Published in final edited form as:

J Acquir Immune Defic Syndr. 2011 April ; 56(4): 333–339. doi:10.1097/QAI.0b013e31820dc0cc.

Low lopinavir plasma or hair concentrations explain second line protease inhibitor failures in a resource-limited setting

Gert U Van Zyl, MBChB, FCPATH(SA) Virol^{*,1}, Thijs E Van Mens², Helen McIlleron, MBChB, PhD³, Michele Zeier, MBChB⁴, Jean B. Nachega, MD, PhD^{4,5}, Eric Decloedt, MBChB³, Carolina Malavazzi, MD⁶, Peter Smith, PhD³, Yong Huang, PhD⁷, Lize Van Der Merwe, PhD^{8,9}, Monica Gandhi, MD, MPH¹⁰, and Gary Maartens, MBChB, FCP, DTM&H³

¹Division of Medical Virology, NHLS Tygerberg and Stellenbosch University, South Africa

²Amsterdam Medical Centre, University of Amsterdam, The Netherlands ³Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, South Africa ⁴Centre for Infectious Diseases and Division of Infectious Diseases, Department of Medicine, Tygerberg Academic Hospital and Stellenbosch University, South Africa ⁵Johns Hopkins Bloomberg School of Public Health, Departments of International Health and Epidemiology, Baltimore, Maryland, USA ⁶Medicins Sans Frontieres, Cape Town, South Africa ⁷Drug Studies Unit, University of California, San Francisco, USA ⁸Biostatistics Unit, Medical Research Council of South Africa, Cape Town, South Africa ⁹Department of Biostatistics, University of Western Cape, Cape Town, South Africa ¹⁰Division of Infectious Disease, University of California, San Francisco, USA

Abstract

Background—In resource-limited settings, many patients, with no prior PI treatment on a second-line, high genetic barrier, ritonavir boosted protease inhibitor (PI) containing regimen have virologic failure.

Methods—We conducted a cross-sectional survey to investigate the aetiology of virologic failure in two public health antiretroviral clinics in South Africa documenting the prevalence of virologic failure (HIV RNA load > 500 copies/ml) and genotypic antiretroviral resistance; and lopinavir hair and plasma concentrations in a nested case-control study.

Results—Ninety three patients treated with a second-line regimen including lopinavir boosted with ritonavir were included, of whom 50 (25 cases, with virologic failure and 25 controls) were included in a nested case control study. Of 93 patients 37(40%) had virological failure, only 2 of which had had major protease inhibitor mutations. The negative predictive values: probability of failure with lopinavir plasma concentration > 1µg/mL or hair concentrations > 3.63ng/mg for virologic failure were 86% and 89%, and positive predictive values of low concentrations 73% and 79%, respectively, whereas all virologic failures with HIV RNA loads above 1000 copies/ml, of patients without protease inhibitor resistance, could be explained by either having a low lopinavir concentration in plasma or hair.

Corresponding author: Gert U. van Zyl, Division of Medical Virology, NHLS Tygerberg and Stellenbosch University, P.O. Box 19063, Tygerberg 7505, South Africa, Telephone: +27219389691, Fax: +27219389361, guvz@sun.ac.za.

Authors contributions: GvZ initiated the investigation. TvM conducted patient recruitment and performed data collection; GM, HM, JBN and MZ provided advice on study design, analytical methods and interpretation of the data; ED provided methods and practical advice on hair collection; MZ and CM organised patient recruitment at the Tygerberg and Khayelitsha sites, respectively and CM provided data on the Khayelitsha patients; GvZ wrote the manuscript draft with the help of GM, JBN, HM and MG; LvdM provided statistical help and advice; MG and YH conducted and developed the hair lopinavir and ritonavir analysis and described of the method and PS performed and validated the plasma lopinavir analysis. All authors approved the manuscript.

Conclusions—Most patients who fail a LPV/r regimen, in our setting, have poor lopinavir exposure. A threshold plasma lopinavir concentration (indicating recent LPV/r use) and/or hair concentration (indicating longer term lopinavir exposure) are valuable in determining the aetiology of virologic failure and identifying patients in need of adherence counselling or resistance testing.

