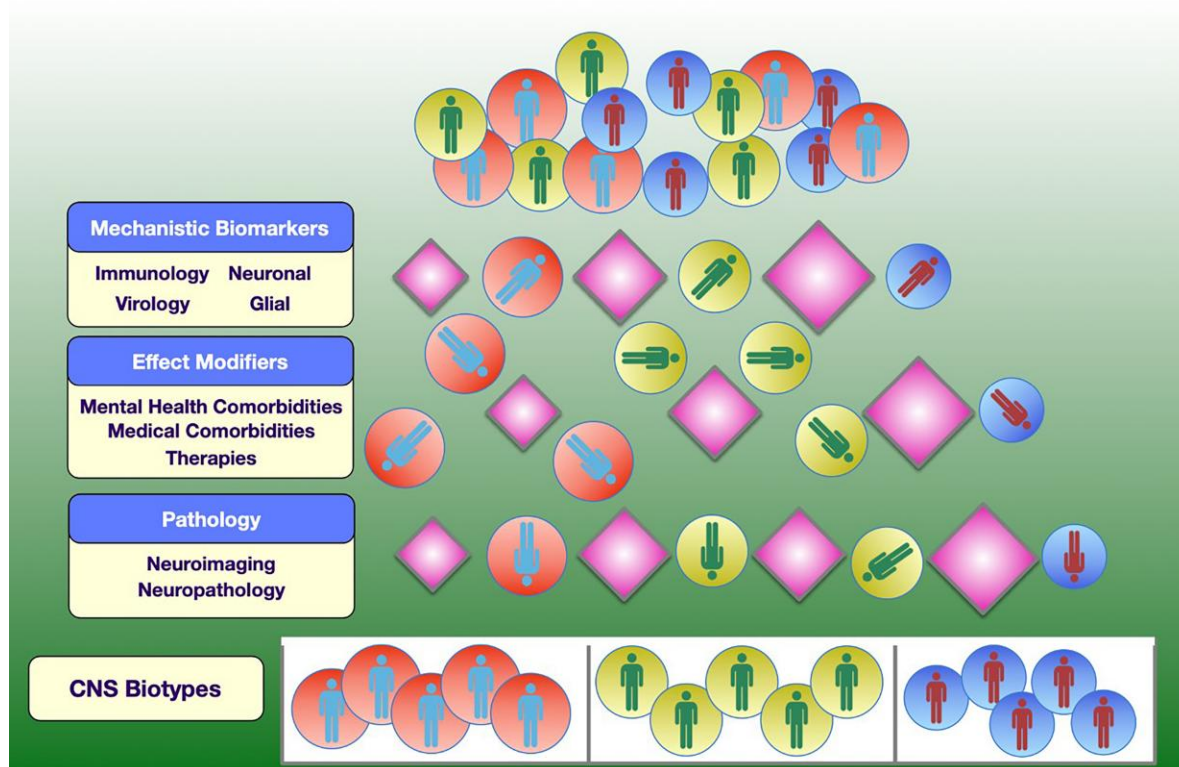
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**Figure 1.** It is likely that different neuropathologies in people infected with human immunodeficiency virus may be generated by different mechanisms and require different treatment strategies. Identifying groups of individuals with similar mechanistic biomarkers, effect modifiers, and neuropathologies may identify people whose pathologies are driven by similar mechanisms that require similar treatment strategies. Abbreviation: CNS, central nervous system.

