Published in final edited form as:

Int Health. 2011 March 1; 3(1): 27–34. doi:10.1016/j.inhe.2010.11.003.

Suboptimal adherence associated with virological failure and resistance mutations to first-line highly active antiretroviral therapy (HAART) in Bangalore, India

Maria L. Ekstrand^{a,b,c,*}, Anita Shet^{b,d}, Sara Chandy^e, Girija Singh^e, Ranjani Shamsundar^f, Vidya Madhavan^g, Shanmugam Saravanan^g, Elsa Heylen^a, and Nagalingeswaran Kumarasamy^g

^aUniversity of California–San Francisco, Department of Medicine, 50 Beale Street, S-1300, San Francisco, CA 94105, USA

^bSt John's Research Institute, Bangalore, India

^cUniversity of California–Berkeley, School of Public Health, Berkeley, CA, USA

^dDepartment of Pediatrics, St John's National Academy of Health Sciences, Bangalore, India

^eDepartment of Medicine, St John's National Academy of Health Sciences, Bangalore, India

^fDepartment of Microbiology, St John's National Academy of Health Sciences, Bangalore, India

^gYRGCARE, Chennai, India

Abstract

This study was conducted to examine the relationship between adherence, viral load (VL) and resistance among outpatients receiving highly active antiretroviral therapy (HAART) in Bangalore, India. In total, 552 outpatients were recruited and VL testing was conducted for all study participants. HIV-1 genotypic resistance testing was performed for 92 participants with a VL ≥ 1000 copies/ml. Interpretation of resistance mutations was performed according to the Stanford database. Past-month adherence and treatment interruptions for >48 h were assessed via self-report. At baseline, 34 participants (6%) reported $<95\%$ past-month adherence and 110 (20%) reported a history of >48 h treatment interruptions. Combining the two adherence measures, 22% of participants were classified as 'suboptimally adherent'. In total, 24% of study participants ($n = 132$) had a detectable VL. Among the 92 samples sent for resistance testing, 68% had at least one nucleoside reverse transcriptase inhibitor (NRTI) mutation, with M184V being the most common

© 2010 Royal Society of Tropical Medicine and Hygiene. Published by Elsevier Ltd. All rights reserved.

*Corresponding author. Tel.: +1 415 597 9160; fax: +1 415 597 9213. mekstrand@gmail.com; maria.ekstrand@ucsf.edu (M. Ekstrand).

Authors' contributions: MLE, GS and SC designed the study protocol; VM, SS and NK conducted the viral genotyping at YRGCARE, and in collaboration with AS and RS interpreted its results; RS supervised all laboratory procedures at St John's Research Institute; EH conducted all statistical analyses; MLE drafted the manuscript, with assistance from NK, AS, SS and VM. All authors read, provided feedback on and approved the final manuscript. MLE is guarantor of the paper.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflicts of interest: None declared.

Ethical approval: The study received ethical clearance from the University of California, San Francisco (UCSF) Committee on Human Research (CA, USA), the St John's Institutional Ethical Review Board (Bangalore, India) and the Indian Committee on Medical Research.

(62%) and with 48% having thymidine analogue mutations. Moreover, 72% had at least one non-nucleoside reverse transcriptase inhibitor (NNRTI) mutation and 23% had three or more NNRTI mutations. Both adherence measures were significantly associated with VL ($P < 0.001$). Suboptimal adherence was significantly associated with resistance mutations ($P < 0.02$). The findings illustrate for the first time the strong association between suboptimal adherence, treatment failure and drug resistance to first-line HAART in India. The predictive value of standard adherence measures was improved by including treatment interruption data. The observed mutations can jeopardise future treatment options, especially in light of limited access to second-line treatments. To develop effective adherence interventions, research is needed to examine culturally-specific reasons for treatment interruptions.

Keywords

HIV/AIDS; Highly active antiretroviral therapy; Adherence; Virological failure; Drug resistance; India

1. Introduction

The evolution of HIV infection from a near-fatal disease to a chronic affliction has led to a shift in the focus of long-term patient management. Given that only a limited number of first-line highly active antiretroviral therapy (HAART) regimens are available at completely or partially subsidised rates in many resource-limited settings, prevention of the development of HIV drug resistance has remained critical for maximising the efficacy and durability of these regimens.

Optimal adherence to antiretroviral regimens is closely associated with achieving and maintaining HIV viral suppression and preventing the development of drug-resistant virus. Missed antiretroviral doses, interruptions in therapy and improper dosing can all lead to HIV drug resistance.¹⁻⁷ Whilst the relationship between adherence and resistance is complex,⁴ much of the literature from the USA suggests that HAART adherence rates of 95% may be needed to suppress viral replication and to avoid development of HIV drug resistance.¹⁻⁸⁻¹³ Achieving and maintaining such a high rate of adherence has presented a challenge for behaviour maintenance in the West, and data from Africa¹⁴ and India¹⁵ also indicate that HAART adherence levels may decline over time in resource-limited settings. As such, adherence is arguably one of the most important predictors both of the long-term success of first-line HAART regimens and of the development of drug resistance.

