HHS Public Access

Author manuscript

Antivir Ther. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

Antivir Ther. 2015 ; 20(1): 73–76. doi:10.3851/IMP2772.

PHYLOGENETIC EVIDENCE OF HIV-1 SEQUENCE EVOLUTION IN SUBJECTS WITH PERSISTENT LOW-LEVEL VIREMIA

Saran Vardhanabhuti[1], **Babafemi Taiwo**[2], **Daniel R. Kuritzkes**[3], **Joseph J. Eron Jr**[4], and **Ronald J. Bosch**[1]

[1]Harvard School of Public Health, Boston, MA

[2]Northwestern University, Chicago, IL

[3]Harvard Medical School, Boston, MA

[4]University of North Carolina, Chapel Hill, NC

Abstract

Background—Persistent low-level viremia (LLV) during the treatment of antiretroviral therapy (ART) is associated with emergent drug resistance mutation (DRM); however insight into its driver is limited. The objectives were to study HIV-1 *pol* sequence evolution in subjects with persistent LLV and evaluate factors associated with sequence changes.

Methods—HIV-1 *pol* sequences from 54 treatment-naive subjects undergoing first-line lopinavir-ritonavir- or efavirenz-containing ART were obtained at pre-ART and end of LLV. HIV-1 sequence evolution was evaluated using phylogenetic analysis and hamming distance calculation. DRMs were interpreted based on the International AIDS Society-USA 2011 update.

Results—Subjects with new DRM during LLV had greater HIV-1 evolution across *pol* from the pre-ART to end of LLV compared to subjects without DRM. Evolution over non-DRM sites was similar between groups. Higher degree of genetic evolution was positively associated with higher HIV-1 RNA levels during LLV, both at DRM and non-DRM sites.

Conclusion—The magnitude of LLV was the primary driver of evolution rate at DRM as well as non-DRM sites. Higher viral load was associated with DRM emergence in these subjects. These findings provide insights that may be applicable to the management of patients with persistent LLV during ART.

Corresponding Author: Saran Vardhanabhuti, 651 Huntington Avenue, FXB547A, Boston, MA 02115. svardhan@sdac.harvard.edu.

Contributions: S.V. and R.J.B designed the study and analyzed data. S.V., B.T., R.J.B wrote the manuscript. S.V., B.T., D.R.K, J.J.R and R.J.B critically reviewed the manuscript.

Potential Conflicts of Interest:

B. T. was a consultant to ViiV, Pfizer, Janssen and GlaxoSmithKline and has received research support from Pfizer and ViiV. D. R. K. is a consultant to Bristol-Myers Squibb, Boehringer-Ingelheim, Gilead, GlaxoSmithKline, InnoVirVax, Koronis, Merck, Tobira, ViiV, VirXSys, ViroStatics and Celera and has received research support from Gilead and Merck. J. J. E. is a consultant to Merck, Gilead, Janssen, ViiV, GlaxoSmithKline and Bristol-Myers Squibb and has received research support from Merck, Bristol-Myers Squibb, ViiV and GlaxoSmithKline. There are no conflicts of interest for the other authors.

Keywords

HIV-1 sequence evolution; drug resistance mutation; low-level viremia; phylogenetic; hamming distance

Introduction

Persistent low-level viremia (LLV), defined as multiple confirmed detectable HIV-1 RNA levels that are < 1,000 copies/mL, are commonly seen in HIV-1 infected subjects undergoing antiretroviral treatment (ART) and their significance to clinical outcome is increasingly recognized [1–5]. Previous studies evaluating drug resistance mutation (DRM) as a binary variable (present or absent) have shown emergence of DRM during persistent LLV, but insight into its drivers is limited [6–10]. We used a phylogenetic model and a modified Hamming distance calculation to quantify sequence evolution in the HIV-1 *pol* gene at both DRM and non-DRM sites of HIV-1 from pre- ART to the end of persistent LLV. We also evaluated factors associated with observed sequence changes.

Methods

Subjects were identified retrospectively from two AIDS Clinical Trials Group (ACTG) studies (A5142 and A5095) of first-line lopinavir-ritonavir- or efavirenz-containing ART [6]. Persistent LLV was defined as at least two VLs > 50 and < 1000 copies/mL over a 24-week period after at least 24 weeks of ART. The end of LLV was the first VL ≤ 50 or ≥ 1000 copies/mL after the LLV period [6]. VL was measured using ultrasensitive Roche Amplicor HIV-1 Monitor assay version 1.0 and/or 1.5. Samples preparation and population sequencing methods were described previously [6].

