Gwamaka E. Mwasakifwa, Cecilia Moore, Dianne Carey, Janaki Amin, Paul Penteado, Marcelo Losso, Poh-Lian Lim, Lerato Mohapi, Jean-Michel Molina, Brian Gazzard, David A. Cooper, Mark Boyd, for the SECOND-LINE Study Group

Relationship between untimed plasma lopinavir concentrations and virological outcome on second-line antiretroviral therapy

AIDS, 2018; 32(3):357-361

Original Manuscript

Concise Communication

Relationship between untimed plasma lopinavir concentrations and virological outcome on second-line antiretroviral therapy.

Gwamaka E MWASAKIFWA[1], Cecilia MOORE[3], Dianne CAREY[1], Janaki AMIN[1, 4], Paul PENTEADO[10], Marcelo LOSSO[5], Poh-Lian LIM[6], Lerato MOHAPI[7], Jean-Michel MOLINA[8], Brian GAZZARD[9], David A COOPER[1], Mark BOYD[1, 2] for **the SECOND-LINE Study Group***

[1]The Kirby Institute, University of New South Wales, Sydney, Australia, [2]University of Adelaide, Lyell McEwin Hospital, Adelaide, Australia, [3]MRC Clinical Trials Unit, London, United Kingdom, [4]Department of Health Systems and Populations, Macquarie University, Sydney, Australia [5]CICAL, Buenos Aires, Argentina, [6]Tan Tock Seng Hospital, Tan Tock Seng, Singapore, [7]University of Witwatersrand, Johannesburg, South Africa, [8]Hopital Saint-Louis, Paris, France, [9]St Stephen's AIDS Trust, London, United Kingdom.[10]Department of Clinical Pharmacology, St Vincent's Hospital, Sydney, Australia.

This study was funded by 2015 Gilead Australia Fellowship Research Grants awarded to Prof Mark Boyd.

Running title: Untimed drug concentration and virological outcome of second-line therapy.

Word count: Manuscript 1,506, Abstract 244.

Corresponding Author: G.E. MWASAKIFWA.

Email: gmwasakifwa@kirby.unsw.edu.au

Abstract

Background: Resource constraints in low and middle-income countries(LMICs) necessitate practical approaches to optimizing antiretroviral therapy outcomes. We hypothesised that an untimed plasma lopinavir concentration(UPLC) at week 12 would predict loss of virological response in those taking lopinavir as part of a second-line antiretroviral regimen.

Methods: We measured plasma lopinavir concentration at week 12 on stored samples from the SECOND-LINE study. We characterised UPLC as: (a) detectable and optimal (≥1000 μg/L); (b) detectable but sub-optimal (≥25 to < 1000 μg/L); (c) undetectable (< 25 μg/L). We used Cox regression to explore relationship between UPLC and loss of virological response over 48 weeks and backwards stepwise logistic regression to explore the relationship between UPLC and other predictors of virological failure(VF) at week 48.

Results: At week 48 we observed VF in 15/32 (47%) and 53/485 (11%) of patients with undetectable and detectable UPLC, respectively, p<0.001. Both suboptimal (adjusted HR 2.94, 95% CI 1.54 - 5.62, p=0.001), and undetectable (adjusted HR 3.55, 95% CI 1.89 - 6.64, p<0.001) UPLC were associated with higher rates of loss of virological response over 48 weeks. In multivariate analysis, an independent association with VF at week 48 and undetectable UPLC was observed after adjustment (OR 5.48, 95% CI 2.23 - 13.42, p< 0.01).

Conclusions: In LMICs implementing a public health approach to ART treatment, untimed plasma drug concentration may provide a practical method for early identification of patients with inadequate medication adherence and facilitate timely corrective interventions to prevent virological failure.

Keywords: HIV, second-line, untimed drug concentration, antiretroviral adherence, virological failure, resistance, LMICs, ART.

