Published in final edited form as:

J Acquir Immune Defic Syndr. 2011 April ; 56(4): 344–348. doi:10.1097/QAI.0b013e31820cf029.

Failure of Initial Therapy with Two Nucleosides and Efavirenz is Not Associated with Early Emergence of Mutations in the C-Terminus of HIV-1 Reverse Transcriptase

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Abstract

It is uncertain how often mutations in the connection or RNase H domains of HIV-1 reverse transcriptase (RT) emerge with failure of first-line antiretroviral therapy. Full-length RT sequences in plasma obtained pre-therapy and at virologic failure were compared in 53 patients on first-line efavirenz-containing regimens from AIDS Clinical Trials Group study A5142. HIV-1 was mostly subtype B (48/53). Mutations in the polymerase but not in connection or RNase H domains of RT increased in frequency between pre-therapy and failure (K103N, $p=0.001$; M184I/V, $p=0.016$). Selection of mutations in C-terminal domains of RT is not common with early failure of efavirenz-containing regimens.

Keywords

HIV-1 drug resistance; HIV-1 reverse transcriptase; RT connection domain; RT RNase H domain; nucleoside and non-nucleoside RT inhibitors; efavirenz

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Potential Conflicts of Interest: R.H. reports having received honoraria or consultant fees from Abbott, Boehringer Ingelheim, Bristol-Myers Squibb, Gilead Sciences, Merck, Schering and Roche, and has received research support (to UCSD) from Abbott, GlaxoSmithKline, Pfizer and Tibotec; S.A.R. reports receiving grant support from Schering-Plough; and J.W.M. reports receiving consulting fees from Gilead Sciences, Merck, and RFS Pharmaceuticals, grant support from Merck, and having an equity interest in RFS Pharmaceuticals; M.D.H. is a paid Data and Safety Monitoring Board member for Boehringer Ingelheim, Tibotec and Pfizer. N.S.-C. reports receiving research grants from Gilead Sciences and Merck. J.H.B. and C.M.L. have no potential conflicts of interest relevant to this article.

Meetings abstract has been presented at:

Presented in part at the XVIII International HIV Drug Resistance Workshop, Fort Myers, Florida, 9–13 June 2009 (abstract 34).

GenBank accession numbers: HM056533-HM056638

INTRODUCTION

Currently recommended antiretroviral therapy (ART) for human immunodeficiency virus type 1 (HIV-1) infection consists of combinations of two nucleoside/nucleotide reverse transcriptase inhibitors (NRTI) with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor. NRTI and NNRTI target HIV-1 reverse transcriptase (RT), an essential enzyme that catalyzes the synthesis of double-stranded HIV-1 DNA from single-stranded genomic RNA. HIV-1 RT is a heterodimeric protein composed of a catalytically active 66 kDa subunit with three domains: polymerase (residues 1–319), connection (residues 320–440) and RNase H (residues 441–560); and a catalytically-inactive 51 kDa subunit that contains only non-functional polymerase and connection domains.¹

Mutations in the polymerase domain of RT are known to confer HIV-1 resistance to NRTI and NNRTI.² Recently, studies have described RT connection and RNase H domain mutations that are more frequent in ART-experienced patients compared to ART-naïve patients.^{3–13} For example, connection domain mutation N348I has been observed to emerge early with failure of zidovudine(ZDV)/lamivudine(3TC)/nevirapine(NVP) and reduces sensitivity to ZDV, didanosine, NVP, efavirenz (EFV), delavirdine, tenofovir and etravirine either as a single mutation or in the context of polymerase domain resistance mutations.^{4,11,12,14,15} Other connection domain mutations, including G333D/E, G335C/D, A360I/V, A365I and A376S have been shown to decrease ZDV susceptibility in the presence of thymidine analog mutations (TAMs),^{5–7} and mutation T369I increases resistance to NNRTI in the presence of NNRTI-resistance mutations.¹⁴

Importantly, genotype-based HIV-1 resistance tests available for clinical use only identify RT mutations in the polymerase domain and some portions of the connection domain. This restricted approach has raised concern that clinically important resistance mutations in RT are being missed.^{13,16,17} To address this concern, we sequenced full-length RT in pre-therapy and virologic failure plasma samples from the 2 NRTI plus EFV arm of AIDS Clinical Trials Group (ACTG) study A5142.¹⁸ Sequences from pre-therapy and time of confirmed virologic failure were compared to assess if mutations in the RT polymerase, connection and RNase H domains emerged at virologic failure. In addition, pre-therapy sequences from patients who did not experience virologic failure were obtained and compared to pre-therapy sequences from patients with virologic failure to assess if polymorphisms in RT predispose to virologic failure of the 2 NRTI plus EFV regimen.

