**TITLE PAGE**

**Title:** Long-term evaluation of residual viremia in a clinical trial of dolutegravir plus lamivudine as maintenance treatment for participants with and without prior lamivudine resistance

**Authors:** DE MIGUEL BUCKLEY, Rosa¹,²; RIAL-CRESTELO, David³,⁴⁺; MONTEJANO, Rocío¹,²; PINTO, Adriana³; JIMENEZ-GONZALEZ, María⁵; LAGARDE, Maria²,⁵; ESTEBAN-CANTOS, Andrés¹,²; ARANGUREN-RIVAS, Paula³; CADIÑANOS, Julen¹,²; BISBAL, Otilia²,³; CASTRO, Juan Miguel¹; SANTACREU-GUERRERO, Mireia³; BERMEJO-PLAZA, Laura³; MORENO, Victoria¹; HERNANDO, Asunción²,³; MARTÍN-CARBONERO, Luz¹,²; RUBIO, Rafael²,³,⁵; DELGADO, Rafael⁴,⁵; ARRIBAS José Ramón¹,²,⁶*, PULIDO, Federico²,³,⁵* on behalf of the ART-PRO, PI16/00837-PI16/00678 study group.

*Contributed equally

#Co-senior authors

¹Infectious Diseases Unit. La Paz University Hospital. IdiPAZ. Madrid, Spain

²Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC).

³HIV Unit. Hospital. University Hospital 12 de Octubre - Imas12, Av. de Córdoba, s/n, 28041 Madrid, Spain.

© The Author(s) 2022. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (https://creativecommons.org/licenses/by-nc-nd/4.0/), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
4. Department of Microbiology. University Hospital 12 de Octubre - Imas12, Av. de Córdoba, s/n, 28041 Madrid, Spain

5. Universidad Complutense School of Medicine. Madrid. Spain

6. Universidad Autónoma de Madrid. Madrid, Spain


Co-Corresponding author: José R. Arribas, MD, Infectious Diseases Unit, Internal Medicine Service, Hospital La Paz, IdiPAZ. Castellana 261, 28046 Madrid, Spain.

E-mail addresses: federico.pulido@salud.madrid.org (F. Pulido), joser.arribas@salud.madrid.org (J.R. Arribas).

Requests for pre-prints:

federico.pulido@salud.madrid.org

José R. Arribas, MD, Infectious Diseases Unit, Internal Medicine Service, Hospital La Paz, IdiPAZ. Castellana 261, 28046 Madrid, Spain. joser.arribas@salud.madrid.org

Source of Funding: Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III PI16/00837-PI16/00678.
KEYWORDS

HIV, residual viremia, dolutegravir plus lamivudine, virologically suppressed, lamivudine resistance, M184V/I, next-generation sequencing, NGS,

ABSTRACT.

In this pilot clinical trial, we evaluated rates of residual replication in persons without lamivudine resistance-associated mutations in proviral DNA population sequencing who switched to dolutegravir plus lamivudine. After 144 weeks there were no signal of changes in residual viremia based on qualitative detection methods, irrespective of past lamivudine resistance.

TEXT

Introduction

Evidence for dolutegravir plus lamivudine treatment in persons with past lamivudine resistance is limited and comes mostly from retrospective cohorts or small prospective studies showing, so far, that past M184V/I mutations do not have a significant negative impact on maintenance of virological suppression and that, when virological failure occurs, it does not involve emerging integrase resistance1–4.

