**SHORT REPORT**

**Darunavir/cobicistat showing similar effectiveness as darunavir/ritonavir monotherapy despite lower trough concentrations**

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**Abstract**

**Introduction:** When darunavir (DRV) 800 mg is boosted with 150 mg cobicistat (DRVcobi), DRV trough concentration (C\text{trough}) is about 30% lower as compared to 100 mg ritonavir (DRV\text{rtv}). DRVcobi shows similar virological efficacy as DRV\text{rtv} when combined with two nucleos(t)ide analogue reverse-transcriptase inhibitors, but it is unknown whether a lower DRV C\text{trough} would undermine the effectiveness of DRVcobi when given as monotherapy (mtDRVcobi).

**Methods:** Prospective observational study on virologically suppressed HIV-infected subjects who switched to mtDRVcobi. Virological failure was defined as two consecutive HIV-RNA >200 copies/mL. Efficacy was evaluated by intention-to-treat (ITT) and on-treatment (OT) analyses, and compared with data from a previous cohort of subjects on mtDRV\text{rtv} conducted at our centre. Plasma DRV C\text{trough} was measured using LC–MS/MS.

**Results:** A total of 234 subjects were enrolled. At week 96, the efficacy rates were 67.8% (CI\text{95}, 61.8 to 73.7) by ITT and 86.9% (CI\text{95}, 78.0 to 87.7) by OT analyses. The corresponding rates in our historical DRV\text{rtv} controls were 67.6% (CI\text{95}, 60.0 to 75.2) and 83.6% (CI\text{95}, 77.2 to 90.0). A total of 135 DRV determinations were performed in 83 subjects throughout the follow-up period, with a median plasma DRV C\text{trough} of 1305 ng/mL (range, 150 to 5895) compared with 1710 ng/mL (range, 200 to 3838) in subjects on monotherapy with DRV\text{rtv} (p = 0.05).

**Conclusions:** DRV C\text{trough} was lower in HIV-infected subjects receiving DRVcobi than with DRV\text{rtv}. However, this did not appear to influence the efficacy of DRVcobi when administered as monotherapy.

**Keywords:** Darunavir; cobicistat; ritonavir; monotherapy; pharmacokinetic; C\text{trough}

**1 | INTRODUCTION**

Cobicistat is a potent and selective human CYP3A inhibitor without anti-HIV activity showing a lower potential for undesirable drug-drug interactions when compared to ritonavir (rtv) [1]. When darunavir (DRV) 800 mg was boosted with cobicistat 150 mg (DRVcobi) once daily in healthy volunteers, DRV exposure was within the limits of bioequivalence for C\text{max} and AUC\text{24h} compared to DRV\text{rtv} but DRV C\text{trough} were about 30% lower [2]. This difference may not be clinically relevant for combined antiretroviral therapy (cART) since DRVcobi has shown similar virological efficacy as DRV\text{rtv} when administered in combination with two nucleos(t)ide analogue reverse-transcriptase inhibitors (NRTIs) [3,4]. However, it remains unclear whether this is also true for DRVcobi when given as monotherapy (mtDRVcobi).

Data derived from both clinical trials and real-life practice suggest that most subjects with long-lasting virological suppression maintain undetectable viraemia 48 to 96 weeks after switching to DRV\text{rtv} monotherapy (mtDRV\text{rtv}). However, mtDRV\text{rtv} is less effective than cART, as transient detectable viral loads (blips) are more frequent [5-8]. Since 2009, protease inhibitor (PI)-based monotherapy is considered as a simplification option in both the Spanish and European guidelines for the use of antiretroviral agents in HIV-1-infected adults without history of failure on prior PI-based therapy and who have had viral load <50 copies/mL for more than 6 months [9,10]. In the clinical practice, DRV\text{rtv} is currently being replaced by DRVcobi, but there are no data about the effectiveness of mtDRVcobi and whether a lower DRV C\text{trough} could impact on efficacy or an increase in the numbers of blips. The aim of this study was to evaluate the efficacy of mtDRVcobi in the daily clinical practice, to analyse the relationship between pharmacokinetic parameters and virological failure, and to compare it with historical data on mtDRV\text{rtv}.

