Spatio-temporal dynamics of drug resistance evolution and persistence of RT-SHIV

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Previous studies have suggested the importance of compartmentalization in the evolution of drug resistance in HIV. Using a nonhuman primate model, we tested how drug resistance establishes in multiple tissues within patients over time.

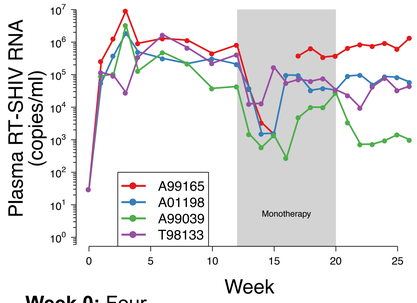
We found that drug resistant virus evolves and spreads through multiple compartments. Although the same viral isolates appear in multiple compartments, the dynamics of evolution differ among them, with the gut being notably fast. We also observed a surprisingly stable population of viral DNA behind rapid changes in viral RNA. Together, these observations suggest that viral dynamics within an individual may be more complex than what is observed only in plasma RNA.

pol sequences were sampled across space and time

Intracellular viral RNA (vRNA) and viral DNA (vDNA) were isolated from three different compartments (PBMC, lymph node (LN), and gut) along with vRNA from plasma at multiple time points before, during and after monotherapy.

~30 single genome sequences were obtained per timepoint per compartment.

Further analysis will focus on animals T98133 (purple) and A99165 (red).

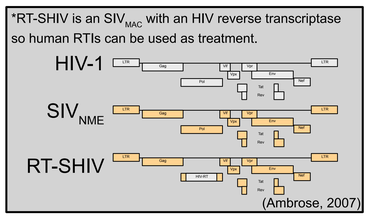


Week 0: Four female pig-tailed macaques infected intravenously with RT-SHIV

Weeks 13-20: EFV or FTC monotherapy was administered daily to select for drug resistant viruses

Weeks 20-26: No treatment

*RT-SHIV is an SIV_{MAC} with an HIV reverse transcriptase so human RTIs can be used as treatment.

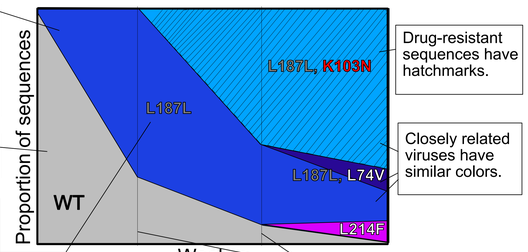


Sequences visualized as dynamical plots

Each color indicates the proportion of single genome sequences with a particular set of mutations (genotype) within the population

Grey indicates wildtype virus (WT)

Mutations are marked on each color block:
Grey: synonymous
White: non-synonymous
Red: drug resistant



Drug-resistant sequences have hatchmarks.

Closely related viruses have similar colors.

The x-axis shows the frequencies of each genotype (y-axis) over time.

Sampled timepoints are shown by vertical lines.

Similar genotypes appear across compartments

The same genotypes (marked by segments of the same color) appear across different compartments at similar time points.

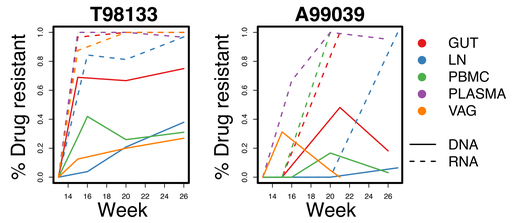
However, some genotypes are found in some but not all compartments.

And some genotypes have different changes in frequency over time in different compartments.

Plasma vRNA sequences do not capture the full evolutionary viral dynamics.

Evolution looks different in vRNA and vDNA

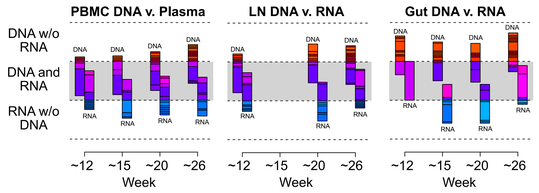
Genotype frequencies change much more rapidly in RNA than DNA. Drug resistance, in particular, spreads much more quickly in vRNA than vDNA.



