Published in final edited form as:

Int J Antimicrob Agents. 2016 August ; 48(2): 158–162. doi:10.1016/j.ijantimicag.2016.04.030.

Random lopinavir concentrations predict resistance on lopinavir-based antiretroviral therapy

Richard Court^{a,*}, Michelle Gordon^b, Karen Cohen^a, Annemie Stewart^c, Bernadett Gosnell^d, Lubbe Wiesner^a, and Gary Maartens^a

^aDivision of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa

^bDepartment of Virology, Nelson R. Mandela School of Medicine, University of Kwazulu-Natal, Durban, South Africa

^cClinical Research Centre, University of Cape Town, Cape Town, South Africa

^dDepartment of Infectious Diseases, Nelson R. Mandela School of Medicine, University of Kwazulu-Natal, Durban, South Africa

Abstract

Considering that most patients who experience virological failure (VF) on lopinavir-based antiretroviral therapy (ART) fail due to poor adherence rather than resistance, an objective adherence measure could limit costs by rationalising the use of genotype antiretroviral resistance testing (GART) in countries with access to third-line ART. A cross-sectional study was conducted in a resource-limited setting at two large clinics in Kwazulu-Natal, South Africa, in patients experiencing VF (HIV-RNA > 1000 copies/mL) on lopinavir-based ART who had undergone GART. Associations between major protease inhibitor (PI) resistance mutations and random plasma lopinavir concentrations were explored. A total of 134 patients, including 31 children, were included in the analysis. The prevalence of patients with major PI resistance mutations was 20.9% ($n = 28$). A random lopinavir concentration above the recommended minimum trough of 1 µg/mL [adjusted odds ratio (aOR) = 5.81, 95% confidence interval (CI) 2.04–16.50; $P = 0.001$] and male sex (aOR = 3.19, 95% CI 1.22–8.33; $P = 0.018$) were predictive of the presence of at least one major PI resistance mutation. Random lopinavir concentrations of <1 µg/mL had a negative predictive value of 91% for major PI resistance mutations. Random lopinavir concentrations are strongly associated with the presence of major PI resistance mutations. Access to costly GART in patients experiencing VF on second-line ART could be restricted to patients

*Corresponding author. Present address: K45 Old Main building, Groote Schuur Hospital, Anzio Road, Observatory, 7925 Cape Town, South Africa. Tel.: +27 21 650 5422. richard.court@uct.ac.za (R. Court).

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.

Competing interests: None declared.

Ethical approval: Ethics approval was granted by the Human Research Ethics Committee of the University of Cape Town (Cape Town, South Africa) [ref. 079/2012] and the Biomedical Research Ethics Committee of the University of Kwazulu-Natal (Durban, South Africa) [ref. BF 068/08]. Informed consent was received from each participant, which included consent for storage of blood samples for further research related to human immunodeficiency virus (HIV), before enrolment into the PCS study.

with lopinavir concentrations above the recommended minimum trough of 1 µg/mL or, in areas where GART is unavailable, be used as a criterion to empirically switch to third-line ART.

Keywords

HIV; Lopinavir pharmacokinetics; Protease inhibitor; Second-line antiretroviral therapy; Antiretroviral resistance; Therapeutic drug monitoring

1. Introduction

In a recent systematic review, rates of virological failure (VF) on second-line protease inhibitor (PI)-based antiretroviral therapy (ART) in resource-limited settings were as high as 38% after 3 years [1]. However, PI resistance was only detected in 18% of isolates from patients with VF [1]. The World Health Organization (WHO) has recommended that countries develop strategies for third-line ART, which has recently been made available in some countries, including South Africa [2]. Considering that most patients failing PI-based ART fail due to poor adherence rather than resistance [3,4], empirically switching patients to third-line ART would be a waste of resources and would not address the reason for VF in most patients. Genotype antiretroviral resistance testing (GART) both to confirm the presence of PI resistance and to guide the choice of an appropriate third-line regimen is expensive (ca. US\$300 per test) [5], and laboratory facilities in resource-limited settings are limited. Predicting the likelihood of PI resistance in patients experiencing VF on second-line ART would limit access to GART to those patients who are most likely to require third-line ART or, in areas where GART is unavailable, could be used as a criterion to empirically switch to third-line ART.

