Re-evaluating signals of viral replication & evolution in lymphoid tissue during ART

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* The research presented here is not the product of any Merck-affiliated study. Conclusions are the authors' own.

Abstract

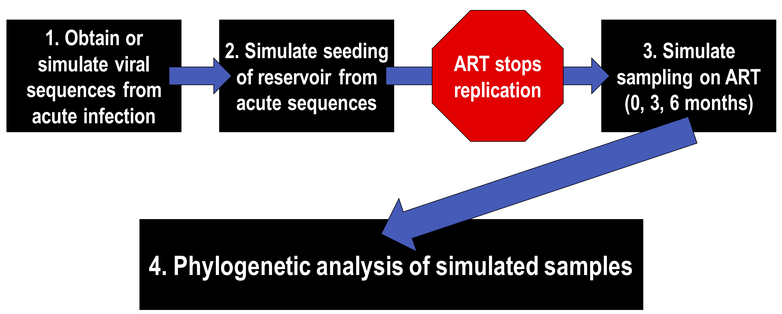
Resting memory CD4⁺ T-cells support a stable latent reservoir of integrated virus; these cells' long lifespan and proliferative capacity contribute to long-term HIV persistence despite antiretroviral therapy (ART).

Whether self-sustaining viral replication – in compartments not sufficiently penetrated by ART – also occurs and contributes to HIV persistence is less clear. A recent study[1] deep-sequenced lymph node and blood samples from three participants during the first six months of ART, finding viral genetic signals of persistent replication and evolution.

We show, however, that these viral genetic signals are expected outcomes of the known multi-phasic decay of infected cells during the first year of ART, and so they do not provide evidence of self-sustaining replication. We use sequence analysis, viral dynamic modeling, and simulations of viral evolution to reach this conclusion.

Investigation of ongoing replication during ART therefore must wait >1 year following the start of ART; earlier analysis is unreliable.

Method overview



Question:
In an idealized scenario where ART stops all viral replication, how often does a misleading signal of viral evolution nonetheless appear?

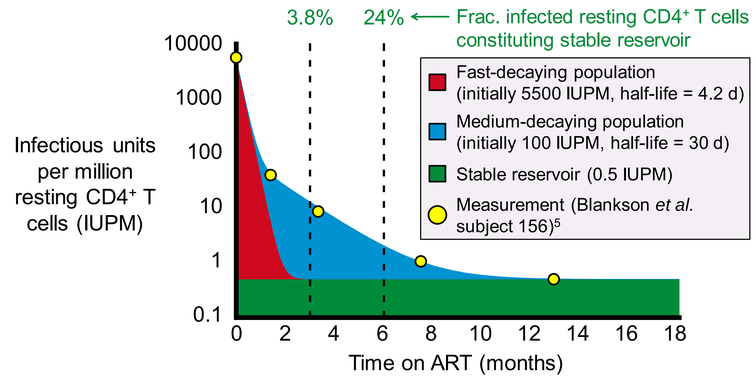
Method detail (see ref. 2 for complete methods)

- **Simulating sequences from acute infection:** Intrahost HIV evolution was simulated using a stochastic model of birth, death, and mutation of infected cells. At a birth event, a newly infected cell has a chance of experiencing mutation at a random site, according to an HKY model-like nucleotide transition matrix. Parameters: Reproductive ratio R_0: 6 (peak period, first 20 days of infection), 1.4 (after peak). Death rate: 1/day. Mutation rate: 3×10^{-5} per site per generation. Sequenced genome length: 587 bp (consistent with ref. 1). Effective population size at carrying capacity: 250 to 4000, 50x higher during peak period. P(site in sequenced region is lethal when mutated): 70%. Rejection sampling was used to exclude runs yielding unrealistic levels of viral genetic diversity or divergence from origin (inconsistent with published values[3]). Summaries reported include >350 replicate simulations per scenario.
- **Obtaining sequences from acute infection:** Three study participants described in ref. 4 had samples collected at least three times within the first four months of infection, with an average of 13 single-virus RNA sequences per time point. Published *gag* sequences were used.
- **Simulating seeding of reservoir from acute sequences:** Actively replicating cells (simulation) or sampled sequences (ref. 4) migrate to reservoir compartments identified using data from ref. 5 (fast-decay, medium-decay, or stable) at rates consistent with compartment sizes.
- **Simulating sampling during ART:** ART was assumed to start 4 months post-infection, consistent with the two fully-suppressed study participants in ref. 1, at which point all birth events cease, but compartment decay continues.
- **Phylogenetic tests of ongoing replication/evolution:** A run is judged to have a misleading appearance of forward evolution if it returns a significantly increasing result ($p < 0.05$) on three tests used in ref. 1: (1) Increase in avg. genetic divergence, measured from most common genotype observed at start of ART, between start of ART and 6 months of ART (Mann-Whitney U test); (2) Positive evolutionary rate by linear regression of divergences over time, using sequences from the start of ART and 3 and 6 months of ART (F-test); (3) Clock-like phylogenetic signal, from root-to-tip regression in ML phylogeny on sequences from start of ART and 3 and 6 months of ART (F-test).
- **Time-structured phylogeny:** Time-resolved phylogenies were estimated using BEAST v2.4.4 with a single-compartment version of the model used in ref. 1. MCC tree displayed.
- **Estimates of selection strength during acute infection** were taken from all articles found to report either selection coefficient or escape rate of one or more CTL escape mutations, based on single-genome sequencing. Escape rate ε (measured in days^{-1}) was converted to a lower bound on selection coefficient for plotting: $s > \varepsilon/(1-\varepsilon)$.

