Variable Replication Competency Following Latency Disruption in CD4+ T Cells

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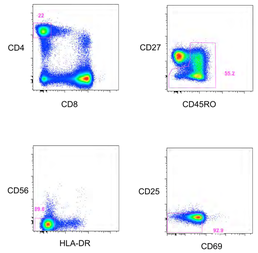
Background. Quantifying the HIV reservoir in treated patients with viral suppression:

HIV Outgrowth Culture (Laird et al. Plos Pathogens 2013 and others):

- **Primary CD4+ T cells cultured in limiting dilution.**
- **Exogenous cells + IL-2 + PHA.**
- **Detect by p24 ELISA or RNA RT-PCR.**

Methods:

1. **Resting memory CD4+ T cells isolated by FACS on PBMC from 25 donors on ART.**



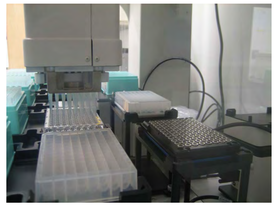
2. **Culture resting memory CD4+ T cells in limiting dilution with anti-CD2,-CD3,-CD28 beads in the presence of either:**
   1. **10^5 MOLT-4/CCR5 + IL-2**

   –OR--

   2. **40 nM Efavirenz**
3. **Collect and replace media every 4 days.**

Methods (cont):

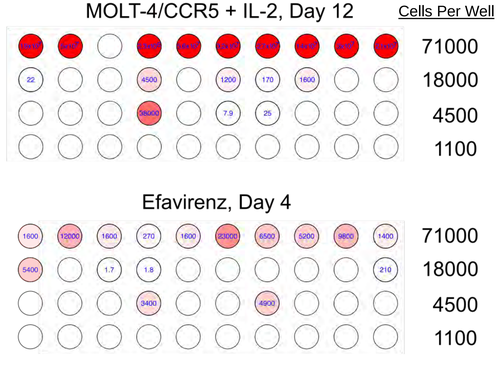
4. **Isolate HIV RNA from media lysates spiked with internal RCAS RNA standard using robot-assisted paramagnetic bead approach and DNAse treatment.**

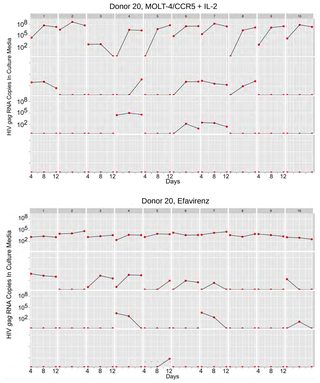


5. **Perform HIV *gag* RNA RT-PCR, RCAS RNA RT-PCR and non-RT *gag* DNA PCR.**
6. **Statistical and graphical analysis performed with R Core and ggplot.**

Results:

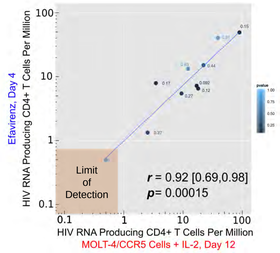
HIV *gag* RNA copies in culture media by culture plate (1st right) or days post stimulation (2nd, and 3rd right). RNA copies in well calculated from RNA copies in RT-PCR reaction, fraction of isolated RNA undergoing RT-PCR, RCAS RNA recovery (mean 63% +- 21%), and fraction of media sampled for RNA isolation. Of 3106 RNA isolations, 3092 had RNA recovery>10% and were included in subsequent analyses. 726 were positive for *gag* RNA, and of these 21 had breakthrough *gag* DNA detected; 7 were excluded as RNA positive due to DNA *gag* copies/RNA copies >1%.

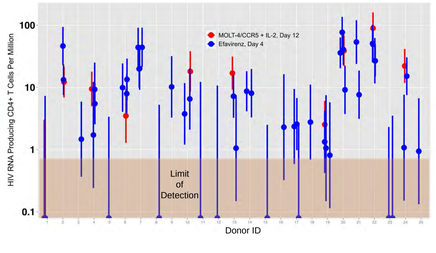




Results (cont):

HIV RNA producing CD4+ T Cells per million can be estimated at day 4 of culture without exogenous cells and viral outgrowth.





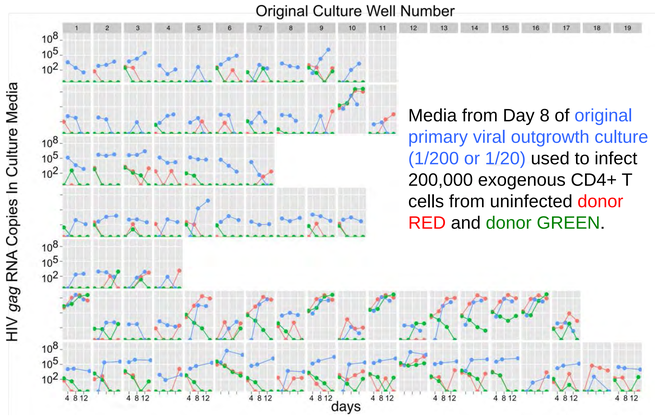
Frequency estimates for HIV RNA Producing Cells Per Million strongly correlate for non-viral outgrowth culture with efavirenz at day 4 and viral outgrowth culture with MOLT-4/CCR5 + IL-2 at day 12. Limit of detection is between 0.5 and 1, determined by input number of cells, and shown as shaded orange. Estimates determined using R Package ELDA (Hu and Smyth, J Immunol Methods, 2009).

On left, Pearson correlation coefficient [95% confidence interval] and p-value shown for correlation, and individual p-values for frequency estimate for each of 10 patients shown.

On right, frequency estimates for viral outgrowth at day 12 or non-viral outgrowth at day 4 shown with 95% confidence intervals. For patients where no HIV RNA was detected, the estimate is plotted at the bottom of the graph.

Conclusions:

1. **Viral outgrowth generally resulted in higher amounts of HIV RNA per positive well compared to viral inhibition cultures, but not higher frequency of positive wells.**
2. **HIV RNA producing CD4+ T cells per million may be estimated at day 4 of a non-outgrowth culture without exogenous cells and in which new rounds of infection are prevented.**
3. **Many wells positive for HIV RNA, even some with increasing RNA copies over time, do not support viral outgrowth in new cultures.**



Media from Day 8 of original primary viral outgrowth culture (1/200 or 1/20) used to infect 200,000 exogenous CD4+ T cells from uninfected donor RED and donor GREEN.