METHODS

Study Design

ACTG A5142 was a phase III, multicenter, randomized, open-label trial among ART-naïve patients consisting of three arms: lopinavir/ritonavir (LPV/r) [Abbott, Abbott Park, IL] plus EFV (Bristol-Myers Squibb, Princeton, NJ), 2 NRTI plus LPV/r and 2 NRTI plus EFV (ClinicalTrials.gov number, NCT00050895).¹⁸ NRTI administered were 3TC plus ZDV (GlaxoSmithKline, Research Triangle Park, NC), stavudine (Bristol-Myers Squibb, Princeton, NJ) or tenofovir disoproxil fumarate (Gilead Sciences, Foster City, CA). Patients' treatment interruptions were recorded. All patients provided written informed consent and the study was approved at each site by an institutional review board or ethics committee.¹⁸

Virologic failure was defined as i) confirmed HIV-1 RNA $<1.0 \log_{10}$ copies/mL reduction from pre-therapy and ≥ 200 copies/mL at/or after week 8 and prior to week 32, ii) lack of suppression to <200 copies/mL by week 32, iii) confirmed rebound >1000 copies/mL after confirmed suppression to <200 copies/mL before week 32, iv) confirmed rebound $>1.0 \log_{10}$ copies/mL from nadir and >1000 copies/mL without confirmed suppression to <200 copies/

mL before week 32, or v) confirmed rebound ≥ 200 copies/mL after confirmed suppression to < 200 copies/mL at/or after week 32. Sixty patients reached protocol-defined virologic failure among 250 patients randomized to 2 NRTI plus EFV in ACTG A5142; 53/60 had stored plasma samples and available sequences from pre-therapy and failure time points with HIV-1 RNA > 450 copies/mL.

One hundred forty-four patients among 190 patients in the 2 NRTI plus EFV arm of A5142 who did not experience protocol-defined virologic failure had an available full-length RT pre-therapy sequence.

Amplification and Sequencing

Viral RNA was extracted from paired pre-therapy/failure plasma samples from 53 patients (QIAamp[®] Viral RNA Mini-kit, Qiagen, Valencia, CA) and converted to cDNA using SuperScript[™] III One-Step RT-PCR System with Platinum[®] Taq High Fidelity (Invitrogen, Carlsbad, CA) and template specific primers. Full-length RT (codons 1–560) was amplified by nested PCR, products were purified with ExoSAP-IT[®] (USB, Cleveland, OH) and bulk sequenced with six bidirectional primers using Big Dye terminator (v.3.1) on an ABI 3100 automated DNA sequencer (Applied Biosystems, Foster City, CA). Sequences were assembled and analyzed using Sequencher 4.9 software (Gene Codes Corporation, Ann Arbor, MI).

Sequences from patients who experienced virologic failure were examined at failure for known NRTI and NNRTI resistance mutations in the polymerase domain using the International AIDS Society-USA (IAS-USA) resistance table² and for novel mutations in the polymerase, connection and the RNase H domains. Sequences from pre-therapy plasma samples among patients who did not experience virologic failure were generated and obtained from Mina John and Simon Mallal.¹⁹ These sequences were analyzed to identify associations between pre-therapy polymorphisms and virologic failure.

Statistical Analysis

Emergence of Mutations at Virologic Failure—Full-length RT sequences at time of failure and pre-therapy from 53 patients who experienced virologic failure were compared for IAS-USA polymerase domain mutations and mutations that occurred more than once in the connection and RNase H domains, using two-sided exact McNemar's test. The same comparison was performed within two subgroups: patients experiencing failure with at least one IAS-USA RT mutation (n=26) and those without any such mutations at failure (n=27). P-values were unadjusted for multiple comparisons.

Associations between Pre-therapy Mutations and Virologic Failure—Pre-therapy sequences from patients who did not experience virologic failure (non-failures, N=144) were compared to 53 pre-therapy sequences from patients who experienced failure for each mutation occurring more than once, using Fisher's exact test (two-sided). P-values were adjusted for multiple comparisons using the Bonferroni correction.

All sequences were compared to the appropriate reference subtype (B, C, D or circulating recombinant form (CRF) AB or AE).

RESULTS

Emergence of RT Mutations at Virologic Failure

HIV-1 subtypes in the 53 patients experiencing virologic failure were predominantly B (48/53); 4/53 were C and 1/53 was CRF AE. RT inhibitor resistance mutations in the

polymerase domain were identified in 26 of 53 (49%) failure samples. Of the 27 patients without resistance mutations, 15 (56%) had interrupted therapy at the time of virologic failure.