Introduction

Optimising second-line antiretroviral therapy(ART) outcome is critical to achieving the global UNAIDS “90–90–90” targets. Worrying trends of increasing second-line regimen failure in low and middle-income countries (LMICs) pose significant challenges to global efforts to achieving these targets [1].

Boosted protease inhibitors (PIs) are the World Health Organisation(WHO) - recommended and preferred anchor drugs for second-line ART regimens [2]. PI-based regimens have demonstrated a characteristic adherence-response relationship [3,4]. While regimen potency is key for virological suppression, and near complete (95%) adherence is critical to assure full and sustained virological suppression, the levels of adherence required for the selection of boosted PI resistance is unknown [5,6]. While high adherence level of 95% has been associated with optimal viral suppression [7], high rates of viral suppression have also been documented among patients with moderate levels of adherence [8,9].

We have previously demonstrated that higher baseline HIV RNA viral load (VL), poor adherence (<100%), greater degrees of study baseline N(t)RTI resistance and ethnicity independently predicted virological failure at week 96 [10].

We decided to extend these observations by assessing whether an early untimed lopinavir drug level could predict the virological outcome (dichotomised to virological suppression, defined as VL< 200 copies/mL and virological failure, defined as VL ≥200 copies/mL at week 48. We were also interested in determining if the independent association between virological failure and 'ethnicity' would remain if we controlled for plasma ART concentration.

We hypothesised that an untimed plasma lopinavir concentration (UPLC) measured at week 12 would predict virological failure at 48 weeks in the SECOND-LINE Study [11].

Participants and trial design

SECOND-LINE was an international, multicentre, open-label, randomised controlled trial comparing ritonavir-boosted lopinavir given with either two or three N(t)RTIs (N(t)RTI group) or with raltegravir (RAL group) as second-line therapy. [11] Of 558 participants, 41 were excluded for either switching off ritonavir-boosted lopinavir prior to week 12 or having an inadequate stored plasma samples at week 12.

Materials and Methods

We retrospectively analysed week 12 plasma lopinavir concentration using stored patient samples obtained from the SECOND-LINE study repository in Sydney, Australia. We measured lopinavir concentration using High-Performance Liquid Chromatography. The method allows for accurate and precise quantitation of samples from 100 µg/L - 15,000 µg/L with the lower limit of detection (LLD) of 25 µg/L. All LPV measurements were untimed. We used minimum target LPV trough concentration for wild-type HIV [11] and LLD of the assay to characterize UPLC into categories.

Study objectives

The primary objective of this study was to investigate the association between untimed detectable (LPV≥25 μg/L) or undetectable (LPV<25 μg/L) plasma LPV at week 12 and virological failure at week 48 (HIV viral load in plasma≥200 copies/mL). Secondary objectives included the association between untimed plasma lopinavir concentration (UPLC) as (a) detectable and optimal (o-UPLC) (≥1000 μg/L); (b) detectable but sub-optimal (s-UPLC) (≥25 to <1000 μg/L); (c) undetectable (u-UPLC) (<25 μg/L) and time to loss of virological response (TLOVR).

Statistical analysis

A chi-square test was used to examine the association between UPLC and virological failure at week 48.Univariate logistic regression was used to assess the association between virologic failure at week 48 and UPLC as well as other correlates of virologic outcome (age, BMI, sex, ethnicity, duration of HIV infection, HIV stage, duration of ART, randomized arm, baseline VL, nadir CD4, baseline CD4, baseline CD8, baseline CD4/CD8 ratio, adherence at week 4, adherence at week 48, baseline resistance (genotypic sensitivity score [GSS]) and HIV subtype). Kaplan-Meier methods and Cox regression models were used to investigate the relationship between UPLC and TLOVR. Statistical analysis was performed using STATA® version 14.2, StataCorp, LLC, Texas, USA. The study was approved by both University of New South Wales and University of Witwatersrand Human Research Ethics Committees.