A simple objective adherence measure could improve the management of patients in VF on second-line ART by identifying which patients are likely failing due to resistance rather than poor adherence. Although vulnerable to the effect of ‘white coat adherence’ [6] and drug–drug interactions, lopinavir plasma concentrations are an objective adherence measure that could be performed on plasma submitted for viral load testing or GART. Several studies have demonstrated the utility of random plasma lopinavir concentrations to predict virological responses on lopinavir-based ART [7,8]. In a small previous pilot study, we described the utility of random plasma and hair lopinavir concentrations to predict the presence of VF and possibly major PI resistance mutations in patients on second-line ART [9]. Hair lopinavir concentrations are an attractive adherence measure as they reflect longer-term adherence, but there are very few laboratories able to perform the assay, obtaining hair samples requires training, and patients may refuse or not have head hair available (hair growth is slower in other areas of the body and the assays have only been tested on head hair). In this study, associations between random plasma lopinavir concentrations and the presence of major PI resistance mutations were investigated in patients with VF on lopinavir-based ART from two large ART clinics in Kwazulu-Natal, South Africa.

2. Materials and methods

2.1. Study population and setting

Participants were initially enrolled as part of the 'Protease Cleavage Site' (PCS) cohort between April 2009 and December 2013 from King Edward hospital (KEH) ART clinic and McCord Hospital ART clinic (also known as 'Sinikithemba') in Durban, South Africa. KEH ART clinic is funded by the South African Department of Health (DoH). 'Sinikithemba' was previously supported by the DoH and the President's Emergency Plan for AIDS Relief before the clinic's closure in June 2012. Patients identified with VF (HIV-RNA > 1000 copies/mL) after a minimum of 6 months of lopinavir-based ART were considered for enrolment in the PCS study, which included GART and adherence counselling. During the study period, the standard South African second-line regimen consisted of lopinavir boosted with ritonavir prescribed every 12 h together with two nucleoside/-tide reverse transcriptase inhibitors (NRTIs). As per WHO guidelines, second-line initiation occurred either for VF on first-line ART or for toxicity/intolerability of first-line drugs. Children aged <3 years or weighing <10 kg at the time of ART initiation were started on lopinavir (Kaletra® syrup) every 12 h with two NRTIs as first-line ART as per national guidelines during the study period [10]. Older children received lopinavir as Kaletra® tablets or syrup.

2.2. Study design

A cross-sectional study was performed including adults and children (defined as ≥ 18 years of age at time of GART) with VF on lopinavir-based ART for a minimum of 6 months in a resource-limited setting. Associations between random lopinavir concentrations and the presence of major PI resistance mutations were explored.

GART was performed at the Hasso-Plattner Laboratory, Nelson Mandela School of Medicine, University of Kwazulu-Natal (Durban, South Africa) using a ViroSeq® HIV-1 Genotyping System (Abbott Molecular Inc., Des Plaines, IL) or a validated in-house assay [11]. The genotype sequences were edited using Sequencher® DNA analysis software [12]. Patients were categorised as having major PI resistance if they had one or more of the following major resistance mutations defined by the Stanford HIV drug resistance database [13]: V32I; L33F; M46I/L; I47V/A; G48V/M; I50V; I54V/T/A/L/M; L76V; V82A/F/T/S; I84V; and L90M. The Stanford HIV drug resistance scoring system for lopinavir was used to categorise the lopinavir resistance profiles as susceptible or as low-, intermediate- or high-level resistance. Viral load testing was performed at the National Health Laboratory Service, Inkosi Albert Luthuli Central Hospital (Durban, South Africa) using NucliSENS easyQ® HIV-1 nucleic acid sequence-based amplification (bioMérieux, Marcy-l'Étoile, France) or Abbott RealTime HIV-1 (Abbott Molecular Inc.) assays.

Random lopinavir concentrations were performed on stored plasma samples remaining after GART at the clinical pharmacology laboratory of the University of Cape Town (Cape Town, South Africa). Lopinavir plasma concentrations were measured using a protein precipitation extraction procedure followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. An AB SCIEX 4000 mass spectrometer (AB SCIEX, Concord, ON, Canada) was operated at unit resolution in the multiple reaction monitoring mode,

monitoring the precursor ions at m/z 629.5 and the product ions at m/z 120.2. A lopinavir-d8 stable isotope was used as an internal standard. The precursor ions of the internal standard were monitored at m/z 637.6 and the product ions at m/z 191.2. The assay was validated over the concentration range of 0.0195–20 $\mu\text{g/mL}$. The intrabatch and interbatch accuracy statistics of the lopinavir assay validation were between 95.0% and 96.4% and between 96.2% and 99.1%, respectively, at high, medium and low quality control concentrations. The coefficient of variation was <3.9%.

