Antiretroviral therapy in second-line: investigating tenofovir-lamivudine-dolutegravir (ARTIST): a randomised controlled trial

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Statistical Analysis Plan for ARTIST Stage 2

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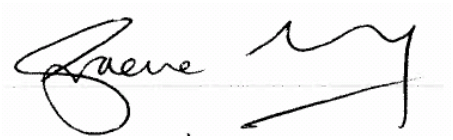

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ARTIST STUDY SUMMARY

As the HIV epidemic matures and more patients are initiated on antiretroviral therapy (ART), focus is on ensuring that those on ART are virologically suppressed. Strategies to support virologic suppression on second-line regimens include the provision of ART that has a low pill burden, good tolerability, a high genetic barrier to resistance, low potential for drug-drug interaction, reduced need for laboratory safety monitoring, and low cost. A fixed-dose combination of tenofovir-lamivudine-dolutegravir (TLD) fulfils these criteria.

Evidence from the NADIA study [1] and the first stage of the ARTIST study [2] suggested that recycling the tenofovir plus lamivudine or emtricitabine (TDF/XTC) backbone from first-line through to second-line could protect the regimen from development of resistance mutations to dolutegravir. This study (ARTIST stage 2) is a phase 2, randomised, double-blind, placebo-controlled, non-comparative trial to determine the proportions of participants achieving virologic suppression when recycling the TDF/XTC backbone with dolutegravir (TLD fixed-dose combination) as a second-line regimen with and without a 14-day lead-in supplementary 50 mg dose of dolutegravir, in those failing a tenofovir-emtricitabine-efavirenz (TEE) first-line regimen. The rationale for the strategy of giving a lead-in supplementary dose of dolutegravir is to compensate for the induction effect of efavirenz on dolutegravir metabolism and transport that persists after efavirenz is stopped.

In ARTIST stage 1, 62 participants were initiated on TLD fixed-dose combination daily with a lead-in supplementary 50 mg dose of dolutegravir taken 12 hours later (to make the dose 50 mg twice daily) for the first 14 days, with continuation of TLD for the duration of the study (48 weeks). Our study findings demonstrated excellent virologic suppression with TLD as a second-line regimen, showing 85% (95% confidence interval [CI], 73 – 93%) achieving plasma viral load <50 copies/mL at 24 weeks, despite substantial baseline nucleoside reverse transcriptase inhibitor (NRTI) resistance [2].

In ARTIST stage 2, 130 participants have been randomised to initiate on TLD fixed-dose combination daily with a lead-in supplementary 50 mg dose of dolutegravir or placebo taken 12 hours later for the first 14 days, with continuation of TLD for the duration of the study (48 weeks). This study is not powered for formal statistical comparison, but the point estimates (and 95% CIs) of the two study arms will be informally compared. The protocol for stage 2 followed the SPIRIT guidelines and has been published [3].

MAIN STUDY PRIMARY AND SECONDARY OUTCOMES

Primary outcome measure

- Plasma viral load <50 copies/mL at 24 weeks

Secondary outcome measures

- Plasma viral load <50 copies/mL at 12 and 48 weeks
- Plasma viral load <400 copies/mL at 12, 24, and 48 weeks
- Plasma viral load <50 copies/mL, <400 copies/mL, and <1000 copies/mL at 4 weeks
- Resistance mutations to dolutegravir by 24 and 48 weeks
- Acquired resistance mutations to NRTIs by 24 and 48 weeks
- CD4 cell count change from baseline at 24 and 28 weeks
- Death by 24 and 48 weeks
- Grade 3 or 4 adverse events by 24 and 48 weeks
- Adverse events leading to treatment discontinuation by 24 and 48 weeks
- Serious adverse events by 24 and 48 weeks

Other outcome measures

- Creatinine change from baseline at 4, 16, and 48 weeks
- Body weight change from baseline at 24 and 48 weeks
- Body mass index change from baseline at 24 and 48 weeks
- Change in sleep assessment from baseline at 2, 4, 8, 12, 16, 20, 24, 36, and 48 weeks
- Change in neuropsychiatric assessment from baseline at 2, 4, 12, 24, and 48 weeks

- Change in neurocognitive assessment from baseline at 2, 4, 12, 24, and 48 weeks
- Adherence to ART as measured by tenofovir-diphosphate concentrations at 12, 24, and 48 weeks

This document describes the statistical analysis plan for ARTIST stage 2.

1. Study information

The number of patients screened and enrolled (or excluded) will be summarised by reasons for exclusion. For participants enrolled in the study, the number of participants discontinued study or lost to follow-up (LTFU) will be tabulated by reasons and last study visits. This information will be summarised in a CONSORT flow diagram, according to the 2010 CONSORT Statement [4].