Results

Our analysis included 517 of 558 participants enrolled into the SECOND-LINE trial who were receiving lopinavir at week 12 and had an adequate stored sample available. At week 48 we observed virological failure in 15/32 (47%) and 53/485 (11%) of patients with undetectable and detectable plasma lopinavir concentrations, respectively, p<0.001. At week 12, 32/517 (6%) had undetectable UPLC, and 485/517 (94%) had detectable UPLC.

Both suboptimal UPLC (adjusted HR 2.94, 95% CI 1.54 - 5.62, p=0.001), and undetectable UPLC (adjusted HR 3.55, 95% CI 1.89 - 6.64, p<0.001) were significantly associated with higher rates of loss of virological response over 48 weeks after adjusting for baseline viral load and randomized arm, **Fig.1**.

In multivariate analysis, an independent association with time to loss of virological response over 48 weeks and undetectable UPLC was observed after adjustment for baseline GSS, baseline VL, baseline BMI, adherence at week 4 and week 48 and ethnicity (OR 5.48, 95% CI 2.23 - 13.42, p< 0.001), **(Table1).**

The association between VF at week 96 and ethnicity observed in our previous analysis (using Asians as comparator group: Whites had OR 2.28; CI 0.65 – 8.2;

p=0.196, Hispanics; OR 3.13; 95%CI 1.21 – 8.13; p = 0.019 and Africans; OR 2.09; CI 0.7 – 6.25; p= 0.185) [10] lost significance with the inclusion of the week 12 UPLC data in the current analysis (Whites as a comparator group: Asians; OR 0.43; CI 0.18 – 1.06; p= 0.368 and Africans; OR 0.59, CI 0.24 –1.45; p =0.247).

Discussion

We observed a significant association between single undetectable UPLC and virological failure among an ethnically diverse cohort of HIV patients randomised to LPV/r as part of a second-line therapy.

Early and objective identification of poor adherence is critical to achieving and sustaining viral suppression. Self-reported adherence for example, while it is cheap and easy to administer, is prone to recall bias and overestimation [12,13]. Underestimation of true adherence and patients' acceptability of medication event monitoring systems(MEMS) has been previously reported [14].

Several studies have demonstrated the relationship between untimed plasma or hair PI concentrations and virological outcome [12–23]. A significant association between a single, low, plasma drug level soon after starting unboosted PI therapy and poor virological outcome[adjusted OR,2.7; CI, 0.10 – 0.72; p<0.001] during the first year of therapy was reported by Alexander et al. [13]. In a retrospective analysis of plasma LPV concentration in 84 patients, Wateba et al reported a significant difference in virological suppression at 3 months among those patients with subtherapeutic (LPV< 3 mg/L), therapeutic concentration (LPV= 3mg/L - 8 mg/L) and toxic concentration (LPV> 8 mg/L), $p < 0.05$ ten days after commencing LPV/r containing regimen [24]. In a cross-sectional analysis of 93 patients treated with LPV/r regimens, low plasma LPV (< 1 μg/ml) had negative predictive value for virologic failure(VL>1000copies/ml) of 92% [17].

In contrast to the above studies, we used untimed plasma LPV at week 12 in a contemporary cohort of 517 patients, in a randomized trial setting, who were receiving LPV/r based, WHO recommended second-line regimens, to predict virological failure at week at 48 with a more stringent definition of virologic failure (VL≥200copies/ml).

Our findings have important clinical implications. Firstly, the measurement of untimed plasma drug concentration may provide a simple and practical method for the identification of patients with inadequate adherence and impending virological failure. This approach might allow early tailored adherence interventions before virologic failure and selection of resistance mutations to facilitate viral re-suppression and optimise treatment outcome [25]. At less than US$50 per sample, one could imagine for instance the development and use of a simple point of care test that reported 'absence' or 'presence' of the drug at any pre-determined level.

