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AntiRetroviral Therapy In Second-line: investigating Tenofovir-lamivudine-dolutegravir (ARTIST): a randomised controlled trial

ARTIST Trial Protocol Version 4.0.

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Summary

As the HIV epidemic matures and more patients are initiated on antiretroviral treatment, focus is on ensuring that those on antiretroviral therapy (ART) are virologically suppressed. The ideal second-line ART regimen should have a low pill burden, good tolerability, low toxicity, is easily monitored, has a high barrier to resistance and that is low cost. A fixed-dose combination of tenofovir, lamivudine and dolutegravir (TLD) fulfils these criteria.

Indirect evidence suggests that recycling the tenofovir + lamivudine or emtricitabine (TDF/XTC) backbone from first through to second line could protect the regimen from the development of resistance mutations to dolutegravir. Stage 1 of the ARTIST trial has a single arm in which 65 participants who are failing a first-line regimen of tenofovir, emtricitabine or lamivudine and efavirenz or nevirapine (TXE or TXN) are initiated on the fixed dose combination of TLD supplemented with a dose of 50 mg dolutegravir taken 12 hours later for the first 14 days. The fixed dose combination of TLD is continued thereafter for the duration of the study (48 weeks). This strategy of giving a lead-in supplemental dose of dolutegravir is to compensate for the inducing effect of efavirenz on dolutegravir metabolism and transport that persists for 2 weeks after efavirenz is stopped. Patients with undetectable HIV viral load (VL) can be safely switched from efavirenz to dolutegravir without the need for dolutegravir dose adjustment as plasma concentrations of efavirenz remain therapeutic for the 3-6 days it takes for dolutegravir concentrations to become therapeutic. However, most patients failing efavirenz-based ART have resistance to both efavirenz and nucleoside reverse transcriptase inhibitors (NRTIs), therefore, sub-therapeutic dolutegravir concentrations could select for resistance mutations to integrase strand transfer inhibitors (InSTIs).

The original study design for Stage 2 of the ARTIST trial was a randomised, open-label, active-controlled trial aiming to determine the virological suppression of patients failing the standard of care first-line regimen of TXE or TXN switching to a dolutegravir based second-line randomised to one of two NRTI backbones: recycled TDF/XTC or zidovudine and lamivudine (which is recommended by the WHO as the preferred second-line ART combination in the absence of genotype resistance tests at switch).

Enrolment for Stage 1 started on August 08, 2019. As of June 17, 2020, 61 participants were enrolled. At week 24, 33 (87%) of 38 participants achieved virological suppression (defined as plasma HIV VL <50 copies/mL). Four of the 5 participants with HIV VL ≥50 copies/mL at 24 weeks had low level viraemia with HIV VL <100 copies/mL. Results at week 12 were consistent with those at week 24, at which point virological suppression was achieved in 41 (82%) of 50 participants. Adverse events graded 3 or 4 occurred in 4 participants, none of which was considered treatment related or led to discontinuation of TLD. Based on these positive preliminary findings, we decided to change the study design for Stage 2 to a Phase II, randomised, double-blind, placebo-controlled trial of TLD fixed dose combination daily with a lead-in supplemental 50 mg dose of dolutegravir versus matching placebo taken 12 hours later for the first 14 days in patients failing a first-line regimen of TXE. The primary endpoint remains the proportion of participants achieving plasma HIV VL <50 copies/mL at 24 weeks.

Another argument for dropping the zidovudine, lamivudine and dolutegravir (ALD) comparison is that there are two ongoing studies investigating recycling of the TDF/XTC backbone with dolutegravir in second-line. Both the Dolutegravir and Darunavir Evaluation in Adults Failing Therapy (D²EFT) study

and the Nucleosides and Darunavir/Dolutegravir In Africa (NADIA) study are non-inferiority trials powered for formal comparisons and will have results available in 2021. Neither study is using the lead-in supplemental dose strategy. Our proposed new Stage 2 would address this key unanswered question of whether a lead-in dolutegravir dose is required to minimize the risk of developing InSTI resistance and treatment failure in patients switching from an efavirenz-based regimen to TLD with raised VL in a Phase II design that could provide important data to supplement the findings of D²EFT and NADIA in informing policy or a Phase III trial.

