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

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ART-PRO is an open-label, single-arm, prospective, pilot clinical trial. Full details of the study have been described elsewhere [5]. In brief, virologically suppressed, integrase strand transfer inhibitor (INSTI)-naive participants were switched to DTG/3TC 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 3TC 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 weeks [6]. The intention-to-treat–exposed (ITT-e) population included all participants receiving ≥1 dose of study medication (United States Food Drug Administration [FDA] snapshot algorithm). Per-protocol analysis excluded those with any deviation to the eligibility criteria. Plasma for human immunodeficiency virus type 1 (HIV-1) RNA quantification was collected at each visit and at study discontinuation. For measurements of plasma HIV-1 RNA <50 copies/mL, qualitative readings of viral load were noted as target detected (TD), and measurements not qualitatively observable as TND. Safety and tolerability outcomes were incidence of adverse events and treatment discontinuations.

For the descriptive analysis, we used median and interquartile range. Differences between groups were assessed with the Kruskal-Wallis and $\chi^2$ tests depending on variable nature. Statistical analysis was performed using R software version 4.1.1 (R Core Team, Vienna, Austria). The study was conducted following all ethical requirements and is registered with ClinicalTrials.gov (NCT03539224).

METHODS

ART-PRO is an open-label, single-arm, prospective, pilot clinical trial. Full details of the study have been described elsewhere [5]. In brief, virologically suppressed, integrase strand transfer inhibitor (INSTI)-naive participants were switched to DTG/3TC 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 3TC resistance. We performed proviral DNA next-generation sequencing (NGS; Illumina Miseq) retrospectively from baseline samples in peripheral blood mononuclear cells.

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RESULTS

Forty-one participants were included, 21 with and 20 without previous 3TC resistance based on historical RNA genotype.
At baseline, 3TC RAMs were detected through proviral DNA NGS with >1% threshold in 27 of 41 (65.9%) participants. The proportion of patients with TND before starting DTG/3TC was 40% in the historical 3TC 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 [5].

At week 144, 37 of 41 (90.2%) had HIV-1 RNA <50 copies/mL: 85.7% in the group with historical 3TC resistance and 95% in the group without history of 3TC 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 3TC RAMs (18/19) and 95% in the group without previous 3TC RAMs (19/20).

The proportion of participants with TND status at week 144 was 88.9% (95% confidence interval [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 = .95, Fisher 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 viremia <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 < .01).

### Table 1. Food and Drug Administration Snapshot at Week 144, Intention to Treat–Exposed Analysis Population (N = 41)

| Outcome | All Participants (N = 41) | Historical Resistance to Lamivudine (n = 21) | No Historical Resistance to Lamivudine (n = 20) | P Value |
|---------|--------------------------|---------------------------------|---------------------------------|---------|
| HIV-1 RNA <50 copies/mL | 37 (90.2) | 18 (85.7) | 19 (95) | .61 |
| HIV-1 RNA ≥50 copies/mL | 0 (0) | 0 (0) | 0 (0) | |
| HIV-1 RNA ≥50 copies/mL in week 144 window | 0 (0) | 0 (0) | 0 (0) | |
| Discontinuation study drug due to lack of efficacy | 0 (0) | 0 (0) | 0 (0) | |
| Discontinuation study drug due to other reasons and last available HIV-1 RNA ≥50 copies/mL | 0 (0) | 0 (0) | 0 (0) | |
| No virologic data at week 144 | 4 (9.8) | 3 (14.3) | 1 (5) | .61 |
| Discontinuation study drug due to AE | 1 (2.4) | 1 (4.8) | 0 (0) | |
| Discontinuation study drug due to other reasons and last available HIV-1 RNA <50 copies/mL | 3 (7.3) | 2 (9.5) | 1 (5) | |

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: AE, adverse event; HIV-1, human immunodeficiency virus type 1.

Overall, 12 participants (6 from the group with history of 3TC resistance) had a total 18 transient viral rebounds. Numerically, transient viral rebounds were lower in the group with history of 3TC resistance (6 vs 12). All persons were repressed 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: 2 protocol violations (persisting M184V mutation on proviral DNA population sequencing, both at week 12), 1 withdrawal due to an adverse event (week 8, insomnia), and 1 person who declined to continue the study (week 48).

### DISCUSSION

In ART-PRO study, past 3TC resistance and/or presence of baseline archived 3TC RAMs did not negatively affect virological suppression, including TND, after 3 years of treatment with DTG/3TC.

We observed high rates of virologic suppression below qualitative detectable levels which, importantly, were comparable in persons with or without past 3TC resistance by visit. Notably, we found that in both the group with historical 3TC resistance and 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 soluble CD14, suggesting that persons with TND may have less residual plasma replication, a reduced reservoir, and inflammation compared with others in whom viral load is qualitatively detectable in some degree [7, 8]. In both the ASPIRE (Pilot Antiretroviral Strategy to Promote Improvement and Reduce Exposure (ASPIRE) study (NCT02263326)) and TANGO (Switch study to evaluate dolutegravir plus lamivudine in virologically suppressed human immunodeficiency virus type 1 positive adults (NCT03446573)) clinical trials, there were no differences in residual viremia between the DTG/3TC and triple-drug therapy arms [9, 10]. However, these studies excluded participants with a history of 3TC resistance. Our study provides additional information on strict virologic control in participants with a history of 3TC resistance.

After 3 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 DTG/3TC as maintenance treatment. In some analysis, a shorter duration of virological suppression or shorter time between M184V detection and switch to DTG/3TC were associated with a higher risk of virological failure [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 5 years of the switch [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 3TC 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 DTG plus 3TC 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 DTG monotherapy where integrase RAMs were selected in cases of virological failure. In our opinion, as discussed elsewhere, when 3TC is paired with a drug with a high barrier to resistance, the possibility of a functional monotherapy is unlikely [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 INSTI-naive 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 the ART-PRO pilot study, we gathered preliminary evidence that DTG plus 3TC was effective in maintaining virologic control, without increases in residual viremia, after 144 weeks despite past historical 3TC resistance and presence of archived 3TC RAMs by NGS. Our results need to be confirmed with a fully powered study, which is currently ongoing (Virologic Outcomes of Lamivudine/Dolutegravir in Virologically Suppressed Subjects With Expected or Confirmed Resistance to Lamivudine [VOLVER], NCT04880785).

Notes

Author contributions. J. R. A., F. P., R. M., M. L., O. B., A. H., and R. D. participated in the conceptualization and design of the study. R. D. M., D. R., M. L., R. M., A. P., O. B., J. C., V. M., L. M., R. R., F. P., and J. R. A. were study investigators and participated in the conduct of the study, including the recruitment and follow-up of participants. A. E., P. A., and R. D. performed DNA sequencing and resistance analysis. L. B., A. H., M. S., and J. M. C. curated data, project administration, and coordination. M. J.-G., R. D. M., and D. R. 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.

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