Table 1 shows polymerase domain mutations that were detected at a higher frequency (3% or greater) at failure than pre-therapy. Only K103N and M184I/V were significantly more frequent at failure than pre-therapy (unadjusted $p=0.001$ and $p=0.016$, respectively). Table 1 also shows connection and RNase H domain mutations that were more frequent at virologic failure than pre-therapy, but none of the changes in frequency were statistically significant (unadjusted p -values ≥ 0.25). All of the pre-therapy mutations listed in Table 1 were also identified in the failure sample from the same patient except for one patient who did not have R358K in the failure sample.

Comparing pre-therapy and failure sequences within the two patient subgroups with ($N=26$) and without ($N=27$) IAS-USA resistance mutations at failure did not identify additional mutations in RT that were significantly more frequent at failure than at pre-therapy.

Associations between Pre-therapy Mutations and Virologic Failure

Of the 144 pre-therapy sequences from non-failures, 139 were subtype B, 3 were subtype C, 1 was subtype D and 1 was CRF AB. Pre-therapy mutations associated with virologic failure, unadjusted for multiple comparisons, were E6D ($p=0.023$), K103R ($p=0.046$) and Q174K ($p=0.015$) in the polymerase domain and Q334H in the connection domain ($p=0.045$) [Table 2]. However, these associations were not significant after correction for multiple comparisons.

DISCUSSION

This study is the first to compare full-length HIV-1 RT sequences in paired plasma samples obtained pre-therapy and at first virologic failure in predominantly subtype B infected patients. The only mutations that were significantly more frequent at virologic failure than pre-therapy were K103N ($p=0.001$) and M184I/V ($p=0.016$) in the polymerase domain, which confer resistance to the study drugs EFV and 3TC, respectively. Other known polymerase domain mutations were not significantly associated with failure although, there were possible trends for K65R ($p=0.13$) and V106I/M ($p=0.13$). Mutations in the RT connection and RNase H domains were not significantly more frequent at virologic failure than at pre-therapy ($p \geq 0.25$), and thus did not contribute to the virologic failure observed.

This study is the largest to date comparing pre-therapy and failure sequences from the same individuals ($N=53$). The only other similar study compared full-length RT sequences in one patient over three years⁴. In this patient, the N348I connection domain mutation was selected by ZDV and/or didanosine therapy⁴ and confers resistance to ZDV, didanosine, NVP, EFV, delavirdine, tenofovir and etravirine.^{4,11,12,14,15} N348I was probably not identified in our study because efavirenz, not NVP, was the NNRTI used for initial randomized therapy in ACTG A5142 and virologic failure was detected early using a sensitive definition of failure.

Other prior analyses of RT connection or RNase H domain mutations have compared sequences from unrelated ART-naïve patients and ART-experienced patients.^{3–13} These studies have identified a number of mutations in the C-terminus of RT that are more frequent in ART-experienced patients compared to ART-naïve including E312Q, G333D/E, G335C/D, N348I, R356K, R358K, A360I/V, A365I, T369I, A371V, A376S and K451R. Our study did not identify these mutations at virologic failure. This may be due, in part, to the strict definition of virologic failure used in ACTG A5142 (see Methods). As a

consequence, the failure samples analyzed were early in the course of virologic breakthrough with limited time on failing therapy for drug-resistant variants to emerge. Nevertheless, our study shows that the first mutations to arise in association with virologic failure in HIV-1 subtype B-infected patients are in the polymerase domain and not frequently in the connection or RNase H domains.

Although our study is the largest pre-therapy-failure comparison of RT sequences, the sample size (N=53 pairs) had limited power to detect mutations that emerge at low frequency. Specifically, the upper bound of the 95% confidence interval for the probability of a mutation that is not detected in 53 patients is 6.7%. Therefore, at alleles where no mutation was observed, there may have been up to 7% of emergent resistance in the study population. Larger sample sizes are needed to exclude connection and RNase H domain mutations that emerge infrequently.

Secondary analyses were performed to assess if pre-therapy RT polymorphisms predispose to virologic failure (Table 2). Polymerase, connection and RNase H domain mutations were not significantly associated with failure after correction for multiple comparisons. To achieve 80% power to detect such an association, assuming a low proportion (e.g. 2%) of the 144 patients that did not experience virologic failure had a specific polymorphism, would require that 14% of the 53 patients that experienced virologic failure have the polymorphism, i.e. a difference in proportion of 12%. As a consequence, our study had power to detect relatively large differences (>10%) in polymorphism frequency between patients experiencing virologic failure and those who did not. Such differences were not detected in our study.

