HIV-1 Diversity After a Class Switch Failure

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Abstract

The purpose of this study is to evaluate whether the choice of a PI- or an efavirenz (EFV)-based HAART initial regimen impacts on the viral diversity after failure from a second, class-switch salvage regimen. Sequential HAART failures after a class switch were identified for which the genotypes showed evidence of signature mutations at each failure. Each second failure was required to be from a viral burden <400 RNA c/ml. Thirteen cases of sequential failure from an initial EFV-containing to a PI-containing regimen (EP), and 19 sequential failures from an initial PI-containing to an EFV-containing regimen (PE) were identified. The persistence of signature mutations from the first failure were evaluated at second failure and compared between the EP and PE groups. Phylogenetic trees were constructed for a subgroup of cases from existing genetic sequence information and branch length analysis was used to determine evidence of viral diversity between groups. For EP sequential therapy, 10 of 12 cases carried forward a key non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation in the second failure compared to 5 of 13 cases for PE sequential therapy ($p = 0.041$). Phylogenetic analysis demonstrated that there was more viral diversity in the PE group as compared to the EP group, consistent with the interpretation that mutations at the second failure added to an ancestral virus closer to baseline rather than to the dominant virus at first failure. The development of HIV viral diversity after multiple HAART failures is determined by the sequence in which the regimens are ordered.

Introduction

THE EXPLOSION OF NEW ANTIRETROVIRAL AGENTS over the past few years have provided physicians with opportunities to extend and improve quality of life for those individuals infected with HIV. This abundance of new agents from the pharmaceutical pipelines has, however, slowed considerably and whether this stable of current antiretrovirals will suffice to maintain the success in stemming progression and mortality until a new round of agents are available is controversial. With this in mind, navigating the complexities of choosing a therapeutic regimen after multiple failures may not only depend on the virus today but also how it evolved under the history of an individual's ART. As a result, modeling has become increasingly important for predicting evolution and selecting therapeutic agents to improve chances of viral suppression.¹⁻³ However, most genetic information is limited to a single point in time for these studies, and no large database exists that follows sequential therapy and failure to therapy over time. Other than specific mutations, the role of history in failure is not known and only inferences about evolutionary pressures can be made from these models.

In many cases when an individual fails a regimen of highly active antiretroviral therapy, the breakthrough virus contains

a signature or primary mutation that can be associated with a particular class or drug in that regimen. When an individual's viral burden rebounds while on antiretroviral therapy, a genotype is performed to determine the dominant viral species and guide the therapy. But the information is incomplete (see Ref. 4). The existence of other minority viral species is not available and only past genotypes may shed some light on other resistant viral species from past therapies. To date, however, the choice of initial therapy has been based on its ability to suppress viral burden in a durable manner, and elevate the CD4 count. The impact on this initial therapeutic choice in future therapies is not clearly understood. This article addresses the question of whether the sequence of class-specific HAART regimens, from first therapy to a salvage regimen, impacts viral diversity and therefore potential therapeutic options after the second failure.

Methods

Design

Patients were included in analyses if they virologically failed a first line therapy, containing either a nonboosted PI (protease inhibitor) or efavirenz (EFV), then had the regimen modified by a class switch to either EFV or a nonboosted

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PI-containing regimen. For inclusion, patients had to have had viral genotypes performed after the first and second failures ($VL > 1000$ RNA c/ml). Patients were not excluded for changes in nucleoside reverse transcriptase inhibitor (NRTI) therapy; however, patients with known interruptions in therapy were excluded to ensure that patients were on continuous therapy and therefore selection pressures were maintained. All patients needed to achieve a viral load < 400 RNA c/ml prior to failure in the second regimen. Median time for first failure for the EP group was 0.71 years and for the second failure 0.59 years. Whereas the median time for first failure for the PE group was 1.42 years, and for the second failure 0.96 years, none of these differences reached significance between groups.

Patient information

Medical records for HIV-1 positive patients followed at the University of Miami/Jackson Memorial Hospital Outpatient Adult HIV Clinics were reviewed electronically ($N = 2662$, received Institutional Approval through the Human Subjects Office) to identify 11 patients who met the criteria as described in the design and verified through chart abstraction. The HIV Drug Resistance Database (<http://hivdb.stanford.edu/index.html>) was used to obtain information on 17 such patients from the ACTG 384 trial (Arms A–D: $N = 489$ available graphs), and on 4 such patients from the Johns Hopkins HIV Clinical Cohort. Comparisons of proportions between groups are based on Fisher's exact test. The key or key mutations are defined in the HIV Drug Resistance Database as well.