Initial adherence to HAART has been found to be excellent in resource-limited countries where government-sponsored free or subsidised HAART medications are made available,¹⁶ with several studies from the African subcontinent reporting acceptable adherence levels in >80% in those who had recently started HAART.¹⁷⁻²⁰ A study in Malawi indicated that, based on self-report, 94% of the participants reported 100% adherence in the past 4 days and also that <80% adherence was significantly associated with a detectable viral load (VL).¹⁷ This confirms that adherence of <80% may be detrimental for long-term treatment success even amongst individuals on fixed-dose combination HAART.

Previous research on HIV-1 drug resistance patterns in South India²¹⁻²⁴ has shown that these vary broadly following the failure of first-line therapy. A variety of key mutations in HIV reverse transcriptase (RT) have been associated with nucleoside reverse transcriptase inhibitor (NRTI) and non-nucleoside reverse transcriptase inhibitor (NNRTI) exposure, which give rise to a diverse range of effects in terms of altered drug susceptibilities, viral replicative capacity and RT biochemistry. Triple-drug regimens containing lamivudine

(3TC) and NNRTI commonly fail with M184V plus one or more NNRTI-associated mutations and subsequent accumulation of thymidine analogue mutations (TAM) and/or, more infrequently, one or more members of the Q151M multinucleoside resistance complex.

Published data on adherence to HAART in India have been scant, with reports primarily from the private sector, using cross-sectional designs,^{25–28} non-standard adherence measures and different recall periods and adherence cut-offs.^{25,28} In previous research,¹⁵ we showed that adherence declined over time and predicted virological failure in a longitudinal cohort of patients recruited in a private clinic setting. It was also demonstrated that the predictive validity of the adherence measures was improved by incorporating information on treatment interruptions, which was the most prevalent form of non-adherence in this setting. However, no data on genotypic resistance mutations were collected, precluding an examination of the relationship between different types of adherence patterns and HIV drug resistance. The current study was designed to meet this need. As part of a prospective study evaluating adherence, the baseline characteristics of a cohort of HIV-infected individuals receiving treatment in Indian private and public clinic settings were analysed with the aim of understanding the inter-relationship of different patterns of HAART adherence, the presence of virological failure and drug resistance mutations that have developed over the course of time while taking fixed-dose combination HAART medications in India.

2. Methods

2.1. Study design and sample

The data presented in this paper were collected as part of an ongoing longitudinal 2-year cohort study of 552 participants recruited from one private and one public hospital in Bangalore, India. Eligibility criteria included being ≥ 18 years of age, capable of communicating verbally in English, Kannada, Tamil or Telugu, being HIV-infected with a history of taking NNRTI-based antiretroviral medication, and willing to participate in all follow-up visits.

Potential participants were referred to the study interviewers by their physician, the outpatient clinic clerk or a screener at outpatient clinic registration. Following referral, participants were brought to a separate room next to the clinic (private clinic patients) or to a nearby study office (public clinic participants) for informed consent and the study interview.

The face-to-face interview was administered by trained study staff every 3 months for 2 years. It lasted approximately 1 h and collected data on a variety of topics, including demographics and health history, medical regimen, adherence behaviours and barriers as well as psychosocial factors. The survey instrument was developed in English, translated into Kannada, Tamil and Telugu, and independently back-translated into English to ensure semantic equivalence.²⁹ Participants had their blood drawn every 6 months by trained staff phlebotomists.

The present analysis includes data from 551 respondents at baseline for whom we had information on their first-line regimen.

2.2. Measures

2.2.1. Demographics—Several demographic characteristics were assessed, including gender, age, marital status, level of education and place of residence.

2.2.2. CD4 cell counts—CD4 cell counts were performed on whole-blood specimens collected in an EDTA tube using a single platform flow cytometry assay (PCA system;

Guava Technologies Inc., Hayward, CA, USA) and the number of cells was reported as cells/ μ l of blood.

2.2.3. Viral load—HIV plasma VLs were determined using a real-time PCR assay with a fluorescein-labelled TaqMan probe for quantitation of HIV particles. The test was developed and its performance characteristics were determined at Molecular Diagnostics and Genetics, Reliance Life Sciences (Mumbai, India). This assay can reliably detect an HIV RNA level of 100 copies/ml of blood.³⁰ As is standard in this setting,^{27,28} a VL of ≥ 1000 copies/ml was selected as the cut-off for resistance testing.

2.2.4. Resistance testing—An HIV-1 drug resistance assay was performed at YRGCARE Infectious Diseases Laboratory (Chennai, India) using an in-house method.³¹ The performance characteristics of this method have been validated³² and the assay is certified by the TAQAS Program (National Serology Reference Laboratory, Australia). Interpretation of the genotype in terms of drug resistance was based on an algorithm established by the online Stanford HIV-1 Sequence Database,³³ and all major clinically relevant RT mutations were analysed.³⁴

2.2.5. Antiretroviral treatment regimen—Participants were asked about their HAART start date as well as their current and past HAART regimens. This was verified by review of current prescriptions or by the patient's medication bottle or blister pack and by chart review. Classification of study participants was based on the regimen they were prescribed during the past month prior to the interview.

2.2.6. Adherence—The present analysis is based on two types of adherence measures, which were combined to create a measure of 'optimal adherence'. (i) Adherence during the past month was measured using a visual analogue scale (VAS).³⁵ The VAS is a horizontal line marked with numbers between 0 and 100 on which the participant indicates the point that best corresponds to the percentage of prescribed pills actually taken in the past month. For the analyses reported here, scores were classified as $<95\%$ vs. $\geq 95\%$. Although multiple self-reported adherence measures are used in this study, the VAS has been found to be the best predictor of virological failure in this setting.¹⁵ (ii) Treatment interruptions: in addition to estimating past-month adherence, participants were asked how many times they had skipped all their medications for >48 h since they started on HAART. This variable was dichotomised as 'zero' versus 'one or more' treatment interruptions.

