People Living with HIV Exhibit Distinct HIV DNA Distribution in Gut Tissues Relative to Gut Damage

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Outline

- Background
 - Viral reservoirs
 - Immunological nonresponders (INR)
- Method
 - Droplet digital PCR (ddPCR)
- Results
- Future plans

Viral reservoirs

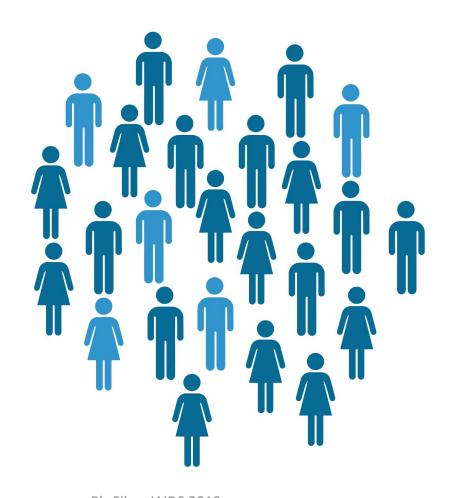
- Virus can persist in multiple forms, in multiple cells and in multiple sites
- Latently infected cells are invisible to the immune system
- A major challenge to HIV cure
- Lymph nodes and gut-associated lymph tissue are major tissue reservoirs rich in viruses and with high frequency of infected cells

Spleen Lymph node Macrophages Lymphocytes Activated Nervous system Resting Resistance to immune killing Astrocyte Response to a specific stimulation Genome integration Transcriptional Adipocytes activity Intact versus defective Provirus sequence proviruses Tissue Cell properties and location

Deeks, Nature Medicine 2021 Zhang, Clin Immunol. 2021. Chun, J Infect Dis. 2002 Zaman, mBIO, 2024 Churchill, Nat Rev Microbiol. 2016

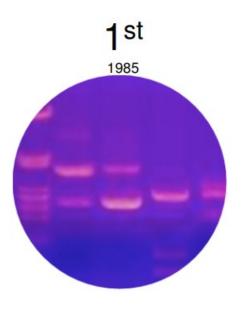
Immunological nonresponders (INR)

- CD4 < 400 cells/uL on continuous ART > 4 years
- 10-30 % of ART treated people living with HIV (PLHIV)
- Increased risk of AIDS and non-AIDS related morbidity and mortality
- Larger viral reservoirs in blood
- Increased immune activation, exhaustion and gut homing
- Etiology possibly related to a defect mucosal barrier
- Higher levels of the enterocyte damage marker iFABP



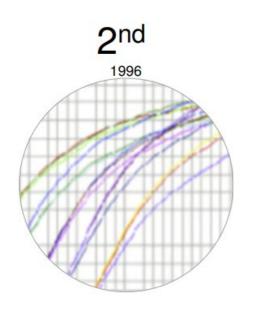
Rb-Silva, JAIDS 2019
Mutoh, Clin Infect Dis 2018
Kelly. PLoS One 2016
Stiksrud JAIDS 2016
Meyer-Myklestad. JID 2021
Lorvik Front Immunol 2021

3rd generation of PCR: Digital PCR (dPCR)



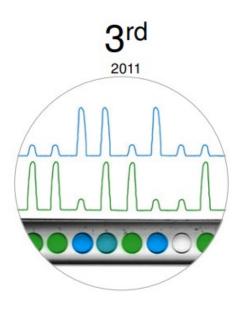
Basic PCRQualitative

-/+ answer



Real-time PCR/ quantitative PCR (qPCR) Absolute or relative quantitation

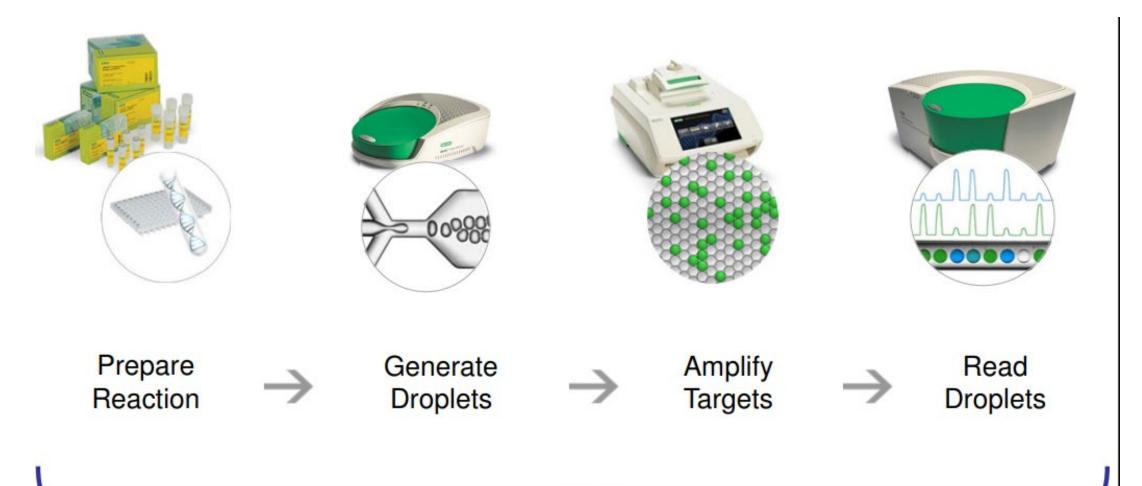
Need for standard curves (absolute)

