Resurgence of HIV-1 Founder Viruses Following Antiretroviral Treatment Interruption

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ABSTRACT

Background
HIV-1 infected subjects, who started antiretroviral treatment in acute infection and were treated for several years, may be able to control HIV-1 replication following treatment interruption.

Methods
Eight Thai participants (7 men, 1 woman (P1)) started antiretroviral treatment days after HIV-1 diagnosis (Fiebig I) and participated in a treatment interruption study after more than 2 years of treatment (median: 1,005 days; range: 899-1,994 days). HIV-1 *pol* sequences (1,791 nt) were obtained from plasma samples following the endpoint-dilution strategy.

Results
Following a median of 26 days (range: 13-48) after treatment interruption, HIV-1 rebounded in all participants. The highest viral load in acute infection was a median of 38,254 copies/ml (range: 11,489-137,044). After treatment interruption, HIV-1 rebounded to a median of 9,358 copies/ml (range: 3,598-20,005) before treatment was re-initiated. We compared HIV-1 *pol* sequences amplified at a median of 3 days (range: 1-5) after HIV-1 diagnosis to sequences obtained a median of 6 days (range: 1-15) after HIV-1 rebound. Sequences from acute HIV-1 infection (n = 15) were used to infer the founder sequence. Most sequences (71%) at HIV-1 rebound were identical to the founder sequence: a median of 11 out of 15 (range: 9-13) sequences were identical, and 91% of sequences (a median of 14 out of 15; range: 12-15) had at most 1 mutation with the founder sequence. Across all participants, mutations were found as singletons unique to a given sequence except for one G-to-A transition that was shared across 3 sequences in one participant (P2) at HIV-1 rebound. There was no evidence of drug resistance mutations, nor any evidence of selection, either positive or negative, at HIV-1 rebound. There was also no evidence of HIV-1 evolution during the 2+ years of treatment as the sequences sampled at HIV-1 rebound were not more divergent from the founder than sequences sampled during acute HIV-1 infection (Mann Whitney test: 0.241< p <0.999).

Conclusions
These results indicate that rebound HIV-1 resulted from the production of viral particles from latently infected CD4+ T cells (possibly clonally expanded during treatment) rather than from a continuous low level viral replication over the treatment years. These results demonstrate that antiretroviral treatment controls HIV-1 replication but is not sufficient to eliminate a viral reservoir that was established only for the first few days of HIV-1 infection.

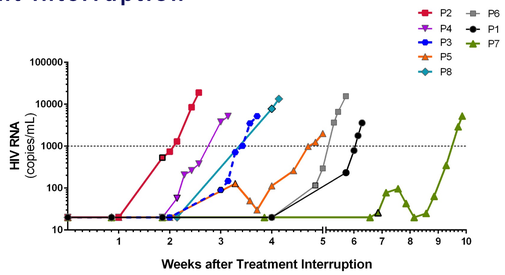
BACKGROUND

Participant	Highest VL Acute phase	Highest VL Rebound	Days after HIV-1 diagnosis Sequencing	Days after HIV-1 diagnosis ART initiation	Days on ART before ATI	Days to VL rebound	Days after rebound Sequencing	Days after rebound ART re-initiation
P1	137,044	3,598	3	5	1,994	40	8	4
P2	52,304	18,953	3	4	1,655	13	5	4
P3	85,265	5,213	1	2	987	21	3	4
P4	14,321	5,169	1	2	1,024	15	11	7
P5	11,489	20,005	2	3	942	23	15	12
P6	18,311	15,598	1	0	899	34	6	4
P7	41,999	5,253	5	5	938	48	21	21
P8	34,509	13,462	3	3	1,716	28	1	1