Phylogenetic analysis

Polyclonal DNA sequence alignments and protein sequence alignments of HIV-1 PR and RT gene sequences (subtype B) for 17 patients from the ACTG 384 clinical trial study⁵ were downloaded from the HIV Stanford database (cases 1–9 and 14–21 from Table 1). Phylogenetic analysis showed evidence of contamination for PID#3 (Table 1) for a sequence that clustered with the HXB2 reference sequence.⁶ The patient was therefore excluded and phylogenetic analysis was carried out for the remaining 16 patients. The length of the protease sequences was 287 bp; the PR sequences start at position 10 in the PR gene (pos 178 in Pol gene). The length of the RT sequences was 628 bp; the RT sequences start at position 113 in the RT gene (pos 578 in Pol gene).

The viral sequences for 8 patients who started on an EFV regimen and switched to a PI regimen after failure (EP group) and 8 patients with the opposite sequence of treatments (PE group) were processed for phylogenetic analysis. Sequences sampled at therapy failure were available for each patient. Ambiguous DNA bases showing bulk PCR sequencing mixtures act as site-specific markers of genetic variation within each host. Ambiguous bases at positions associated with drug resistance were resolved by selecting the base that codes for the drug resistant amino acid. For ambiguous bases at positions not associated with drug resistance, the codon that codes for the amino acid in the protein file was selected. (If more than one codon could be chosen, the most frequent codon at that position was selected by looking within the patients' sequences, and at all patient sequences or the consensus sequence if a within-patient resolution was not found.) Gap only columns at the beginning or end of sequences were

eliminated from the alignment. A maximum likelihood tree was created for the PR and the RT set of sequences by using the DNAML program from the Phylip package, version 3.68. The subtype B reference sequence HXB2 was used as the outgroup. A rate heterogeneity alpha parameter of 0.5 was chosen and the trees were inferred using the slow computation option. Branch length distances for sequences from the same patient between therapy failures were computed from the tree. Statistical analysis was performed on the branch lengths using SPSS 17.

Results

Table 1 shows the composite data for all 32 patients with drug class switch failures. Subjects 1 to 13 first received an EFV-containing regimen that was changed after failure to a PI-containing regimen (EP), whereas subjects 14 to 32 first received a PI-containing regimen that was changed after failure to an EFV-containing regimen (PE). The first and second regimens are shown for each patient in the columns labeled 1st HAART and 2nd HAART, respectively. The key mutations as determined by genotype after the first failure are in the third column. The column designated by key mutations 2nd failure is divided into two. The subcolumn labeled "from 1st HAART" shows the mutations carried forward from the first failure to which the mutations in the 2nd failure (the right subcolumn) were added. When EFV is the initial therapy, 12 of 13 patients had a key NNRTI mutation upon failing the first HAART regimen (bold type in Table 1). In 10 of 12 cases, this mutation was present in the second failure (83%). In 13 of the 19 cases where a PI-containing regimen was used as the initial regimen, a key PI-resistance mutation was found at first HAART failure (bold type in Table 1). In these 13 cases, only 5 had a key PI-mutation in the viral genotype onto which the failed second regimen mutations added (38%). This difference in the persistence of a key mutation from first to second regimen failure between EFV or PI containing first regimens is significant at the $p = 0.041$ level.