In the ART-PRO study we investigated the efficacy of dolutegravir plus lamivudine without excluding persons with past lamivudine resistance as long as there were no lamivudine resistance-associated mutations in proviral DNA population genotyping at baseline. Given the exploratory nature of this pilot study, we investigated the long-term efficacy of this combination using conventional limits (<50 copies/mL) and a more stringent measure of virologic suppression using target not detected (TND).
Methods

ART-PRO is an open-label, single-arm, prospective, pilot clinical trial. Full details of the study have been described elsewhere6. Briefly, virologically suppressed, INSTI-naïve participants were switched to dolutegravir plus lamivudine if proviral DNA population (Sanger) sequencing did not detect the presence of M184V/I or K65R/E/N mutations. Participants were classified according to prior history of lamivudine resistance. We performed proviral DNA next-generation sequencing (NGS, Illumina Miseq) retrospectively from baseline samples in peripheral blood mononuclear cells. The primary endpoint was efficacy at 48 weeks. Secondary endpoints included efficacy at 96 and 144 weeks6. Intention-to-treat-exposed (ITT-e) population included all participants receiving ≥1 dose of study medication (US FDA snapshot algorithm). Per protocol analysis excluded those with any deviation to the eligibility criteria. Plasma for HIV-1 RNA quantification was collected at each visit and at study discontinuation. For measurements of plasma HIV-1 RNA <50 copies/mL, qualitatively readings of viral load were denoted as target detected (TD) and measurements not qualitatively observable as target not detected (TND). Safety and tolerability outcomes were incidence of adverse events and treatment discontinuations.

For the descriptive analysis we used median and interquartile ranges. Differences between groups were assessed with the Kruskal-Wallis and chi-squared tests depending on variable nature. Statistical analysis was performed using R software (version 4.1.1; R Core Team [2020], Vienna, Austria).

The study was conducted following all ethic requirements and is registered with ClinicalTrials.gov, NCT03539224.
Results

Forty-one participants were included, 21 with and 20 without previous lamivudine resistance based on historical RNA genotype. At baseline, lamivudine resistance-associated mutations were detected through proviral DNA NGS with >1% threshold in 27/41 (65.9%) participants. The proportion of patients with TND before starting DTG/3TC was 40% in the historical lamivudine resistance group and 44.4% in the group without historical resistance, excluding participants with blips >50 copies/mL. Other demographic characteristics were well balanced between groups.

At week 144, 37/41 (90.2%) had HIV-1 RNA<50 copies/mL: 85.7% in the group with historical lamivudine resistance and 95% in the group without history of lamivudine resistance, ITT-e analysis, FDA Snapshot. (Table 1) Efficacy in the per protocol population was 94.9% (37/39): 94.7% in the group with previous lamivudine RAMs (18/19) and 95% in the group without previous lamivudine RAMs (19/20).

The proportion of participants with TND status at week 144 was 88.9% (95% CI: 67.2-96.9) in the group with historical mutation and 84.2% (95% CI: 62.4-94.5) in the group without historical mutation (p=1, Fisher’s exact test). Throughout the visits there was no difference between groups in the proportion of patients with TND (Figure 1). In the overall analysis of the participants with viremias below 50 copies/mL, the rate of TND increased from 42.1% at baseline to 86.5% at week 144 (difference: 44.4% [95% CI: 23.03-60.53; p<0.01).

Overall, twelve participants (6 from the group with history of lamivudine resistance) had a total 18 transient viral rebounds. Numerically, transient viral rebounds were
lower in the group with history of lamivudine resistance (6 vs. 12). All persons re-suppressed on study treatment.

Through week 144, there were no cases of virological failure nor selection of new resistance mutations. Four participants prematurely discontinued the study, all had HIV RNA<50 copies/mL at the time of discontinuation: two protocol violations (persisting M184V mutation on proviral DNA population sequencing, both at week 12), one withdrawal due to an adverse event (week 8, insomnia) and one person who declined to continue the study (week 48).

**Discussion:**

In ART-PRO study past lamivudine resistance and/or presence of baseline archived lamivudine RAMs did not negatively affect virological suppression, including TND, after three years of treatment with dolutegravir plus lamivudine.