2.3. Statistical analysis

Stata v.13.0 (StataCorp LP, College Station, TX) was used to perform the statistical analysis. Multivariate logistic regression was performed to identify associations with the presence of at least one major PI resistance mutation. The following variables were included a priori in the multivariate model: age; sex; duration on lopinavir; viral load at the time of VF; and lopinavir concentration $\geq 1 \mu\text{g/mL}$, which is the minimum recommended trough for lopinavir [14].

2.4. Ethics

This study was reviewed and approved by the Human Research Ethics Committee of the University of Cape Town and the Biomedical Research Ethics Committee of the University of Kwazulu-Natal. Informed consent was received from each participant, which included consent for storage of blood samples for further research related to human immunodeficiency virus (HIV), before enrolment into the PCS study.

3. Results and discussion

A total of 164 patients fulfilled the eligibility criteria for entry into the study; 20 were excluded as their GART could not amplify, 1 was excluded due to a contaminated GART and 9 had missing data. Thus, 134 patients, including 31 children, were included. Of the 31 children, 6 were below the age of 3 years and were therefore initiated on lopinavir as first-line ART. All children weighed $>10 \text{ kg}$ at the time of ART initiation. Patient characteristics at the time of VF of lopinavir-based ART are shown in Table 1. In total, 28 patients (20.9%), including 16 men, harboured major PI resistance mutations. Moreover, 19 patients (14.2%) demonstrated high-level PI resistance and 9 patients (6.7%) had reduced susceptibility to lopinavir. The most common NRTI backbone was zidovudine and didanosine (50.7%), which reflects the national ART treatment guidelines during the study period [15]. Table 2 illustrates the range of lopinavir concentrations in patients harbouring at least one major PI resistance mutation. The mean random lopinavir concentration in patients with at least one major PI resistance mutation was 9.13 $\mu\text{g/mL}$ (standard deviation 7.52 $\mu\text{g/mL}$). A scatterplot of lopinavir concentrations versus the number of major PI resistance mutations is shown in Fig. 1. Supplementary Figs 1 and 2 illustrate the frequency of major PI mutations in patients with a random lopinavir concentration $\geq 1 \mu\text{g/mL}$ and $<1 \mu\text{g/mL}$ respectively. Table 3 shows the factors associated with the presence of at least one major PI resistance mutation. On multivariate analysis, the strongest association with the presence of at least one major PI resistance mutation was a random lopinavir concentration above the recommended trough ($\geq 1 \mu\text{g/mL}$) [adjusted odds ratio (aOR) = 5.81, 95% confidence interval (CI) 2.04–16.50; $P=$

0.001], followed by male sex (aOR = 3.19, 95% CI 1.22–8.33; $P = 0.018$). There was an association of duration on lopinavir-based ART with PI resistance in the univariate analysis, but this association was not significant in the multivariate analysis. Table 4 describes the diagnostic utility of a random lopinavir concentration to predict the presence of PI resistance in a patient in VF on lopinavir-based ART. Of the 64 patients with a random lopinavir concentration $\geq 1 \mu\text{g/mL}$ (Table 1), 42 had no major PI resistance mutations, accounting for the low positive predictive value (PPV) of 34% (95% CI 23–47%). However, the negative predictive value (NPV) of a random lopinavir concentration $<1 \mu\text{g/mL}$ to exclude the presence of a PI resistance mutation was 91% (95% CI 82–97%).

The strongest factor associated with major PI resistance mutations was random lopinavir concentrations. This novel finding, if confirmed, could be a useful surrogate marker for resistance. To our knowledge, this is the first study in a resource-limited setting that has demonstrated a well-powered association between random lopinavir concentrations and the presence of PI resistance. These findings build on those of a small recent pilot study which showed that low random lopinavir concentrations were associated with VF on second-line ART [9]. In patients with VF on second-line ART, a random lopinavir concentration $\geq 1 \mu\text{g/mL}$ is predictive of at least one major PI resistance mutation, whilst a random lopinavir concentration $<1 \mu\text{g/mL}$ predicts an absence of PI resistance.

