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P1

Women with HIV have a lower frequency of monocyte reservoir reactivation compared to men with HIV

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There are known sex differences in HIV infection, latency, and cognitive outcomes. Women are overrepresented in cohorts of HIV controllers while also having higher rates of cognitive dysfunction. HIV persists in monocytes and contribute to neurological issues as infected monocytes cross the blood-brain barrier and infect brain macrophages. Therefore, we sought to assess if there were sex-differences in latency and reactivation of the monocyte reservoir in a large cohort of virally-suppressed people with HIV (vsPWH). Viral reservoirs were assessed in 176 vsPWH (56% female, 66% Black). Monocytes and CD4s were isolated from PBMCs and used in cell specific intact proviral DNA (IPDA) and quantitative viral outgrowth assays (QVOA). Monocyte IPDAs and monocyte-derived macrophage (MDM)-QVOAs were completed on 176 and 75 vsPWH, respectively. CD4-IPDAs and CD4-QVOAs were completed on 173 and 27 vsPWH, respectively. T cell contamination was assessed by flow cytometry and TCRβ qPCR, and mathematically removed from monocyte signal. 94% of vsPWH had detectable provirus in monocytes (median[md]= 58 copies[cp]/1e6) with 42% having detectable intact provirus (md=17cp/1e6). No sex differences were observed in the frequency or absolute quantity of provirus in monocytes. 24% of vsPWH had reactivatable monocyte reservoirs. Notably, while the infectious units per million values did not differ between sexes, the frequency of reactivation was significantly lower in women compared to men (13% vs 32%, $\chi 2$=4.4, P=0.036). No sex differences were observed in HIV DNA or reactivation frequency in CD4s. Our data suggest that the monocyte reservoir is less likely to reactivate in women with HIV despite having equivalent levels of HIV DNA as men. Further work is needed to determine if tissue resident macrophage reservoirs are also less likely to reactivate in women. These findings suggest that though women have greater cognitive dysfunction, this may not be due to reactivation of monocyte reservoirs.

P2

Hydrogen sulfide supplementation protects microglial cells against HIV Tat-induced ferroptosis

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The HIV Tat (Transactivator of transcription) protein plays a crucial role in HIV pathogenesis, not only by facilitating viral transcription but also by contributing to comorbidities such as NeuroHIV. HIV Tat interacts with key proteins involved in cellular compensatory pathways, exacerbating neuroinflammation and neurodegeneration in the central nervous system. Its effects have been shown to dysregulate microglial ferroptosis in vitro models, HIV transgenic rats, and postmortem brains of HIV-positive individuals. Ferroptosis is fundamentally characterized by the interplay between cellular insults that trigger lipid peroxidation – marked by increased expression of acyl-CoA synthetase long-chain family member 4 (ACSL4) – and the antioxidant systems that counteract this damage. The solute carrier family 7-member 11-glutathione peroxidase 4 (GPX4) antioxidant system represents a critical target for mitigating ferroptotic death. Hydrogen sulfide has emerged as a key endogenous signaling molecule with therapeutic potential in the nervous and cardiovascular systems. In this study, pretreatment with sodium hydrosulfide (NaHS), a hydrogen sulfide donor, significantly restored GPX4 levels in mouse BV2 microglial cells exposed to HIV Tat (100 ng/mL, 48 h). NaHS also demonstrated a concentration-dependent increase in ferritin heavy chain 1 while reducing ACSL4 expression. Also, NaHS (100 μM) maintained stable levels of 4-hydroxynonenal, a reactive electrophile involved in lipid peroxidation. Flow cytometric analysis revealed that NaHS effectively reduced HIV Tat-induced reactive oxygen species production, and it significantly attenuated HIV Tat-induced lactate dehydrogenase release in BV2 cells, indicating cytoprotective effects. These

findings highlight the potential of NaHS in modulating redox homeostasis and protecting against HIV Tat-induced ferroptosis in microglial cells, underscoring its therapeutic relevance in NeuroHIV.

P3

Making a better handle: Designing a computational tool for improving CRISPR-associated HIV-1 proviral targeting via sgRNA tracrRNA design

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In 2023, the human immunodeficiency virus type 1 (HIV-1) pandemic affected 39 million individuals worldwide. A latent reservoir of infected cells but not actively producing infectious HIV-1, poses a challenge for modern therapies, such as anti-retroviral therapy (ART). Although there is currently no cure, gene editing strategies based on a clustered regularly interspaced short palindromic repeats (CRISPR) system have demonstrated the ability to target and excise/inactivate the HIV-1 provirus by utilizing two or more guide RNAs (gRNAs) to direct cleavage via CRISPR-associated protein 9 (Cas9) endonuclease to desired sites within the viral genome. sgRNA gene editing delivery systems include a trans-activating CRISPR RNA (tracrRNA/handle), which interacts between the single guide RNA (sgRNA) spacer and Cas9 nuclease. Changes to these tracrRNA's have shown to increase editing or cleavage efficiency and include secondary structure, poly T tract, uridine removal, and a super stable loop. The composition and structure of the tracrRNA, is a critical constraint in a sgRNA's effectiveness, therefore tracrRNA modifications of sgRNAs targeting the proviral HIV-1 genome can increase editing efficiency. Our strategy is to construct a sgRNA tracrRNA library which includes modifications previously mentioned and validate sgRNA efficiency and off-target effects in targeting the HIV-1 provirus via high throughput screening. Our RNASculpt tool creates this library of tracrRNA sequences with secondary structures resembling a user given reference Cas9 tracrRNA. It accomplishes this by mutating the reference sequence and utilizing ViennaRNA -fold and -distance to predict a secondary structure and a similarity value between reference and mutated sequence. Results demonstrated RNASculpt created a library of Cas9 tracrRNAs with a suggested similarity value and structure. Use of this tool can allow for a more efficient and faster approach in creating and optimizing distinct sgRNAs, aiding in the future steps of selecting vector components in the delivery of HIV-1 gene editing machinery.

P4

Regulation of Circular RNAs in PBMCs from People with HIV and Human Cerebral Organoids: Implications for HIV-Associated Neurocognitive Disorders

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Circular RNAs (circRNAs) are a newly identified class of single-stranded RNAs characterized by their head-to-tail covalent joining. Although their functions remain largely undefined, circRNAs are currently being explored in various biomedical fields. Recent studies have characterized the differential expression of circRNAs in peripheral blood mononuclear cells (PBMCs) during the early stages of HIV-1 infection. After primary infection, HIV-1 enters the central nervous system (CNS) within days, establishing viral reservoirs that contribute to the development of HIV-associated neurocognitive disorder (HAND), a condition affecting approximately 42% of individuals living with HIV. Our preliminary microarray-based profiling of circRNA expression in PBMCs from 19 virally suppressed women living with HIV (WLWH), with or without HAND, revealed a set of differentially expressed circRNAs that correlate with the neurocognitive status of the HIV-1 donors. To further investigate these findings, we manipulated the

expression levels of selected circRNAs and analyzed their effects in a latently infected T cell line (JLat10.6). Notably, circ_000780, derived from the FAM107B gene of unknown function, was consistently downregulated in nearly all donor samples as well as in JLat10.6 cells. Overexpression of this circRNA led to reactivation of latent HIV-1 in JLat10.6 cells. We also adopted a 3D cellular model using cerebral organoids (COs) infected with HIV-1 to examine the circRNA profile in HIV-1 infected COs. Our results suggest that the circRNA expression in HIV-1 infected COs - whether in the presence or absence of antiretroviral therapy (ART) - presents a distinct profile compared to uninfected COs, warranting further investigation. Interestingly, circ_0007556, which has been shown to serve as the template for amyloid beta (Aβ) peptide transcription, appeared to be upregulated in HIV-1 infected COs, independent of ART treatment. Together, these findings suggest that the regulation of circRNAs by HIV may contribute to the complex mechanisms underlying HIV-associated neurocognitive diseases.

P5

CRISPR-Cas9-Mediated Genome Editing via AAV Vectors Suppresses HSV-1 Infection and Reactivation in 2D, 3D Culture Models, and a Latent Rabbit Keratitis Model

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Herpes simplex virus type 1 (HSV-1) is a significant cause of morbidity, including HSV-1 keratitis, a major global cause of blindness, and latent infections in the trigeminal ganglia. Although current antiviral treatments alleviate symptoms, no cure exists for HSV-1, particularly for latent infections. In this study, we developed a CRISPR-Cas9-based gene editing approach targeting HSV-1's ICP0 and ICP27 genes, both critical for viral replication and latency. Using Vero cells, 3D human induced pluripotent stem cell-derived cerebral organoids (COs), and a latent rabbit model of HSV-1 keratitis, we tested the effectiveness of AAV-CRISPR-Cas9 vectors expressing Staphylococcus aureus Cas9 (SaCas9) and paired guide RNAs (gRNAs). We observed a significant reduction in HSV-1 replication in infected Vero cells and in COs, with ICP0- or ICP27-targeting constructs leading to substantial viral suppression and reduced viral rebound in COs with latent infections. Furthermore, in the rabbit model, AAV-SaCas9 vectors delivered through corneal scarification reduced viral shedding by over 50%. With intravenous administration, viral shedding was completely eliminated in 92% of treated rabbit eyes, and the trigeminal ganglia in the IV administered rabbits showed reduced HSV-1 DNA and RNA expression. These findings demonstrate the potential of CRISPR-Cas9-mediated gene editing as an effective strategy for treating both acute and latent HSV-1 infections, including HSV-1 keratitis, and highlight its promise for eradicating the latent viral reservoir.

P6

The Human Neurotropic Polyomavirus, JC Virus, Small Tumor Antigen Promotes S Phase Entry and Cell Cycle Progression

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JC virus (JCV), a ubiquitous human polyomavirus, causes a devastating disease of the human brain known as progressive multifocal leukoencephalopathy in immunocompromised individuals, including AIDS and cancer patients, and in those who are treated with immunomodulatory drugs (Crohn's and Multiple Sclerosis disease patients). JCV also has oncogenic potential previously demonstrated in tissue culture and animal

models. The early coding region of JCV encodes two major oncogenic proteins, large T antigen (LT-Ag) and small t antigen (Sm t-Ag), due to the alternative splicing of early pre-mRNA transcripts. Although LT-Ag is known to play critical roles in cell transformation by targeting the key cell cycle regulatory proteins, including pRb and p53, such a role for Sm t-Ag in this process remains largely unknown. Sm t-Ag is a small protein consisting of 172 amino acids, 82 of which are shared with N-terminal sequences of LT-Ag. In the current study, we investigated whether Sm t-Ag affects the cell cycle progression in cells constitutively expressing this protein through which it contributes to cell transformation. Such studies revealed that it indeed facilitates S phase entry and exit when cells are released from G0/G1 growth arrest. Examination of the cell cycle stage-specific expression profiles of the selected cyclins and cyclin-dependent kinases, including those active at the G1/S and G2/M transition states, also demonstrated a higher level of early expression of these regulators, such as cyclin B, cyclin E, and Cdk2. In addition, analysis of the impact of Sm t-Ag on the growth-promoting pathways, including those active in the PI3K/Akt/mTOR axis, showed substantially higher levels of the phosphorylated-Akt, -Gsk3-b and -S6K1 in Sm t-Ag-positive cells. Collectively, our results demonstrate that Sm t-Ag promotes cell cycle progression by activating the growth-promoting pathways through which it most likely contributes to the LT-Ag-mediated cell transformation.

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P7

Cannabidiol (CBD) treatment modulates the cortex and striatum transcriptome of EcoHIV infected mice and reverses virus mediated cognitive impairment.

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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 chronically EcoHIV infected mice to investigate the effects of CBD administration on brain transcriptome profiles, behavioral outcomes and virus load. Our studies show that infected mice treated with CBD present better behavioral outcomes than those untreated. Furthermore, virus load was reduced in the brain of EcoHIV infected mice treated with CBD. RNA sequencing in prefrontal cortex and striatum identified transcriptional signatures in HIV infected mice compared to uninfected controls that included dysregulation of immune-related genes and genes related to synaptic transmission and other neuronal functions. The dysregulation of some of these genes and pathways was restored by CBD treatment. Our data indicates that CBD can have beneficial effects in mitigating some HIV deleterious effects in the brain.

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P8

CRISPR-engineered stimulation of IL-15 signaling improves NK cell effector function

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Like many other immune cell types, NK cells show impaired effector activity during chronic HIV infection. This leads to their inability to efficiently target and eliminate infected cells and persistence of the HIV reservoir. Recently, adoptive NK therapy involving activated or engineered NK cells has become a promising cancer immunotherapy approach. However, its use for HIV is still in early research and developmental stages. NK cells primarily rely on IL-15 signaling for the development and maintenance of

populations physiologically, and dysregulated IL-15 signaling is observed in people living with HIV. IL-15 binds to IL-15Ra intracellularly and is then trans-presented by myeloid cells to IL-2b/common gamma receptor complex expressing NK cells. We are currently investigating two strategies to boost IL-15 signaling in NK cells, anticipating improved proliferative and effector abilities and leading to increased clearance of HIV reservoirs. 1) Utilizing the CRISPR-activation (CRISPRa) platform to induce IL-15/IL-15Ra expression in NK cells and 2) CRISPR-knockout of CISH gene, a negative regulator of IL-15-induced JAK/STAT signaling. CRISPRa-IL-15/IL-15Ra-treated primary NK cells showed significantly increased transcription levels of IL-15 and IL-15Ra via RT-qPCR and a marked increase in surface expression of IL-15Ra via flow cytometry. This led to improved proliferation, metabolic activity, and cytotoxicity of CRISPRa-IL-15/IL-15Ra compared to CRISPRa-control treated NK cells. Similar effects were observed in CRISPR-CISH-KO-treated NK cells. These proof-of-concept data enabled the initiation of currently ongoing in vivo studies with the long-term goal of developing adoptive engineered NK therapy for HIV.

P9

Retroviral infection of human neurospheres and use of stem Cell EVs to repair cellular damage

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HIV-1 remains an incurable infection that is associated with substantial economic and epidemiologic impacts. HIV-associated neurocognitive disorders (HAND) are commonly linked with HIV-1 infection; despite the development of combination antiretroviral therapy (cART), HAND is still reported to affect at least 50% of HIV-1 infected individuals. It is believed that the over-amplification of inflammatory pathways, along with release of toxic viral proteins from infected cells, are primarily responsible for the neurological damage that is observed in HAND; however, the underlying mechanisms are not well-defined. In recent years, neurospheres derived from induced pluripotent stem cells (iPSCs) have been utilized to model the effects of different neurotropic viruses. Here, we report the generation of neurospheres from iPSC-derived progenitor cells and we show that these cultures are permissive to retroviral (e.g. HIV-1, HTLV-1) replication. In addition, we also examine the potential effects of stem cell derived extracellular vesicles (EVs) on HIV-1 damaged cells as there is abundant literature supporting the reparative and regenerative properties of stem cell EVs in the context of various CNS pathologies. We also observed substantial increases in TNFα, IL-8, and IL-1β after HIV-1 infection, and EVs from either iPSC or MSC EVs restored the expression of TNFα, IL-8 and IL-1β to levels that were comparable to the uninfected control. We identified multiple protein and RNA markers associated with regulations of cytokines, including identification of at least two EV-associated lncRNAs (i.e. AC120498.9, ADIRF-AS1) whose secondary structures contained sequences of double-stranded RNA ≥ 30 bp in length that led us to examine their interactions with different RNA binding proteins including PKR. Taken together, this data indicates that EVs are capable of exerting both neuroprotective and anti-inflammatory effects in HIV-1 infected neurospheres.

P10

The Hidden Role of VZV Reactivation in Neurodegenerative Diseases and Stroke

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Varicella Zoster Virus (VZV) infects over 95% of the global population. Following primary infection, it causes chickenpox, after which the virus establishes lifelong latency in neuronal ganglia. Under conditions such as aging or immunosuppressive events (e.g., medications, stress), VZV can reactivate, leading to the painful skin rash known as shingles (zoster). Increasing evidence from large-scale epidemiological studies suggests that VZV reactivation may also play a significant role in the development of severe neurological

diseases, including vascular dementia, Alzheimer's disease (AD), and stroke. However, a major challenge in confirming VZV's causative role has been the absence of detectable infectious virions in affected tissues. In this study, we identified a novel mechanism where VZV-induced extracellular vesicles (EVs) contribute to these neurological diseases, even in the absence of infectious virions. Specifically, we demonstrated that non-infectious EVs derived from plasma of zoster patients can trigger prothrombotic and proinflammatory responses months after rash resolution and antiviral treatment. Additionally, EVs from VZV-infected human trigeminal neurons were found to impair the phagocytic activity of macrophages and microglia towards Aβ42, exacerbating amyloid accumulation in AD models. Analysis of EV content revealed a single VZV protein (immediate early 62) and several miRNAs, which are under investigation as potential causal agents. These findings introduce a novel mechanism by which VZV may contribute to neurologically linked diseases, even without the presence of infectious virions in the affected tissues.