Keywords

Lopinavir; medication adherence; resource-limited settings; protease inhibitor resistance mutations; plasma concentration; hair concentration

Introduction

Adult HIV infected patients in resource-limited settings who qualify for antiretroviral therapy (ART) are started on a first-line non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimen^{1, 2}. Lamivudine, a nucleoside reverse transcriptase inhibitor (NRTI) commonly used in first-line regimens, and NNRTIs both have low genetic barriers to resistance – hence a single mutation confers a high level of resistance³⁻⁵. Therefore, if patients do not have adequate adherence to this first-line regimen, antiretroviral resistance can arise rapidly, resulting in virologic failure⁶ and necessitating a switch to second-line therapy agents. The recommended second-line ART regimen in resource-limited settings is one composed of dual NRTIs and a ritonavir (RTV) boosted protease inhibitor, typically Lopinavir/ritonavir (LPV/r)^{2, 7}.

LPV/r has a high genetic barrier to resistance⁸. However both LPV/r and didanosine, which until recently was one of the NRTIs recommended in combination second-line regimens, are commonly associated with gastrointestinal side effects,^{9, 10} which can limit this regimen's tolerability.

The prevalence of failure in patients on a second-line regimen has been reported to be as high as 33% in South African patients on LPV/r-based regimens¹¹. Unlike virological failure on an NNRTI regimen very few patients who fail a LPV/r-based regimen have resistance to LPV/r, provided they have not had prior exposure to protease inhibitors¹¹⁻¹³. This suggests that most second-line virologic failures are due to inadequate adherence rather than viral resistance¹³. Since the appropriate management of patients, failing second-line therapy depends on the underlying aetiology of failure, it is important to distinguish inadequate drug exposure (likely due to adherence lapses) from antiretroviral resistance. The identification of patients with poor drug exposure can limit unnecessary genotypic antiretroviral resistance testing (GART), which is costly, enabling GART to be reserved for those who fail despite adequate drug exposure. This selective use of GART could aid in the choice of the next optimal regimen, either through using currently available drugs, or by guiding the choice of third-line regimen agents, once newer antiretrovirals (ARVs) become accessible in resource-limited settings.

Self-report or pill counts are the adherence measurements used most often by ART programmes in resource-limited settings, but both measures tend to overestimate adherence¹⁴. Measuring drug concentrations or therapeutic drug monitoring, has the potential to provide an objective estimate of adherence. Trough or random lopinavir plasma concentrations reflect recent adherence as the plasma half-life of lopinavir (LPV) used in a LPV/r formulation is 5-6 hours, whereas LPV concentrations in hair specimens estimates longer term exposure. Recently, hair specimens have been used to measure antiretroviral drug exposure, using techniques of liquid chromatography coupled with tandem mass

spectrometry¹⁵, and protease inhibitor concentrations in hair have been shown to strongly correlate with antiretroviral success¹⁶.

Methods

We conducted a cross sectional study of virologic failure in adult patients on a second-line LPV/r containing regimen. Patients were consecutively enrolled at Tygerberg Hospital Family Clinic and Ubunthu Clinic in Khayelitsha, Cape Town. Adult patients on a second-line LPV/r containing regimen, for at least one month, who gave informed consent, were eligible for inclusion into the study. Basic demographic information, blood specimens for HIV-1 RNA load and LPV plasma concentrations, and hair specimens for lopinavir and ritonavir measurement were collected. Genotypic resistance testing was done in patients with HIV-1 RNA loads > 500 copies/ml. Genotypic resistance testing was funded as part of a larger descriptive study and was performed at the Tygerberg Laboratory. However as we had limited funding available and hair analysis was only available at a remote laboratory in San Francisco, California, we chose a nested case control design as the most efficient strategy to evaluate the value of hair and plasma lopinavir concentrations. The recruitment target was 25 cases and 25 controls. Patients were included in the nested case-control study, based on having an appropriate matched control, matched by age and gender in the clinic. Patients were individually matched for gender and paired with the closest available age match; with a window of not more than 5 years we could include 19 patients only; another 6 patients were included on the basis of a age difference no more than 8 years. Patients were only excluded from the case-control study on the basis of not having a suitable match or having insufficient specimens for testing.