2. Baseline characteristics

Baseline characteristics will be described in each study arm (tabulated as below). Categorical characteristics will be described using numbers and percentages. Continuous characteristics will be described using medians and interquartile ranges (IQRs). We will describe means (standard deviations) for continuous variables that are normally distributed.

Table 1. Demographic and Baseline Characteristics.

	Lead-in DTG (N = 65)	Placebo (N = 65)
Age, years – median (IQR)		
Female sex – no (%)		
Weight, kg – median (IQR)		
BMI, kg/m^2 – median (IQR)		
CD4 cell count, $cells/mm^3$ – median (IQR)		
Viral load, log_{10} copies/mL – median (IQR)		
Time receiving first-line ART, years – median (IQR)		
Previously received stavudine or zidovudine – no (%)		
NRTI genotypic resistance* – no (%) †		
Two fully active NRTIs#		
Resistance to one NRTI*		
Tenofovir, not XTC		
XTC, not tenofovir		
Efavirenz genotypic resistance* – no (%) †		
TFV-DP concentration - median (IQR)		

DTG = dolutegravir; IQR = interquartile range; BMI = body-mass index; ART = antiretroviral therapy; NRTI = nucleoside reverse transcriptase inhibitor; XTC = lamivudine or emtricitabine; TFV-DP = tenofovir diphosphate.
*Resistance was classified with the Stanford algorithm, with a score of ≥15 indicating at least low-level resistance.
†Denominators indicate the numbers of participants with available viral sequences. #Both NRTIs had a Stanford score <15 indicating susceptibility or only potential of low-level resistance.

3. Analysis of the primary endpoint

The primary endpoint is the proportion of participants achieving virologic suppression (defined as plasma viral load [VL] <50 copies/mL) at 24 weeks. We will describe the proportion (and 95% CI) of participants achieving a VL <50 copies/mL at 24 weeks in each study arm. The visit window for the primary endpoint is +/- 6-weeks. No formal between-group comparison will be made for the primary endpoint as we do not have sufficient power.

We will use the modified intention to treat (ITT) analysis according to the Food and Drug Administration (FDA) Snapshot algorithm for analysing virologic failure [5]. All participants who receive ≥1 dose of the study medication will be part of the analysed cohort. The FDA Snapshot algorithm regards those with measured VL ≥50 copies/mL, discontinued study/study drug due

to lack or loss of efficacy, missing VL within the visit window, intolerance or adverse event because of any drug in the regimen requiring switch, and drug substitution not permitted by the protocol as failures [5]. LTFU will be regarded as failure. Stopping or switching because of dolutegravir or NRTI intolerance or adverse events will be regarded as failure. Switching for reasons of stopping contraception, wish to become pregnant, becoming pregnant, transfer out for nonclinical reasons, and death from non-HIV and nondrug causes (as assessed by the study investigator) will not be regarded as failure and will be excluded from the population.

4. Analysis of secondary efficacy endpoints and time to event analysis

A modified ITT analysis at 12 and 48 weeks will be performed to describe the proportions (and 95% CIs) of participants achieving a VL <50 copies/mL (using the FDA Snapshot algorithm as described above) in each study arm. A secondary analysis will describe the proportions (and 95% CIs) of participants achieving a VL <400 copies/mL at 12, 24, and 48 weeks using the modified ITT analysis and the FDA Snapshot algorithm in each study arm. We will describe the median VL (and IQR) and the proportions (and 95% CIs) of participants achieving a VL <50 copies/mL, a VL <400 copies/mL, and a VL <1000 copies/mL, at 4 weeks using the modified ITT analysis and the FDA Snapshot algorithm in each study arm.

The proportions of participants with a VL <50 copies/mL at each VL testing timepoint (measured at weeks 4, 8, 12, 16, 20, 24, 36, and 48) will be presented with 95% CIs in each study arm. The visit window is +/- 16-days around each visit except week 24 which has a window of +/- 6-weeks. A graph that plots proportions (with 95% CIs) of participants with VL <50 copies/mL at each VL testing timepoint in each study arm will be presented. We will tabulate VL outcomes at each study visit (virologic suppression [defined as a VL <50 copies/mL or a VL <400 copies/mL] and virologic non-suppression) using percentages and 95% CIs. If the proportion suppressed is 0 or 100%, a one-sided 97.5% CI will be estimated. The median time to virologic suppression (and IQR) will be described and a Kaplan Meier survival analysis will be performed to describe this outcome.

We will describe CD4 cell count measured at weeks 24 and 48 using medians and IQRs, and describe change in CD4 cell count from baseline.

Between-group differences for secondary endpoints will be analysed with Chi-squared tests (or Fisher's exact tests if the number in any cell is ≤5) for binary data and with Wilcoxon rank sum tests for continuous data.

