

## PE11

### Quantification and replication competency of HIV-1 following latency disruption in CD4+ T-cells

Hataye J.<sup>1</sup>, Casazza J.<sup>1</sup>, Ambrozak D.<sup>1</sup>, Boritz E.<sup>1</sup>, Yamamoto T.<sup>2</sup>, Douek D.<sup>1</sup>, Koup R.<sup>1</sup>

<sup>1</sup>National Institute of Allergy and Infectious Diseases, Vaccine Research Center, Bethesda, United States, <sup>2</sup>Osaka University, World Premier International Immunology Frontier Research Center (IFReC), Osaka, Japan

**Background:** The size of the latent reservoir in a patient with ART induced HIV suppression can be estimated by viral outgrowth in a limiting dilution culture of activated CD4+ T cells. A culture well containing HIV is typically detected with p24 ELISA, but recently HIV RNA RT-PCR has been shown to be more sensitive. This allowed us to determine the proportion of cells producing viral RNA that resulted in replication competent virus.

**Methods:** Resting memory CD4+ T cells from 9 virally suppressed patients were stimulated with beads coated with antibodies against CD2, CD3, and CD28, and plated in limiting dilution in two conditions: 1) 100,000 MOLT-4/CCR5 cells per well and IL-2 were added on day 1 to facilitate viral outgrowth, or 2) the reverse-transcriptase inhibitor efavirenz was present immediately on day 0 to suppress viral replication, with no exogenous cells or IL-2 added. Culture media was collected and replaced every 4 days, and the viral RNA isolated and then quantified by real time HIV gag RT-PCR. The frequency of HIV RNA producing cells was estimated using the R package for Extreme Limiting Dilution Analysis.

**Results:** The frequency of HIV RNA producing cells following latency disruption was strongly correlated under viral outgrowth vs. viral suppression conditions. In most positive wells under viral suppression, viral RNA was detectable by day 4; some were followed by an increase while others decreased. In some outgrowth wells, the amount of HIV RNA on days 8 and 12 greatly exceeded that in comparable wells in the suppression assay. Culture supernatant from positive outgrowth wells was used to infect new cultures of activated, allogeneic CD4+ T cells. In 2 experiments, each utilizing a different donor, 35%(27/78) and 14%(11/78) of original positive outgrowth wells supported viral growth.

**Conclusions:** While HIV gag RNA RT-PCR with a concentrated viral suppression culture was as sensitive for quantifying the frequency of HIV RNA producing cells as a viral outgrowth assay, much HIV RNA recovered in the outgrowth wells, including many wells that had increasing amounts of viral RNA over time, did not represent replication-competent virus.