2.2.6.1. 'Sub/optimal adherence'—To gain a more comprehensive measure of adherence, the VAS and treatment interruption scores were combined into a new adherence variable, in which participants with both a VAS score of $\geq 95\%$ and no treatment interruptions were classified as 'optimally adherent', with a score of 1. Participants who reported either a history of treatment interruptions or $<95\%$ adherence in the last month were classified as 'suboptimally adherent' and were assigned a score of 0. Two subgroup analyses were also conducted in which suboptimal participants were further divided into (i) those who were classified as non-adherent according to one of the two measures and (ii) those who were non-adherent according to both adherence measures.

2.3. Data analysis

Univariate analysis of categorical variables consisted of frequencies and cross-tabulations, with χ^2 tests to assess the significance of the associations. Differences between groups of continuous variables were examined by *t*-test or the Mann–Whitney *U*-test. Analyses were performed in SPSS for Windows v15.0.1 (SPSS Inc., Chicago, IL, USA).

Odds ratios of detectable VL (i.e. VL $\geq$ 100 copies/ml) and the presence of mutations for optimally and suboptimally adherent patients were calculated using SAS for Windows v9.2 (SAS Institute Inc., Cary, NC, USA). All significance levels reported are two-sided.

3. Results

3.1. Demographics

Table 1 shows the demographic information and medical history of the participants.

3.1.1. Adherence—At baseline, 34 study participants (6%) reported taking <95% of their medications in the past month, whilst 110 (20%) reported a history of at least one treatment interruption >48 h. This replicates the findings of a previous cohort¹⁵ showing that treatment interruptions are the most common form of non-adherence in this setting. Combining these two measures, 22% ($n = 123$) of the sample was classified as ‘suboptimally adherent’, i.e. classified as non-adherent on one or both measures.

3.1.2. Viral load and resistance mutations—In total, 132 study participants (24%) had a detectable VL (median 8850 copies/ml, interquartile range 1175–147 688 copies/ml). Moreover, 18% of the samples ($n = 101$) had a VL $\geq$ 1000 copies/ml and were sent for viral genotyping. Plasma from 9 of these samples could not be amplified, resulting in a final sample of 92 samples for resistance testing. Genotypic mutational patterns are listed in Table 2. RT drug resistance-associated mutations were observed in 86% of the samples, NRTI resistance mutations were identified in 68% and NNRTI resistance mutations in 72%. Of the NRTI mutations, M184V was the predominant mutation (65%), followed by TAMs (44; 48%). Of the 48% with TAMs, the majority were TAM-2 pathway mutations (34/44; 77%), 66% (29/44) were TAM-1 mutations and 43% (19/44) had a mixture of both TAM-1 and TAM-2. No insertions or deletions in the RT gene were observed. Y181C (37%) was the predominant NNRTI mutation, followed by K103N (26%) and G190A (18%).

3.1.3. Adherence, virological failure and resistance mutations—As seen in Table 3, all adherence measures were significantly associated with VL, with 42% of suboptimally adherent patients compared with only 19% of optimally adherent patients showing virological failure ($P < 0.001$). These results replicate and extend our earlier private clinic findings¹⁵ suggesting that both the VAS and the measure of treatment interruptions are also valid measures in public healthcare settings.

Adherence was also significantly associated with the development of resistance mutations in the subsample of participants experiencing virological failure, but only when history of treatment interruptions was considered, either alone or in combination with past-month adherence. The association was strongest when both measures were combined, with 87% of ‘suboptimally adherent’ patients having at least one mutation ($P < 0.02$). No mutations were associated with occasional non-adherence alone as measured by the VAS. However, examining the relationship between treatment interruptions and specific mutations showed that participants who reported one or more treatment interruptions were significantly more likely to have at least one TAM-1 pathway mutation (45% vs. 20%; $P < 0.01$), with M41L/LM mutations being the most strongly associated (38% vs. 14%; $P < 0.01$), compared with participants who reported no treatment interruptions.

4. Discussion and conclusions

These findings demonstrate for the first time in India a strong association between suboptimal adherence, treatment failure and drug resistance among patients on first-line

HAART, reinforcing the need to understand better and to reduce culturally-specific adherence barriers both in private and public healthcare settings.

The past-month adherence rates reported here are excellent and are comparable with those in previous studies both in India^{15,26} and in other resource-limited settings,^{15,26,36,37} using similar time frames. However, as shown both in our previous work¹⁵ and in other studies, adherence levels frequently decline over time,^{26,38,39} suggesting that patients may require assistance to maintain optimal levels over the long-term. Although promising and culturally-specific strategies remain to be identified in future research, they are likely to include programmes implemented at multiple levels, including structural (such as shortening clinic wait times, decentralisation of antiretroviral therapy clinics, prescriptions for >30 days of medication), family- and peer-based support, as well as the use of interventions focusing on individual issues, including mental health, and strategies that tailor regimens and prescription refill times to individual lifestyles. Finally, given the recent rapid increase in the use of mobile phone technology in India, future intervention programmes may want to examine the use of phones both as reminders to take medication and to refill prescriptions as well as for counselling to help remove adherence barriers. Given that South Indian HAART patients are a diverse group, each with his or her unique circumstances, it is unlikely that one intervention will fit the needs of all patients, but rather it may be necessary to equip adherence counsellors with a menu from which they and the patients can choose the most appropriate strategies for each situation.

