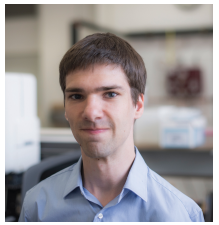
HIV genetic diversity and compartmentalization in lung and blood of individuals on long-term cART



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HIV persistence and compartmentalization within-host tissues

- During untreated HIV, viral populations evolve, disseminate into tissues, enter the reservoir, and persist despite ART[1].
- While HIV persistence in blood is well-studied, less is known about HIV persistence in tissues, including in the **lung**.
- Proviruses persisting in lung could exist as distinct *i.e.*, **compartmentalized** viral populations from those in blood due to:
  - ***founder effects, followed by restricted gene exchange between compartments.*** *If limited HIV sequences are seeded into tissues during untreated infection, but there is limited gene exchange with blood, these founder strains would evolve "locally", yielding distinct viral populations to those in blood.*
  - ***Differential clonal expansion/contraction of reservoir cells in blood and lung.*** *This would lead to different proviral populations only in terms of frequencies.*
- Achieving a better understanding of blood/tissue compartmentalization, including to what extent blood proviral composition reflects that in tissue, will inform the design of curative approaches against HIV.

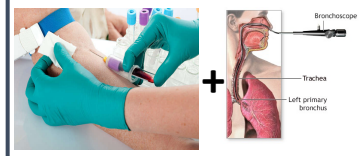
Objective

To characterize blood/lung proviral diversity and genetic compartmentalization in PLWH on long-term cART

Samples

Paired buffy coat ("blood") and bronchoalveolar lavage ("lung") specimens from **nine** PLWH undergoing bronchoscopy

Matched sample collection



Buffy coat ("blood")

Bronchoalveolar lavage ("lung")

DNA and RNA extraction

For DNA

+

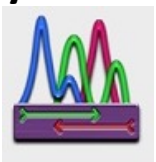
For RNA

Amplification & Sanger sequencing

HIV *nef* via limiting dilution PCR

+

Sequence analysis and QC



Hypermutated, recombinant, and sequences with other defects were excluded

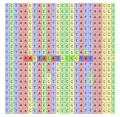
Phylogenetic analysis

Between-host phylogeny

+

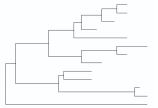
7,500 Within-host phylogenies

Tests of genetic compartmentalization



Distance-based (K_{ST}, AMOVA)

+



Tree-based (CC, SM)

Subgenomic single-genome proviral sequences (*nef*) were collected from matched blood and lung from nine individuals with suppressed viremia on ART. For two participants, HIV RNA sequences were also collected from available longitudinal pre-ART plasma. Markov chain Monte Carlo (MCMC) methods were used to infer 7,500 within-host phylogenies per participant. Genetic compartmentalization was assessed using Hudson, Boos and Kaplan's nonparametric test (K_{ST}), Analysis of Molecular Variance (AMOVA), Slatkin-Maddison (SM), and the Correlations coefficient (CC) test, with the latter two conditioned over all trees.

[1]Cohn *et al*. Cell Host Microbe. 2020 Apr 8; 27(4): 519–530. PMID: 32272077.

Results

Table 1. Participant information and sampling

ID	Sex	Years on ART prior to proviral sampling	Intact proviral lung sequences Total N (distinct n; %)	Intact proviral blood sequences Total N (distinct n; %)	pre-ART plasma HIV RNA sequences[a] Total N (distinct n; %)
1	M	8	29 (29; 100%)	88 (64; 72%)	-
2	M	15	31 (13; 42%)	60 (50; 83%)	-
3	M	0.6	6 (6; 100%)	75 (60; 80%)	-
4	M	18	42 (7; 17%)	78 (53; 68%)	91 (65; 71%)
5	M	5	6 (6; 100%)	91 (35; 38%)	-
6	M	12	43 (18; 42%)	126 (56; 44%)	211 (99; 47%)
7	M	10	4 (4; 100%)	79 (57; 72%)	-
8	F	9	14 (2; 14%)	47 (44; 91%)	-
9	M	3	8 (7; 88%)	55 (40; 73%)	-
Total			**183 (92; 50%)**	**699 (459; 66%)**	**302 (164; 54%)**

Participants were a median 60 (IQR, 51-62) years of age and had maintained viremia suppression on ART for a median of 9 (IQR, 4-13) years.

[a]Plasma HIV sequences were collected from three longitudinal pre-ART timepoints from participants 4 and 6. Pre-ART plasma was not available for the other participants.