P11

Ethanol-Induced Alternative Splicing of Mcl-1 in Human Neural Progenitors and Organoids: Implications for Alcohol Toxicity and Brain Development

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Alternative splicing and the expression of splice variants in the brain play a key role in modulating protein functions, which may influence behaviors associated with alcohol dependence and neurotoxicity. However, the development of suitable in vitro human culture models that accurately mimic brain development remains essential for studying these effects. Recent advancements in induced pluripotent stem cell (hiPSC) culture techniques and 3D brain organoid systems have enabled the creation of both 2D and 3D models, offering promising platforms to explore the impact of alcohol on brain development and function. In this study, we generated and characterized neural progenitor cells (NPCs) and human cerebral organoids (hCOs) from hiPSCs to investigate the effects of alcohol on brain development. Our findings reveal that ethanol exposure at different stages of hCOs development significantly impacts organoid viability and growth. Early-stage exposure led to complete loss of viability, while later exposure resulted in reduced organoid size and severe structural damage. Histological analysis further demonstrated the complete loss of neuronal cultures in mature hCOs. We also observed that hiPSCs, NPCs, and immature hCOs exhibited heightened sensitivity to alcohol-induced toxicity, while more mature hCOs showed increased resistance. In line with our previous studies, which demonstrated that ethanol exposure induces alternative splicing of Mcl-1 (a key mediator of neurotoxicity in the developing fetal brain), this study used semi-quantitative and quantitative analyses to assess Mcl-1 pre-mRNA splicing. We found that ethanol exposure significantly decreased the Mcl-1L/Mcl-1S ratio in hiPSCs and NPCs, with no significant effect on mature hCOs. These results underscore the critical role of Mcl-1 in alcohol-induced neurotoxicity and suggest that the Mcl-1L splice variant confers a protective effect against ethanol toxicity in hiPSCs and NPCs. This discovery highlights a novel mechanism that contributes to the increased sensitivity of neural progenitors to alcohol-induced toxicity.

P12

Antiretroviral drugs may affect autophagic processes in primary human astrocytes differently, contributing to HIV-NCI

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Despite the great success of antiretroviral therapy (ART), HIV associated Neurocognitive Impairment (NCI) remains a significant comorbidity, decreasing quality of life and increasing risk of mortality for people with HIV (PWH). ART itself may contribute to HIV-NCI by impacting astrocyte autophagy. Autophagy removes unwanted/ damaged material from the intracellular environment in a lysosome-dependent manner. Astrocytes, abundant CNS cells with crucial functions, maintain homeostasis, in part,

by autophagy. Dysregulated astrocyte autophagy contributes to neurodegenerative diseases, but few studies have examined ART effects on astrocyte autophagy in the context of HIV-NCI. We treated primary human astrocytes for 24h or 7 days daily with Tenofovir+Emtricitabine+Dolutegravir (ART), and performed LC3 and p62 turnover assays by Western blotting (WB). ART significantly inhibits LC3 and p62 turnover at both time points (n=9-10, $p<0.05$), indicating autophagy is inhibited. P62 is one autophagy adaptor that mediates mitophagy, the targeted degradation of damaged mitochondria. Mitophagy is vital for homeostasis. Since p62 turnover is inhibited, we hypothesized that ART also inhibits mitophagy. We examined mitophagy in astrocytes after 24h or 7 days daily ART. There is a trend (24h), and significant decrease (7 days daily; n=10, $p<0.05$) in BNIP3L/Nix homodimer. There is no change in Cytochrome C oxidase subunit 2, PINK1, or PARKIN by WB. Mitochondrial mass, measured by flow cytometry, was unchanged (n=10). We are characterizing mitophagy further with a HaloTag-based mitophagy reporter. Astrocytes express the transduced HaloTag reporter properly in the mitochondria, and the HaloTag enzyme is functional. These data highlight that antiretroviral drugs may have distinct effects on autophagic processes in astrocytes, which may alter their homeostasis. Understanding the impacts of ART on astrocyte autophagy is important for improving ART for PWH who have, by necessity, long-term exposure to antiretrovirals. It also facilitates development of new therapies for HIV-NCI, a burdensome comorbidity, that may include autophagy modulation.

P13

A Scalable Platform for Producing and Preserving Stem Cell Extracellular Vesicles with Reparative Properties

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Extracellular vesicles (EVs), particularly those derived from mesenchymal stem cells (MSCs), offer novel solutions to many challenges faced by the biopharmaceutical manufacturing industry. EVs from stem cells are natural carriers of nucleic acids, proteins, and lipids and can modulate intercellular communication and contribute to tissue regeneration. Relative to traditional stem cell-based therapies, EVs present fewer safety concerns due to their inability to divide and differentiate, reduced immunogenicity, and enhanced stability. To date, the reparative properties of MSC EVs were studied across various pathologies, demonstrating their suitability for a wide range of future therapeutic applications. However, there is a need to develop and standardize robust methodologies for the production of well-characterized, functional MSC EVs. We recently coupled our large-scale, 3D biomanufacturing capabilities with our well-established EV isolations protocols to mass-produce functional EVs from immortalized MSCs. Isolated EVs undergo stringent quality control assays including Nanoparticle Tracking Analysis (NTA) to assess particle concentration and size distribution, western blot to assess expression of EV-associated markers, and sterility testing. We have also performed extended characterization assays, including mass spec proteomic analysis and multiplex surface marker profiling to better assess the expression of EV-associated molecular cargo. Our data shows clear distinctions in the biochemical and physical properties of EVs derived from MSCs relative to EVs derived from non-MSCs. We evaluated EV functionality in vitro using physiologically relevant 2D and 3D cell types including primary skin cells, immune cells, and cells of the central nervous system (CNS). Our assay readouts include EV uptake, cell viability, immune activation, angiogenesis, and expression of apoptotic proteins, inflammatory cytokines, and cell cycle regulators. Our data demonstrates MSC EVs are able to promote cell migration, reduce inflammation, and reverse cellular death across multiple cell types. Overall, this data demonstrates our ability to reproducibly manufacture MSC EVs. Furthermore, highlighting the ability of these EVs to reduce cellular damage and promote reparative phenotypes. Collectively, MSC EVs represent an innovative and scalable solution with high potential to revolutionize the future of next generation therapeutics and biopharmaceuticals.

P14

Gene expression dynamics in neurons and astrocytes following increased dosing of frontline antiretrovirals in an iPSC neuroglial model

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While antiretroviral therapy (ART) has markedly improved life expectancy for people with HIV, they remain disproportionately affected by neurocognitive dysfunction. Accumulating evidence indicates that ART may contribute to neuronal injury and sustained neuroinflammation through chronic glial activation in the setting of prolonged viral suppression. Studying these effects in humans remains challenging due to limited access to CNS tissue samples and the limitations of rodent models in capturing HIV-specific cellular responses. To address this, we employed a human induced pluripotent stem cell (iPSC)-derived coculture model consisting of excitatory cortical neurons (iNs) and astrocytes (iAs) to assess the functional impact of commonly prescribed antiretroviral drugs (ARVs). The iAs-iNs cocultures were exposed to increasing concentrations of ARVs, guided by their reported peak plasma levels in the human CNS (Cmax), for 10-14 days. Neuronal function was assessed via multi-electrode array, astrocytic function was evaluated through glutamate reuptake assay, and transcriptomic changes were profiled using single-nuclei RNA sequencing. ARV exposure resulted in dose-dependent cytotoxicity; notably, higher concentrations led to increased cell death and suppressed spontaneous neuronal firing. Additionally, iAs in iAs-iNs cocultures exhibited functional impairment, including significantly reduced glutamate reuptake. Transcriptomic profiling revealed a greater degree of transcriptional alteration in iAs than in iNs. Unexpectedly, the different doses of ARVs led to distinct gene expression profiles, rather than dose-dependent increases in the same pathways. Together, these findings highlight the vulnerability of iPSC-derived neuroglial networks to ARVs and underscore the need to understand the functional impact of ART in the CNS of people with HIV.

P15

Role of endolysosomal oxidative stress in HIV-1 Tat-induced neuronal injury

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HIV-1 Tat continues to contribute to development of HIV-associated neurocognitive disorders (HAND) in ART era. Among many other underlying mechanisms, Tat-induced oxidative stress in cytosol, mitochondria, and nucleus plays an important role in its neurotoxic effect. However, little is known about the role of endolysosome specific oxidative stress in the neurotoxic effect of Tat, especially when endolysosomes represent as early steps of exogenous Tat-induced synaptodendritic impairment. Consistent with our previous work that Tat enters neuronal endolysosome and induces endolysosome de-acidification, we demonstrated that Tat induces endolysosome membrane leakage and increased endolysosome oxidative stress, an early event prior to mitochondrial dysfunction. In the present studies, we test the hypothesis that Tat-induced lysosomal oxidative stress leads to endolysosome damage and synaptodendritic impairment. We developed an endolysosome-targeted hydrogen peroxide sensor, using which we showed that Tat induces increase in endolysosomal oxidative stress likely by activating endolysosome-resident NOX2. Such Tat-induced endolysosomal oxidative stress could be sensed by endolysosome-resident ROS sensor TRPM2. Significantly, inhibiting NOX2 or blocking TRPM2 attenuates Tat-induced endolysosome damage and dendritic impairment. Thus, our findings suggest that endolysosomal oxidative stress plays an early and important role in Tat-induced neuronal injury.

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P16

Neuroinvasion of highly pathogenic avian influenza A(H5N1) viruses of clade 2.3.4.4b

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Highly pathogenic avian influenza (HPAI) A(H5Nx) viruses of the A/Goose/Guangdong/1/1996 (gs/GD) lineage have demonstrated pronounced neurotropism in wild mammals including dolphins, polar bears, and seals as well as in experimental animal models including ferrets and mice. The distribution of clade 2.3.4.4b A(H5N1) virus in the brain and the CNS of infected animals is distinct from that of earlier gs/GD lineage viruses, although the driving factors are unknown. Access to the brain via the olfactory bulb and olfactory nerve is the conventional theory of neuroinvasion for HPAI influenza viruses; however, clade 2.3.4.4b viruses isolated from the brain of dolphins challenge this theory. The purpose of this study was to understand the enhanced neurotropic potential and neuropathogenesis of HPAI A(H5N1) viruses of clade 2.3.4.4b through examination of the susceptibility of human neuronal cells and the route of movement from intranasal infection to the brain. Infections (1, 0.1, 0.01 MOI) of human (ReNcell) differentiated neurons from the ventral mesencephalon with H5N1 (clade 1 and 2.3.4.4b) viruses demonstrated severe neurodegeneration and cytotoxicity with cell death observed as early as 24hpi. No infection was observed with A(H1N1) (pandemic, seasonal) virus. Interestingly, hCMEC/D3 (blood-brain barrier) could sustain viral replication with all viruses tested. Ferrets infected with A/lesser scaup/GA/W22-145E/2022 (H5N1; clade 2.3.4.4b) showed severe neurological symptoms, including seizures, inflammation in the neck, unbalanced movement, and hemiplegia. Preliminary histology data showed a mixture of positive and negative viral antigens in the olfactory bulb. These data suggest that the neuropathogenesis of A(H5N1) clade 2.3.4.4b viruses is driven by rapid virus dissemination with multiple routes of neuroinvasion

P17

Extracellular vesicle-associated HIV Tat is associated with cognitive impairment and learning deficiencies

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Background: The HIV-Tat protein is produced despite virological control with antiretroviral therapy (ART) and is present in the sera of persons with HIV (PWH). As Tat is both neurotoxic and activates latent virus it is an important target for adjunctive therapeutic development. Quantifying Tat is important for monitoring responses to anti-Tat therapeutics. Here we quantify Tat in the sera of PWH via extracellular vesicle (EV)-ELISA and correlate the presence of Tat to neurologic disease. We also determined the cellular origin of Tat-associated EVs and investigated the sub-vesicle location of Tat in EVs.

Methods: EVs were isolated from transfected cells and sera from PWH on ART (n=204) and HIV negative controls (n=21) via differential ultra-centrifugation. EVs were quantified using nanoparticle tracking and confirmed biochemically. Tat within EVs was visualized with super-resolution microscopy (dSTORM). Tat presence was correlated to cognition using global deficit score (GDS), overall T-score, and individual domain T-scores. EVs containing Tat from sera were captured by immunoprecipitation and immunoblotted for cellular origin markers.

Results: Only a fraction of Tat released from transfected cells was associated with EVs where it is primarily localized within the lumen. Hence capture of Tat-associated EVs by ELISA required membrane disruption. EV-associated Tat was present in 24.5% (50/204) of PWH and in no (0/21) controls. Presence of EV-associated Tat in sera correlated with clinical impairment by GDS (P=0.034) and overall T-score (P=0.046), which was driven by impairment in the learning domain (P=0.0094; all Fisher's exact). One third of PWH with Tat had learning impairment compared to 12% without Tat. EVs containing Tat also contained CD4 suggesting the EVs originated from HIV-infected T cells.

Conclusion: Tat is produced despite suppressive ART. EV-associated Tat in the sera of PWH correlates with cognitive impairment. The EV-ELISA for Tat detection may be useful for monitoring responses to anti-Tat therapeutics.

P18

HIV-Nef in serum is a biomarker for microvascular disease and causes endothelial cell damage and neurotoxicity

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Currently available antiretroviral therapies (ART) have no effect on the production of HIV transcripts once the virus is integrated in the chromosome. We previously found HIV-1 RNAs are present in extracellular vesicles (EVs) derived from cerebrospinal fluid (CSF) and plasma in persons with HIV (PWH) who are well controlled on ART. EVs are small membrane bound vesicles that are released from every cell type, they are heterogeneous in size and cargo and are reflective of their cell of origin. Thus, EVs can be utilized to enhance the resolution of pathogen detection as they may contain viral cargo. In our recently published study of 85 PWH, we found that EV-associated viral RNA correlated with neurocognitive deficits and the transcripts were enriched in the CSF. In our current work, a high-resolution viral detection assay was used to assess the relationship between transcription from latent viral reservoirs and brain MRI volumetrics. Analysis shows the HIV-1 nef in serum correlated with brain atrophy, neuronal damage, and small vessel disease as measured by brain MRI. This was confirmed using immunohistochemistry on postmortem brain tissue which showed perivascular immunostaining for Nef in glial cells. In vitro experiments suggest that Nef protein can damage brain endothelial cell tight junctions using a cell impedance assay and can also cause neurotoxicity. Our findings suggest that despite adequate control of HIV infection with ART, HIV-Nef is expressed in perivascular glial cells which may cause microvascular damage and neurotoxicity. It is also released in EVs which may serve as a biomarker. These findings suggest a relationship between latent viral transcription and CNS complications of long-term disease and point to novel mechanisms that contribute to dysfunction in infected individuals that can be targeted to achieve a functional cure.

P19

Prophylactic effect of a small molecule, C381 in alleviating HIV Tat-induced microglial activation and neuroinflammation

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HIV Tat (transactivator of transcription), a viral protein, plays a crucial role in driving microglial activation, creating a proinflammatory milieu that contributes to neuroinflammation and neurodegeneration. Previous studies have implicated mitochondrial dysfunction, impaired mitophagy, and cytotoxicity as key mechanisms underlying HIV Tat-induced microglial activation. Targeting these pathways could offer potential therapeutic strategies to mitigate HIV-associated neuroinflammation. This study investigated the protective effects of a small molecule C381 against HIV Tat-induced microglial activation and neuroinflammation. Mouse primary microglial cells (MPMs) were pretreated with C381 (30 μM) for one

hour, followed by exposure to HIV Tat (50 ng/ml) for 24 hours. Microglial activation, mito/autophagy, and lysosomal function markers were assessed using western blot analysis, while proinflammatory cytokine expression was evaluated via qPCR. Mitochondrial metabolic changes (oxygen consumption rate- OCR, and extracellular acidification rate- ECAR), mitochondrial membrane potential (MMP), and mitochondrial ROS was evaluated using the seahorse assay, and staining with JC1 dye, and MitoSox, respectively. HIV Tat exposure led to increased expression of activation markers (CD11B), mitophagy regulators (DLP1, PINK1), and autophagy markers (Beclin1, LC3B-II, p62), resulting in mitochondrial dysfunction. Exposure of mouse primary microglia to HIV Tat caused decreased MMP, increased mitochondrial ROS, and decreased OCR, and ECAR. Interestingly, pretreatment of MPMs with C381 resulted in restoration of MMP, ROS, OCR as well as ECAR. Notably, C381 pretreatment attenuated microglial activation, restored mito/autophagy flux, and improved mitochondrial function. These findings suggest that C381 has significant therapeutic potential in alleviating HIV Tat-mediated microglial activation and neuroinflammation, offering a promising strategy for mitigating HIV-associated neurotoxicity.

P20

Brincidofovir Inhibits Epstein-Barr Virus and Related Gammaherpesvirus in Human and Nonhuman Primate Cells

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Multiple sclerosis (MS) is a progressive neuroinflammatory disease characterized by areas of demyelinated lesions that are disseminated in space and time in the central nervous system. Neuroinflammatory diseases, including MS, have multifactorial etiologies associated with genetic susceptibilities, dysregulated immune responses, and environmental triggers. Examples of environmental triggers include viruses such as Epstein-Barr virus (EBV). EBV is of growing interest for its potential role in neurodegenerative diseases such as MS and it has been hypothesized that the reactivation of EBV can lead to the onset of MS symptoms in predisposed individuals. Brincidofovir (BCV) is broad-spectrum antiviral treatment that has proven to be effective against double-stranded deoxyribonucleic acid (dsDNA) viruses, such as smallpox and EBV. The effects of BCV on EBV reactivation were evaluated in vitro using EBV-infected spontaneous lymphoblastoid cell lines (SLCLs) and EBV-infected peripheral blood mononuclear cells (PBMCs) derived from MS patients and healthy controls. In addition, a B lymphoblastoid cell line and PBMCs from common marmosets (Callithrix jacchus) naturally infected with an EBV-related gammaherpesvirus (Callitrichine herpesvirus 3, CalHV-3) were used to measure BCV efficacy in a nonhuman primate model. BCV significantly inhibited gammaherpesviruses, with decreased lytic and latent viral transcript expression, particularly in those individuals who were shown to reactivate EBV. These results suggest that BCV may be a useful antiviral for inhibiting EBV activity in MS patients and suggests a method for selecting MS patients for possible inclusion in an anti-herpesvirus clinical trial due to their ability to reactivate EBV. Additionally, this work further validates the utility of CalHV-3 in marmosets as a translational model for the investigation of successful EBV-targeting therapeutics.