In summary, this study of full-length RT did not identify mutations in the connection or RNase H domains associated with virologic failure in predominantly HIV-1 subtype B infection. These findings suggest that full-length RT sequencing is not essential for management of failure first-line efavirenz-containing regimens when failure is detected early, although analyses of larger datasets are needed before firm conclusions can be drawn.

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The following were pharmaceutical representatives: T. George (Bristol-Myers Squibb); S. Brun, K.W. Garren, R. Rode (Abbott Laboratories); J.F. Rooney, M. Poblentz, M. Hitchcock (Gilead Sciences).

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Acknowledgments

We thank Mina John and Simon Millal (Royal Perth Hospital-Perth, Australia) for providing pre-therapy RT sequences, the study sites and their personnel, and the patients for their participation in the study.

Sources of Financial Support: Supported by grants (R01AI081571, AI064086, AI27670, AI36214, AI69432, and a Virology Support Subcontract from the ACTG Central Group Grant U01AI38858) from the National Institute of Allergy and Infectious Diseases, National Institute of Health, and the Pitt AIDS Research Training Program (NIH/ NIAID 5T32AI065380-04).

REFERENCES

1. Wang J, Smerdon SJ, Jager J, et al. Structural basis of asymmetry in the human immunodeficiency virus type 1 reverse transcriptase heterodimer. *Proc. Natl. Acad. Sci. USA*. 1994 Jul 19; 91(15): 7242–7246. [PubMed: 7518928]
2. Johnson VA, Brun-Vezinet F, Clotet B, et al. Update of the Drug Resistance Mutations in HIV-1. *Top HIV Med*. 2008 Dec; 16(5):138–145. [PubMed: 19106428]
3. Cane PA, Green H, Fearnhill E, et al. Identification of accessory mutations associated with high-level resistance in HIV-1 reverse transcriptase. *AIDS*. 2007 Feb 19; 21(4):447–455. [PubMed: 17301563]
4. Hachiya A, Kodama EN, Sarafianos SG, et al. Amino acid mutation N348I in the connection subdomain of human immunodeficiency virus type 1 reverse transcriptase confers multiclass resistance to nucleoside and nonnucleoside reverse transcriptase inhibitors. *J Virol*. 2008 Apr; 82(7):3261–3270. [PubMed: 18216099]
5. Hachiya A, Shimane K, Sarafianos SG, et al. Clinical relevance of substitutions in the connection subdomain and RNase H domain of HIV-1 reverse transcriptase from a cohort of antiretroviral treatment-naïve patients. *Antiviral Res*. 2009 Jun; 82(3):115–121. [PubMed: 19428602]

