Well-Seeded Reservoirs in Gut Are Associated With Tertiary Lymphoid Structures With Activated ISR

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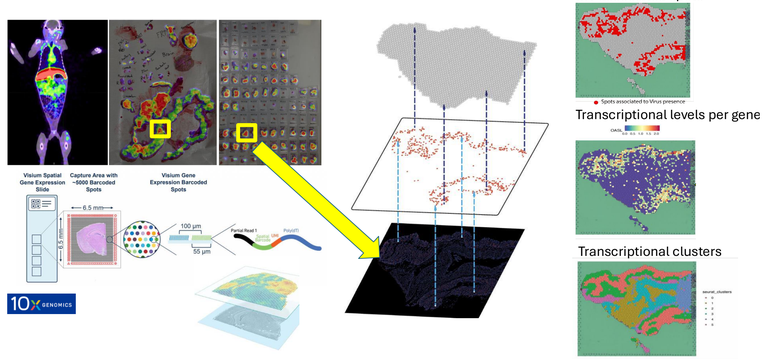
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Background

Despite effective antiretroviral therapy (ART), HIV-1 persistence is the major obstacle to a functional cure. Thus, understanding the tissue microenvironment during ART of the reservoirs that lead to a rapid viral rebound after treatment failure or analytical treatment interruption (ATI) is key.

Methods

We have developed immunoPET/CT-guided spatial transcriptomics, combined with immunofluorescence detection of viral proteins and viral sequencing using the SIV/rhesus macaque model. With this approach we can find and study foci of viral replication in tissues of all animals. Here we compare the local neighborhood of the rebound eclipse-phase foci (4-6 days post-ATI) from animals initiating ART 4 days (early-seeded reservoir with short lifespan) or 10 weeks (well-seeded reservoir) after high-dose challenge, as well as early-seeded tissues from animals on ART for 3 or 7 weeks. Gut-associated tissues containing infected cells foci were identified with a Copper-labelled probe against viral envelope. Subsequently, sections were validated by SIV proviral PCR, evaluated by immunofluorescence to localize SIV proteins, and characterized by 10x Visium spatial transcriptomics.

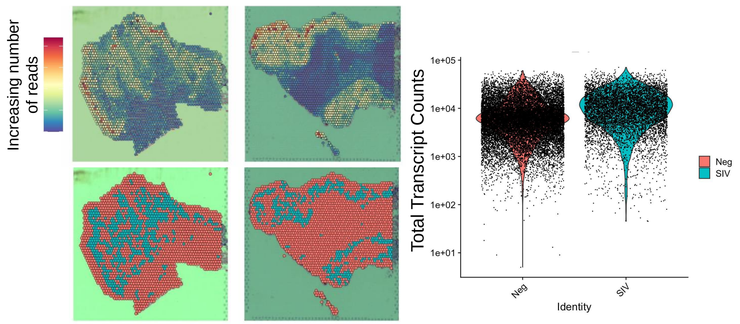


Schematic of our immunoPET/CT-guided spatial transcriptomics and IF-spatial transcriptomics bioinformatic integration. Briefly, PET/CT-guided necropsies detect “hot” tissues and “hot” tissue sections. Adjacent sections are used for spatial transcriptomics using 10X Visium poly-A based appraoch and virus detection is attained with IF. We deploy bioinformatic approaches to tag Visium spots with viral presence and perform general and virus-specific spatial transcriptomics.

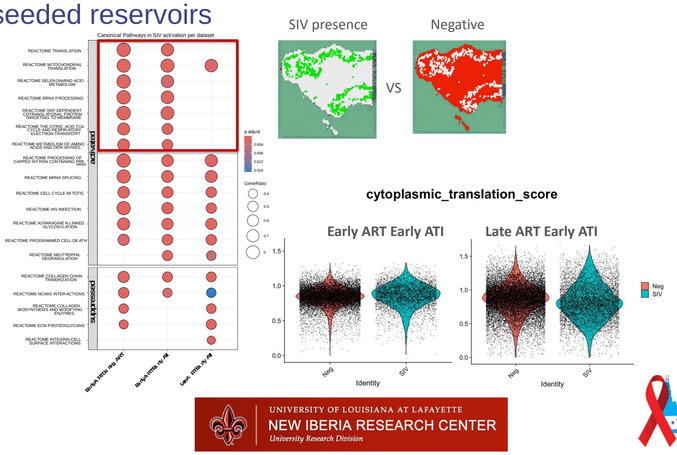
Persistent reservoirs in the gut possibly establish a *Virus Microenvironment (VME)* in TLS that is characterized by the activation of stress responses.

Results

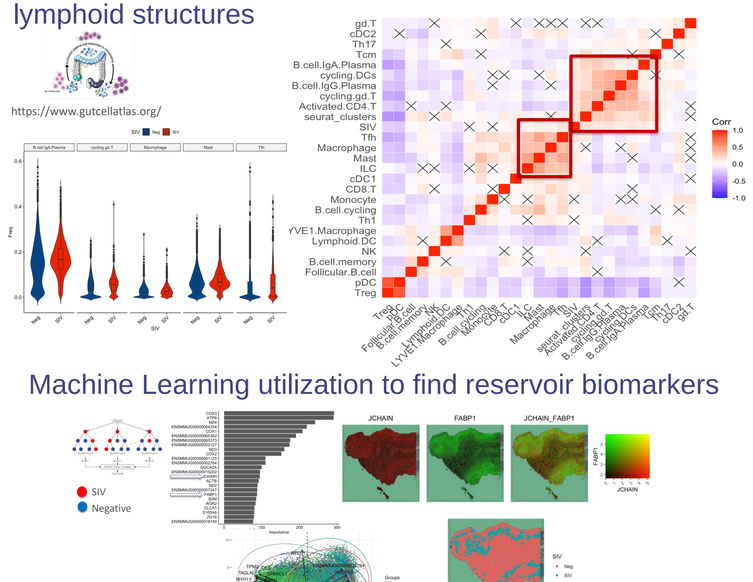
SIV Presence associated with higher transcriptional levels



Differentially expressed pathways are consistent with activation of integrated stress response (ISR) in well-seeded reservoirs



Late ART reservoir possible association with tertiary lymphoid structures

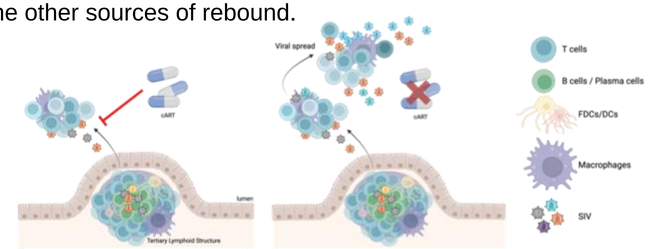


Machine Learning utilization to find reservoir biomarkers

Conclusions

Hypothesis: The early rebounding reservoir that conforms a robust recovery of viremia might be established in viral sanctuaries defined by a **VIRUS MICROENVIRONMENT** with activation of stress responses.

These populations reside in gut luminal areas that favor viral production and are consistent with the first source of rebounding viruses that trigger activation of the other sources of rebound.



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