1. Background and rationale

As the HIV epidemic matures, focus is turning to the last 90 in the 90-90-90 strategy: aiming for 90% of those on treatment to be virally suppressed by 2020 (1). Antiretroviral (ARV) drug resistance necessitates a change in regimen and is a major obstacle to achieving this target. Resistance results not only in poor outcomes for individuals, but also compromises public health treatment strategies, interferes with efforts to reduce transmission and financially burdens healthcare systems (2).

By 2020, 25.6 million people could be receiving ARVs in Sub-Saharan Africa, and up to 3 million (15.6%) will be on second-line treatment (under conditions of perfect retention, immediate switching and universal viral load (VL) monitoring, as is done in the South African context) (3). This proportion is expected to increase further by 2030 (3). The reasons for this potential growth in demand for second-line treatment are diverse, but include the fact that the cohort on ARVs is maturing; about half the patients on ARVs worldwide started treatment before 2010 (3) and as patients remain on treatment long-term, their cumulative incidence of disengagement increases (4). This population of patients with poor engagement has a large requirement for second-line treatment, as 30% who then restart first-line treatment have been shown to have resistance mutations (5). Challenges with antiretroviral therapy (ART) adherence while on treatment also contribute to first-line ART failure and requirement for second-line.

1.1. Dolutegravir as second-line

Currently in the Western Cape, the standard of care for first-line treatment is the fixed dose combination of tenofovir, emtricitabine and efavirenz (TDF/FTC/EFV – TXE regimen, where X can stand for emtricitabine (FTC) or lamivudine (3TC) interchangeably) unless there are contraindications, and the first choice second-line is zidovudine, lamivudine and lopinavir/ritonavir (AZT/3TC/LPV/r) (6). This guidance is in-line with World Health Organization (WHO) recommendations (7).

Those requiring second-line treatment often have health facility and regimen-related barriers to engaging with treatment, but second-line options are less ‘adherence-friendly’ than the first-line ARVs that have already failed. Second-line options are currently 2.5 to 3 times more expensive than WHO recommended first-line treatment (3,8), require additional monitoring (haemoglobin for AZT toxicity and cholesterol and triglycerides for LPV/r) (6), require a more burdensome twice daily dosing (9) and are poorly tolerated (6,9). The gastro-intestinal side effects of the protease inhibitors (PIs) make adherence difficult, increasing the risk of the development of virologic failure and contributing to the reluctance of patients

and clinicians to switch to second-line.

The WHO recommends regimens that have low toxicity, low cost, high genetic barriers to resistance, high potency, and usefulness across different populations (10). Dolutegravir (DTG), an integrase strand transfer inhibitor (INSTI), fulfils many of these criteria: it has good tolerability and has been shown to have a lower risk of discontinuation in a regimen with two nucleoside reverse transcriptase inhibitors (NRTIs), has a low pill burden with once daily dosing (11,12), as well as a lower potential for drug-drug interactions than the current standards of care (EFV and LPV/r) (12). At $75 per person per year, a new generic fixed dose formulation of DTG with TDF and 3TC is cheaper than both current first and second-line options (13). A major transition to newer drugs, including DTG, could represent savings up to US$3 billion globally by 2025 (14) and allow more people to be on ART within available resource limits (15).

Multiple studies have shown DTG to be a more efficacious option for first-line compared with EFV (SPRING-1 and SINGLE), raltegravir (SPRING-2) and darunavir (DRV)/ritonavir (r) (FLAMINGO) (11). As a second-line regimen it has proved superior to raltegravir with an optimised backbone in patients with resistance to at least two ARV classes, irrespective of the backbone drugs (SAILING) and has been shown to be effective with twice daily dosing at achieving viral suppression even in the presence of resistance to other InSTIs (VIKING, VIKING-3 and VIKING-4).

