Limited Evolution of Drug Resistance in HIV-1 Infected Patients with Persistent Low-level Viremia on a Stable Antiretroviral Regimen for Greater Than One Year

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Abstract

Background: Achieving and maintaining suppression of HIV-1 RNA levels below the limit of detection is the goal of antiretroviral therapy (ART). Unfortunately, many treated HIV-infected patients do not achieve or maintain this goal. The virologic threshold for changing ART in the setting of detectable viremia and stable CD4+ T-cell counts has not been established. Some studies have suggested clinical and immunologic benefits can be maintained in patients with low-level viremia on ART though the sustainability of these benefits may be limited by the accumulation of drug resistance mutations (DRM). We investigated the frequency with which new DRM develop during prolonged low-level viremia in a group of HIV+ patients followed in the NIAID-CC HIV/AIDS program.

Methods: We conducted a study of antiretroviral experienced HIV+ patients with stable, detectable viral replication (<10,000 copies/ml) persisting over at least 12 months with no change in ART. Viral RT and PR genotype sequences from at least two samples separated by at least 12 months were analyzed using commercial population-based genotypes (Visible Genetics) to quantify the development of new drug resistance mutations; phylogenetic analyses were also performed on the composite sequences. Additionally, CD4+ T-cell counts and HIV-1 RNA levels were assessed to identify changes in immunologic and virologic parameters over time.

Results: We identified 47 patients with stable low-level viremia from a total of 196 viremic patients on ART (24%). Overall the patients included represented a treatment-experienced group with an average of 5 RT mutations (range 1-10) and 5 PR mutations (range 0-10) seen on initial genotyping. Genotypes were obtained an average of 802 days apart (range 406-2010 days). Comparison of population genotypes obtained at the start and after at least one year of persistent viremia revealed very few clinically significant drug resistant mutations. Nucleotide sequence identity was on average 98% (range 95-99%) for paired sequences available for 300 patients when compared by ClustalW. Phylogenetic analysis of the population based nucleotide sequence performed on 18 genotypes from 7 of these patients showed little genetic change, with mean divergence of 0.37% (range 0-1.1%) in the *pro-pol* region during the period of observation. Additionally, no clinically significant declines in immune parameters were noted in this group.

Conclusion: In a select group of patients, stable low-level viremia was not associated with accumulation of new clinically significant resistance mutations.

Introduction

Achieving and maintaining suppression of HIV-1 RNA levels below the limit of detection is the goal of antiretroviral therapy (ART). Unfortunately, many treated HIV-infected patients do not achieve or maintain this goal. The virologic threshold for changing ART in the setting of detectable viremia and stable CD4+ T-cell counts has not been established. Some studies have suggested clinical and immunologic benefits can be maintained in patients with low-level viremia on ART though the sustainability of these benefits may be limited by the accumulation of drug resistance mutations (DRM). The evolution of DRM during viremia and the characteristics which contribute to individual variation in DRM susceptibility are incompletely understood.

Methods

Patient Selection:

- HIV positive patients with persistent low-level viremia on a stable antiretroviral (ARV) regimen for greater than 1 year were retrospectively identified from the NIAID-CC HIV/AIDS Intramural Research Clinic database (CRIMSON)
- 196 patients screened; 47 met the inclusion criteria for the study (below)
- A comparison group of 15 patients with rising viremia (HIV-1 bDNA >100 copies/ml with a positive slope persisting over at least 6 months on a stable ARV regimen) were also analyzed

Inclusion Criteria:

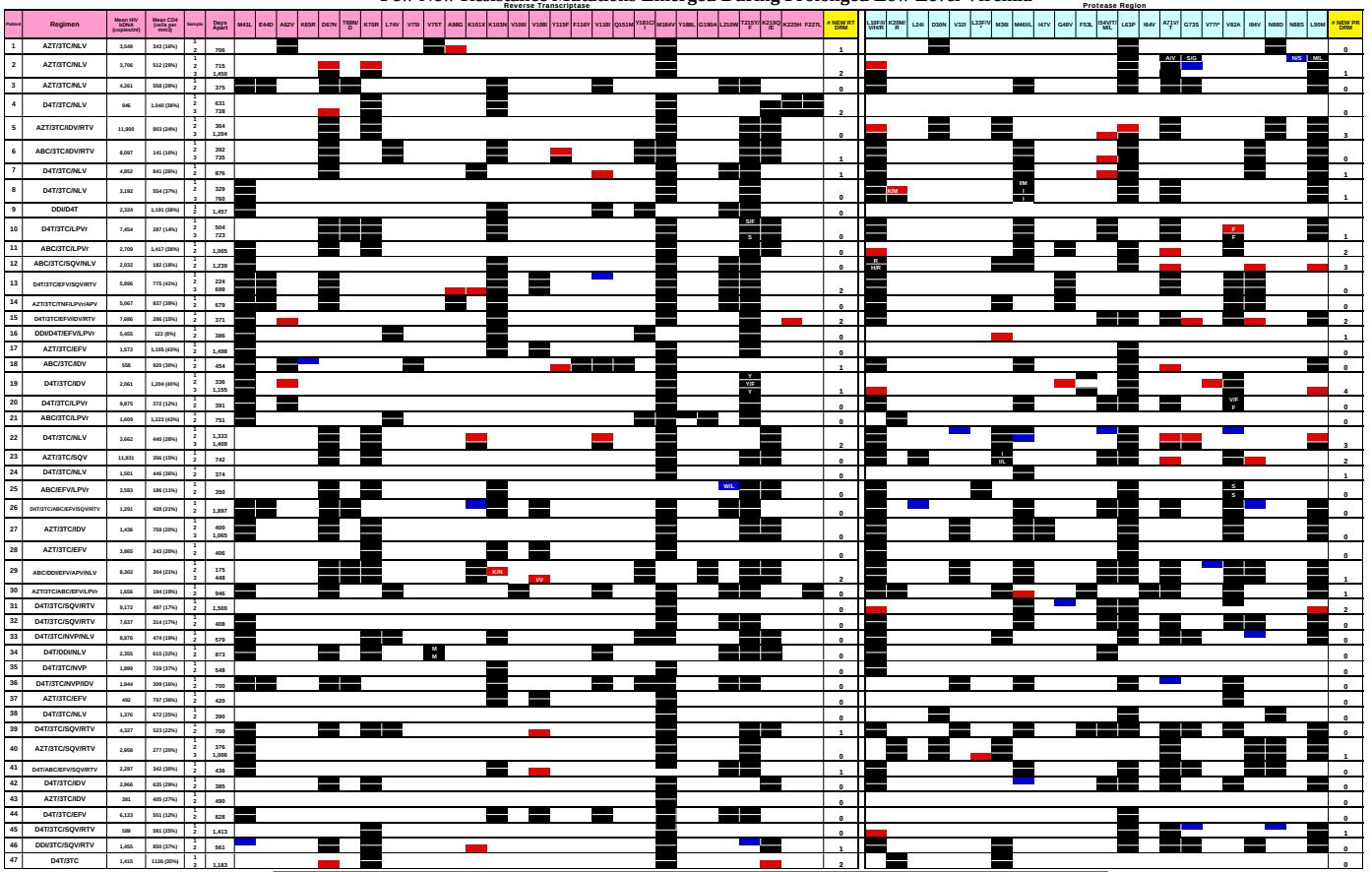
- HIV positive patients on a stable ARV regimen for at least 8 weeks, with no change in ART for at least 1 year
- Persistently elevated HIV bDNA > 100 copies/ml and < 10,000 copies/ml for at least 60 weeks
- Slope of viral load plotted over time not statistically significantly different from zero ($p \geq 0.05$)
- Plasma samples available at least 365 days apart, with the first sample available from at least 8 weeks after the last ARV change

Analysis:

- Collection of historical information including pre-treatment viremia, ARV experience and current ARV regimen, preexisting DRM (if available)
- Comparison of population genotyping (Visible Genetics) of early and late data point samples
- Comparison of sequences by ClustalW and phylogenetic analysis on select samples

Results

Few New Resistance Mutations Emerged During Prolonged Low Level Viremia



	PERSISTENT VIREMIA	RISING VIREMIA
	MEAN (range)	MEAN (range)
Age	46 years (29-61)	43 (27-56)
Duration of HIV Infection	15 years (7-20)	13 years (2-22)
Total Duration of ARV Therapy	11 years (4-18)	10 years (3-15)
Duration on ARV Regimen Studied	802 days (406-2010)	586 days (154-1370)
Pre-treatment HIV RNA	5.28 log copies/ml (4.00-5.91)	5.29 log copies/ml (4.18-5.88)
Previous ARV Exposure		
# Agents By Class: NRTI	4 (2-6)	5 (2-6)
# Agents By Class: NNRTI	1 (0-2)	1 (0-2)
# Agents By Class: PI	2 (1-4)	3 (0-5)
Mean CD4 Count On Current ARV Regimen	594 cells/mm3 (123-1223)	592 cells/mm3 (273-1094)
Change in CD4 Count During Period of Observation	-4 cells/month (-54 to +28)	-4 cells/month (-17 to +5)
Number of Major DRM on Baseline Genotype		
Reverse Transcriptase	5 (1-10)	3 (1-6)
Protease	5 (0-10)	2 (0-8)
Number of New Major DRM Arising During Observation		
Reverse Transcriptase	0.5 (0-2)	1.4 (0-3)
Protease	0.7 (0-4)	2.2 (0-10)
ClustalW Sequence Identity	98% (95-99%)	97% (94-99%)

Discussion

- In a select group of HIV-1 positive patients with persistent viremia on a stable ARV regimen for >1 year:
  - The development of new drug resistance mutations (DRM) based on population genotyping was rare:
    - New Reverse Transcriptase DRM/patient: mean < 1, range 0-2
    - New Protease DRM/patient: mean < 1, range 0-4
  - Sequence comparisons in 30 patients revealed significant similarities with mean percent identity 98% (range 95-99%)
- Etiology of low level viremia is unknown
  - Viral RNA on therapy was significantly lower than pre-therapy, likely excluding adherence as explanation for partial suppression
  - Hypersusceptibility phenomena (NNRTI or amprenavir) could not explain partial suppression
- Reasons for limited emergence of new resistance mutations are uncertain
  - Resistance profiles revealed that many resistance sites, including TAMS, remained wild type, suggesting resistance possibilities were not exhausted
    - 15/47 patients had wild type RT T215
  - It is possible that in these patients' viruses, significant declines in HIV-1 fitness would be associated with acquisition of additional mutations, and so additional mutations did not emerge
  - Analysis remains limited as population based genotyping used in this study will only detect most common circulating virus.
  - Further analysis of viral, immune, and clinical characteristics may identify patients in whom changes in antiretroviral therapy may be delayed.