Viral Dynamics of HIV-1 Rebound Viremia: A Pilot Study

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Background

When combination antiretroviral therapy (cART) is initiated in chronic HIV-1 infection and then discontinued, the virus invariably rebounds to near pre-treatment levels. The HIV-1 virus populations arising upon treatment interruption reflect the functional latent reservoir and are the actual viruses that curative strategies need to interdict. Here, we aim to employ single genome sequencing (SGS) on plasma samples taken shortly after treatment interruption to characterize the clonality and kinetics of virus arising from latency.

Definition: ***Rebound Founder Virus*** **(RFV): the virus(es) arising from latently-infected cells upon treatment interruption.**

Signficance:

- ✧ **RFV reflect the size, diversity, and rate at which latently infected cells are activated from the functional latent reservoir**
- ✧ **RFV are the targets of immune pressure and curative strategies**

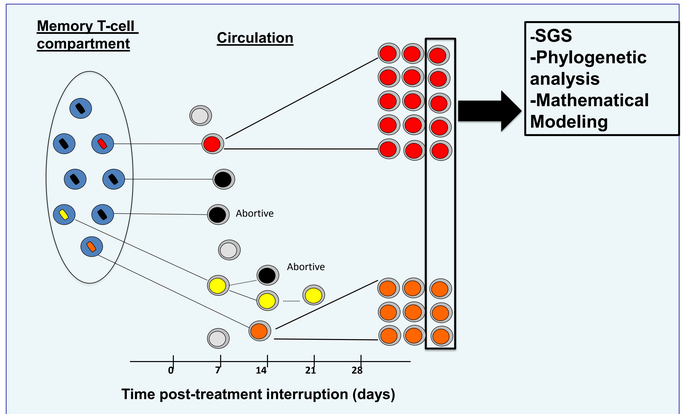
Pilot Study Aims:

1. **Characterize clonality and kinetics of virus arising from latency after treatment interruption of cART initiated in chronic infection.**
2. **Utilize viral dynamics to quantitate, characterize the functional latent HIV-1 reservoir.**
3. **Characterize baseline rebound viral kinetics for analysis of curative interventions and to track immune pressure.**

Methods:

- ✧ **Subjects: 4 Subjects with pre-existing samples from prior treatment interruption (TI) trial with cART initiation in chronic infection and >2 years virus suppression on cART**
- ✧ **SGS performed on first 4 samples with detectable viremia post-TI.**

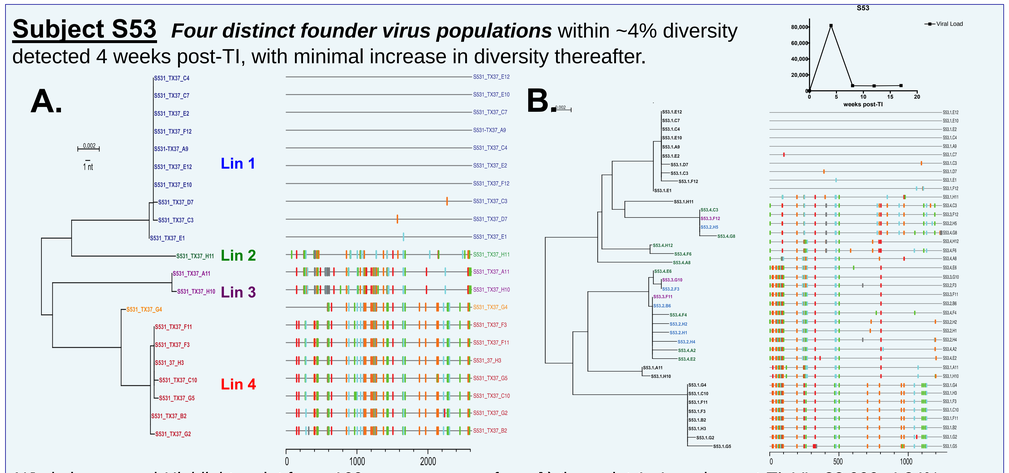
Model of RFV Identification Strategy



Rebound Founder Virus (RFV): In samples taken shortly after cART interruption, can we use SGS/modeling to infer the sequences of the viruses initiating rebound viremia, or ***Rebound Founder Virus(es)***

