ARTIST Trial Statistical Analysis Plan: STAGE 1

Zidovudine, lamivudine and dolutegravir (AXD) Relative to Tenofovir, lamivudine and dolutegravir (TXD) In Second Line Antiretroviral Therapy (ARTIST) Trial: a randomised controlled trial

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1. ARTIST Summary

As the HIV epidemic matures and more patients are initiated on antiretroviral treatment, focus is on ensuring that those on antiretroviral therapy are virologically suppressed. Strategies to support virological suppression on second-line include the provision of ART that has a low pill burden, good tolerability, low toxicity, is easily monitored, and that is low cost. A fixed-dose combination of tenofovir, lamivudine and dolutegravir (TDF/3TC/DTG: TXD) fulfils these criteria.

Indirect evidence suggests that recycling the TDF/3TC backbone from first through to second line could protect the regimen from the development of resistance mutations to DTG. This study will be a randomised, open-label, active-controlled trial aiming to determine the virological suppression of patients failing the standard of care first-line regimen (TDF/emtricitabine(FTC) or lamivudine (3TC)/NNRTI) who have been switched to a DTG based second line with a recycled TDF/3TC backbone. It will comprise two stages, with stage one enrolling 65 participants, and providing a supplemental dose of DTG 50mg for 14 days to compensate for the enzyme-inducing effect of the discontinued EFV. Stage two will randomise the participants to receive the fixed-dose TXD without supplemental DTG (65 participants) or the World Health Organization-recommended second-line regimen (zidovudine/3TC/DTG – 65 participants). This study is not powered for formal statistical comparison, but the point estimates (and 95% confidence intervals) of the two arms in Stage 2 will be informally compared. This document describes the statistical analysis plan for stage 1.

2. Study information

The number of patients screened and enrolled or excluded will be summarised by reason for exclusion. For the patients enrolled in the study, the number of patients discontinued or lost to follow up will be tabulated by reason and last study visit. This information will be summarised in a CONSORT flow diagram, as per the 2010 CONSORT Flow Diagram template (1).

3. Baseline characteristics

Patients will be described with respect to baseline characteristics. Categorical characteristics will be described using numbers and percentages. Continuous characteristics will be described using medians and interquartile ranges for non-parametric distribution or means and standard deviations for parametric distribution.

4. Analysis of the primary efficacy endpoint

The primary endpoint of the ARTIST trial is the proportion of patients achieving viral load (VL) suppression (defined as a VL result <50 copies/mL) at 24 weeks

The statistical analysis will describe the proportion (and 95% confidence interval) of participants who achieve a suppressed VL at the week 24 visit.

We will use a modified intention to treat analysis (mITT) according to the FDA snapshot analysis algorithm for analysing virological failure: 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 HIV RNA ≥ 50 copies/ml, those with missing HIV RNA within the visit window, intolerance or adverse event due to any drug in the regimen requiring switch, and those with drug substitution not permitted by the protocol as failures (2). LTFU will be considered failure. Stopping or switching due to DTG or NRTI intolerance or adverse events will be regarded as failure. Switching for reasons of stopping contraception or wish to become pregnant, or becoming pregnant, transfer out for non-clinical reasons and death from non-HIV and non-drug causes (as assessed by the study doctor) will not be regarded as failure.

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

A modified intention to treat analysis at 12 and 48 weeks will be performed to describe the proportion (and 95% confidence interval) of patients achieving a suppressed VL (defined above). A secondary analysis will also describe the proportion (and 95% confidence interval) of participants who achieve a VL<400 copies/mL at 12, 24 and 48 weeks using the modified intention to treat analysis as described above.

The proportion of participants with a suppressed VL at each time point will be presented with 95% confidence intervals. The median time to suppression will be described and a Kaplan Meier survival analysis will be performed.

We will describe the week 24 and 48 CD4 counts using medians and IQRs.

A graph that plots percentage (with 95%CI) of participants with VL < 50 copies/mL at each VL testing timepoint will be presented. We will also tabulate outcomes at each visit (including suppressed, not suppressed, loss to follow-up).