A limitation of random lopinavir concentrations as an adherence measure is that they only provide a ‘snapshot’ of recent adherence as the plasma half-life of lopinavir in the currently used lopinavir/ritonavir combination is 5–6 h [9]. Random lopinavir concentrations may overestimate adherence if a patient only takes his/her pills in the days leading up to the clinic visit, so-called ‘white coat adherence’ [6]. In addition, the sensitivity and specificity of a random lopinavir concentration $\geq 1 \mu\text{g/mL}$ to predict the presence of at least 1 major PI resistance mutation is modest (79% and 60% respectively), and the positive predictive value is low (34%) indicating that random lopinavir concentrations are not useful as rule in tests, but the high negative predictive value of a random lopinavir concentration $<1 \mu\text{g/mL}$ may be a useful rule out test for PI resistance. Despite these limitations, a random plasma lopinavir concentration $\geq 1 \mu\text{g/mL}$ was strongly predictive of PI resistance in this study. An affordable semi-quantitative lopinavir assay, categorising lopinavir concentrations as either above or below $1 \mu\text{g/mL}$, could be set up and performed on plasma samples sent to the laboratory for GART prior to performing sequencing; only those samples with lopinavir concentrations $\geq 1 \mu\text{g/mL}$ would then be submitted for GART. In areas where GART is unavailable, random lopinavir concentrations could be performed on residual plasma from samples submitted for viral load testing after second-line ART failure has been diagnosed; empirical switches to third-line ART could be limited to patients with lopinavir concentrations $\geq 1 \mu\text{g/mL}$.

In patients on second-line ART with VF, a random lopinavir concentration $<1 \mu\text{g/mL}$ could exclude patients from costly GART owing to its high NPV, and rather indicate the need for enhanced adherence support, which achieved high rates of virological suppression in a study of VF on second-line ART [16].

Lopinavir hair concentrations estimate adherence over ca. 1 month [9] and therefore may provide a better estimate of adherence than plasma concentrations. Unfortunately,

measurement of lopinavir hair concentrations is not widely available and this remains a research tool. We recently showed that adherence assessed by pharmacy refills, which is an inexpensive and easily implementable adherence measure, is strongly associated with the presence of VF on second-line ART [17]. Our group has also shown that adherence assessed by pharmacy refills predicted PI resistance [18]. Disadvantages of pharmacy refill include requiring a pharmacy system with accurate dispensing records as well as an inability to differentiate between non-adherence and other forms of treatment interruptions [19]. Larger studies are required to compare the use of adherence assessed by pharmacy refills with random lopinavir concentrations as predictors of PI resistance on second-line ART.

The finding in this study that men were at higher risk of PI resistance could be explained by their lower ART adherence, which drives resistance, as reported in several studies [20,21]. However, the sex ratio in this cohort is unbalanced. The number of men ($n = 91$) is more than twice as high as the number of women ($n = 43$), which may have enhanced the association of PI resistance with male sex. Our group has shown that longer duration on lopinavir was a strong predictor of PI resistance in a different cohort with a longer duration of PI exposure [18]. The shorter duration on lopinavir and the smaller sample size in the current cohort may explain the weaker association found between lopinavir duration and PI resistance.

The prevalence of major PI resistance in the current cohort (20.9%) is consistent with reports of patients on second-line lopinavir-based ART from a recent systematic review [22]. Earlier studies in Africa suggested a low prevalence of major PI resistance mutations [3,4,23], although there is evidence to suggest this may be increasing [22,24]. One study from a resource-limited setting (India) described a high prevalence (73%) of resistance in patients with VF on second-line ART, but many of these patients were on indinavir and several had prior exposure to unboosted PIs, thus their findings are unlikely to be generalisable [25].

This study had several limitations. First, the study was cross-sectional with a small sample size. Furthermore, the number of children in the cohort was very small and the study was therefore underpowered to look for associations between random lopinavir concentration and PI resistance in children alone. Second, some of the children would have been on first-line ART, the results of which may not be applicable to all patients on second-line ART. Third, one of the study sites ('Sinikithemba') was a state-aided non-government organisation and the results therefore may not be generalisable to all public sector ART clinics. Fourth, we had no information regarding other medications that patients were taking at the time of GART, which may have resulted in low lopinavir concentrations because of drug– drug interactions.