Figure 1: Relation between distinct and overall sequences and between-host phylogeny: **A)** Participants differed markedly in their distribution of distinct and identical (*i.e.* duplicate) proviral *nef* sequences (**Table 1**). This can be visualized by plotting each participant's cumulative number of distinct sequences by the total number of sequences collected. **B)** Overall, there was no consistent pattern in the frequency of distinct sequences recovered from blood versus lung (Wilcoxon matched pairs signed rank test p>0.99). This suggests that one compartment is not inherently more likely to harbor clonally-expanded populations than the other. **C)** A phylogenetic tree inferred from all intact HIV sequences confirmed that each participant formed a monophyletic clade with branch support values of 100%, and all participants harbored HIV subtype B. Overall, participant 3 exhibited the highest within-host diversity (calculated as the mean patristic or "tip-to-tip" distance between all pairs of distinct sequences) whereas participant 9 exhibited the least diversity (*inset*). Although clinical histories are not available for all participants, these observations are consistent with participant 3 having had HIV for at least 15 years prior to suppressing viremia on ART, whereas participant 9 initiated ART shortly following diagnosis (early ART limits HIV evolution and diversity[2]).

[2]Massanella *et al*. Lancet. 2021 May;2(5):e198-e209. PMID: 34841369.

Figure 1

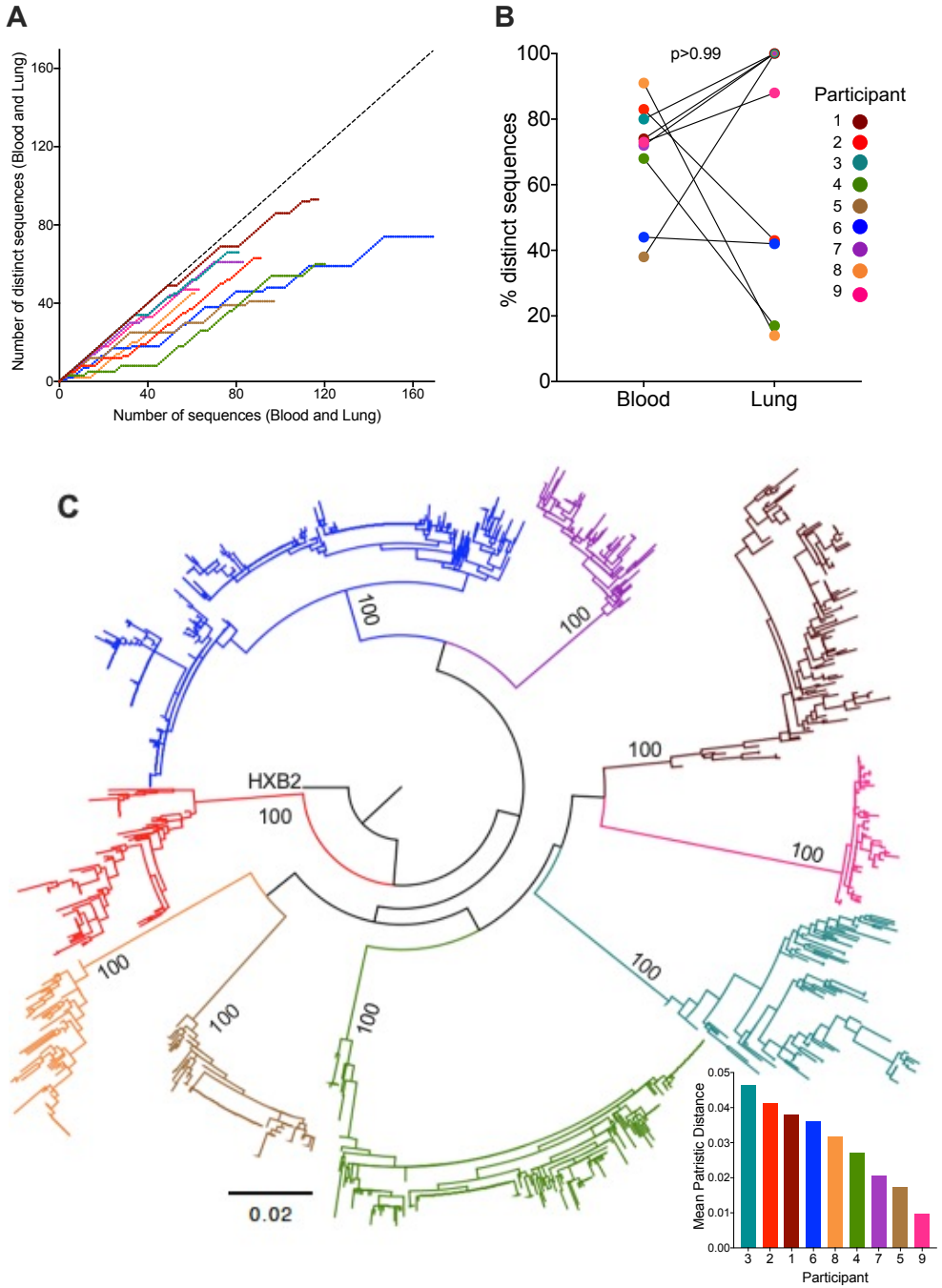


Figure 2

Limited evidence of genetic compartmentalization in blood and lung

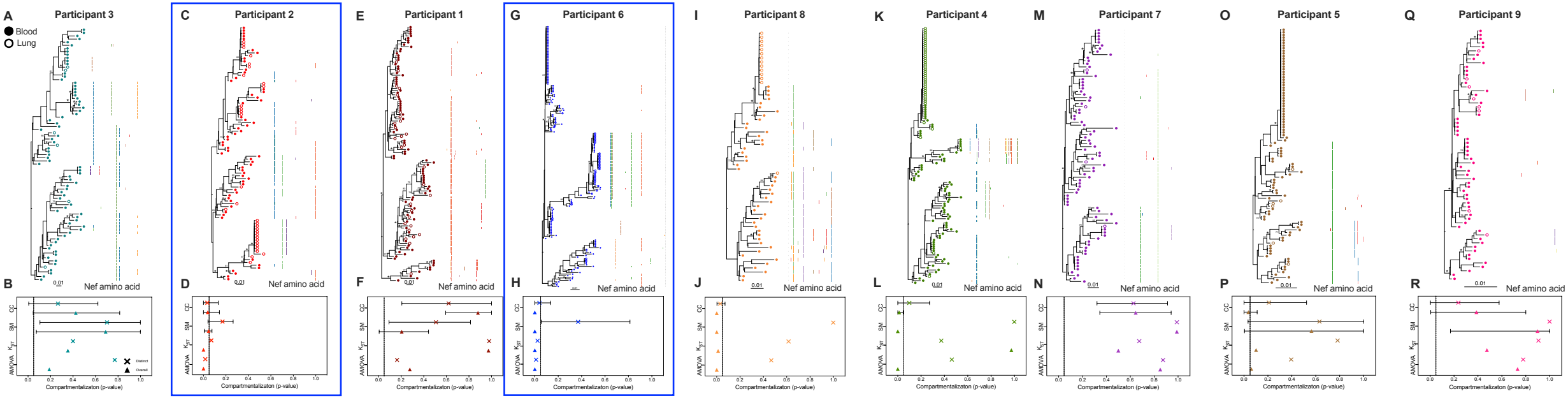