Secondly, even with an ethnically diverse population, ethnicity or racial categories are weak proxies for interrogating differential virologic outcomes with contemporary, potent, highly forgiving ART regimens. While it may be tempting to explain higher rates of virological failure by ethnically-determined drug distribution and metabolism, we have demonstrated that virological failure in the SECOND-LINE study was more likely simply a marker of poor adherence.

Strategies to optimise adherence will be critical to the long-term success of ART programs worldwide. While third-line ART is mentioned in WHO guidelines, it is mainly aspirational in LMICs and its optimal composition not well grounded in clinical science.

The study has some weaknesses. Plasma ART concentration can be influenced by sex, age, BMI, drug-drug interactions, drug-food interaction, disease state, drug transporters and genetic polymorphism [26–28] .

We measured LPV/r at a single time point thus limiting our ability to interrogate inter-personal and intra-personal variability [29] of the plasma LPV concentrations. In some individual cases, we were unable to analyse the relationship between plasma lopinavir concentration and virological outcome due to missing or inadequate plasma samples. These phenomena partly explain the imperfect association between UPLC and virological suppression observed in our current analysis.

Conclusions

In LMICs, where a public health approach to the provision of HIV treatment is widely implemented, single untimed LPV concentration offers a practical method for adherence stewardship, optimising treatment outcome to boosted PI-based therapy and ensuring sustainability of ART treatment programs. This may be even more attractive if a simple point-of-care technology could determine the absence or presence of LPV were available. Further study using untimed LPV or other PI plasma concentration to optimise virological outcome deserves further research in prospective clinical trials.

Acknowledgements

We thank the SECOND-LINE Study participants and their partners, families, and carers for participation in the study. We also thank all the staff from all centres participating in the trial and laboratory staff at HIV Immunovirology (Biobank) Laboratory, St Vincent's Hospital Centre for Applied Medical Research, St Vincent's Hospital, Sydney. We thank Prof Ross Norris, Scientific Head of the Clinical Pharmacology & Toxicology and General Biochemistry Laboratory, St. Vincent's Hospital, Sydney. We thank Gilead for study support.

SECOND-LINE study group:

Writing Committee: G Mwasakifwa, C Moore, D Carey, J Amin, M Losso, P-L Lim, L Mohapi, J-M Molina, B Gazzard, D Cooper, M Boyd. Protocol steering committee; W Belloso, MA Boyd, DA Cooper, J Elliott, S Emery, B Gazzard, E Gotuzzo, A Humphries, A Kamarulzaman, E Kedem,N Kumarasamy, P Li, P-L Lim, MH Losso, JS Madero, P Mallon,L Mohapi, J-M Molina, C Nwizu, P Phanuphak, S Kerr, H Teppler,O Van de Steen, C Woodward, M Wolff. Project team: C Abela, J Amin, M Arriaga, N Berthon-Jones, MA Boyd, DA Cooper, K Courtney-Vega, S Emery, N Espinosa, H Haskelberg, S Hough, A Martin, W Lee, CL Moore, K Passadee, J Taylor, M Valdovinos, J Zhou. Site investigator: W Belloso, C Binits, V Biter, A Casiro, P Chetchotisakd,J Contarelli, S Foulkes, B Gazzard, E Gotuzzo, A Kamarulzaman,E Kedem, N Kumarasamy, C Lee, N Leerattanapecth, P Li, P-L Lim,G Lopardo, MH Losso, M Luna, S Lupo, JS Madero, OG Messina,L Mohapi, J-M Molina, C Nwizu, C Perez, P Phanuphak, R Salazar,J Sanchez, D Smith, CT Soo, K Supparatpinyo, JFA Villanueva, MJ Wolff, R Wood. Site staff: E Betina Angel, R Aramburo, JV Bazalar, SR Borja,R Cabello, A Clarke, G Copertari, DO David, M Delfino, J Echevarria,S Ferret, N Grace, LAG Hernandez, R Kaplan, T Khotphuwieng, S Kumar, A La Rosa, AL Loh, S Man, P Mootsikapun, LK Nim, RG Northland,SFS Omar, S Poongulali, A Rodriguez, A Rojas, D Salami, M Sanchez,J Sarangapany, P Sugandhavesa, C Maor, C Higgs, M Tan, L Trape,W-Y Chung, J Aploon, R Lourens, C Lai Fong, M Valdovinos, G Viloria,C Wongvoranet, HIV Immunovirology (Biobank) Laboratory staff, St. Vincent’s Hospital Centre for Applied Medical Research. Data and safety monitoring board: R Gulick, D Dunn, M Dolan. Endpoint reviewer: S Pett.