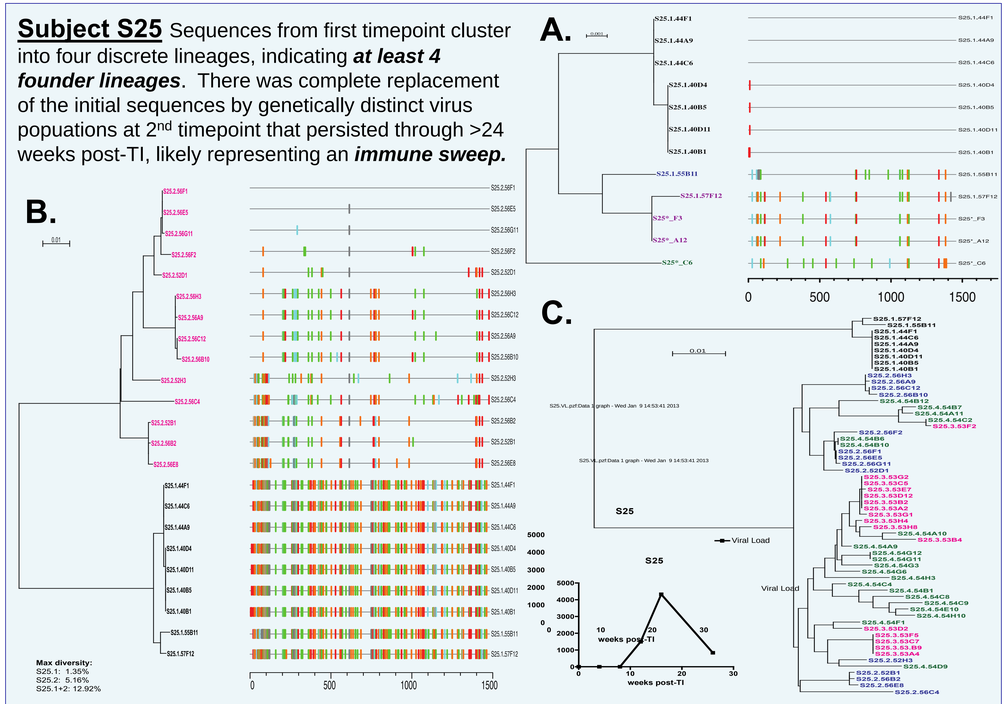
Results

Subject S53 *Four distinct founder virus populations* within ~4% diversity detected 4 weeks post-TI, with minimal increase in diversity thereafter.



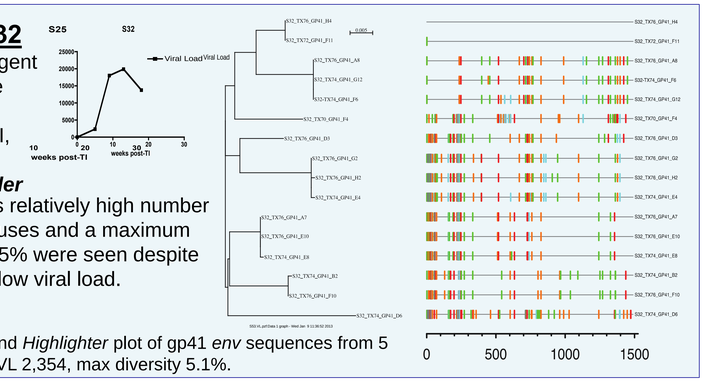
NJ phylogeny and *Highlighter* plot for gp160 *env* sequences from **A)** timepoint 1, 4 weeks post-TI, VL: 82,000, 4.24% max diversity. 4 discrete lineages (labeled Lin 1-4) with recombinant sequences in orange. Sequence depth allows 95% CI sensitivity to detect virus comprising >10% **B)** Timepoints 1-4, with later sequences (timepoints 2-4, in color) interdigitated within earlier sequences (in black).