**2 | MATERIALS AND METHODS**

This prospective observational study was carried out at the Virgen del Rocío University Hospitals in Spain. All subjects
who maintained virological suppression ≥6 months and who switched to mtdRV_cobi once daily from January 2015 to January 2016 were included. Subjects with previous virological failure (VF) while on a PI-containing regimen were included if the genotypic resistance tests showed no major (I47V, I50V, I54M/L, L76V and L84V) or ≤3 minor resistance mutations associated with reduced susceptibility to DRV according to the 2014 International AIDS Society criteria [11]. The prescription of mtdRV_cobi was based on the criteria of the attending physicians as part of their daily clinical practice based on the encouraging results of several clinical trials [12-17] and personal experience on boosted-PI monotherapy [18-28], with the objective of avoiding toxicity associated with nucleoside analogues, increasing adherence, and to augment the cost-effectiveness of therapy [29]. Inclusion was not dependent on CD4+ T cell counts, hepatitis C virus (HCV) coinfection, laboratory parameters or the presence of viral blips during the previous 12 months.

In our hospital, mtdRV_cobi was not prescribed in case of pregnancy; hepatitis B coinfection or for concomitant use with drugs having potential adverse interactions with DRV_cobi pharmacokinetics [30]. This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee for Clinical Research of the Virgen del Rocío University Hospital. All subjects provided written informed consent to use their anonymized data and to perform plasma drug monitoring.

2.1 | Endpoints, follow-up and assessments

The primary clinical endpoint was treatment effectiveness, assessed as the percentage of subjects with virological suppression after 48 and 96 weeks according to intention-to-treat (ITT) analysis (non-complete/missing = failure). Virological failure (VF) was defined as two consecutive confirmed plasma HIV-RNA >200 copies/mL, or a single HIV-RNA level >200 copies/mL if followed by a loss to follow-up. A cut-off level of 200 copies/mL was chosen as a more accurate measurement of VF since values <200 copies/mL suffer high variability and the risk of emerging resistance is believed to be relatively low [31,32]. An additional estimation of virological failure rates using 50 copies/mL as criteria for VF was made to compare with other studies. As a secondary outcome, virological efficacy was assessed using on-treatment (OT) analysis, where subjects who discontinue therapy for any reason, as well as those who are lost to follow-up, are not considered. In addition, a pharmacological sub-study was performed in which the association between plasma levels and treatment outcome was analysed. As reference, efficacy data and pharmacological results were compared with those of a historical cohort of 150 subjects who started mtdRV rtv at our centre [8].

Subject assessments were performed at baseline and every 3 months thereafter, including adherence (subject self-report and pharmacy records), adverse events (AEs), biochemical and haematological profiles, flow cytometric counts of CD4+ T cells and plasma HIV-RNA levels (COBAS AmpliPrep/COBAS TaqMan HIV-1 test, version 2.0). AEs and abnormal laboratory findings were evaluated according to a standardized toxicity grade scale (AIDS Clinical Trials Group) [33]. Genotypic resistance tests were performed on subjects with VF when viral load levels were sufficient. Subjects who missed two consecutive scheduled visits were considered lost to follow-up.

2.2 | Blood sampling and determination of DRV concentrations

Blood samples were drawn 24 h (±30 min) after the previous DRV_cobi dose taken after standard breakfast and processed within an hour after collection. Plasma was separated and stored at −80°C until assayed. Separation was performed on a Phenomenex Luna C18 (5 μm, 150 x 2 mm) analytical column. The mobile phase was composed of 2 mM ammonium acetate 0.1% formic acid and acetonitrile 0.1% formic acid. DRV was extracted from the plasma by protein precipitation, using acetonitrile containing a deuterated internal standard. Plasma DRV concentrations were determined using LC–MS/MS based on an adapted method [34] with standard curves that were highly linear over the range of 50 to 10,000 ng/mL and an intra- and inter-assay precision and accuracy of <15%.