As in our previous cohort study, >48 h treatment interruptions remain the most common form of non-adherence in this setting, suggesting that it may be most useful for adherence interventions in India to focus on facilitating uninterrupted adherence rather than only targeting occasional missed doses. Research is needed to understand better the barriers to timely prescription refills and medication adherence during holidays, family celebrations and other events that may make uninterrupted medication consumption difficult.

The association between the VAS measure of adherence and treatment outcome is consistent with our previous research both in India¹⁵ and elsewhere.^{37,39,40} In addition, the results show that the predictive value of such standard adherence measures was improved by including data on treatment interruptions. This is consistent with our previous research, which showed that adding information on treatment interruptions improved the ability of the VAS to predict virological failure.¹⁵

The data in this paper extend our previous findings by demonstrating a significant association between adherence and antiretroviral drug resistance. However, this association is significant only when measuring adherence in the form of treatment interruptions, either alone or as part of the combined 'optimal adherence' measure. In contrast, data on past-month adherence alone was not associated with resistance in this sample. This finding further underscores the importance of developing a better understanding of the structural, interpersonal and individual barriers to long-term uninterrupted adherence so that they can be addressed in future adherence interventions. Given the lack of widely available second-line HAART in this setting, this may be the best option for minimising further drug resistance and maintaining the efficacy of first-line drugs in India and other resource-limited settings.

The frequency of RT mutations observed in this study is somewhat lower than that reported previously^{24,41,42} in India. This may be due either to geographical differences in the prevalence of resistance or to the fact that all cases in the present paper were defined by VL, whilst treatment failure was identified at either immunological or clinical failure in the prior studies. This may have allowed an accumulation of mutations as patients continued their

failing therapy while their CD4 levels declined. If so, it reinforces recommendations⁴³ to make VL testing a part of routine treatment even in resource-constrained settings. Not surprisingly, M184V was the predominant mutation in this sample and its prevalence was comparable with that described for HIV patients failing HAART⁴⁴ in other settings. This can likely be attributed to the wide use of 3TC in India in first-line regimens owing to its practicality and low toxicity. This is also in accordance with what has been reported in other studies among treated patients in India^{42,45} as well as in other studies of subtype C, which included 3TC as a NRTI backbone.^{46,47}

TAMs were the next most frequently observed NRTI mutation (48%), but again at a lesser frequency than previous reports from India.^{24,41} Most of the patients developed TAM-2 pathway mutations that confers less NRTI cross-resistance and hence might still benefit from abacavir (ABC), tenofovir (TDF) and didanosine (DDI) for their subsequent treatment. Although subtype C isolates have been reported to have a higher tendency to develop K65R, ^{48,49} in contrast we observed a lower rate of K65R (3%) compared with global reports. However, the findings here are comparable with other Indian studies, with a slightly higher rate than observed in Mumbai⁴⁵ but somewhat lower than that observed in Chennai.^{24,41}

Among the low genetic barrier NNRTI mutations, Y181C was the predominant mutation, followed by K103N, which is similar to what has been reported previously both in India^{24,41,42,45} and globally.^{50,51}

Interpretation of these findings is somewhat limited by a lack of comprehensive records of adherence since initiation of therapy, which may at least partly explain why some participants who reported optimal adherence in the past month and no treatment interruptions were still failing therapy and developing resistance mutations. Although it is possible that these patients may have been infected with drug-resistant virus, this is unlikely to account for all mutations given the low rate of transmitted resistance in India, which has ranged from 0% to 14% in previous studies.^{31,52-57} It is also likely that some of the participants may have missed occasional doses more than a month ago, which were not captured by our measures.

These results indicate that participants with higher VLs (VL $\geq$ 1000 copies/ml) were taking HAART for a significantly longer duration than those with a VL < 1000 copies/ml (30 months vs. 18 months, respectively). This is an expected finding in a setting where VL monitoring is not routine and where second-line HAART options are severely restricted. However, treatment failure is also likely to occur with longer periods of HAART exposure with increasing accumulation of minor viral populations over time.^{41,58}

In conclusion, given that only a limited number of first-line HAART regimens are available at free or greatly reduced rates in India as well as in many other resource-limited settings, prevention of the development of HIV drug resistance is critical. To accomplish this, it is necessary to understand better culturally-specific structural, interpersonal and individual factors that drive treatment interruptions and to design feasible, effective and sustainable interventions to enhance uninterrupted adherence. Until more treatment options are widely available in resource-limited settings, this is the best strategy for maximising the efficacy and durability of first-line regimens.

Acknowledgments

The authors gratefully acknowledge the assistance of the Bangalore-based Prerana study team for their dedication and the hard work to recruit and retain the study participants while providing them with a safe environment in which to discuss these often sensitive topics. The authors dedicate this paper to the participants, who gave so generously of their time and shared their stories, even when this was difficult.

Funding: This research was supported by grant R01MH067513 from the National Institute of Mental Health (NIMH) (Bethesda, MD, USA).