There is growing interest in using a biotyping approach to better understand CNS complications in the setting of treated HIV infection (Figure 1). Biotypes are defined as groups of individuals who are similar based on a combination of biomarkers and linked to specific adverse CNS outcomes and/or underlying mechanisms. This approach has long been used in the field of psychiatry to define biologically distinct disease phenotypes based on underlying mechanisms, and to capture such heterogeneity [9–13]. For example, one of the first large-scale National Institute of Mental Health–funded studies was the Bipolar Schizophrenia Network on Intermediate Phenotypes Consortium Study, which drew on neurobiological heterogeneity rather than clinical diagnoses to delineate subgroups independent of their clinical phenomenology [14]. Similar methods can be applied to neurodegenerative disease, for example, Alzheimer disease, in which biological changes such as amyloid deposition may occur decades before cognitive symptoms manifest [15]. Biotyping has led to remarkable changes in the neurodegenerative field. In June 2021, for the first time in 18 years, a new therapy, aducanumab, was approved for Alzheimer disease, and several more are in the pipeline for approval from the United States Food and Drug Administration. Other fields where biotyping

approach have been successfully implemented outside HIV include cancer-related cognitive impairment [16], autism spectrum disorder [17], and depression-predicted response to treatment [18].

Such an approach could include relevant virologic, inflammatory, immune activation, and tissue damage biomarkers, as well as pathologic findings, with the goal of identifying PWH with common mechanisms underlying their neuropathogenesis that might benefit from a specific adjunctive therapy in addition to suppressive ART. Here, we explore immunologic, virologic, and neuropathologic data that could be incorporated into biotype analyses, to guide future research efforts in this setting.

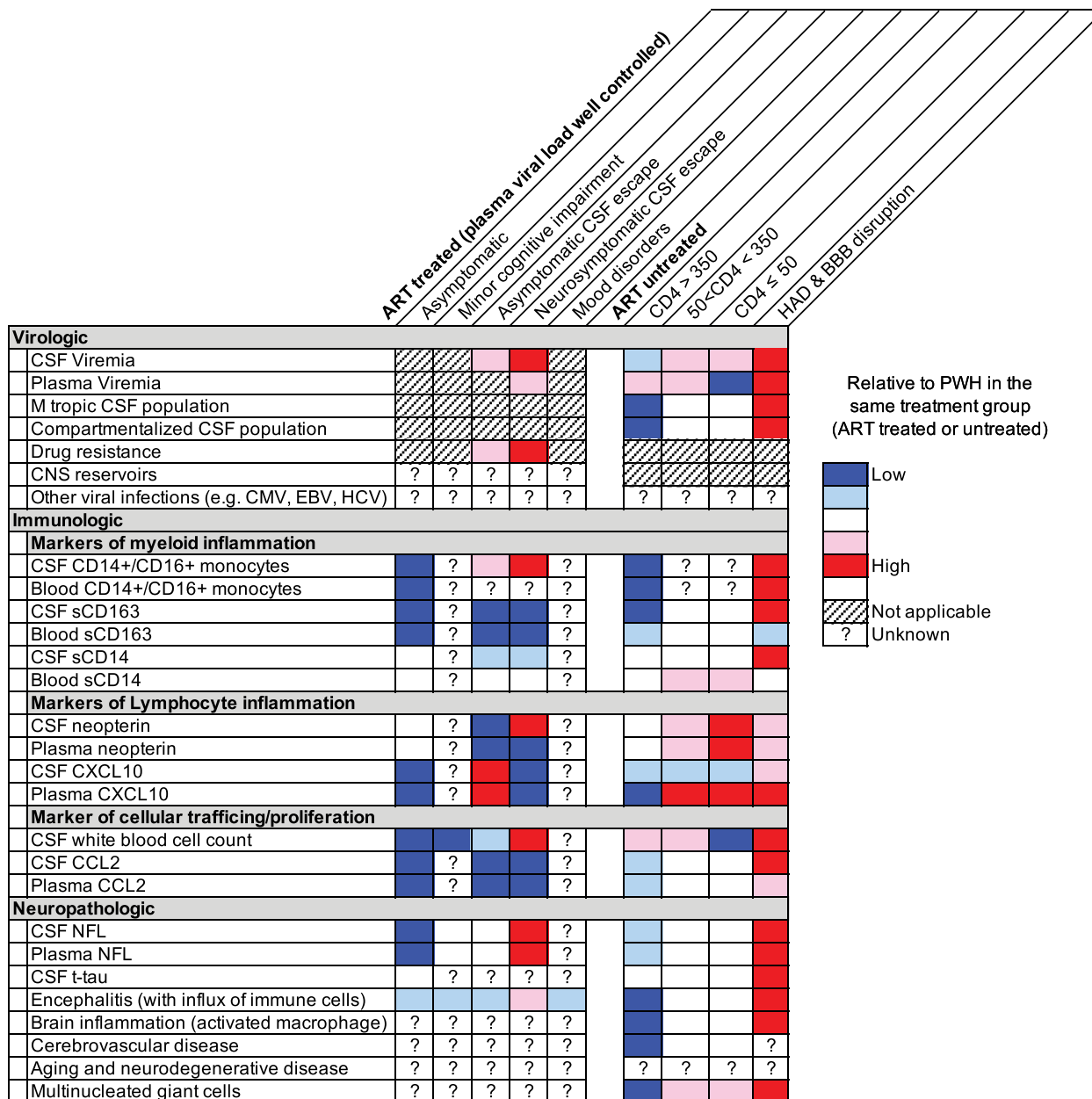
### **SOLUBLE AND CELLULAR BIOMARKERS (INFLAMMATION, IMMUNE ACTIVATION, AND NEURONAL INJURY)**