Because of the small amount of data, we wanted to determine if another method in evaluating the difference in HAART sequencing would demonstrate a similar difference. Longitudinal viral nucleotide sequences for 8 patients who started on an EFV-containing regimen and 8 patients who started on a PI-containing regimen and who met study inclusion criteria were downloaded from the Stanford HIV Data Base (ACTG 384 trial) and processed for phylogenetic analysis. Shown in Fig. 1 are the trees for RT genes and PR genes for EFV to PI (Fig. 1A and C), and PI to EFV (Fig. 1B and D) sequenced therapies, respectively. The taxa in each tree is labeled first by the PID, then the sampling time in weeks after start of initial therapy taken as zero time. Patient sequences clustered in subtrees representing the evolutionary relatedness of these sequences. For subtrees that contain more than three sequence times, the first failure is given by the earliest time shown following zero time, and the second failure is shown by the next sampling time after the first failure. For visual purposes the branches have been colored for the RT tree for the EFV to PI therapeutic sequence, and for protease tree for the PI to EFV therapeutic sequence. The red indicates key mutations developed while patients received EFV; green, while they received a PI; and blue, prior to therapy (no key mutations in the connecting branches). Notice that for the RT

TABLE 1. VIRAL DIVERSITY AFTER SEQUENTIAL HAART FAILURE

PID ^a	1st HAART	Key mutations 1st failure	2nd HAART	Key mutations 2nd failure	
				From 1st HAART ^c	From 2nd HAART
EFV ^b to PI					
1	EFV,ddI,d4T	74V, 75I, 103N^d , 238N	NFV, ZDV,3TC	75I, 103N	30N, 184V
2	EFV,ZDV,3TC	71T, 103N , 184V	NFV,ddI,d4T	71T, 179D	
3	EFV,ddI,d4T	103N , 184V, 188L	NFV, ZDV,3TC	—	71V
4	EFV,ddI,d4T	71V, 67V, 75I, 100I, 103N	NFV, ZDV,3TC	103N , 225H	30N, 184V
5	EFV,ddI,d4T	103N , 225H	NFV, ZDV,3TC	103N , 225H	184V
6	EFV,ddI,d4T	103N , 225H	NFV, ZDV,3TC	103N , 225H	184V
7	EFV,ddI,d4T	71V, 103N , 215Y	NFV, ZDV,3TC	71V, 103N , 215Y	88S, 184V
8	EFV,ddI,d4T	103N	NFV, ZDV,3TC	103N	30N, 184V
9	EFV,ddI,d4T	101E	NFV, ZDV,3TC	101E, 190A	184V
10	EFV,ZDV,3TC	41L, 103N , 184V, 215Y	NFV, ABC,TDF,3TC	41L, 103N , 184V, 215Y	30N, 46L/I, 88D, 67N, 210W
11	EFV, ZDV, 3TC	67N, 70R, 103N , 219E, 118I, 184V	LPV/RTV, TDF, 3TC	67N, 70R, 103N , 219E, 118I, 184V	215F/I
12	EFV,ZDV,3TC	101E, 184V, 190S , 215Y,	LPV/RTV, d4T, 3TC	101E, 184V, 190S , 215Y	41L, 44D, 67N
13	EFV,d4T,3TC	67N/G, 101E, 190S	IDV/RTV ABC, 3TC	67N/G 101E 190S	82A, 184V
PI to EFV					
14	NFV,ddI,d4T	46I , 88S	EFV,ZDV,3TC	—	67G, 103N, 184V, 190E
15	NFV,ddI,d4T	30N , 74V	EFV,ZDV,3TC	—	67N, 103N, 184V, 230L, 238E
16	NFV,ddI,d4T	30N , 67N	EFV,ZDV,3TC	—	103N, 184V, 188H
17	NFV,ZDV,3TC	90M , 184V	EFV,ddI,d4T	—	103N, 188C
18	NFV,ZDV,3TC	101, 184V	EFV,ddI,d4T	10I	103N, 225H
19	NFV,ZDV,3TC	46I , 63P	EFV,ddI,d4T	—	20R, 103N, 188H
20	NFV,ZDV,3TC	30N , 184V	EFV,ddI,d4T	30N , 184V	103N
21	NFV,ZDV,3TC	184V	EFV,ddI,d4T	—	—
22	NFV,ZDV,3TC	36I, 184V	EFV,ddI,d4T	184V	103N, 225H
23	IDV,ZDV,3TC	10I, 46I , 63P, 82F, 67N, 70R, 184V, 219Q	EFV,ddI,TDF	10I, 46I , 63P, 82F, 90M , 67N, 70R, 184V, 219Q	101E 190A
24	NFV,ZDV,3TC	20R, 36I, 90M , 184V	EFV,ddI,TDF	20R, 36I	103N
25	NFV,ZDV,3TC	71V, 90M , 184V	EFV, d4t,TDF,3TC	71V, 90M , 184V	(65R)
26	NFV,d4T,3TC	184I/V	EFV,ddI,TDF	—	103N
27	IDV/RTV, ZDV,3TC	24I, 36I, 54V, 71V, 46I/L, 82A/S , 67N, 70R, 184V, 215S/V, 219Q	EFV,ddI,TDF	24I, 36I, 67N, 70R 184V, 219Q	103N, 100I
28	SQV, ZDV,3TC	46I , 84V, 90M , 54V, 71V, 41L, 67N, 184V, 210W, 215Y	EFV,ZDV,TDF	46I, 84V, 90M , 54V, 71V, 41L, 67N, 184V, 210W, 215Y	103N, 190A
29	NFV,ZDV,3TC	30D/G, 36I, 54V 71A/G, 184V	EFV,d4T,ddi,3TC	10I, 71T, 90M , 184V	103N, 225H
30	NFV,ZDV,3TC	10I, 63T, 64L, 71T, 77I, 90M , 184V	EFV,ZDV,3TC,ABC	184V	10R
31	NFV,d4T,3TC	10F, 30N /D, 71V 88D, 41M/L, 67N, 70R, 219Q	EFV,TDF,3TC	30N /D, 71V, 88D, 41L, 67N, 70R, 219Q	101Q, 103N, 215S
32	NFV,ZDV,3TC	184V	EFV,ZDV,3TC,ABC	184V	103N