2.3 | Statistical analysis

Categorical and quantitative variables were compared using the χ² test, Student’s t-test or Mann-Whitney nonparametric test, according to their distribution. Time-to-event analyses were performed by Kaplan-Meier survival curves. Both the intra- and inter-subject variability in drug concentrations was measured using the coefficients of variation (CV) of the available values from each subject. Pharmacokinetic data were compared with those of the historical DRV rtv cohort [8], where 587 samples from 119 subjects were analysed. Statistical analyses were performed using the IBM software (SPSS v. 23.0, Chicago, USA), and p-values <0.05 were considered significant.

3 | RESULTS AND DISCUSSION

A total of 234 subjects were included in the study with a median follow-up of 96 weeks (IQR: 58 to 96; range, 24 to 96). Baseline characteristics are described in Table 1. Before switching to mtdDRV_cobi, 175 (74.8%) subjects were on monotherapy as maintenance regimen (144 on DRV rtv and 31 on LPV rtv), while 48 (20.5%) and 11 (4.7%) subjects were on triple and dual therapy, respectively.

One hundred and fifty-four (65.8%) subjects had an earlier VF while receiving non-boosted PI, including 30 (12.8%) subjects who had experienced a previous VF on a DRV rtv-based regimen caused by treatment withdrawal. Genotypic resistance tests before switching to mtdDRV_cobi was available for 127 subjects who had shown VF, including all 30 subjects who had failed to DRV rtv, showing no major resistance mutations to DRV in any case.

3.1 | Efficacy and safety

The Kaplan-Meier estimations of treatment effectiveness by ITT analysis were 82.5% (CI95, 77.6 to 87.3) and 67.8% (CI95, 61.8 to 73.7) at week 48 and 96, respectively, while the historical control data with mtdDRV rtv were 82.7% (CI95, 76.7 to 88.7) and 67.6% (CI95, 60.0 to 75.2) respectively. In an OT
Table 1. Baseline characteristics of the study population (n = 234)

| Parameter | Value |
|-----------|-------|
| Male, no. (%) | 178 (76.1) |
| Age (years), M (IQR) | 49.5 (43 to 54) |
| Weight (kg), M (IQR) | 71.5 (62.5 to 83) |
| BMI kg/m², M (IQR) | 25 (22 to 27) |
| Nadir CD4⁺ T cells/µL, M (IQR) | 150 (52 to 248) |
| CD4⁺ T cells/µL, M (IQR) | 662 (512 to 837) |
| Zenith HIV-RNA log₁₀ copies/mL, M (IQR) | 4.8 (4.1 to 5.3) |
| Previous CDC C stage, no (%) | 66 (28.2) |
| Risk factor for HIV, no. (%) | Previous intravenous drug use | 96 (41) |
| Homosexual contact | 62 (26.5) |
| Heterosexual contact | 66 (28.2) |
| Other | 10 (4.3) |
| Chronic hepatitis C, no. (%) | 40 (17.1) |
| Cirrhosis no. (%) | 8 (3.4) |
| Months on treatment, M (IQR) | 141 (92 to 195) |
| Months with undetectable HIV-RNA, M (IQR) | 85 (50 to 119) |
| Presence of blips in the previous 12 months, no. (%) | 22 (9.4) |
| Previous failure on protease inhibitors, n (%) | 154 (65.8) |
| Previous ART regimens | Monotherapy regimens | 175 (74.8) |
| DRV<sub>rtv</sub>, monotherapy | 144 (61.5) |
| LPV<sub>rtv</sub>, monotherapy | 31 (13.2) |
| Dual therapy regimens | ATV + 3 TC | 11 (4.7) |
| DRV<sub>rtv</sub>, + 3TC | 7 (2.9) |
| Others | 1 (0.42) |
| Triple therapy regimens | 3 (1.2) |
| CKD-EPI mL/min/1.73 m², M (IQR) | 99.1 (83.2 to 105.8) |
| CKD-EPI < 60 mL/min/1.73 m², no. (%) | 3 (12) |

