

## RESEARCH BASED TEMPLATE

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### **Identifying targets for HIV in resident-memory CD4<sup>+</sup> T cells in human anogenital tissues**

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**Background:** Despite effective anti-retroviral therapy, HIV remains a global epidemic. New infections occur predominantly through sexual transmission, where the virus encounters a heterogenous population of tissue resident-memory CD4<sup>+</sup> T cells (CD4<sup>+</sup> TRM) and persists in these cells under anti-retroviral therapy as latent reservoirs. However, we do not know how and in which T cell subsets these latent reservoirs are established during sexual transmission. Finding and eliminating the latent reservoir is the largest hurdle against a cure. Therefore, in our study, we aim to characterise CD4<sup>+</sup> TRMs in human anogenital tissues and identify productively or latently infected subsets during initial infection.

**Methods:** We applied a 24-colour flow cytometry panel specifically designed for phenotyping human anogenital CD4<sup>+</sup> TRMs (PMID: 37772977) to characterise the CD4<sup>+</sup> T cells isolated from epithelial and underlying mucosal layers (dermis & lamina propria) in various human anogenital tissues. We explored the phenotypic heterogeneity via unsupervised learning algorithms.

**Results:** CD4<sup>+</sup> TRMs expressing CCR5 are proportionally enriched in epithelium. CCR5<sup>+</sup> CD4<sup>+</sup> T cells densely populate vagina and colorectum compared to labial skin. Regulatory-like CD39<sup>+</sup>TIGIT<sup>+</sup> CCR5<sup>+</sup>CD4<sup>+</sup> T cells and resting TRM-like CD69<sup>+</sup>CD127<sup>+</sup>CCR5<sup>+</sup>CD4<sup>+</sup>T cells present in labia, vagina, and large bowel, both phenotypes have been associated with latent reservoirs. The CD39<sup>+</sup>TIGIT<sup>+</sup> subset was disproportioned over the resting memory CD69<sup>+</sup>CD127<sup>+</sup> subset under heightened CD4<sup>+</sup> T cell activation (R=0.52, p=0.004), which is associated with tissue inflammation and higher susceptibility of HIV.

**Conclusion:** Heterogeneous HIV-susceptible CD4<sup>+</sup> T cells are present in human anogenital tissues including epithelial layers. We are currently optimising a CCR5-tropic dual-fluorescence virus that will enable us to identify the productive and latent virus following infection of these anogenital tissue CD4<sup>+</sup> T cells. Understanding their roles in the establishment of an early HIV latent reservoir are vital for developing targeted treatment.

*This study is funded in part by the NHMRC2027984- CIA Nasr.*