References

1 Ajose O, Mookerjee S, Mills EJ, Boulle 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.

2 World Health Organization. *Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach.* ; 2016. http://www.ncbi.nlm.nih.gov/books/NBK374294/ (accessed 4 Mar2017).

3 Bangsberg DR, Moss AR, Deeks SG. Paradoxes of adherence and drug resistance to HIV antiretroviral therapy. *J Antimicrob Chemother* 2004; **53**:696–699.

4 Wyl V von, Klimkait T, Yerly S, Nicca D, Furrer H, Cavassini M, *et al.* Adherence as a Predictor of the Development of Class-Specific Resistance Mutations: The Swiss HIV Cohort Study. *PLoS ONE* 2013; **8**. doi:10.1371/journal.pone.0077691

5 Bangsberg DR. Less Than 95% Adherence to Nonnucleoside Reverse-Transcriptase Inhibitor Therapy Can Lead to Viral Suppression. *Clin Infect Dis* 2006; **43**:939–941.

6 Friend J, Parkin N, Liegler T, Martin JN, Deeks SG. Isolated lopinavir resistance after virological rebound of a ritonavir/lopinavir-based regimen. *AIDS Lond Engl* 2004; **18**:1965–1966.

7 Paterson DL. Adherence to Protease Inhibitor Therapy and Outcomes in Patients with HIV Infection. *Ann Intern Med* 2000; **133**:21.

8 Shuter J, Sarlo JA, Kanmaz TJ, Rode RA, Zingman BS. HIV-infected patients receiving lopinavir/ritonavir-based antiretroviral therapy achieve high rates of virologic suppression despite adherence rates less than 95%. *J Acquir Immune Defic Syndr 1999* 2007; **45**:4–8.

9 Bangsberg DR, Acosta EP, Gupta R, Guzman D, Riley ED, Harrigan PR, *et al.* Adherence–resistance relationships for protease and non-nucleoside reverse transcriptase inhibitors explained by virological fitness: *AIDS* 2006; **20**:223–231.

10 Boyd MA, Moore CL, Molina J-M, Wood R, Madero JS, Wolff M, *et al.* Baseline HIV-1 resistance, virological outcomes, and emergent resistance in the SECOND-LINE trial: an exploratory analysis. *Lancet HIV* 2015; **2**:e42–e51.

11 Group S-LS. Ritonavir-boosted lopinavir plus nucleoside or nucleotide reverse transcriptase inhibitors versus ritonavir-boosted lopinavir plus raltegravir for treatment of HIV-1 infection in adults with virological failure of a standard first-line ART regimen (SECOND-LINE): a randomised, open-label, non-inferiority study. *The Lancet* 2013; **381**:2091–2099.

12 Liechty CA, Alexander CS, Harrigan PR, Guzman JD, Charlebois ED, Moss AR, *et al.* Are untimed antiretroviral drug levels useful predictors of adherence behavior? *AIDS* 2004; **18**:127–129.

13 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.

14 Gonzalez-Serna A, Swenson LC, Watson B, Zhang W, Nohpal A, Auyeung K, *et al.* A single untimed plasma drug concentration measurement during low-level HIV viremia predicts virologic failure. *Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis* 2016; **22**:1004.e9-1004.e16.