M (IQR), Median (interquartile range); CDC, Centers of Disease Control; HCV, hepatitis C virus; ART, antiretroviral therapy; DRV<sub>rtv</sub>, ritonavir-boosted darunavir; LPV<sub>rtv</sub>, ritonavir-boosted lopinavir; ATV, atazanavir; 3TC, lamivudine; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

Although somewhat less effective than triple therapy, more than 90% of virologically suppressed subjects switching to DRV<sub>rtv</sub>, maintained virological control in the clinical practice, even in subjects with previous VF- on PI-based regimens when no major resistance mutations for DRV were present [7,8]. As it has been reported from various studies, monotherapy has a higher frequency of blips, although this has not been related to a higher frequency of VF. Moreover, in patients who failure on monotherapy with boosted PI, no
resistance mutations have been found. and the introduction of analogues has been enough to control the infection again [35-37]. These results, the benefits of regimens lacking the toxicity of nucleoside analogues, the low incidences and grades of the AEs, and to save up in antiretroviral drug costs [29] support the use of mtDRVrtv in clinical practice.

Recently, Ciaffi et al. reported a rate of virological failure as high as 21% at week 48 with DRV- or lopinavir-based monotherapy as second-line maintenance treatment for HIV-1-infected patients in sub-Saharan Africa [38]. Almost certainly these results were due to the fact that only 80% of the patients had a viral load $<50$ copies/mL at baseline.

Currently, DRVrtv is being replaced by DRVcobi in spite of an about 30% lower DRV Ctrough as observed in healthy volunteers. To the best of our knowledge, this is the first study addressing the effectiveness of mtDRVcobi. Our results show that the DRV Ctrough is about 22% lower for DRVcobi than the concentrations observed from our historical data with mtDRVrtv. However, these concentrations remain above sixfold the protein binding-adjusted EC$_{90}$ for wild-type HIV-1 ($200$ ng/mL) and above threefold the protein binding-adjusted EC$_{50}$ for resistant HIV-1 ($550$ ng/mL) [39] in most subjects. Two and 13 subjects had a Ctrough below of 200 and 550 ng/mL, respectively, of whom only two subjects had VF, presenting a DRV Ctrough of 463.82 and 353.83 ng/mL. While the intra-subject variability in DRV Ctrough was similar for both regimens, the inter-subject variability appeared to be higher in the case of DRVcobi, although the number of subjects is insufficient to draw conclusions on this issue. Nonetheless, the effectiveness was similar to that observed previously with mtDRVrtv [7,8]. The frequency of AEs in the present study was below 2%, which can in part be explained by the large proportion of subjects who were on a DRVrtv-based regimen before switching to mtDRVcobi.

Our study has some limitations. First, blood samples for drug monitoring in the present study was not as frequent as in the earlier study, as in the last years the blood samples for the control of the HIV infection can be collected in primary health centres from where the samples are transferred in the same morning to our hospital. However, the analysis of pharmacokinetics was not the primary aim of the present work and still a considerably high proportion of patients representing the overall population could be analysed. Furthermore, although the number of subjects with VF are low, the data obtained from those subjects who were virologically suppressed show that the lower DRV concentrations observed when boosting with cobicistat appear not to impact on treatment outcome. Second, data from a previous cohort on mtDRVrtv was used as reference, since the present study is a prospective observational study that does not include controls. Still, both data sets were obtained from one single cohort including subjects seen at one single centre and by the same physicians, following a unique protocol. Therefore, the data can be regarded as comparable.
 According to the data obtained in our study, it seems that the change ritonavir to cobicistat does not affect the efficacy of monotherapy with DRV, regardless of the lower plasma C trough achieved.

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COMPETING INTERESTS
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AUTHORS’ CONTRIBUTIONS
1. LFLC, AGV and MTR conceptualized and designed the study.
2. LFLC obtaining funding for the study.
3. LFLC, PV and NE contributed to provision of study materials or subjects.
4. AGV and TFM collected darunavir plasma levels.
5. AGV and MTR collected, assembled the data and managed the database.
6. AGV, MTR and LFLC analysed and interpreted the data.
7. AGV, MTR and LFLC drafted the article.
8. NE and PV critically reviewed the article for important intellectual content.
9. All authors approved the final version of the article.

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