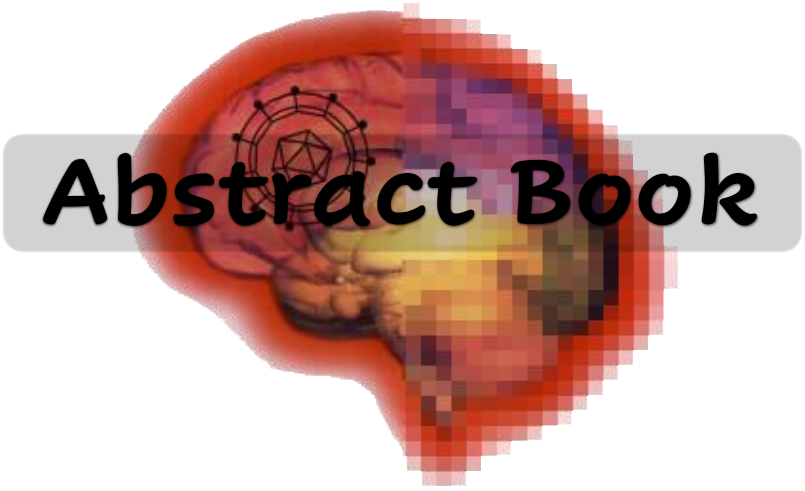
18th International Symposium on NeuroVirology

Held jointly with the

2022 Conference on HIV in the Nervous System



Abstract Book

October 11-14, 2022

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P1

CD4DIM CD8BRIGHT T cells home to the brain and mediate HIV neuroinvasion

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CD4dim CD8bright T cells are a mature population of CD8+ T cells that upon activation up regulate CD4dimly on their surface. Expression of CD4 on these cells suggest that they can be an additional source of HIV neuroinvasion and persistence in the brain. We used HIV infected NOD/SCID/IL-2rcγ(-/-) (NSG) humanized mice to track CD4dim CD8bright T cell homing to the brain and define their role in HIV dissemination into the brain. We report here that CD4dim CD8bright T cell are found in the brain at a median frequency of 2.6% and in the spleen at median frequency of 7.6 % of CD3+ T cells. In the brain, 10-20% of CD4dim CD8bright T cells contain integrated provirus which is infectious as demonstrated by viral outgrowth assay. CD4dim CD8bright T cells in the brain exhibited significantly higher expression of the brain homing receptors, CX3CR1 and CXCR3 in comparison to their single positive CD8+ T cell counterpart. Blocking lymphocyte trafficking into the brain of humanized mice, via anti-VLA4 and anti-LFA1 antibodies, reduced CD4dimCD8bright T cell trafficking into the brain by 60% and diminished brain HIV proviral DNA by 72%. Collectively, our findings demonstrate that CD4dim CD8bright T cells can home to the brain and support productive HIV replication. These studies also reveal for the first time that CD4dim CD8bright T cells are capable of HIV neuroinvasion and are a reservoir for HIV.

P2

Astrocytes emerge as a prominent cell type harboring rebounding SIV following cART cessation

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Although combination antiretroviral therapy (cART) maximally suppresses HIV replication, cART cessation dramatically causes viral rebound. This viral rebound provides an opportunity to understand cellular sources of rebounding virus in key sanctuary sites of HIV under cART, including the CNS. Using a cART cessation model of SIV mac239 and archived tissue, we assessed the cellular sources of rebounding virus. Out of ten total rhesus macaques, seven were infected with SIVmac239, while three were uninfected controls. Remaining macaques were either untreated and developed AIDS (n=3), or were treated with cART regimen FTC, TDF, & DTG or RAL for six months (n=3) or two months (n=1). cART was then stopped and the brain was harvested at either four (n=1) or fourteen days (n=2) post-cART cessation. One animal was kept fully suppressed for two months before necropsy. Frontal, occipital, temporal lobes, midbrain, hindbrain, and cerebellum regions were assessed for SIV using viral RNA/DNA scope and co-stained for microglia/macrophages (Iba-1, CD68/163/206) and astrocytes (GFAP) using fluorescence microscopy. We found that fluorescent detection of RNAscope can be quantified by a halo of RNA signal surrounding the nucleus, while DNAscope can be quantified by a punctate bright fluorescent signal co-localized with nuclear DAPI staining. Further, although microglia/macrophages expressed the highest levels of viral RNA in AIDS, astrocytes exhibited elevated levels of viral RNA following cART cessation. As expected, we detected increased DNAscope signal in cART suppressed tissues compared to AIDS and cART cessation, with higher DNA signal in myeloid cells localized to the frontal lobe compared to the cerebellum or occipital lobe. These findings demonstrate that astrocytes contribute to rebounding virus following cART cessation. These studies also have implications for directing therapies that can block viral egress from the CNS following cART cessation.

P3

Differential expression of circular RNAs in PBMCs of African America Women living with HIV-1: potential biomarkers of HAND

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Circular RNAs (circRNAs) are a new class of head-to-tail joined single-stranded RNAs with poorly defined functions and are currently under investigation in several biomedical fields. Recently, differential expression of circRNAs in peripheral blood mononuclear cells (PBMCs) has been characterized during the early stages of HIV-1 infection. HIV enters the Central Nervous System (CNS) within days after peripheral infection, resulting in the establishment of viral reservoirs in the CNS and chronic neuroinflammation that persists even with successful antiretroviral treatment (ART) and leads to HIV-associated neurocognitive disorder (HAND), a condition affecting close to 42% of HIV positive individuals. A recent meta-analysis study has also reported that studies in a cohort with a high proportion of women had higher HAND prevalence. Here we studied the circRNAs profile in PBMCs from eight African American women living with HIV (WLWH) on ART and affected by HAND. The circRNAs profiles from those HAND donors were compared with an additional eleven donors with HIV on ART but no neurological complications and six healthy donors. The circRNA transcriptomes were profiled through a Human Circular RNA Array on total RNA extracted from participants' PBMCs. The levels of the top differentially expressed HAND-associated circRNAs were further verified by qRT-PCR. Our results suggest that the circRNA expression of PBMCs from WLWH on ART and affected by HAND possesses a distinct profile than those with no neurological disease that warrant further investigation of their biomarker potential for HAND development.

P4

A targeted sgRNA library-based approach for selection of CRISPR/Cas9-gRNA pairs used in an HIV-1 cure strategy

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Human immunodeficiency virus type 1 (HIV-1) persistence has been attributed to the latent viral reservoir of integrated proviral DNA in tissues including the peripheral blood, lymphoid tissue, brain, gut, and likely other tissues. An in silico prediction algorithm trained on a dataset of patient-derived LTRs isolated from PBMCs led to the discovery of broad-spectrum selected molecular guide RNA (gRNA) targets (SMRT) gRNAs. SMRT gRNAs are predicted to cleave 100% of patient-derived LTR samples as well as a dataset of publicly available patient sequences, which would lead to inactivation or excision of the integrated provirus. In vitro study of the SMRT gRNAs revealed high cell viability and cleavage activity through flow cytometry and fluorescent microscopy in TZM-bl, P4R5, and J-Lat cell systems. Sequences of HIV-1 LTR have been obtained from an independent set of patient-derived tissue samples from the NNTC in the brain and spleen with Illumina NextSeq. The PBMC-derived SMRTs showed >90% predicted efficacy for the

brain sequences. Furthermore, the Multiple Lentiviral Expression System (MULE) is currently being used to engineer lentiviral constructs to deliver Sa or Sp Cas9 and targeted gRNAs to cells. These lentiviruses will be produced in a library approach to simultaneously measure the ability of thousands of gRNAs to silence HIV-1 expression. Accumulation of this data as well as delivery of a library of gRNAs will allow analysis of Sa and Sp Cas9 enzyme efficiency, gRNA specificity – including consideration of the effect of a leading G nucleotide in the protospacer, and on- and off-target cleavage efficacy.

P5

Persistent interferon induction and neuroimmune dysregulation after HIV-1 viral infection in human stem cell derived microglia and cerebral organoids

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Human Immunodeficiency Virus type-1 (HIV-1) Associated Neurocognitive Disorder (HAND) affects up to half of HIV-positive patients and causes long-term neurological consequences, including dementia. There are no effective therapies for HAND due to a lack of understanding of the pathophysiology of HIV-induced glial and neuronal functional deficits in humans. To address this, we developed a model of HIV-1 central nervous system (CNS) infection using human induced pluripotent stem cells (hiPSC)-derived mixtures of microglia, neurons, and astrocytes in both 2D cultures and 3D brain organoids to model viral infection of human microglial cells and identify neurological consequences. hiPSCs were differentiated into primitive microglial precursor cells (PMPs), assayed for expression of CD4 and CCR5 receptors, and infected with HIV-1 virus in a 2D monoculture. We detected HIV p24 production in culture supernatant by ELISA in a time-dependent manner after infection with two different CCR5-tropic replication competent virus strains (JRFL and YU2). Key inflammatory cytokines associated with neurocognitve impairment in patients with HAND, such as CXCL10 and M-CSF, were found upregulated after infection. To further elucidate our findings, we used RNAseq to analyze whole-genome transcriptome response and discovered a robust interferon response in conjunction with upregulation of selected inflammatory cytokines. The induced expression of interferon-stimulated genes such as ISG15, RSAD2, and CXCL10 was confirmed by quantitative RT-PCR for both JRFL and YU2 infection and demonstrated persistence of the interferon response. Infected PMPs were then added to human microglia-containing cortical organoids and were shown to integrate into the organoid with viral gene expression persisting for at least two weeks. Ongoing efforts are focused on delineating underlying molecular and cellular mechanisms of HIV infection in the human CNS model. The observation of robust interferon-stimulated responses in HIV-1 infected microglia may provide novel insights into the mechanisms of HAND and provide novel routes for therapy development.

P6

Potentially beneficial effects of cannabidiol (CBD) in EcoHIV infection in culture and mice

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Cannabidiol (CBD), a non-psychoactive cannabis component, is freely available and widely used for relieving pain and anxiety commonly experienced by people living with HIV (PLWH) and it has been described as having several anti-inflammatory effects. However, its long-term effects on HIV infection and progression of HIV neurocognitive disease remain largely undetermined. Here, we use in vitro models and EcoHIV infected mice to investigate the effects of CBD on virus load, inflammatory markers, brain

transcriptional profiles, and behavioral outcomes. Following kinetic and dose optimization studies, our preliminary results show a reduction of EcoHIV viral load in monocyte/macrophage-derived RAW 264.7 and microglia-derived SIM-A9 cell lines and in primary murine macrophages pre-treated with CBD. Virus load was also reduced in the brain of EcoHIV infected mice pretreated with 30 mg/kg of CBD. This effect was accompanied by altered transcriptional signature in brain tissue compared to untreated infected mice that included down-regulation of immune-related genes TNF-α, CXCL10 and C3. However, CBD treatment of EcoHIV mice had no significant effect on neurocognitive disease in these animals. Our data indicate that CBD can have beneficial effects in mitigating some HIV effects in vitro and in vivo. Supported by grants R01 DA052844-01 and U01 DA053629 from the National Institute on Drug Abuse (NIDA), NIH

P7

Generation of iPSC-derived 3D Neurospheres for Modeling Retroviral Infection and EV-mediated Repair

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Improvements in biotechnology and advances in stem cell research have provided novel platforms for CNS disease modeling. HIV-1 remains incurable and HIV-associated neurocognitive disorders (HAND) are commonly associated with HIV-1. Despite the development of cART, HAND still affects up to 50% of HIV-1 patients. It is believed that over-amplification of inflammation and release of toxic viral proteins are responsible for the neurological damage observed in HAND; however, the underlying mechanisms require further study. Here, we report the generation of iPSC-derived neurospheres and show that mature neurospheres are composed of at least 3 CNS cell types. Our data also shows that neurospheres are permissive to HIV-1 infection in both short-term (acute) and long-term (latent) cultures that can be maintained for up to six months (1). In addition, we examine the functional effects of stem cell extracellular vesicles (EVs) on HIV-1 infected neurospheres, as there is abundant literature supporting their reparative properties in the CNS (2,3). Our data suggests that stem cell EVs may modulate neuroprotective, anti-apoptotic, and anti-inflammatory properties. We believe the mechanism of action is driven by EV-associated cargo, including long non-coding RNAs, reparative cytokines, and kinases that assist in the removal of cell cycle blocks. Collectively, this demonstrates the feasibility of iPSC-derived neurospheres for modeling HIV-1 infection and highlights the potential of stem cell EVs for rescuing cellular damage. References: 1) Branscome et al. Scientific Reports. 2022 Feb 7;12(1):2019. doi: 10.1038/s41598-022-05848-x. 2) Branscome et al. J Neuroimmune Pharmacol. 2020 Sep;15(3):520-537. doi: 10.1007/s11481-019-09865-y. 3) Branscome et al. Front Cell Dev Biol. 2020 Jun 10;8:455. doi: 10.3389/fcell.2020.00455.

P8

Modeling HIV-1 infection dynamics in cells of a myeloid lineage to better understand HIV/HBV co-infection

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HIV and HBV cause chronic viral infections and coinfected individuals have a 5-6 fold increased risk for developing cirrhosis and hepatocellular carcinoma (HCC) over mono-infection. The basis for this difference is not clear, but HIV-infected T cells and liver macrophages likely contribute to an altered liver microenvironment, exacerbating disease. This effect may be mediated by the effect of viral proteins that are still produced because of the integration of HIV-1 proviral DNA from infected cells. The heterogenous liver macrophage population, including Kupffer cells and human monocyte-derived macrophages (hMDMs), are likely reservoirs for HIV as viral RNA and DNA have been detected in patient-derived livers. This project examined hMDMs and liver macrophages isolated from peripheral blood and resected liver tissue, respectively. These cells were infected with HIVADA, a R5 tropic virus. The number of p24 containing cells and formation of multi-nucleated giant cells, a hallmark of HIV infection in tissues, particularly the brain, was evaluated using high content imaging on the CX7 High Content scanner. Changes in virion production were evaluated by measuring p24 levels in supernatant using AlphaLISAs. Our results show that an increasing number of infected cells correlates with increased viral production over time and production of multi-nucleated giant cells. We have also showed that liver macrophages derived from patient samples are susceptible to HIV-1ADA infection, and that treatment with anti-retroviral Biktarvy results in reduced p24 levels. Furthermore, treatment of hMDMs with HBV proteins known to be chronically present in infected individuals resulted in altered p24 production and percentage of p24 positive cells, which may occur in a serotype-specific manner. This data is a critical step in establishing a baseline model of HIV infection dynamics to further evaluating changes in HIV infection in cells of a myeloid origin.

P9

Zoster-associated Prothrombotic Plasma Exosomes and Increased Stroke Risk

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Herpes zoster (HZ, shingles) caused by varicella zoster virus (VZV) reactivation increases stroke risk for up to one-year post-HZ. The underlying mechanisms are unclear, however, the development of stroke distant from the site of zoster (e.g. thoracic, lumbar, sacral) that can occur months after resolution of rash points to a long-lasting, virus-induced soluble factor(s) that can trigger thrombosis and/or vasculitis. Herein, we investigated the content and contributions of circulating plasma exosomes from HZ and non-HZ patient samples. Compared to non-HZ exosomes, HZ exosomes; (1) contained proteins conferring a prothrombotic state to recipient cells, and (2) activated platelets leading to the formation of platelet-leukocyte aggregates. Three-month post-HZ exosomes yielded similar results and also triggered cerebrovascular cells to secrete the proinflammatory cytokines, IL-6 and IL-8. These results can potentially change clinical practice through addition of antiplatelet agents for HZ and initiatives to increase HZ vaccine uptake to decrease stroke risk.

P10

Chronic immune activation and gut barrier dysfunction is associated with neuroinflammation in ART-suppressed SIV+ rhesus macaques

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BACKGROUND: HIV-associated neurocognitive disorders (HAND) affect ~30% of virally suppressed people with HIV (PWH), suggesting that HAND pathogenesis may be driven by mechanisms other than direct viral replication in the brain including chronic systemic inflammation. However, the precise viral dependent and independent changes to the brain of virally suppressed PWH remains unclear. METHODS: Here we characterized the CNS reservoir and immune environment of SIV-infected rhesus macaques during acute (n=4), chronic (n=12) or ART-suppressed SIV-infection (n=11). Multiplex immunofluorescence for markers of SIV-infection (RNA/DNAscope) and immune activation was performed on frontal lobe and matched gut tissue. CNS and gut inflammation were also measured in an SIV-uninfected model of chronic colitis, validated to mimic SIV-induced gut damage, to determine the effect of gut damage on neuroinflammation independent of SIV-infection. RESULTS: SIV-infected animals contained viral DNA+ cells that were not reduced in the brain or gut by ART ($P<0.05$), supporting the presence of a stable viral reservoir in these compartments. SIV-infected animals had heightened levels of activated astrocytes and microglia producing antiviral (Mx1 and/or TGF-β1) and oxidative stress markers (SOD1) as well as reduced blood-brain barrier (BBB) integrity than uninfected animals, and these dysfunctions were not abrogated by ART ($P<0.05$ for all). Interestingly, measures of CNS immune activation and BBB integrity correlated with gut, but not CNS, viremia and immune activation in virally suppressed animals, supporting the role of systemic inflammation as a contributor to neuroinflammation. Furthermore, SIV-uninfected animals with experimentally induced gut damage showed a similar immune activation profile in the brain to animals with SIV, supporting the role of chronic gut damage as an independent source of neuroinflammation. CONCLUSIONS: We show that ART-suppressed SIV-infected rhesus macaques exhibit impaired BBB integrity and heightened microglial and astrocyte activation which is associated in part with viral reservoirs and immune activation in the gut.