Patients whose hair was too short to cut using two fingertips to hold it were excluded from hair analysis. The study was approved by the Stellenbosch University Committee for Human Research.

Hair was collected from the occipital area, as it has the lowest variability in hair growth rate^{17, 18}. At least 25 strands (3-4 mm tuft) of hair was cut as close to the scalp as possible. The distal end was clearly marked with tape since the proximal part of the specimen is expected to yield concentrations most reflective of recent exposure. The hair specimens were wrapped in aluminum foil to avoid excessive light exposure and stored at room temperature in a plastic bag with desiccant until analysis. For any particular patient all specimens (HIV viral load, genotypic resistance test, hair LPV concentration and plasma LPV concentration) were collected concurrently at a single clinic visit.

Laboratory methods

For viral load testing we used the NucliSENS EasyQ HIV-1 version 2.0 (bioMérieux Boxtel, Netherlands). For the purpose of this study we defined virologic failure as having a viral load > 500 HIV-1 RNA copies/ml plasma since this is the sensitivity threshold for GART to be performed using the in-house assay. Antiretroviral resistance testing was done by an in-house validated genotypic PCR and sequencing method, which has been described in prior publications^{19, 20}.

Lopinavir (LPV) plasma concentration was measured by tandem liquid chromatography-mass spectrometry (University of Cape Town, Clinical Pharmacology laboratory). As it was logistically not possible to collect trough specimens for LPV concentration measurement, a random specimen was collected, along with the time of the last dose.

Hair LPV and ritonavir concentrations were measured by liquid chromatography and tandem mass spectroscopy (LC/MS/MS) at the University of California, San Francisco using a

modified method^{15, 16}. The LPV and RTV in each hair specimen was extracted with MeOH/FTA(9/1), then analysed by the API-5000 LC/MS/MS machine. Approximately 2mg of proximal hair was analysed. The other conditions were identical to the published methods paper by the same group¹⁵.

Statistics

The statistical analysis was performed using STATA version 10.0. For comparison of the medians of matched data, the Wilcoxon signed rank test was used and for categorical data Fisher's exact test was used. To describe the association between LPV plasma or hair concentrations and failure we used conditional logistic regression to calculate odds ratios and 95% confidence intervals. We selected LPV hair and plasma cut-off concentrations with receiver operating characteristics, choosing cut-offs that maximised the sum of sensitivity and specificity. When investigating the number of cases and controls categorised according to a cut-off LPV plasma or hair concentration we defined a LPV concentration above the cut-off, which indicates adequate therapy exposure, as 'negative' and a concentration below this cut-off, which would be expected to predict virologic failure, as 'positive'.

Results

From the first study site, Tygerberg Hospital Family Clinic, we identified 99 patients on a LPV/r second-line regimen in the clinic database, of whom we were able to ask 77 patients to join the study; of them 70 were eligible and agreed to participate. From the second study site, Ubunthu Clinic, Khayelitsha, we recruited patients attending two sequential clinic visits dedicated to patients failing a second-line LPV/r regimen; 25 patients were asked to join and all 24 were included as they were eligible and agreed to participate.

In total 94 patients were thus included and specimens, demographic- and clinical information were collected. In one patient viral load testing failed and was thus excluded. Thirty seven out of the 93 patients (40%; 95% Confidence Interval: 30-50%) had virologic failure, defined as having an HIV-1 RNA load of > 500 copies per ml. Seven patients were taking rifampicin-containing anti-tuberculosis therapy, 5 of these patients had virologic failure, 2 of whom were not using appropriately adjusted doses of LPV/r. Although more patients on concurrent treatment for tuberculosis failed than those not on TB therapy, this difference was not statistically significant (Fisher's exact test: $p=0.11$). Resistance testing was unsuccessful in 4 of the 37 patients with virologic failure. Of the 33 patients who had resistance tests, 28 patients had no resistance to any of the antiretroviral drugs in their current regimen and 5 patients had resistance mutations to the current regimen. Only two patients had major PI resistance mutations. Both patients also had NRTI mutations (see Table 1).