The need to identify immune biomarkers associated with lasting CNS damage is urgent. Ongoing viral replication in the CNS generates elevated levels of soluble and cellular biomarkers, which decline after ART initiation [19, 20]. Such biomarkers typically do not return to normal despite ART initiation [21–23] but can reach near-normal levels in PWH treated during the earliest phases of HIV infection [24]. Whether this is because they experience less damage related to HIV replication,

retain better immune function [24–26], have a smaller CNS reservoir [25, 26], or/and some other factor is unknown. While the specific molecular pathways controlling biomarkers in the CNS are unresolved, biomarkers are often generated by specific cell types and can provide hints about the mechanisms that influence their expression. In this section, we describe soluble and cellular biomarkers that may be used in biotype analyses (see also Figure 2).

Soluble biomarkers in plasma and cerebrospinal fluid (CSF) have many features that make them attractive for incorporation

in biotype analyses including lower costs than other assays (eg, imaging and viral sequencing) and accessibility in clinical settings. Most importantly, the data linking soluble biomarkers with neuropathogenesis are substantial. For example, CSF neopterin, a biomarker of myeloid activation, is elevated during untreated HIV infection and reaches the highest levels during HIV-associated dementia (HAD) [27]. Similarly, ART-treated, virologically suppressed PWH with mild cognitive impairment and without significant comorbidities had higher levels of both neopterin and neurofilament light (NFL) in CSF than PWH



**Figure 2.** Virologic, immunologic and neuropathologic biomarkers that may be informative for biotype analyses. This subset includes biomarkers that vary greatly across pathologic states associated with untreated and/or treated infection. The utility of these biomarkers is difficult to assess due to a lack of relevant data and/or nonstandardized data collection.

without cognitive impairment [28]. Extensive data also show that elevation of 2 other plasma biomarkers of myeloid activation, soluble (s)CD163 and sCD14, in plasma are associated with cognitive impairment [29]. The association between sCD163 levels and cognitive impairment remains strong after ART initiation, with sCD163 levels declining in people who remain cognitively unimpaired but remaining elevated in impaired people [30]. Importantly, sCD163 and sCD14 are small components of a larger set of relevant biomarkers (eg, MMP9 [matrix metalloproteinase 9], CCL2, MCP-1 [monocyte chemoattractant protein 1], TNF $\alpha$  [tumor necrosis factor- $\alpha$ ], CXCL10, sCD14, sCD163, and sCAMs [soluble vascular cell adhesion molecule]) that have been proposed to be associated with biological mechanisms that influence CNS complications in PWH [20], and might define certain CNS biotypes.