P11

Disrupted Interferon type I signaling in peripheral monocytes from cognitive impaired patients and human brain organoids

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Patients with HIV (PWH) develop neurocognitive disorders, driven by monocytes infiltrating into the brain, and neuronal dysfunction, despite the effectiveness of antiretroviral therapy. Type I interferon signaling, performs important functions in the central nervous system (CNS) such as microglia activation, synaptic plasticity, and cognitive function. We hypothesize that disrupted IFN-I signaling triggers monocyte infiltration and cognitive decline. We measured Interferon alpha (IFNa) in the plasma of controls, HIV-positive, and Alzheimer's disease (AD) patients by ELISA and cytokine array. Then, we selected peripheral blood mononuclear cells (PBMCs) from a subgroup of patients and measured interferon alpha/beta receptor 1 (IFNAR1) in CD14+ monocytes by flow cytometry. Finally, we co-cultured patient-derived monocytes with human brain organoids. IFNa1 levels are slightly higher in the plasma of PWH cognitive impaired and significantly higher Alzheimer's disease patients ($p=0.035$), compared to controls. In a cytokine array of plasma from PWH ($n=48$), higher levels of IFNa2 were detected in the plasma of cognitive impaired ($n=21$) patients, compared to normal ($n=27$) patients ($p=0.022$). Flow cytometry revealed that the percentage of IFNAR1+ CD14+ monocytes are decreased in PBMCS from HIV-positive ($p=0.020$) and AD patients ($p=0.011$) compared to controls. The statistically significant decrease in surface IFNAR1 levels was highly influenced by monocytes from male participants ($p=0.029$). Western blotting of PBMCs revealed that Interferon Regulatory Factor 3 (IRF3) is phosphorylation and active in PBMCs from PWH compared to controls ($p=0.036$). Upon co-culture of human brain organoids with these monocytes, we observed a decrease in IFNAR1 expression in the organoids, and significant activation of IRF3 which subsequently decreased with cognitive impairment. Co-culture of patient-derived HIV-infected monocytes with brain organoids also triggered amyloid beta1-42 secretion, which was not observed in untreated organoids. Thus, disrupted IFN-I signaling in monocytes may contribute to CNS infiltration, neuropathology, and cognitive decline in both PWH and Alzheimer's disease patients.

P12

HIV infection induces foam cell formation via the caspase-1 pathway, contributing to HIV-associated atherogenesis

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Chronic HIV infection is primarily associated with inflammation driving comorbidities such as cardiovascular disease (CVD), including HIV-related atherogenesis. Caspase-1 activation in leukocytes has been observed in HIV infection; however, whether caspase-1 activation and the downstream pro-inflammatory cytokines interleukin-1beta (IL-1β) and interleukin-18 (IL-18) contribute to chronic inflammation is still undetermined. Here, we focused on investigating the underlying mechanism of HIV-induced caspase-1 activation in monocyte/macrophages and its atherogenic role. Peripheral blood mononuclear cells from 25 people with HIV (PWH) and 25 people without HIV were examined for the ability to form foam cells. We found a significant increase in foam cell and activation of caspase-1 pathway in ex vivo cells treated with oxLDL from PWH compared to the control group ($P<0.0001$). We showed similar results in vitro with PBMCs infected with HIV-ADA exhibiting increased foam cells formation and activation of the NLRP3 pathway compared to uninfected cells. Inhibition of NLRP3 activity using MCC950 reduced the effect of HIV/oxLDL in the foam cell formation process. Together, these results highlight the possible role of the NLRP3/caspase-1 inflammasome pathway in HIV-accelerated

atherogenesis. The understanding of the mechanism of HIV-associated CVD will help to better design and develop novel therapeutic interventions for treatment/prevention.

P13

Deficient B cell responses correspond with encephalitis in SIV-infected macaques

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Simian immunodeficiency virus (SIV) infection in rhesus and pigtail macaques (Macaca mulatta and M. nemestrina) are both widely used models for AIDS pathogenesis. The course of infection in these species physiologically resembles HIV infection in humans, and the immune response and disease progression similarly vary widely between individuals. We hypothesized that defective adaptive immune responses lead to poor clinical outcomes, including central nervous system (CNS) disease. Here we demonstrate that deficient B cell responses are associated with the development of SIV encephalitis (SIVE) in infected macaques. SIV-specific antibody responses were assessed by Western blot and ELISA using plasma and CSF samples from the terminal timepoint (50 - 547 days) of 40 macaques, 20 of which developed SIVE. Immunohistochemistry (IHC) of peripheral lymph nodes and spleen was performed using antibodies against CD3, CD4, CD8a, CD20, IgM, and IgG and counterstaining with hematoxylin. While SIV-specific antibody responses vary between individuals, there was a marked deficiency in the development of IgG antibody responses in animals that subsequently developed encephalitis (n = 13 pigtail, 7 rhesus). In contrast, IgM responses were present regardless of outcome. IHC of lymph nodes and spleen from encephalitic macaques showed a reduced number of strongly IgG positive cells and loss of tissue architecture despite similar levels of T cell populations compared to SIV infected macaques without encephalitis. These findings demonstrate that the development of functional adaptive immune responses is necessary to protect the CNS and an important determinant of pathological outcomes of SIV infection. In particular, the development of appropriate B cell responses after inoculation closely correlates with protection from SIVE. Understanding the role of B cells in protection from SIV pathogenesis may help address ongoing cognitive dysfunction and chronic inflammatory pathology in people with HIV.

P14

Antiretroviral drugs inhibit astrocyte autophagy in a drug-specific manner

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Antiretroviral therapy (ART) has dramatically improved the lifespan of people with HIV (PWH). Yet, HIV associated Neurocognitive Impairment (HIV-NCI) still develops. It decreases quality of life, and is an independent risk factor for mortality. ART itself may contribute to HIV-NCI. Clinical studies showed relationships between NCI and specific antiretroviral drugs, drug classes, and duration of antiretroviral exposure. Several studies demonstrated antiretroviral toxicity on different CNS cells. The mechanisms by which ART may contribute to HIV-NCI are incompletely characterized, but may relate to effects on astrocyte autophagy. Astrocytes, abundant CNS cells with many crucial functions, maintain their homeostasis, in part, by autophagy. Autophagy is a highly regulated, intracellular proteolytic process that removes damaged and toxic organelles and macromolecules from the intracellular environment using a specialized double-membrane vesicle (termed autophagosome) that sequesters cargo for lysosomal degradation. Dysregulated astrocyte autophagy contributes to neurodegenerative diseases. ART may contribute to HIV-NCI by impacting autophagy. Several antiretroviral drugs affect autophagy in CNS cells, although these studies were not of astrocytes. We show that two antiretroviral regimens inhibit autophagy in primary human astrocytes with drug-specific effects. We treated primary human astrocytes with Tenofovir+Emtricitabine plus either Raltegravir (ARTral) or Dolutegravir (ARTdol) for 24h or 7 days

daily. Lysates were collected for Western blotting analyses of LC3-II and p62, and RNA for qRT-PCR of p62, macroautophagy markers. We found that ARTral decreases autophagosome biogenesis without impacting degradation, while ARTdol decreases both biogenesis and degradation. Neither ARTral or ARTdol were toxic, as demonstrated by LDH assays. These data highlight that antiretroviral drugs may have distinct effects on astrocyte autophagy, which may impact homeostasis. Understanding the impacts of ART on astrocytes and on autophagy is important for improving ART, and for development of therapies for HIV-NCI, a highly burdensome comorbidity for PWH who have, by necessity, long-term exposure to antiretroviral drugs.

P15

Intact HIV proviruses persist in the central nervous system despite viral suppression with antiretroviral therapy

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HIV persistence in blood and tissue reservoirs represents the major barrier to HIV cure and is a possible cause of comorbid disease. HIV is known to infect the central nervous system (CNS); however, to date the size and replication competent nature of the CNS reservoir is unclear. Here we employed a droplet digital PCR assay to detect total HIV DNA and the intact proviral DNA assay (IPDA) to provide the first quantitative assessment of the intact and defective HIV reservoir in well-characterised brain tissues from autopsies. Further phenotypic characterisation of brain reservoir cells was provided by in situ hybridisation (DNAscope) targeting HIV DNA and laser capture microdissection and PCR of CD68+ brain cells. HIV DNA was present at similar levels in brain tissues from untreated viremic or antiretroviral (ART)-suppressed individuals (n=36; median: 22.3 vs 26.2 HIV pol copies/106 cells), reflecting a stable CNS reservoir that persists despite therapy. Furthermore, 9/12 viremic and 5/8 virally suppressed individuals also harboured intact proviruses in the CNS (13.5 vs 4.63 intact copies/106 cells). CNS and peripheral reservoirs harboured a similar frequency of intact proviruses (~20% of proviruses). In situ hybridisation (DNAscope) identified the presence of HIV DNA in brain myeloid cells and sequences of proviruses isolated from purified brain myeloid cells compartmentalised relative to those from matched peripheral lymphoid tissue reservoirs, indicating that the CNS harbours a distinct reservoir. Thus, here we provide the first evidence of an intact, potentially replication competent, HIV reservoir in the CNS of virally suppressed people living with HIV.

P16

Regional analyses of intact and defective HIV proviruses in the brain

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BACKGROUND: Currently no scalable cure for HIV exists due to the presence of long-lived and stable viral reservoirs in blood and tissue sites throughout the body that are not eradicated by treatment with antiretroviral therapy (ART). We recently demonstrated the first evidence of an intact, potentially replication competent HIV reservoir in the frontal lobe of people with HIV (PWH) who were duly suppressed with ART. Here we extend on these findings to provide the first characterization of the HIV intact and defective reservoir throughout multiple regions in the brain. METHODS: The total (HIV pol), intact and defective (intact proviral DNA assay, IPDA) HIV proviral reservoir was quantified in frozen human frontal lobe, cerebellum and basal ganglia brain tissue from viremic (i.e. untreated) or virally-suppressed PWH and HIV-seronegative controls (National NeuroAIDS Tissue Consortium USA, n=19) by droplet digital PCR based approaches. RESULTS: HIV pol DNA was detected in all brain regions regardless of ART treatment status demonstrating the widespread presence of HIV DNA in the brain that is not influenced by long-term ART treatment. Higher levels of HIV pol DNA were present in the frontal lobe relative to the basal ganglia (median = 49.08 vs. 10.17 HIV pol copies/106 cells, $p>0.05$) and cerebellum (49.08 vs. 8.80 HIV pol copies/106 cells, $p=0.0078$) in virally-suppressed PWH. Furthermore, virally-suppressed PWH also harbored intact proviruses in the basal ganglia and cerebellum (2.79 vs. 1.50 intact copies/106 cells), supporting the presence of likely replication competent genomes in these brain regions. CONCLUSION: Here we provide the first characterization of intact and defective HIV proviruses across multiple regions of the brain, demonstrating that stable and potentially replication competent HIV reservoirs can persist throughout the brain despite ART treatment. This highlights the great importance of considering the CNS in HIV cure.

P17

Evaluating integrated HIV-1 quasispecies using Near Full Length sequencing in the context of neuroHIV

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The integrated human immunodeficiency virus type 1 (HIV-1) provirus forms a stable but latent viral reservoir in various tissues. It has been shown that both replication competent and defective proviral

sequences exist in the viral reservoir. At least some defective proviruses are able to produce viral proteins. While many individuals achieve undetectable levels of replicating virus as a result of anti-retroviral therapy (ART), current methods for monitoring viral load may not reflect the full landscape. Droplet digital PCR (ddPCR), along with near full-length (NFL) amplification and third-generation sequencing (TGS) are valuable tools to evaluate the absence or presence of genes which may contribute to chronic HIV-1 neuropathogenesis and disease. Sequencing the PBMC compartment has shown an approximate 30 percent failure of amplification of the viral genomic regions capable of encoding viral accessory proteins. We hypothesized that the reason for such differential results could be due to accumulation of replication incompetent defective proviruses. Given these observations, we have used NFL strategies to target provirus and examine the full spectrum of integrated proviruses within the PBMC compartment of individuals in context of cognitive impairment from the Drexel patient cohort and between the spleen and brain from the National NeuroAIDS Tissue Consortium (NNTC) Cohort. Amplicon analyses were conducted utilizing the MinION Nanopore TGS platform. Initial results showed ART-suppressed individuals had mostly defective virus as the predominant species. Future analyses will apply the NFL strategy to examine the potential role of defective genomes in the emergence of neuroHIV.

P18

Advances in artificial intelligence for studying HIV genetic variation in the brain

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Recent advances in artificial intelligence (AI) have greatly accelerated fields in many scientific disciplines from physics to biology. Neural networks, particularly as applied to language modeling, have shown to be particularly adept at understanding genomic languages as well or better than human languages. These models are also advantageous in that they can pre-learn from large quantities of unlabeled data to leverage the information present in smaller datasets typical in neuroHIV. This is accomplished through a process called transfer learning in which knowledge is shared between related tasks. To this end, we have developed an ever-expanding suite of HIV specific AI tools. First, we have released Quasipore, a nanopore basecaller built specifically for HIV. This AI tool dramatically increases the single-molecule level accuracy of the nanopore long read sequencer when processing HIV sequence. With no change to the chemistry or library preparation, this basecaller can reduce miscalled bases to fewer than 1:10,000 bp, allowing for single molecule analysis of HIV. Second, we have recently released HIV-BERT, a Bidirectional Encoder Representations from Transformers for HIV. This transformer model has been pre-trained on all UniProt proteins to learn the general language of known proteins, then it was further refined on large collections of HIV proteins to learn the specific ‘dialect’ of HIV proteins. HIV-BERT has demonstrated an ability to predict response to antiretroviral therapy, cellular tropism, and body site at state-of-the-art accuracy levels. The tool can also be used for alignment-free grouping of sequences by similar function. This presentation will discuss how these, and other technologies, can accelerate the study of HIV in the brain.

P19

Unexpected Roles of Toll-Like Receptor Adaptors TRIF and Mal/ MyD88 in Microglia Response to Neurotropic Murine Beta-Coronaviruses

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The current COVID-19 pandemic highlights the impact of beta-coronaviruses (β-CoVs) in human health. Human endemic CoVs, as well as the three highly pathogenic β-CoVs (SARS-, MERS-, and SARS-CoV-2) cause acute, mild to severe, and fatal respiratory tract illnesses. SARS-CoV-2, however, is associated with frequent neurologic symptoms despite unclear direct neuroinvasive properties. Murine β-CoVs mouse hepatitis virus (MHV)-JHM and -A59 strains are experimental models of acute encephalitis and chronic demyelination. Microglia are the resident innate immune cell of the central nervous system (CNS), functioning in maintaining homeostasis, immunosurveillance, and initiating immune responses upon infection. The role of microglia in β-CoV neuropathogenesis is poorly understood. While they are required for protection against lethal murine CoV encephalitis, microglia do not restrict SARS-CoV-2 infection in the K18-human ACE2 transgenic mouse model. Importantly, microglia contribute to neuroinflammation in mice infected with either murine CoVs or SARS-CoV-2. The innate immune sensors that determine how microglia influence host protection and disease progression in response to β-CoV infections remain ill-defined. Here, we systematically assess the role of Toll-like Receptor (TLR) adaptor proteins TIR-domain-containing adapter-inducing interferon-β (TRIF) and Myeloid differentiation primary response 88 (MYD88) in antiviral response and neuroinflammation following infection with MHV-A59 and -JHM in microglia. Surprisingly, murine CoV replication was drastically reduced in TLR - adaptor deficient microglia. This reduction was most prominent in cells lacking MyD88, with viral titers virtually undetectable upon MHV-JHM challenge. This inhibition correlated with significantly reduced double-stranded RNA intermediates and viral nucleocapsid protein expression. Moreover, TRIF was shown to negatively regulate several pro-inflammatory molecules, such as IL-6, MIP-1α, and MIP-1β during β-CoV infection. Our data highlight novel, unexpected roles of TLR adaptors as negative regulators of the antiviral response in β-CoV-infected microglia. This recontextualizes the often-assumed protective nature of TLR adaptors, as they may facilitate neuropathogenesis in a virus-specific manner.

P20

Empirically Identified Neuropsychological Impairment Subtypes in a Predominantly Black Cohort of People Living with HIV

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Introduction: HIV-associated neurocognitive disorders (HAND) have long been recognized as heterogeneous, and there is increasing support for the presence of multiple cognitive phenotypes among people living with HIV (PWH). Communities of color, who are disproportionately burdened by HIV, are underrepresented in this work. Thus, we used an empirical approach to identify HAND phenotypes and their correlates in a predominantly Black cohort of people with well-managed HIV. Methods: 301 adult PWH (ages 25-73, 88% Black, 98% CART-treated) in the Clinical and Translational Research Support

Core (CTRSC) Cohort of the Temple/Drexel Comprehensive NeuroHIV Center (CNHC) completed comprehensive neuropsychological assessments. Neuropsychological measures adjusted for age, sex, education, and race/ethnicity were entered in a latent class analysis (LCA). Demographic and clinical characteristics were compared across classes. Results: LCA identified five classes: intact cognition (12%), executive weakness (26%), mild motor slowing (18%), learning/memory impairment (26%), and global impairment (18%). Classes differed in education, antiretroviral history, and comorbidities. The executive weakness class had lower educational attainment, longer antiretroviral duration, and higher lifetime cocaine use. The motor slowing class had more delayed ART initiation, higher current depressive symptoms, and higher vascular risk burden. The memory impairment class had higher lifetime alcohol use. Finally, the globally impaired class had lower educational attainment, higher vascular risk burden, and greater functional difficulties. Conclusions: We identified five cognitive phenotypes with distinct clinical characteristics among PWH. Results are generally consistent with prior findings of motor, memory, and globally impaired HAND phenotypes. Notably, the executive weakness group is novel and highlights the importance of assessing higher-order, untimed executive functions. Cognitive phenotypes clarify the nature of and contributors to HAND and identify potential treatment targets, especially in the communities most affected by neuroHIV. Future work will include more Hispanic/Latino PWH and will examine biopsychosocial mechanisms and longitudinal outcomes of these HAND phenotypes.