Of the 93 patients, studied, 50 were included in the nested case-control study: 25 cases (with virologic failure) and 25 controls. For 19 matched pairs an age match with an age difference between 1-5 years were available, another 6 pairs were included who had an age difference of 6-8 years. The group ages were well-matched as the mean age difference between the case and control groups was 0.3 years. The results of the cases and control patients are summarised in Table 2. LPV plasma concentrations, LPV hair concentrations and RTV hair concentrations were lower in patients with virological failure compared to controls. Patients with virologic failure also had a shorter duration of therapy and significantly lower concurrent CD4 counts than controls (Table 2).

Although we recruited 25 cases and controls, hair specimens were available in 19 cases and 19 controls only, as many patients had shaven their hair too short (Table 2). LPV plasma concentrations and LPV hair and RTV hair concentrations were compared in patients with or

without virological failure. There was a clear separation of LPV hair and plasma concentrations between patients with or without virologic failure. However RTV hair concentrations (Table 2) were not clearly separated between those with and without virologic failure. The odds ratio and 95% confidence interval (95% CI) of virological failure for each 5 µg/ml decrease in LPV plasma concentration was 2.1 (95% CI: 1.2-3.6) and 71.9 (95% CI: 0.6-8518.2) for each 5 ng/mg decrease in LPV hair concentration. In post-hoc analysis the inclusion of hair LPV concentrations, in addition to plasma LPV concentrations provided a better model – as evident from a lower score according to Akaike's information criterion (AIC) of 11.5 versus 26.4. Receiver operating characteristic optimal cut-offs (that maximised the sum of sensitivity and specificity for predicting failure) were a LPV plasma concentration below 1 µg/ml and hair LPV concentration below 3.63 ng/mg (see table 3). These LPV plasma and LPV hair concentrations had respective calculated negative predictive values (NPV) of 86% and 89%, and calculated positive predictive values (PPV) of 73% and 79% when the prevalence of virologic failure is 40%. A scatterplot of lopinavir hair and plasma concentrations is shown in Figure 1. Thirty one percent patients had viral loads > 1000 copies, at this prevalence and viral load threshold, the calculated NPV for LPV plasma and hair concentrations were 92% and 96%, respectively. LPV plasma concentrations were not affected by timing of the blood specimens as there was no association with taking the last LPV/r dose the previous day or the same morning and having a concentration below or above the cut-off (Fisher's exact test: $p=1.0$). Amongst patients with virological failure, 6 had LPV plasma concentrations above the cut-off (that is false negative LPV concentrations – see Table 3). Three of the 6 patients with high LPV plasma concentrations had low LPV hair concentrations, suggesting a mechanism for failure (good short-term exposure, but poor long-term exposure). Two others had viral loads below 1000 copies/ml and could be excluded from the false negatives as classified by LPV plasma or hair concentrations by changing the viral load threshold to 1000 copies (see Table 3). The only remaining patient with failure despite high LPV plasma concentrations, number 99, had significant PI and NRTI resistance (see Table 3 and Table 1). Also at a viral load threshold of 1000 copies/ml the only remaining patient with failure despite a high LPV-hair concentration (Table 3) had a lopinavir plasma concentration below the limit of detection – suggesting omission of recent doses as an explanation of failure. Of the case-control group, the only patients with concurrent rifampicin therapy for tuberculosis were cases whose LPV plasma and hair concentrations were both below the respective cut-offs.