^aCases 1–9, 14–21 are from ACTG 384 (<http://hivdb.stanford.edu/index.html>). Cases 10–13, 22–28 from the Miami Cohort. Cases 29–32 from the Hopkins Cohort.

^bABC, abacavir; ddI, didanosine; EFV, efavirenz; IDV, indinavir; LPV, lopinavir; NFV, nelfinavir; PI, protease inhibitor; r, ritonavir; SQV, saquinavir; d4T, stavudine; 3TC, lamivudine; TDF, tenofovir; ZDV, zidovudine.

^c“—” in the col represents either minor mutations or wild type and the same for both initial and second failures.

^dBold mutations represent major mutations that were in the first failure.

FIG. 1. Phylogenetic tree analysis from sequential therapeutic failures: Sequential failures from EFV-containing first regimen followed by a PI-containing regimen (EP) are shown in (A) and (C), and failures from PI-containing first regimen followed by EFV-containing regimen (PE) are shown in (B) and (D). The RT gene is shown in A and B, and the PR gene in C and D, for EP and PE therapy sequencing, respectively. A and D were color coded; the baseline sequence is *blue*, *red color* represents the EFV branch onto which the key mutation added, *green color* represents the NFV branch onto which the key mutation added. Each branch is annotated by the patient identifier (Table 1), and the week failure occurred following the initiation of therapy (zero time). The construction of the phylogenetic trees is described in the Methods section.

gene for EFV to PI, the PI branch added to a branch that had a key EFV mutation in 7 of the 8 cases. For the PR gene, the second failure added to the first failure branch in only one of the 8 cases (Table 1; PID 14–21). Branches prior to a bifurcation for the second failure were colored based upon whether a key first failure mutation could be inferred from the second genotype (see Table 1: from 1st HAART subcolumn).

To confirm whether the sequence of therapy after failure impacted on development of viral sequences, we evaluated whether the branch length from first to second failure was shorter than from baseline to second failure. Virus at second failure that is on the same path as the virus at first failure implies that evolution proceeded from the detectable (majority) virus at first failure. Virus at second failure on a separate path from that of the first failure virus implies that the evolution did not proceed from the majority viral strain that was sampled at first failure, but from a minority strain that is in most cases closer to the ancestral virus at baseline. The graph in Fig. 2 shows the branch length difference between baseline to second failure and first to second failure for the protease and RT genes for the different therapies. The graph shows that when patients receive an EFV containing regimen followed by a PI containing regimen the difference in branch lengths is greater than zero, whereas for the reverse order, the branch length difference is less than zero. The negative result follows from the fact that the first and second failures occur on separate branches. The association between branch length distances and treatment sequence was significant at the $p = 0.016$ level using a repeated measures ANOVA.