P21

Subcortical Gray and Ventricular Atrophy in a Cohort with Well-Controlled HIV Defines Specific Biotypes

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Background: People with HIV (PWH) may develop neurocognitive dysfunction and brain atrophy despite adequate antiretroviral therapy. While restricted viral replication and systemic risk factors have been implicated, it remains unknown if they result in specific clinical and pathological biotypes. We used brain volumetrics and neurocognitive testing to identify Neuro-HIV biotypes. Methods: Participants in a natural history study underwent MRI and neuropsychological testing. Subcortical gray matter (SGM) and ventricular volumetrics were derived from FreeSurfer. Volumes were transformed into percentiles after adjustment for age, sex, skull size, and MRI scanner by a calculator developed by Potvin et al. Correlation coefficients between percentile and clinical variables were calculated. Logistic regression assessed effects of dichotomized clinical variables on binary percentiles. Results: Our cohort included 209 PWH (159 male, 50 female) and 64 controls (34 male, 30 female). Subsets with the worst 20th percentile of SGM or ventricular atrophy were identified. There were no demographic differences between individuals within the SGM atrophy subset and the rest of the cohort. For PWH with SGM atrophy, correlations controlled for age, sex, and IQ showed associations between SGM percentile and overall T-score (r=0.41, p=0.008), executive (r=0.43, p=0.004), learning (r=0.37, p=0.015), memory (r=0.38, p=0.014), motor (r=0.36, p=0.021), and global deficit score (r=-0.37, p=0.015). SGM percentile also correlated with total cholesterol (r=-0.44, p=0.014). For ventricular measurements, a significantly higher proportion of females had atrophy, so results were stratified by sex. Males with HIV and ventricular atrophy were associated with performing >0.5 standard deviations below the cohort average for speed of information processing (OR=2.46, CI=1.16-5.22, p=0.018) and motor (OR=3.48, CI=1.62-7.56, p=0.001). Discussion: PWH who have brain atrophy

can be grouped into those with SGM or ventricular atrophy. These groups are associated with distinct risk factors and neurocognitive deficits.

P22

Coronavirus infection in chemosensory cells

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Clinical manifestation of human coronaviruses (HCoVs) related diseases are mostly related to the respiratory system, although secondary complications such as headache, anosmia, ageusia and myalgia, have been reported. There are limited studies exploring human coronaviruses (HCoVs) infection and replication in chemosensory cells associated with ageusia and anosmia. Here we characterized HCoV-OC43 and SARS-CoV-2 infection in two types of chemosensory cells, olfactory and taste cells, with their unique molecular and histological characteristics. We first assessed HCoV-OC43 infection in in vitro cultured human olfactory epithelial cells (hOECs) and fungiform taste papilla (HBO) cells. Interestingly, while both cell types were susceptible to HCoV-OC43 infection, viral replication rates were significantly reduced in HBO cells compared to hOECs. More interestingly, while culture media from hOECs cells was able to produce secondary infection in Vero cells, there was very limited secondary infection from HBO cells, suggesting that HBO cells were not able to release infectious virus. on the other hand, unlike HCoV-OC43, SARS-CoV-2 showed comparable levels of viral infection rates in both hOECs and HBO cells. Furthermore, our RT-qPCR based gene array studies revealed that several key genes involved in taste and olfactory functions were significantly altered by SARS-CoV-2 infection. There results may suggest a possible mechanism associated with chemosensory symptoms, such as anosmia and ageusia in patients infected with SARS-CoV-2.

P23

Regulation of OPRM1 pre-mRNA splicing by HIV-1 and morphine

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Opioids, such as morphine, activate the mu opioid receptor (MOR) encoded by Opioid Receptor Mu 1 (OPRM1) gene. The human OPRM1 pre-mRNA undergoes extensive alternative splicing events and capable of expressing more than two dozen of isoforms. However, characterization of OPRM1 signaling is generalized, and only one isoform (MOR-1) has been referred in most studied. Here, we investigated the molecular impact of morphine and HIV-1 on regulation of OPRM1 pre-mRNA splicing in in vitro and in vivo models. Our results suggested that morphine treatment specifically induces the alternative splicing of MOR-1X isoform among the other isoforms analyzed in neuronal cells. Interestingly, alternative splicing and expression of MOR-1X isoform was also induced in postmortem brain tissues obtained from people with HIV (PWH). Additionally, treatment of control rats with morphine induced alternative splicing of MOR-1X in the brain regions involved in the reward pathways. More interestingly, HIV-1 transgenic (HIV-1Tg) rats, showed an additive induction of MOR-1X isoform with the exposure to morphine. To further assess the possible role of HIV secretory proteins in alternative splicing of OPRM1 gene, we analyzed the impact of HIV-1 Tat, gp120 and Nef proteins on alternative splicing of MOR-1X isoform. While the Tat and gp120 had no visible effects, treatment of neurons with Nef induced MOR-1X alternative splicing that was comparable to treatment with morphine. Moreover, expression of MOR-1X isoform in HEK cells revealed an altered cellular signaling mediated by this isoform compared to MOR-1. Altogether, our results

suggest that HIV-1 may alter MOR isoform expression by amplifying the rate of MOR-1X alternative splicing induced by morphine.

P24

Single cell gene expression profiling in CSF of patients with neurological symptom of the postacute sequelae of SARS-CoV2 infection

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Since the onset of the coronavirus disease 2019 (COVID-19) pandemic, there have been reports of patients experiencing a wide variety of health problems experienced four or more weeks after initial coronavirus infection that is referred to as postacute sequelae of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (PASC). Neurologic manifestations associated with COVID-19 have also emerged such as fatigue and cognitive impairment, however the pathogenic mechanisms associated with disease development remains unknown. Since various acute and chronic viral infection may cause immunological alterations such as chronic activation and infiltration of inflammatory cells into the central nervous system (CNS) that underlie the pathogenesis of these neurologic disorders, it is important to determine if immunological 'signatures' can be defined that reflect events in the CNS of patients with neurologic symptoms of PASC. In this study, we examined the single cell gene expression profiling by using single cell RNA sequencing platform from cells of cerebrospinal fluid (CSF) and peripheral blood mononuclear cells of subjects with post-COVID-19 neurologic syndrome (post-COVID: n=6) compared to healthy normal donors (ND: n=5). Analysis of lymphocyte distribution, the post-COVID group showed an increased ratio of subsets of CD4+ T cells including cytotoxic T cells and regulatory T cells in the CSF, compared to ND group. In addition, an increase of B cell plasmablasts was detected in the CSF of a subset of the post-COVID group, which was also confirmed by flow cytometric analysis. The single cell gene expression analysis showed different gene expression patterns in CSF CD4+ T cells including memory and regulatory CD4+ T cells, compared to ND group. These results demonstrated that patients with neurologic symptoms of PASC may have chronic immune dysregulation consistent with what is observed in other virus-associated neuroinflammatory diseases. These studies also highlight the importance of single cell immune profiling in CSF.

P25

HIV-1 Vpr induces caspase-dependent GSDME activation and pyroptosis in human neurons

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The inflammasome-pyroptosis axis (IPA) is a molecular pathway involving activation of inflammasomes and the gasdermin family of proteins, resulting in inflammatory regulated cell death, termed pyroptosis. We have previously reported that exposure to HIV-1 Viral Protein R (Vpr) induces the IPA in microglia and contributes to the pathogenesis of HIV-associated neurocognitive disorder (HAND). Given that Vpr induces cell death in neurons, we hypothesized that Vpr-mediated neuronal death involved the IPA. We

exposed human neurons to recombinant Vpr and observed features consistent with pyroptosis, including induction of caspase-1 cleavage, SYTOX green uptake, and LDH release. In parallel, beta-III-tubulin (a marker of neuronal health) was degraded following Vpr exposure. Additionally, apoptotic caspase-3 and its pyroptotic substrate gasdermin E (GSDME) were activated in neurons following exposure to Vpr. Confocal microscopy indicated GSDME accumulated at the cell membrane following Vpr exposure, suggesting it mediated lytic cell death. Pre-treatment of neurons with the caspase-1 inhibitor VX-765 prevented Vpr-mediated death, and reduced activation of caspase-3 and GSDME, suggesting that caspase-1 is an apical caspase in the pathway. To validate these in vitro findings, we examined brain tissue from HAND patients. GSDME and caspase-1 were upregulated in HAND brains, while beta-III-tubulin was diminished. Additionally, co-localization of active-caspase-3 and GSDME immunoreactivity was detected in neurons, indicative of GSDME-dependent pyroptosis. Collectively, these data indicate Vpr exposure activates a caspase-1-caspase-3-GSDME pathway that leads to neuronal pyroptosis. Our findings reveal a novel pathway of neuronal injury and death, which contributes to HAND pathogenesis and represents potential therapeutic targets for treatment of HAND.

P26

HIV-1 induced neuropathogenesis reflected neurocognitive dysfunction and mood disorders in the novel humanized microglial mouse model

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Effective antiretroviral treatment has lessened the severity but not the frequency of Human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND), ranging from elevated levels of anxiety and depression to deficits in learning and memory. A relevant animal model is needed to better understand the HIV brain infection and molecular mechanisms of HAND. However, HIV-1 specificity to human cells precludes the use of animal models. We recently developed a novel humanized glial mouse reconstituted with both the human immune system and microglia to understand the molecular mechanisms and brain infection of HAND. HIV infection in humanized microglial mice mirrored the neuropathology of human disease, such as the presence of multinucleated giant cells, astrogliosis, and myelin and synapto-dendritic loss. Reinforcement of the model for the studies of neuroHIV also requires the display of behavioral deficits as seen in humans. To these ends, the deficits in memory and learning and mood disorders like anxiety and depression were analyzed using the novel object recognition (NOR), elevated plus-maze (EPM), open field test (OFT), and sucrose anhedonia test. HIV-infected mice revealed memory deficits with a significantly lower recognition index of the novel object in NOR. EPM showed memory deficits with higher latency to enter the closed arm after repeated trials, and elevated levels of anxiety indicated by lower bouts of head dips. OFT displayed higher anxiety levels and a slower rate of habituation as suggested by a lower percent change in stereotypical grooming and less time spent in the center. A significant level of depression with lower sucrose preference due to anhedonia was evident. Transcriptomic analysis of HIV-infected mouse brains revealed glial activation and pro-inflammatory response to viral infection. Thus, the humanized glial mouse enables studies of neuroHIV in presence of HIV infection.

P27

SARS-CoV2 infected mice display signs of neurological sequelae

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Increasing epidemiological evidence indicates that ~80% of individuals infected with SARS-CoV2 develop Covid-19 and experience neurological sequelae characterized by etiological factors including cognitive and neuropsychiatric deficits. Biological factors intertwined with the overactivity of the CNS inflammatory response during Covid, impact the functional status of the CNS. For example, the bidirectional relationship between CNS inflammation and dysregulation of the kynurenine (KYN) pathway (KP), and its metabolites, such as kynurenic acid (KYNA) and quinolinic acid (QUIN) in Covid patients is triggered by inflammation-induced indoleamine 2,3-dioxygenase (IDO). This indicates that dysregulation of critical KP metabolites and CNS inflammation by SARs-CoV2 may underlie maladaptive changes in the CNS. Therefore, it is critical to elucidate the interplay between SARS-CoV2-induced KP dysregulation, associated networks, and CNS inflammation during Long-Covid. We tested the hypothesis that mice infected with SARS-CoV2 will develop clinical signs and Covid related neurological outcomes like those observed in Covid patients. Our data demonstrates that mice inoculated with mouse adapted SARs-CoV2 develop Covid related neurological sequelae at one-month post-infection. We observed and quantified changes in encephalitis-like behavior, alterations in motor activity, and in learning and memory, that was different between sexes. In parallel, we found that the neurological outcomes to be associated with increased levels of neuroinflammation and dysregulation of KP metabolites. We expect that mice surviving 12 months post-infection to develop an exacerbated neurological phenotype that is correlated to KP dysregulation and CNS inflammation.

P28

The Role of Dissociable Neuromorphometric Profiles in Adults Living with HIV

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INTRODUCTION. People living with HIV (PLWH), despite having achieved viral suppression via combination antiretroviral therapy, remain at greater risk than their uninfected peers for developing cognitive impairment. We conducted a cross-sectional investigation of neuromorphometric changes associated with HIV-disease and HIV-associated neurocognitive disorders (HAND), respectively. METHODS. High-resolution structural magnetic resonance images were acquired from 104 PLWH (mean age = 56.2, 26% female at birth, 64% Black) and 46 HIV-uninfected controls (mean age = 57.3, 33% female at birth, 52% Black) using a 3-Tesla Siemens Magnetom Trio equipped with a 12-channel head coil (n = 88) or a 3-Tesla Siemens Prisma-Fit scanner and 20-channel coil (n = 62). Participants were administered a comprehensive set of neuropsychological tests for the determination of HAND diagnoses using Frascati criteria. Scans were preprocessed using fMRIPrep 20.2.6, and whole-brain voxel based morphometry analysis was performed using the CAT12 toolbox in SPM. Additional vertex-wise estimates of gray matter volume (GMv) and cortical thickness (CT) were determined via surface-based analysis in FreeSurfer v6.0, and subsequent region-of-interest (ROI) analyses were performed using general linear models in R/RStudio. RESULTS. Voxelwise analyses revealed trends of cortical atrophy among PLWH in frontal, temporal, and cerebellar cortex. Surface and ROI analyses showed additional evidence for frontal atrophy in PLWH irrespective of global impairment. Lower CD4+ T lymphocyte nadir corresponded to lower GMv and CT in left caudal ACC and right temporal pole among PLWH, and cognitive impairment among PLWH was

associated with decreased right cerebellar white matter volume at p < .001. DISCUSSION. Despite the heterogeneous nature of the studied population, there is mounting evidence for a distinguishable neuromorphometric profile linking chronic infection to cognitive decline. These findings have implications for the establishment of a noninvasive biomarker for HAND among PLWH. Future studies should further investigate the relationship between cerebellar atrophy and HAND.

P29

Impact of CD4 Nadir on Neurocognitive Trajectories within People Living with HIV

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Introduction: Human immunodeficiency virus type (HIV) type 1 (HIV-1)-associated neurocognitive disorders (HAND) persist in the combination antiretroviral therapy (cART) era. CD4 nadir is a well-established predictor of cognition cross-sectionally, but its impact on longitudinal neurocognitive (NC) trajectories is unclear. The few studies on this topic examined trajectories of global cognition, rather than specific NC domains. The current study examined CD4 nadir in relation to domain-specific NC decline. Methods: HIV+ adults (n=132; 328 total visits; ages 25-68; 38% CD4 nadir <200; 92% undetectable viral load, 98% on cART, current mean CD4=686, mean time post-nadir=6.1 years) from the Temple/Drexel Comprehensive NeuroHIV Center (CNHC), Clinical and Translational Research Support Core (CTRSC) Cohort were administered comprehensive NC assessments longitudinally (average follow-up time=4.9 years). Principal component analysis (PCA) was utilized to determine NC domains. Longitudinal NC trajectories were examined using linear mixed modeling. Results: PCA found three NC domains: (1) motor speed/executive function, (2) visuoconstruction/visuospatial memory, and (3) verbal fluency. CD4 nadir was not associated with change in motor speed/executive function or visuoconstruction/visuospatial memory, but showed an association with change in verbal fluency (p=.015). Specifically, those with CD4 nadir <200 demonstrated increasing verbal fluency over time (p=.003), whereas those with CD4 nadir >200 demonstrated stable verbal fluency (p=.69). Current CD4 improved significantly in both groups (p<.001). Conclusion: While low CD4 nadir has been associated with weaker neurocognition among people living with HIV, the results of this study suggest that low CD4 nadir does not lead to ongoing cognitive decline. Furthermore, verbal fluency may improve over time in those with lower CD4 nadir, possibly reflecting the beneficial cognitive effects of long-term treatment and immune reconstitution. These findings can provide reassurance to individuals who have experienced low CD4 nadir and are fearful about disease progression. Future studies should incorporate brain structure trajectories in relation to CD4 counts.

P30

PINCH post-translational modifications in glioma progression, and chemotherapeutic sensitivity in GBM depends on p53 genotype

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Gliomas are the most common adult CNS tumors and despite treatment efforts including surgery, radiation, and chemotherapy, essentially all low-grade gliomas progress to glioblastoma multiforme (GBM). Surgical resection is usually not possible and the recurrence rate of GBM is almost 100% after 9 months with a

median survival of approximately 15 months due to its high capacity for invasion and resistance to therapy. Thus, improved therapeutic approaches are needed to decrease recurrence and prevent progression of astrocytoma into GBM. Gliomas with IDH1/2 mutation have also been reported to accumulate Tau protein in higher proportion than wild type IDH and to increase Tau expression with tumoral malignancy in IDH mutated cases, thus TAU could be used as a biomarker for glioma progression. The PINCH protein is expressed in the mature CNS in many neurodegenerative pathologies such as Alzheimer, Parkinson Disease, HIV infection and in gliomas. PINCH-mediated signaling involves cell migration, spreading, and survival pathways which are all critical events in cancer progression. In fact, increased PINCH expression is related to poor prognosis in colorectal, pancreatic and breast cancer. Our new data link PINCH expression with the anti-oncogenic gene p53, suggesting that the two proteins work in concert in progression to glioblastoma (GBM). p53 is mutated in approximately 80% of gliomas leading to p53 pathway deregulation that contributes to chemotherapy resistance. Our data show that PINCH is dramatically increased in brain cancers as a function of grade of malignancy. We also observed a PINCH post-translational modification linked to p53 mutation in glioma cell lines. Since PINCH expression levels and post-translational modifications are linked to vulnerability of GBM cells to therapeutic intervention, findings from these studies will provide valuable data for potential adjuvant therapies for GBM and possibly other cancers.