P21

Reactivation of Epstein Barr Virus in Cells from Multiple Sclerosis Patients and Healthy Controls

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Epstein-Barr virus (EBV) is a ubiquitous gammaherpesvirus that has been identified as a possible trigger for multiple sclerosis (MS). The reactivation of EBV in vivo is thought to be one possible mechanism for disease progression in MS. A combinatorial treatment of sodium butyrate (NaB) and tetradecanoyl phorbol acetate (TPA) was used in vitro to reactivate EBV in peripheral blood mononuclear cells (PBMCs) and EBV infected spontaneous lymphoblastoid cell lines (SLCLs) derived from MS patients and healthy controls. Treatment with NaB and TPA significantly increased viral lytic and latent mRNA expression in both SLCLs and PBMCs, demonstrating successful reactivation. The reactivation of EBV in PBMCs significantly correlated with the ability to generate an SLCL. These results suggest that the ability of EBV to reactivate is not universal, despite the virus' ubiquitous prevalence. Thus, assessing viral reactivation may be a valuable tool for cohort selection in studies of antivirals which target EBV in MS.

P22

HIV-1 Tat dysregulates glial glutamate transporter expression in vitro and in vivo

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Since the implementation of accessible anti-retroviral therapy (ART), the most severe acute manifestations of human immunodeficiency virus type 1 (HIV-1) infection have largely been mitigated. However, various comorbidities persist, including HIV-1-associated neurocognitive disorders (HAND). While the most severe forms of HAND have been reduced alongside a decrease in immune deficiency, less severe forms of HAND remain and are estimated to affect 15 to 50% of PWH. This persistence of cognitive impairment in the context of viral suppression suggests other mechanisms underlying central nervous system (CNS) symptoms. The HIV-1 Transactivator of transcription (Tat) is detectable in the cerebrospinal fluid of PWH and has been well characterized for its neurotoxic effects. The body of literature on Tat toxicity is centered on the use of the 86-amino acid (86aa; Tat86) form. Due to the prominence of the 101aa sequence in PWH, we aim to establish a model utilizing Tat101 to examine Tat-induced glutamate toxicity and potential therapeutic targets. We show that treatment of glial cell cultures with low concentrations of recombinant Tat101 recapitulates glutamatergic mechanisms of toxicity. Tat101 exposure downregulates astrocytic EAAT2 protein in culture, validating EAAT2 as a therapeutic target for Tat-induced glutamate toxicity. Further, Tat101 injected pre-frontal cortex (PFC) tissue also exhibits downregulation of EAAT2 transcripts independent of previously reported transcriptional modulation. Conversely, Tat101-exposed microglia exhibit a trend toward upregulation of the cystine-glutamate antiporter xCT. Future studies will extend these findings in a lentiviral transduction system to model how expression of Tat from integrated DNA in a small population of glial cells, similar to the CNS reservoir of individuals on ART, can influence glutamatergic dysfunction and, ultimately, cognitive impairment in vivo.

P23

Defining differences in CRISPR gene-editing repair pathways in HIV-1-targeted cells

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CRISPR gene editing of the integrated HIV-1 proviral DNA creates double-strand breaks within the provirus which are preferentially repaired by the host's double-stranded break (DSB) repair proteins involved in the nonhomologous end-joining (NHEJ) repair pathway. The repair of these breaks introduces mutations and indels that inhibit replication of the HIV-1 proviral DNA. Though these repair proteins are known to be involved in CRISPR-mediated HIV-1 proviral DNA editing, less is known about their expression in cells targeted by HIV-1. In addition, little is known about how activation influences the expression of these proteins and whether their expression levels affect the efficiency of CRISPR editing. Here, we sought to assess the expression of NHEJ repair proteins namely Ku70, LIG4 and DNA-PKcs in CD4 T cells (Jurkats) and microglia (HMC3). To do this, western immunoblot densitometry analysis of these proteins was performed using extracts derived from CD4 T cells and microglia under resting and activated states. The results show that CD4 T cells and microglia exhibit differences in their expression of Ku70, LIG4 and DNA-PKcs under resting and activated states. Of note, CD4 T cells expressed higher levels of LIG4 compared to microglia. However, activation did not impact expression of Ku70 and LIG4 expression in microglia. Our goal is to map out the expression profile of all NHEJ repair proteins in HIV-1-targeted cells and assess the impact of cellular activation on the expression of these proteins. Since these NHEJ repair proteins are involved in CRISPR-mediated HIV proviral DNA editing, we plan on determining the impact of NHEJ repair protein expression differences on CRISPR editing efficiency.

P24

CSF and Plasma Biomarkers of Neuroinflammation Predict Neurocognitive Performance in HIV: A Longitudinal Analysis

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This study examined the relationships between cerebrospinal fluid (CSF) and plasma biomarkers of inflammation and neurodegeneration, and their association with neurocognitive performance in HIV infection. Data from 79 participants (50 from A5090 and 29 from A736), predominantly male (94%) with a median age of 45 years, were analyzed. Significant positive correlations were observed between blood plasma and CSF levels of neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), and tumor necrosis factor-alpha (TNFα) at both baseline and week 24. Neurocognitive performance, measured by NPZ-8 scores, showed modest improvement from baseline (median -0.5) to week 24 (median -0.4). Linear regression analyses identified significant relationships between plasma interleukin-6 (IL-6) and interferon gamma-induced protein 10 (IP-10) levels and NPZ-8 scores at baseline, while CSF TNFα levels were significantly associated with NPZ-8 scores at week 24, after controlling for sex and other covariates. Correlation analyses of HIV-related and Alzheimer's-related biomarkers revealed significant interactions, particularly between GFAP and several Alzheimer's biomarkers (amyloid-beta 42 [Aβ42], phosphorylated tau 181 [P-Tau181], and phosphorylated tau 217 [P-Tau217]) in CSF at week 24. These findings suggest complex relationships between inflammatory markers, neurodegeneration, and cognitive function in HIV, with potential implications for understanding the pathophysiology of HIV-associated neurocognitive disorders and their relationship to aging-related neurodegenerative processes.

P25

Activation of the lysosomal cation channel controls oligodendrocyte actin polymerization and prevents bictegravir-mediated inhibition of oligodendrocyte maturation

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Antiretroviral therapy (ART) has led to a reduction in the most severe forms of HIV-associated neurocognitive disorders (HAND); however, cognitive impairment continues to persist in approximately 30-50% of people living with HIV. White matter (WM) alterations persist despite peripheral suppression by ART, suggesting that antiretrovirals (ARV) may directly contribute to WM structural and functional deficits. Work in our laboratory has revealed that the frontline integrase strand transfer inhibitor, bictegravir (BIC), inhibits oligodendrocyte maturation and remyelination through lysosomal de-acidification; however, this blockade is not complete and there are oligodendrocytes that are able to mature. Therefore, we sought to determine whether, despite expression of mature myelin lineage markers, oligodendrocytes exposed to BIC exhibit any deleterious alterations. Using our well-established cell culture system of OPC purification and differentiation, we demonstrate that proteolipid protein (PLP)+ oligodendrocytes treated with BIC exhibit significantly shorter processes and less complex branching compared to controls. Conversely, cells treated with the lysosomal cation channe TRPML1 agonist MLSA1, previously shown to prevent BIC-mediated inhibition of oligodendrocyte maturation, had significantly greater morphologic complexity. To determine how BIC might affect oligodendrocyte process extension, we first sought to examine how TRPML1 activation influenced this function. Activation of TRPML1 results in a significant increase in the ratio of F/G-actin and phalloidin staining intensity, indicative of actin polymerization, an essential step in oligodendrocyte process outgrowth. TRPML1 has been shown to control actin polymerization via activation of the Rac1/PAK pathway, a known master regulator of actin polymerization, in OPCs. We demonstrate that BIC treatment significantly decreases the phosphorylation of PAK1, suggesting that this ARV disrupts process extension by perturbation of the actin cytoskeleton in oligodendrocytes. Taken together, our work confirms that Rac1/PAK cytoskeletal rearrangement is regulated by TRPML1 in maturing oligodendrocytes which is disrupted by BIC, a previously unknown mechanism by which BIC impacts oligodendrocyte maturation.

P26

Pharmacologic induction of Wnt/β-catenin and AMP-activated protein kinase signaling alleviates ART-induced mitochondrial stress

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People with HIV (PWH) experience numerous non-AIDS co-morbidities (NACs) such as metabolic syndrome, diabetes, bone loss, cardiovascular disease and neuroinflammation. NACs is likely attributed to cellular stress associated with HIV infection and/or antiretrovirals (ART). Our group and others have demonstrated that ART induces mitochondrial dysfunction, cell cycle arrest and DNA damage, increased inflammatory cytokine production and dysregulation of gene expression in immune cells such as monocyte-derived macrophages (MDMs). In this study, we evaluated targetable mechanisms to alleviate mitochondrial stress observed in the presence of ART. Efavirenz (EFV), while is not used clinically, it is associated with a great deal of neurotoxicity, allowing us to assess targetable mechanisms of mitochondrial dysfunction. We treated primary human PBMCs with EFV both without and in combination with metformin and lithium chloride. Metformin engages the AMP-activated protein kinase signaling while lithium chloride activates Wnt/β-catenin signaling. In the presence of EFV, we observed an increase in mtDNA copy number, increased membrane polarization via mitotracker red staining, and a decrease in oxygen consumption rate (OCR). When PBMCs were treated with EFV, in the presence of either metformin or lithium chloride, OCR was increased, suggesting that metformin and lithium chloride were rescuing the

toxic effects of EFV. Our studies suggest that engaging both the Wnt/β-catenin and AMP-activated protein kinase signaling pathways have beneficial effects to reverse EFV-mediated mitochondrial stress.

P27

iPSC-Derived Brain Cells and Brain Organoids to Model Polysubstance Use in the Context of HIV

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Background: Both drugs of abuse (DOA) and HIV infection can deregulate brain cells crosstalk and therefore break homeostatic signals, leading to interesting parallels between HIV-1 neuropathogenesis, and the toxic effects induced by consuming DOA.

Methods: iPSC-derived astrocytes (iA), dopaminergic and cortical neurons (iDOPA, iCORT), microglia iMG, and cerebral brain organoids containing microglia (MCOs) were used to model brain homeostasis. iMG were infected with macrophage-tropic HIV-1 (AD8) or a single round HIV-1. Cultures were treated on day 1 and 3 post-infection with METH, oxycodone (OXY) or combination of both drugs (polysubstance) and compared to untreated controls. A decrease/increase in the HIV RNA-to-DNA ratio analyzed by qPCR was used to measure the extent of the drug effects on viral replication. Western blot and microscopy were used to evaluate the impact of drugs on iA, iMG and neuronal markers of activation or degeneration.

Results: DOA did not modify HIV expression in iMG monocultures. METH increased HIV replication in iMG + iA cocultures, but no effects were observed with OXY or polysubstance. Microscopy and Western blot analysis revealed that METH induced iA activation, increasing GFAP and AQP4 expression and reducing Nurr1 expression. qPCR analysis of iDOPA + iMG/HIV cocultures exposed to the DOA did not reveal changes in HIV expression. In tricultures where astrocytes were also present, DOA increased HIV expression. qPCR analysis of the iCORT + iA + iMG triculture exposed to HIV-1 and treated with DOA showed that METH and more dramatically polysubstance can increase HIV replication (5-fold increase). Similarly, in HIV-infected MCOs, OXY and polysubstance increased HIV replication.

Conclusions: The impact of DOA on HIV replication is mediated by cell-cell interactions. DOA exposure has different effects on HIV replication based on the types of cells in co-culture systems. In contrast, microglia monocultures are insufficient to study DOA's impact on HIV replication.

P28

Cocaine increases myeloid HIV replication and inflammation via dopamine-associated and dopamine independent mechanisms

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Cocaine use disorder is highly prevalent in people with HIV (PWH) and has a greater negative impact on disease and neurocognitive function these individuals than the general population. The mechanism(s) by which cocaine and HIV exacerbate neurological disease are not clear. Cocaine acts acutely to increase dopamine levels in CNS. Our data show that dopamine levels associated with the use of cocaine and other addictive substances, increase HIV replication in human macrophages and inducible pluripotent stem cell-derived microglia (iMg) while also exacerbating inflammation in these cells. The effects of dopamine on HIV replication seem to be mediated, at least in part, by activation of dopamine receptors, as they are inhibited by the pan-dopamine receptor antagonist, Flupenthixol. Dopamine seems to act via a non-canonical, Ca^{2+} signaling pathway that increases surface CCR5 expression, mediating increased HIV entry. However, our recent data indicate that in microglia, cocaine may act through a second, non-dopamine related effect that also increases HIV replication. iMg were inoculated with HIV in the presence of cocaine

(HIV + Coc) show an increase in p24Gag and the percentage of infected iMg. This effect was blocked by inhibition of the sigma-1 receptor (S1R), but not by inhibition of dopamine receptors. Further, S1R agonists independently increased p24 secretion, and immunofluorescence staining showed increased S1R in p24- cells and reduced S1R in p24+ cells in HIV+Coc cultures relative to cultures infected with HIV alone. Single-cell RNA sequencing revealed Coc decreases host antiviral and innate immune responses, while increasing genes associated with the unfolded protein response. These data indicate that cocaine use by PWH promotes increased viral replication and neuropathogenesis via two distinct pathways in human myeloid cells and suggest that therapeutics targeting CNS infection in individuals who use cocaine may need to engage multiple signaling pathways in order to be effective.

P29

Characterizing mechanisms by which buprenorphine may mitigate HIV-NCI

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HIV-associated neurocognitive impairment (HIV-NCI) affects up to 50% of people with HIV (PWH) despite viral suppression with antiretroviral therapy (ART), and negatively impacts their quality of life. However, there are currently no treatments for HIV-NCI. The pathogenesis of HIV-NCI is mediated in part by transmigration of HIV exposed/infected mature monocytes across the blood brain barrier (BBB), bringing virus into the central nervous system (CNS) where it can infect other brain parenchymal cells and mediate neuroinflammation. We previously showed using an EcoHIV-infected mouse model of HIV-NCI, that buprenorphine, an opioid agonist therapy used to treat opioid use disorder, inhibited or reversed cognitive impairment that occurs with infection. These effects were associated with decreased monocyte entry and HIV DNA burden within the CNS of infected mice. However, understanding of mechanisms by which buprenorphine may mitigate HIV-NCI remains limited. We are examining the impact of buprenorphine on CCL2-mediated transmigration of mature monocytes across an in vitro model of the human BBB. We found decreased mature monocyte transmigration upon buprenorphine treatment with uninfected (21% reduction, n=8), HIV-exposed/infected (17% reduction, n=3), and HIV-exposed/infected cells treated with ART (47% reduction, n=2), suggesting buprenorphine may mitigate HIV-NCI in part by limiting entry of these cells into the CNS. We are also characterizing buprenorphine's impact on integrin-mediated binding of mature monocytes to adhesion proteins on the peripheral side of the BBB, an early step in transmigration. We found decreased adhesion of HIV-infected/exposed monocytes to ICAM-1 (33% reduction, n=6), and VCAM-1 (30% reduction, n=2). We found that buprenorphine also reduces CXCL12-mediated uninfected monocyte adhesion to ICAM-1 (34% reduction, n=3). This suggests buprenorphine's effects may be generally applicable to chemokines that mediate monocyte entry into the CNS in the context of HIV-NCI. Our findings suggest buprenorphine limits a key HIV neuropathogenic mechanism and indicate its potential for treating HIV-NCI.

P30

Astrocytes exhibit pro-viral inflammatory responses in a model of CNS HIV infection

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HIV currently infects over 38 million people worldwide. Despite the high efficacy of antiretroviral therapies, HIV persists via transcriptionally silent latent infection and long-lived viral reservoirs in tissue sites such as the central nervous system (CNS). Viral persistence in the CNS leads to sustained neuroinflammation, which in turn contributes to the development of a spectrum of deficits in memory, learning, and/or motor functions referred to as HIV-associated neurocognitive disorders (HAND), observed

in 40%–50% of people living with HIV (PLWH). Microglia, the resident macrophages of the CNS, are a key population of HIV-susceptible cells in this niche. Astrocytes are also impacted by HIV infection through abortive integration of the viral genome, PRR sensing of viral components, and through indirect activation by neighboring infected cells. Because microglia are challenging to model in vitro, the dynamics of HIV replication in microglia as well as the immune response of microglia and neighboring astrocytes to HIV infection remain poorly understood. Using human induced pluripotent stem cell-derived microglia (iMg) and astrocytes (iAst) we show that HIV readily replicates in iMg but not iAst. During HIV infection, microglia fail to activate canonical intracellular anti-viral pathways or secrete pro-inflammatory cytokines, likely due to the capacity of HIV to evade detection by innate immune sensors. Surprisingly, we have shown that coculture of iAst with iMg leads to robust increases in HIV replication, suggesting that iAst act to facilitate HIV replication in iMg. iAst exposed to infected iMg produce the pro-inflammatory cytokines TNFα and IL-6. These cytokines are known to activate NF-kB dependent transcriptional regulation, which in turn can induce transcription of the integrated HIV genome. Indeed, pharmacological inhibition of NF-kB signaling significantly reduces HIV replication in iMg-iAst cocultures. Overall, our data suggest that astrocyte-driven neuroinflammation can promote NF-kB signaling and replication of HIV in microglia. Further, we have shown that activation of iAst into the observed pro-inflammatory state is associated with dysregulated lysosomal trafficking in iMg following HIV infection. Given the associations of IL-6, TNFα, and dysregulated cellular metabolism with increased neurocognitive injury in PLWH, this signaling axis may be a viable target of adjunctive immunotherapies for individuals susceptible to HAND.