The use of biomarkers of lymphoid and myeloid activation is complicated by the fact that they do not always change in tandem across stages of untreated HIV infection and after ART initiation, likely reflecting the divergent biology of myeloid and lymphoid cells and raising the possibility that these cells contribute to CNS pathogenesis in different ways [20]. For example, biomarkers of lymphoid activation correlate closely with HIV RNA levels in CSF, whereas markers of myeloid activation are better correlated with neuronal injury (eg, NFL levels in CSF) [20]. A growing body of evidence from human studies suggests a link between monocytes and poor neurological outcomes in humans [31–33]. Of particular interest is the intermediate (activated) CD14<sup>+</sup>CD16<sup>+</sup> monocyte subset that develops from classical monocytes [34] and represents <10% of total monocytes [35]. Consistent with them having a role in inflammation and T-cell activation, intermediate monocytes have been shown to express higher levels of antigen presentation molecules than other monocyte subsets [36, 37] and produce high levels of inflammatory cytokines when stimulated [35]. A study examining 2 cohorts of virally suppressed women with HIV found that higher proportions of intermediate monocytes in the blood were associated with worse global cognitive function, executive function, and processing speed, and also predicted neurocognitive impairment 12 months later [33]. Interestingly, expression of CCR2 was significantly increased on peripheral intermediate monocytes in individuals with HIV-associated cognitive disorder (HAND) compared to PWH with normal cognition, irrespective of ART status, viral load, and current or nadir CD4<sup>+</sup> T-cell count [38, 39]. A single-arm, 24-week, open-label clinical trial of cenicriviroc, a dual CCR2 and CCR5 antagonist, was associated with improved neurocognitive performance and decreased plasma markers of myeloid activation in virally suppressed PWH [6]. These data potentially link changes in myeloid activation to cognitive performance, although the study was small and not randomized.

The mechanistic link between activated monocytes and HAND remains unclear. Studies of humans have observed

that HAND is associated both with the proportion of activated monocytes in the blood [32] and with the proportion of those cells that are HIV infected [31]. Similarly, studies of nonhuman primates (NHPs) suggest that the effect of intermediate monocytes on cognition may be due to them facilitating viral seeding of the brain [40–43], but more recent studies in NHPs [44] and humans [45, 46] suggest that HIV is primarily trafficked into the CNS by infected CD4<sup>+</sup> T cells. The importance of CD4<sup>+</sup> T cells in seeding the brain and generating neuropathogenesis is further supported by a study observing that 12 weeks after initial simian human immunodeficiency virus (SHIV) infection, SHIV was detected in CD4<sup>+</sup> T cells but not in other cell types in the brain [47].

We now appreciate that T cells likely contribute to the neuropathogenesis of HIV in many ways. This paradigm shift is related to the realization that, although T-cell entry into the CNS is generally restricted [48, 49], high numbers of T cells can be observed in untreated PWH during acute infection [50, 51], in the setting of CD8<sup>+</sup> T-cell encephalitis [52] and in people with HAD [53], but also in treated PWH with suppressed HIV RNA in plasma but with symptomatic [54, 55] or asymptomatic CSF escape [56]. Furthermore, the concentration of HIV-infected cells [57] in the CSF of ART-treated PWH is associated with neurocognitive impairment. The mechanisms by which T cells contribute to neuropathogenesis are likely multiple. During CSF escape, CD4<sup>+</sup> T cells are often the primary source of HIV in the CSF [56, 58]. In contrast, during CD8<sup>+</sup> T-cell encephalitis, CD8<sup>+</sup> T cells in the brain create a highly inflammatory environment [52]. These results indicate that T cells can influence neuropathogenesis both by producing HIV and contributing to inflammation.

Based on published data, 3 non-mutually exclusive “immune vector models” have been proposed to contribute to the natural history of HIV infection [20]. The *lymphocyte vector model* develops during primary HIV infection and increases as blood CD4<sup>+</sup> T-cell count declines, reaching a peak in the mid-range of blood CD4<sup>+</sup> T-cell count, then falling as the counts decline further [20]. This pattern mirrors leukocyte and HIV RNA dynamics in the CSF. The *macrophage vector model* involves a gradual increase in sCD14, MCP-1, and neopterin concentrations with falling CD4<sup>+</sup> T-cell count [20]. This vector appears to be associated with a noninflammatory type of CNS injury. The *mixed vector model* involves disruption of the blood-brain barrier (BBB), resulting in increased concentration of many inflammatory biomarkers. While the cause of disruption is unclear, this model is associated with an increase in all sets of biomarkers and with more severe CNS injury.