P31

Probing HIV, anti-retroviral drug, and nicotine interactions via CEST-MRI

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Background The Chemical Exchange Saturation Transfer (CEST) effect occurs when certain compounds to exchange proton saturation with water molecules. Because Magnetic Resonance Imaging (MRI) observes water protons throughout the body, researchers can use these concepts in tandem (CEST-MRI). Through CEST-MRI, researchers can probe the body in vivo for compounds of interest (e.g. metabolites, drugs) present in previously unobservable locations/concentrations. This is accomplished via indirect observation, saturating compounds at their characteristic chemical offsets ($\Delta\omega$) and observing the associated attenuation of water signal. One interesting application of CEST-MRI occurs while imaging of anti-retroviral drugs (ARVs), which constitute modern treatment plans for the human immunodeficiency virus (HIV). Using CEST-compatible ARVs (e.g. 3TC, with liable hydroxyl and amino protons), imaging and treating HIV progression are on track to become one and the same. Despite the successes of ARVs, combinational anti-retroviral therapy (cART) constitutes treatment, not a cure. Because HIV can hide from ARVs behind the blood-brain barrier (BBB), patients still deal with symptoms, including HIV-associated neurological diseases (HAND) and neuroinflammatory ARV side-effects. To increase patient quality of life, we must fine-tune cART, continually assessing the efficacy of treatment and identifying complicating factors. Tobacco usage may be one complicating factor, modulating drug efficacy in a regime requiring consistency: nicotine modulates metabolic absorption of ARVs, altering pharmokinetics (PK), while ARVs modulate metabolic absorption of nicotine. Goal: Use CEST to study/track HIV-associated metabolites and investigate HIV-ARV- nicotine interactions. Aim 1: Use CEST-MRI to observe ARV-nicotine effect on glutamate and myoinositol to guide the design of drug delivery modalities. Hypothesis: Glutamate and myoinositol can be measured with CEST in vivo to reflect HIV-ARV-nicotine interactions and reflect HIV-ARV-nicotine induced neuropathologies. Aim 2: ART/Nicotine metabolites will be acquired in vivo via CEST-MRI for infected mice to study possible interactions. Hypothesis: Nicotine-ARV mice will have different HIV suppression than ARV-only mice.

P32

Toll-Like Receptors Dictate Macrophage Responses to Beta-coronavirus Infection

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The emergence of SARS-CoV-2 as a highly pathogenic beta-coronavirus (β-CoV) highlights the critical need to investigate cell-type specific responses to infection. Among the three pathogenic β-CoVs identified since 2002, only SARS-CoV-2 is frequently associated with central nervous system (CNS) involvement, but without sound data on neuroinvasiveness. Murine CoVs are the prototype of the β-CoV genus and provide experimental models of viral neuropathogenesis, with lung and liver involvement, depending on the virus strain. Remarkably, β-CoV-induced immunopathology in lung and brain correlates with exacerbated, non-protective, monocyte/macrophage infiltration in both humans and mice. Macrophages may play important roles in antiviral defense, but also likely contribute to pathogenesis. A current gap in knowledge is to determine macrophage's innate immune sensors and pathways that sense β-CoV infection. Here, we investigated the role of toll-like receptors (TLRs) and their adaptors TIR-domain-containing adapter-inducing interferon-β (TRIF) and myeloid differentiation factor 88 (MyD88) in response to two neurotropic murine β-CoV strains: the highly neurovirulent MHV-JHM and the neuroattenuated MHV-A59. We infected immunocompetent (wild-type) and immune deficient (lacking either TLRs or their adaptors) macrophages with MHV-JHM and -A59 strains. Virus production and cytokine/chemokine secretion were determined using plaque assays and enzyme-linked immunosorbent assays (ELISAs), respectively. Activation of intracellular signaling pathways was evaluated via infrared immunoblot analysis. Viral replication was severely impaired in the absence of TRIF and TLR2, suggesting their involvement as negative regulators of the anti-viral response. Mechanistically, we identified upregulation in expression of the cytoplasmic RNA sensor melanoma differentiation-associated protein 5 (MDA5) in the absence of TRIF or TLR2. Interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and interleukin -1β (IL-1β) secretion was dependent on the TLR2/MyD88 pathway. Understanding the mechanisms through which these receptors and adaptors function during β-CoV infection will prove crucial for developing novel antivirals to limit complications associated with macrophage-driven CoV immunopathology, including COVID-19.

P33

Broad-spectrum selected molecular gRNA target (SMRT) guide RNAs demonstrate efficacy in latent HIV-1-infected cells

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CRISPR/Cas9 gene-editing has emerged as a potential therapeutic strategy to achieve an HIV-1 cure. Using an in silico prediction algorithm, a set of broad-spectrum selected molecular gRNA target (SMRT) gRNAs were designed that were predicted to cleave 100 percent of patient-derived PBMC LTR sequences. A novel fluorescence system including the NL4-3 HIV-1 molecular clone that encodes GFP and a Cas9 expression system that encodes RFP was used with the aforementioned custom anti-HIV-1 SMRT gRNAs. Results from these experiments indicated that when the Cas9 system was active, GFP expression could be reduced by as much as 98 percent. Fluorescence microscopy and flow cytometry results indicated that gRNAs targeting the more conserved TAR region of the LTR yielded the greatest reduction in HIV-1 gene expression. Furthermore, a highly sensitive beta-galactosidase system in TZM-bl cells indicated that at least

97 percent of LTR-driven gene expression was reduced when multiple SMRT gRNAs were used. Finally, the latently infected T cell line, J-Lat 10.6, was transduced with a lentiviral vector encoding Cas9 and a SMRT gRNA to more accurately model latency. These results indicated that a TAR-targeting gRNA, SMRT1, and a Tat-targeting gRNA, TatA, were the most effective at preventing J-Lat 10.6 cells from reactivating from latency. These results have demonstrated that our custom SMRT gRNAs possess broad-spectrum cleavage activity and could contribute to HIV-1 treatment strategies or possibly even a cure at some point in the future. Future studies will examine the off-target effects of these broad-spectrum gRNAs in vitro and in vivo using GUIDE-Seq.

P34

Chronic cocaine exposure aggravates the learning and memory deficits in the long-term HIV Tat-expressing mice involving genome-wide alterations of DNA methylation and gene expression in hippocampus

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HIV infection of the central nervous system causes HIV-associated neurocognitive disorder (HAND) in up to 50% HIV-infected individuals. Cocaine use facilitates the HAND progression. HIV infection and cocaine use both alter epigenetic DNA methylation. However, it is unknown whether the impact of cocaine use on HAND involves changes of epigenetic DNA methylation. In this study, we first took advantage of the doxycycline inducible and brain-specific HIV Tat transgenic mouse model (iTat) of HAND and characterized effects of chronic cocaine exposure and long-term Tat expression on HAND-associated neurology and neuropathology. We found that cocaine exposure worsened the learning and memory of iTat mice, accompanied by dendritic spine swelling, increased synaptophysin expression, and diminished microglia and astrocyte activation. These effects were more pronounced in female and ageing mice. We then employed single-base resolution whole genome bisulfate sequencing and RNA sequencing and identified 14,838 hypermethylated CpG-related differentially methylated regions (DMR) and 15,800 hypomethylated CpG-related DMR that were linked to 52 down- and 127 up-regulated genes by cocaine and Tat in hippocampus. We further uncovered these genes to be mostly enriched at the neuronal function- and cell morphology- and synapse formation-related ECM-receptor interaction pathway, and to be linked to behavioral and pathological changes altered by cocaine and Tat. Eight mostly affected genes included four in microglia Ift172, Eif2ak4, Pik3c2a, and Phf8, two in astrocytes Garem1 and Adgrb3, and two in neurons Dcun1d4 and Adgrb3. These findings demonstrated for the first time that chronic cocaine use and long-term Tat expression interactively contributed to HAND neurology and neuropathology through genome-wide changes of DNA methylation and gene expression and suggest that targeting epigenetic changes could potentially serve as a new therapeutic strategy to treat cocaine use disorder in people living with HAND.

P35

Assessment of antiretroviral toxicity in primary myeloid cells

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HIV antiretrovirals (ARVs) have increased the life span of people living with HIV (PLWH) to one that approaches that of their seronegative counterparts. However, the toxicities associated with chronic use of ARVs also contribute to HIV related co-morbidities such as heart disease, bone loss and HIV associated neurocognitive disorders (HAND). These comorbidities result in an overall lower quality of life in PLWH compared to sero-negative individuals. We assessed here the effects of cART, independent of HIV, on primary human monocyte-derived macrophages (MDMs). We report that Triumeq (abacavir/dolutegravir/lamivudine) skewed alternative MDMs toward an inflammatory nonsenescent

phenotype. Both Atripla (efavirenz/emtricitabine/tenofovir) and Triumeq caused mitochondrial dysfunction, specifically efavirenz and abacavir. Additionally, transcriptome sequencing (RNA-seq) demonstrated that both Atripla and Triumeq caused differential regulation of genes involved in immune regulation and cell cycle and DNA repair. To establish an in vivo model, we orally administered combination Atripla and Triumeq to adult hu-PBMC-NSG mice and demonstrate via mass spectrometry that ARVs, can all be detected in the plasma, and all but lamivudine and emtricitabine were detected in the brain, spleen, kidneys, heart, lungs, liver, bone tissue, proximal and distal colon, and cervical lymph nodes. Further, Triumeq reduced viral load achieving up to 100% inhibition of vRNA in tissues from several mice. Finally, while both vRNA and vDNA were reduced, the ratio varied in different tissues where the distal colon, spleen and kidneys had more vDNA relative to vRNA suggesting more latently infected cells than productively infected cells. Together these studies demonstrate ARVs impact MDMs, independent of HIV, and suggest that efficacy of ARVs tissue penetration is associated with breath of HIV reservoir /latency

P36

HIV-1 Infection: virus and host wrestle for control of the SUMOylation system

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The conjugation of small ubiquitin-like modifier (SUMO) proteins to substrates is a well-described post-translational modification that regulates protein activity, subcellular localization, and protein-protein interactions for a variety of downstream activities. SUMO proteins are also important in anti-viral immunity, opposing viral replication and mediating interferon-dependent anti-viral mechanisms. Thus, pathogens have evolved to exploit the host SUMOylation machinery to ensure viral persistence and pathogenesis. During infection, the human immunodeficiency virus type 1 (HIV-1) manipulates the host SUMOylation machinery to ensure its viral replication in CD4+ T cells, however, whether HIV-1 manipulates the SUMO paralogs to control its replication and/or latency in glial cells like microglia is unclear. Microglia are the main HIV-1 target cells in the CNS and constitute an important reservoir for viral pathogenesis. In microglial cells, the co-repressor COUP-TF interacting protein 2 (CTIP2) recruits a multi-enzymatic chromatin-modifying complex and establishes a heterochromatic environment at the HIV-1 promoter, leading to HIV-1 silencing. Studies have shown that post-translational modifications (PTMs), including phosphorylation and SUMOylation, mediate CTIP2's interactions with other proteins and complexes. Similarly, tripartite motif-containing protein 28 (TRIM28), a known SUMO E3 ligase, associates with CTIP2 in the heterochromatin complex to inhibit HIV-1 viral replication. While SUMOylation and phosphorylation have been implicated in regulating the activity of CTIP2 and TRIM28 in the context of T-cell signaling events and immune responses, respectively, the impact of PTMs in the initiation and establishment of HIV-1 latency in microglia remains largely unknown. To this end, we hope to demonstrate the effects of HIV-1 infection on the host SUMOylation system, particularly when it comes to HIV latency in microglia .

P37

Transcriptional activity of brain-derived HIV long terminal repeat sequences from virally suppressed people with HIV

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The CNS is a reservoir of HIV. Recently we demonstrated that the HIV reservoir in the brain of virally suppressed people with HIV (PWH) consists of a pool of both intact and defective HIV proviruses. We and others have demonstrated that HIV long terminal repeats (LTR) isolated from the CNS of viremic PWH are functional and are distinct to those isolated from matched peripheral tissues of the same individual. Further, CNS-derived LTRs from viremic PWH demonstrated a lower basal transcriptional activity to lymphoid-derived LTRs. Despite the presence of intact potentially replication competent proviral genomes, whether the LTRs are functional and transcriptionally active is unclear. To determine if LTRs from the CNS of virally suppressed PWH are potentially functional, LTRs have been isolated from CNS tissue from virally suppressed PWH by single genome amplification. Transcription factor binding site and phylogenetic analyses were performed. While we have previously demonstrated clear compartmentalisation of sequences between the CNS and lymphoid compartments for viremic PWH, compartmentalisation was not observed in virally suppressed PWH. Additionally, sequence analysis of LTRs from the CNS of virally suppressed PWH have intact core promoter regions (TATA, Sp1, NF-κB) which differed from that observed in viremic PWH. LTRs isolated from virally suppressed PWH were inducible with HIV tat and PMA and current latency reversal agents. Characterisation of the in vivo activity of HIV brain-derived LTRs isolated above is ongoing. These findings support the presence of a potentially transcriptionally competent inducible pool of HIV in the brain of virally suppressed PWH. Therefore, understanding the function of HIV LTRs derived from intact proviral genomes in CNS may inform the role of the CNS reservoir in ongoing disease and as a barrier to HIV cure.

P38

Altering chromatin to improve CRISPR cleavage of HIV-1 provirus

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Even with effective therapy there is still no cure for human immunodeficiency virus type 1 (HIV-1), in part due to its inability to eliminate the latent viral reservoir that harbors integrated virus. CRISPR/Cas9 cleavage can excise or inactivate this reservoir by using gRNAs specifically targeting the transactivation response element (TAR) regions in the long terminal repeat (LTR) of the integrated provirus. However, no gRNAs have yet demonstrated 100% target cleavage. One explanation is that the TAR region is obstructed by a nucleosome (Nuc-1) in latently infected cells, which could prevent gRNAs from binding to target sequences. We hypothesize that CRISPR-Cas9 cleavage can be enhanced by loosening the chromatin architecture, making target sequences more accessible for gRNAs. We propose a strategy that utilizes sub-therapeutic doses of latency reversal agents (LRAs) as chromatin remodeling tools to relax chromatin and dissociate nucleosomes from the DNA sequences they obstruct. Previous analyses using integrative genomic data showed that DNA availability at CRISPR editing sites was significantly less than that required for endogenous gene expression. We showed that latency reversal using HDACis was dose-dependent in a latently infected T-cell model (J-Lat 10.6 cells). Beta-gal analysis revealed that a combination of a TAR-specific gRNA CRISPR treatment and low-dose histone deacetylase inhibitor (HDACi) significantly reduces LTR-driven transcription in TZM-bl cells. Genome-wide, Unbiased Identification of DSBs Enabled by Sequencing (GUIDE-Seq) analysis also showed altered gRNA on- and off-target edits depending on the mode of cell activation. Future studies will examine the effectiveness of this strategy in J-Lat 10.6 cells using different gRNA/HDACi combinations at increasing low-dose concentrations.

P39

Cocaine upregulates mitochondria-derived vesicular pathway for selective removal of oxidized cargo

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Mitochondria are dynamic organelles that have a central role in cellular metabolism. Our preliminary data show that astrocytes, when exposed to cocaine, increase the OXPHOS activity leading to generation of mitochondrial reactive oxygen species (mROS). Conversely, the increased generation of mROS was not accompanied by mitochondrial depolarization, mitophagy or cell death. Based on these observations, we perceived another mechanism of steady-state removal of oxidized mitochondrial protein complexes facilitated astrocytic activation, proliferation and survival and may disrupt the neuron-glia signaling, thereby contributing to synaptic impairment in the central nervous system during cocaine abuse. The current work focused on identifying the mitochondrial quality control mechanism in astrocytes responsible for cocaine-induced changes. Mitochondrial quality control is an essential process required to clear the accumulation of unfolded, oxidized or otherwise damaged proteins and lipids from the organelle. As the "energy powerhouse", mitochondria depend on several different pathways that continually survey for damage. Apart from the well-characterized pathways including constitutive mitochondrial proteolysis and mitophagy, we uncovered a novel mitochondrial quality control pathway, conserved from bacteria, in which mitochondria release small, mitochondrial-derived vesicles (MDVs). In this context, we observed rapid formation of MDVs in astrocytes exposed to cocaine, suggesting MDV formation as an essential housekeeping mechanism and a first line of defense against cocaine-induced toxicity in astrocytes.

P40

HIV-1 Tat-mediated microglial ferroptosis involves the ACSL4/miR204-5p signaling axis

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Despite the efficacy of combination antiretroviral therapy (cART) in controlling viremia, CNS continues to harbor viral reservoirs. The persistence of low-level virus replication in the CNS leads to the accumulation of early viral protein such as HIV-1 Tat (Transactivator of transcription. In this study, we sought to examine a novel cell death pathway – ferroptosis, in Tat-exposed microglia. Our results showed that exposure of mouse primary microglial cells to HIV-1 Tat resulted in an induction of ferroptosis, which was characterized by increased levels of acyl-CoA synthetase 4 (ACSL4), oxidized phosphatidylethanolamine, lipid peroxidation, the labile pool of iron, ferritin heavy chain-1, mitochondrial outer membrane rupture, elevated proinflammatory cytokines and a concomitant decrease in glutathione peroxidase-4 levels. Both pharmacological inhibition (ferrostatin-1 and deferoxamine) and gene silencing approaches using ACSL4 siRNA further validated the critical role of ferroptosis in HIV-1 Tat-mediated microglial activation and neuroinflammation. Bioinformatics analyses identified miR-204 as an upstream modulator of ACSL4, which was further confirmed using argonaute immunoprecipitation. These in vitro findings were also validated in the prefrontal cortices, striatum, and hippocampus of HIV-1 transgenic rats. Overall, this study underscores a novel role of the miR-204/ACSL4 axis in HIV-1 Tat-mediated ferroptosis underlying microglial activation and neuroinflammation.