P31

Wnt7a regulates inflammasome activation in resident brain cells

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HIV infection is associated with chronic neuroinflammation, which contributes to neurodegenerative processes in the central nervous system (CNS). Resident myeloid cells, including microglia and macrophages, play a central role in this inflammation by secreting neurotoxic inflammatory mediators. However, the mechanisms regulating neuroinflammation within different CNS cell types remain poorly understood. Our previous work demonstrated that differentiation of monocytes into macrophages in the presence of the morphogen Wnt7a generates a macrophage subset with reduced surface expression of CD14, CD163, and CD11b, but increased production of IL-1β and IL-6. Additionally, Wnt7a expression was lower in postmortem brain tissue from individuals with HIV-associated dementia (HAD) compared to HIV-negative controls. Here, we investigate the role of Wnt7a in neuroinflammation, specifically its regulation of the NLRP3 inflammasome in CNS cells. Using monocyte-derived macrophages (MDMs), iPSC-derived microglia and astrocytes, and cerebral organoids, we show that Wnt7a overexpression increases NLRP3, IL-1β, IL-18, and IL-6 in MDMs, iMicroglia, and neurons but not in astrocytes. Conversely, lentiviral-mediated Wnt7a knockdown reduces these inflammatory markers in MDMs. These findings provide insight into inflammasome activation in CNS cells, with implications for understanding HIV-associated neuroinflammation and neuropathogenesis.

P32

Analysis of chromatin accessibility across the HIV-1 integrated provirus as a predictive strategy for gRNA selection

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Despite advances in antiretroviral therapeutics, human immunodeficiency virus type 1 (HIV-1) persists via the latent viral reservoir that harbors the integrated provirus. Utilization of CRISPR/Cas9 gene editing to excise or inactivate the integrated provirus has been proposed as a curative strategy to combat latency. We have developed a computational pipeline to design gRNAs that specifically target important functional regions of the integrated provirus while accounting for HIV-1 sequence diversity. We have found that gRNAs which target sites critical for latency maintenance and reactivation, such as an NF-kB binding site and the transactivation response element (TAR) located in the HIV-1 long terminal repeats (LTR), were the most effective in reducing HIV-1 proviral expression and reactivation in vitro. While we predict that the accessibility required for Cas9-mediated editing is less than what is required for gene expression, we have not explored how the chromatin state at specific target sites impacts editing efficiency with our gRNAs. As studies have shown that epigenetic interference can impede CRISPR/Cas cleavage of target DNA, we are interested in further investigating chromatin accessibility across the length of the integrated provirus. To this end, we have compiled a number of publicly available HIV-1 ATAC-seq and ChIP-seq datasets from both lymphoid and myeloid cell types. Assessment of the chromatin state across the proviral genome under various conditions will allow us to use this information as a predictive measure for gRNA selection in future experiments. We will also analyze these datasets alongside our own ATAC-seq and ChIP-seq results to determine how cleavage events resulting from different gRNAs impact the proviral chromatin environment on successful targeting. Future experiments will explore whether the use of latency reversal agents to alleviate epigenetic repression at specific targets sites enhances editing efficiency.

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P33

Human Pegivirus alters immune and transcriptomic profiles in the brain and blood of Parkinson's Disease patients

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Parkinson's disease (PD) is a neurodegenerative disorder with both genetic and environmental factors contributing to onset and progression. Viral infections have emerged as potential environmental triggers that influence PD pathology. Using ViroFind, an unbiased platform for whole virome sequencing, along with RT-qPCR we identified Human Pegivirus (HPgV) in 5/10 (50%) of PD brains, but in 0/14 of the age- and sex-matched controls suggesting a potential association with PD. HPgV-brain positive PD patients showed increased neuropathology by Braak stage and Complexin-II levels, while those positive in the blood had higher IGF-1 and lower pS65-Ubiquitin, supporting disruption in metabolism or mitophagy in response to HPgV. RNAseq revealed altered immune signaling in HPgV-infected PD samples, including consistent suppression of IL4 signaling in both the brain and blood. Longitudinal analysis of blood samples showed a genotype-dependent viral response, with HPgV titers correlating directly with IL4 signaling in a LRRK2-genotype dependent manner. YWHAB was identified as a key hub gene in the LRRK2-genotypic response, which exhibited an altered relationship with immune related factors, including NFKB1, ITPR2, and LRRK2 itself in HPgV+ PD patients. These results suggest a role for HPgV in shaping PD pathology, and highlight the complex interplay between viral infection, immunity and neuropathogenesis.

P34

Neuroinflammation and Glymphatic Dysfunction in HIV-Associated Neurocognitive Disorders

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The glymphatic system facilitates the exchange of interstitial and cerebrospinal fluid through perivascular spaces in the brain, a process driven by aquaporin-4 (AQP4) water channels on astrocyte end feet. Mislocalization of AQP4 from the end feet to the soma can reduce interstitial fluid flow, leading to extracellular waste accumulation, including hyperphosphorylated Tau, a hallmark of Alzheimer's disease and, in some cases, human immunodeficiency virus (HIV). Approximately 50% of people with HIV (PWH) experience HIV-associated neurocognitive disorders (HAND), a spectrum of cognitive and motor impairments ranging from mild deficits to severe dementia. Limited studies show increased AQP4 expression in brain homogenates from the mid-frontal gyrus of PWH with symptomatic HAND, suggesting AQP4 function and localization may affect cognitive status. Dysregulation of AQP4 via adenosine A2a Receptor (A2aR) signaling has been observed in other neuroinflammatory diseases, where A2aR activation triggers PKA/PKC-mediated phosphorylation, leading to AQP4 internalization, mislocalization, and reduced glymphatic function. Disruptions in this clearance system can exacerbate neuroinflammation, oxidative stress, and neuronal dysfunction, all of which are implicated in HAND progression. Using NanoString's GeoMx Digital Spatial Profiler with the Neuroscience Protein Panel, we identified protein expression changes suggesting neuroinflammation in PWH, further supporting the role of AQP4 dysregulation in HAND pathology. Specifically, we observed altered expression of inflammatory markers linked to gliosis and blood-brain barrier dysfunction, processes that may contribute to glymphatic impairment. These findings implicate inflammatory pathways in glymphatic dysfunction and impaired clearance of toxic proteins. Thus, AQP4 mislocalization and post-translational modifications, potentially driven by neuroinflammation, may contribute to HAND and its associated cognitive decline. Targeting A2aR signaling and inflammatory pathways may offer potential therapeutic strategies for mitigating glymphatic dysfunction and neurodegeneration in HAND.

P35

Endosomal mechanisms of SARS-CoV-2 spike S1–induced inflammation and senescence in human astrocytes

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SARS-CoV-2, the virus responsible for COVID-19, has been linked to persistent neurological and cognitive impairments, collectively termed neuro-PASC. These long-lasting symptoms can arise even in mild cases, though the precise underlying mechanisms remain elusive. Recent studies suggest that COVID-19 may accelerate brain aging and induce cellular senescence, potentially contributing to neurodegenerative processes. In this study, we explored the role of the SARS-CoV-2 spike protein subunit S1 in promoting a senescence-like state in human astrocytes. Our findings reveal that S1, but not a mutant version lacking the multibasic motif (RRAR), triggers an increase in IL-6 and CCL2/MCP1 secretion, elevates P16 protein levels, and enhances SA-β-gal activity,Äîhallmarks of cellular senescence. Moreover, S1 disrupts endolysosomal integrity, causing increased pH, structural alterations, and membrane permeability, leading to the release of galectin-3 and cathepsin D. Mechanistically, we identified toll-like receptor 7 (TLR7) as a central mediator of S1-induced endolysosome damage and cellular senescence. S1 directly interacts with endolysosomal TLR7. Activation of TLR7 alone mimics S1's effects, whereas TLR7 knockdown significantly mitigates S1-induced cellular senescence. These findings underscore the critical role of the multibasic motif in S1-mediated astrocyte dysfunction and reveal TLR7 as a key player in these processes. Targeting this pathway could offer new therapeutic strategies for addressing the neurological complications associated with COVID-19.

P36

The effects of methamphetamine on uninfected and HIV-infected mature monocytes during CXCL12 and CCL2-mediated adhesion, chemotaxis, and blood-brain barrier transmigration

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Globally, 39.9 million people are currently living with HIV. Antiretroviral therapy (ART) has greatly increased the lifespan and quality of life of people with HIV (PWH). Despite viral suppression with ART, PWH are at an increased risk for several comorbidities including HIV-associated neurocognitive impairment (HIV-NCI), a spectrum of neurocognitive deficits. HIV-NCI affects 15-50% of PWH and currently there are no treatments. Additionally, approximately 13-30% of PWH use methamphetamine (MA), which is associated with higher rates of HIV-NCI. Our lab and others showed that intermediate, or mature, monocytes (CD14+CD16+) preferentially transmigrate across the blood-brain barrier (BBB) to CXCL12 or CCL2. Intermediate monocytes are preferentially infected by HIV and are more abundant in peripheral blood of PWH relative to people without HIV. Using in vitro maturation and HIV infection of mature monocytes, we characterized the effects of MA on steps of transmigration, including cell adhesion, matrix chemotaxis, and the entire process using our in vitro human BBB model. Our data demonstrate that MA increases fold change (FC), compared to baseline, of adhesion of uninfected mature monocytes treated with CCL2 to ICAM-1 relative to CCL2 alone; the average FC to baseline for CCL2 was 2.33, compared to MA+CCL2 which was 2.86 (n=18). Our data also demonstrate that MA increases the chemotaxis of uninfected (n=7) and HIV-infected mature monocytes (n=10) to CXCL12 or CCL2, the FC to baseline was 2.96 for CXCL12 and MA+CXCL12 was 3.46 (uninfected) and 2.75 for CXCL12 and 3.32 for MA+CXCL12 (HIV-infected). MA also increases BBB transmigration of mature monocytes to CXCL12, FC of CXCL12 alone was 1.50 and of MA+CXCL12 was 1.70 (n=10), or to CCL2, FC to CCL2 alone was 1.50 and to MA+CCL2 was 2.40 (n=10). MA may contribute to HIV-NCI by increasing monocyte entry into the brain, resulting in exacerbated neuroinflammation and reseeding of viral reservoirs.

P37

The role of inflammation, the kynurenine pathway, and blood-brain barrier disruption in depression pathogenesis in people with HIV

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Depression is the most common neuropsychiatric comorbidity in people with HIV, with a prevalence of 30-50%, nearly twice that of the general population. Depression in PWH is associated with increased morbidity and mortality, underscoring the importance of understanding its neurobiological mechanisms in PWH. HIV infection induces chronic inflammation and kynurenine pathway (KP) activation despite viral suppression with antiretroviral therapy. How HIV-associated chronic inflammation and KP activation drive depression pathogenesis remains unclear. We hypothesize that HIV-associated inflammation and KP metabolites mediate depression pathogenesis by disrupting the blood-brain barrier (BBB) and altering CD14+CD16+ monocyte function, both implicated in depression and HIV neuropathogenesis. We found that plasma from PWH (n=6) increased permeability of a human BBB model nearly 2-fold compared with plasma from PWoH (n=5, p=0.02). Treatment of the BBB model with quinolinic acid (n=2), a KP metabolite elevated in PWH and associated with depression, dose-dependently increased BBB permeability (100 nM = 1.3-fold; 200 nM = 2.6-fold; 300 nM = 2.9-fold; 400 nM = 3.8-fold). We are conducting a cross-sectional study recruiting PWH and people without HIV (PWoH), with and without depression. Participants undergo assessments for depression severity, anhedonia, and other psychosocial measures. Using plasma and PBMC from participants, we evaluate peripheral KP activity, plasma markers of BBB damage, BBB permeability, and CD14+CD16+ monocyte transmigration. Multivariate analyses will assess associations of KP metabolites, BBB permeability, and PBMC transmigration with depression/ anhedonia severity

among PWH and PWoH. Additionally, we examine how KP metabolites affect BBB integrity and CD14+CD16+ monocyte function in vitro. We analyzed plasma from these participants, for whom we are blinded, and found that they cluster into low, mid, and high plasma concentrations of sE-selectin (n=92) and sICAM-1 (n=76), markers of brain endothelial damage. These findings suggest that plasma mediators in PWH contribute to BBB disruption and potentially mediate depression in PWH.

P38

A BSL-2 pre-clinical murine model to investigate henipavirus in vivo biology and candidate therapeutics

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Nipah virus (NiV) and Hendra virus (HeV) are members of the Henipavirus genus known to cause severe neurological and/or respiratory disease. Due to the high post-infection fatality rate and the lack of approved therapeutics for human use, these viruses are classified as biosafety level 4 (BSL-4) agents, presenting significant practical barriers both to the study of NiV and HeV pathogenesis and the evaluation of candidate countermeasures. To address this limitation, we present data describing a novel BSL-2 model of authentic henipavirus infection in mice, using a recombinant form of the non-pathogenic henipavirus, Cedar virus (rCedV). Although wild-type mice are highly resistant to infection with henipaviruses, we have recently shown that luciferase expressing rCedV (rCedV-Luc) can establish a transient infection in IFNAR-KO mice, but without pathology or mortality. In this study, we have improved this model via use of recombinant CedVs that express the attachment glycoprotein (G) and fusion glycoprotein (F) from NiV (rCedV-NiV-Luc) and HeV (rCedV-HeV-Luc). Notably, these glycoproteins, which are not known virulence genes, alter the cellular tropism of rCedV to better emulate that of NiV and HeV. Through longitudinal BLI, we demonstrate that these novel rCedVs display a in vivo tissue tropism in IFNAR-KO mice that more closely emulates authentic NiV and HeV infection, although pathogenesis remains absent. However, by further impairing the host interferon response using STAT1 deficient mice (STAT1-KO), we show that infection with rCedV-NiV-Luc and rCedV-HeV-Luc leads to clinical signs of neurological disease and mortality, with high penetrance. Furthermore, through qPCR analysis and immunohistochemistry we confirm the presence of virus in the brain and lung tissue of animals that exhibit disease signs. Overall, these data suggest that infection of STAT1-KO mice with rCedVs can serve as a novel BSL-2 pre-clinical platform for the investigation of candidate therapeutics and studies of henipavirus pathogenesis mechanisms.

P39

SUMO Proteomic Analyses Identify HIV Latency-Associated Proteins in Microglia

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Microglial cells are the main HIV target cells in the brain and constitute an important reservoir for latent infection and a major obstacle in achieving a cure for chronic HIV infection. Therefore, the mechanisms involved in HIV latency and viral reactivation warrant further studies to identify targets of HIV reservoir formation and clearance. SUMOylation (small ubiquitin-related modification) is a post-translational modification that involves the covalent attachment of SUMO proteins to target substrates. The dynamic process regulates several aspects of cellular functions, including protein stability, subcellular localization, and cellular signaling. SUMOylation has significant implications in immune regulation for viral infections as it has been shown to regulate the activity of toll-like receptors (TLRs) and transcription factors like NF-κBA. Additionally, several viruses like, herpesviruses, influenza, and HIV have evolved mechanisms to hijack the host SUMOylation system to enhance their replication and to evade host immune responses. However, host cells can use SUMOylation to mount an antiviral response by modifying key immune factors

that limit viral replication. This dichotomous role of SUMOylation makes the SUMO system a critical area of research. To further explore this relationship, we performed proteomic analysis using an innovative cellular model of HIV latency in microglia. Here, we report that global SUMO conjugation is significantly increased in HIV latently infected microglia, with global SUMO conjugation levels decreasing following HIV reactivation. Microglia with latent HIV and upon HIV reactivation present unique proteomic landscapes. Through our experiments targeting SUMOylation with a SUMO-specific inhibitor as a therapeutic strategy for viral infections, we observed a significant reduction in HIV reactivation in a dose-dependent manner. Together, these studies highlight the importance of SUMOylation in cellular processes, its emerging role in innate immunity, and its implications in latent HIV infection. Our work suggests that targeted modulation of SUMOylation may offer new therapeutic strategies for treating viral infections.