Biomarkers of neuronal damage are also of interest. For example, elevated NFL, reflecting injury to axons, is detected in the CSF during primary HIV infection and in untreated PWH with low CD4<sup>+</sup> T-cell counts, but rarely during treated HIV infection with higher CD4<sup>+</sup> T-cell counts [22, 59–61].

Very high levels of NFL are found in PWH with HAD [22, 62], and elevated levels can be found years before manifestation of dementia symptoms and often correlate with markers of immune activation [63]. Although NFL decreases after ART initiation, levels remain slightly elevated compared to controls without HIV [60, 64, 65]. Levels of NFL in CSF are very sensitive to ongoing neuronal injury, both in untreated PWH with HAD and those on ART with symptomatic CSF escape; NFL levels measured in plasma correlate with those in the CSF in the absence of ART, but more studies are needed to determine if they also decline in response to ART [66–68]. Both CSF and plasma NFL measures might be useful in biotype analysis.

In conclusion, neurocognitive impairment in untreated people likely involves systemic and local immune activation and neuronal damage, but we do not know yet which biomarkers (or *vectors*) might play the most important role in pathogenesis and at what stage of disease. Markers of myeloid activation seem to be particularly relevant in the context of CNS dysfunction. Importantly, these associations seem to persist regardless of HIV RNA and CD4<sup>+</sup> T-cell count, but no consistent pathogenic association has yet been established between neuroinflammation and neurocognitive impairment in ART-suppressed PWH. Because sCD163 and sCD14 in plasma, as well as neopterin and MCP-1 in CSF, have been linked to neurocognitive impairment across several studies, they may be valuable targets for mitigating poor outcomes in neuroHIV, but they are not specific to the CNS. While these markers are useful for correlating with different diseases, their utility for biotyping remains unclear in the setting of HIV. Finally, emerging single-cell and spatial omics technologies and epigenetic tools for characterizing the immune state of the CNS can be applied to blood, CSF, and brain tissue, and discoveries from these methods should shed further light on neuroimmune pathogenesis and neuronal circuitry in PWH that will further inform biotypes in PWH [69, 70]. Going forward, researchers should identify biomarkers to facilitate a precision medicine approach that stratifies individuals into subgroups based on their unique immune and injury signatures, which might inform underlying mechanisms and therapeutic targets.

## VIRAL NEUROPATHOGENESIS

Detectable HIV RNA in the CSF is a ubiquitous feature of untreated HIV and a defining feature of several pathogenic states affecting PWH on ART [71, 72]. These include neurosymptomatic CSF viral escape [54, 55], secondary CSF escape [73], and HIV encephalitis [74]. A mechanistic link between HIV viremia and neuropathogenesis is implied by the fact that these pathogenic states often resolve when ART is initiated that suppresses both systemic and CSF replication [54, 55, 75]. Recent studies [45, 57] suggest that even when PWH are ART suppressed, the presence of HIV-infected cells in the CSF is

associated with impaired cognition and/or neuroinflammation [57]. However, ART intensification studies have failed to reduce intrathecal immune activation and residual low-level viral load in CSF in ART-suppressed PWH, suggesting that this is not due to ongoing HIV replication in the CNS [76, 77]. This lack of viral replication is further supported by the fact that drug-resistant variants rarely evolve in the CNS during ART. In the absence of ART, severe cognitive impairment is associated with extensive replication in the CNS [53], but the connection between mild impairment and viral replication has not been established. HIV replication in the CNS is influenced by several parameters including CSF white blood cells, tropism of HIV variants, drug concentration in the CNS, drug resistance mutations, and the presence of CNS coinfections (Figure 2). These variables are attractive candidates for incorporation into biotype analyses aimed at better characterizing the relationship between HIV replication and neuropathogenesis.