P41

Amyloid-beta (Aβ) and HIV cooperate in facilitating HIV neurocognitive impairment and Alzheimer's Disease in mouse models

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Antiretroviral therapy (ART) controls HIV replication and extends patients' lives. Nevertheless, half of HIV patients on ART suffer chronic neurocognitive impairments (HIV-NCI). HIV-NCI severity increases with age overlapping Alzheimer's Disease (AD) and raising possible risk of co-pathogenic interactions during HIV-NCI and AD progression. We investigated these interactions in mice using mouse-tropic HIV (EcoHIV) that causes NCI; exogenous oligomeric Aβ; transgenic (Tg) mice expressing human APP and PS1 genes with familial AD-linked mutations (TgAPP/PS1); and C57BL/6 mice expressing humanized native Aβ (B6JhAbeta), a model for late-onset AD (LOAD). Aβ-HIV co-pathogenesis was tested by intranasal delivery of subtoxic doses of oligomeric Aβ (oAβ) to acutely EcoHIV infected C57BL and in EcoHIV infected pre-symptomatic TgAPP/PS1 and LOAD mice. We tested EcoHIV infection by QPCR for viral DNA and RNA in periphery and brain; NCI in radial arm water maze and fear conditioning; hippocampal LTP dysfunction by electrophysiology ex vivo; amyloid levels by ELISA, dot blots, and immunohistochemistry; apoptosis by TUNEL, brain gene dysregulation by RNAseq; and brain protein expression by Western blot. Subtoxic oAβ caused memory dysfunction in acutely EcoHIV-infected compared to untreated infected or oAβ treated uninfected mice. Subtoxic oAβ but not PBS added to hippocampus slices from acutely EcoHIV infected asymptomatic mice caused LTP dysfunction. Acute EcoHIV infection of young, normally asymptomatic TgAPP/PS1 mice facilitated hippocampal LTP dysfunction and neuronal apoptosis, memory deficits, brain gene dysregulation, and increased amyloid compared to uninfected mice. Chronic infection of young LOAD mice with EcoHIV caused hippocampal LTP and memory dysfunction by 6 months of age compared to same-age uninfected control LOAD. Our data suggest that factors involved in HIV-NCI and AD progression, when present in the same organism, functionally interact to accelerate and worsen brain disease. These results are relevant to aging HIV patients on ART and to HIV patients at risk for AD.

P42

Assessment of integrated HIV-1 proviral DNA defectiveness in the brain

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Human immunodeficiency virus type 1 (HIV-1) virus forms a stable but transcriptionally silent viral reservoir in regions of the brain. However, the nature of these integrated proviruses are poorly understood as many studies only examined a small collection of sequences from individuals. To this end, a collection of brain sections and matched spleen samples were selected from the National NeuroAIDS Tissues

Consortium (NNTC). After genomic DNA isolation, they were subjected to long amplification utilizing a near-full length (NFL) amplification strategy that completely encompasses all genes in the HIV genome. The amplicons were sequenced using the third-generation sequencing (TGS) nanopore sequencer generating thousands of reads per sample. These reads were cleaned utilizing Quasipore, a new deep learning basecaller specifically trained for HIV sequence analysis generating reads with fewer than 1 error per 10kb. Finally, the high-quality reads were examined using BamSlicer, an in-development tool for exploring reads at the single molecule level. This examination revealed a spectrum of mutations from single base changes to large deletions. Future work will explore how these mutations impact the function of the virus and relate to neurocognitive impairment.

P43

Characteristics of asymptomatic neuroinvasive coronavirus infection in outbred and inbred mice

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We are developing a model to investigate the potential long-term neurological complications resulting from mild/asymptomatic COVID-19 in young children, employing OC43 infection in mice as a surrogate system. Human coronavirus OC43 is similar to SARS-CoV-2, the causative agent of COVID-19, in both its genetics and disease pathogenesis, but it can be handled in the more widely accessible biosafety level two facilities. Thus, we infected young inbred C57BL/6 and outbred CD-1 mice with low doses of OC43 to model mild/asymptomatic neuroinvasive coronavirus infection. Eight cohorts of male and female mice were inoculated intranasally at postnatal day 19 with 3x105 TCID50 of OC43 and observed for disease symptoms, including lethargy, hunched posture, fur quality, and weight change for up to nine days. No symptoms were observed in either mouse strain, and there was no significant impact on weight. At 4- and 9-days post-infection (dpi), mice were euthanized, brains were removed and dissected by region for RNA extraction, and viral RNA (vRNA) for the nucleocapsid gene was quantified by RT-qPCR. There was evidence of neuroinvasion through the olfactory system (olfactory bulb, olfactory tract, piriform cortex) and hippocampus. The time course of neuroinvasion differed by sex and mouse strain. Males of both strains showed comparable levels of vRNA at 4 and 9 dpi in the olfactory bulb (OB), but male CD-1 mice had significantly more vRNA in the olfactory tract (OT) overall. Female CD-1 mice had less vRNA in the OB at 9 than 4 dpi, indicating clearance that was not observed in C57 mice. We have demonstrated neuroinvasion by a coronavirus in an asymptomatic context in both inbred and outbred mice, providing a tool to model and study long-term complications of asymptomatic/mild COVID-19. Next, we will use immunostaining to establish a more detailed timeline and tropism of neuroinvasion in our mouse models.

P44

CNS-derived IgG from HIV infected individuals contains a distinct glycosylation signature that enhances antibody penetration of the blood brain barrier: relevance for HIV cure strategies

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The CNS is a latent reservoir for HIV in the era of combination anti-retroviral therapy(cART). Despite some success in targeting reactivated reservoirs with broadly neutralizing antibodies following cART cessation, only ~0.1% of antibodies reach the CNS due to the blood brain barrier(BBB), thus decreasing the efficacy of antibody (Ab)-mediated therapeutics targeting this reservoir. A better understanding of how to target Abs to the CNS is therefore needed. One potential strategy is to delineate the composition of

antibody Fc glycosylation in the CNS and reverse engineer anti-HIV IgGs to mimic these properties to increase delivery to this compartment. In conjunction with the National Neuro-AIDS Tissue Consortium (NNTC), we acquired eight matched plasma and CSF samples from HIV infected individuals and isolated IgGs. We characterized the glycosylation pattern of these Abs and found that there was a statistically significant decrease in IgG sialylation and trends towards lower fucose in CSF-derived IgG compared to plasma-derived IgGs. This finding was confirmed by lectin blotting and mass spectrometry. To assess the efficacy of CNS penetration of Abs, we first biochemically and genetically manipulated monoclonal antibodies to have less sialylation and less fucose, respectively, labeled them with Cy5, and modeled their ability to penetrate the CNS using an in vitro BBB model. We found increased BBB entry of these Abs compared to those that were unmodified. We next tested the original NNTC-derived CSF IgG with a decrease in sialylation and fucose (n=7) and found that 6/7 of these antibodies displayed an increased entry when compared to plasma derived IgGs. Together, our studies demonstrate that a distinct CNS-derived IgG glycosylation profile allows for increased BBB penetration or retention, suggesting that bioengineering anti-HIV specific Abs with lower sialylation/low fucose glycosylation pattern can enhance Ab presence in the CNS and is a viable option in therapy targeting the CNS.

P45

Induction of neuroinflammatory reactive astrocyte polarization in primary human cells

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In the context of neurodegenerative pathology, activated M1 microglia promote neuroinflammation through secretion of pro-inflammatory mediators. Exposure to a specific array of microglia-secreted cytokines, TNF-α, IL-1α, and C1q, activates the NF-kappaB pathway and alters transcriptional activity of astrocytes, inducing conversion to a cytotoxic inflammatory "A1" reactive astrocyte phenotype. Non-reactive astrocytes are essential for neuronal survival, synaptic support, and blood-brain barrier functional integrity. Upregulation of pro-inflammatory gene expression, downregulation of neurotrophic and synaptogenic factors, and secretion of neurotoxic signals by A1 or A1-like astrocytes positions them as a potential contributor to various pathologies. The consequences of A1 polarization on sustaining barrier function has yet to be fully examined, but may have etiological implications for diseases associated with impaired barrier permeability. Frequently identified by upregulation of complement component 3 (C3), A1-like astrocytes have recently been characterized in neurodegenerative disorders like Alzheimer's disease and multiple sclerosis (MS), with polarization correlating with disease progression and severity. To elucidate cellular and molecular consequences of neurotoxic astrocytes, and to investigate their relevance to neuroviral pathologies, an in vitro model is necessary. Primary human fetal astrocytes are more physiologically representative than cell lines; however, variations in fetal gene expression can present possible challenges. We found that primary human astrocytes treated with TNF-α, IL-1α, and C1q, at both single and repeated dosages, undergo an A1-like phenotypic polarization that can be identified by upregulated expression of C3 and its cleavage products. Further investigation using co-culture barrier models and treatment with pathogenic proteins may provide insights into the role of neurotoxic reactive astrocytes in neuroviral pathologies.

P46

Microglia-associated synaptodendritic damage in post-mortem HIV-1+ individuals with HAND

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Progressive synaptodendritic dysfunction, characterized by alterations in neuronal and dendritic spine morphology, has been recognized as one of the fundamental neural mechanisms underlying HIV-associated neurocognitive disorder (HAND). Microglia, which serve as a persistent HIV-1 viral reservoir in the brain, are critical for brain development and maturation, as well as synaptic plasticity. However, whether microglial activation and/or dysfunction underlie the profound synaptodendritic alterations observed in HAND has yet to be elucidated. To address this knowledge gap, measures of microglia proliferation and neuronal damage were evaluated in the dorsolateral prefrontal cortex (dlPFC) of nine post-mortem HIV-1 seropositive individuals with HAND. First, consistent with previous reports, HIV-1 mRNA expression was co-localized with the microglial marker, Iba1; observations which provide additional support for microglia as a viral reservoir for HIV-1. Fundamentally, microglia in the dlPFC were proliferated, as evidenced by co-localization of Iba1 and Ki67. Second, we critically tested the utility of the chimeric HIV (EcoHIV) to model the microglial proliferation observed in post-mortem HIV-1 seropositive individuals with HAND. Eight weeks after EcoHIV infection, pronounced microglial proliferation and synaptodendritic dysfunction was observed in EcoHIV, relative to control, rats. Regression analyses demonstrated the relationship between microglial proliferation and measures of synaptodendritic dysfunction, whereby two variables representing neuronal and dendritic spine morphology account for 68.6% of the variance in the number of co-localized Iba1 and Ki67 cells [$F(2,11)=9.8$, $p \leq 0.005$, $r=0.828$). Collectively, the proliferation and dysfunction, induced by chronic HIV-1 viral protein exposure, may underlie the profound synaptodendritic alterations in HIV-1. Understanding the neural mechanisms underlying HIV-1 associated synaptodendritic dysfunction affords a key target for the development of novel therapeutics for HAND. Funded by NIH Grants: DA013137, DA056288, MH106392, NS100624.

P47

Functional cure of HIV brain disease in chronically EcoHIV infected mice by therapeutic vaccination with gag-pol mosaic vaccine

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Introduction. Antiretroviral therapy in HIV infected people controls virus replication and partially restores immune competence but fails to prevent chronic HIV diseases including HIV-associated neurocognitive impairment. Therapeutic vaccination has been shown to improve anti-HIV immune responses but its effects upon chronic HIV complications are unknown. We tested this therapeutic approach in conventional mice infected by chimeric HIV, EcoHIV, that develop anti-HIV T cell responses, but like HIV infected people, can neither clear virus nor control HIV brain disease. Methods. To boost T cell responses in chronically EcoHIV-infected mice, we used mosaic DNA vaccines expressing widely conserved regions of HIV Gag and Pol, pSM2.HIVconsv1+ pSM2.HIVconsv2. EcoHIV burdens in T cells, macrophages, and microglia were determined by QPCR, anti-HIV T cell responses by Elispot to HIV Gag and Pol peptides, brain gene dysregulation by RNAseq, synaptodendritic damage by confocal microscopy, HIV localization in the brain by RNAscope, and cognitive function by radial arm water maze. Results. Chronically EcoHIV infected mice receiving control plasmid had anti-HIV T cell responses in periphery and brain, but they carried viral DNA and RNA in lymphocytes, macrophages, and microglia; showed broad dysregulation of genes

involved in immune, neuronal, and metabolic functions in striatum, showed reduced dendritic arbors in hippocampus and cortex, and exhibited impaired memory. Vaccination of cognitively impaired mice with pSM2.HIVconsv1+ pSM2.HIVconsv2 enhanced HIV-specific T cell responses, reduced HIV burdens systemically and in the brain including in isolated microglia, and reversed brain disease at the levels of gene dysregulation, synaptodendritic injury, and cognitive impairment. Conclusions. These findings indicate that therapeutic pSM2.HIVconsv1+ pSM2.HIVconsv2 vaccination induced protective T cell responses in chronically infected mice that reduced HIV below pathogenic levels and allowed recovery from chronic brain disease. To our knowledge, this is the first demonstration that functional cure of HIV chronic brain disease through therapeutic vaccination is feasible.

P48

Peripheral Inflammation May Indicate AD Risk in People with HIV

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BACKGROUND: Aging with HIV presents new complications like risk of Alzheimer's disease (AD). Inflammation is one possible pathogenic mechanism in AD and raises the question of how low-grade chronic inflammation characteristic among people with HIV (PWH) may influence AD-related pathogenesis. We assessed whether peripheral inflammation relates to cerebrospinal fluid (CSF) markers of AD pathology in PWH. METHODS: Participants included 78 PWH aged 25−70 years (mean age=46.48 [SD=9.71]; 81% male, 62% White; 35.6% virally undetectable) from the National NeuroAIDS Tissue Consortium (NNTC) who had data on plasma-based inflammatory markers (MCP-1, TNFα, IL-6, IP-10, sCD14, sCD163) and the CSF-based AD pathology markers of Aβ42, p-Tau181 and neurofilament light (NfL; marker of axonal integrity). All biomarkers were measured by commercial immunoassay. A series of linear regressions examined the relationships between each plasma inflammatory biomarker and each CSF AD-associated marker. Analyses were adjusted for age, HIV RNA, CD4 count, and substance use diagnoses due to their significant relationships with AD biomarkers. RESULTS: Inflammatory markers most consistently related to Aβ42 levels. Specifically, higher MCP-1, TNFα, IL-6 and sCD163 levels showed small/moderate and significant (β=-0.24 to -0.31; ps<.05) relationships with lower Aβ42 levels (indicative of greater Aβ42 pathology). The strongest relationship was between higher MCP-1 and higher NfL levels (β=0.38; p<.005). IL-6 inversely related to p-Tau181 (β=-0.26, p=0.022). CONCLUSIONS: Our results show that higher peripheral inflammation relates to more advanced CSF-derived Aβ and NfL pathology among PWH and point to the possibility that the higher levels of inflammation in PWH put them at higher risk for AD pathology. Longitudinal studies with PWH and HIV-negative controls are needed to further probe these preliminary findings. These correlations give promise to the use of blood-based inflammatory markers as an alternative to invasive lumbar punctures to indicate AD risk among PWH.

P49

Efficacious Excision of HIV-1 mRNA from Mixed Glia In Vitro

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Mixed glia are infiltrated with HIV-1 viral proteins early in the course of infection leading to the development of a persistent viral reservoir in the central nervous system. Modification of the HIV-1 genome using gene editing techniques, including CRISPR/Cas9, have shown great promise towards eliminating HIV-1 viral reservoirs; whether these techniques are capable of removing HIV-1 viral proteins from mixed glia, however, has not been systematically evaluated. Herein, the efficacy of adeno-associated virus 9 (AAV9)-CRISPR/Cas9 gene editing for eliminating HIV-1 mRNA from cortical mixed glia was evaluated. In vitro cell culture techniques were utilized to isolate and grow sex-specified mixed glia from neonatal (Postnatal Day (PD) 1 to PD 3; n=9; Male: n=5, Female: n=4) and adolescent (PD 35; n=5; Male: n=3,

Female: n=2) HIV-1 Tg rats. A within-subjects experimental design was utilized to treat male and female HIV-1 Tg mixed glia with varying doses (0, 0.9, 1.8, 2.7, 3.6, 4.5, or 5.4 μL) of CRISPR/Cas9 for 72 hours. Expression of HIV-1 mRNA was evaluated using in situ hybridization, whereby cells were hybridized with probes specific for HIV-1 viral proteins. Preliminary results show significant expression of HIV-1 mRNA, independent of biological sex, in the absence of CRISPR/Cas9 treatment (i.e., 0 μL). At the highest does (i.e., 5.4 μL) of CRISPR/Cas9, significant excision of HIV-1 mRNA was observed in both neonatal and adolescent HIV-1 Tg rats; albeit individual variability in the excision efficacy was noted. Collectively, these proof-of-concept observations support the susceptibility of mixed glia to efficacious gene editing via AAV9-CRISPR/Cas9. Funded by NIH Grants: DA013137, DA056288, MH106392, NS100624.

P50

Host restriction factor profile in brain is associated with clinical status and HIV-1 burden

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Abstract: HIV-encoded DNA, RNA, and proteins persist in the brain despite effective antiretroviral therapy with undetectable plasma and CSF viral RNA levels, often in association with neurocognitive impairments. Although the determinants of lentivirus persistence have garnered attention, the expression and regulation of host restriction factors for HIV-1 and SIV in the brain remain unknown. Here, we investigated the transcriptomic profile of antiretroviral host restricting genes, by RNAseq that were confirmed by quantitative RT-PCR in brain tissues from persons without HIV-1 infection (HIV[-], n = 10), HIV-1 infection without pre-mortem brain disease (HIV[+], n = 10), with neurocognitive disorders (HIV[+]/HAND, n = 10), and neurocognitive disorders with encephalitis (HIV[+]/HIVE, n = 10). Increased expression of host restriction factor genes was observed in brains of HIV[+] individuals with HAND/HIVE in association with brain viral load. We also analyzed the expression of established SIV-associated host restriction factors in the brains of SIV-infected Chinese rhesus macaques at different stages of ART treatment. We observed a diminished antiviral immune response among ART-treated SIV-infected animals although ART interruption induced expression of several host restriction factors. Thus, the brain displays a distinct expression profile of host restriction factors that are associated with neurological status as well as viral burden in the brain while ART interruption can influence the brain's profile of host restriction factors that might contribute to altered disease outcomes.