P40

Use of subtherapeutic latency reversal conditions to improve CRISPR/Cas9 cleavage of the HIV-1 provirus

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Human immunodeficiency virus type 1 (HIV-1) is a retrovirus that integrates into the host cell's genome as a provirus, establishing a persistent reservoir of infected cells. As the host cell transitions to a quiescent phenotype, the provirus enters a period of latency, characterized by low levels of viral transcription and virion production. Proviral latency is regulated in large part by a myriad of host factors that establish either a repressive or permissive chromatin environment at the 5' long terminal repeat (LTR) of the provirus, particularly positioning and modification of 5' LTR nucleosomes nuc-0 and nuc-1. Proviral chromatin has been targeted as part of HIV-1 cure strategies Shock and Kill as well as Block and Lock, which use small molecule latency reversal agents (LRAs) and latency promoting agents (LPAs) to either promote or suppress viral gene expression, respectively. This has had limited success in vivo. Another strategy, CRISPR/Cas9 gene editing of the 5' LTR, aims to target the integrated proviral sequence directly. Cuts made by the Cas9 endonuclease are repaired through nonhomologous end joining (NHEJ), which is highly error-prone and can result in deleterious insertions and deletions (indels) or excision events. This strategy has been nearly 100% effective in silico and in plasmid transfection models, but the efficacy reduces by nearly 50% in latently-infected J-Lat 10.6 T cells. We hypothesize that nuc-0 and nuc-1 impede binding and cleavage by the CRISPR/Cas9 system, thus reducing its efficacy in latently-infected cells. We propose the "Tickle and Tweeze" strategy, which incorporates elements of Shock and Kill and CRISPR/Cas9 gene editing. Herein, we aim to use subtherapeutic doses of LRAs to reduce nucleosome affinity for proviral DNA, promoting CRISPR/Cas9 activity without inducing transcription from the provirus. Our goal is to determine how subtherapeutic latency reversal conditions affect CRISPR/Cas9 activity and NHEJ-induced indels and excision events.

P41

HIV antiretroviral drugs trigger stress granule formation via integrated stress response in oligodendrocytes

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Most people with HIV (PWH) in the United States are virally suppressed due to antiretroviral therapy (ART); however, cognitive and behavioral symptoms persist in some individuals with HIV-associated neurocognitive disorder (HAND) along with underlying white matter abnormalities. In fact, thinning of the largest white matter track, the corpus callosum, correlates with duration of ART suggesting that ART drugs may contribute to this pathology. We have previously demonstrated that select ART drugs, such as elvitegravir (EVG), prevent the maturation of oligodendrocytes (OLs) and remyelination. Differentiating OLs treated with EVG activates the Integrated Stress Response (ISR) and co-treatment with the ISR inhibitor ISRIB rescues differentiation. During ISR activation, stress granules (SGs) often form, sequestering proteins, mRNAs and translation machinery. While they have yet to be thoroughly studied in OLs, the chronic presence of SGs in neurons is thought to accelerate neurodegeneration in a variety of diseases. Here we show that differentiating OLs treated with the ART drugs bictegravir (BIC) and EVG leads to formation of cytoplasmic SGs. Drug removal after 2 hours leads to rapid decrease in SG presence, demonstrating their dynamic nature. ISRIB co-treatment with BIC and EVG prevents SG formation, indicating the SGs form canonically via the ISR. Ex vivo analysis of mice treated with BIC, and a small cohort of PWH on ART revealed the presence of SGs within OLs of the corpus callosum and cortical white matter, respectively; In post-mortem tissue of PWH with and without HAND, we observed an increased percent of OLs with SGs and number of SGs per OL in PWH with HAND compared with neurocognitively normal individuals. These findings suggest that SG formation in OLs may contribute to persistent white matter pathology in PWH with HAND, and implicate SGs as a potential therapeutic target for improving outcomes for PWH on ART.

P42

The effect of oligomer amyloid-β on memory deterioration in the preclinical stage of HIV-infected mice

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Background: Despite effective antiretroviral therapy, 50% of people living with HIV (PLWH) develop cognitive and memory deterioration. As life expectancy grows with PLWH, aging becomes one of the mental health risk factors of neurodegenerative disease, Alzheimer's disease (AD). To assess how oligomer Aβ (oAβ) promotes neurocognitive impairment in PLWH and accelerates disease development, we employed the preclinical phase of HIV mouse models with a subclinical dose of oAβ.

Methods: C57BL/6 and transgenic APP/PS1 mice were infected with EcoHIV-1 by intraperitoneal injection (IP). After 8 days of infection, a subclinical dose of oAβ was given to C57BL/6 mice by intranasal administration (IN). Brain oAβ was measured using ELISA. After 10 days after infection with oAβ administration, hippocampal long-term potentiation (LTP) and behavioral tests were performed to assess cognitive and memory function. Aβ-42 expression assay, apoptosis, gene changes, and amyloid pathology are used for molecular analysis.

Results: LTP was impaired with oAβ after 10 and 30 days of EcoHIV infection in each group of C57BL/6 mice. Administration of a subclinical dose of oAβ to preclinical EcoHIV-infected mice accelerated cognitive and memory deterioration. In parallel, apoptotic cell death was observed positive to TUNEL assay and cleaved caspase-3. Aβ plaques were not observed in C57BL/6 while observed in APP/PS1 mice in the

preclinical stage. LTP of APP/PS1 mice significantly declined in EcoHIV-1 infected mice after 10 days of IP injection with behavioral change. EcoHV-1 infected APP/PS1 mice accumulated significantly higher levels of oAβ but no changes in the viral levels in the brain and spleen.

Conclusions: Combining subclinical dose of oAβ to preclinical phase of EcoHIV-infected mice accelerated the deterioration of memory function in C57BL/6 mice and induced cell death in the brain. Infecting EcoHIV-1 in APP/PS1 mice for 10 days by IP induced more microglia cell response around the Aβ plaques.

P43

TRPML1-redox sensing regulates gp120-mediated changes to levels of cellular iron and reactive species

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Increased levels of reactive species and endolysosome dysfunction are implicated in the pathogenesis of neurodegenerative disorders, including HIV-1 associated neurocognitive disorders (HAND). Others and we have shown that HIV-1 envelope glycoprotein 120 (gp120) changes in endolysosome functions, increases levels of intracellular iron, changes levels of reactive species, and increases neuronal injury and death. However, the mechanisms by which HIV-1 gp120 impairs endolysosome function and triggers disruption in intracellular iron and the reactive species interactome remain unclear. Endolysosome-resident cation channels including transient receptor potential mucolipin-1 (TRPML1), that functions as a redox sensor, have been implicated in the neurotoxic actions of HIV-1 gp120. Here, using SH-SY5Y human neuroblastoma cells we found that HIV-1 gp120 increased levels of endolysosome reactive oxygen species (ROS), lipid peroxidation (LPO) and protein oxidation, and decreased levels of hydrogen sulfide (H2S), glutathione (GSH) and endolysosome ferrous iron (Fe^{2+}); all these effects were blocked by Ned-19, an analog of nicotinic acid adenine dinucleotide phosphate that inhibits TRPML1. Additionally, HIV-1 gp120-induced increases in levels of intracellular reactive species led to TRPML1 activation and oxidation-induced endolysosome Fe^{2+} release into the cytosol; effects blocked by the antioxidants N-acetyl cysteine and Trolox as well as Ned-19 or TRPML1 knockdown. HIV-1 gp120-induced increases in cytosolic Fe^{2+} and ROS levels and decreases in cytosolic H2S levels were blocked by Ned-19. Thus, inhibition of aberrant TRPML1 activity may represent a promising strategy against HAND and other diseases where iron and redox imbalances have been implicated. (We gratefully acknowledge funding from 5P20GM139759, R01MH119000, and 2R01DA032444.)

P44

Gender-specific gut mycobiome alteration promotes psychosocial behavior and cognition deficits in EcoHIV-infected female mice

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Background: HIV-associated neurobehavioral disorders remain prevalent despite antiretroviral therapy, necessitating novel therapeutic strategies. Emerging evidence suggests that gut fungal dysbiosis contributes to neuroimmune dysfunction, yet its role in HIV-associated psychosocial and cognitive deficits remains unclear. Here, we identify a gender-specific gut fungal signature linked to EcoHIV-induced psychosocial and cognitive impairments and demonstrate the therapeutic potential of Pachyman, a β-glucan with immunomodulatory properties.

Methods: EcoHIV-infected female C57BL/6 mice were treated with Pachyman or vehicle. Psychosocial and cognitive functions were assessed using the open field test, elevated plus maze, three-chamber social

interaction test, and novel object recognition test. Flow cytometry was used to analyze immune cell populations in the colonic lamina propria. ITS2 rRNA gene sequencing and metabolomic analysis were conducted to profile gut fungal composition and metabolic pathway alterations.

Results: EcoHIV infection led to a significant reduction in the fungal species Malassezia globosa in female mice, which strongly correlated with impaired social interaction. Pachyman treatment reversed social avoidance ($P < 0.01$) and cognitive deficits ($P < 0.01$) without affecting locomotor or anxiety-related behaviors. Mechanistically, EcoHIV increased Dectin-1+ macrophages in the colon ($P < 0.01$), a change reversed by Pachyman ($P < 0.05$). Notably, Dectin-1+ macrophages correlated with social avoidance ($P = 0.008$, $r = 0.5209$) and Malassezia globosa abundance ($P = 0.0074$, $r = 0.5285$). EcoHIV infection also disrupted key immune-related metabolic pathways, which Pachyman treatment normalized.

Conclusion: These findings reveal a previously unrecognized role of the gut mycobiome in HIV-associated psychosocial behavior and cognitive deficits, particularly in female subjects, and position Pachyman as a promising candidate for integrative therapeutic interventions. Further investigation is warranted to explore the translational potential of targeting gut fungi–immune interactions in HIV-associated neuropsychiatric and cognitive disorders.

P45

Characterization of SIV Reservoirs in the Central Nervous System of SIV-Infected Rhesus Macaques Following AAV9-CRISPR/Cas9 Therapy

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HIV-1 reservoirs in the central nervous system (CNS) present a major barrier to achieving a cure, as viral persistence and associated neuroinflammation can contribute to HIV-associated neurocognitive disorders (HAND), even under suppressive combination antiretroviral therapy (cART). Our previous work demonstrated that CRISPR/Cas9 gene editing can excise SIV genomes from infected rhesus macaques. However, the regional distribution of SIV proviruses in the CNS and the efficacy of this approach across different brain areas remain poorly understood. In this study, ten adult rhesus macaques were intravenously infected with barcoded SIVmac239 and treated daily with cART for over 20 months. Five animals received an intravenous infusion of AAV9-delivered CRISPR/Cas9 with dual guide RNAs targeting conserved regions in the Psi and Gag genes. The remaining five received AAV9-CRISPR/Cas9 lacking gRNAs as controls. One week post-infusion, animals underwent analytical treatment interruption (ATI). Upon viral rebound, necropsies were performed, and 19 nervous system tissues (15 CNS and 4 PNS) were collected post-perfusion for virological and molecular analyses. Droplet digital PCR (ddPCR) revealed widespread presence of SIV and Cas9 DNA across sampled tissues. In some brain regions, intact SIV proviral DNA was detected using the intact proviral DNA assay (IPDA), confirming the persistence of viral reservoirs in the CNS. In treated animals, successful excision of proviral DNA was confirmed via PCR and Sanger sequencing. These findings demonstrate that AAV9-CRISPR/Cas9 targeting of SIV can mediate proviral excision in diverse CNS regions, supporting its potential as a curative strategy for eliminating viral reservoirs in the brain.

P46

Impact of HIV on Resting-state Connectivity in People with HIV with a History of Methamphetamine Use

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Methamphetamine (METH) use is a significant public health concern with high rates of use worldwide. We need to better understand how METH impacts brain function to develop intervention strategies in patient

care. Prior research suggests that both HIV and METH use can individually impact the integrity of functional magnetic resonance imaging (fMRI) resting-state default mode and salience networks that underly cognitive activities. METH use and HIV have each been tied to changes in the connectivity between specific brain regions, including the dorsolateral prefrontal cortex (DLPFC) and the anterior cingulate cortex (ACC). It is possible that changes in connectivity levels due to HIV and METH use history interact, complicating network integrity that supports cognitive activity. Using the SPM-based CONN toolbox, we assessed seed-to-seed functional connectivity to identify differential patterns stratified by HIV infection and METH use history. Participants from the Translational Methamphetamine AIDS Research Center (TMARC) cohort with adequate resting-state MRI data were included (n=127): persons without HIV or history of METH use (n=39); persons with HIV (n=29); persons with METH use history (n=34); persons with HIV and history of METH use (n=24). We found that people with HIV (including those with METH use history) had greater connectivity between the ACC and DLPFC relative to those in those without HIV despite history of METH use ($F(3, 127) = 4.01$, $p=0.009$). We did not find an effect of METH use history or an interaction between HIV and history of METH use. Findings suggest that there was a general HIV effect, which had a greater impact on the connectivity of brain regions that are important for cognitive control and attention with or without a history of METH use. While we did not observe a dual effect of METH use and HIV status further examining the combined effects of HIV and METH are recommended.

P47

The role of osteopontin (OPN/SPP1) in midbrain dopaminergic neuronal circuits of HIV-infected humanized mice

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OPN/SPP1 (Osteopontin/ secreted phosphoprotein 1), a multifunctional protein associated with inflammation, is highly expressed in the CNS and elevated in people living with HIV (PWH). While accumulating evidence from our laboratory suggests neuroprotective roles for OPN/SPP1, its molecular mechanisms in CNS-specific functions remain largely unknown. Clinical studies report that a subset of PWH have alterations in dopaminergic circuits, which have also been demonstrated in non-infectious transgenic HIV rodents that exhibit altered motivational and apathetic behavior. While the influence of HIV and OPN/SPP1 on dopaminergic signaling has been individually investigated, their synergistic impact on these circuits is underexplored. Based on results from mouse PET-neuroimaging and ongoing behavioral studies, we hypothesize that systemic inflammation after HIV infection impairs dopaminergic circuit signaling, and that local OPN/SPP1 expression and activity is required for proper dopaminergic signaling and homeostatic maintenance. We investigated the impact of systemic HIV infection and global OPN/SPP1 knockdown on the expression of tyrosine hydroxylase (TH), TSPO, local OPN/SPP1 and Ctip2 in humanized NOD/Scid IL-2y (hu-NSG) mice. Preliminary findings reveal robust expression of OPN/SPP1 in a subset of cells in the midbrain region near the ventral tegmental area (VTA). As expected, there was a trend toward increased OPN/SPP1 expression in HIV-infected mice which was not observed in those with OPN knockdown. While there was no effect of HIV infection or OPN/SPP1 on TH+ cell numbers in the midbrain, a trend toward reduced TH expression in the OPN knockdown groups irrespective of infection status was observed. Lastly, there were no significant differences in TSPO expression in glial cells in the midbrain regions or in Ctip2 expression in the striatum, cortex, and hippocampus between the four groups. Additional data collection is ongoing to understand the relationship between viral load, marker gene expression to identify the OPN-expressing cells of the midbrain regions and other brain regions that comprise specific dopaminergic circuits.

P48

Exploring the Impact of HIV and Osteopontin on Hippocampal Vascular Density

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Despite the lifesaving benefits of antiretroviral therapy (ART), HIV-infected individuals remain at significantly increased risk for comorbid cerebrovascular pathologies, including atherosclerosis, vasculitis, and aneurysms. In this regard, the incidence of stroke in PWH is double that of uninfected people. Putative mechanisms contributing to the elevated risks for stroke include HIV-mediated injury to the blood-brain-barrier, immune activation and pathologic inflammation. Osteopontin/secreted phosphoprotein 1 (OPN/SPP1) is a proinflammatory phosphoprotein upregulated in the CNS during HIV infection. Collectively, our published data has demonstrated neuroprotective properties of OPN/SPP1. Under healthy conditions, OPN/SPP1 is expressed at low levels in endothelial cells and increases following vascular injury to promote healing. While this response has been reported to be beneficial in the short term, other data suggests that chronic overexpression of OPN/SPP1 can contribute to vascular dysfunction. While the effects of OPN/SPP1 on the vascular system has been studied in humans, the combined influence of HIV infection remains unexplored. We used HIV-infected NSG-hCD34 humanized mice to examine the combined effects of HIV and OPN/SPP1 on vascular density in the hippocampus. Brains from unengrafted/OPN+, Buffer/OPN+, Buffer/OPN-, HIV/OPN+, and HIV/OPN- mice were coronally sectioned. Tissue sections encompassing the dorsal and ventral hippocampus were collected and stained for collagen IV. The average blood vessel density in the dorsal and ventral hippocampus was quantified for each cohort. Our findings suggest that HIV/OPN+ mice exhibit increased hippocampal vascular density compared to controls. Specifically, a significant difference in vascular density was observed among the groups in the ventral hippocampus ($F(1,7) = 7.944$, $p = 0.0258$). Interestingly, OPN/SPP1 and HIV-encoded proteins, Tat and Nef, are known to promote angiogenesis, hence our results suggest a potential synergistic role of HIV and OPN/SPP1 in driving vascular changes.

P49

Effect of Kinases in Extracellular Vesicles from HIV-1-Infected Cells on Bystander Cells

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As of 2023, there were 39.9 million people living with Human Immunodeficiency Virus type 1 (HIV-1). Although great strides have been made in treatment options for HIV-1, and our understanding of the HIV-1 life cycle has vastly improved since the start of this global health crisis, a functional cure remains elusive. One of the main barriers to a cure is latency, which allows the virus to persist despite combined antiretroviral therapy (cART). Recently, we have found that exosomes, which are small, membrane-enclosed particles released by virtually all cell types and known to mediate intercellular communication, caused an increase in RNA Polymerase II loading onto the HIV-1 promoter. This resulted in the production of both short- and long-length viral transcripts in infected cells under cART. This current study examines the effects of exosome-associated kinases on bystander cells. The phospho-kinase profiling of exosomes revealed differences in the kinase payload of exosomes derived from uninfected and HIV-1-infected cells, with CDK10, GSK3β, and MAPK8 having the largest concentration differences. These kinases were shown to be biologically active and capable of phosphorylating substrates, and they modulated changes in the cell cycle dynamics of exposed cells. Given the relevance of such effects for the immune response, our results implicate exosome-associated kinases as new possible key contributors to HIV-1 pathogenesis that affect bystander cells. These findings may guide new therapeutic avenues to improve the current antiretroviral treatment regimens.