Figure 2: Within-host phylogenies and compartmentalization results: Each within-host nucleotide alignment was first reduced to distinct sequences only. The best-fitting substitution model for each dataset was then determined via jModelTest. Next, MCMC methods were used to build a random sample of phylogenies for each participant. Two parallel runs with MCMC chains of five million generations, sampled every 1,000 generations, were performed in MrBayes. The first 25% of iterations were discarded as burn-in, yielding 7,500 phylogenies per participant. Identical sequences were then grafted back on, to generate two sets of trees for analysis: first, trees containing distinct sequences *per compartment* (*i.e.* if a sequence was present in both blood and lung, it would be included twice in the tree) and second, trees containing all within-host sequences collected. The tree with the highest-likelihood, midpoint rooted is displayed for each participant. Node support values are shown as posterior probabilities, where those ≥70-89% are reported adjacent to the respective node, and those ≥ 90% are marked with asterisks. The plot to the right of each tree shows *amino acid* differences with respect to the top sequence in phylogeny. Compartmentalization tests were run two ways: first, restricting to only distinct sequences per compartment ("*distinct*" analysis), and second, including all sequences ("*overall*" analysis). P-values of the resulting AMOVA, K_{ST}, SM, and CC compartmentalization tests are shown below each tree, with "x" and triangle symbols denoting p-values from the distinct and overall analyses, respectively. For SM and CC tests, symbols show mean p-value averaged over all 7,500 within-host trees; bars represent the 95% HPD interval of the p-value.