4. Conclusion

Random lopinavir concentrations are an objective adherence measure that could assist with the management of patients with VF on second-line ART. Larger prospective studies in resource-limited settings are required to validate the utility of random lopinavir concentrations to predict PI resistance in patients with VF on second-line ART.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: MG was funded by a grant from the South African National Research Foundation [Thuthuka WIR 66446]. GM was supported in part by the National Research Foundation (NRF) of South Africa [UID no. 85810]; the grant-holder acknowledges that opinions, findings and conclusions or recommendations expressed in any publication generated by NRF-supported research are that of the author(s) and that the NRF accepts no liability whatsoever in this regard. Research reported in this publication was also supported by the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) [Award nos. UM1 AI068634, UM1 AI068636 and UM1 AI106701]. Overall support for the International Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT) was provided by the NIAID [U01 AI068632], the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) and the National Institute of Mental Health (NIMH) [AI068632]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Reference

1. Ajose O, Mookerjee S, Mills EJ, Boule A, Ford N. Treatment outcomes of patients on second-line antiretroviral therapy in resource-limited settings: a systematic review and meta-analysis. *AIDS*. 2012; 26:929–938. [PubMed: 22313953]
2. World Health Organization. Consolidated ARV guidelines. Geneva, Switzerland: WHO; 2013. <http://www.who.int/hiv/pub/guidelines/arv2013/art/thirdlineart/en/index2.html> [accessed 22 October 2015]
3. Wallis CL, Mellors JW, Venter WDF, Sanne I, Stevens W. Protease inhibitor resistance is uncommon in HIV-1 subtype C infected patients on failing second-line lopinavir/r-containing antiretroviral therapy in South Africa. *AIDS Res Treat*. 2011; 2011:769627. [PubMed: 21490784]
4. El-Khatib Z, Ekstrom AM, Ledwaba J, Mohapi L, Laher F, Karstaedt A, et al. Viremia and drug resistance among HIV-1 patients on antiretroviral treatment: a cross-sectional study in Soweto, South Africa. *AIDS*. 2010; 24:1679–1687. [PubMed: 20453629]
5. Levison J, Wood R, Scott C, Ciaranello A, Martinson N, Rusu C, et al. The clinical and economic impact of genotype testing at first-line antiretroviral therapy failure for HIV-infected patients in South Africa. *Clin Infect Dis*. 2013; 56:587–597. [PubMed: 23087386]
6. Feinstein A. On white-coat effects and the electronic monitoring of compliance. *Arch Intern Med*. 1990; 150:1377–1378. [PubMed: 2369237]
7. Alejandro, G.; Swenson, L.; Nohpal, A.; Watson, B.; Auyeung, K.; Montaner, J., et al. Untimed drug levels and resistance in patients experiencing low-level HIV viraemia. 22nd Conference on Retroviruses and Opportunistic Infections (CROI); 23–26 February 2015; Seattle, Washington. International Antiviral Society–USA/CROI Foundation; 2015. [abstract 117]
8. Alexander CS, Asselin JJ, Ting LSL, Montaner JSG, Hogg RS, Yip B, et al. Antiretroviral concentrations in untimed plasma samples predict therapy outcome in a population with advanced disease. *J Infect Dis*. 2003; 188:541–548. [PubMed: 12898441]
9. van Zyl GU, van Mens TE, McIlleron H, Zeier M, Nachega JB, Decloedt E, et al. Low lopinavir plasma or hair concentrations explain second-line protease inhibitor failures in a resource-limited setting. *J Acquir Immune Defic Syndr*. 2011; 56:333–339. [PubMed: 21239995]
10. Department of Health, Republic of South Africa. Guidelines for the management of HIV in children. 2nd. 2010. http://familymedicine.ukzn.ac.za/Libraries/Guidelines_Protocols/2010_Paediatric_Guidelines.sflb.ashx [accessed 23 October 2015]
11. Hirsch MS, Gu HF, Schapiro JM, Hammer SM, Johnson VA, Kuritzkes DR, et al. Antiretroviral drug resistance testing in adult HIV-1 infection: 2008 recommendations of an International AIDS Society–USA panel. *Clin Infect Dis*. 2008; 47:266–285. [PubMed: 18549313]
12. Gene Codes Corporation. [accessed 06 June 2016] Sequencher DNA analysis software. <https://www.genecodes.com>