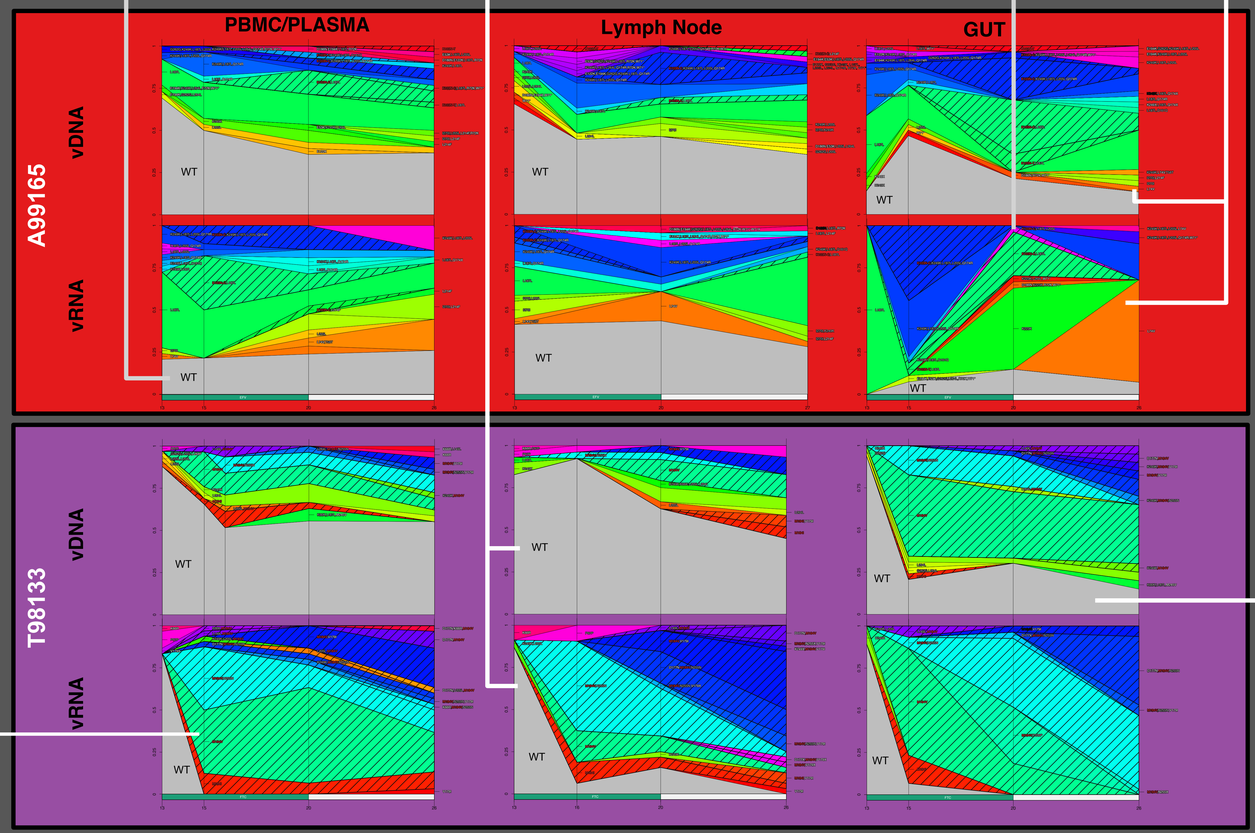
vDNA not only changes slowly, but also encodes a **relatively stable** proportion of WT virus.

A small percentage of vDNA encodes most vRNA

Genotypes at low or undetectable frequencies in the vDNA account for the bulk of the sampled vRNA.



In the plots above, the diversity of vDNA and vRNA is compared within compartments. Each timepoint is represented by two bars: the left bar represents vDNA and the right bar represents vRNA. Each color shows a different genotype. Bars in purple represent genotypes found in both vRNA and vDNA, and bars in red or blue represent genotypes found in vDNA or vRNA alone, respectively.



Gut dynamics appear faster in both vRNA and vDNA

There is a higher degree of population structure (assessed by F_{ST}) between different time points in gut vRNA compared to blood or LN vRNA across all macaques.

vDNA in the gut retains a smaller proportion of WT virus than vDNA in other compartments.

Together, these observations suggest that in the gut, **viral replication cycles may be shorter,** or selective pressures may be greater.

Conclusions

Most circulating viruses are encoded by a small amount of vDNA.The vDNA population is stable relative to vRNA, and maintains a significant WT population (except the gut).

Viruses with the same mutations appear across compartments, suggesting a strong role of migration in intrahost evolution.

Different strains often predominate in different compartments underscoring the need to integrate spatial understanding into treatment and research.