P51

VZV infection of human olfactory epithelium causes amyloid deposition in the absence of increases in proinflammatory cytokines.

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Varicella zoster virus (VZV) reactivation, manifesting as herpes zoster, increases the risk of dementia. Further, VZV infection shares similar pathologies with Alzheimer's disease (AD), including amyloid accumulation, neuroinflammation, and cognitive impairment. In a parallel body of literature, prior to a

diagnosis of AD, smell loss and early pathological changes (including amyloid deposition) in the olfactory system are present that subsequently spread to the entorhinal cortex and hippocampus, where late AD pathology develops. Because VZV can infect the olfactory epithelium (OE) during reactivation from trigeminal ganglia, we hypothesize that VZV infection of the OE leads to amyloid deposition and a proinflammatory environment, thereby accelerating AD progression. To test this hypothesis, we cultured healthy nasal epithelial cells from 3 human donors, characterized cell composition by immunofluorescence (IF), measured VZV DNA by qPCR following OE culture infection, quantitated proinflammatory cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, and TNFα) and amyloid-beta (Aβ) peptides in conditioned supernatant by multiplex ELISAs, and determined amyloid deposition by IF. Results show that OE cultures contained immature olfactory sensory neurons, microvillar cells, and horizontal basal cells. Following infection, cultures had increasing amounts of VZV DNA 5, 7, and 9 days later, demonstrating permissiveness to VZV infection. Surprisingly, compared to uninfected culture supernatant, VZV-infected culture supernatant had decreased cytokines that may contribute to ineffective viral clearance. In addition, VZV-infected culture supernatant had decreased Aβ40 and Aβ42, suggestive of a potential accumulation of intracellular amyloid. Indeed, increased amyloid staining was seen in VZV-infected OE cultures. These findings suggest that VZV can potentially contribute to established olfactory pathologies seen in AD and, by extension, accelerate AD progression.

P52

Human iPSC-derived astrocytes as a model for JCV infection in the brain

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JC virus (JCV), a human-selective polyomavirus, is responsible for progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system (CNS). JCV normally resides in a latent state in the kidneys of more than half of the adult population, but in rare cases of severe and/or selective immune suppression, the virus can replicate in astrocytes and oligodendrocytes in the brain. This infection results in rapid demyelination due to lytic infection in oligodendrocytes as well as alterations of astrocyte phenotypes. Some data suggest that astrocytes may be infected prior to oligodendrocytes but there is currently no relevant model to study JCV infection of astrocytes. Using astrocytes derived from human induced pluripotent stem cells (hiPSCs), we have developed an in vitro model of JCV infection in the brain. We show that astrocytes support viral DNA replication, gene expression and the formation of virus particles as shown by qPCR, immunofluorescence analysis and transmission electron microscopy. Furthermore, we perform for the first time an in-depth proteomic characterization of JCV-infected astrocytes and show a dysregulation of proteins involved in DNA replication, the cell cycle, and the DNA damage response, mirroring what is known for other polyomaviruses. Using our hiPSC-derived CNS model, we hope to pave the way towards the identification of putative targets for antiviral therapies and biomarkers of disease onset.

P53

The Role of DNA Methylation in Regulating the Expression of NKG2DLs in HIV-Infected Microglia

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Human immunodeficiency virus type-1 (HIV) establishes a reservoir within the central nervous system (CNS) within the first 1-2 weeks of infection. Microglia are a major HIV reservoir in the CNS as they have the capacity to produce infectious HIV virions and maintain a latent viral reservoir despite current antiretroviral therapy (ART) treatment regimens. Interestingly, an innate effector immune cell called natural killer (NK) cells are present at elevated levels within the CNS during HIV infection. The interactions

between HIV-infected microglia and NK cells and mechanisms influencing these interactions are still not well understood. MHC class I polypeptide-related sequence (MIC) A and B are surface proteins related to MHC molecules, but function as stress-induced ligands that interact with activating receptor NKG2D on NK cells. MIC A expression has been found to be reduced on T cells during HIV infection. Therefore, it is possible that HIV downregulates the expression of MIC A/B or other NKG2DLs on microglial cells to avoid NK cell killing. Downregulation of NKG2D ligands (NKG2DLs) could be the result of increased DNA methylation, which has been found in other immune cell-related genes during HIV infection. Preliminary data suggest that a latently infected cell line (HC69) of microglia has reduced expression of MIC A/B compared to uninfected (C20) and productively infected (HC69) microglia. Using bisulfite sequencing, qRT-PCR, and flow cytometry, we predict that the expression of other NKG2DLs will be reduced in latently infected microglia due to DNA methylation in the promoter regions of NKG2DL genes. Our studies probe novel epigenetic factors involved in NK cell recognition of infected microglia and suggest the role of methylation in maintaining a reservoir of latently infected microglia in the CNS. This research could inform future studies aimed at leveraging NK cells for shock and kill strategies in the CNS.

P54

Detection of human Polyomavirus JC in pediatric gliomas

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Gliomas are primary neoplasms with poorly understood etiology. Viral infections seem to be among the risk factors associated with this kind of tumor. We focused our attention on JC polyomavirus (JCPyV), a small naked DNA virus, recognized as the etiological agent of the progressive multifocal leukoencephalopathy (PML). Since JCPyV has been proposed to play a role in oncogenesis of several brain tumors, the presence of JCPyV was searched in 34 pediatric patients affected by gliomas by quantitative polymerase chain reaction (qPCR). Then nested PCRs were performed for amplification of the N and C-terminal region of large tumor antigen (LTAg), a protein encoded by the early genome region, known to be involved in viral replication and cellular transformation, Non-Coding Control Region (NCCR) which contains origin of replication, promoter and enhancer for genes expression and Viral protein 1 (VP1), a capsid protein involved in attachment, adsorption, and penetration. JCPyV DNA was detected in 5/34 (14.7%) tumor biopsies with a viral load mean value of 1.9x104. nPCR reported the presence of the N-terminal region of the LTAg while the C-terminal was not found in any sample. An archetype structure of the NCCR was observed in the 5 samples even if in most cases, either the Mad-1 or the Mad-4 NCCRs have been found associated with human brain neoplasms. VP1 sequence was not detected in any sample. Our results confirm the presence of JCPyV DNA in human brain tumors and its possible association with tumor development. However, further studies are warranted to better understand JCPyV's role in tumorigenesis in pediatric brain tumors.

P55

Analysis of Viral Immune Signatures in Chronic Neurological Diseases from Extracellular Vesicles in Cerebrospinal Fluid

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Extracellular vesicles (EVs) are released from virtually all cell types, and may package many inflammatory factors and, in the case of infection, viral components. As such, EVs can play not only a direct role in the development and progression of disease, but they can also be used as biomarkers. We analyzed the EVs from the cerebrospinal fluid (CSF) of healthy volunteers (HVs) and patients with a variety of chronic neurologic diseases of both known viral and non-viral etiologies including Multiple Sclerosis (MS), HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), HTLV-1-infected asymptomatic carriers (ACs), and other neurological diseases (ONDs), to investigate the surface repertoires of CSF EVs during disease. Significant increases in CD8+ and CD2+ EVs were found in HAM/TSP patient CSF samples compared to other clinical groups, consistent with the immunopathologically-mediated disease associated with CD8+ cells in the CNS of HAM/TSP patients. Furthermore, CD8+, CD2+, CD44+, and CD40+ EVs were significantly increased in the CSF from patients with viral infections compared to those without. These data suggest that CD8+ and CD2+ CSF EVs may be important as: 1) potential biomarkers for viral-mediated neurological diseases, and 2) as possible meditators of areas of the disease process in infected individuals.

P56

CSF Escape in the SIV/Macaque Model of HIV CNS Disease

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Although antiretroviral therapy (ART) suppresses viral replication in people living with HIV (PLWH), latently infected CNS microglia remain an elusive target, posing a barrier to cure. The SIV pigtailed macaque model of HAND consistently recapitulates progressive stages of HIV infection culminating in encephalitis in >90% of juvenile males lacking the Mane-A*084 allele. With antiretroviral therapy consisting of dolutegravir, tenofovir and emtricitabine started at 12 days post inoculation, SIV-infected macaques become virologically suppressed in plasma after an average of 50 days of treatment. Similarly, levels of CSF SIV RNA are typically below the limit of detection with ART. However, 4 of 22 SIV-infected macaques on this ART regimen developed CSF escape with SIV RNA (peak > 1,000 copies/mL) in consecutive CSF samples without concurrent sustained plasma viremia. Prior to CSF escape, SIV RNA was not detected in either CSF or plasma for >100 days. ART was stopped in one animal with CSF escape to track rebound viral load kinetics. In this animal, compared with treated SIV animals without CSF escape, CSF viral load reached the highest level of 2.3 X 10 5 copies SIV RNA/mL 14 days post-ART withdrawal. In contrast, in animals without CSF escape, CSF SIV RNA levels were lower, ranging from 166 to 7.5x10 4 SIV RNA copies/mL at peak after stopping ART. The development of CNS escape in this SIV/ART model enables study of this documented phenomenon in PLWH. Further examination of enhanced CSF viral rebound following analytic treatment interruption (ATI) within this CSF escape model will be a crucial step in defining where and how latent SIV/HIV can be reactivated in CNS compartments.

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HIV-1 accelerates Neurogranin loss in brain organoid model of HIV-1 neuropathogenesis

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Synaptodendritic damage is the neuropathological hallmark of HIV-1-associated neurocognitive disorders (HAND). We have previously identified that Neurogranin (Nrgn), a post-synaptic protein, is dysregulated in brain of HIV-1-positive individuals with HAND. However, it remains to be determined the mechanisms underlying this dysregulation. Here we sought to investigate Nrgn dysregulation in neurons using our novel

triculture brain organoids model. We first assessed Nrgn mRNA levels in frontal cortex (FC) tissues from HIV-1-positive (n=49) individuals with and without HAND in comparison to neurocognitively normal individuals (n=22). We found that Nrgn mRNA levels are significantly lower (by 2.5-fold) in HIV-1-positive individuals in 75.5% of cases (37 out of 49). We further compared the expression of Nrgn to the dendritic marker microtubule-associated protein 2 (MAP2) to verify whether Nrgn dysregulation correlates with dendritic damage. We verified that the Nrgn/MAP-2 ratios of colocalization staining intensity is significantly reduced in HIV+/HAND- (p=0.0009) and HIV+/HAND+ (p=0.0001) samples compared to control (n=4). Additionally, we found that mRNA levels of Nrgn correlate with MAP2 (positive correlation coefficient=0.5658), suggesting that Nrgn dysregulation in HIV+-individuals parallels dendritic loss. To study the mechanisms in a physiologically relevant model, we leveraged brain organoid technology by incorporating infected microglia to recapitulate the HIV-infected brain microenvironment. We found that Nrgn expression decreased (2.7-fold, p=0.0056) as early as 5 days after the incorporation of HIV-infected microglia compared to mock. Accordingly, HIV-infection caused significant decrease of Nrgn immunostaining independently of neurodegeneration. Our results indicate that Nrgn loss parallels dendritic simplification but precedes neurodegeneration, which suggests that Nrgn loss is an early molecular hallmark of HAND. Nrgn loss can potentially cascade into the cognitive impairment observed in HAND. Further studies are being conducted to examine the host and viral factors involved in Nrgn dysregulation and its contributions to the morphological and functional changes in neurons.

P58

COVID-19 is associated with neuroinflammation and suppression of brain peroxisomes

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Background: COVID-19, the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is associated with encephalopathy in over 30% of infected patients, although the neuropathogenic mechanisms remain uncertain. COVID-19 is associated with inflammation in the central nervous system (CNS), termed neuroinflammation. Recent evidence suggest SARS-CoV2 may also suppress critical peroxisome proteins. We investigated the neural cell tropism and host responses during SARS-CoV-2 infection in multiple experimental platforms. Methods: Viral quantitation, cell tropism and associated host innate immune responses were examined in autopsied brains from persons with COVID-19 and matched controls, primary human neural cells, and hamsters infected intranasally with SARS-CoV-2 using ddPCR/RT-PCR, immunodetection, multiplex ELISA, Western blotting, and plaque assays. Results: Analyses of brain tissue from COVID-19 patients revealed upregulation of inflammatory genes (e.g., IL8, IL18, CXCL10, NOD2) and induction of pro-inflammatory cytokines (GM-CSF, IL-18). Peroxisome gene and protein expression, including PMP70, PEX3 and PEX14, was suppressed in the brainstem of COVID-19 patients. SARS-CoV-2 spike and NSP3 proteins were immunodetected in astrocytes and neurons in the brain of a COVID-19 patient, with concurrent viral RNA detection in four of twelve patients. Additionally, SARS-CoV-2 productively infected cultured human astrocytes in vitro. In SARS-CoV-2 infected hamsters, viral RNA was detected in the olfactory bulb and neuroimmune responses were observed in the olfactory bulb and cerebrum (e.g., Cxcl10, Il18, Gsdmd, and Ninj1). Pex3 gene expression, and catalase protein levels were suppressed in the cerebrum of infected hamsters. Immunohistochemical staining of the brains from infected hamsters revealed increased Iba-1 immunoreactivity and suppressed PMP70 immunostaining in

the central corpus callosum. Conclusions: SARS-CoV-2 infects the brain with productive infection of astrocytes, albeit with minimal persistent infection. Neuroinflammation and upregulation of inflammatory cytokines in response to SARS-CoV2 infection was observed in all experimental platforms and was associated with suppression of peroxisomes.

P59

Host Factors associated with COVID-19 Severity and Neurological Mechanisms in a Hispanic Population

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Severe Acute Respiratory Virus (SARS-CoV-2) causes coronavirus 2019 disease (COVID-19) ranging from asymptomatic to severe disease. We hypothesize that host factors will determine COVID-19 severity. After IRB approval, a total of 121 men and women ages 21-80 yrs were recruited in Puerto Rico. Plasma and PBMCs samples were collected from COVID-19 positive unvaccinated (n=39) and vaccinated (n=11) patients, including negative unvaccinated (n=56) and vaccinated (n=15) controls. Unvaccinated COVID-19 patients were stratified based on symptomatology as follows: asymptomatic/mild (n=18), moderate (n=13), and severe (n=8). Vaccinated participants were stratified as COVID-19 positive and negative controls. DNA was isolated from PBMCs of participants and Global Diversity Arrays were used. Quantitative proteomics was performed in plasma using Tandem Mass Tag (TMT) labeling, Proteome Discoverer, and Limma Statistics. Genomics and Proteomics results were analyzed in IPA. Cytokines were quantified in plasma using a human cytokine array. Astrocyte and neuron-derived exosomes were quantified in plasma by flow cytometry. Preliminary genomics results show that among the top 10 pathways in enrichment analysis for genes with variants of ≥0.50 allele frequency in patients with severe COVID-19 include: Axonal guidance, CREB, and synaptogenesis signaling. Similarly, proteomics revealed that synaptogenesis signaling was inhibited in severe COVID-19. Other mechanisms such as lipid and metabolic regulators, acute phase response, iron homeostasis, and atherosclerosis signaling were deregulated across COVID-19 disease severities. Levels of IL-1Ra, IP-10, RANTES, TNFa, and MIP-1a were increased in COVID-19 patients in a severity-dependent manner, whereas PDGF-BB levels were decreased in COVID-19 patients. Astrocyte-derived exosomes were increased in the plasma of COVID-19 patients. In vaccinated participants, COVID-19 infection increased levels of Aminopeptidase N, Hemoglobin subunits beta and delta, Alpha-1-acid glycoprotein 1, Haptoglobin, Carbonic anhydrases 1 and 2, and IP-10 in plasma. This study uncovers potential host predictors of COVID-19 severity and mechanisms associated with neurological consequences in Hispanics.

P60

Monoclonal antibody improves paralysis outcome in a delayed-treatment mouse-model of Enterovirus D68-induced paralysis

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Enterovirus D68 (EV-D68) causes mild to severe upper respiratory illness and is associated with a poliomyelitis-like syndrome (acute flaccid myelitis, AFM) which predominately affects children. EV-D68 was infrequently reported prior to 2014 but has caused several worldwide outbreaks in recent years. There are currently no vaccines or proven therapy to prevent or treat EV-D68-associated neurologic illness. Since most patients seek medical attention only after the onset of AFM, we utilized an outbred neonatal mouse model of AFM to examine the effects of anti-EV-D68 specific antibody treatment initiated after development of clinical paralysis. For these experiments we used a chimeric anti-EVD68 monoclonal antibody – designated 15C5-Chmra – which has a mouse Fab fragment containing sequences derived from those originally described by Zheng et al. and a human Fc fragment. In our clinical model, we found that 15C5-Chmra significantly improved paralysis score and survival against 2014 and 2016 EV-D68 strains compared to untreated controls. It also significantly reduced viral titer in the spinal cord and muscle in our clinical treatment model. We further analyzed the spread of virus from the initiating limb and found that treatment prevents the development of paralysis in other limbs but does not reverse paralysis which was present at the time of treatment. This work, along with the work of others, suggests that monoclonal antibody treatment could effectively treat AFM and that initiating treatment soon after onset of paralysis will improve outcomes.

