Opioid Use Disorder Associates With Reduced HIV Reservoirs but Elevated Inflammation

Benjamin Bone[1], Rian Shah[1], Rajendra Pahwa[1], Hansel Tookes[1], David Forrest[1], Allan Rodriguez[1], Suresh Pallikkuth[1] and Savita Pahwa[1]
[1]Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL, USA,

Background

- **HIV persistence** during suppressive antiretroviral therapy (ART) is maintained by **long-lived cellular reservoirs** (1, 2) whose size, composition, and transcriptional activity are **shaped by the host immune environment** (3)**.**
- **Opioid use disorder** (**OUD**), which is increasingly driven by synthetic opioids such as fentanyl, is common among people with HIV (PWH) and is **associated with immune dysregulation, chronic inflammation, and altered myeloid and lymphoid function** (4, 5).
- The impact of OUD on HIV reservoir characteristics under ART remains poorly defined and we aimed to investigate that here.

- **Hypothesis:** OUD is associated with heightened systemic inflammation that alters the cellular immune landscape and modulates HIV reservoir size, composition, and transcriptional activity during ART.

People with controlled HIV and opioid use disorder were observed to have **smaller total** and **defective HIV reservoirs** but **heightened systemic inflammation** and **distinct immune-reservoir interactions during ART.**

Aims and Methods

Aims:

- **Define how OUD associates with HIV reservoir size, composition, and transcriptional activity under ART.**
- **Determine whether OUD associates with systemic inflammation in people with HIV on ART.**
- **Evaluate adaptive and innate cellular correlates of reservoir and inflammatory phenotypes.**

Cohort:

We leveraged samples from the R01 supported **OPIS study** (4), investigating vaccine responses in PWH with and without OUD. Urine toxicology confirmed opioid usage.

Table 1: Participant demographics

		OUD+	OUD-
	N	**30**	**29**
Race	**White**	**77%**	**38%**
	Black/African American	**13%**	**59%**
	Other	**10%**	**3%**
Sex	**Male**	**63%**	**55%**
	Female	**37%**	**45%**
Median CD4+ count (cells/uL)		**687 (557-887.6)**	**642.6 (419.9-904.4)**
Median viral load (copies/mL)		**20 (LOD) (20-332)**	**20 (LOD) (20-96)**
Median age (years)		**42 (38.25-53.25)**	**54 (48-58)**
Median duration of ART (years)		**2 (1-4)**	**16 (10-26)**

Methods:



Intact proviral DNA assay (IPDA) to measure HIV reservoir in CD4+ T cells with parallel cell-associated RNA measurements to determine transcriptional activity.



Multiplex Luminex analyses of participant blood plasma to quantify inflammatory markers.



High dimensional flow cytometry to measure activation induced marker (AIM) expression on CD4+ and CD8+ T cells in response to HIV Gag peptide.



Single cell transcriptional and surface protein marker profiling of whole PBMCs via 10X CITE-seq.

Results – Reservoir data

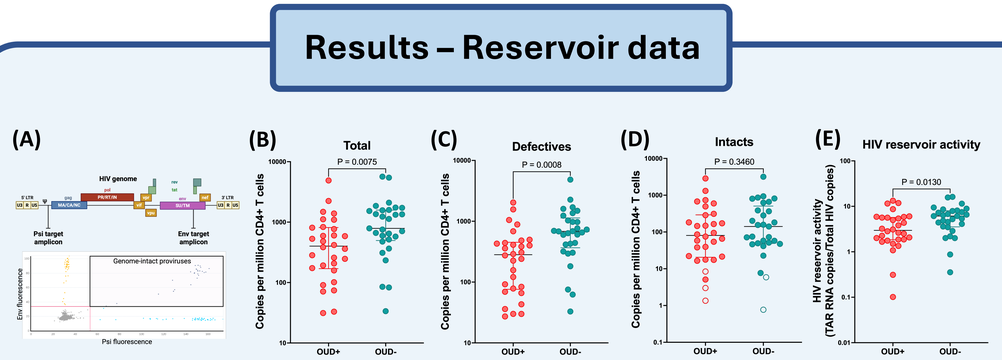


Figure 1: Opioid use disorder associates with altered HIV reservoir composition and reduced transcriptional activity.
(A) Schematic demonstrating that genome-intact proviruses are determined by double positivity through digital PCR assay of amplicons targeting the *psi*, and *env* regions of the provirus. Absolute measurements of Total **(B)**, Defective **(C)**, and Intact **(D)** proviruses. HIV reservoir transcriptional activity is presented in **(E)**, calculated as cell-associated HIV TAR copies normalised to total HIV DNA copies per million $CD4^+$ T cells. Mann Whitney U tests were used for comparisons, with p-values, medians, and IQRs annotated. Empty circle data points represent participants where the copy number was below the limit of detection.

Plasma cytokines

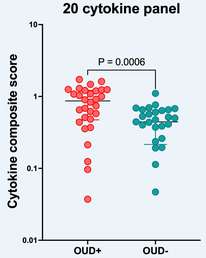


Figure 2: Opioid use disorder is associated with heightened systemic inflammation.
A composite plasma cytokine score was generated for all participants by aggregating their normalised plasma concentrations of inflammatory and immune activation markers measured by multiplex Luminex assay. A Mann Whitney U test was used for the comparison, with p-value, median, and IQR annotated.