6. Analysis of participants who do not virologically suppress

6.1. Description of participants by outcome (suppressed vs unsuppressed)

We will describe the following participant characteristics comparing those who were virologically suppressed and those not suppressed at week 24 and week 48.

- Age (median and IQR)
- Sex (proportion male)
- NRTI resistance at baseline (proportion) (3):
    - 2 fully active NRTIs (both with a Stanford score <15 indicating only the potential of low-level resistance or susceptible)
    - 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)
- Baseline VL (median and IQR)
- Baseline CD4 count (median and IQR)
- Prior exposure to AZT or D4T (proportion)
- Summary of concomitant medications known to interact with DTG (number and proportion of participants prescribed each noted medication within the preceding 24 or 48 weeks)

A secondary analysis will also describe these characteristics categorising participants into those with a VL<400 copies/mL and those with a VL >400 copies/mL at 24 and 48 weeks.

6.2. Description of the development of dolutegravir and new NRTI resistance

In participants who do not suppress their VL after week 12 or who suppress and have a rebound VL >50 copies/mL, a repeat VL will be performed after two weeks and those who do not show a VL decline will have a genotypic antiretroviral resistance test performed if sufficient nucleic acid amplification permits this (resistance tests will be performed at the time of failure and on their baseline sample).

The proportion of patients who develop DTG resistance mutations and emergent NRTI mutations by 24 and 48 weeks on second-line TXD will be described. The individual mutations and Stanford Scores (3) will also be described.

6.3. Analysis of adherence factors

Description of adherence

For the participants who do not suppress on TXD at 24 and 48 weeks, markers of adherence will be described.

- The TFV-DP concentrations will be described at weeks 0, 12, 24, 36 and 48, for participants who fail at any time point (these concentrations will be conducted retrospectively)
    - The concentration will be categorised according to Anderson et al (4)
        - < 350 fmol/punch (equivalent of - men: <1.2 doses per week. women: <0.6 doses per week)
        - 350-700 fmol/punch (men: 1.2 - 3.2 doses per week. women: 0.6 - 2.0 doses per week)
        - 700-1250 fmol/punch (men: 3.2-6 doses per week. women: 2.0-5.3 doses per week)
        - > 1250 fmol/punch (men: >6 doses per week. women: >5.3 doses per week)

- Median time (and IQR) since last treatment dose will also be described for these participants at each time point
- The medication possession ratio (based on pharmacy refill data) will be described.
    - The medication possession ratio will be defined as (5,6):

    Number of days ARV dispensed/ Number of days between first and last ARV pick-up (excluding the ART dispensed at last pick)
    - This will be calculated for each visit interval and presented as an MPR per interval and used to produce an average MPR per month for the total time in the trial. The maximum MPR for a particular visit interval is 100%, and any values greater than 100% will be noted as '100%'.

Analysis of adherence

The TFV-DP concentrations (median and IQR, as well as the proportion of participants in each concentration category described by Anderson et al (4) will be described at week 24 for all participants (regardless of failure).

The medication possession ratios (median and IQR) will also be described for all participants at week 24 (this will be defined as: number of days ARV dispensed/number of days between first and last ARV pick-up (excluding the ART dispensed at last pick up (5,6)).

These measures will be compared between those with suppressed VL and those not suppressed at week 24

7. 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 proportion of participants who develop serious adverse events, ACTG grade 3-4 adverse events (laboratory or clinical) or who require discontinuation of any of the ARVs in the regimen, by week 48. We will describe the numbers and proportions of each type of event.

We will describe the laboratory safety bloods (creatinine at weeks 4, 16 and 48 and full blood count and differential at weeks 4, 8, 12 and 24) using medians and IQRs at each time point.

We will describe the proportion of participants in each treatment arm who develop DTG-related adverse events, including depressive symptoms, symptoms of anxiety and insomnia. We will describe the counts and proportions of each type of event, as well as the time to development of the event and the severity where available.

