Predicting HIV Sequences that Arise after Analytic Treatment Interruption

Mary F. Kearney[1], Ann Wiegand[1], Wei Shao[2], Michael Lederman[3], Rajesh T. Gandhi[4], Brandon Keele[2], Jonathan Z. Li[5]

[1]HIV DRP, NCI-Frederick; [2]Frederick National Laboratories for Cancer Research; [4]Case Western Reserve University; [4]Massachusetts General Hospital; [5]Brigham and Women's Hospital, Harvard Medical School

Background

- Our understanding is still limited as to the source and diversity of rebounding HIV after an ATI
- Clonal proliferation of HIV-infected cells may be a key contributor to viral persistence, but it has not been demonstrated that these cells are the source of viral rebound after stopping ART.
- The ability to predict which HIV variants are likely to rebound would provide insights into the HIV reservoir and has implications for the design of curative interventions

Objectives

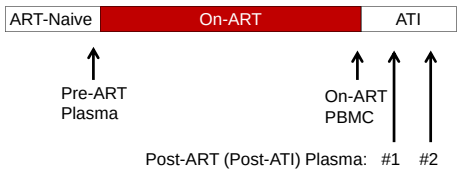
To determine the phylogenetic relationships between HIV populations from the following samples in participants undergoing an Analytic Treatment Interruption (ATI):

- **Pre-ART plasma**
- **On-ART cell-associated DNA and RNA**
- **Post-ART (post-ATI) plasma at two time points 1-2 months apart**

Methods

Data Sources, Study, and Subject Selection

- 10 participants of prior ACTG ATI studies (384, A5095, A5024, A5068, A5170, A5197) with available pre-ART and post-ATI plasma
  - 7 participants had available on-ART PBMCs, mostly at the time of treatment interruption
  - The second post-ATI plasma time point occurred a median of 4 weeks after the first post-ATI time point



- Single-genome sequencing was performed on a 1180bp region of HIV *pol* that includes PRO and RT
- A median of 27 single-genome sequences were obtained for each sample type
- Sequence diversity by average pairwise distance (APD)
- HIV population structure and divergence was evaluated by phylogenetic analysis and a test for panmixia

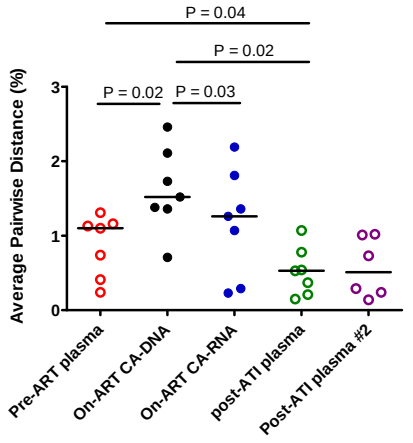
Results

Description of Participants and Samples

- Participants had a median pre-ART and pre-ATI CD4+ counts of 541 and 800 cells/mm^3
- Participants on ART for a median of 4 yrs (Q1, Q3: 3.8, 4.5 yrs) prior to the ATI
- First viremic time point occurred a median of 4.5 weeks after ATI at a median viral load of 6,386 copies/mL

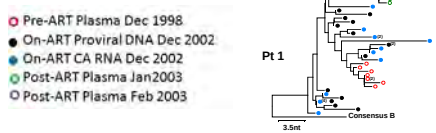
Result #1: Highest Sequence Diversity was Found in the On-ART Cell-Associated DNA

Friedman Test P<0.01

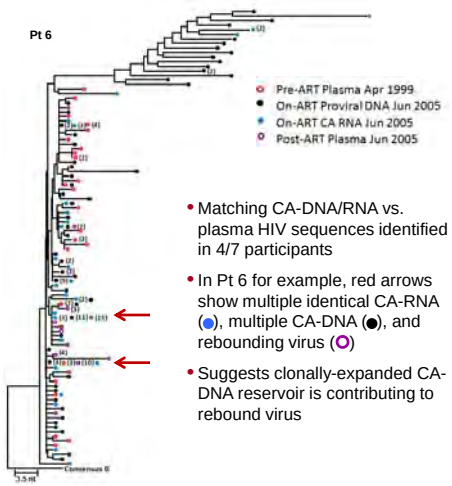


Result #2: The HIV Cell-Associated RNA Population is also Diverse, Suggesting that Expression During ART is not Rare

- No significant difference in CA-DNA and CA-RNA populations by tests for panmixia.
- Pt 1 for example, the range of CA-RNA (●) nearly matches that of CA-DNA (●)
- Diversity is only slightly lower in CA-RNA than in CA-DNA (e.g., Pt 1 APD DNA = 1.4%, APD RNA = 1.3%)

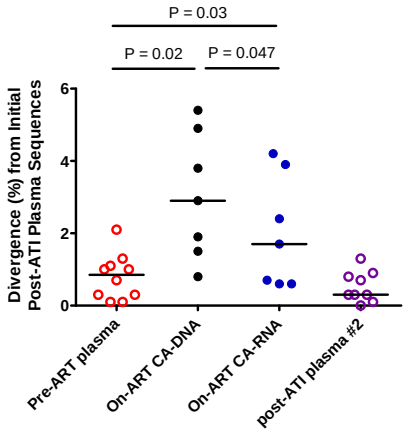


Result #3: Probable Expanded Clonal Populations of CA-DNA and CA-RNA Give Rise to Rebounding Virus after ATI

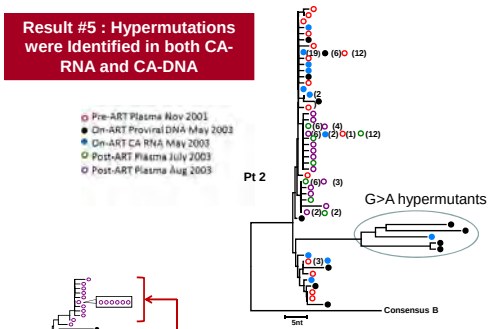


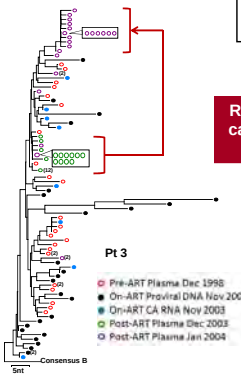
- Matching CA-DNA/RNA vs. plasma HIV sequences identified in 4/7 participants
- In Pt 6 for example, red arrows show multiple identical CA-RNA (●), multiple CA-DNA (●), and rebounding virus (○)
- Suggests clonally-expanded CA-DNA reservoir is contributing to rebound virus

Result #4: Rebounding Virus is More Similar to Pre-ART Plasma HIV than On-ART CA-DNA or CA-RNA



Result #5 : Hypermutations were Identified in both CA-RNA and CA-DNA





Result #6: Population Shifts can Occur in the 1-2 Months after Viral Rebound

- Viral population shifts can occur in the 1-2 months after rebound
- In Pt 1 and 3 for example, there is a shift in population structure post-ART by test for panmixia (p<0.001)

Conclusions

- Viral sequence diversity is high in both the cellular HIV DNA and RNA populations during ART
- Probable expanded clonal populations of cellular HIV DNA can give rise to rebounding virus after treatment interruption
- Rebounding virus is most similar to that found in the pre-ART plasma population than the on-ART CA-DNA or RNA
- Hypermutated HIV DNA does not prevent viral RNA expression
- Within 1-2 months after viral rebound, population shifts can occur, suggesting rebound from multiple cellular sources

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