Inflammatory markers measured:

- IFABP
- sCD163
- **sCD25**
- **IL-10**
- IL-17a
- IL-22
- D-Dimer
- VCAM-1
- ICAM-1
- LBP
- hsCRP
- **TNFa**
- **IL-1b**
- **IL-6**
- **sTNFR-I**
- **sTNFR-II**
- **CCL2/MCP1**
- **IL-8**
- IL-1a
- **sCD14**

Highlighted markers were significantly elevated in OUD+

HIV-specific responses

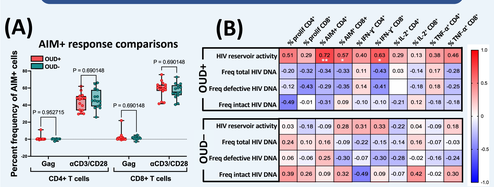


Figure 3: HIV-specific T cell responses correlate with reservoir activity in OUD+ but not OUD- individuals.
HIV-specific CD4+ and CD8+ T cell responses were measured by proliferation, activation induced marker (AIM) expression, and intracellular cytokine staining following HIV Gag stimulation, normalising to the unstimulated (media) control. **(A)** Frequencies of AIM+ CD4+ and CD8+ T cells following stimulation. Mann Whitney U tests were performed and P-values annotated. **(B)** Relationships between HIV-specific CD4+ CD8+ responses and reservoir data were investigated through Spearman rank correlations. Spearman R values presented in matrix. Significant correlations are annotated (* = p<0.05 ** = p<0.01).

Single-cell CITE-seq

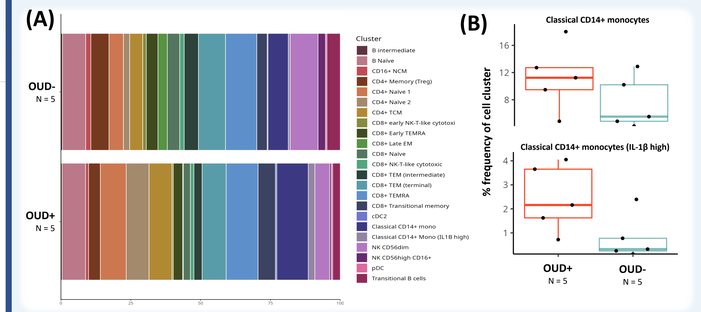


Figure 4: Pilot single-cell CITE-seq analysis identifies trends for monocyte compartment shifts in individuals with opioid use disorder.
PBMCs from a subset of the OPIS participants (OUD+ N=5; OUD- N=5) were profiled using 10X Genomics CITE-seq. Cell phenotype annotation was informed by Azimuth as well as manual evaluation of transcriptomic and surface protein features. A total of 58,708 cells were analysed (OUD+ 30,909 cells; OUD- 27,799 cells). The relative abundance of the identified immune populations were determined (A) with per participant values presented for the classical monocyte populations as boxplots. Boxplots demonstrate trends in difference, but these were not determined to be significant by Wilcoxon-rank sum test.

Conclusions

- Individuals with OUD exhibited reduced total and defective HIV DNA and dampened reservoir transcriptional activity.
- OUD was associated with systemic inflammation, reflected by higher composite plasma cytokine score.
- HIV-specific T cell response magnitudes were similar between OUD statuses; however positive correlations were observed between immune response measures and reservoir activity in OUD+ individuals.
- Pilot CITE-seq analysis suggests trends towards expansion of classical and IL-1β high inflammatory monocyte states in OUD+, which could contribute to systemic inflammation observed.
- **Together these findings indicate that OUD is associated with a pro-inflammatory immune environment and altered interactions between host immunity and the HIV reservoir, highlighting inflammation-linked immune remodelling as a potential modifier of HIV persistence during ART.**

Future Directions

- Our ongoing studies are investigating the cellular and molecular mechanisms linking opioid-associated inflammation to altered immune-reservoir interactions.

References:
(1) Finzi D *et al.* Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. Science. 1997 Nov 14;278(5341):1295-300. doi: 10.1126/science.278.5341.1295. PMID: 9360927.
(2) Siliciano JD *et al.* Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. Nat Med. 2003 Jun;9(6):727-8. doi: 10.1038/nm880. Epub 2003 May 18. PMID: 12754504.
(3) Armani-Tourret M *et al.* Immune targeting of HIV-1 reservoir cells: a path to elimination strategies and cure. Nat Rev Microbiol. 2024 Jun;22(6):328-344. doi: 10.1038/s41579-024-01010-8. Epub 2024 Feb 9. PMID: 38337034; PMCID: PMC11131351.
(4) Dang C *et al.* Opioids exacerbate inflammation in people with well-controlled HIV. Front Immunol. 2023 Nov 1;14:1277491. doi: 10.3389/fimmu.2023.1277491. PMID: 38022645; PMCID: PMC10646416.
(5) Liang X et al. Opioid System Modulates the Immune Function: A Review. Transl Perioper Pain Med. 2016;1(1):5-13. PMID: 26985446; PMCID: PMC4790459.

Contact details:
Presenter: Benjamin Bone PhD – brb173@med.miami.edu
PI: Savita Pahwa MD – spahwa@med.miami.edu

Acknowledgements:
Funding support was provided from NIH through R01 grant (**R01DA051202**) to Savita Pahwa. We acknowledge and thank the study participants.