We will describe the count and proportion of women of child-bearing potential who become pregnant within the study period, and describe the outcomes of the mother and of the newborn child.

We will describe the count and proportion of mortality (all-cause) and the count of specific causes, in the study period.

8. Evaluation of the pharmacokinetic sub-study

A pharmacokinetic sub-study will be conducted on 12 participants in stage 1. Trough concentrations of DTG at day 3, 7, 14, and 28 and off-treatment concentrations of EFV at day 0, 3, 7, and 14 will be measured.

The median (and IQR) residual EFV concentrations in the first 14 days will be presented. The geometric mean ratio (GMR) (with 90% CI) of DTG trough concentrations will be presented as:

- Day 3 vs day 28
- Day 7 versus day 28
- Day 14 versus day 28

The proportion of patients with DTG trough concentrations above the protein adjusted 90% inhibitory concentration (PA-IC_{90}) value at all time points will be described. All metrics will be stratified by *CYP2B6* metaboliser genotype.

9. Pre-specified subgroup analysis relating to the primary endpoint, secondary efficacy endpoints and time to event analysis

The proportion (and 95% confidence interval) of participants who achieve a suppressed VL by 24 and 48 weeks and the time to suppression (Kaplan Meier survival curves) will be described stratified by NRTI resistance present at baseline (3):

- 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)

This stratification is to illustrate any difference in failure or time to failure between groups, although statistical comparisons are likely to be underpowered.

A secondary analysis will also describe the proportion (and 95% confidence interval) of participants who achieve a VL<400 copies/mL at 24 and 48 weeks, and the time to suppression (Kaplan Meier survival curves), stratified by NRTI resistance at baseline.

10. Sensitivity analyses

A sensitivity analysis of the proportion of participants achieving VL suppression at week 24 will be performed including only those participants still in the study and still receiving the study drug at these time points. The following participants will be excluded from this sensitivity analysis:

- Participants who had TFV-DP concentrations < 350fmol/punch at the time point, indicating poor adherence, will be removed from analysis.
- Participants who were lost to follow up (regarded as failure in the mITT analysis) or missing VL within window
- Participants who stopped or were changed from the study drug by 24 weeks for reasons other than failure of the regimen (regarded as failure in the mITT analysis)
- Patients excluded from the mITT analysis for reasons described in section 4 above, will similarly be excluded from this analysis.

11.Protocol deviations

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

12. Publication

The Statistical Analysis Plan will be published online (updated on clinicaltrials.gov) before locking the database.

13. References

1. Moher D, Schulz KF, Altman DG. The CONSORT Flow Diagram. CONSORT Transparent Reporting of Trials. 2010.
2. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and research (CDER). Human Immunodeficiency Virus-1 Infection : Developing Antiretroviral Drugs for Treatment Guidance for Industry Human Immunodeficiency Virus-1 Infection : Developing Antiretroviral Drugs for Treatment Guidance for Industry. 2015.
3. Stanford University. Release Notes for HIVdb, HIVseq and HIValg [Internet]. HIV Drug Resistance Database. 2019. Available from: https://hivdb.stanford.edu/page/release-notes/
4. Anderson PL, Liu AY, Castillo-mancilla JR, Gardner EM, Seifert SM, Mchugh C, et al. Intracellular Tenofovir-Diphosphate and Emtricitabine-Triphosphate in Dried Blood Spots following Directly Observed Therapy. Antimicrob Agents Chemother. 2018;62(1):1–13.
5. Grossberg R, Zhang Y, Gross R. A time-to-prescription-refill measure of antiretroviral adherence predicted changes in viral load in HIV. J Clin Epidemiol. 2004;57(10):1107–10.
6. McMahon JH, Jordan MR, Kelley K, Bertagnolio S, Hong SY, Wanke CA, et al. Pharmacy adherence measures to assess adherence to antiretroviral therapy: Review of the literature and implications for treatment monitoring. Clin Infect Dis. 2011;52(4):493–506.