15 Gandhi M, Ameli N, Bacchetti P, Gange SJ, Anastos K, Levine A, *et al.* Protease Inhibitor Levels in Hair Samples Strongly Predict Virologic Responses to HIV Treatment. *AIDS Lond Engl* 2009; **23**:471–478.

16 Durant J, Clevenbergh P, Garraffo R, Halfon P, Icard S, Del Giudice P, *et al.* Importance of protease inhibitor plasma levels in HIV-infected patients treated with genotypic-guided therapy: pharmacological data from the Viradapt Study. *AIDS Lond Engl* 2000; **14**:1333–1339.

17 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 1999* 2011; **56**:333–339.

18 Di Giambenedetto S, De Luca A, Villani P, Bacarelli A, Ragazzoni E, Regazzi M, *et al.* Atazanavir and lopinavir with ritonavir alone or in combination: analysis of pharmacokinetic interaction and predictors of drug exposure. *HIV Med* 2008; **9**:239–245.

19 Winston A, Bloch M, Carr A, Amin J, Mallon PWG, Ray J, *et al.* Atazanavir trough plasma concentration monitoring in a cohort of HIV-1-positive individuals receiving highly active antiretroviral therapy. *J Antimicrob Chemother* 2005; **56**:380–387.

20 Prasitsuebsai W, Kerr SJ, Truong KH, Ananworanich J, Do VC, Nguyen LV, *et al.* Using Lopinavir Concentrations in Hair Samples to Assess Treatment Outcomes on Second-Line Regimens Among Asian Children. *AIDS Res Hum Retroviruses* 2015; **31**:1009–1014.

21 Olds PK, Kiwanuka JP, Nansera D, Huang Y, Bacchetti P, Jin C, *et al.* Assessment of HIV antiretroviral therapy adherence by measuring drug concentrations in hair among children in rural Uganda. *AIDS Care* 2015; **27**:327–332.

22 Gandhi M, Ameli N, Bacchetti P, Anastos K, Gange SJ, Minkoff H, *et al.* Atazanavir Concentration in Hair Is the Strongest Predictor of Outcomes on Antiretroviral Therapy. *Clin Infect Dis* 2011; **52**:1267–1275.

23 Clevenbergh P, Mouly S, Sellier P, Badsi E, Cervoni J, Vincent V, *et al.* Improving HIV Infection Management Using Antiretroviral Plasma Drug Levels Monitoring: A Clinicians Point of View. *Curr HIV Res* 2004; **2**:309–321.

24 Wateba M, Billaud E, Dailly E, Jolliet P, Raffi F. Low initial trough plasma concentrations of lopinavir are associated with an impairment of virological response in an unselected cohort of HIV-1-infected patients. *HIV Med* 2006; **7**:197–199.

25 Garone DB, Conradie K, Patten G, Cornell M, Goemaere W, 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. *South Afr J HIV Med* 2013; **14**:170–176.

26 Bellusci CP, Rocco C, Aulicino P, Mecikovsky D, Curras V, Hegoburu S, *et al.* Influence of MDR1 C1236T polymorphism on lopinavir plasma concentration and virological response in HIV-1-infected children. *Gene* 2013; **522**:96–101.

27 Cressey TR, Lallemant M. Pharmacogenetics of antiretroviral drugs for the treatment of HIV-infected patients: An update. *Infect Genet Evol* 2007; **7**:333–342.

28 Fletcher CV, Jiang H, Brundage RC, Acosta EP, Haubrich R, Katzenstein D, *et al.* Sex-Based Differences in Saquinavir Pharmacology and Virologic Response in AIDS Clinical Trials Group Study 359. *J Infect Dis* 2004; **189**:1176–1184.

29 Acosta EP, Kakuda TN, Brundage RC, Anderson PL, Fletcher CV. Pharmacodynamics of human immunodeficiency virus type 1 protease inhibitors. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2000; **30 Suppl 2**:S151-159.