P61

Characterizing subsets of HIV-infected and uninfected CD14+CD16+ monocytes that contribute to neuropathogenesis

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HIV-associated neurocognitive impairments (HIV-NCI) affect 15-40% of people with HIV (PWH) despite antiretroviral therapy (ART). HIV enters the CNS early, often before people are aware they are infected. One mechanism of CNS infection is the transmigration of HIV-infected CD14+CD16+ intermediate (mature) monocytes across the blood-brain barrier (BBB). Mature monocytes are increased in the blood of PWH, are most susceptible to HIV infection compared to other monocytes, and preferentially transmigrate across the BBB to CCL2. To characterize functional properties of mature monocytes and the effects of HIV infection on these cells, we performed single cell RNA-sequencing (scRNA-seq) of uninfected and HIV-infected mature monocytes and showed that both populations formed 9 monocyte clusters. Expression of genes from migratory, inflammatory, or neurotoxic molecular pathways in each cluster compared to other clusters, was analyzed. This demonstrated two groups of clusters in both uninfected and HIV-infected monocytes: those with increased expression of genes in these pathways (Group 1) and those with decreased or minimal expression (Group 2). We hypothesize that uninfected and HIV-infected Group 1 mature monocytes with high gene expression in migratory and inflammatory pathways contribute to HIV neuropathogenesis more than Group 2 monocytes. We propose that Group 1 monocytes preferentially transmigrate across the BBB and produce more inflammatory mediators and ROS that are major mediators of HIV neuropathogenesis. We demonstrated that uninfected Group 1 monocytes show higher median fluorescence intensities (MFI's) of cell-surface proteins ALCAM, CD52, CD86, and SDC2 found in our scRNA-seq analyses of Group 1 than those in Group 2. They also produce more ROS. We will continue to characterize the functional properties of Group 1 compared to Group 2 monocytes to assess their potential

contributions to HIV neuropathogenesis. The goal is to develop novel interventional strategies to block their entry into the brain, thereby reducing damage and subsequent NCI.

P62

Analysis of the Effect of HDAC Inhibitor, TSA, on the Expression and Stability of JC Virus LT-Ag

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JC virus (JCV), a human polyomavirus, infects most of the human population and remains latent, usually in the kidneys and B-cells. JC Virus infection in most individuals is asymptomatic but under immunosuppressive conditions, JC virus reactivates to infect oligodendrocytes and astrocytes resulting in a disease known as progressive multifocal leukoencephalopathy (PML). This disease is often fatal and there is no cure as of now. The JCV genome is functionally divided into three regions: an early coding region, a late coding region, and a non-coding regulatory region. Large tumor antigen (LT-Ag), a nuclear phosphoprotein essential for viral replication, is encoded by the viral early region. LT-Ag is critical to various mechanisms of viral persistence, including driving infected cells into the S phase by binding to and inactivating Rb protein function. This disrupts the pRb's ability to bind to the E2F transcription factors, therefore inducing S phase entry. Trichostatin A (TSA) is a histone deacetylase inhibitor (HDACi) known to affect many cellular processes, including growth arrest, differentiation, autophagy, and apoptosis. To test whether TSA has any effect on the expression and stability of LT-Ag, oligodendroglioma cells (TC620) were transfected with a LT-Ag expressing plasmid and treated with various concentrations of TSA. We observed a significant decrease in the level of LT-Ag in the TSA-treated cells compared to untreated ones. In addition, microscopic analysis of TSA-treated cells clearly showed a more branched-out morphology compared to controls, showing that TSA has a pronounced effect on cell morphology. Moreover, the analysis of the cell cycle stage-specific CDKs including CDK1 and CDK2 displayed a significant reduction in their expression in the cells treated with TSA, analogous to LT-Ag. This suggests that CDKs may play a role in the stability of LT-Ag through its phosphorylation. However, more evidence is needed to further validate such a conclusion.

P63

Tracking fluorescently labeled anti-HIV IgG in the brain as a CNS-specific cure strategy

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The Central Nervous System (CNS) is an important latent reservoir for HIV and thus its targeting is critical for a viable cure strategy. Long-term HIV suppression without combined anti-retroviral therapy (cART) or cure strategies should consider approaches that can also target HIV in the brain. Anti-HIV antibodies delivered to the brain can be a potential approach to complement cART cessation therapeutics or cure strategies. However, due to the blood brain barrier, delivery of antibodies to the CNS is limited to about 0.1% or less of injected antibodies and therefore efficacy of antibodies targeting the brain is low. We set out to better characterize antibody delivery to the brain in NSG-humanized mice using several imaging techniques including the In Vivo Imaging System (IVIS) for live imaging, light sheet microscopy for whole organ analysis, and conventional fluorescence microscopy for defining cellular uptake/delivery of antibody. Cy5 labeled Gamunex (pooled human IgG) or bispecific anti-HIV antibody PGT121-Transferrin was injected intraperitoneal (IP) and at 48hrs, prior to necropsy, Cy5 levels were measured in the brain of live mice. We found signal in the brain of animals that received Cy5-labeled antibodies but not in animals that received a PBS injection. We assessed the cy5 signal via light sheet microscopy and observed an integrated network of antibody delivery to the brain via the vasculature therein. Using pre-injection IgG to make a

standard curve, we measured antibody concentration within an array of brain tissues. Using OCT sections we stained for astrocytes, microglia, and macrophages and found that the antibody was only found in microglia/macrophages, likely due to their abundance of Fc receptors. Together, these studies demonstrate the efficacy of antibody penetration into the brain as a viable approach in future studies to specifically target the HIV reservoir in the brain.

P64

COVID-19 neuroinflammation is associated with CSF IgG glycosylation signature and auto-reactivity

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Neuroinflammation contributes to neurological complications in both mild and severe cases of COVID-19. The mechanism(s) and/or correlates of COVID-19 neuroinflammation is not entirely clear. IgG glycosylation patterns can characterize inflammation and severity of diseases, including neuroinflammatory diseases. We defined CSF IgG glycosylation pattern among COVID-19 patients and determined their association with neuropathology and neuroinflammatory mediators of deceased COVID-19 patients. Specifically, we characterized CSF-derived IgG glycosylation patterns through glycoproteomic analysis from 11 deceased, unvaccinated COVID-19 patients (median age 69 [IQR 61-77], 7 males, 4 females) and assessed CSF soluble and cellular markers of neuroinflammation through multiplex immunoassays and histologic brain neuropathology. SARS-CoV-2 was undetectable in all CSF samples. 10 out of 11 donors had anti-SARS-CoV-2 IgGs in the CSF. Inflammatory glycosylation profiles exhibited decreased proportions of galactosylation and sialylation. Inflammatory glycan patterns positively correlated with CSF neuroinflammatory markers (ANNA-1, neopterin, sCD14, sCD163, TNFalpha, IFNgamma, IL-8, RANTES, MCP-1), while anti-inflammatory patterns were inversely associated with anti-Spike IgG levels. These correlations were confirmed by a Spearman rank-sum bootstrap of 25,000 simulations. Lastly, CSF IgG antibodies were self-reactive, as demonstrated in an ex vivo mouse brain slice. Together these data suggest autoimmunity as a source of neuroinflammation and brain damage following SARS-CoV-2 infection and describe the potential for using bulk IgG glycosylation patterns as a potential marker of neuroinflammation.

P65

HIV Tat-mediated microglial activation and neuroinflammation: Role of lncRNA Xist-miR-124-CCL2 axis

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HIV-1 and its proteins, such as Tat (transactivator of transcription), mediate neurotoxic effects in microglia by releasing proinflammatory cytokines and chemokines, in turn, affecting neuronal health and leading to HAND. Recent reports reveal that long noncoding (lnc) RNAs can regulate gene expression by serving as competitive endogenous RNAs (ceRNAs) involving binding of miRNAs and affecting the downstream target genes. Reports on the role of lncRNAs in the context of HIV-1-mediated microglial activation and neuroinflammation remain scant. Herein, we sought to determine the involvement of lncRNA Xist (X-inactive Specific Transcript) in mediating the activation of BV2 cells exposed to HIV-1 Tat. Our findings demonstrated that HIV-1 Tat increased the expression of lncRNA Xist and concomitantly downregulated the expression of miR-124 in BV2 cells. We also found that exposure to HIV-1 Tat upregulated the expression of the chemokine CCL2 and microglial activation marker, Iba1, in BV2 cells. Gene silencing study using lncRNA Xist siRNA further underscored the role of lncRNA Xist/miR-124/CCL2 axis in HIV-1 Tat-mediated activation of BV2 cells. Bioinformatics analyses also identified a potential miR-124 novel binding site for lncRNA Xist and the chemokine CCL2. The binding of lncRNA Xist with miR-124 was determined using dual luciferase assay, argonaute immunoprecipitation, and RNA immunoprecipitation assays. We also validated the in vitro findings in vivo doxycycline-inducible Tat mice model. Overall, our findings suggest that HIV-1 Tat upregulates the expression of lncRNA Xist, which, in turn, sponges miR-124, leading ultimately to increased expression of the target gene CCL2 and culminating to microglia activation and neuroinflammation.

P66

Endogenous IFNβ Affects Neuronal Synapses in the Presence and Absence of Chronic CNS Exposure to HIV-1

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Infection with HIV-1 continues despite effective combination anti-retroviral therapy (cART) to compromise the brain and cause HIV-associated neurocognitive disorders (HAND) in approximately half of the HIV positive population. Our laboratory utilizes a transgenic mouse (tg) model of HIV-associated brain injury expressing the viral gp120 envelope protein under the control of a modified GFAP promoter in astrocytes (HIVgp120tg mice). This model recapitulates key neuropathological features of human HIV brains, including neuronal damage, differential gene expression and memory-related behavioral deficits. HIV-1 infection and transgenic expression of gp120 both activate the innate immune system, including the production of the type I interferons (IFN) α and -β. In this study, we investigated the role of endogenous IFNβ by cross-breeding IFNβ knock-out (KO) with HIVgp120tg mice. At 1.5, 6 and 9 months of age 3 females and 3 males per genotype (IFNβKO-gp120tg, IFNβKO, IFNβ wild-type (WT)-gp120tg and IFNβWT non-tg) were sacrificed, immediately transcardially perfused and the brains were analyzed for differential gene expression. QRT-PCR analysis confirmed in all age groups the lack of induction of several ISGs, such as IRF7, in IFNβKO- compared to IFNβWT-gp120tg brains. Immunofluorescence staining of sagittal brain sections for neuronal and glial markers was performed on 9 months-old mice and neuropathological analysis employed deconvolution microscopy or quantification of fluorescence. IFNβ-deficient gp120tg and non-tg controls both displayed significantly reduced Synaptophysin (SYP) positive

presynaptic terminals and MAP-2 positive neurites compared to IFNβWT non-tg controls, although injury in the presence of gp120 was similar to that in the respective IFNβWT counterparts. Altogether, our study suggests a significant role of endogenous IFNβ in the quantitative regulation of neuronal presynaptic terminals and neurites but also that it is insufficient to prevent over time HIVgp120-induced injury.

P67

Segregated functional domain organization of HIV-1 Tat and Rev overlapping reading frames dictated by codon selection pressure between viral regulatory proteins

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Human immunodeficiency virus type 1 (HIV-1) displays one of the highest mutation rates amongst viruses. Mutations arising in HIV-1 protein coding regions may either be synonymous or nonsynonymous, and the latter may alter the amino acid sequence in a given protein. Transcriptional regulation of HIV-1 is not currently targeted by ART thus allowing nonsynonymous mutations to enhance or repress transcription-related pathogenesis at the cellular level. This has been noted in HIV-1-encoded proteins, Tat and Rev, which regulate viral transcription. While HIV-1 hijacks transcriptional machinery of the host cell, Tat initiates highly processive transcription of the proviral genome and Rev regulates nuclear export of incompletely spliced viral transcripts. Both Tat and Rev mechanisms work in tandem for efficient mRNA production and trafficking and reside in overlapping reading frames in the HIV-1 genome. Prior models of HIV-1 open reading frame distributions have shown Tat and Rev to have segregated functional domain organization as well as viral fitness attributed to tat codon selection. Therefore, we sought to characterize the structure of Tat and Rev functional domain organization and codon selection pressures in the Drexel Medicine CARES patient cohort. The profiles of tat and rev genes were reconstructed for viral quasispecies from 137 clinical isolates and were used to calculate selection pressure for overlapping codons in each protein. Calculation of mean log2 dN/dS ratios revealed opposing selection pressures between tat and rev overlapping regions. Segregated domain organization was apparent in tat and rev overlapping regions, with tat experiencing overall higher diversifying selection throughout. Tradeoffs between tat and rev codon selection in overlapping regions elucidate how viral genetic variation affects and may lead to dysfunction of overlapping gene products.

P68

Characterizing microglia phenotypes during HIV latency and reactivation

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Antiretroviral Therapy (ART) effectively reduces HIV-1 replication, however 15-60% of patients receiving ART still experience HIV-1-associated neurocognitive disorders (HAND). Our hypothesis is that periodic emergence of HIV from latency is the cause for neurological complications. To study HIV-1 latency and

associated microglia phenotypes, we have previously used a coculture model with Induced Pluripotent Stem cells (iPSC) derived microglia (iMG), infected with single round HIV-1 containing a short-lived GFP and neurons. We have shown neurons can suppress HIV reactivation in iMGs, when reactivated using high dose poly (I:C) or TNFα, compared to iMGs cultured without neurons. Even within the cultures of pure iMGs, a fraction of the cells entered latency in vitro upon infection. These latently infected cells can be reactivated using TNFα, as quantified by both HIV-1 transcripts and HIV-1 GFP expression. Performing scRNA-sequencing and antibody sequencing (AbSeq) on these iMGs, and classifying the functionally HIV latent and reactivated populations, we were able to correlate HIV RNA expression levels to the expression of different microglia specific surface protein markers. The HIV-reactivated populations were enriched for pro-inflammatory markers, co-stimulatory CD80, CD86 and Fc receptors, CD16, CD32 and CD64. By contrast, the HIV latent iMGs had reduced expression of each of the pro-inflammatory markers and had an increased levels of "anti-inflammatory" or tissue-supportive markers like CD115, CD195 and CD163. Additionally, we also noted, surface expression of HLA-DR (MHCII) and CD40L reduced with increasing HIV expression. Thus, we conclude that the iMGs with latent HIV could be skewed to a reduced "immune-activated" state when compared to iMGs with reactivated HIV. We are currently extending these experiments to include iPSC derived neurons and astrocytes in a triculture system with HIV-1-infected microglia and are comparing the phenotypes of iMGs to microglia obtained from the brains of HIV-1 infected humanized mice and reconstituted iPSC derived organoids.

P69

HIV induces AQP4 dysfunction via A2aR leading to HAND due to glymphatic failure

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The glial-lymphatic or glymphatic fluid clearance system promotes the exchange of interstitial fluid (ISF) and cerebrospinal fluid through the arterial perivascular spaces into the brain. This process is facilitated in part by aquaporin-4 (AQP4) water channels located primarily on astrocyte end feet abutting endothelial cells of the blood brain barrier. Changes in expression levels or mislocalization of AQP4 from astrocytic end feet to the soma can lead to decreased ISF flow leading to buildup of extracellular waste products like hyperphosphorylated Tau (pTau). pTau accumulation is a neuropathological hallmark in Alzheimer's disease (AD) and in some people with human immunodeficiency virus (HIV). Approximately 50% of people with HIV (PWH) suffer from HIV-associated neurocognitive disorders (HAND), which is a spectrum disorder linked to cognitive and motor decline in PWH. Limited studies have shown that in HIV CNS infection that expression levels of AQP4 in brain homogenates from the mid-frontal gyrus of PWH with symptomatic HAND were significantly increased compared to those with asymptomatic HAND, which raises the question if AQP4 function and subcellular localization may contribute to cognitive status. Studies in other neuroinflammatory diseases have shown dysregulation of AQP4 through the adenosine A2aR (A2aR) signaling. A2aR activation leads to PKC-mediated inhibitory phosphorylation of AQP4 (Ser180, Ser276) that is proposed to contribute to channel internalization, mislocalization and decreased expression. . Therefore, it is possible that common mutations in aqp4, subcellular mislocalization, dysfunction, expression levels or post-translational modifications contribute to HAND. Therefore the cognitive changes we see in HAND maybe due to changes in AQP4 may contribute by decreasing clearance of toxic aberrant proteins and HIV mechanistically alters AQP4 in part via dysregulation of A2aR.

P70

Neuronal deletion of nSMase2 reduces amyloid production and directly protects neurons

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Viral infections of the brain are known to accelerate the production and deposition of A-beta peptides. Although multiple publications have shown that A-beta peptides are associated with EVs, compared with the total amount of A-beta present in plasma, CSF, or supernatants from cultured neurons, relatively little A-beta is associated with EVs. The involvement of EVs has largely been inferred by pharmacological inhibition or deletion of neutral sphingomyelinase-2 (nSMase2), a key regulator for the biogenesis of at-least one population of EVs. We used a Cre-Lox system to selectively delete nSMase2 from pyramidal neurons in APP/PS1 mice (APP/PS1-SMPD3-Nex1) and found a ~70% reduction in A-beta deposition at 6 months of age and ~35% reduction at 12 months of age. Brain ceramides increased in APP/PS1 mice were similar to Wt in APP/PS1-SMPD3-Nex1 mice, suggesting that elevated brain ceramides predominantly involved neuronal expressed nSMase2. Reduced levels of PSD95 and deficits of long-term potentiation in APP/PS1 mice were normalized in APP/PS1-SMPD3-Nex1 mice. In contrast, elevated levels of IL-1beta, IL-8 and TNF-alpha in APP/PS1 mice were not normalized. The size of liquid ordered membrane microdomains were increased in APP/PS1 mice, as was the amounts of APP and BACE1 localized to these domains. Pharmacological inhibition of nSMase2 activity reduced the size of the liquid ordered membrane microdomains, reduced the localization of APP with BACE1 and reduced the production of A-beta1-40 and Abeta1-42. Very little A-beta was associated with EVs in all conditions tested. We also found that nSMase2 directly protected neurons from the toxic effects of oligomerized A-beta and preserved neural network connectivity despite considerable A-beta deposition. These data demonstrate that nSMase2 plays a role in the production of A-beta by stabilizing the interaction of APP with BACE1 in liquid ordered membrane microdomains, and inhibition or genetic deletion of nSMase2 can protect neurons.