P50

Extracellular vesicle purification methods identify distinct infectious particles released from HIV-1-infected T-cells

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This study investigates the overlapping biophysical, biochemical, and functional characteristics of extracellular vesicles (EVs) associated with human immunodeficiency virus type-1 (HIV-1) that challenge conventional size definitions. Using differential ultracentrifugation (DUC), five fractions (2K to 167K(L)) were isolated from HIV-1-infected T-cells. While 2K to 167K(S) exhibited heterogeneous size distributions with median diameters exceeding 100 nm, 167K(L) uniquely contained small EVs averaging below 50 nm. Synchronized cells study revealed large infectious particles in 2K pellet, containing amphisome markers and viral components. In contrast, 167K(L) EVs tested positive for CD63, HSP70, and HIV-1-associated proteins. Notably, membrane-bound viral integrase (HIV-1 IN) in 167K(L) EVs was detected only after detergent treatment, confirming vesicular encapsulation. Single-particle super-resolution microscopy confirmed colocalization of CD63, HIV-1 IN, and envelope glycoprotein (Env) on 167K(L) particles. Strikingly, 167K(L) EVs demonstrated infectivity, which was significantly diminished upon anti-CD63 immunodepletion, implicating surface CD63 in their infectious mechanism. To our knowledge, this represents the first identification of infectious sub-50 nm HIV-1 particles (smHIV-1) via EV isolation methods. In summary, these findings redefine the biophysical boundaries of HIV-1 virions, suggesting that viral particles smaller than previously recognized contribute to infection, with profound implications for understanding viral transmission and pathogenesis.

P51

Gene expression and chromatin accessibility of HIV-1 infected microglial model system reveals a new immediate inflammatory response with altered transcriptional landscape

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HIV-associated neurocognitive disorders (HAND) continue to be a significant burden to people with HIV-1 (PWH) despite antiretroviral therapy. Microglia are targeted by HIV-1 at early time points following infection and can contribute to chronic infection in the central nervous system. Microglia play a central role in promoting neuroinflammation and HAND symptoms seen in PWH. Exploring the transcriptional changes in microglia following HIV-1 infection could reveal mechanisms underlying the development of HAND. iPSC-derived microglia (iPSC-MG) were infected with HIV-1 BaL and longitudinal samples on days 1, 2, 4, 6, 8 post-infection (dpi) were collected for bulk RNA-seq (TruSeq), while ATAC-seq (OmniATAC) was performed on samples from 1 and 4 dpi. The nf-core Nextflow pipelines (rnaseq and atacseq) were used for sequencing data analyses. Differentially expressed genes (DEGs) analysis was performed with DESeq2, and differential accessible regions (DARs) with DiffBind. For enrichment clusterProfiler was used with the MSigDB Hallmark gene set. For transcription factors (TF) activity evaluation, diffTF was used in classification mode, coupling ATAC-seq and RNA-seq data to infer TFs activity. The integration of RNA-seq (1 or 8 dpi) with ATAC-seq (1 or 4 dpi) data uncovered common enriched pathways and transcriptional activity, for both time-points comparisons. The intersection of DEGs with promoters-DARs showed enrichment for Hallmark processes including inflammatory response, TNFα signaling via NF-κB and IFN-α response. Analyzing TFs classified as activators identified TFs previously linked to neurodegeneration processes, such as ZEP1, BACH1, ATF3 and DDIT3. These TFs could impact HAND symptoms seen in PWH. In this study, we utilized a multi-omics approach, merging transcriptomics with chromatin accessibility data to define the transcriptional network responses in microglia post HIV-1

infection. HIV infection triggered the rapid onset of inflammatory profiles that may contribute to neurodegeneration in PWH. The mechanisms identified here may shed light on the transcriptional alterations leading to HAND.

P52

Regional and Epigenetic Microglial Responses to HSV-1 Infection: Implications for Neurodegeneration

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Herpes simplex virus type 1 (HSV-1) infection has been implicated in neuroinflammation and Alzheimer's disease (AD) pathology. Using a physiologically relevant model of intranasal HSV-1 infection, we investigated the regional and temporal microglial responses to primary HSV-1 infection in wild-type (WT) and 5xFAD (AD-susceptible mice). In WT mice, HSV-1 infection induced dystrophic microglia, with multiple regions of the brain exhibiting sustained microglial activation even after viral clearance. Notably, even regions with minimal viral antigen, such as the olfactory bulb, mounted an acute inflammatory response, suggesting intrinsic regional vulnerability to infection-driven neuroinflammation. In 5xFAD mice, primary HSV-1 infection altered microglial transcriptional profiles, shifting them towards an AD-like inflammatory phenotype. RNA sequencing of CD11b+ microglia revealed increased expression of Cxcr4, linked to tau pathology, and reduced levels of Csf1, a key antiviral mediator. Furthermore, microglia exhibited increased lipid droplet accumulation and reduced phagocytosis of infected cells, correlating with Aβ accumulation in the brainstem and hypothalamus. To investigate the long-term impact of HSV-1 infection, we performed single-cell multiome (snRNA/ATAC-seq) analysis in WT mice, identifying a unique HSV-1-responsive microglial subpopulation characterized by inflammatory gene signatures (e.g., Stat1, Irf4). Chromatin accessibility analysis further demonstrated epigenetic reprogramming, suggesting that HSV-1 infection drives innate immune memory, potentially leading to chronic neuroinflammation and microglial dysfunction. These findings suggest that HSV-1 may exacerbate neurodegenerative processes by impairing microglial function. Ongoing studies will define the epigenetic regulatory networks underlying these alterations, providing insight into novel therapeutic targets for preventing HSV-1-associated neurodegeneration and AD risk.

P53

West Nile-like viruses in unusual Hosts in Nigeria: Consequences for the Nervous System

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The West Nile virus is spreading across most of the world and transmitted to vertebrae hosts by infected mosquito vectors. While birds are the primary reservoirs of the virus, various other vertebrate hosts are known to harbor the virus. The identification of these unusual hosts is important in the understanding of the epidemiology of the virus. In this study, we screened several animal wildlife species in Nigeria from different parts of the country and observed seroprevalence to West Nile-like viruses in all species examined. This included primates (74%), bats (62%), squirrels (27%), pangolins (24%), pigeons (20%) and cattle egret (19%) respectively. A preliminary immnunohistochemical investigation shows West-Nile and related flavivirus E protein localization in several organ systems including the brain of bats, and in the kidney, heart, and liver of cattle egrets and pigeons. PCR analysis of the West Nile virus shows over 60% positivity in the serum of primates while sequencing revealed >99% homologue to the lineage 1 reference sequence on NCBI database. We are currently planning a molecular screening of the brain to discriminate for viral identification within the flavivirus family for West Nile, Usutu, Dengue, and Japanese encephalitis viruses amongst others in these unusual hosts. Data from this work will be presented at the conference.

P54

Cocaine Leverages Antiviral and Unfolded Protein Responses to Accelerate HIV Infection of iPSC-Microglia via Sigma-1

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Cocaine (Coc) use is comorbid among individuals living with HIV; however, the exact mechanisms through which Coc influences HIV infection remain uncertain. We showed that dopamine levels induced by stimulant use increase HIV replication in macrophages and microglia. However, it is uncertain whether Coc acts solely via dopamine or if it has other distinct effects on HIV. To assess this, we inoculated human-induced pluripotent stem cell-derived microglia (iMg) with HIV +/- Coc. We evaluated changes in the percentage of infected iMg as well as p24Gag secretion using high-content imaging and AlphaLISA. Coc increased p24 secretion and the number of infected iMg. These effects persisted even in the presence of antiretroviral therapy. The inhibition of dopamine receptors did not diminish the impact of Coc, but inhibition of sigma-1 receptor (S1R) did block the effect. S1R is a chaperone protein targeted by Coc, and S1R agonists independently increased p24 secretion, suggesting that Coc acts through S1R rather than dopaminergic pathways. Immunofluorescence staining showed increased S1R in p24- cells and reduced S1R in p24+ cells in HIV+Coc cultures, and showed that HIV+Coc promotes S1R movement to the nuclear envelope and endoplasmic reticulum. Single-cell RNA sequencing (scRNAseq) validated by qPCR and Western Blot revealed Coc reduces host antiviral response genes and enhances XBP1-driven unfolded protein response, suggesting Coc reduces the host response against viral replication and enhances viral protein folding. Multiplex cytokine panel supported this showing reduced antiviral cytokines (e.g. IL-12) and increased pro-survival cytokines (e.g. IL-4). To confirm these responses are possibly driven by the bystander cells exposed to HIV and Coc, we segregated our scRNAseq clusters into HIVhigh vs HIVlow clusters and found increased S1R and XBP1 in the HIVlow clusters. Future studies in mixed culture systems will assess changes in HIV infection dynamics, neuronal/glial health, and function in response Coc.

P55

Cerebrospinal fluid cytokine expression in virologically controlled PWH and associations with neurological measures

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Background: The persistence of HIV expression in the brains of people with HIV (PWH) is a well-described phenomenon, and the implications for the neurological health of patients is of concern. Studies have shown progressive neurocognitive dysfunction in PWH despite virological suppression in blood with ART. The mechanisms by which HIV contributes to these negative outcomes are not fully understood but include neuroinflammation. The aim of this study was to interrogate the role of cytokines in this pathology.

Methods: CSF was collected from a large cohort of virologically controlled PWH (n=141 visits) and a set of age- and sex-matched HIV-negative controls (HC) (n=30 visits). Participants underwent brain MRI and neuropsychological testing. Levels of thirty CSF cytokines were measured with a multiplex magnetic bead panel. T-tests and Spearman's correlations were used for statistical analysis.

Results: Fourteen cytokines were significantly elevated in PWH compared to HC. Among those elevated were IP-10 ($p<0.001$), G-CSF ($p<0.001$), TNF-α ($p=0.001$), and IL-10 ($p=0.006$). MRI data (n-69 PWH) showed that MCP-1/CCL-2, IP-10, and EGF negatively correlated with gray matter volume ($\rho=-0.28/p=0.03$, $\rho=-0.30/p=0.02$, $\rho=-0.33/p=0.01$ respectively) and positively correlated with CSF volume

(ρ=0.27/p=0.04, ρ=0.37/p=0.004, ρ=0.33/p=0.01 respectively), and IP-10 and VEGF positively correlated with MRI lesion volume (ρ=0.29/p=0.03, ρ=0.28/p=0.03 respectively). Interestingly, several cytokines had positive correlations with neuropsychological testing measures in PWH (n=84), including MIP-1α (Executive function: ρ=0.34, p=0.002; Attention/working memory: ρ=0.22, p=0.04; Overall T-score: ρ=0.29, p=0.008) and IL-6 (Verbal fluency: ρ=0.24, p=0.02; Psychomotor speed: ρ=0.22, p=0.04; Overall T-score: ρ=0.22, p=0.04). Seven cytokines had concurrent significant positive correlations with CSF Aβ42/Aβ40 ratios and negative correlations with CSF pTau181/Aβ42 ratios.

Conclusions: CSF cytokines in PWH are expressed at higher levels than in HC. Our results indicate that this differential expression has complex implications for neurological outcomes, with potentially both protective and deleterious effects in the CNS in the setting of HIV infection.

P56

HIV Modulates APP Isoform Expression and Aβ Accumulation in Human Cerebral Organoids: A Novel Link to Neurocognitive Dysfunction

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Human immunodeficiency virus (HIV) continues to present a significant global health challenge, with chronic infection leading to a range of long-term complications, including HIV-associated neurocognitive disorders (HAND). Neuroinflammation observed in HAND shares similarities with Alzheimer's disease (AD), but the molecular connections between HIV infection and AD-like pathology remain poorly understood. AD is characterized by the accumulation of amyloid-beta (Aβ), which is driven by the processing of amyloid precursor protein (APP) and the formation of neurofibrillary tangles composed of insoluble tau protein. APP exists in three major isoforms,ÄîAPP695, APP751, and APP770,Äîwhose expression is regulated by alternative splicing. The isoforms containing the KPI domain (APP751 and APP770), which are encoded by exon 7 of the APP gene, are associated with amyloidogenic processing and are elevated in AD. However, the role of HIV in modulating APP isoform splicing and Aβ accumulation remains unclear. This study investigates the expression of APP isoforms in 2D and 3D human cerebral organoid (hCO) models to assess the impact of HIV on APP splicing and Aβ accumulation. Our results reveal a significant increase in total APP expression and Aβ accumulation in hCOs following HIV-1 infection. Notably, HIV-1 infection led to increased splicing of the APP751 and APP770 isoforms, accompanied by a reduction in APP695 levels. Furthermore, U87MG cells overexpressing APP751 and APP770 isoforms showed increased Aβ production, as confirmed by western blot and immunocytochemical analysis. These findings suggest a novel mechanism through which HIV may influence the post-transcriptional regulation of APP, contributing to Aβ accumulation in the brain during HAND.

P57

Machine learning-derived neuroHIV biotypes: preliminary outcomes from the NIH-sponsored MIAAD-NHIV initiative

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Brain health disorders (BHDs) affect a substantial proportion of people with HIV (PWH), including those who achieve viral suppression with antiretroviral therapy (ART). Significant heterogeneity exists in the clinical expression of BHDs among PWH, which hampers prevention and treatment efforts. This symposium will introduce the MIAAD-NHIV initiative, an international collaboration to leverage data from

past and ongoing NIH-sponsored research projects to investigate the heterogeneity of BHDs in virally suppressed PWH. The MIAAD-NHIV initiative applies the analytic strengths of machine learning methods to identify actionable mechanisms underlying BHDs in PWH that could be targeted to treat BHDs among PWH. The session will review the conceptual underpinnings, the design and analytic strategy, and preliminary findings of the MIAAD-NHIV initiative.

P58

Alzheimer's Disease-like Pathology in HIV-Infected Humanized Mice and Regulation by Osteopontin/Secreted Phosphoprotein-1

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Chronic neuroinflammation associated with microglial activation is a hallmark pathological component of Alzheimer's Disease (AD). Increased binding of PET-ligands used in neuroimaging to quantify the expression of translocator protein (TSPO), a marker for microglial activation, is found in both AD, and in HIV-infected humanized mice (hu-mice), a model for neuroHIV . Moreover, we reported that TSPO signaling is amplified in hu-mice with knockdown of osteopontin/secreted phosphoprotein-1 (OPN/SPP1), a key regulatory protein of inflammation that is overexpressed in the brain and CSF in AD, neuroHIV, multiple sclerosis, Parkinson's and other neurodegenerative diseases. Given the shared neuroinflammatory signature between AD and neuroHIV, we hypothesized that chronic and persistent infection promotes high levels of OPN/SPP1 that are in the short-term, neuroprotective but in the long-term may be detrimental. To determine if similar pathological processes present in AD are in neuroHIV, and whether OPN/SPP1 regulates them, we conducted a comprehensive quantitative immunocytochemical analysis of amyloid peptides, ApoE, and p-Tau in the brains of chronically infected NSG-hCD34 hu-mice. A multi-label immunofluorescence approach using antisera against AD-associated proteins, glia and neurons was developed. With GFAP reactivity as an indicator of astrogliosis, the extent of its association with ApoE lipoprotein aggregates was quantified. We used high-resolution hyperscan multi-channel LED imaging combined with IMARIS analytic tools to quantify and characterize the morphology and spatial dynamics of ApoE-associated lipoparticles, beta-amyloid, p-Tau, NeuN, and GFAP. Preliminary findings suggest that p-Tau expression is elevated in HIV-infected mice irrespective of OPN/SPP1 expression levels compared to uninfected mice with OPN/SPP1 knockdown. In contrast, ApoE is increased in HIV-infected mice, but not when OPN/SPP1 expression is suppressed. Moreover, OPN/SPP1 knockdown decreases beta-amyloid expression, while GFAP expression is increased. These data provide evidence that chronic neuroinflammation in HIV infection alters similar endogenous pathways implicated neuronal injury and degeneration seen in AD.

P59

Immune and Viral Signatures from CSF Extracellular Vesicles in Multiple Sclerosis and HAM/TSP

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Background: The development of Multiple Sclerosis (MS) involves a combination of genetic and environmental factors, but the exact etiological agent(s) remain unclear. In contrast, human T-cell lymphotropic virus type 1 (HTLV-1) associated myelopathy (HAM/TSP) is a chronic, progressive

neurologic disease caused by HTLV-1 infection. Both diseases share many clinical features. Extracellular vesicles (EVs) are known to be indicators and mediators of health and disease, particularly in viral infection. Therefore, we hypothesized that EV signatures would be distinctive and informative in MS and HAM/TSP. The objective of this study was to identify distinctive EV repertoires and cargo from HAM/TSP, MS, and healthy volunteer (HV) cerebrospinal fluid (CSF).