5. Analysis of participants who do not virologically suppress

5.1. Description of participants by outcome (suppressed versus unsuppressed)

We will describe the following characteristics comparing participants who are virologically suppressed (achieving a VL <50 copies/mL and a VL <400 copies/mL) and those not suppressed (VL ≥50 copies/mL and VL ≥400 copies/mL) at 24 and 48 weeks.

- Age (median and IQR)
- Sex (proportion female)
- NRTI resistance at baseline (proportion) using the Stanford algorithm for prediction of drug-susceptibility, with a score of ≥15 indicating at least low-level resistance [6]:
    - 2 fully active NRTIs (both with a Stanford score <15 indicating susceptibility or only potential of low-level resistance)
    - 1 fully active NRTI (one with a Stanford score <15 and one with a Stanford score ≥15)
    - No fully active NRTIs (both with a Stanford score ≥15 indicating at least low-level resistance to both NRTIs)
- Baseline VL (median and IQR)
- Baseline CD4 count (median and IQR)
- Previous ART duration (median and IQR)
- Prior exposure to AZT or D4T (proportion)
- Tenofovir-diphosphate (TFV-DP) concentrations at 24 and 48 weeks (proportion in each concentration category) described in details in section 5.3 below

5.2. Description of treatment-emergent dolutegravir and NRTI resistance

VL measurement is obtained at baseline, 4, 8, 12, 16, 20, 24, 36, and 48 weeks. If any VL after week 12 is ≥50 copies/mL, or if there is less than one log decline in VL from baseline, or if VL is suppressed at any timepoint and subsequently rebounds to ≥50 copies/mL, intensive adherence counselling is performed, and VL measurement is repeated after two weeks. If there is no decline and the repeat VL is ≥500 copies per mL, a genotypic resistance testing is performed on a new sample at this timepoint and on the baseline sample.

The number and proportion of participants who develop dolutegravir resistance mutations and emergent NRTI mutations by 24 and 48 weeks in each study arm will be described. The individual mutations and Stanford scores [6] will also be described. We will describe the number and proportion of participants who had virologic failure (defined as having two consecutive VLs >1000 copies/mL after 12 weeks) by 24 and 48 weeks in each study arm.

5.3. Analysis of adherence

TFV-DP concentrations on dried blood spots are conducted as an objective measure of adherence. Proportions in each concentration category will be described at 24 and 48 weeks for participants who are not virologically suppressed (VL ≥50 copies/mL and VL ≥400 copies/mL) and compared with those who achieved virologic suppression (VL <50 copies/mL and VL <400 copies/mL) in each study arm.

TFV-DP concentrations will be categorised using the threshold defined by Anderson et al [7].

- <350 fmol/punch (men: <1.2 doses per week and women: <0.6 doses per week)
- 350 – 700 fmol/punch (men: 1.2 – 3.2 doses per week and women: 0.6 – 2.0 doses per week)
- 700 – 1250 fmol/punch (men: 3.2 – 6 doses per week and women: 2.0 – 5.3 doses per week)
- >1250 fmol/punch (men: >6 doses per week and women: >5.3 doses per week)

TFV-DP concentrations (median and IQR) and the proportion of participants in each category using the threshold defined by Anderson et al [7] will be described at baseline, 12, 24, and 48 weeks for all participants (regardless of virologic success or failure).

6. Analysis of secondary safety and tolerability endpoints

Secondary safety and tolerability analyses will be performed, using the all-patients-treated approach (excluding participants who were randomised but never started on the study medication). We will describe the numbers and proportions of participants in each study arm who develop serious adverse events, laboratory and/or clinical Grade 3-4 adverse events (graded according to the Division of AIDS [DAIDS] criteria [8]), and discontinuation of any drug in the TLD regimen by 24 and 48 weeks.

We will describe creatinine at weeks 4, 16, and 48 using medians and IQRs at each time point. We will describe weight (and BMI) at weeks 24 and 48 using medians and IQRs, and describe change in weight (and BMI) from baseline.

We will describe the numbers and proportions of participants in each study arm who develop insomnia (measured with the insomnia severity index [9] at baseline, 2, 4, 8, 12, 16, 20, 24, 36, and 48 weeks), and describe change in insomnia severity index scores from baseline. We will describe the numbers and proportions of participants in each study arm who develop anxiety and depressive symptoms (measured with the brief symptoms inventory anxiety subscale [10] and the centre for epidemiology studies depression scale [11] at baseline, 2, 4, 12, 24, and 48 weeks), and describe change in scores from baseline. We will describe the time to development of such events and the severity where available. We will describe the median and IQRs of neurocognitive assessment scores in each study arm (measured with the Simioni symptom questionnaire [12] and the cognitive assessment tool rapid version [13] at baseline, 2, 4, 12, 24, and 48 weeks), and describe change in scores from baseline.

