Barcoded SHIV Model Enables In-Vivo Tracking of bnAb-Sensitive and -Resistant Variants During bnAb Administration at ART Initiation

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Background: Despite the success of antiretroviral therapy (ART) in suppressing HIV replication, lifelong treatment is required due to persistent reservoirs that rebound upon ART interruption. ART initiation is a critical window when viral replication ceases but antigen levels remain high, offering an opportunity to target infected cells. Broadly neutralizing antibodies (bnAbs) given at ART initiation have shown promise, but mechanistic insights are lacking. To interrogate this, we used a barcoded SHIV.CH505.v2 model containing V3 glycan bnAb-sensitive (wildtype, WT) and resistant (escape mutant, EM) viruses, enabling clonotype tracking over time. This design enables intra-animal comparisons that control for inter-animal variability to interrogate how bnAbs influence reservoir seeding, evolution, and reactivation.

Methods: Rhesus macaques were intravenously infected with barcoded WT and EM SHIV.CH505.v2, each containing non-overlapping libraries of >6,000 clonotypes. Plasma viral RNA was sequenced via barcode-targeted amplicon sequencing and analyzed using a custom pipeline to assess barcode diversity and clonotype distribution during acute infection and ART initiation. Since plasma viral loads decline near ART initiation and barcoded provirus in the $CD4^+$ reservoir remains rare after acute infection, single-round amplicon PCR is limited by low template in both settings. As such, we are optimizing a nested PCR approach to improve barcode recovery from these low-input samples.

Results: WT and EM viral stocks replicated comparably, with WT showing a modestly faster replication rate. All animals established productive infection, with peak viremia of ~6log copies/mL at 2 weeks post-infection followed by rapid decline to undetectable by 6 weeks after ART initiation. Sequencing at days 7, 14, and 21 post-infection revealed median WT:EM proportions of 0.27, 0.40, and 0.60, respectively. Plasma barcode sequencing during acute infection recovered hundreds to thousands of barcodes per animal, reflecting high inoculum diversity of both WT and EM viruses.

Conclusion: Our barcoded SHIV.CH505.v2 WT/EM system enables precise, high-throughput quantification of viral population dynamics before and during ART *in vivo*. Through initial validation of plasma barcode sequencing, we establish this model as a robust intra-animal platform for dissecting bnAb mechanisms at ART initiation and, long term, their impact on reservoir seeding, clearance, and rebound dynamics.