We observed high rates of virologic suppression below qualitative detectable levels which, importantly, were comparable in persons with or without past lamivudine resistance by visit. Notably, we found that in both, the group with historical lamivudine resistance and in the group without, the proportion of patients with TND increased progressively and significantly up to week 144. TND, although not currently used for decision-making in clinical practice, is linked to reduced levels of residual HIV-RNA replication as measured by single copy assay, HIV-DNA and sCD14, suggesting that persons with TND may have less residual plasma replication, a reduced reservoir and inflammation compared with others in whom VL is qualitatively detectable in some degree\(^7,8\). In both ASPIRE and TANGO clinical trials there were no differences in residual viremia between the DTG/3TC and triple-drug
therapy arms\textsuperscript{9,10}. However, these studies excluded participants with a history of lamivudine resistance. Our study provides additional information on strict virologic control in participants with a history of lamivudine resistance.

After three years of follow-up, we have not observed a single case of virological failure. Several cohorts have suggested that a historical M184V mutation does not affect the efficacy of dolutegravir plus lamivudine as maintenance treatment. In some analysis, a shorter duration of virological suppression or shorter time between M184V detection and switch to dolutegravir plus lamivudine were associated with a higher risk of virological failure\textsuperscript{11,12}. However, a recent study analyzing with what to date is the largest cohort of persons receiving this treatment in this context did not find that the M184V mutation was associated to virological failure, including when the mutation was detected within five years of the switch\textsuperscript{13}.

Rather than duration of virological suppression or time since the mutation was last detected prior to the switch, we used baseline proviral DNA population sequencing to select participants in whom we could expect that lamivudine RAMs would not be present at such a significant proportion to put virologic control at risk. While there are still evident gaps of knowledge, it is however reassuring that an increasing number of persons have received dolutegravir plus lamivudine in this context and, when virologic failure has occurred, there has been no case of emergent integrase RAMs. This is important because it is distinctive from dolutegravir monotherapy where integrase RAMs were selected in cases of virological failure. In our opinion, as discussed elsewhere, when lamivudine is paired with a drug with a high barrier to resistance the possibility of a functional monotherapy is unlikely.\textsuperscript{6}
Our study has some limitations. First and foremost, the limited sample size, natural to a proof-of-concept clinical trial, precludes the generalizability of our results. ART-PRO included only integrase-naïve participants, an unlikely scenario in coming years. Using proviral DNA as an exclusion criterion could be debatable, given that this technique is usually not available in most settings and that we still have limited understanding of the clinical significance or archived RAMs, especially minority variants.

In conclusion, in ART-PRO pilot study we gathered preliminary evidence that dolutegravir plus lamivudine was effective in maintaining virologic control, without increases in residual viremia, after 144 weeks despite past historical lamivudine resistance and presence of archived lamivudine-RAMs by NGS. Our results need to be confirmed with a fully powered study, which is currently ongoing (VOLVER study, NCT04880785).

Conflicts of interest

Rosa de Miguel. Grants from Fondo de Investigaciones Sanitarias (Rio Hortega fellowship CM17/00064), during the conduct of the study; personal fees (speaker fee) and non-financial support from Janssen, non-financial support and personal fees (speaker fee) from ViiV, non-financial support and personal fees (speaker fee) from Gilead, outside the submitted work.

David Rial-Crestelo. Personal fees from Gilead Sciences Inc, non-financial support and personal fees from Janssen Cilag, grants and personal fees from ViiV Healthcare, outside the submitted work.
Rocio Montejano. Grants from Instituto Salud Carlos III -Fondo social Europeo (Juan Rodes 18/00039), during the conduct of the study; personal fees from Viiv Health care, Gilead y Janssen Cilag, outside the submitted work.

Adriana Pinto. Honoraria for lectures and for congress activities from both Viiv Healthcare and Gilead Sciences.

Maria Lagarde. Personal fees from Gilead, Viiv Health and MSD outside the submitted work.

Andrés Esteban-Cantos. Granted by a PFIS fellowship from Instituto de Salud Carlos III-Fondo Social Europeo (FI17/00194).

Paula Aranguren. Personal fees from Viiv Healthcare, outside the submitted work.