Methods: Surface repertoires of cerebrospinal fluid (CSF) EVs from individuals with MS (n=55) and HAM/TSP (n=20) were compared to HV (n=13) with refinements of two flow cytometry bead-based methodologies: 1) a commercially available multiplex assay (MPA; Miltenyi), and 2) a customizable capture technology called Nanovials (NVs; Partillion). Additionally, CSF EVs were characterized by size and concentration by microfluidic resistive pulse sensing (MRPS; Spectradyne Arc), and for RNA cargo by RNASeq.

Results: Numbers of CSF EVs and particles (65-400 nm) did not differ between clinical groups (~$5x10^8$ p/mL), however, MPA and NV results showed that individuals with HAM/TSP had significantly elevated EV-associated immune markers including CD8, CD2, and HLA-ABC, but not CD4 compared to MS and HVs. Additionally, RNASeq detected specific viral-associated transcripts in CSF EVs.

Conclusions: HTLV-1-mediated neurological disease showed increased CD8+, CD2+, and HLA-ABC+ CSF EV signals, which is consistent with the known CD8-associated immunopathology in HAM/TSP. This was reinforced with orthogonal analysis using NVs. Furthermore, we showed detection of discrete viral RNAs in CSF EVs, which may play a role in disease pathogenesis or be able to be used as biomarkers.

P60

TLR7 mediates HIV-1 Tat-induced cellular senescence in human astrocytes

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Tat is an important pathogenic factor of HIV-associated neurocognitive disorders (HAND) even with ART, affecting latent infection of HIV and contributing to accelerated aging and neuroinflammation. As a secreted viral protein, Tat is known to enter endolysosomes of CNS cells through receptor-mediated endocytosis, and we have shown that extracellular Tat enters astrocytes via endocytosis, and that Tat induces endolysosome dysfunction and remains trapped in endolysosomes for extended periods of time without being fully degraded. Furthermore, Tat accumulated in endolysosomes is functionally intact, and that upon release from endolysosomes into cytosol, Tat enters nucleus and induces HIV-1 LTR transactivation. Significantly, blocking endolysosome-resident TLR7 attenuates Tat-mediated HIV-1 LTR transactivation. Emerging evidence indicates that Tat induces cellular senescence, which could contribute to accelerated aging, neuroinflammation, and the development of HAND; However, the underlying mechanism is not clear. Given that endolysosome dysfunction is strongly linked to cellular senescence, we tested the hypothesis that Tat induces endolysosome dysfunction and cellular senescence via TLR7 in human astrocytes. We demonstrated that Tat interacts with TLR7 via its arginine-rich basic domain, and such an interaction results in endolysosome dysfunction and the development of a senescence-like phenotype including cell cycle arrest, enhanced SA-β-gal activity, and increased release of senescence-associated secretory phenotype (SASP) factors (IL-6, IL-8, and CCL2). Thus, we provide compelling evidence that Tat-induced endolysosome dysfunction drives the development of cellular senescence in human astrocytes. Our findings also highlight the novel role of TLR7 in the development of cellular senescence and suggest that TLR7 represents a novel therapeutic target against cellular senescence and the development of HAND.

P61

CRISPR antiviral inhibits neurotrophic JC polyomavirus in two- and three-dimensional models through dual-gRNA excision by SaCas9

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Progressive multifocal leukoencephalopathy (PML) is a demyelinating disorder of the central nervous system caused by the lytic proliferation of JC polyomavirus (JCV) at the expense of myelin-producing oligodendrocytes. JCV proliferates during immune compromise, spreading from its primary latency site in the kidney to the brain where it causes the irreversible damage characteristic of PML. For immunosuppressed patients, PML is fatal and untreatable. This study addresses the JCV treatment gap through the use of CRISPR technology. It illustrates the efficacy of a dual-gRNA directed, SaCas9 enacted CRISPR antiviral targeting the LTAg and VP1 regions of the JCV genome. Using bioinformatic analysis and cell cloning, optimal gRNAs were rendered to specifically target three critical regions of the JCV genome while minimizing off-target risk to the human host. Of the seven combinations compared, the dual-gRNA construct simultaneously targeting early and late regions of the JCV genome produced the greatest reduction in viral load. The efficiency of this dual-gRNA CRISPR antiviral was tested using stable cell lines (inoculated through puromycin-selection) as well as cerebral organoids (treated with lentivirus-packaged CRISPR construct). In both two- and three-dimensional cell culture models, the linking span of the JCV genome was confirmed to be excised on Sanger sequencing, resulting in fewer genomic copies on qPCR, inhibited protein expression on western blot and immunofluorescent imaging, and impaired infectivity of viral progeny on adoptive transfer. This supports the potential of this treatment as both a prophylactic and a therapeutic. Exclusively explored in vitro, the primary limitation of this work is its absence of a multi-system model. Advancement to a more translatable preclinical model(i.e., small mammal) is a necessary future direction to assess systemic delivery, dose optimization, and general safety.

P62

EV68-228-N monoclonal antibody treatment halts progression of paralysis in a mouse model of EV-D68 induced acute flaccid myelitis

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In 2014, 2016, and 2018, infection with Enterovirus D68 (EV-D68) was associated with outbreaks of a poliomyelitis-like paralytic syndrome, called acute flaccid myelitis (AFM). While only a small fraction of patients infected with EV-D68 developed AFM, this subgroup of patients does not typically seek treatment until after the onset of neurological symptoms. There are currently no approved human monoclonal antibody therapies or vaccines available for EV-D68. Here we show that a monoclonal antibody, EV68-228-N, can quickly stop the progression of paralysis in a mouse model of AFM, even when treatment is initiated after the onset of paralysis. We found that EV68-228-N effectively halted the progression of paralysis when tested against both 2014 and 2016 EV-D68 isolates in an immunocompetent mouse model of AFM. All animal experiments were conducted in a blinded fashion. The IC50 of EV68-228-N against 2014 and 2016 EV-D68 isolates was confirmed in vitro to be less than 330 ng/ml, and EV68-228-N was found to be equally effective at neutralizing 2018 and 2022 viral isolates without any evidence of emerging resistance. We further show that, following infection with EV-D68, mice treated with EV68-228-N have more surviving motor neurons in the spinal cord's lumbar enlargement than control treated animals. Taken together this work suggests that EV68-228-N treatment has the potential to halt the progression of paralysis

in AFM patients who present at the clinic with neurologic symptoms and that EV68-228-N will retain neutralization potential against emerging EV-D68 isolates.

P63

A Cooperative Editing of Viral and Cellular Genomes Involved in the Early Stage of Viral-Host Interaction by CRISPR-Cas9, Eliminating HIV-1 Replication in In Vitro Cell Models

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HIV-1 remains a major challenge due to genomic heterogeneity, latent reservoirs, and host genome integration, making eradication difficult. Current therapies suppress replication but fail to achieve a complete cure. To address this, we developed a combination treatment strategy using CRISPR-Cas9 gene editing with ribonucleoprotein (RNP) delivery to target both viral and host factors essential for HIV-1 infection. We focused on CCR5, a critical co-receptor for viral entry, and Mannosyl oligosaccharide glucosidase (MOGS), an enzyme crucial for glycosylation of the HIV-1 envelope protein, which is necessary for virion maturation and infectivity. Disrupting MOGS impairs viral glycan processing, resulting in the production of non-infectious virions upon latency reversal, while CCR5 knockout prevents viral entry. Finally, we targeted the HIV-1 LTR-Gag region to directly disrupt viral replication, effectively eliminating any remaining virus escaping earlier stages. Our results, using HIV-p24 ELISA and GFP infection assays, demonstrate for the first time that combining CCR5 and MOGS knockout drastically reduces primary HIV infection in TZM-bl cells. HIV-p24 levels decreased by 99.06%, from 72.6587 ng/ml in controls to 0. 6811 ng/ml in treated samples, as CCR5 knockout blocked viral entry. Adding HIV LTR-Gag CRISPR therapy further reduced HIV-p24 to 0 ng/ml by eliminating any virus that escaped CCR5 targeting. Secondary infection assays showed that either CCR5 and MOGS, or CCR5, MOGS, and LTR-Gag knockouts, reduced HIV-p24 levels by 99.96% and 99.91%, respectively, from 74.0920 ng/ml to 0.0278 ng/ml and 0.0670 ng/ml. These results emphasize the synergistic effect of combination therapy by blocking entry, impairing virion maturation, and eliminating residual virus. In conclusion, this finding highlights the potential of the CRISPR-Cas9 gene editing approach to effectively inhibit HIV-1 infection in cell culture, paving the way for applications in animal models and clinical settings.

P64

Characterization of the potential sumoylation sites, Lys108 and Lys164, of JC virus ORF4 protein: Evidence for their involvement in the targeting of PML-NBs and their reorganization

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The human neurotropic polyomavirus, JC virus (JCV), causes a devastating central nervous system disease known as progressive multifocal leukoencephalopathy (PML) by specifically infecting oligodendrocytes and astrocytes. JCV late coding region transcripts undergo a cis- and/or trans-splicing to produce several splice variants, encoding numerous regulatory or structural proteins. We have recently reported discovering and characterizing four novel open-reading frames from this region, encoding ORF1, ORF2, ORF3, and ORF4 proteins. Among those, the ORF4 protein was determined to be the only JCV protein that targets unique protein complexes in the nucleus, known as PML-NBs, and reorganizes their distribution pattern. We believe that by doing so it most likely alters their function and allows JCV to replicate more efficiently in the infected cells. PML-NBs are dynamic protein complexes consisting of various permanently (PML, hDaxx, SP100, and ATRX) and transiently (p53, SMC5/6, HP1, etc.) recruited members and have been recently implicated in playing critical roles in intrinsic and innate immunity against viral infections. Sumoylation is an essential feature of a protein that is recruited to the PML-NB complexes. Prediction studies revealed that the ORF4 protein contains two potential sumoylation sites within its structure (Lys108

and Lys164), suggesting a role for sumoylation in ORF4 functions. Our initial mutational studies demonstrated a partial or complete loss of function of the ORF4 protein in its PML-NB-targeting and reorganization activities, providing evidence that sumoylation plays an essential role in the recruitment of this protein into the PML-NBs and perhaps in inhibiting their function in JCV infection cases. We are currently in the process of further evaluating the significance of these sites in ORF4 functions utilizing in vitro and in vivo model systems.

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P65

Dopamine Dysregulation and Neuroinflammation in HIV and Methamphetamine Induced Neuropsychiatric Dysfunction

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Public health in the US is facing two overlapping epidemics, namely, substance use disorder and HIV infection. The use of methamphetamine (METH) in the US is on the rise, with a 43% increase in past-year METH use from 2016-2019. Amphetamines induce neurotoxicity through a variety of mechanisms, including excitotoxicity, neuroinflammation, oxidative stress, and mitochondrial damage. Clinically, amphetamines are associated with cognitive decline and psychiatric disorders like depression, psychosis, and anxiety. METH use is a prominent risk factor for HIV infection and correlates with poor adherence to antiretrovirals. HIV infection is associated with neuropsychiatric deficits, loss of dopaminergic neurons, neuroinflammation, and increased oxidative stress. The loss of dopaminergic neurons in HIV infection may play a critical role in interactions with METH, as METH's biological and psychostimulatory effects are primarily mediated through dopamine. Inflammation has been shown to be associated with depression as well as cognitive impairment. Moreover, in people living with HIV receiving treatment, neuroinflammation not only persisted but also correlated with cognitive changes. Given the public health impact of the METH use and HIV epidemics, there is a critical need to understand the mechanisms driving concomitant HIV and METH associated neuropsychiatric disorders. My present research uses EcoHIV, a murine HIV model, to infect mice which have received either saline or METH treatment. Then, a series of behavioral tests are performed. Our results suggest sex-specific behavioral changes in tests which measure cognition, and depression. Further, we have assessed neuroinflammation and oxidative stress associated with infection and/or METH use, linking these behavioral changes with their possible causative mechanisms. These findings will help to guide future research which will focus on repurposing and creating novel therapeutics for use in patients with concomitant HIV infection and METH use disorder. This work was supported by the NIH awards DA059849, DA050528, MH128022, HL126559, DA060085, and NS141704.

P66

HIV Infection of BBB Pericytes Modulates Gap Junction-Mediated Crosstalk with Endothelial Cells

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Antiretroviral therapy has significantly reduced the morbidity and mortality of HIV-1 disease progression, yet HIV-Associated Neurocognitive Impairments (NCI) persist with over 50% of patients experiencing mild to severe degrees of cognitive decline. Recent studies show that blood-brain barrier (BBB) impairment

is a consistent feature of these neurocognitive disorders, resulting from HIV-1's disruption of communication between BBB cells. Pericytes, crucial for maintaining homeostasis of the cerebrovascular microenvironment, can be productively infected with HIV-1. Given their pivotal role and susceptibility to HIV-1, we hypothesize that HIV-1 infection of pericytes disrupts BBB endothelial cell integrity through dysfunctional signaling via gap junctions and hemichannels, potentially exacerbating the development of HIV-associated NCI. To this aim, we employed a co-culture model with primary human BBB pericytes and microvascular endothelial cells in which pericytes were infected with HIV-1 for 3 or 7 days, exhibiting active and latent viral phenotypes. Analysis of co-cultures confirmed a dysregulation of BBB integrity in both actively and latently infected cultures. Furthermore, differential regulation of key gap junction genes and proteins was observed in actively and latently infected pericytes as well as co-cultured endothelial cells. The novel findings of this study also revealed enhanced functional gap junction communication between HIV-infected pericytes and endothelial cells at the time of both active and latent infection. In-progress studies are focusing on the mechanistic outcomes of this increased signaling to endothelial cells and how this contributes to BBB damage. This is important as further studies on the cellular and molecular mechanisms driving the pathogenesis of NCI could provide potential targets for future treatments and interventions.

P67

Impact of NLRP6 inflammasome in the combined effect of HIV Tat and ethanol-mediated neuroinflammation in astrocytes

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Nearly half of people living with HIV consume alcohol, placing them at a 2–3-fold higher risk of developing HIV-associated neurocognitive disorders (HAND), a major public health concern. HIV Tat-induced neuroinflammation is linked to the activation of brain cells, including astrocytes, which release proinflammatory chemokines and cytokines, contributing to cognitive dysfunction. This study investigates the molecular mechanisms driving neuroinflammation in the context of HAND pathogenesis among HIV-infected individuals who consume alcohol. Recent findings from our lab identified NLRP6 (NOD-like receptor family pyrin domain-containing 6) as a key player in HIV Tat- and ethanol-induced neuroinflammation, regulated by microRNAs (miRNAs). Given that alcohol use exacerbates HIV-associated neuroinflammation, we aimed to explore the epigenetic regulation of NLRP6-mediated neuroinflammation in astrocytes exposed to HIV Tat and ethanol. Mouse primary astrocytes (MPAs) exposed with HIV Tat (50 ng/mL) and ethanol (50 mM) exhibited significant upregulation of astrocyte activation markers (GFAP) and NLRP6 cooperatively. This combined exposure also increased NLRP6 downstream signaling mediators, including caspase-1 and proinflammatory cytokines IL1β and IL18. Notably, silencing NLRP6 expression inhibited these effects, confirming its crucial role in HIV Tat- and ethanol-mediated neuroinflammation. Furthermore, a microarray analysis identified miR-339 as significantly downregulated miR in ethanol-exposed astrocytes. qPCR validation further revealed decreased expression of miR-339 in MPAs exposed to HIV Tat and ethanol. Argonaute immunoprecipitation assays confirmed that miR-339 directly targets the 3'UTR of NLRP6 mRNA. Overexpression of miR-339 in MPAs exposed to HIV Tat and ethanol further validated its role in epigenetically regulating NLRP6 inflammasome signaling. In summary, our findings highlight the role of miR-339 in regulating NLRP6 inflammasome signaling and associated neuroinflammation in HIV Tat- and ethanol-exposed astrocytes. These results provide novel insights into the epigenetic mechanisms underlying HAND pathogenesis in the context of alcohol use.

P68

iPSC-Derived Microglia-Integrated Forebrain Organoids: A Novel Model for HIV Infection in the CNS

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Microglial integration into brain organoids provides a model to study neuro-immune interactions during neurodevelopment and pathologic processes such as CNS viral infections. Here, we describe a novel model of integration of human iPSC-derived microglia (iMg) to human iPSC-derived forebrain organoids and demonstrate the capacity of integrated iMg to harbor productive HIV replication. We also present data suggesting that the iMg integration enhances neuronal maturation. Human forebrain organoids were generated from two independent iPSC lines and were matured for 45–55 days before they were used in a series of integration experiments. Briefly, common myeloid progenitors were differentiated from iPSCs of adult human fibroblasts and further differentiated into iMg. Organoid-iMg integration was performed by coculturing organoids on a layer of iMg for 10 days in organoid media containing M-CSF and IL-34. For viral transduction, 1 ng/mL of the microphage-tropic HIV-ADA strain was added to either ongoing organoid-iMg cocultures or iMg-integrated organoids transferred to new plates. Robust HIV infection was achieved within 5–7 days, with nearly all IBA-1 positive cells displaying HIV p24 marker. Productive HIV infection was confirmed by qPCR and p24 alphalisa. We also demonstrated that the productive HIV infection could be transferred by coculturing infected organoids over a layer of uninfected iMg or with uninfected iMg-integrated organoids. Overall, we achieved robust iMg integration of total cells in the iMg-integrated organoids. The microglia in iMg-integrated organoids, without HIV infection, displayed ramified morphology. qPCR analysis of iMg-integrated organoids revealed a strong correlation between markers of neuronal differentiation (Nestin and Ctip2) and the myeloid marker CD14 ($r > 0.7$, $p < 0.001$), and this correlation did not change in the presence of HIV. These results demonstrate the suitability of our model to study neuro-immune interactions in the presence of HIV infection and developmental studies of the CNS.