Figure 3

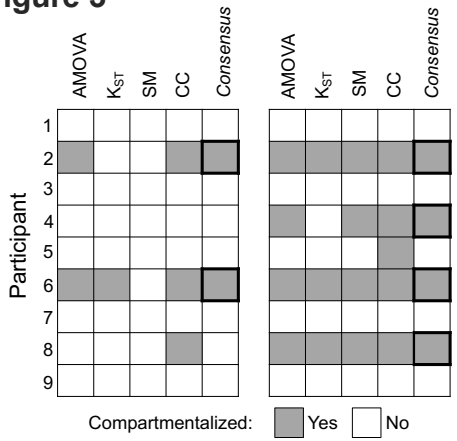


Figure 3: Compartmentalization summary: When considering only distinct sequences per compartment, only participants 2 and 6 exhibited strong support for compartmentalization across blood and lung. When considering all within-host sequences, four of nine participants exhibited strong support for genetic compartmentalization (participants 2, 4, 6 and 8). By contrast, five of the nine participants (1,3,5,7 and 9) exhibited no strong evidence of genetic compartmentalization regardless of inclusion of identical sequences.

Figure 4

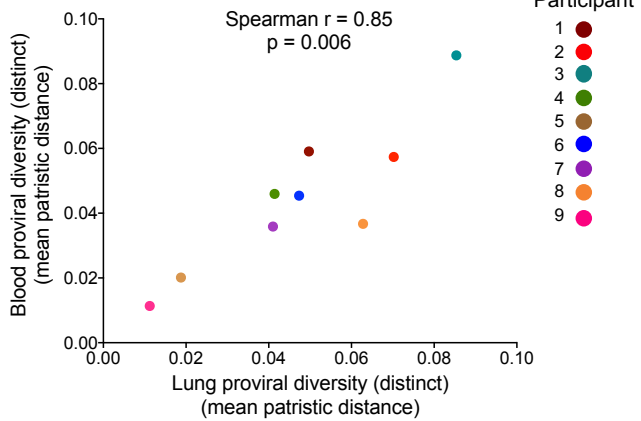


Figure 4: Strong correlation between blood and lung overall proviral diversity: Values represent mean within-host patristic (tip-to-tip) distances between all pairs of distinct sequences per compartment. Despite inter-individual variability in the overall numbers of sequences recovered per participant, the within-host diversity of unique proviral sequences recovered from blood correlated significantly with the diversity of those recovered from lung, indicating that overall HIV diversity in blood generally reflects that in lung.

On-ART proviral diversity put in context with plasma HIV RNA diversity pre-ART

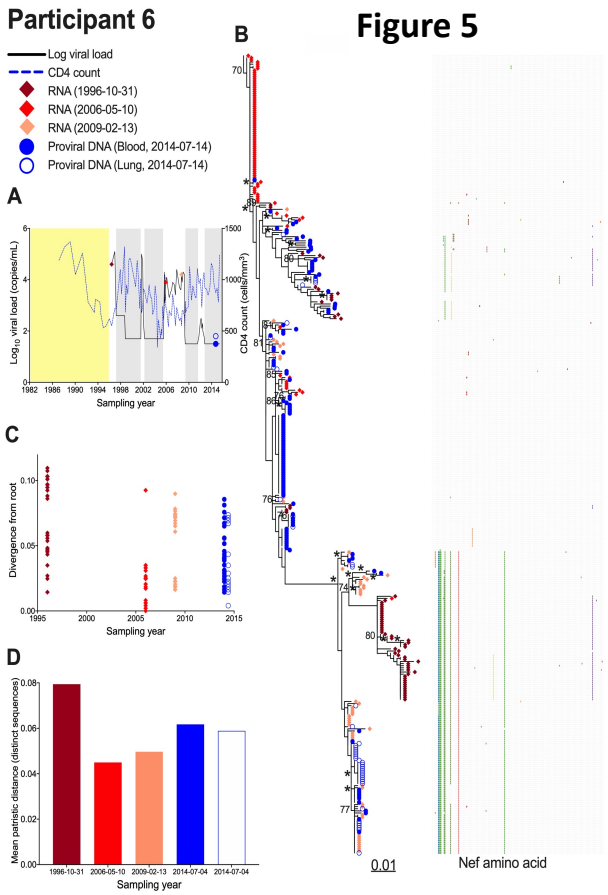
Results and Conclusion

Participant's clinical history (5A):
Diagnosed with HIV in 1982, initiated ART in 1997. ART interrupted in 2002, 2006-2009, 2012. Plasma HIV RNA (1996, 2006, 2009), and proviral (2014) sequences collected.

Tree rooted based on 2006-2009 ART interruption (5B): Ideally, one would root the tree at a position that represents the most recent common ancestor (MRCA) of the dataset. Instead, tree rooted at the location that maximized the correlation between root-to-tip distances and sampling time of plasma sequences collected in 2006 and 2009 **(5C)**, reason being, 2006 sequences would have represented HIV populations newly-emerging from the individual's reservoir, which then evolved during the subsequent three years.