Discussion

We observed a very high rate of virologic failure (40%) in patients on a second-line LPV/r-based regimen in our setting, which was even higher than another study from South Africa that reported a failure a prevalence of 33%¹¹. Virologic failure increases the risk of disease progression and death²¹. Poor drug exposure, most likely due to inadequate adherence, was the major cause of antiretroviral failure in our study. However, two of the 33 patients who had resistance tests had major protease inhibitor resistance mutations, despite the fact that this was their first protease inhibitor (PI) exposure. The two patients with major protease inhibitor mutations also had NRTI mutations. As expected, the CD4 count was lower in patients with virologic failure compared to controls. Patients with virologic failure had a shorter history of LPV/r therapy than controls, this could however not be explained by insufficient time to allow suppression as the shortest second-line therapy duration was 2 months and the interquartile range was 9-21.5 months. As there are currently no third-line options available in most resource-limited settings, patients are often retained on the second-line regimen irrespective of having virological failure. PI resistance mutations in patients on a boosted PI regimen usually occur at a low rate with moderate levels of adherence²². Therefore although the rate of acquisition of PI resistance mutations is low, it is likely that the number of patients with significant PI resistance will accumulate over time in resource-

limited settings, resulting in a growing number of patients needing a third-line regimen. The use of better tolerated ARVs, as is recommended in the current updated World Health Organization's guidelines ⁷, may improve adherence and therefore decrease the proportion of failures.

LPV plasma and hair concentrations and RTV hair concentrations were all significantly lower in patients with virological failure relative to controls. We found cut-off LPV drug concentrations for plasma and hair that could discriminate between failure and non-failure. Using a viral load failure criterion of 1000 copies/ml, a LPV plasma concentration $< 1 \mu\text{g/ml}$ and LPV hair concentration of $< 3.63 \text{ ng/mg}$ had negative predictive values for virologic failure of 92% and 96%, respectively. In a further exploration of the false negative cases (patients with either a high LPV plasma concentration or hair concentration, whilst having virologic failure), all cases except one could be explained by having poor drug exposure, as was evident from either having a low LPV plasma or hair concentration, the remaining case of failure had major protease inhibitor and NRTI resistance.

We therefore propose a diagnostic algorithm (Figure 2) that includes viral load measurement, random LPV plasma and hair concentration measurement to identify patients who are likely to have virologic failure due to genotypic resistance and not poor adherence. At the outset of the study we defined virologic failure as > 500 copies per ml as this allowed us to do resistance testing. However a criterion of sustained viral load above 1000 copies/ml is used to define failure in the South African public sector, and although a viral load between 50-1000 copies/ml would warrant clinical attention, such as adherence counselling, it would not indicate the need for a therapy change, in patients on a high genetic barrier second-line regimen. At a viral load threshold of 1000 copies/ml, a random LPV plasma concentration $< 1 \text{ mg/ml}$ or hair concentration $< 3.63 \text{ ng/mg}$ due to their excellent negative predictive value could exclude patients with poor drug exposure from unnecessary, costly antiretroviral resistance testing. Indeed, patients with a LPV or hair concentration below the threshold can be targeted for adherence interventions with repeat viral load testing at a follow-up visit. Although GART could be performed at HIV-1 RNA loads of below 1000 copies, commercial assays are only licensed to detect resistance above this threshold and the intensive investigation of patients, treated with such a high genetic-barrier regimen with low-level viraemia may not be cost effective. In the algorithm we suggest only analyzing LPV concentrations in hair and plasma in patients with viral loads above 1000 copies/ml. The proposed algorithm will be less costly than GART for patients failing second-line regimens as the cost of a plasma LPV concentration measurement is about 27 US\$ and hair measurement 40 US\$, whereas GART typically cost from 250 to 530 US\$ in the South African setting. The benefit of using a random plasma concentration and a hair concentration, in combination, is that a low plasma concentration would indicate *recent* poor adherence and a low hair concentration, measured in a 1 cm hair specimen, *average* poor adherence over a period of about one month, as hair grows at about 1-1.5 cm per month, even when the recent adherence as indicated by a high plasma LPV concentration may be adequate (but too short to suppress viral replication). These tests therefore could also provide some insight in the adherence patterns of patients. A low *random* plasma concentration, in the absence of trough measurements, proved to be informative as most patients with virologic failure had very low random plasma LPV concentrations.