Within the HIV-1 *pol* sequence, we interrogated 987 nucleotide positions (329 amino acids: protease codon 1–99 and reverse transcriptase codon 1–230). There were 46 DRM sites including 31 reverse transcriptase and 15 major protease mutation sites based on the International AIDS Society-USA 2011 update [11]. For each subject, paired HIV-1 sequences (pre-ART and the final sequence of LLV) were used to characterize HIV-1 sequence evolution using two different approaches.

1. Phylogenetic analysis calculated nucleotide substitution rates for each subject based on Tamura-Nei (TN93) model. Pairwise TN93 distances were computed and normalized by follow-up time using PolEvolution scripts in HyPhy package [12]. TN93 model was selected because it corrects for biases in unequal base composition and differences in transition/transversion rates seen in nucleotide sequence evolution of HIV-1 [12].
2. Modified Hamming distance [13] measured the percentage mismatch in nucleotides and amino acids between HIV-1 sequences obtained pre-ART and the end of LLV. To accommodate mixture amino acids, i.e. for partially matched and complete mismatch nucleotides or amino acids, the distance was assigned a value of 0.5 and 1, respectively. This distance is normalized by the sequence length but does not take into account the time span between the two isolates.

In the HyPhy package, global nucleotide substitution rates were compared between groups using a likelihood-ratio test (LRT). Differences in Hamming distances between groups were compared using Wilcoxon rank-sum tests. Metrics of HIV-1 viremia [6] and the magnitude of sequence evolution (nucleotide substitution rates and Hamming distances) were correlated using Spearman's rank coefficient. In addition to the methods described above, we also calculated synonymous (dS) and non-synonymous (dN) substitution rates based on the method of Nei and Gojobori [14] using SNAP (Synonymous and Non-synonymous Analysis Program) v1.1.1 [15–17].

Results

Fifty-four subjects had *pol* sequence data available before treatment and at the end of LLV (median pre-ART VL = 5.1 $\log_{10}$ copies/mL, [25^{th}, 75^{th} quantiles]: 4.7, 5.7). The median duration of follow-up between pre-ART and the end of LLV was 78 weeks (25^{th}, 75^{th}: 64, 104) and median duration of LLV was 32 weeks (25^{th}, 75^{th}: 24, 40). New resistance mutations were detected during LLV in 20/54 (37%) subjects. The 20 subjects with new DRM during LLV had greater HIV-1 sequence evolution (nucleotide substitution rate [95% CI]:5.1×10^{-3} substitutions/site/year [4×10^{-3} – 6×10^{-3}]) from pre-ART to final LLV sequence compared to subjects without new DRM (3.9×10^{-3} [3×10^{-3} – 4×10^{-3}]) across the *pol* sequence (P=0.011). Greater sequence evolution in those with new DRM was also observed when analyzing by initial regimen (efavirenz + nucleoside analogs (NRTIs): 4.5×10^{-3} [4×10^{-3}–6×10^{-3}] vs. 1.4×10–3 [1×10^{-3}–2×10^{-3}], p<0.001; lopinavir/ritonavir + NRTIs: 7.2×10^{-3} [5×10^{-3}–9×10^{-3}] vs. 4.1×10^{-3} [3×10^{-3}–5×10^{-3}], p=0.002). Over non-DRM sites, global nucleotide substitution rates were similar between new DRM vs. non-DRM groups (3.9×10^{-3} [3×10^{-3}–5×10^{-3}] vs. 3.7×10^{-3} [3×10^{-3}–4×10^{-3}], P=0.68).

Restricting to 20 subjects who developed DRM during LLV, 10 subjects who had VL > 200 copies/mL at the time DRM was detected had much higher nucleotide substitution rate across *pol* sequence from pre-ART to the end of LLV compared to those with VL < 200 copies/mL at the time of DRM detection (6.9×10^{-3} [6×10^{-3}–8×10^{-3}] vs. 2.9×10^{-3} [2×10^{-3}–4×10^{-3}], P<0.001).