Discussion

The main finding from this study supports the idea that the class of antiretroviral agents used in sequential therapy determines the viral diversity. By using the key resistance mutations as markers for determining the persistence of viral species, the data demonstrate that initial therapy with efavirenz-based regimens produce robust resistant species onto which failure from a nonboosted PI-based regimen adds. This is in distinction to individuals who fail a nonboosted PI-based regimen followed by a second regimen containing efavirenz. In this case, the data show in most cases EFV mutations add to a dominant viral species that no longer contained the key PI mutation. These data do not rule out the possibility that other

minority species exist that carry both the PI and NNRTI mutations, or other mutations that genotyping may miss. Alternatively, because of how genotyping is performed, the existence of two mutations does not mean that they are necessarily on the same viral species when there is no drug pressure. However, in the current study, the fact that the viral outgrowth occurred on a suppressive regimen that contained both mutations suggests they are on the same virus. If they were not, the viral species that did not contain the resistance mutation would be suppressed.

The data from the ACTG-384 study in the Stanford HIV Database provided an opportunity to generate phylogenetic trees that permitted further investigation of the drug-sequencing hypothesis. The branch length differences shown in Figure 2 strongly suggest that the sequence of therapy impacts on viral diversity of the dominant breakthrough virus. We note that the consistency of the results of the two approaches we considered—one based only on the proportion of patients who carried a mutation from the first failure to the second, and the other based on phylogenetic analysis—strengthens these findings despite the relatively small sample size.

One explanation for the impact of sequence of therapy on diversity is the fitness of the dominant viral species. A number of studies have demonstrated that a K103N mutation has little effect on viral fitness,^{7,8} whereas protease inhibitor signature mutations exact a fitness cost.^{9,10} Our results would arise from addition of the new mutations at failure of the second regimen to the most fit virus. Numerous *in vitro* studies have looked at the fitness of an HIV virus with mutations that occur after the failure for the therapies shown in Table 1.^{9–15} In general, these studies support the idea that mutations from the second failure did add to the most fit virus. Time from first to second failure was not significantly different between the two arms and would not account for the differences in diversity (data not shown).

Although the current preferred PI-containing therapies are ritonavir-boosted, these data demonstrate that the order of different antiretroviral class regimens in sequential therapeutic failures may be important in the development of resistant virus. Moreover, this study has relevance in developing countries since nonboosted PIs continue to be used (see, for example, Ref. 16.) Because longitudinal data was available on these patients, we were able to control for a number of variables not available in large databases. Knowing the therapy history and whether the patient has been on continuous therapy is important in understanding evolutionary pressures. Evaluating genotypes after failure from a nondetectable viral burden provided an opportunity to study the addition of the signature mutations by diminishing the possibility of recombination.^{17–19} Predictive programs draw their power from statistical evaluation of large databases of point in time genotypes. Because the patient specific detail is not available from these databases, there will always be inherent scatter that may confound predictions. The possibility that resistant mutations can be dispersed among different virions by appropriate ordering of sequential therapy may preserve therapeutic options in salvage therapy that are not available when all the key mutations are on a single virion.²⁰

Treatment of HIV continues to be complex and based on numerous factors. The role for informatics in guiding physicians in therapeutic decision-making will continue to expand. Combining across large longitudinal databases will grow in

FIG. 2. Branch length differences are greater for EP than PE therapy sequencing: The difference between the branch length from zero time to 2nd failure, and time from 1st failure to 2nd failure (as measured from the time point tips) is shown for each panel in Figure 1. Horizontal lines represent the mean values for each set of points.

importance and will be needed in order to generate sufficient statistical power to address questions such as the one poised in this current work.

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Author Disclosure Statement

There is no conflict of interest relevant to this article for any of the authors.