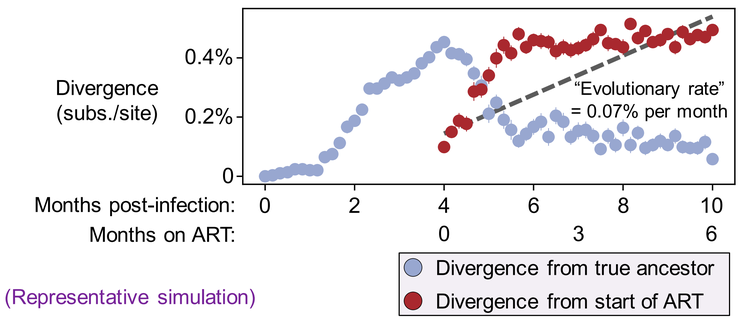
Results

1 HIV reservoir undergoes major population shift in first 6 to 12 months of ART

- During early ART, recently infected cells tend to decay first
- Infected resting cell population shifts towards "older" virus
- Modeling question: Can this shift masquerade as "viral evolution"?



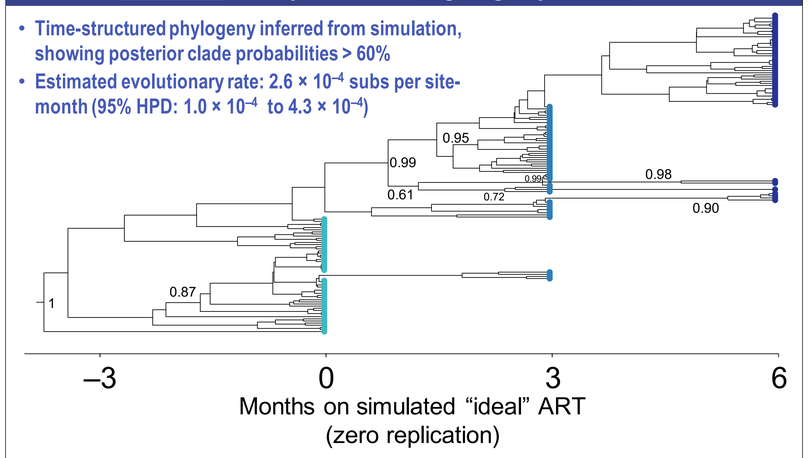
2 This population shift causes samples taken during first 6 months of ART to undergo reversion to ancestral state



(Representative simulation)

3 Strong selection occurring prior to ART makes this reversion masquerade as ongoing replication & evolution

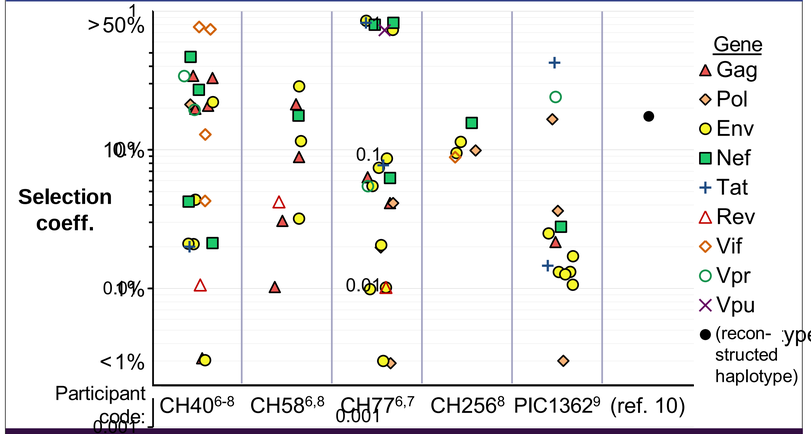
- Time-structured phylogeny inferred from simulation, showing posterior clade probabilities > 60%
- Estimated evolutionary rate: 2.6×10^{-4} subs per site-month (95% HPD: 1.0×10^{-4} to 4.3×10^{-4})



4 Misleading signals of replication/evolution appear in early ART up to half the time if strong selection occurred pre-ART

Input parameters		Fraction of simulations passing tests for replication & evolution ($p < 0.05$)	
Selection coeff. in acute infection	Frac. sites under selection	Passing at least one test, not contradicted by others	Passing all three tests
0	—	1%	0.2%
5%	1%	5%	4%
5%	3%	11%	10%
20%	**1%**	**45%**	**41%**
20%	**3%**	**54%**	**50%**
Sequences from ref. 4	Pt. "B"	22%	4%
	Pt. "H"	9%	0%
	Pt. "OU"	92%	27%

5 Patients experience multiple strong selective sweeps during acute infection (review of published escape mutations)



Conclusions

- Currently available viral genetic sequences do not support inference that self-sustaining ("$R_0 > 1$") viral replication persists during suppressive ART.
- Studies investigating this question should use sequences collected more than a year after initiation of ART.
- Where possible, population genetic studies of viral evolution should be conducted with reference to models of viral dynamics and literature on growth/decay of subpopulations.

References

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