We obtained similar findings using modified Hamming distance calculation. Hamming distance changes across the *pol* sequence (i.e., % median mismatch [95% CI]) from pre-ART to final LLV sequences were greater in subjects with new DRM compared to subjects without DRM in both nucleotides 1.4 % [1.1%, 1.8%] vs. 1.1 % [0.7%, 1.4%]; P=0.02, and amino acids 1.6 % [1.1%, 1.9%] vs. 1.0 % [0.5%, 1.2%]; P=0.001. Nucleotides and amino acid changes over non-DRM sites were similar in both groups (1.1% [0.9%, 1.7%] vs. 1.0 % [0.7%, 1.4%]; P=0.11 and 1.2 % [0.8%, 1.6%] vs. 1.0 % (0.6%, 1.4%]; P=0.25, respectively).

Without exclusion of DRM positions, synonymous substitution rates (dS) were similar between subjects with new DRM vs. subjects without DRM (median dS [95% CI]: 0.018 (0.006- 0.040) vs. 0.020 (0.006 – 0.025), p=0.54 but non-synonymous substitution rate (dN) was higher in subjects with new DRM: 0.003 (0.003 – 0.005) vs. 0.001 (0 – 0.003), p=0.03. With exclusion of DRM positions, synonymous and non-synonymous substitution rates were

both similar between DRM vs. non-DRM groups (dS: 0.016 (0.007 – 0.034) vs. 0.017 (0.006 – 0.023), p=0.48 and dN: 0.000 (0 – 0.002) vs. 0.001 (0–0.003), p=0.75). Additionally, we did not detect any difference in synonymous substitution rates over non-DRM sites based on initial regimens (p=0.16).

As shown in Table 1, higher rates of nucleotide substitution across the *pol* region were associated with higher HIV-1 RNA levels during LLV (first, minimum, maximum and HIV-1 RNA area under the curve, all P<0.05, r=0.31 to 0.55).

Restricting to non-DRM sites, nucleotide substitution rate was also positively correlated with HIV-1 RNA levels during LLV (P<0.05 for each metric, r=0.27 to 0.51) except maximum VL which did not reach statistical significance [P=0.07, r=0.25]). Similar results were seen when modified Hamming distance was used in the association analyses. Age, sex, race, pre-ART VL, pre-ART CD4 and duration of LLV were not associated with HIV-1 sequence evolution during LLV.

Similarly in Table 2, synonymous and non-synonymous substitution rates were associated with higher HIV-1 RNA levels across *pol* sequence and restricting to non-DRM sites. In particular, non-synonymous substitution rate across *pol* sequence was strongly associated with first VL during LLV.

Discussion

By using continuous metrics of HIV sequence changes, we separately examined HIV-1 evolution at DRM and non-DRM sites in patients experiencing LLV during first-line ART. Analogous methods have been used to quantify HIV evolution rates within versus outside cytotoxic T-lymphocyte-targeted regions [12]. To our knowledge, ours is the first study to apply these methods to LLV and also interrogate sequence changes at non-DRM sites. We found greater HIV-1 evolution from the pre-ART to the final sequence of LLV in subjects who developed DRMs compared to subjects who did not develop DRM driven by the evolution at the DRM sites. Among subjects with emergent DRM, those with DRM detected at VL > 200 copies/mL had much greater sequence changes compared to those with DRM detected at VL < 200 copies/mL. These results were consistent using phylogenetic analysis and modified Hamming distances. The magnitude of LLV was the primary driver of evolutionary rate at DRM as well as non-DRM sites. This agrees with the strong association between higher VL and DRM emergence in these subjects [6]. Additionally, a prior study also found a relationship between low-level viremia (time adjusted AUC of VL) and sequence evolution in the *env* region among patients from whom virus was recovered by coculture of peripheral blood mononuclear cells after 2 years of plasma suppression [18]. The methods employed in this study should be applied to larger populations and a broader array of regimens to further characterize HIV-1 evolution kinetics that promote new DRM during LLV. Nevertheless, the study provides insights that may be applicable to the management of patients with persistent LLV during ART.

Acknowledgements

We thank Sergei Kosakovsky Pond for providing HyPhy scripts, PolEvolution, and making these available to adapt for our analysis. We would like to thank Evgenia Aga and Katie Mollan for database and analytic support, Michael Hughes and Laura Smeaton for reviews of the manuscript, and wish to credit and thank the investigators and lab personnel who generated the sequence data.

This work was supported by the AIDS Clinical Trials Group under the National Institute of Allergy and Infectious Diseases grants AI-68636, AI-68634, Harvard CFAR (P30 AI060354), ACTU (AI69423), UNC Center for AIDS Research (AI50410), CTSA (RR025747).