Sustained replication in the CNS (CSF or brain cells) can be identified based on the presence of viral lineages in the CNS that are genetically distinct (compartmentalized) from lineages in the blood [78]. Sequence analyses of virus in the CNS of untreated people with HAD [53] frequently observe viral lineages that are genetically distinct from those in the blood; such lineages are less frequent in early infection but emerge in some PWH within the first year after HIV transmission [51]. Studies of compartmentalization are typically performed by sequencing viral RNA or DNA from the blood and CNS (CSF or brain tissue) of untreated people [79]. Similarly, viral RNA can be sequenced from people on ART and experiencing CSF escape. Such analyses have observed that viral drug resistance is often found in the setting of neurosymptomatic CSF escape [55, 80] and has also been observed in asymptomatic CSF escape [56]. Drug concentrations can be lower in the CNS, with some drugs being particularly poor at crossing the BBB [81, 82]. This creates a situation in which partially drug-resistant variants may replicate in the CNS despite being well suppressed in the periphery (ie, CSF escape). More recently, sequence analyses have examined viral DNA in CSF cells and brain tissue collected from individuals on suppressive ART [83].

An additional potential consequence of sustained replication in the CNS is a shift in tropism from T cells to macrophages. The blood and lymphoid organs typically contain a high frequency of CD4<sup>+</sup> T cells, and viruses isolated from the blood have the nearly universal ability to efficiently infect CD4<sup>+</sup> T cells and inefficiently infect myeloid lineage cells (ie, T-cell tropism [84–87]). In contrast, in the CNS where CD4<sup>+</sup> T cells are typically rare and myeloid lineage cells are common, some HIV variants have been observed to be well-adapted to infecting myeloid lineage cells (ie, macrophage-tropic) [53, 88–93]. Most of these macrophage-tropic lineages have been observed in untreated people late in disease with high viral loads and severe neurologic involvement [53, 88, 90, 94–96]. In contrast,

variants in the CSF early in infection typically have a poor to moderate ability to infect macrophages [51] and do not reach the level of true macrophage-tropic variants from the CNS of people with HAD. The evolution of macrophage tropism has been shown to also generate variants with an increased ability to infect cells expressing a low density of CD4 [53, 89, 92, 93], making it possible to assess macrophage tropism using a cell line with inducible levels of CD4 [97]. Astrocytes are another cell type within the brain that has been reported to be infected by HIV, typically via cell-to-cell contact with HIV-infected T cells (reviewed in [98, 99]), and observed to produce virus after cytokine stimulation [100–103]. However, there is also evidence that HIV-1 detected within astrocytes may be due to phagocytosis rather than infection [104].

In ART-suppressed PWH, HIV-1 may continue to contribute to neuropathogenesis by producing HIV proteins. Currently available ART regimens can prevent the formation of infectious viral particles but cannot prevent formation of viral transcripts and proteins. Surprisingly, in a subset of PWH, the intranuclear protein Tat can be detected in the CSF even when virus cannot be detected in blood or CSF [105]. Furthermore, HIV transactivating region sequences and Tat protein have been detected in exosomes derived from the CSF of patients on long-term ART [106]. The detection of Tat in CSF has also been associated with cognitive impairment [106].

One of the most poorly understood factors thought to contribute to HIV neuropathogenesis is the presence of other infectious agents in periphery or the CNS and the accompanying lymphocytes. Herpes zoster infection in the periphery has been associated with neuropathogenesis and elevated CSF white blood cells in untreated people [107] and in people on ART with CSF escape [73]. Similarly, measures of anti-cytomegalovirus immunoglobulin G in blood, prior systemic syphilis infection, and pulmonary tuberculosis have all been associated with cognitive dysfunction and/or depression in the setting of HIV [108–111]. It is uncertain whether neuropathogenesis in these conditions relates to heightened systemic immune activation with secondary CNS effects, or presence of various pathogens in the CNS. The use of next-generation sequencing may present new opportunities for addressing this issue and incorporating the resulting data in biotype analyses.