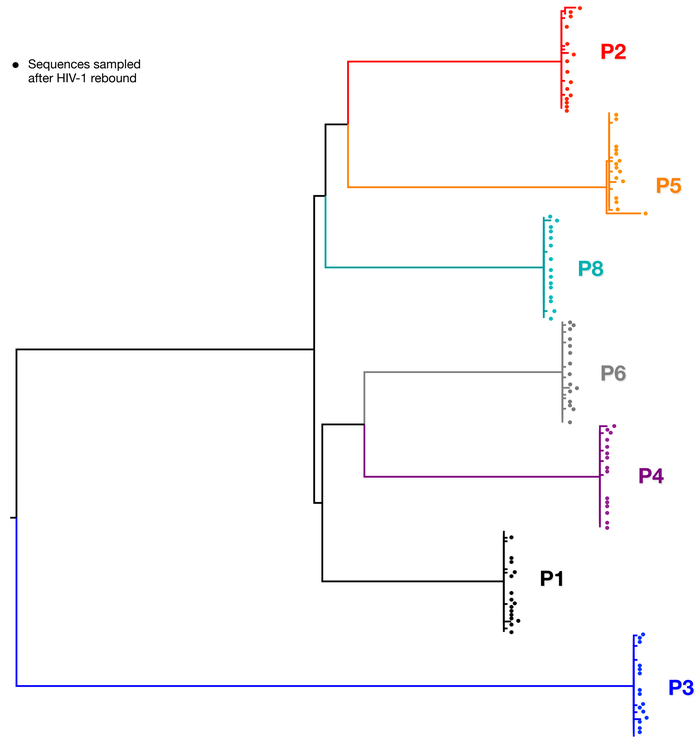
- Eight participants were diagnosed with HIV-1 infection during the Fiebig I phase and started antiretroviral treatment a few days later:
- Acute HIV-1 infection:
  - The highest viral load ranged between 11,489 and 137,044 (median = 38,254) copies/ml
  - Participants started treatment up to 5 (median = 3.5) days after HIV-1 diagnosis
  - Treatment during 2.5 to 5.5 (median = 2.8) years before treatment interruption
- HIV-1 rebound:
  - Viral rebound occurred between 13 and 48 (median = 26) days after HIV-1 diagnosis
  - The highest viral load ranged between 3,598 and 20,005 (median = 9,358) copies per ml
- ART re-initiation:
  - When 2 VL > 1000 copies/ml 1 week apart, CD4 < 350, clinical ARS, CDC B or C
  - Participants reinitiated treatment between 1 and 21 (median = 4) days after HIV-1 rebound

RESULTS

HIV-1 rebound occurred between 13 and 48 (median = 26) days after treatment interruption

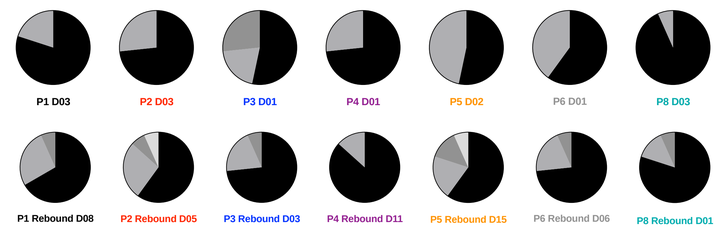


Sequences sampled during acute HIV1 infection and after viral rebound were intermingled in phylogenetic trees



Phylogenetic tree based on HIV-1 *pol* nucleotide sequences. Maximum Likelihood tree based on 1,791 nucleotides (1st & 2nd pos.: HKY+G; 3rd pos.: TIM+G; Garli v.2.01). Fifteen sequences were amplified from plasma samples by endpoint-dilution during acute infection and at HIV-1 rebound.

Most sequences at HIV-1 rebound were identical to the founder sequence



Comparison to founder sequence: Identical | 1 mutation | 2 mutations | >2 mutations

- The founder sequence was defined as the most recent common ancestor of all sequences sampled during acute HIV-1 infection
- After HIV-1 rebound, a median of 11 out of 15 (range: 9-13) sequences (71%) were identical to the founder
- 91% of sequences (a median of 14 out of 15; range: 12-15) had at most 1 mutation with the founder sequence
- Across all participants, mutations were found as singletons unique to a given sequence except for one G-to-A transition that was shared across 3 sequences in one participant (P2) at HIV-1 rebound
- No evidence of selection, either positive or negative, at HIV-1 rebound (tested with three methods)

CONCLUSION

- Most sequences at HIV-1 rebound were identical to the founder sequence
- No evidence of drug resistance mutations
- No evidence of HIV-1 evolution during ART
- The sequences sampled at HIV-1 rebound were not more divergent from the MRCA than sequences sampled during acute HIV-1 infection
- These results suggest that HIV-1 resulted from the production of viral particles from latently infected cells rather than from uninterrupted low level viral replication
- These results are in agreement with the clonal expansion of CD4+ T cells during ART
- Antiretroviral treatment was not sufficient to eliminate a viral reservoir that was established only for the first few days of HIV-1 infection.

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