Our study was explorative with a few limitations: 1) the cross-sectional design did not enable us to longitudinally monitor the effect of lopinavir exposure on virologic failure; 2) due to logistical reasons we measured random LPV concentrations rather than trough concentrations, which could have been more accurate; 3) our study was limited by small sample size; 4) a final limitation is that stable patients are referred out to primary clinics

which could result in a selection bias with a higher LPV failure rate in the study population compared to other settings, as problematic patients are often retained in care.

Conclusion

We found a very high prevalence of virologic failure amongst adult patients in our South African setting on a LPV/r based regimen. The majority failed due to poor drug exposure, most likely related to poor adherence, as was evident from either a low lopinavir plasma or hair concentration. Therefore the use of plasma and hair LPV concentrations could be valuable in diagnosing the cause of virologic failure and allow targeted GART only in those patients where failure is not explained by poor drug exposure.

Acknowledgments

Funding: The South African Department of Health for funding through a Comprehensive Care and Management and Treatment Plan (CCMT) Grant. JBN is supported by The United States National Institutes for Allergy and Infectious Disease (NIAID/NIH), Division of AIDS (DAIDS): K23 AI 068582-01; The European Developing Countries Clinical Trial Partnership (EDCTP) Senior Fellowship Award: TA-08-40200-021 and the Wellcome Trust Southern Africa Consortium for Research Excellence (SACORE): WT087537MA. GM is supported by the National Research Foundation, South Africa.

Other acknowledgements: Dr Hans Prozesky, Department Medicine, Stellenbosch University and Tygerberg Academic Hospital who manages the Tygerberg Infectious Diseases Clinic Database as part of a PEPFAR funded project; Dr Jantjie Taljaard, Department Medicine, Stellenbosch University and Tygerberg Academic Hospital for supporting the study at the Infectious Diseases Clinic; Dr Shaheed Matthee, Ubunthu Clinic Khayelitsha and Department of Health, Western Cape Province, and Dr Gilles van Cutsem, Ubunthu Clinic Khayelitsha and Mediciens Sans Frontieres, South Africa for supporting the study

Dr Ruth Greenblatt, MD. Division of Infectious Disease, University of California, San Francisco, USA for involvement in development of the hair drug analysis method.

Prof Wolfgang Preisner, MD, PhD for advice on manuscript preparation.

References

1. World Health Organization. Scaling up antiretroviral therapy in resource-limited settings: Guidelines for a public health approach. WHO; Geneva: 2002.
2. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach, 2006 revision. WHO; Geneva: 2006.
3. Quiros-Roldan E, Airoldi M, Moretti F, et al. Genotype resistance profiles in patients failing an NNRTI-containing regimen, and modifications after stopping NNRTI therapy. *J Clin Lab Anal*. 2002; 16(2):76–78. [PubMed: 11948795]
4. Delaugerre C, Rohban R, Simon A, et al. Resistance profile and cross-resistance of HIV-1 among patients failing a non-nucleoside reverse transcriptase inhibitor-containing regimen. *J Med Virol*. Nov; 2001 65(3):445–448. [PubMed: 11596076]
5. Frost SD, Nijhuis M, Schuurman R, Boucher CA, Brown AJ. Evolution of lamivudine resistance in human immunodeficiency virus type 1-infected individuals: the relative roles of drift and selection. *J Virol*. Jul; 2000 74(14):6262–6268. [PubMed: 10864635]
6. Gardner EM, Sharma S, Peng G, et al. Differential adherence to combination antiretroviral therapy is associated with virological failure with resistance. *AIDS*. Jan 2; 2008 22(1):75–82. [PubMed: 18090394]
7. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: Recommendations for a public health approach, 2010 revision. WHO; Geneva: 2010.
8. Mo H, King MS, King K, Molla A, Brun S, Kempf DJ. Selection of resistance in protease inhibitor-experienced, human immunodeficiency virus type 1-infected subjects failing lopinavir- and ritonavir-based therapy: mutation patterns and baseline correlates. *J Virol*. Mar; 2005 79(6):3329–3338. [PubMed: 15731227]