P69

West Nile Virus Elicits Different Microglial Responses in Various Brain Regions

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West Nile Virus (WNV) is a Flavivirus that is spread to human usually through mosquito bites. It can invade the CNS after passing through the circulatory system where it can cause death in humans. In an effort to better understand how WNV reacts in the brain, we infected 8 week old female C57BL/6 female mice via footpad injection and monitored their health over a 14 day period before collection and dissection of different regions of the brain. We noted that the whole brain had a peak response to WNV 9 days following footpad injection. This includes inflammation, interferon, and microglial responses. The cortex region showed a particularly exaggerated response at day 9 as compared to the other measured regions of the brain, the midbrain, hindbrain, and cerebellum. In addition, microglial activation was noted as elevated at all timepoints measured, 5, 7, 9, and 14 days following injection. We noted that anti-inflammatory microglial reaction was elevated in all region, but showed a distinct drop in reaction in the cortex from 7 days post infection onward. Over course of these experiments, we also tracked weight and behavior of the mice and noted that both began to shows signs of decline 9 days following WNV induction in mice that would become sick. Our data replicates what is seen in the greater body of human data where a smaller fraction of subjects experience notably detrimental side effects from WNV infection. Furthermore, the 9th days post infection seems to be a critical timepoint to determine the overall response to WNV.

P70

Disrupted IFN-1 Signaling but Unaltered Phagocytosis in Cognitive Impaired Hispanics with HIV on ART

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People with HIV (PWH) develop HIV-associated neurocognitive disorders marked by infiltrated monocytes, inflammation, and neuronal dysfunction. Despite effective antiretroviral therapy, there is no treatment for cognitive decline. Type 1 interferon (IFN-1) signaling is important for synaptic plasticity and cognitive function. We hypothesized that disrupted IFN-1 signaling negatively affects monocyte function and triggers cognitive decline. Plasma IFN-alpha (IFN-a) and beta (IFN-b) and neurofilament light chain (NFL) levels were measured in PWH stratified by cognitive status, Alzheimer's disease (AD) patients with mild cognitive impairment and HIV-negative controls by ELISA (N=28). IFN-a receptor 1 (IFNAR1) levels in blood mononuclear cells were measured by flow cytometry. CD14+ monocytes (N=23) were isolated from peripheral blood mononuclear cells (PBMCs) and tested for phagocytic activity using a zymosan photometric assay. Cognitive impaired PWH showed slightly elevated plasma IFN-a1 levels, while AD patients had significantly higher levels of IFN-a1 (p=0.03) compared to controls and NFL compared to PWH (p=0.004) with and without cognitive impairment and controls (p=0.005). Plasma NFL levels were significantly higher in males with HIV compared to controls (p=0.02), but no differences were detected in women with HIV. Plasma IFN-b significantly decreased in men and women with HIV (p=0.001). A lower percentage of IFNAR1+CD14+ monocytes was detected in PWH (p=0.02) and AD patients (p=0.01), which was significantly lower in men (p=0.03) but not in women. However, phagocytic activity was similar in monocytes across all the groups. Disrupted IFN-1 signaling may contribute to monocyte phenotype shifts, recruitment to the brain, and neurodegeneration, processes which may not involve deficits in phagocytosis for removal of protein aggregates. Differential IFN-a and IFN-b plasma levels and biological sex differences warrant further investigation for future diagnostic and therapeutic approaches.

P71

Determining viral factors crucial for JC polyomavirus cell tropism and central nervous system pathogenesis

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JC Polyomavirus (JCPyV) infects 40-90% of the adult population in a benign kidney infection. However, in a subset of immunocompromised individuals it causes a rapid and often fatal infection known as Progressive Multifocal Leukoencephalopathy (PML). There are no cures for PML and those who survive the initial infection are left with severe lifelong complications. Much of the process the virus must undergo to travel to the brain and establish infection is currently unknown. It was previously thought that mutations in the non-coding control region (NCCR) of the virus were necessary for the virus to enter and establish infection in the central nervous system (CNS) however recent findings in our lab indicate the situation may be more complex. We recently sequenced the virus from a patient with acute JC-associated meningoencephalitis, which showed no rearrangements in the NCCR. The patient had no apparent brain lesions, but high levels of JCPyV in the cerebral spinal fluid. After treatment the patient had complete neurological recovery unlike typical PML patients. This has prompted us to hypothesize that JCPyV is entering the CNS prior to mutating the NCCR and these mutations are acquired once in the CNS to better infect glial cells. To test this, we have developed novel primary cell culture methods to model the renal and neuronal environments using JCPyV with rearranged and non-rearranged NCCRs. The knowledge gained from these studies will aid in understanding how JCPyV changes from an asymptomatic virus harbored in the kidney to a deadly neuro pathogen.

P72

Exploring Benzodiazepines as a Unique Viral Reactivator in HIV-1 Infected Human Monocyte Derived Macrophages

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HIV-1's persistent reservoirs pose a significant challenge in developing a permanent cure, with replication-competent provirus hiding in long-lived cells, evading antiretroviral therapy (ART). A unique and poorly understood aspect of this challenge is the role of HIV-1-infected myeloid cells, which can cross the blood-brain barrier, contributing to the central nervous system (CNS) reservoir. These cells are implicated in the development of HIV-associated neurocognitive disorders (HAND), complicating the virus's persistence. While research has mainly focused on T-cells, studies on myeloid cells suggest their epigenetic regulation differs, with important implications for neuroHIV. The effect of substance misuse, especially benzodiazepines (BDZ), on myeloid cells and neurocognition remains under-explored. Our prior studies have shown that BDZs disrupt latency in T-cells, yet their impact on HIV-infected myeloid cells is not well understood. In experiments using human monocyte-derived macrophages (hMDMs) to model brain-resident myeloid cells, we observed that infection and ART exposure did not reduce the number of infected macrophages over time, though p24 Gag production decreased significantly. However, proviral transcription remained elevated, and ChIP analysis revealed active transcription at the integrated LTR, suggesting myeloid cells are governed by a distinct viral control mechanism, different from the transcriptional latency seen in CD4+ T-cells. Upon BDZ exposure, viral production rebounded, an effect not seen with T-cell LRAs. supporting the hypothesis that HIV-infected myeloid cells may exist in a "semi-quiescent" state with a unique epigenetic profile. Histone 3 (H3) modification of the LTR during semi-quiescence and subsequent re-activation were also different than what is seen in T-cells. This suggests that HIV and BDZ exposure may be altering the epigenetics of the host genome. Disrupting this state with BDZ could provide a potential shock-and-kill strategy and insights into worsened HAND symptoms and cognitive decline in BDZ-using PLWH.

P73

The Use of CBD and Its Synthetic Analog HU308 in HIV-1-Infected Myeloid Cells

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Currently, there is no cure for human immunodeficiency virus type 1 (HIV-1) infection. However, combined antiretroviral therapy (cART) aids in viral latency and prevents the progression of HIV-1 infection into acquired immunodeficiency syndrome (AIDS). cART has extended many lives, but people living with HIV-1 (PLWH) face lifelong ailments such as HIV-associated neurocognitive disorders (HAND) that range from asymptomatic HAND to HIV-1-associated dementia. HAND has been attributed to chronic inflammation and low-level infection within the central nervous system (CNS) caused by proinflammatory cytokines and viral products. These molecules are shuttled into the CNS within extracellular vesicles (EVs), lipid bound nanoparticles and are released from cells as a form of intercellular communication. Here we explore the use of cannabidiol (CBD), as a promising and potential therapeutic for HAND patients, and a similar synthetic molecule, HU308. The data shows that both CBD and HU308 decrease non-coding and coding viral RNA (TAR and env) as well as proinflammatory cytokines as IL-1β and TNF-α mRNA. This decrease in viral RNA occurs in vitro differentiated primary macrophages, in EVs released from HIV-1-infected cells monocytes, and infected neurospheres, and in vivo, a humanized mouse model of HIV-1 infection demonstrated a decrease in circulating viral RNA with HU308 treatment. Overall, CBD or HU308 may be a viable option to decrease EV release and associated cytokines which would dampen the virus spread and may be used in effective treatment of HAND in combination with cART.

P74

Neuro-microglial immune underlying HIV and substance use comorbidity

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Neuroinflammation significantly contributes to HIV-associated neurocognitive disorders (HANDs), with 25-50% of people living with HIV (PLWH) exhibiting symptoms. Chronic, low-level expression of HIV proteins and a proinflammatory state in the central nervous system (CNS) are thought to cause synaptodendritic injuries, leading to neurobehavioral and cognitive declines. Potent CNS stimulants such as Methamphetamine and Cocaine use are frequently co-morbid with HIV and lead to exacerbated neuroinflammation and cognitive impairment. The drugs have shown to promote excitotoxicity, immune signaling, oxidative stress responses, and mitochondrial dysfunction, all of which result to microglial activation and subsequent neuronal damages. Damaged or stressed neurons release altered metabolic products known as damage-associated molecular patterns (DAMPs) inducing sterile inflammatory responses in CNS. Our lab is interested in understanding mechanism of neuro-microglial communication that underlies excitotoxicity. Our current study investigates innate immune sensing of neuronal DAMPs produced by over-stimulated neurons in microglia. Through genome screening and mechanistic studies, we previously identified a cellular protein called polyglutamine binding protein 1 (PQBP1) as a PRR to the HIV-1 capsid PAMP and demonstrated that the PRR-PAMP engagement initiates cGAS signaling. Recently, a neuronal activity-regulated immediate-early gene, Arc (activity-regulated cytoskeleton-associated protein), was shown to form HIV Gag capsid-like structures. This protein is evolutionarily related to retrotransposon Gag protein, and the assembled Arcs can be transferred between cells via extra cellular vesicles. Our preliminary results indicate that overstimulation or stressing of neurons elevates the production of Arc-capsid containing EVs. And co-culturing of human induced pluripotent stem cell (iPSC)-derived microglia with either Arc-capsid containing EVs induce cGAS signaling. We hypothesize that analogous to HIV-1, incoming Arc-capsid is recognized by PQBP1 to induce cGAS signaling. We envision that cGAS sensing of both HIV-1 and Arc capsids in the infected microglia fuels the exacerbated CNS inflammation underlying the comorbidity.

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HIV-1 infection activates innate sensing in microglia through the inflammatory cGAS-STING pathway

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A significant proportion of people living with HIV/AIDS (PLWH) develop HIV-associated neurocognitive disorders (HAND). While combination antiretroviral therapy (cART) has substantially reduced morbidity and mortality, chronic neuroinflammation persists in PLWH, yet the underlying cellular mechanisms remain unclear. Microglia play a central role in HIV-associated neuroinflammation, producing inflammatory cytokines and metabolites that contribute to neurotoxicity. Here, we show that the GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway is a key activator of intracellular innate immunity in microglia, which is not controlled by cART. We find that markers associated with cGAS/STING inflammation are present on HIV positive post- mortem human brain tissues under cART. To investigate this mechanism, we used iPSC-derived microglia (iMGL), monocyte-derived microglia (MD-Mgl), and microglia-containing cerebral organoid (CO-iM) models to study the interaction between HIV particles and recruitment and activation of innate sensing machinery in microglia. HIV-infected iMGLs activate the cGAS/STING pathway following capsid integrity loss, whereas cGAS/STING KO iMGLs do not. Multiplex ELISA and RT-qPCR revealed that cGAS activation from HIV-infected iMGL and MD-Mgl leads to the production of pro-inflammatory cytokines/chemokines which in turn contribute

to the skewing of iPSC-derived astrocytes into the neurotoxic phenotype. In addition, we observed compromised neuronal function after challenging neurons with supernatants harvested from infected microglia where we found disruption of neurons measured by number of connections and quantification of synaptic markers such as PSD95, Synapsin1 and TUBB3. Finally, by leveraging cyclic multiplex immunofluorescence and CO-iMs, we find that the activation of cGAS/STING in microglia results in strong skewing of astrocytes into inflammatory-associated phenotypes and signals of compromised neuronal function. Our findings demonstrate that the cGAS/STING pathway in microglia is a key driver of HIV-induced neuroinflammation, related to HAND pathogenesis by disrupting brain homeostasis.

P76

Enterovirus D68 2A protease causes nuclear pore complex dysfunction and motor neuron toxicity

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Acute flaccid myelitis (AFM) is a polio-like paralytic disease of children. It is caused by enterovirus infection, and increased circulation of Enterovirus D68 (EV-D68) has been associated with multiple worldwide outbreaks of AFM in recent years. AFM has been attributed to infection of spinal motor neurons by EV-D68, however the mechanisms linking infection to toxicity are unknown. The enterovirus life cycle involves the disruption of nucleocytoplasmic transport, allowing RNA binding proteins that typically reside in the nucleus to participate in viral replication in the cytoplasm, and disrupting the nuclear export of host mRNA to favor viral translation and replication. Similar deficits in nucleocytoplasmic transport have been implicated in neurodegenerative disorders, most notably the motor neuron disease Amyotrophic Lateral Sclerosis (ALS). We therefore hypothesized that enterovirus-induced nucleocytoplasmic transport dysfunction contributes to the selective motor neuron toxicity that occurs in AFM. Here, we demonstrate that the EV-D68 2Apro and 3Cpro proteases cleave six nucleoporins, but that this disrupts the expression of 17 nucleoporins in total. The activity of 2Apro, but not 3Cpro, disrupts the permeability barrier of the nuclear pore complex and prevents active transport protein cargoes but not the export of RNA. In an induced pluripotent stem cell (iPSC)-derived spinal motor neuron model of AFM, inhibition of 2Apro with telaprevir is neuroprotective, independent of its potential antiviral effects. These findings elucidate the role of the nuclear pore complex in AFM pathogenesis, and suggest possible targets for neuroprotective therapies.

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Chronic Cocaine Exposure Alters SIV Latency in the Periphery but not the Central Nervous System

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Substance use disorders among people with HIV (PWH) can severely impact HIV infection and have been associated with increased severity of HIV neuropathology. Cocaine is a commonly used drug among PWH, with an estimated 5-15% of PWH having a cocaine use disorder and is implicated in the pervasiveness of HIV-associated cognitive impairment. Utilizing a simian immunodeficiency virus (SIV) model in rhesus macaques (RM, n=12), we assessed the effects of chronic cocaine exposure on SIV infection in both the periphery and central nervous system (CNS). Six RM were given daily intramuscular cocaine starting 30 days prior to inoculation with SIVmac251, and were weaned off cocaine by 158 days post infection (dpi). All 12 RMs were placed on a daily-injectable ART regimen at 42 dpi and euthanized at 300 dpi while still on suppressive ART. Plasma and CSF was collected throughout the study to monitor SIV viral load. At the terminal time point, tissues from both the periphery and CNS were collected for SIV DNA, SIV RNA, and SIV reservoir measurements. Cocaine had minimal effects on the plasma and CSF viral loads, and no

significant difference was observed in SIV DNA and RNA measurements in both the CNS and peripheral tissue. Cocaine exposure did not alter the size of the macrophage reservoir in any compartment. In contrast, cocaine exposure resulted in significantly larger reactivable CD4+ T cell reservoirs in all compartments, despite lower levels of intact SIV genomes in this cell type. This suggests that long-term cocaine exposure can lead to reduced reservoir seeding in the periphery while likely altering the quiescent state of the CD4+ T cell population resulting in more robust reactivation ex vivo.

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Establishing Multicellular Blood-Brain Barrier Infection Models for Antiviral Drug Discovery

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With low vaccination rates in endemic countries, tick-borne encephalitis virus (TBEV) poses a risk to the population who, upon infection, can develop severe complications, like encephalitis. Access to the central nervous system (CNS) by neurotropic viruses can be achieved via the blood-brain barrier (BBB), a critical cellular unit protecting the brain parenchyma from toxins and pathogens in circulation. While the impermeable characteristics of the BBB are critical for brain protection, they are also obstacles for the treatment of brain infections with antivirals. Our objective is to establish in vitro BBB models that would serve as tools to identify BBB-permeable antivirals and to investigate the effect of TBEV infection on the BBB. We developed a transwell insert BBB model with human brain microvascular endothelial cells (HBMEC) and pericytes (HBMVP). Due to the versatility of the insert model, we could co-culture the barrier cells with various target cell lines, including human neural stem cell line HNSC.100-differentiated astrocytes. Immunofluorescence staining of HBMEC (CD31) and HBMVP (NG2) markers provided visual confirmation of barrier formation on the insert membrane. Fluorometric assays, revealed the HBMEC-HBMVP co-culture decreased monolayer permeability in comparison to HBMEC monocultures, highlighting the importance of cellular crosstalk in BBB tightness. To validate our model as a screening tool for BBB-permeable antivirals, we tested antiretrovirals with varying CNS-Penetration-Effectiveness (CPE) scores. Preliminary data reveals our BBB model permits the passage of nevirapine (high CPE), but not saquinavir (low CPE) demonstrating that our in vitro model mimics in vivo BBB selectivity. Initial in vitro BBB infection experiments reveal TBEV crosses a co-culture of HBMEC and HBMVP with no increase in permeability. Utilization of our in vitro models may not only help in expediting the development of therapeutics against neurotropic viruses but may also provide insight into the TBEV-induced cellular responses responsible for promoting encephalitis.


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