6. Kemp SD, Shi C, Bloor S, et al. A novel polymorphism at codon 333 of human immunodeficiency virus type 1 reverse transcriptase can facilitate dual resistance to zidovudine and L-2',3'-dideoxy-3'-thiacytidine. *J. Virol.* 1998 Jun; 72(6):5093–5098. [PubMed: 9573280]
7. Nikolenko GN, Delviks-Frankenberry KA, Palmer S, et al. Mutations in the connection domain of HIV-1 reverse transcriptase increase 3'-azido-3'-deoxythymidine resistance. *Proc Natl Acad Sci U S A.* 2007 Jan 2; 104(1):317–322. [PubMed: 17179211]
8. Ntemgwa M, Wainberg MA, Oliveira M, et al. Variations in reverse transcriptase and RNase H domain mutations in human immunodeficiency virus type 1 clinical isolates are associated with divergent phenotypic resistance to zidovudine. *Antimicrob Agents Chemother.* 2007 Nov; 51(11):3861–3869. [PubMed: 17724152]
9. Roquebert B, Wirden M, Simon A, et al. Relationship between mutations in HIV-1 RNase H domain and nucleoside reverse transcriptase inhibitors resistance mutations in naive and pretreated HIV infected patients. *J. Med. Virol.* 2007 Mar; 79(3):207–211. [PubMed: 17245724]
10. Santos AF, Lengrubner RB, Soares EA, et al. Conservation patterns of HIV-1 RT connection and RNase H domains: identification of new mutations in NRTI-treated patients. *PLoS ONE.* 2008; 3(3):e1781. [PubMed: 18335052]
11. Yap SH, Sheen CW, Fahey J, et al. N348I in the connection domain of HIV-1 reverse transcriptase confers zidovudine and nevirapine resistance. *PLoS Med.* 2007 Dec.4(12):e335. [PubMed: 18052601]
12. Waters JM, O'Neal W, White KL, et al. Mutations in the thumb-connection and RNase H domain of HIV type-1 reverse transcriptase of antiretroviral treatment-experienced patients. *Antivir Ther.* 2009; 14(2):231–239. [PubMed: 19430098]
13. von Wyl V, Ehteshami M, Demeter LM, et al. HIV-1 Reverse Transcriptase Connection Domain Mutations: Dynamics of Emergence and Implications for Success of Combination Antiretroviral Therapy. *Clin Infect Dis.* 2010 Jul 28.
14. Gupta S, Fransen S, Paxinos EE, et al. Combinations of mutations in the connection domain of human immunodeficiency virus type 1 reverse transcriptase: assessing the impact on nucleoside and nonnucleoside reverse transcriptase inhibitor resistance. *Antimicrob Agents Chemother.* 2010 May; 54(5):1973–1980. [PubMed: 20194692]
15. Sluis-Cremer N, Moore K, Radzio J, et al. N348I in HIV-1 reverse transcriptase decreases susceptibility to tenofovir and etravirine in combination with other resistance mutations. *AIDS.* 2010 Jan 16; 24(2):317–319. [PubMed: 20010074]
16. Gotte M. Should we include connection domain mutations of HIV-1 reverse transcriptase in HIV resistance testing. *PLoS Med.* 2007 Dec.4(12):e346. [PubMed: 18052605]
17. Roquebert B, Marcelin AG. The involvement of HIV-1 RNase H in resistance to nucleoside analogues. *J Antimicrob Chemother.* 2008 May; 61(5):973–975. [PubMed: 18325896]
18. Riddler SA, Haubrich R, DiRienzo AG, et al. Class-sparing regimens for initial treatment of HIV-1 infection. *N Engl J Med.* 2008 May 15; 358(20):2095–2106. [PubMed: 18480202]
19. John M, Heckerman D, James I, et al. Adaptive Interactions between HLA and HIV-1: Highly Divergent Selection Imposed by HLA Class I Molecules with Common Supertype Motifs. *J Immunol.* 2010 Mar 15; 184(8):4368–4377. [PubMed: 20231689]

Table 1RT Mutations More Frequent at Virologic Failure than at Pre-therapy (N=53 pairs)^a

Domain	Mutation	% Pre-therapy(N) ^b	% Failure(N) ^b	p-value ^c
Polymerase	K65R	0(0)	7.6(4)	0.13
	V90I	0(0)	3.8(2)	0.50
	K101E	0(0)	5.7(3)	0.25
	K103N	1.9(1)	23(12)	0.001
	V106I/M	1.9(1)	9.4(5)	0.13
	V179D	3.8(2)	7.6(4)	0.50
	M184I/V	1.9(1)	15(8)	0.016
	Y188H	0(0)	3.8(2)	0.50
Connection	G190S	0(0)	5.7(3)	0.25
	R358K	7.6(4)	11(6)	0.63
	A376S	1.9(1)	5.7(3)	0.50
RNase H	M/T377L	13(7)	17(9)	0.50
	V467I	17(9)	23(12)	0.25
	K530R	4.1(2)	8.2(4)	0.50

^a Mutations $\geq 3\%$ more frequent at virologic failure compared to pre-therapy.^b Total N=53 except K530R (N=49, 4 subtype C sequences were excluded from analysis because they differed from subtype B consensus).^c Exact McNemar's test (two-sided).

Table 2Associations of Pre-Therapy RT Mutations with Virologic Failure^a

Domain	Mutation	Controls ^b	Failures	p-value ^e
		N=144 ^c	N=53 ^d	
		% (N)	% (N)	
Polymerase	E6D	3 (4)	12 (6) ^f	0.023
	K103R	1 (2)	8 (4)	0.046
	Q174K	1 (2)	10 (5)	0.015
Connection	Q334H	6 (9)	16 (8)	0.045

^a Mutations with $p < 0.05$ listed; not corrected for multiple comparisons.^b Controls did not reach protocol-defined virologic failure.^c For the control group, subtype C sequences (N=3) were excluded for analyses of codon 334 because they differed from subtype B consensus.^d For the failure group, subtype C sequences (N=4) were excluded from analysis at codon 334 because they differed from subtype B consensus. One CRF AE sequence was excluded at codon 174 for the same reason.^e Fisher's exact test (two-sided) not corrected for multiple comparisons. No significant associations were found after correction for multiple comparisons (Bonferroni).^f Total N=52, missing first 16 amino acids for one sample.