Tracking HIV Clones: The Barcode Project

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Introduction:
In order to eliminate the viral reservoir, it is important to understand the forces that drive reservoir dynamics. With the advent of next generation sequencing, it has been possible to study selection pressures exerted on the viral landscape in patients. These in vivo studies suggest that the reservoir is more dynamic than previously appreciated with waves of reservoir contraction and expansion that are poorly understood. We hypothesize the virus itself induces reservoir contraction and expansion. To test this hypothesis, we monitored proviral expansion and contraction over time using a barcoded virus in an ex vivo model.

Method:
In order to capture reservoir expansion and contraction, we generated a high diversity barcoded (BC) HIV by inserting 12 random nucleotides between the env and nef genes. We infected primary unstimulated CD4 T cells with this BC HIV and monitored the infection over 9 days. Using inverse PCR technology at limiting dilution, we identified proviral clones and linked the BC to specific integration sites. To enhance throughput, we then developed methods to track the number of clones over time without limiting dilution PCR. To do this, we performed PCR with and without primer ID followed by sequencing of the barcoded region. We also developed a bioinformatic pipeline with filters designed to remove false barcodes that were generated by sequencing errors.

Results:
Our BC HIV showed similar potency to wild-type HIV. Consistent with our hypothesis, we detected a steady increase in the percentage of proviral clones over time in culture with ~ 26% proviral clones by day 9. Interestingly only 1% of the resting T cells were dividing by eFluor consistent with the hypothesis that the infected cells were dividing more than the uninfected cells.

We also tracked HIV death of the infected cells overtime revealing that the infected cells die more rapidly than the uninfected. Taken together this suggests that the infected cells turnover more than the uninfected cells.

Using primer ID we also developed a pipeline to identify clones without limiting dilution PCR to increase throughput. Our first challenge was to overcome sequencing errors which artificially increases the number of BCs. We found by grouping BCs-PrimerID pairs with similar sequences (i.e. less than 2-4 nucleotide differences) we could identify the original BC-PrimerID pair. We validated this technique by running a single barcode through the same methodology and pipeline demonstrating it was possible to identify the original sequence. We are in the process of validating this technique using a mixture of diverse barcodes with a defined number of repeated sequences.

Conclusion: Tracking resting HIV infected cells with a barcoded viruses demonstrates these cells are induced to divide as well as die faster than uninfected cells.