9. Gathe J, da Silva BA, Cohen DE, et al. A once-daily lopinavir/ritonavir-based regimen is noninferior to twice-daily dosing and results in similar safety and tolerability in antiretroviral-naïve subjects through 48 weeks. *J Acquir Immune Defic Syndr*. Apr 15; 2009 50(5):474–481. [PubMed: 19225400]
10. Barreiro P, Corbaton A, Nunez M, Gonzalez-Lahoz J, Soriano V. Tolerance of didanosine as enteric-coated capsules versus buffered tablets. *AIDS Patient Care STDS*. Jun; 2004 18(6):329–331. [PubMed: 15294082]
11. El-Khatib Z, Ekstrom AM, Ledwaba J, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. *AIDS*. Jul 17; 24(11):1679–1687. [PubMed: 20453629]
12. Arribas JR, Delgado R, Arranz A, et al. Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and 2 nucleosides for maintenance therapy of HIV: 96-week analysis. *J Acquir Immune Defic Syndr*. Jun 1; 2009 51(2):147–152. [PubMed: 19349870]
13. Riddler SA, Haubrich R, DiRienzo AG, et al. Class-sparing regimens for initial treatment of HIV-1 infection. *N Engl J Med*. May 15; 2008 358(20):2095–2106. [PubMed: 18480202]
14. Berg KM, Arnsten JH. Practical and conceptual challenges in measuring antiretroviral adherence. *J Acquir Immune Defic Syndr*. Dec 1.2006 43 1:S79–87. [PubMed: 17133207]
15. Huang Y, Gandhi M, Greenblatt RM, Gee W, Lin ET, Messenkoff N. Sensitive analysis of anti-HIV drugs, efavirenz, lopinavir and ritonavir, in human hair by liquid chromatography coupled with tandem mass spectrometry. *Rapid Commun Mass Spectrom*. Nov; 2008 22(21):3401–3409. [PubMed: 18837069]
16. Gandhi M, Ameli N, Bacchetti P, et al. Protease inhibitor levels in hair strongly predict virologic response to treatment. *AIDS*. Feb 20; 2009 23(4):471–478. [PubMed: 19165084]
17. Cassani M, Da Re N, Giuliani L, Sesana F. Experience with hair testing in the clinical biochemistry laboratory of Ca' Granda Niguarda Hospital, Milan, Italy. *Forensic Sci Int*. Jan 17; 1997 84(1-3):17–24. [PubMed: 9042706]
18. Kosuge K, Uematsu T, Araki SI, Matsuno H, Ohashi K, Nakashima M. Comparative dispositions of ofloxacin in human head, axillary, and pubic hairs. *Antimicrob Agents Chemother*. May; 1998 42(5):1298–1302. [PubMed: 9593174]
19. van Zyl GU, Claassen M, Engelbrecht S, et al. Zidovudine with nevirapine for the prevention of HIV mother-to-child transmission reduces nevirapine resistance in mothers from the Western Cape, South Africa. *J Med Virol*. Jun; 2008 80(6):942–946. [PubMed: 18428139]
20. van Zyl GU, van der Merwe L, Claassen M, et al. Protease Inhibitor Resistance in South African Children With Virologic Failure. *Pediatr Infect Dis J*. Sep 24.2009
21. Pujades-Rodriguez M, Balkan S, Arnould L, Brinkhof MA, Calmy A. Treatment failure and mortality factors in patients receiving second-line HIV therapy in resource-limited countries. *JAMA*. Jul 21; 304(3):303–312. [PubMed: 20639564]
22. Bangsberg DR, Moss AR, Deeks SG. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. *J Antimicrob Chemother*. May; 2004 53(5):696–699. [PubMed: 15044425]

Figure 1. Scatterplot of Lopinavir hair (ng/mg) and plasma concentrations ($\mu\text{g/mL}$) in patients with virologic failure (triangles) and non-failure patients (open circles). The dashed lines indicate the respective concentration cut-offs: LPV plasma concentration of 1 $\mu\text{g/mL}$ and LPV hair concentration of 3.63 ng/mg.

Figure 2.