13. Stanford University. [accessed 06 June 2016] HIV Drug Resistance Database. 2014. <http://hivdb.stanford.edu/DR/PIResiNote.html>
14. la Porte C, Back D, Blaschke T, Boucher C, Fletcher C, Flexner C, et al. Updated guideline to perform therapeutic drug monitoring for antiretroviral agents. *Rev Antivir Ther*. 2006; 3:4–14.
15. Department of Health, Republic of South Africa. [accessed 5 October 2015] Standard treatment guidelines and essential medicines list for South Africa. 2008. <http://www.kznhealth.gov.za/edlphc2008.pdf>
16. Garone DB, Conradie K, Patten G, Cornell M, Goemaere E, Kunene J, et al. High rate of virological re-suppression among patients failing second-line antiretroviral therapy following enhanced adherence support: a model of care in Khayelitsha, South Africa. *S Afr J HIV Med*. 2013; 14:166–169.
17. Court R, Leisegang R, Stewart A, Sunpath H, Murphy R, Winternheimer P, et al. Short term adherence tool predicts failure on second line protease inhibitor-based antiretroviral therapy: an observational cohort study. *BMC Infect Dis*. 2014; 14:664. [PubMed: 25472544]
18. Cohen, K.; Stewart, A.; Kengne, A.; Leisegang, R.; Coetsee, M.; Maharaj, S., et al. A clinical prediction rule for PI resistance in resource-limited settings. 22nd Conference on Retroviruses and Opportunistic Infections (CROI); 23–26 February 2015; Seattle, Washington. International Antiviral Society– USA/CROI Foundation; 2015. [abstract 604]
19. Berg KM, Arnsten JH. Practical and conceptual challenges in measuring antiretroviral adherence. *J Acquir Immune Defic Syndr*. 2006; 43(Suppl 1):S79–S87. [PubMed: 17133207]
20. Teresa B, Testa L, Chiesa E, Adorni F, Clara A, Castelnovo B, et al. Outcome of a second-line protease inhibitor-containing regimen in patients failing or intolerant of a first highly active antiretroviral therapy. *J Acquir Immune Defic Syndr*. 2000; 24:115–122. [PubMed: 10935686]
21. Nachega JB, Hislop M, Dowdy DW, Lo M, Omer SB, Regensberg L, et al. Adherence to highly active antiretroviral therapy assessed by pharmacy claims predicts survival in HIV-infected South African adults. *J Acquir Immune Defic Syndr*. 2006; 43:78–84. [PubMed: 16878045]
22. Hosseinipour MC, Gupta RK, van Zyl G, Eron JJ, Nachega JB. Emergence of HIV drug resistance during first- and second-line antiretroviral therapy in resource-limited settings. *J Infect Dis*. 2013; 207(Suppl 2):S49–S56. [PubMed: 23687289]
23. Levison JH, Orrell C, Gallien S, Kuritzkes DR, Fu N, Losina E, et al. Virologic failure of protease inhibitor-based second-line antiretroviral therapy without resistance in a large HIV treatment program in South Africa. *PLoS One*. 2012; 7:e32144. [PubMed: 22427821]
24. van Zyl GU, Liu TF, Claassen M, Engelbrecht S, de Oliveira T, Preiser W, et al. Trends in genotypic HIV-1 antiretroviral resistance between 2006 and 2012 in South African patients receiving first- and second-line antiretroviral treatment regimens. *PLoS One*. 2013; 8:e67188. [PubMed: 23840622]
25. Saravanan S, Vidya M, Balakrishnan P, Kantor R, Solomon SS, Katzenstein D, et al. Viremia and HIV-1 drug resistance mutations among patients receiving second-line highly active antiretroviral therapy in Chennai, Southern India. *Clin Infect Dis*. 2012; 54:995–1000. [PubMed: 22323567]

Highlights

- Most patients fail second-line antiretroviral therapy (ART) owing to poor adherence rather than resistance.
- Predictors of protease inhibitor (PI) resistance in patients with second-line virological failure (VF) are needed.
- We found random lopinavir (LPV) concentrations to be the strongest predictor of PI resistance.
- Other factors associated with PI resistance were male sex and duration on LPV.
- Genotype antiretroviral resistance testing could be limited to patients in VF with random LPV concentrations $\geq 1 \mu\text{g/mL}$.

Fig. 1.
Range of lopinavir concentrations (µg/mL) versus number of major protease inhibitor resistance mutations.