References

1. Bangsberg DR, Hecht FM, Charlebois ED, Zolopa AR, Holodniy M, Sheiner L, et al. Adherence to protease inhibitors, HIV-1 viral load, and development of drug resistance in an indigent population. *AIDS*. 2000; 14:357–66. [PubMed: 10770537]
2. Harrigan PR, Hogg RS, Dong WW, Yip B, Wynhoven B, Woodward J, et al. Predictors of HIV drug-resistance mutations in a large antiretroviral-naïve cohort initiating triple antiretroviral therapy. *J Infect Dis*. 2005; 191:339–47. [PubMed: 15633092]
3. King MS, Brun SC, Kempf DJ. Relationship between adherence and the development of resistance in antiretroviral-naïve, HIV-1-infected patients receiving lopinavir/ritonavir or nelfinavir. *J Infect Dis*. 2005; 191:2046–52. [PubMed: 15897990]
4. Lucas GM. Antiretroviral adherence, drug resistance, viral fitness and HIV disease progression: a tangled web is woven. *J Antimicrob Chemother*. 2005; 55:413–6. [PubMed: 15722389]
5. Parienti JJ, Massari V, Descamps D, Vabret A, Bouvet E, Larouze B, et al. Predictors of virologic failure and resistance in HIV-infected patients treated with nevirapine- or efavirenz-based antiretroviral therapy. *Clin Infect Dis*. 2004; 38:1311–6. [PubMed: 15127346]
6. Roge BT, Barford TS, Kirk O, Katzenstein TL, Obel N, Nielsen H, et al. Resistance profiles and adherence at primary virological failure in three different highly active antiretroviral therapy regimens: analysis of failure rates in a randomized study. *HIV Med*. 2004; 5:344–51. [PubMed: 15369509]
7. Sethi AK, Celentano DD, Gange SJ, Moore RD, Gallant JE. Association between adherence to antiretroviral therapy and human immunodeficiency virus drug resistance. *Clin Infect Dis*. 2003; 37:1112–8. [PubMed: 14523777]
8. Arnsten JH, Demas PA, Farzadegan H, Grant RW, Gourevitch MN, Chang CJ, et al. Antiretroviral therapy adherence and viral suppression in HIV-infected drug users: comparison of self-report and electronic monitoring. *Clin Infect Dis*. 2001; 33:1417–23. [PubMed: 11550118]
9. Bangsberg DR, Perry S, Charlebois ED, Clark RA, Roberston M, Zolopa AR, et al. Non-adherence to highly active antiretroviral therapy predicts progression to AIDS. *AIDS*. 2001; 15:1181–3. [PubMed: 11416722]
10. Gifford AL, Bormann JE, Shively MJ, Wright BC, Richman DD, Bozzette SA. Predictors of self-reported adherence and plasma HIV concentrations in patients on multidrug antiretroviral regimens. *J Acquir Immune Defic Syndr*. 2000; 23:386–95. [PubMed: 10866231]
11. Haubrich RH, Little SJ, Currier JS, Forthal DN, Kemper CA, Beall GN, et al. The value of patient-reported adherence to antiretroviral therapy in predicting virologic and immunologic response. California Collaborative Treatment Group. *AIDS*. 1999; 13:1099–107. [PubMed: 10397541]
12. Paterson DL, Swindells S, Mohr J, Brester M, Vergis EN, Squier C, et al. Adherence to protease inhibitor therapy and outcomes in patients with HIV infection. *Ann Intern Med*. 2000; 133:21–30. [PubMed: 10877736]
13. Stephenson J. AIDS researchers target poor adherence. *JAMA*. 1999; 281:1069. [PubMed: 10188643]
14. Gill CJ, Hamer DH, Simon JL, Thea DM, Sabin LL. No room for complacency about adherence to antiretroviral therapy in sub-Saharan Africa. *AIDS*. 2005; 19:1243–9. [PubMed: 16052078]
15. Ekstrand ML, Chandy S, Heylen E, Steward W, Singh G. Developing useful highly active antiretroviral therapy adherence measures for India: the Prerana study. *J Acquir Immune Defic Syndr*. 2010; 53:415–6. [PubMed: 20190588]
16. Nachega JB, Mills EJ, Schechter M. Antiretroviral therapy adherence and retention in care in middle-income and low-income countries: current status of knowledge and research priorities. *Curr Opin HIV AIDS*. 2010; 5:70–7. [PubMed: 20046150]
17. Ferradini L, Jeannin A, Pinoges L, Izopet J, Odhiambo D, Mankhambo L, et al. Scaling up of highly active antiretroviral therapy in a rural district of Malawi: an effectiveness assessment. *Lancet*. 2006; 367:1335–42. [PubMed: 16631912]