Proposed algorithm to investigate the cause of virologic failure of a Lopinavir boosted with low dose ritonavir (LPV/r) containing second-line regimen in resource-limited settings. LPV concentrations in plasma and hair will only be analysed when the viral load is >1000. This would allow the differentiation of patients who are failing due to poor adherence versus probable genotypic resistance.

*: Asterisk indicating the tests that are done at the ‘viral load’ visit.

Table 1
Patients with clinically significant resistance mutations to current regimen

Patient number	PI mutations	NRTI mutations	Plasma HIV-RNA load	LPV – plasma	LPV-hair
99	I54V, V82A	M184V	13 000	10.3	NAV
32	L10F, L23F, M46I, I54V, T74S, V82A	D67N, T69N, K70R, M184V, T215F, K219Q	5 500	NAV	NAV
12	T74S	D67N	940	BLD	0.97
74	T74S	D67N, T69N	3700	NAV	NAV
109	None	M41L, M184V T215FIST	35 000	BLD	NAV

Footnote:

Plasma HIV RNA load: copies per ml
 LPV - plasma: LPV-plasma concentration in µg/ml
 LPV-hair: LPV hair concentration in ng/mg hair
 BLD: Below limit of detection (<0.2. µg/ml)
 NAV: Not available

Table 2
Descriptive statistics of demographic and clinical characteristics of patients included in the case-control study

Descriptive statistics: Median(IQR) (except for gender)			
Factor	Non - Failure (n=25) [VL< 500 copies/ml]	Failure (n=25) [VL> 500 copies/ml]	P-value
Age (years):	38(34-43)	36(30-46)	NA (matching criterion)
Gender	Female =16	Female =16	NA (matching criterion)
CD4 count (cells/ μ l)	297(236-544)	168(105-227)	0.0069
Log ₁₀ VL (Log ₁₀ copies/ml)	< 1	4.1(3.0-5.5)	NA (matching criterion)
BMI	24.5(22.1-26.1)	24.1(21.3-29.6)	0.322
Duration of LPV therapy (months)	28(13-44)	11.5(9-21.5)	0.0217
LPV- plasma (μ g/ml)	10.2(6.31-12.70)	0(0-0.81)	0.0037
LPV-hair (ng/mg)	8.36(5.63-12.13) (n=19)	0.97(0.27-3.15) (n=19)	0.0009
RTV-hair (ng/mg)	0.81(0.46-1.22) (n=19)	0.13(0.04-0.54) (n=19)	0.0084

Footnote:

IQR: interquartile range

VL: HIV-1 RNA load

BMI: Body mass index

LPV: Lopinavir

RTV: Ritonavir

NA: not applicable

P values are from Signed rank test.

Table 3
Lopinavir plasma and hair concentrations as predictors of virologic failure

LPV plasma concentration < 1µg/ml as predictive of virological failure using an HIV RNA cut-off of 500 copies/ml			
LPV plasma concentration	HIV RNA Load < 500 copies/ml	HIV RNA Load > 500 copies/ml	Total
<1µg/ml	1	19	20
>1µg/ml	24	6	30
Total	25	25	50
LPV plasma concentration < 1µg/ml as predictive of virologic failure using an HIV RNA cut-off of 1000 copies/ml			
LPV plasma concentration	HIV RNA Load < 1000 copies/ml	HIV RNA Load > 1000 copies/ml	Total
<1µg/ml	4	16	20
>1µg/ml	27	3	30
Total	31	19	50
LPV hair concentration < 3.63 ng/mg as predictive of virologic failure using an HIV RNA cut-off of 500 copies/ml			
LPV hair concentration	HIV RNA Load < 500 copies/ml	HIV RNA Load > 500 copies/ml	Total
<3.63 ng/mg	2	16	18
>3.63 ng/mg	17	3	20
Total	19	19	38
LPV hair concentration < 3.63 ng/mg as predictive of virologic failure using an HIV RNA cut-off of 1000 copies/ml			
LPV hair concentration	HIV RNA Load < 1000 copies/ml	HIV RNA Load > 1000 copies/ml	Total
<3.63 ng/mg	5	13	18
>3.63 ng/mg	19	1	20
Total	24	14	38