Table 1

Characteristics of 134 patients with virologic failure on lopinavir-based antiretroviral therapy

Male/female	91 (68%)/43 (32%)	
Adult (>18 years)	103 (77%)	
Age range (years)	Adults (22-56)	
	Children (1-18)	
Median no. of months on lopinavir	20.0 (IQR: 12.8 to 34.2)	
HIV subtype	Subtype C	
VL at genotype (log₁₀ copies/mL)	Mean: 5.6 (SD: 6.2) Median: 4.6 (IQR: 4.0 to 5.2)	
NRTI backbone	AZT + DDI	68 (51%)
	TDF + 3TC	21 (16%)
	D4T + 3TC	12 (9%)
	AZT + 3TC	8 (6%)
	Other [*]	25 (19%)
No. with 1 major protease inhibitor mutations	28 (21%)	Adults: 23 (22.3%)
		Children: 5 (16.1%)
Predicted protease inhibitor resistance^{**}	High	19/28 (68%)
	Intermediate	7/28 (25%)
	Low	2/28 (7%)
No. with lopinavir concentration 1 µg/mL	64 (48%)	

NRTI: Nucleoside/tide reverse transcriptase inhibitors; AZT: zidovudine; DDI: didanosine; TDF: tenofovir; 3TC: lamivudine; D4T: stavudine; ABC: abacavir; LPV: lopinavir
IQR: Interquartile range; SD: Standard deviation

^{*} DDI+3TC, ABC+3TC, D4T+DDI, ABC+D4T, TDF+DDI, ABC+DDI, AZT+DDI+3TC, LPV only

^{**} Stanford category for lopinavir resistance

Table 2

Comparison of random lopinavir concentrations in 28 patients harbouring at least one major protease inhibitor (PI) resistance mutation

Age (years)	Sex	No. of major PI mutations	Lopinavir concentration µg/mL)
2	M	3	7.90
3	M	3	6.20
6	F	4	27.60
13	M	1	BLQ
18	F	5	10.90
36	F	1	BLQ
41	M	1	BLQ
38	M	2	BLQ
34	M	2	7.77
36	F	3	0.37
47	F	3	2.56
56	M	3	4.74
34	M	3	11.30
48	M	3	20.30
44	M	4	0.99
33	F	4	2.46
35	F	4	4.46
43	F	4	7.83
41	M	4	7.91
35	M	4	8.77
40	F	4	10.30
39	M	4	11.30
40	M	4	13.20
43	M	4	15.70
45	F	4	16.00
32	F	4	16.60
52	F	4	17.80
43	M	4	22.90

BLQ, below level of quantification (the lower limit of quantification was 0.0781 µg/mL).
Grey shading indicates children.

Table 3

Factors associated with at least one major protease inhibitor resistance mutation in 134 participants with virological failure on lopinavir-based antiretroviral therapy

	<i>n</i>	Univariate analysis			Multivariate analysis		
		OR (95% CI)	<i>P</i> -value	aOR (95% CI)	<i>P</i> -value		
Age at GART	Per 10-year increase	1.26 (0.90–1.75)	0.174	1.27 (0.91–1.77)	0.163		
Sex	Male	3.90 (1.64–9.28)	0.002	3.19 (1.22–8.33)	0.018		
	Female	Ref.		Ref.			
VL at time of GART (copies/mL)	100 000	0.93 (0.40–2.27)	0.916	0.93 (0.34–2.54)	0.892		
	<100 000	Ref.		Ref.			
Duration on lopinavir (years)	2	2.20 (0.95–5.12)	0.068	2.17 (0.84–5.62)	0.111		
	<2	Ref.		Ref.			
Lopinavir concentration (µg/mL)	1	5.59 (2.09–14.93)	0.001	5.81 (2.04–16.50)	0.001		
	<1	Ref.		Ref.			

OR, odds ratio; CI, confidence interval; aOR, adjusted odds ratio; GART, genotype antiretroviral resistance testing; VL, viral load.

Table 4

Diagnostic utility of a random lopinavir concentration of 1 µg/mL to predict protease inhibitor resistance

		95% CI
Sensitivity	79%	59% to 92%
Specificity	60%	50% to 70%
Positive likelihood ratio	1.98	1.46 to 2.69
Negative likelihood ratio	0.36	0.17 to 0.73
Positive predictive value	34 %	23% to 47%
Negative predictive value	91%	82% to 97%

CI: Confidence interval