We will describe the number and proportion of women of child-bearing potential who become pregnant within the study period (48 weeks), and describe the outcomes of the mother and of the newborn child.

We will describe the number and proportion of deaths (all-cause) within the study period (48 weeks) and report specific causes of death.

7. Subgroup analyses related to the primary endpoint, secondary efficacy endpoints, and time to event analysis

We will describe the proportions (and 95% CIs) of participants who achieve a VL <50 copies/mL at 24 and 48 weeks and the time to virological suppression (Kaplan Meier survival curves), overall and in each study arm, stratified by NRTI resistance present at baseline [6]:

- 2 fully active NRTIs (both with a Stanford score <15)
- 1 fully active NRTI (one with a Stanford score <15 and one with a Stanford score ≥15)
- No fully active NRTIs (both with a Stanford score ≥15)

A secondary analysis will describe the proportions (and 95% CIs) of participants who achieve a VL <400 copies/mL at 24 and 48 weeks and the time to suppression (Kaplan Meier survival curves), overall and in each study arm, stratified by NRTI resistance at baseline.

8. Sensitivity analyses

A sensitivity analysis at weeks 12, 24, and 48 will describe the proportions (and 95% CIs) of participants who achieve a VL <50 copies/mL and those who achieve a VL <400 copies/mL in each study arm. This sensitivity analysis will include only those still in the study and receiving the study drug. The following participants will be excluded from this sensitivity analysis:

- Participants who have evidence of poor adherence (low TFV-DP concentration <350 fmol/punch)
- Participants who are LTFU or missing a VL within the visit window

- Participants who stop or were changed from the study drug for reasons other than failure of the regimen (i.e., for lack or loss of efficacy, intolerance, or adverse event because of any drug in the regimen requiring switch)
- Participants excluded from the modified ITT analysis for reasons described in section 3 above

9. Protocol deviations

A list of all protocol deviations (major and minor) will be compiled. These will be defined according to the relevant trial Standard Operating Procedures, and in accordance with the guidance of the Human Research Ethics Committee at the University of Cape Town. These will be presented as supplementary material in the trial publication.

10. Publication

This statistical analysis plan will be published online (updated on clinicaltrials.gov) before locking the database for analysis.

References

1. Paton NI, Musaazi J, Kityo C, et al. Dolutegravir or darunavir in combination with zidovudine or tenofovir to treat HIV. *New Engl J Med*. 2021;385(4):330-341.
2. Keene CM, Griesel R, Zhao Y, et al. Virologic efficacy of tenofovir, lamivudine and dolutegravir as second-line antiretroviral therapy in adults failing a tenofovir-based first-line regimen: a prospective cohort study. *AIDS (London, England)*. 2021;35(9):1423.
3. Zhao Y, Keene C, Griesel R, et al. AntiRetroviral Therapy In Second-line: investigating Tenofovir-lamivudine-dolutegravir (ARTIST): protocol for a randomised controlled trial. *Wellcome Open Research*. 2021;6(33):33.
4. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *Trials*. 2010;11(1):1-8.
5. Human immunodeficiency virus-1 infection: developing antiretroviral drugs for treatment. Rockville, MD: Food and Drug Administration, Center for Drug Evaluation and Research. [November 2015]. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/human-immunodeficiency-virus-1-infection-developing-antiretroviral-drugs-treatment.
6. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis*. 2006;42(11):1608-1618.

7. Anderson PL, Liu AY, Castillo-Mancilla JR, et al. Intracellular tenofovir-diphosphate and emtricitabine-triphosphate in dried blood spots following directly observed therapy. *Antimicrob Agents Chemother*. 2018;62(1):e01710-17.
8. U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1. [July 2017]. https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf
9. Morin CM, Belleville G, Bélanger L, Ivers H. The Insomnia Severity Index: psychometric indicators to detect insomnia cases and evaluate treatment response. *Sleep*. 2011;34(5):601-608.
10. Derogatis LR, Unger R. Symptom checklist-90-revised. *The Corsini encyclopedia of psychology*. 2010:1-2.
11. Radloff LS. The CES-D scale: A self-report depression scale for research in the general population. *Applied psychological measurement*. 1977;1(3):385-401.
12. Simioni S, Cavassini M, Annoni J-M, et al. Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *Aids*. 2010;24(9):1243-1250.
13. Joska J, Witten J, Thomas K, et al. A comparison of five brief screening tools for HIV-associated neurocognitive disorders in the USA and South Africa. *AIDS and Behavior*. 2016;20(8):1621-1631.