P71

Neuronal deletion of nSMase2 reduces amyloid production and directly protects neurons

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Viral infections of the brain are known to accelerate the production and deposition of A-beta peptides. Although multiple publications have reported that A-beta peptides are associated with EVs; compared with the total amount of Amyloid-beta present in plasma, CSF, or supernatants from cultured neurons, relatively little Amyloid-beta is associated with EVs. The involvement of EVs has largely been inferred by pharmacological inhibition or deletion of neutral sphingomyelinase-2 (nSMase2), a key regulator for the biogenesis of at-least one population of EVs. We used a Cre-Lox system to selectively delete nSMase2 from pyramidal neurons in APP/PS1 mice (APP/PS1-SMPD3-Nex1) and found a ~70% reduction in Amyloid-beta deposition at 6 months of age and ~35% reduction at 12 months of age. Brain ceramides increased in APP/PS1 mice were similar to Wt in APP/PS1-SMPD3-Nex1 mice, suggesting that elevated brain ceramides predominantly involved neuronal expressed nSMase2. Reduced levels of PSD95 and

deficits of long-term potentiation in APP/PS1 mice were normalized in APP/PS1-SMPD3-Nex1 mice. In contrast, elevated levels of IL-1beta, IL-8 and TNF-alpha in APP/PS1 mice were not normalized. The size of liquid ordered membrane microdomains were increased in APP/PS1 mice, as was the amounts of APP and BACE1 localized to these domains. Pharmacological inhibition of nSMase2 activity reduced the size of the liquid ordered membrane microdomains, reduced the localization of APP with BACE1 and reduced the production of Amyloid-beta1-40 and Amyloid-beta1-42. Very little Amyloid-beta was associated with EVs under all conditions tested. We also found that nSMase2 directly protected neurons from the toxic effects of oligomerized Amyloid-beta and preserved neural network connectivity despite considerable Amyloid-beta deposition. These data demonstrate that nSMase2 plays an important role in the production of A-beta by stabilizing the interaction of APP with BACE1 in liquid ordered membrane microdomains, and inhibition or genetic deletion of nSMase2 can protect neurons.

P72

Characterizing the role of monocytes in HIV neuropathogenesis

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HIV associated Neurocognitive Impairment (HIV-NCI) affects 15-40% of people with HIV (PWH) despite viral suppression with ART. Understanding the pathogenesis of HIV-NCI is key to developing specific therapies. HIV-NCI is mediated, in part, by transmigration across the blood brain barrier (BBB) of infected CD14+CD16+ monocytes. In the CNS, CD14+CD16+ monocytes contribute to infection and activation of parenchymal cells, resulting in production of neurotoxic host and viral factors that cause neuronal damage. We showed that HIV infection mediates increased transmigration of CD14+CD16+ cells across an in-vitro BBB model to CCL2, a chemokine elevated in the CNS of PWH. As a result of HIV infection some cells harbor viral DNA (HIV+), and other cells do not, but are exposed to viral factors (HIVexp). Mechanisms by which infected CD14+CD16+ monocytes mediate the development and progression HIV-NCI have not been characterized longitudinally. We hypothesize that transmigration of CD14+CD16+ monocytes, particularly HIV+ CD14+CD16+ monocytes, across the BBB contributes to HIV-NCI pathogenesis of PWH on ART. In this study, we are isolating PBMC from 70 PWH on ART from the Manhattan HIV Brain Bank cohort at two timepoints 24 months apart to analyze CCL2-induced transmigration of monocytes and of HIV+ and HIVexp PBMC across our in-vitro BBB model. We demonstrated that the CD14+CD16+ monocytes from PWH with cognitive impairment transmigrated in greater numbers than those from PWH with normal cognition at both timepoints. We showed that CCR2, the receptor for CCL2 was higher on CD14+CD16+ monocytes in those with cognitive impairment as compared to those with normal cognition at timepoint 1. These results will be correlated with cognitive domains and indicators of neuronal health obtained by MRS cross-sectionally and longitudinally. This work will determine the role of CCL2-mediated transmigration of CD14+CD16+ monocytes in the context of HIV-NCI progression and may identify a biomarker for HIV-NCI.

P73

Characterizing the effects of methamphetamine on the mature monocyte transcriptome: Implications for contribution to HIV neuropathogenesis

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HIV neurocognitive impairment (HIV-NCI) manifests as a spectrum of cognitive and motor deficits and behavioral changes that affects 15-40% of people with HIV (PWH) despite successful viral suppression with antiretroviral therapy. HIV enters the CNS and results in inflammation and neuronal damage that contribute to HIV-NCI. One mechanism of entry is through the transmigration of HIV infected CD14+CD16+ (mature) monocytes across the blood brain barrier (BBB) in response to chemokines including CCL2, which are elevated in the CNS. The use of methamphetamine (meth), a potent CNS stimulant, is a growing public health issue that intersects with the HIV epidemic. Chronic meth use results in neurocognitive impairment, and studies have shown that some PWH who use meth have increased severity of HIV-NCI. Our laboratory demonstrated that meth treatment of mature monocytes increases transmigration of to CCL2 across an in vitro human BBB model. To characterize mechanisms that mediate this, we performed RNA sequencing of primary human HIV infected mature monocytes with and without meth treatment. We found that genes related to extracellular matrix (ECM) degradation and cytoskeletal rearrangement, such as MMP9, CFL1 and GSN, were upregulated. We chose some targets to validate the sequencing results and correlate changes in protein levels by western blot analysis, ELISA, and flow cytometry. We demonstrated increased expression and/or secretion of MMP9 (matrix metalloproteinase 9), which degrades the ECM and facilitates extravasation, and of the actin binding protein gelsolin, which promotes migration. Preliminary data also show a potential increase in CD109, which promotes tumor metastasis, and CD151, a tetraspanin involved in signaling cascades that result in cell adhesion, motility, and activation. Increased expression of these molecules can, in part, mediate increased monocyte recruitment into the CNS, contributing to neuropathogenesis. Our goal is to identify potential novel therapeutic targets to mitigate HIV-NCI for PWH who use methamphetamine.

P74

The characterization of EVs released from HIV-1 infected monocytes with CBD and HU308 treatment

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Over 38 million people were living with human immunodeficiency virus-1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), in 2021. Currently, there is no cure for HIV-1 infection; however, combined antiretroviral therapy (cART) prevents the disease progression into AIDS and promotes viral latency. cART has extended many lives, but people living with HIV-1 (PLWH) face lifelong ailments such as HIV-associated neurocognitive disorders (HAND), which encompasses a variety of central nervous system (CNS) disorders that range from asymptomatic to early onset Alzheimer's disease. HAND has been attributed to chronic inflammation and low-level HIV-1 infection within the CNS. Previous studies have shown viral products and pro-inflammatory cytokines move throughout the CNS within extracellular vesicles (EVs), lipid-bound nanoparticles released from cells as a form of intercellular communication. This study looks at the impact of cannabidiol (CBD), a promising therapeutic for HAND patients, and a similar synthesized molecule, HU308, on the EVs released from HIV-1 infected cells and 3D model neurospheres. The data shows that CBD and HU308 decrease viral products and pro-inflammatory cytokines both intra-

and extra-cellularly within HIV-1 infected cells and neurospheres. The mechanism of suppression is specific and affects the classical exosomal secretion pathway. Overall, CBD and HU308 may be a viable option in combination with cART as HAND therapeutics.

P75

The impact of EcoHIV infection in cocaine seeking behaviors

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Substance use disorder (SUD) is highly comorbid with HIV infection though the underlying neurobiology of this relationship is poorly understood. SUD is characterized by a high propensity to relapse. Preclinical research on the neurobiobehavioral outcomes of progressive HIV infections may yield crucial information to improve SUD prognosis, including reducing the risk of relapse in people living with HIV (PLWH). To model progressive HIV, adult male and female C57BL6J mice were inoculated with EcoHIV, a chimeric HIV-1 which infects murine cells by replacing envelope glycoprotein gp120 with gp80. Five weeks after inoculation, mice were trained in a cocaine conditioned place preference (CPP) paradigm with 2 cocaine (10mg/kg, i.p.) conditioning sessions, followed by 4 days of extinction training. Cocaine and stress-induced reinstatement were assessed following administration of cocaine or yohimbine, an α2 adrenergic antagonist, to determine EcoHIV effects. After the behavioral test, the effect of EcoHIV on neuronal activation by cocaine was assessed. Putative neuronal activation in the prefrontal cortex (PFC) and nucleus accumbens (NAc) was assessed by quantitative analysis of the expression of the immediate early gene, cFos. Data indicated that EcoHIV and sham-inoculated mice exhibited similar cocaine CPP and extinction. However, EcoHIV mice showed significantly increased cocaine- and yohimbine-induced reinstatement ($p<0.05$). cFos expression was increased in PFC and NAc by cocaine in all mice. However, cocaine interacted with EcoHIV infection to increase cFos expression selectively within the infralimbic PFC (IL) and NAc shell subregions. In summary, EcoHIV infected mice exhibited escalated cocaine and yohimbine-induced reinstatement of cocaine seeking compared to controls. These findings may have implications for factors precipitating risk for relapse in PLWH. Progressive EcoHIV infection may dysregulate IL and NAcShell activity to facilitate the cocaine seeking and reinstatement. Ongoing research will characterize how EcoHIV infection dysregulates activity within the IL-NAcShell pathway during cocaine reward learning and relapse.

P76

Neutral sphingomyelinase-2 regulates late stage of HIV biogenesis

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Modification of lipid composition in plasma membrane following HIV-infection facilitates the formation of membrane curvature required for viral assembly and budding. Ceramide is an important lipid involved in the formation of membrane curvature and maintaining stability by promoting a lamellar-hexagonal phase transition of polar head group, and saturated fatty acid tails create highly ordered membrane microdomains known as a lipid rafts. Neutral sphingomyelinase-2 (nSMase2) is a sphingomyelin hydrolase that generate ceramide from sphingomyelin. Here, we provide the first evidence that nSMase2 was colocalized with HIV-Gag, directly bound to HIV-Gag in lipid rafts where the late stage of HIV-biogenesis occurred. HIV-infection increased the expression and the activity of nSMase2 with corresponding increase of multiple ceramides in HIV-infected cells. Inhibition of nSMase2 by phenyl(R)-(1-(3-(3,4-dimethoxyphenyl)-2,6-

dimethylimidazo[1,2-b]pyridazin-8-yl)pyrrolidin-3-yl)-carbamate (PDDC) or molecular interference resulted in impaired Gag processing, decreased HIV-replication, and the production of non-infectious virions with morphological misshaping, many of which appeared to have incomplete membrane closure. NSMase2 was incorporated into virons, and virions released from PDDC-treated cells exhibited significantly different lipid compositions compared to virions released from untreated cells. Impaired Gag-processing by PDDC in HIV-infected cells induced endolysosomal accumulations of HIV-Gag with endolysosomal stress, followed by apoptotic cell death. Inhibition of nSMase2 by PDDC in HIV-infected humanized mice (CD34+ NSG-mice and BLT-mice) resulted in a linear decrease of plasma HIV to below detectable limits (5 of 7 CD34+ NSG mice; 3 of 5 BLT-mice). PDDC-treated mice that achieved viral loads below detectable limits did not exhibit rebound when inhibitor was withdrawn. In contrast, ART-treated mice were below detectable limits within 10 weeks of treatment (6 of 7 CD34+ NSG-mice; 4 of 5 BLT-mice), and all showed viral rebound following cessation of ART. These findings suggest that nSMase2 as a critical regulator for late stage of HIV biogenesis that can be therapeutically targeted for HIV cure.

P77

Intervention in arachidonic acid cascade and eicosanoid production: sex dependent benefits in HIVgp120tg mouse model for HAND

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Combination antiretroviral therapy (cART) has transformed HIV infection from a terminal disease to a manageable chronic health condition, extending patients' life expectancy to that of the general population. However, the incidence of HIV-associated neurocognitive disorders (HANDs) has persisted despite virological suppression. Patients with HIV display persistent signs of immune activation and inflammation despite cART. The arachidonic acid (AA) cascade is an important immune response system responsible for both pro- and anti-inflammatory processes. Here, we report the presence of inflammatory eicosanoids in the brains of a transgenic mouse model of NeuroHIV that expresses soluble HIV-1 envelope glyco-protein in glial cells (HIVgp120tg mice). Additionally, we report that the effect of LTC4S knockout in HIVgp120tg mice resulted in the sexually dimorphic transcription of COX- and 5-LOX-related genes. Furthermore, the absence of LTC4S suppressed ERK1/2 and p38 MAPK signaling activity in female mice only. The mass spectrometry-based lipidomic profiling of these mice reveals beneficial alterations to lipids in the brain. Given the availability and popularity of pharmaceuticals aimed at both COX (ibuprofen, naproxen, aspirin) and 5-LOX (zileuton, pranlukast, montelukast, zafirlukast) activity; combined with their relative safety and affordability, it is a tempting prospect to add these drugs into the daily drug regimen of the HIV positive population. Targeting the AA cascade may hold potential in the treatment of neuroinflammation observed in NeuroHIV and HANDs.

P78

HIV Innate Sensing in Microglia Activates the cGAS/STING Pathway of Inflammation

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A significant percentage of people living with HIV/AIDS (PLWHA) exhibit HIV associated neurocognitive disorders (HAND). With the development and improvement of combination antiretroviral therapies (cART) there was a substantial decrease of the death and morbidity rates of PLWHA. Despite the cART progress, PLWHA still present a high prevalence of chronic neuroinflammation, where the cellular mechanism(s) that drive such inflammation have not been entirely defined. Certain models of neuroinflammation induced

by HIV propose that the activation of microglia from infection results in the production of inflammatory cytokines and metabolites with nefarious outcomes to the brain. Here, we hypothesize that the GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) pathway of viral "innate sensing" is a major mechanism of intracellular innate immunity in microglia under current cART regimens. We find that markers associated with cGAS/STING inflammation are present on HIV positive post mortem brain tissues, including under cART. Further, we find that HIV-challenged iPSC-derived microglia (hMGL) activate the cGAS/STING innate sensing pathway by forming cytoplasmic complexes of Polyglutamine Binding Protein 1 (PQBP1) and cGAS on HIV particles soon after capsid integrity loss. We find that cGAS activation in hMGL is responsible for the production of metabolites that, in turn, skew iPSC-derived astrocytes into pro-inflammatory associated phenotypes. Further, by differentiating iPSCs into brain organoids containing microglia, astrocytes, and neurons and by performing cyclic multiplexed immunofluorescence we find a high level of HIV infected microglia, which results in a strong activation of astrocytes into inflammatory-associated phenotypes. These observations support a model where the activation of the cGAS/STING innate sensing machinery in microglia plays an important role in the disruption of normal brain homeostasis by inflammatory processes and contributing to HAND.

P79

Honokiol reverses HIV-associated neurocognitive disorders in a SCID mouse model

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Regardless of combined antiretroviral therapy (ART), mild HAND still commonly occur due to the incompetence of ART to eradicate brain HIV, and consequential persistent viral infection in the brain. Pathologically HAND appears to correlate with immune dysregulation, and ongoing presence of neurotoxic HIV proteins, which lead to progressively deteriorated neurocognitive function. Eventually, more severe forms of neurocognitive impairment, such as dementia may take place, and, in aging people with HIV (PWH) comorbidities likely increase susceptibility to HAND and accelerate progression. Therefore, adjunctive therapies need to be developed that reduce or eliminate brain HIV or limit cognitive impairment. Honokiol is known to exhibit anti-inflammatory and neuro-protective effects. CN is a synthetic Honokiol analogue and was administered in a SCID HAND mouse model in order to determine its ability to reverse cognitive and pathological features of HAND. Mice (n=24) were firstly intracerebrally inoculated with HIV-infected human monocyte derived macrophages (MDMs). Control group of mice (n=12) were injected with uninfected MDMs. Then half of the HAND mice (n=12) were administrated 3mg of CN intraperitoneally daily for 6 days, the other HAND mice were given saline. Cognition was assessed through object recognition testing (ORT) among three groups of mice before and after treatment. Mice were sacrificed after 1 week of treatment and the severity of neuroinflammation, including astrogliosis (GFAP), dendritic arborization (MAP2), microgliosis (MHCII+/CD45+), was evaluated by both histopathology and flow cytometry. Honokiol treatment reversed ORT abnormalities. Flow cytometry revealed that the percentage of activated astrocytes were significantly decreased in CN-treated HAND mice compared to untreated HAND mice. Additionally, CN also largely restored the changes of neuronal dendritic arborization compared to untreated HAND mice. Interestingly, CN promoted mouse mononuclear phagocyte infiltration into the CNS. Future investigations will focus on characterizing the molecular and immunological profiling of monocytes and microglia in this HAND mouse model.

P80

Development of Murine Models of Sleep Deficits in HIV-associated Neurocognitive Disorder

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Despite the widespread use of combined antiretroviral therapies (cART), HIV-associated Neurocognitive Disorder (HAND) afflicts nearly 50% of people living with HIV (PLH) with broad, deleterious impacts on quality of life. Simultaneously, almost 70% of individuals with managed chronic HIV infection exhibit behavioral aspects of insomnia, such as difficulty falling asleep, difficulty staying asleep, poor sleep quality, and low daytime energy levels, a rate 2.5-fold higher than the population at large. These comorbidities in tandem comprise a vicious cycle in PLH, for whom the physical, mental, and cognitive damage induced by poor-quality sleep is compounded by similar detriments induced by HIV infection. Despite the high incidence of sleep disorders in PLH and their significant impact on cognition, little basic research has examined the role of sleep in HAND and less yet has explored the interplay of potential therapeutics on sleep. Using the EcoHIV-infected mouse model for HAND, we found that EcoHIV-infected mice exhibited disrupted sleep during the early rest period and increased sleep fragmentation, similar to what has been reported in PLH but not yet described in murine models. JHU083, a novel glutamine antagonist shown to enhance cognitive function in HAND, ameliorated the EcoHIV infection-induced abnormal sleep phenotypes. To extend the application of these findings, we are evaluating sleep phenotypes in the humanized bone marrow-liver-thymus (BLT) mouse model of HIV-infection, to assess the role of sleep disturbances in HAND and evaluate the effect of cognition-enhancing HIV therapeutics on sleep. Successfully creating and characterizing these sleep disturbance models would allow the broader HIV community to test sleep abnormalities and evaluate new therapeutics.