Inferences based on rooted tree (5C, 5D):
HIV RNA sequences circulating in 1996 were diverse (5D) and widely divergent from MRCA (5C); this was a result of ~15 years of uncontrolled within-host HIV evolution. Sequences emerging in plasma in 2006 less diverse and divergent from root; swarm of HIV variants emerged from reservoir following ART interruption, including variants that had circulated in plasma prior to 1996. Viral populations continued to evolve until 2009. Proviral sequences sampled 5 years later on suppressive ART were broadly similar to HIV RNA populations that replicated between 2006-2009, in terms of root-to-tip divergence, with slightly higher diversity.

Figure 5



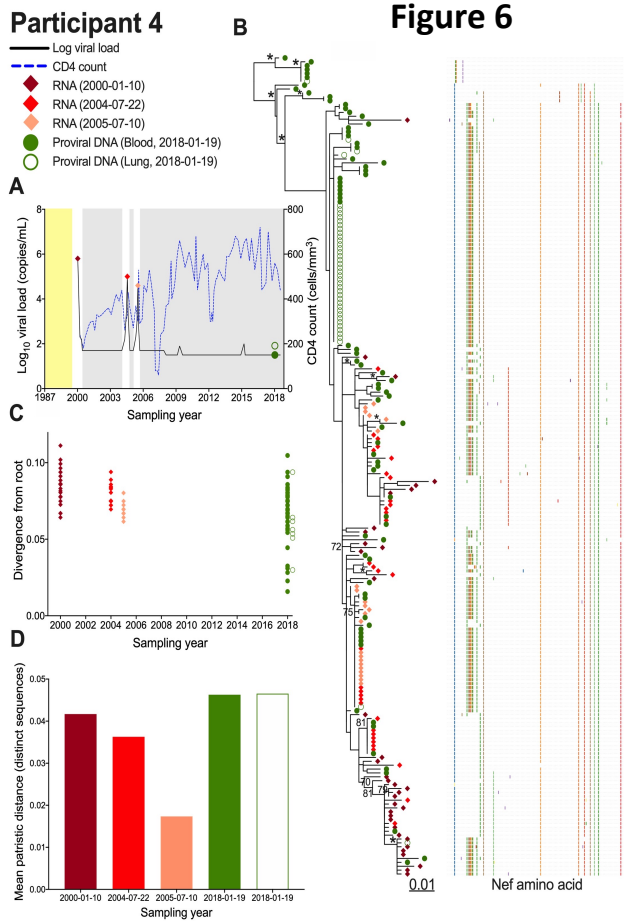
Key observations: Proviral pool was substantially re-seeded by variants that emerged and evolved during the 2006-2009 treatment interruption. HIV RNA sequences from 1996 featured a distinct subclade that was quite divergent from the root, that contained only a single sequence from one other timepoint, a 2006 plasma sequence. This suggests that representatives of this clade, which were quite abundant in plasma just prior to first ART initiation in 1996, did not persist in abundance in the reservoir.

Participant's clinical history (6A):
Diagnosed with HIV in 1987, initiated ART in 2000. Viremic episodes in 2004, 2005. Plasma HIV RNA (2000, 2004, 2005), and proviral (2018) sequences collected.

Tree rooted using outgroup (6B): As there were no longitudinal plasma samples available where substantial within-host HIV evolution would have occurred, we used an outgroup (HXB2) to root the phylogeny.

Inferences based on rooted tree (6C, 6D):
Proviral sequences collected after ~18 years on suppressive ART interspersed throughout the whole phylogeny and were closest to the root of the tree (6B). They exhibited a far broader range of divergences from root, were also more diverse, compared to HIV RNA sequences from 2000 (6C. 6D). Proviral pool contained sequences that had been archived prior to 2000. Divergence and diversity of plasma HIV RNA sequences emerging in 2004/2005 following ART interruptions, representing rebounding HIV in plasma after release from reservoir, was smaller than that seen in 2000, and vastly smaller than seen in overall proviral pool.

Figure 6



Key observation: Replication-competent reservoir sequences largely "date" to around the time of ART initiation, whereas the overall proviral pool (including defective proviruses) contains sequences much older than this.

Overall conclusions

Results reveal limited genetic compartmentalization between proviruses persisting in blood and lung on suppressive ART.

Presence of shared HIV sequences is consistent with migration of clonally-expanded reservoir cells between sites.

Eradication strategies will need to contend with genetically diverse HIV in blood and tissues.