References

1. Laprise C, de Pokomandy A, Baril JG, Dufresne S, Trottier H. Virologic failure following persistent low-level viremia in a cohort of HIV-positive patients: Results from 12 years of observation. Clin Infect Dis. 2013; 57:1489–1496. [PubMed: 23946221]
2. Cohen C. Low-level viremia in HIV-1 infection: consequences and implications for switching to a new regimen. HIV Clin Trials. 2009; 10:116–124. [PubMed: 19487182]
3. Sungkanuparph S, Groger RK, Overton ET, Fraser VJ, Powderly WG. Persistent low-level viraemia and virological failure in HIV-1-infected patients treated with highly active antiretroviral therapy. HIV Med. 2006; 7:437–441. [PubMed: 16925729]
4. Swenson LC, Min JE, Woods CK, Cai E, Li JZ, Montaner JS, Harrigan PR, Gonzalez-Serna A. HIV drug resistance detected during low-level viraemia is associated with subsequent virologic failure. AIDS. 2014
5. Gonzalez-Serna A, Min JE, Woods C, Chan D, Lima V, Montaner JS, Harrigan PR, Swenson LC. Performance of HIV-1 Drug Resistance Testing at Low Level Viraemia and Its Ability to Predict Future Virologic Outcomes and Viral Evolution in Treatment-Naive Individuals. Clin Infect Dis. 2014
6. Taiwo B, Gallien S, Aga E, Ribaudo H, Haubrich R, Kuritzkes DR, et al. Antiretroviral Drug Resistance in HIV-1–Infected Patients Experiencing Persistent Low-Level Viremia During First-Line Therapy. J Infect Dis. 2011; 204:515–520. [PubMed: 21791652]
7. Li JZ, Gallien S, Do TR, Martin JN, Deeks S, Kuritzkes DR, et al. Prevalence and Significance of HIV-1 Drug Resistance Mutations among Patients on Antiretroviral Therapy with Detectable Low-Level Viremia. Antimicrob. Agents Chemother. 2012; 56(11):5998–6000. [PubMed: 22890763]
8. Aleman S, Soderbarg K, Visco-Comandini U, Sitbon G, Sonnerborg A. Drug resistance at low viraemia in HIV-1-infected patients with antiretroviral combination therapy. AIDS. 2002; 16:1039–1044. [PubMed: 11953470]
9. Karlsson AC, Younger SR, Martin JN, Grossman Z, Sinclair E, Hunt PW, et al. Immunologic and virologic evolution during periods of intermittent and persistent low-level viremia. AIDS. 2004; 18:981–989. [PubMed: 15096800]
10. Delaugerre C, Gallien S, Flandre P, Mathez D, Amarsy R, Ferret S, et al. Impact of low-level-viremia on HIV-1 drug-resistance evolution among antiretroviral treated-patients. PLoS ONE. 2012; 7:e36673. [PubMed: 22590588]
11. Johnson VA, Calvez V, Gunthard HF, Paredes R, Pillay D, Shafer R, et al. 2011 Update of the drug resistance mutations in HIV-1. Topics in HIV Medicine. 2011; 19:156–164.
12. Hightower GK, May SJ, Pérez-Santiago J, Pacold ME, Wagner GA, Little SJ, et al. HIV-1 Clade B pol Evolution following Primary Infection. PLoS ONE. 2013; 8:e68188. [PubMed: 23840830]
13. Mollan K, Daar ES, Sax PE, Balamane M, Collier AC, Fischl MA, et al. HIV-1 amino acid changes among participants with virologic failure: associations with first-line efavirenz or atazanavir plus ritonavir and disease status. J Infect Dis. 2012; 206:1920–1930. [PubMed: 23148287]
14. Nei M, Gojobori T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. 1986; 3:418–426. [PubMed: 3444411]
15. [Accessed on March 12, 2014] SNAP v1.1.1 Perl version. http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html.

16. Rhee SY, Gonzales MJ, Kantor R, Betts BJ, Ravela J, Shafer RW. Human immunodeficiency virus reverse transcriptase and protease sequence database. Nucleic Acids Research. 2003; 31(1):298–303. [PubMed: 12520007]

17. Korber, B. HIV signature and sequence variation analysis. In: Rodrigo, Allen G.; Learn, Gerald H., editors. Computational Analysis of HIV Molecular Sequences. Vol. Chapter 4. Dordrecht, Netherlands: Kluwer Academic Publishers; p. 55-72.