Subject S25 Sequences from first timepoint cluster into four discrete lineages, indicating ***at least 4 founder lineages***. There was complete replacement of the initial sequences by genetically distinct virus popuations at 2nd timepoint that persisted through >24 weeks post-TI, likely representing an ***immune sweep.***



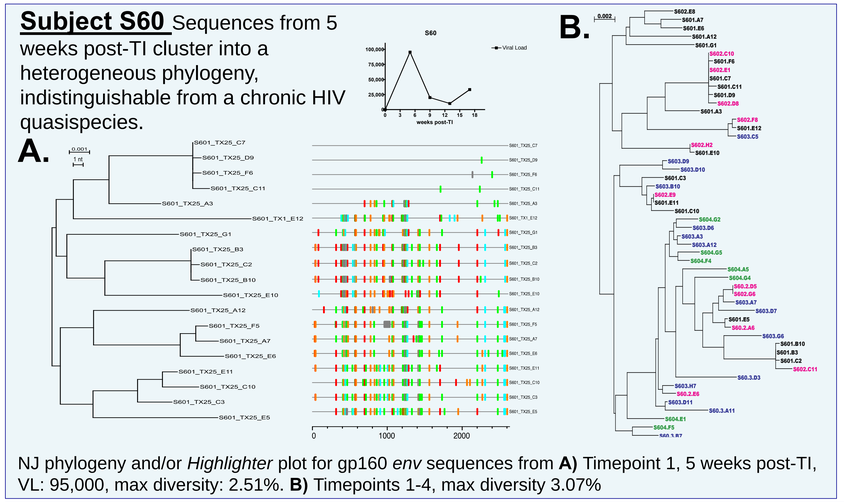
NJ phylogeny and/or *Highlighter* plot of gp41 *env* sequences from **A)** Timepoint 1: 12 wk post -TI, VL 1496, 1.35% max diversity, lineages color-coded. **B)** gp41 *env* sequences from timepoints 1&2 show 2 discrete lineages, timepoint 1 in black, timepoint 2 in pink. Timepoint 2 sequences with 5.16% max diversity. Greater than 10% diversity between timepoint 1 & 2 lineages **C)** Timepoints 1-4: 2 discrete lineages persist, lineage from first timepoint replaced by sequences from timepoints 2-4. Mitochondrial D-loop sequencing confirmed patient identity in all samples.

Subject S32 Multiple divergent lineages were detected at 5 weeks post-TI, indicating ***at least 8 founder viruses***. This relatively high number of founder viruses and a maximum diversity of > 5% were seen despite the relatively low viral load.



NJ phylogeny and *Highlighter* plot of gp41 *env* sequences from 5 weeks post-TI, VL 2,354, max diversity 5.1%.

Subject S60 Sequences from 5 weeks post-TI cluster into a heterogeneous phylogeny, indistinguishable from a chronic HIV quasispecies.



NJ phylogeny and/or *Highlighter* plot for gp160 *env* sequences from **A)** Timepoint 1, 5 weeks post-TI, VL: 95,000, max diversity: 2.51%. **B)** Timepoints 1-4, max diversity 3.07%

Conclusions

- ✧ **We can use single genome sequencing to identify and characterize rebound founder virus populations in post-TI plasma samples**
- ✧ **We detect variable, and often discrete, numbers of RFV in first weeks after TI**
  - ✧ **4 subjects ≥ 4, 4, 8, 10 rebound founder virus populations.**
  - ✧ **Greater number of founder viruses initiating rebound viremia than hypothesized**
  - ✧ **These data begin to describe baseline from which to measure curative strategies**
- ✧ **We can identify immune sweeps in rebound virus populations**
  - ✧ **Suggests significant immune pressure in first weeks post-TI**
  - ✧ **Further studies planned to characterize immune pressure present at rebound**
- ✧ **Rebound virus populations expand rapidly**
  - ✧ **More quickly than hypothesized**
  - ✧ **More frequent sampling may better measure rate of expansion**