References

1. Beerenwinkel N and Drton M. A mutagenetic tree hidden Markov model for longitudinal clonal HIV sequence data. *Biostatistics* 2007;8:53–71.
2. Foulkes AS and DeGruttola V. Characterizing classes of antiretroviral drugs by genotype. *Stat Med* 2003;22:2637–2655.
3. Ribeiro RM and Bonhoeffer S. Production of resistant HIV mutants during antiretroviral therapy. *Proc Natl Acad Sci USA* 2000;97:7681–7686.
4. Grant PM and Zolopa AR. The use of resistance testing in the management of HIV-1-infected patients. *Curr Opin HIV AIDS* 2009;4:474–480.
5. Shafer RW, Smeaton LM, Robbins GK, De Gruttola V, Snyder SW, D'Aquila RT, *et al.*: Comparison of four-drug regimens and pairs of sequential three-drug regimens as initial therapy for HIV-1 infection. *N Engl J Med* 2003;349:2304–2315.
6. Learn GH, Jr., Korber BT, Foley B, Hahn BH, Wolinsky SM, and Mullins JI. Maintaining the integrity of human immunodeficiency virus sequence databases. *J Virol* 1996;70:5720–5730.
7. Collins JA, Thompson MG, Paintsil E, Ricketts M, Gedzior J, and Alexander L. Competitive fitness of nevirapine-resistant human immunodeficiency virus type 1 mutants. *J Virol* 2004;78:603–611.
8. Cong ME, Heneine W, and Garcia-Lerma JG. The fitness cost of mutations associated with human immunodeficiency virus type 1 drug resistance is modulated by mutational interactions. *J Virol* 2007;81:3037–3041.
9. Mammano F, Trouplin V, Zennou V, and Clavel F. Retracing the evolutionary pathways of human immunodeficiency virus type 1 resistance to protease inhibitors: Virus fitness in the absence and in the presence of drug. *J Virol* 2000;74:8524–8531.
10. Martinez-Picado J, Savara AV, Sutton L, and D'Aquila RT. Replicative fitness of protease inhibitor-resistant mutants of human immunodeficiency virus type 1. *J Virol* 1999;73:3744–3752.
11. Iglesias-Ussel MD, Casado C, Yuste E, Olivares I, and Lopez-Galindez C. *In vitro* analysis of human immunodeficiency virus type 1 resistance to nevirapine and fitness determination of resistant variants. *J Gen Virol* 2002;83:93–101.
12. Koval CE, Dykes C, Wang J, and Demeter LM. Relative replication fitness of efavirenz-resistant mutants of HIV-1: Correlation with frequency during clinical therapy and evidence of compensation for the reduced fitness of K103N + L100I by the nucleoside resistance mutation L74V. *Virology* 2006;353:184–192.
13. Resch W, Ziermann R, Parkin N, Gamarnik A, and Swannstrom R. Nelfinavir-resistant, amprenavir-hypersusceptible strains of human immunodeficiency virus type 1 carrying an N88S mutation in protease have reduced infectivity, reduced replication capacity, and reduced fitness and process the Gag polyprotein precursor aberrantly. *J Virol* 2002;76:8659–8666.
14. Wainberg MA. The impact of the M184V substitution on drug resistance and viral fitness. *Expert Rev Anti Infect Ther* 2004;2:147–151.
15. Wang J, Dykes C, Domaoal RA, Koval CE, Bambara RA, and Demeter LM. The HIV-1 reverse transcriptase mutants G190S and G190A, which confer resistance to non-nucleoside reverse transcriptase inhibitors, demonstrate reductions in RNase H activity and DNA synthesis from tRNA(Lys, 3) that correlate with reductions in replication efficiency. *Virology* 2006;348:462–474.
16. Pujades-Rodriguez M, O'Brien D, Humblet P, and Calmy A. Second-line antiretroviral therapy in resource-limited settings: The experience of Medecins Sans Frontieres. *AIDS* 2008;22:1305–1312.
17. Charpentier C, Nora T, Tenaillon O, Clavel F, and Hance AJ. Extensive recombination among human immunodeficiency virus type 1 quasispecies makes an important contribution to viral diversity in individual patients. *J Virol* 2006;80:2472–2482.
18. Nora T, Charpentier C, Tenaillon O, Hoede C, Clavel F, and Hance AJ. Contribution of recombination to the evolution of human immunodeficiency viruses expressing resistance to antiretroviral treatment. *J Virol* 2007;81:7620–7628.
19. Shriner D, Rodrigo AG, Nickle DC, and Mullins JI. Pervasive genomic recombination of HIV-1 *in vivo*. *Genetics* 2004;167:1573–1583.
20. Kolber MA. A K103N resistance mutation is not an absolute contraindication to adding efavirenz to a salvage regimen: A case report. *AIDS* 2005;19:1337–1338.

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