18. Bajunirwe F, Arts EJ, Tisch DJ, King CH, Debanne SM, Sethi AK. Adherence and treatment response among HIV-1-infected adults receiving antiretroviral therapy in a rural government hospital in Southwestern Uganda. *J Int Assoc Physicians AIDS Care (Chic Ill)*. 2009; 8:139–47.
19. Bell DJ, Kapita Y, Sikwese R, van Oosterhout JJ, Lalloo DG. Adherence to antiretroviral therapy in patients receiving free treatment from a government hospital in Blantyre, Malawi. *J Acquir Immune Defic Syndr*. 2007; 45:560–3. [PubMed: 17558333]
20. Ramadhani HO, Thielman NM, Landman KZ, Ndosi EM, Gao F, Kirchherr JL, et al. Predictors of incomplete adherence, virologic failure, and antiviral drug resistance among HIV-infected adults receiving antiretroviral therapy in Tanzania. *Clin Infect Dis*. 2007; 45:1492–8. [PubMed: 17990233]
21. Bloor S, Kemp SD, Hertogs K, Alcorn T, Larder BA. Patterns of HIV drug resistance in routine clinical practice: a survey of almost 12000 samples from the USA in 1999. *Antivir Ther*. 2000; 5(Suppl 3):132.
22. Calvez V, Mouroux M, Descamps D, Izopet J, Delaugerre C, Yvon A, et al. Occurrence of thymidine-associated mutations in naive patients treated more than 6 months by stavudine/lamivudine bi-therapy combination and tri-therapies including stavudine/didanosine or stavudine/lamivudine. *Antivir Ther*. 2000; 5(Suppl 3):40–1.
23. Wallis CL, Mellors JW, Venter WD, Sanne I, Stevens W. Varied patterns of HIV-1 drug resistance on failing first-line antiretroviral therapy in South Africa. *J Acquir Immune Defic Syndr*. 2010; 53:480–4. [PubMed: 19801944]
24. Vidya M, Saravanan S, Uma S, Kumarasamy N, Sunil SS, Kantor R, et al. Genotypic HIV type-1 drug resistance among patients with immunological failure to first-line antiretroviral therapy in south India. *Antivir Ther*. 2009; 14:1005–9. [PubMed: 19918105]
25. Cauldbeck MB, O'Connor C, O'Connor MB, Saunders JA, Rao B, Mallesh VG, et al. Adherence to anti-retroviral therapy among HIV patients in Bangalore, India. *AIDS Res Ther*. 2009; 6:7. [PubMed: 19400929]
26. Sarna A, Pujari S, Sengar AK, Garg R, Gupta I, Dam J. Adherence to antiretroviral therapy & its determinants amongst HIV patients in India. *Indian J Med Res*. 2008; 127:28–36. [PubMed: 18316850]
27. Shah B, Walshe L, Saple DG, Mehta SH, Ramnani JP, Kharkar RD, et al. Adherence to antiretroviral therapy and virologic suppression among HIV-infected persons receiving care in private clinics in Mumbai, India. *Clin Infect Dis*. 2007; 44:1235–44. [PubMed: 17407045]
28. Sharma M, Singh RR, Laishram P, Kumar B, Nanao H, Sharma C, et al. Access, adherence, quality and impact of ARV provision to current and ex-injecting drug users in Manipur (India): an initial assessment. *Int J Drug Policy*. 2007; 18:319–25. [PubMed: 17689381]
29. Marín, G.; Marín, BV. *Research with Hispanic populations*. Sage Publications; Newbury Park, CA: 1991.
30. Palmer S, Wiegand AP, Maldarelli F, Bazmi H, Mican JM, Polis M, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol*. 2003; 41:4531–6. [PubMed: 14532178]
31. Balakrishnan P, Kumarasamy N, Kantor R, Solomon S, Vidya S, Mayer KH, et al. HIV type 1 genotypic variation in an antiretroviral treatment-naïve population in southern India. *AIDS Res Hum Retroviruses*. 2005; 21:301–5. [PubMed: 15943572]
32. Saravanan S, Vidya M, Balakrishnan P, Kumarasamy N, Solomon SS, Solomon S, et al. Evaluation of two human immunodeficiency virus-1 genotyping systems: ViroSeq 2.0 and an in-house method. *J Virol Methods*. 2009; 159:211–6. [PubMed: 19490976]
33. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis*. 2006; 42:1608–18. [PubMed: 16652319]
34. Johnson VA, Brun-Vezinet F, Clotet B, Gunthard HF, Kuritzkes DR, Pillay D, et al. Update of the drug resistance mutations in HIV-1. *Top HIV Med*. 2008; 16:138–45. [PubMed: 19106428]
35. Giordano TP, Guzman D, Clark R, Charlebois ED, Bangsberg DR. Measuring adherence to antiretroviral therapy in a diverse population using a visual analogue scale. *HIV Clin Trials*. 2004; 5:74–9. [PubMed: 15116282]