Julen Cadiñanos was supported by a Río Hortega fellowship from Instituto de Salud Carlos III-Fondo Social Europeo (CM19/00059) and has received speaker fees from Gilead.

Otilia Bisbal. Grants from Viiv Health care, scholarship for expert courses by MSD and speaker fees from Gilead outside the submitted work.

Mireia Santacreu. Advisory fees, speaker fees and grant support: Viiv.

Victoria Moreno. Personal fees from Viiv Health Care, personal fees from Gilead Sciences, personal fees and non-financial support from Janssen Cilag, personal fees from Merck Sharp & Dohme, outside the submitted work.

Luz Martín Carbonero. Advisory fees and speaker fees: Viiv, Gilead, Janssen, MSD.
Rafael Rubio. Personal fees from ViiV Health Care, personal fees from Gilead Sciences, personal fees from Janssen Cilag, personal fees from Merck Sharp & Dohme, outside the submitted work.

Rafael Delgado. Advisory fees and speaker fees: GSK, ViiV, Gilead.

Jose R Arribas. Advisory fees, speaker fees and grant support: Viiv, Janssen, Gilead, MSD, Aelix.

Federico Pulido. Advisory fees, speaker fees and grant support: ViiV, Gilead, Janssen, MSD, Thera.

The remaining authors have none to declare.

ACKNOWLEDGEMENTS

This study was funded by Fondo de Investigaciones Sanitarias, Instituto de Salud Carlos III PI16/00837-PI16/00678. Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC) CB21/13/00039.

J.R.A., P.P., RM, ML, OB, AH, and RD participated in the conceptualization and design of the study.

R.D.M, D.R., ML, RM, AP, OB, JC, VM, LM, RR, FP and JRA were study investigators and participated in the conduct of the study, including the recruitment and follow-up of participants.

AE, PA, and RD performed DNA sequencing and resistance analysis.

LB, AH, MS and JMC curated data, project administration, and coordination.
MJ-G, RDM and DR were involved with formal data analysis.

J.R.A. and F.P. were responsible for funding acquisition and supervision of all the processes of the trial.

All authors participated in the drafting and review of the manuscript.

The authors thank the study participants; their families and care-givers; investigators and site staff who participated in the study.

REFERENCES


4. Reynes J, Montes B, Tuaillon E, Meftah N, Fernandez C. Virological efficacy and tolerability of dual therapy maintenance with Dolutegravir plus Lamivudine In...


Table 1. FDA-snapshot at week 144, Intention to treat-exposed (ITT-e) analysis population (n=41).

<table>
<thead>
<tr>
<th></th>
<th>All participants (n=41)</th>
<th>Historical resistance to lamivudine (n=21)</th>
<th>No historical resistance to lamivudine (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 RNA &lt;50 copies/mL</td>
<td>37 (90.2)</td>
<td>18 (85.7)</td>
<td>19 (95)</td>
<td>0.61</td>
</tr>
<tr>
<td>HIV-1 RNA ≥50 copies/mL</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>HIV-1 RNA ≥50 copies/mL in W144 window</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Discontinuation Study Drug due to Lack of Efficacy</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Discontinuation Study Drug due to other reasons and last available HIV-1 RNA ≥50 copies/mL</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>No virologic data at W144</td>
<td>4 (9.8)</td>
<td>3 (14.3)</td>
<td>1 (5)</td>
<td>0.61</td>
</tr>
<tr>
<td>Discontinuation Study Drug Due to AE</td>
<td>1 (2.4)</td>
<td>1 (4.8)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Discontinuation Study Drug due to other reasons and last available HIV-1 RNA &lt;50 copies/mL</td>
<td>3 (7.3)</td>
<td>2 (9.5)</td>
<td>1 (5)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AE: adverse event.
**Figure 1.** Proportion of participants with HIV RNA <50 copies/mL target not detected and <50 copies/mL target detected by visit.