Variables relating HIV replication and/or persistence with neuropathogenesis (Figure 2) present several challenges for incorporation into biotype analyses. First, all require CSF or brain tissue. Second, sequencing analyses of compartmentalization and drug resistance are limited by the quality of sequences available. Thus, sequencing data incorporated into biotype analyses will require metadata characterizing how the data were generated and extensive quality assurance. Third, given that the genetic determinants of macrophage tropism have not been identified (reviewed by [112]), assessment requires endpoint dilution polymerase chain reaction, cloning of full

length viral *envs* or genomes, and assessment of viral entry into macrophage or a surrogate low CD4<sup>+</sup> T-cell line—a set of steps unlikely to be performed on a grand scale. Finally, detection of HIV proteins and transcripts is still a research tool not available in clinical laboratories and is not widely performed. These issues will need to be considered to incorporate some types of virologic data into biotype analyses. In contrast, other virologic variables such as the concentration of viral RNA and HIV-infected cells in the blood and CSF and the presence of other viral infections may be assessed on a wide range of participants using highly standardized methods.

## INCORPORATING PATHOLOGY IN HIV BIOTYPING

Modern clinical practice iteratively integrates laboratory-based observations with clinical phenotypes to define appropriate diagnostic categories. Similarly, an iterative process involving neuropathologic study has informed scientific knowledge of HIV in the brain since the inception of the pandemic. For example, neuropathology demonstrated that only half of PWH who died with dementia had HIV encephalitis, suggesting that more research was needed to connect clinical phenotypes with underlying neuropathologic findings [113]. Subsequently, analysis of CD68<sup>+</sup> macrophage accumulation in brain tissue found that cognitive performance was correlated with the quantity of infiltrating brain macrophages in the pretherapeutic era [114]. However, the mechanistic connection between infected macrophages and neurodegeneration remained elusive. Further studies showed that HIV neuropathogenesis is much more complex including the release of cell-derived neurotoxic molecules, the breakdown of the BBB, glial cell activation, release of cytokines and chemokines, and neurodegeneration [2, 115].

Importantly, the neuropathology of HIV is (likely) different in PWH with ART-mediated viral suppression. A large study of 589 brains collected in the National NeuroAIDS Tissue Consortium found that HAND was associated with a variety of nonspecific, noninfectious pathologies and minimal non-diagnostic changes, suggesting that histologic tools centered on virally induced, grossly inflammatory pathologies, so useful in earlier stages of the pandemic, did not have the same efficacy in elaborating the neuropathogenesis of HAND in the current era of ART [116].

Nevertheless, neuropathology is still important to the understanding of HAND neuropathogenesis, as it can reveal important relationships between HAND, HIV, and an increasingly prevalent spectrum of other brain pathologies and smaller-scale neuroimmune perturbations that cannot be directly visualized by clinical means. An example of this is seen in our understanding of the relationship between HIV infections and large-artery cerebrovascular disease, as arterial walls in the circle of Willis are not easily visualized by neuroradiology

[117, 118]. For example, increased wall thickness predicted decreased global t-scores and decrements in several cognitive domains in PWH [119]. This implicates that arteriosclerosis is an important contributor to cognitive outcomes in the ART era and is associated with traditional risk factors such as hypertension and diabetes, as well as exposures to protease inhibitor-based ART [120, 121]. Another important pathological feature to consider is amyloid deposition, a phenomenon for which there are excellent premortem manners of investigation, including positron emission tomography (PET) ligand studies and well-developed biomarkers [122–124]. In one study, the earliest cortical deposition of amyloid was better predicted by duration of HIV than by age, suggesting that HIV-associated accelerated aging and brain biologic age are more important determinants of incipient Alzheimer-type neurodegeneration than simple chronological age [125]. The mechanisms by which HIV leads to increased amyloid deposition involved inhibition of its breakdown, increased production, and increased aggregation when complexed with HIV-Tat protein.