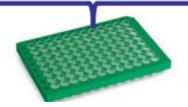


Digital (dPCR)Absolute quantitation

No need for standard curves, copies/μl

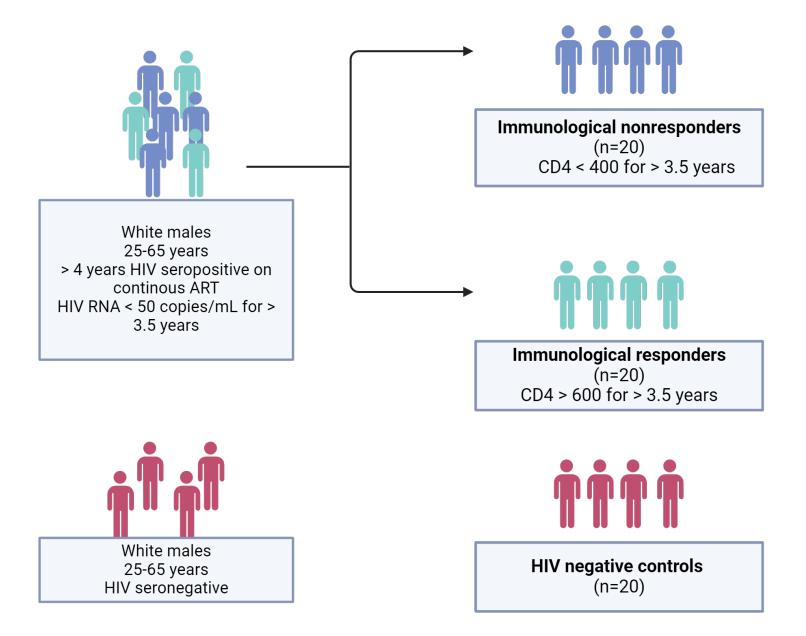
Droplet digital PCR (ddPCR) workflow

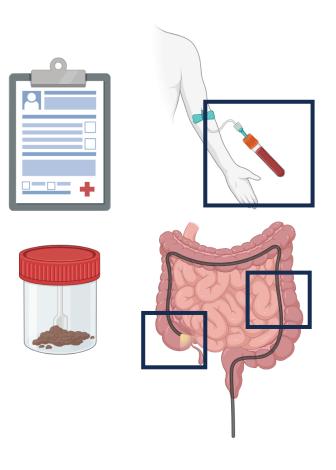




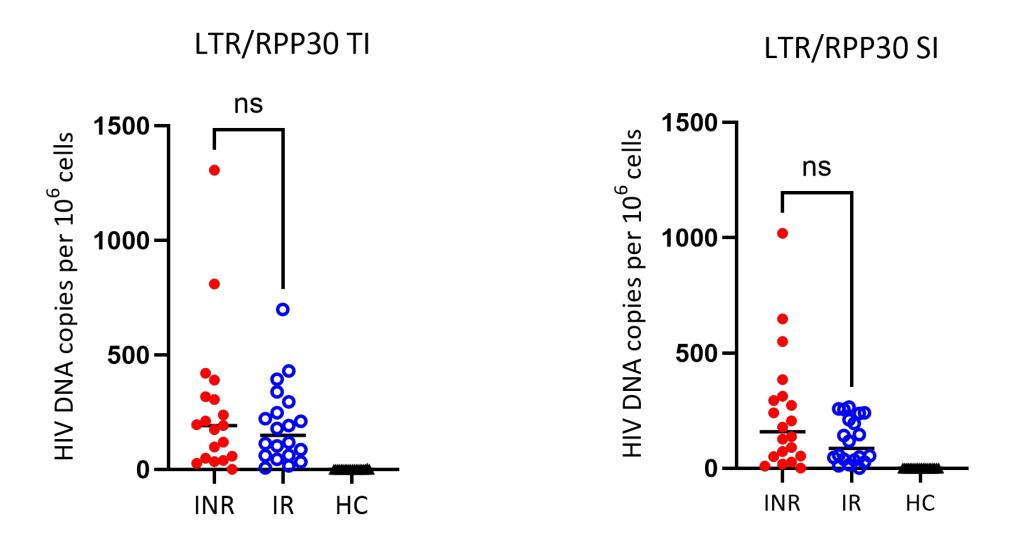


Cross sectional trail

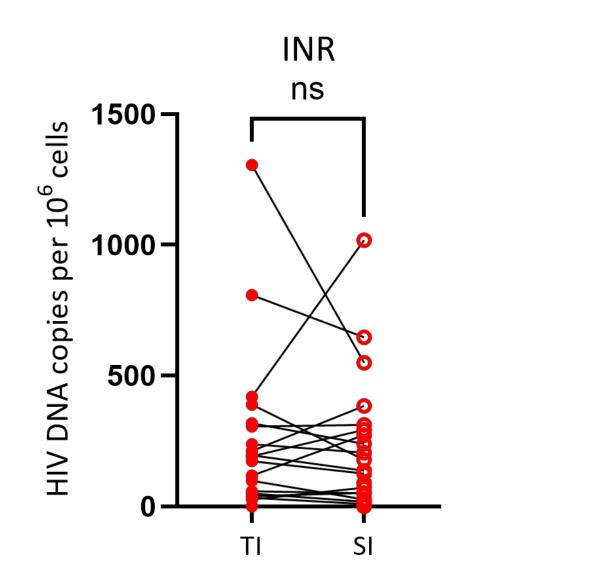


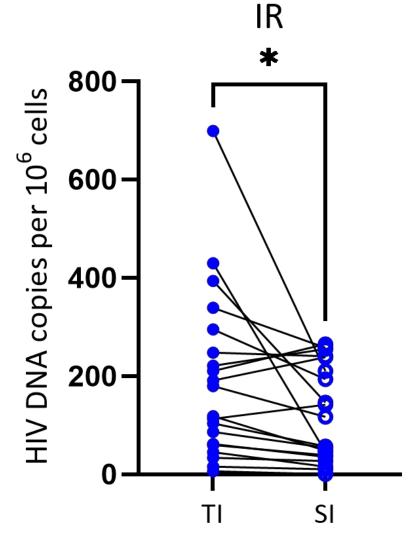


Viral reservoirs in terminal ileum and sigmoid colon

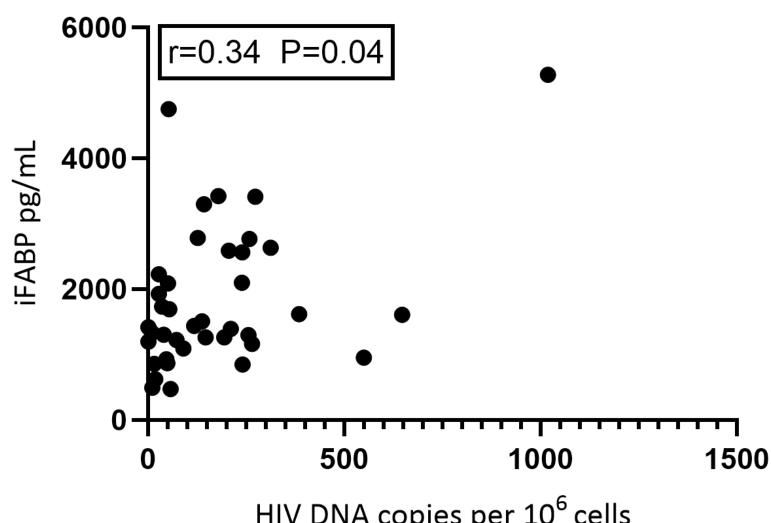


Immune responders have smaller reservoirs in colon





A possible link between mucosal damage and viral reservoirs in colon



HIV DNA copies per 10⁶ cells

Future plans

- Other ways of characterizing the reservoir and immunological subpopulations
 - Single nuclear RNA-sequencing (snRNAseq)
- Evaluate the role of the viral reservoir as a prognostic marker of CD4 count
 - 7-year follow-up





Hanne Hestdal Gullaksen Mari Kaarbø Dag Henrik Reikvam Asle Medhus Anne Ma Dyrhol-Riise





Norwegian Society for Medical Microbiology Blix foundation People living with HIV participating in our studies HIV negative controls included from the Dep.of Gastroenterology