36. Mills EJ, Nachega JB, Buchan I, Orbinski J, Attaran A, Singh S, et al. Adherence to antiretroviral therapy in sub-Saharan Africa and North America: a meta-analysis. *JAMA*. 2006; 296:679–90. [PubMed: 16896111]
37. Oyugi JH, Byakika-Tusiime J, Charlebois ED, Kityo C, Mugerwa R, Mugenyi P, et al. Multiple validated measures of adherence indicate high levels of adherence to generic HIV antiretroviral therapy in a resource-limited setting. *J Acquir Immune Defic Syndr*. 2004; 36:1100–2. [PubMed: 15247564]
38. Laurent C, Diakhate N, Gueye NF, Toure MA, Sow PS, Faye MA, et al. The Senegalese government's highly active antiretroviral therapy initiative: an 18-month follow-up study. *AIDS*. 2002; 16:1363–70. [PubMed: 12131213]
39. Oyugi JH, Byakika-Tusiime J, Ragland K, Laeyendecker O, Mugerwa R, Kityo C, et al. Treatment interruptions predict resistance in HIV-positive individuals purchasing fixed-dose combination antiretroviral therapy in Kampala, Uganda. *AIDS*. 2007; 21:965–71. [PubMed: 17457090]
40. Walsh JC, Mandalia S, Gazzard BG. Responses to a 1 month self-report on adherence to antiretroviral therapy are consistent with electronic data and virological treatment outcome. *AIDS*. 2002; 16:269–77. [PubMed: 11807312]
41. Kumarasamy N, Madhavan V, Venkatesh KK, Saravanan S, Kantor R, Balakrishnan P, et al. High frequency of clinically significant mutations after first-line generic highly active antiretroviral therapy failure: implications for second-line options in resource-limited settings. *Clin Infect Dis*. 2009; 49:306–9. [PubMed: 19522657]
42. Sen S, Tripathy SP, Patil AA, Chimanpure VM, Paranjape RS. High prevalence of human immunodeficiency virus type 1 drug resistance mutations in antiretroviral treatment-experienced patients from Pune, India. *AIDS Res Hum Retroviruses*. 2007; 23:1303–8. [PubMed: 17961120]
43. Smith DM, Schooley RT. Running with scissors: using antiretroviral therapy without monitoring viral load. *Clin Infect Dis*. 2008; 46:1598–600. [PubMed: 18419496]
44. Wainberg MA. Increased fidelity of drug-selected M184V mutated HIV-1 reverse transcriptase as the basis for the effectiveness of 3TC in HIV clinical trials. *Leukemia*. 1997; 11(Suppl 3):85–8. [PubMed: 9209307]
45. Deshpande A, Jauvin V, Magnin N, Pinson P, Faure M, Masquelier B, et al. Resistance mutations in subtype C HIV type 1 isolates from Indian patients of Mumbai receiving NRTIs plus NNRTIs and experiencing a treatment failure: resistance to AR. *AIDS Res Hum Retroviruses*. 2007; 23:335–40. [PubMed: 17331042]
46. Grossman Z, Vardinon N, Chemtob D, Alkan ML, Bentwich Z, Burke M, et al. Genotypic variation of HIV-1 reverse transcriptase and protease: comparative analysis of clade C and clade B. *AIDS*. 2001; 15:1453–60. [PubMed: 11504976]
47. Kantor R, Zijenah LS, Shafer RW, Mutetwa S, Johnston E, Lloyd R, et al. HIV-1 subtype C reverse transcriptase and protease genotypes in Zimbabwean patients failing antiretroviral therapy. *AIDS Res Hum Retroviruses*. 2002; 18:1407–13. [PubMed: 12512512]
48. Doualla-Bell F, Avalos A, Brenner B, Gaolathe T, Mine M, Gaseitsiwe S, et al. High prevalence of the K65R mutation in human immunodeficiency virus type 1 subtype C isolates from infected patients in Botswana treated with didanosine-based regimens. *Antimicrob Agents Chemother*. 2006; 50:4182–5. [PubMed: 17015626]
49. Trotta MP, Bonfigli S, Ceccherini-Silberstein F, Bellagamba R, D'Arrigo R, Soldani F, et al. Clinical and genotypic correlates of mutation K65R in HIV-infected patients failing regimens not including tenofovir. *J Med Virol*. 2006; 78:535–41. [PubMed: 16555278]
50. Hanna GJ, Johnson VA, Kuritzkes DR, Richman DD, Brown AJ, Savara AV, et al. Patterns of resistance mutations selected by treatment of human immunodeficiency virus type 1 infection with zidovudine, didanosine, and nevirapine. *J Infect Dis*. 2000; 181:904–11. [PubMed: 10720511]
51. Wallis, C.; Bell, C.; Boulme, R.; Sanne, I.; Venter, F.; Papathanasopoulos, M., et al. Emerging ART drug resistance in subtype C: experience from the 2 clinics in Johannesburg, South Africa; 14th Conference on Retroviruses and Opportunistic Infections; Los Angeles, CA. Alexandria, VA. 25–28 February 2007; 2007. Conference on Retroviruses and Opportunistic Infections
52. Deshpande A, Recordon-Pinson P, Deshmukh R, Faure M, Jauvin V, Garrigue I, et al. Molecular characterization of HIV type 1 isolates from untreated patients of Mumbai (Bombay), India, and

- detection of rare resistance mutations. *AIDS Res Hum Retroviruses*. 2004; 20:1032–5. [PubMed: 15585093]
53. Eshleman SH, Hudelson SE, Gupta A, Bollinger R, Divekar AD, Gangakhedkar RR, et al. Limited evolution in the HIV type 1 pol region among acute seroconverters in Pune, India. *AIDS Res Hum Retroviruses*. 2005; 21:93–7. [PubMed: 15665648]
54. Iqbal HS, Solomon SS, Madhavan V, Solomon S, Balakrishnan P. Primary HIV-1 drug resistance and polymorphic patterns among injecting drug users (IDUs) in Chennai, Southern India. *J Int Assoc Physicians AIDS Care (Chic Ill)*. 2009; 8:323–7.
55. Kandathil AJ, Kannangai R, Abraham OC, Rupali P, Pulimood SA, Verghese VP, et al. The frequency of HIV-I drug resistance mutations among treatment-naïve individuals at a tertiary care centre in south India. *Int J STD AIDS*. 2009; 20:522–6. [PubMed: 19625581]
56. Lall M, Gupta RM, Sen S, Kapila K, Tripathy SP, Paranjape RS. Profile of primary resistance in HIV-1-infected treatment-naïve individuals from Western India. *AIDS Res Hum Retroviruses*. 2008; 24:987–90. [PubMed: 18593351]
57. Neogi U, Prarthana BS, Gupta S, D'Souza G, De Costa A, Kuttiatt VS, et al. Naturally occurring polymorphisms and primary drug resistance profile among antiretroviral-naïve individuals in Bangalore, India. *AIDS Res Hum Retroviruses*. 2010; 26:1097–101. [PubMed: 20836706]
58. Hong S, Cao J, Tu YT. Evolution of HIV-1 in a patient population failing multiple-drug therapy. *Microbiol Immunol*. 2009; 53:535–9. [PubMed: 19703248]

