Poster #414

# Impact of SARS-COV-2-Mediated CD4+ T Cell Activation on HIV DNA Persistence In-Vivo

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## Introduction

The impact of COVID-19-mediated T-cell activation on the expansion of the HIV-1 reservoir remains unknown. As a result, we sought to determine the impact of SARS-CoV-2 infection on expansion of HIV-1-infected CD4+ T cells and changes in cellular HIV burden following COVID.

We previously demonstrated that people with HIV (PWH) on ART experience high rates of detectable low-level, transient plasma HIV-1 RNA (“viral blips”) following COVID-19, but the impact of SARS-CoV-2 infection and associated increase in immune activation and inflammation on HIV reservoirs remains unknown.[1] Using a surface activation induced marker assay (AIM) and ddPCR, we compared frequencies of SARS-CoV-2 specific, AIM+ CD4+ T cells and cell-associated HIV-1 DNA levels from participants in the LIINC and SCOPE cohort pre and post COVID-19 infection to test the hypothesis that expansion of SARS-CoV-2-responsive CD4+ T cell harboring HIV drive proliferation of the HIV reservoir.

## Methods

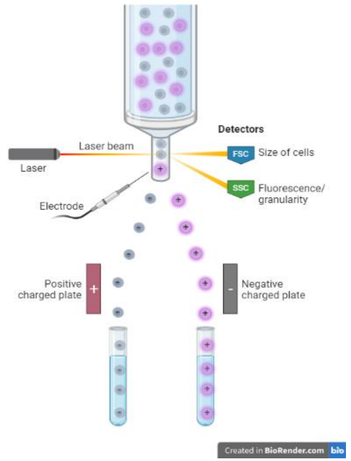
We collected PBMC pre- and post-SARS-CoV-2 infection from 5 PWH on suppressive ART with samples available prior to and approximately 2 months following acute COVID-19 leveraging the UCSF SCOPE and LIINC cohorts.

We performed FACS sorting following 12 hours of stimulation with a CD4+ T cell-optimized overlapping SARS-CoV-2 peptide pool.[2] Live CD4+ T cells expressing the following combination of surface markers were collected followed by DNA extraction optimized for small cell number inputs:

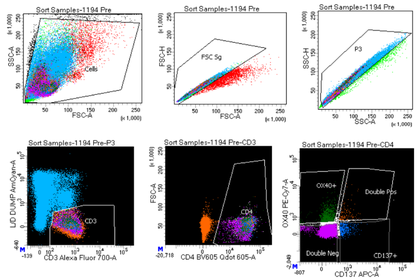
OX40L+ CD137(4-1BB)+
OX40L+ CD137-
OX40L- CD137+
OX40L- CD137-

Ref: 1: Peluso et al. Clinical Infectious Diseases, 2021
2: Crotty S, Science 2021

### Activation Induced Surface Marker Sorting




### Gating Strategy




### HIV-1 DNA Quantitation

HIV-1 DNA from CD4+ T cell AIM subsets was quantified using a highly sensitive ddPCR assay that detects LTR-Gag region across broad Group M subtypes.

## Results

### Change in SARS-CoV-2 AIM+ CD4+ T cell Percentages Following COVID-19

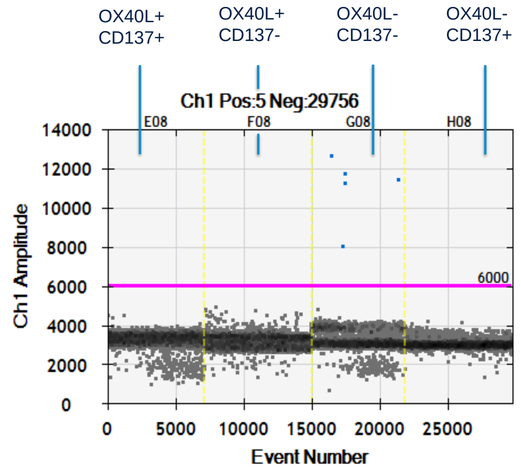
| Pt | Timepoint | OX40L+ CD137+ | OX40L+ CD137- | OX40L- CD137+ | OX40L-CD137- |
|---|---|---|---|---|---|
| P1 | Pre | 263 (0.03%) | 7603 (1%) | 1090 (0.14%) | 750000 (98.8%) |
| | Post (M0) | 1457 (0.18%) | 15487 (1.87%) | 1738 (0.21%) | 806000 (97.7%) |
| | $Log_2$ FC | 2.6 | 0.9 | 0.6 | -0.02 |
| P2 | Pre | 121 (0.03%) | 5442 (1.6%) | 342 (0.10%) | 331000 (98.2%) |
| | Post (V1) | 1639 (0.39%) | 24619 (5.81%) | 4998 (1.18%) | 396321 (93.6%) |
| | $Log_2$ FC | 3.7 | 1.9 | 3.6 | -0.1 |
| P3 | Pre | 257 (0.07%) | 6507 (1.75%) | 925 (0.25%) | 365000 (97.9%) |
| | Post (M0) | 980 (2.29%) | 13666 (3.19%) | 1272 (0.30%) | 412000 (96.3%) |
| | $Log_2$ FC | 5.0 | 0.9 | 0.3 | -0.02 |
| P4 | Pre | 1287 (0.21%) | 10180 (1.69%) | 6324 (1.05%) | 584736 (97.1%) |
| | Post (M0) | 6997 (3.06%) | 77851 (3.41%) | 15097 (0.66%) | 2200000 (96.2%) |
| | $Log_2$ FC | 3.9 | 1.0 | -0.7 | -0.01 |
| P5 | Pre | 9710 (1.49%) | 62393 (9.58%) | 32777 (5.04%) | 546114 (83.9%) |
| | Post (M1) | 15919 (0.74%) | 288243 (13.4%) | 43806 (2.04%) | 1800000 (83.8%) |
| | $Log_2$ FC | -1.0 | 0.5 | -1.3 | -0.002 |

### HIV-1 DNA Copies/$10^6$ in SARS-CoV-2 AIM+/- CD4+ T Cells

| Pt | Timepoint | OX40L+ CD137+ | OX40L+ CD137- | OX40L- CD137+ | OX40L-CD137- |
|---|---|---|---|---|---|
| P1 | Pre | * | 9444 | Not Detected | * |
| | Post (M0) | * | Not Detected | | Not Detected |
| P2 | Pre | * | 16666 | * | Not Detected |
| | Post (V1) | * | Not Detected | * | Not Detected |
| P3 | Pre | Not Detected | * | * | Not Detected |
| | Post (M0) | * | 18889 | Not Detected | Not Detected |
| P4 | Pre | Not Detected | Not Detected | Not Detected | 4569 |
| | Post (M0) | Not Detected | Not Detected | Not Detected | 957 |
| P5 | Pre | * | Not Detected | * | Not Detected |
| | Post (M1) | Not Detected | * | Not Detected | Not Detected |

* Insufficient cell yields to quantify HIV DNA

### Example of ddPCR HIV-1 quantitation within sorted CD4+ T cells




## Conclusions

- COVID-19 in PLWH led to a modest SARS-CoV-2-specific CD4+ T cell response in 5/6 participants with HIV-1 on ART as determined by AIM assay.
- We were unable to detect HIV-1 DNA in AIM OX40+/CD137+ CD4+ T cells following SARS-CoV-2 infection, likely due to low numbers of AIM+ cells following stimulation.
- Future studies with more participants and larger numbers of PBMC will be necessary to fully understand the impact SARS-CoV-2 antigen-driven proliferation or maintenance of the HIV-1 reservoir.

### Acknowledgments

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