18. Gunthard HF, Frost SD, Leigh-Brown AJ, Ignacio CC, Kee K, Perelson AS, et al. Evolution of envelope sequence of human immunodeficiency virus type 1 in cellular reservoirs in the setting of potent antiviral therapy. J Infect Dis. 1999; 73(11):9404–9412.

Table 1

Association of HIV-1 sequence evolution with subjects' baseline characteristics and HIV-1 metrics during low-level viremia.

	Nucleotide Substitution Rate (substitutions/site/year)			Nucleotide Hamming Distance (%mismatch)		
	***pol* sequence**	**DRM sites**	**Non-DRM sites**	***pol* sequence**	**DRM sites**	**Non-DRM sites**
Sex	(0.56)	(0.29)	(0.71)	(0.34)	(0.89)	(0.24)
Race	(0.96)	(0.62)	(0.97)	(0.24)	(0.10)	(0.53)
Age (years)	(0.13)	(0.29)	(0.15)	(0.37)	(0.49)	(0.37)
Pretreatment VL (log_{10} copies/mL)	(0.32)	(0.07)	(0.68)	(0.33)	(0.05)	(0.79)
Pretreatment CD4 (cells/mm^3)	(0.83)	(0.93)	(0.59)	(0.55)	(0.98)	(0.29)
Length of low-level viremia (weeks)	(0.31)	(0.11)	(0.38)	(0.54)	(0.11)	(0.98)
First VL during LLV (copies/mL)	0.55 (<0.0001)	0.48 (<0.001)	0.51 (<0.0001)	0.61 (<0.0001)	0.46 (<0.001)	0.56 (<0.0001)
Minimum VL during LLV (copies/mL)	0.40 (<0.001)	0.39 (<0.01)	0.33 (<0.0001)	0.52 (<0.05)	0.42 (<0.01)	0.48 (<0.01)
Maximum VL during LLV (copies/mL)	0.32 (0.0001)	0.34 (<0.001)	0.25 (<0.0001)	0.57 (0.07)	0.48 (<0.05)	0.50 (<0.05)
Time adjusted area under the curve (copies/mL)	0.36 (<0.0001)	0.39 (<0.0001)	0.27 (<0.0001)	0.60 (<0.05)	0.51 (<0.01)	0.52 (<0.01)

Displayed are Spearman correlations (with significance levels in parentheses). For sex and race and other baseline characteristics, only significance levels are shown (based on Kruskal-Wallis or Wilcoxon rank-sum tests).

DRM, drug resistance mutation; LLV, low-level viremia

Table 2

Association of HIV-1 sequence synonymous (dS) and non-synonymous (dN) substitution rates with subjects' baseline characteristics and HIV-1 metrics during low-level viremia.

	***Pol* sequence**		**Non-drm sites**	
	dS	**dN**	**dS**	**dN**
Sex	(0.64)	(0.99)	(0.97)	(0.84)
Race	(0.44)	(0.98)	(0.39)	(0.44)
Age (years)	(0.08)	(0.44)	(0.11)	(0.37)
Pretreatment VL (log_{10} copies/mL)	(0.35)	(0.05)	(0.43)	(0.80)
Pretreatment CD4 (cells/mm^3)	(0.60)	(0.83)	(0.78)	(0.22)
Length of low-level viremia (weeks)	(0.92)	(0.22)	(0.69)	(0.54)
First VL during LLV (copies/mL)	0.46 (<0.01)	0.52 (<0.001)	0.45 (<0.0001)	0.38 (<0.001)
Minimum VL during LLV (copies/mL)	0.29 (<0.05)	0.39 (<0.05)	0.29 (<0.01)	0.29 (<0.05)
Maximum VL during LLV (copies/mL)	0.31 (<0.05)	0.35 (<0.05)	0.32 (<0.01)	0.30 (<0.05)
Time adjusted area under the curve (copies/mL)	0.29 (0.05)	0.40 (<0.05)	0.30 (<0.01)	0.27 (<0.05)

Displayed are Spearman correlations (with significance levels in parentheses). For sex and race and other baseline characteristics, only significance levels are shown (based on Kruskal-Wallis or Wilcoxon rank-sum tests).

DRM, drug resistance mutation; LLV, low-level viremia