Table 1Demographic characteristics and medical history of study participants ($n = 551$)

Characteristic	VL < 1000 copies/ml ($n = 450$)	VL $\geq$ 1000 copies/ml ($n = 101$)
Male	68% (307)	67% (68)
Age (years) [mean (range)]	37 (18–70)	40 (22–75) **
Marital status		
Married	72% (322)	64% (65)
Never married	11% (50)	8% (8)
Widowed/divorced/separated	17% (78)	28% (28) *
Education		
<10 years	46% (206)	36% (36)
10 years	27% (122)	34% (34)
>10 years	27% (122)	31% (31)
Residency		
Bangalore City	66% (299)	60% (61)
Karnataka (outside Bangalore)	24% (109)	27% (27)
Other	9% (42)	13% (13)
Months on HAART [mean (range)]	18 (1–52)	30 (1–175) ***
Months on current regimen [mean (range)] ^b	12 (0–47)	15 (0–80)
Current HAART regimen		
3TC + d4T + NVP	35% (158)	26% (26)
3TC + AZT + NVP	51% (228)	49% (49)
Other NVP-based	0.2% (1)	2% (2) *
3TC + d4T + EFV	6% (29)	4% (4)
3TC + AZT + EFV	7% (32)	10% (10)
FTC + TDF + EFV	0.4% (2)	6% (6) ***
AZT monotherapy	0% (0)	1% (1) *
PI-based regimen	0% (0)	3% (3) ***
CD4 cell count (cells/mm ³) [median (IQR)] ^c	348 (222–476)	218 (103–353) ***

VL: viral load; HAART: highly active antiretroviral therapy; 3TC: lamivudine; d4T: stavudine; NVP: nevirapine; AZT: zidovudine; EFV: efavirenz; FTC: emtricitabine; TDF: tenofovir; PI: protease inhibitor; IQR: interquartile range.

^aData are % (n) unless otherwise stated.

^bData missing for 2 people ($n = 549$).

^cData missing for 1 person ($n = 550$).

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$.

Table 2Genotypic mutational patterns among patients failing first-line therapy ($n = 92$)

Mutation	% (n)
NRTI-associated mutations	
≥1 NRTI mutation	68% (63)
Non-TAMs	
M184V/MV/I/IM	65% (60)
E44D/DE/A/K	9% (8)
L74V	4% (4)
V75M	4% (4)
V118I	4% (4)
T69D/DN	3% (3)
K65R	3% (3)
Q151M	2% (2)
TAMs	
TAM-1 pathway	
T215Y	21% (19)
M41L/LM	25% (23)
L210W	3% (3)
TAM-2 pathway	
T215F	12% (11)
D67N/DN	27% (25)
K70R/KR/E	22% (20)
K219E/Q	13% (12)
Patients with TAMs	48% (44)
Patients with TAMs + K65R	1% (1)
NNRTI-associated mutations	
≥1 NNRTI mutation	72% (66)
≥ 3 NNRTI mutations	23% (21)
Y181C/CY/I/V	37% (34)
K103N/KN/R	26% (24)
K101E/EK/Q/KQ	14% (13)
G190A/AG	18% (17)
V108I	15% (14)
A98G	12% (11)
V106M/MV	8% (7)
V90I	4% (4)
Y188L	4% (4)
E138K/EK	3% (3)
Patients with ≥2 NNRTI/NRTI mutations	67% (62)
Patients without NRTI and NNRTI mutations	24% (22)

NRTI: nucleoside reverse transcriptase inhibitor; TAM: thymidine analogue mutation; NNRTI: non-nucleoside reverse transcriptase inhibitor.

Table 3

Association between adherence, virological failure and drug resistance

Adherence	Outcome of interest			
	Detectable VL ^a		Any resistance-associated mutation ^b	
	%	OR (95% CI)	%	OR (95% CI)
Treatment interruptions				
Yes	43 ***	3.1 (2.0–4.9)	86 *	2.8 (1.0–8.6)
No	19		68	
Past-month adherence				
<95%	62 ***	5.9 (2.9–12.5)	84	1.9 (0.5–8.7)
≥95%	21		74	
Suboptimal adherence ^c				
Yes	42 ***	3.2 (2.1–4.9)	87 **	3.6 (1.3–10.9)
No	19		65	

VL: viral load; OR: odds ratio.

^aDetermined for the total sample (*n* = 551)^bDetermined for the sample who underwent viral genotyping (*n* = 92).^cSuboptimal adherence is <95% adherence or treatment interruption.*
P ≤ 0.05**
P < 0.02***
P < 0.001.