In summary, human tissue analysis offers highly sensitive and specific diagnostics, allows detection and more specific localization of diverse pathologies, and enables stronger querying of pathogenesis and mechanisms underlying various biotypes. This is important since blood and even CSF are imperfect windows into the brain and direct visualization of brain tissue is currently the only way to confirm our observations. While usually available only at the time of death, and thus subject to the typical biases of autopsy populations, it remains a crucial tool to refine existing biotypes, albeit in a retrospective manner. New developments in high-resolution mapping of brain cell composition and function using single-nucleus and single-cell omics approaches hold promise to reveal novel cell types, targets of infection, and perturbations in neuronal circuitry underlying neuroHIV, with large-scale efforts underway (<https://scorch.igs.umaryland.edu/>). Furthermore, neuroimaging using PET ligands for detection of viral reservoirs, glial cell activation, synaptic density, amyloid and tau, neurotransmitter receptors such as dopamine and serotonin, high-resolution magnetic resonance imaging techniques, and novel methods of CSF analyses provide hope that the neuropathology of the brain might be studied by corollary studies available in living humans.

## CONCLUSIONS

As part of this workshop, the Virology, Immunology and Neuropathology Working Group has identified a list of immunologic, virologic, and neuropathological biomarkers that could be used to create biotypes in the field of neuroHIV (Figure 2). These efforts will require the development of new, highly quantitative methods, coordination of biospecimen collection and processing procedures, and standardized data generation to identify biotypes that are robust and reproducible

and that distinguish varying neurobiology between diverse populations. These biotypes might map onto our existing understanding of CNS outcomes (eg, cognitive impairment, depression), and likely will be influenced by many factors, such as source of sampling, stage of infection, treatment status, race/ethnicity, age, sex/gender, comorbidities, and many more. These unique biotypes should be continuously refined as new knowledge becomes available.

Importantly, factors other than HIV need to be carefully considered and are difficult to exclude in many studies, unless appropriate controls are included (ie, participants who have similar risk and lifestyle factors). For example, a study including PWH with suppressed plasma HIV RNA and well-matched controls without HIV found similar levels of myeloid activation in blood, suggesting that factors other than HIV drive myeloid activation and inflammation and may thereby contribute to CNS complications [126]. Likewise, people without HIV on preexposure prophylaxis with high-risk sexual behavior often have increased levels of inflammatory biomarkers in blood and CSF [127], suggesting that lifestyle factors or ART exposure may explain some of the findings in treated PWH. Thus, the use of appropriate controls (with similar risk factors and demographic profiles) will be crucial.

Biotypes should be reproducible across cohorts and longitudinally within an individual and creating platforms for data harmonization and standardization across studies (which include source of samples, various assays, platforms, protocols) will be important. Data transparency is also important, and all data should be made publicly available. This is particularly important for multiomics data. Having reference laboratories would be a good practice to standardize assays and serve as repository of reagents. Finally, all neuroHIV-related biotype data should incorporate the guiding principles of findability, accessibility, interoperability, and reusability (FAIR) to allow optimum reuse of the data.

Although the biotyping approach may prove to enhance diagnosis and treatment decisions, some challenges need to be considered that might hinder its clinical translation. These include the clinical diversity of mental health disorders, the technical complexity, costs, and the need for specialized training for laboratory staff, in addition to ethical concerns such as protecting the privacy and security of participants' data and maintaining health equity.

The primary challenge is determining whether these individuals represent distinct biotypes with distinct underlying mechanisms. Examination of individuals across a range of suppression levels reveals the range of possible phenotypes. Unsuppressed individuals also show a range of phenotypes that may result from distinct underlying mechanisms.

In conclusion, understanding the impact of HIV on cognition and mental health from a personalized, brain-based perspective could lead to the development and implementation of maximally effective intervention strategies. Additionally, better understanding the impact of HIV in this way could provide crucial insights on the ways in which it affects the



trajectory of individuals' mental health and cognitive functioning. More research and interdisciplinary discussions are needed to successfully implement these methods to the field of HIV.

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