In the DAWNING study, DTG with two NRTIs (at least one fully active) was superior in safety and efficacy to the current standard of care (LPV/r with two NRTIs) as second-line treatment, achieving 82% viral suppression (VL<50 copies/mL) compared to 69% of the LPV/r patients (16). This provides compelling evidence to consider DTG as the preferential second-line option. The WHO now supports the use of DTG as second-line for its superiority in viral suppression, quicker CD4 recovery, tolerability and safety profile, and suggests that both adults and children failing a non-nucleoside reverse transcriptase inhibitor (NNRTI) or PI based first line regimen should be given DTG as the preferred second-line option (12). The South African public sector introduced a second-line regimen of ALD for patients failing TXE in January 2020 (46).

1.2. Choice of NRTI backbone

1.2.1 DTG Resistance

DTG selects for the R263K mutation and sequential mutations along the same pathway that reduce the efficacy of DTG. However the R263K mutation may reduce viral replicative capacity and reduce the magnitude of viral rebound (11,17). DTG has a very high barrier to resistance, but resistance has been described using DTG monotherapy as maintenance in the DOMONO study (three patients) (18) and the MONCAY study (two patients) (19). In both studies, this resistance was delayed and tended to occur 6-12 months after switch (18,19).

DTG resistance has also been described in patients on DTG as second-line. The DAWNING and SAILING studies each found two patients who developed InSTI (including R263K) mutations (17,20). There have also been reports of the development of InSTI resistance in treatment-naïve patients initiating TDF/3TC/DTG (TLD regimen) (21,22), with the R263K mutation as a pathway to the development of

resistance (22). This potential for the development of resistance means that the backbone must be carefully considered in order to ensure the durability of a DTG-based second-line regimen.

1.2.2 Optimised backbone

The DAWNING study showed superiority of DTG with an optimised backbone over a PI second-line and the results can be applied when there is at least one fully active NRTI (16). Thus the WHO recommends the use of an optimised backbone to improve the durability of DTG regimens (2,10,12). However, optimisation requires genotypic resistance testing, and in the DAWNING study genotyping was used to ensure the selection of at least one fully active NRTI (16). This testing is not widely available in low and middle income countries (LMICs) (23), and where it is, its use is restricted by cost to second-line failures only. Without genotypic testing to determine the optimum regimen, the WHO recommends AZT (ALD regimen) as the first choice backbone for second-line if failing a regimen containing TDF or abacavir (ABC) (12).

1.2.3 Recycling TDF and XTC (3TC or FTC) in a second-line backbone

However, there are two arguments for recycling TDF and 3TC or FTC as the backbone for a DTG-based second-line (TLD regimen) instead of using an optimised backbone. Firstly AZT is less well tolerated, requires twice daily dosing, needs more intensive monitoring and is less efficacious than TDF if there is no resistance to either drug (6,9).

Secondly, it is well established that NRTI resistance impairs viral fitness in both in vitro and clinical studies, particularly the M184I/V mutations (3TC and FTC). In the EARNEST trial, the NRTI/LPV/r combination outperformed the LPV/r/raltegravir and LPV/r monotherapy arms (86%, 81% and 78% suppression respectively) in patients who were no longer responding to a first-line combination containing two NRTIs, even though 95% of the enrolled patients had at least one NRTI mutation and 59% had no active NRTIs (24). Similar results were found in the SELECT and the SECOND LINE trials (25,26), showing that compromised NRTIs could be recycled with a boosted PI.

Oliveira et al showed that under DTG drug pressure in vitro, only the wild type virus was able to acquire R263K resistance mutations and that K65R (TDF) and M184I/V mutations protected against the development of DTG resistance (an additional advantage over raltegravir and elvitegravir, which were not afforded this protection) (2). In this study the effect of the combination of K65R and M184I/V mutations was not assessed, but as these mutations do not compensate each other it is suspected that this combination will have the same protective effect together as separately (2).

While patients have developed DTG resistance mutations on DTG monotherapy (18), clinical studies suggest that resistance does not develop if DTG is paired with even an inactive backbone. DTG with 3TC as dual therapy was found to be non-inferior to triple ART and no resistance was found (GEMINI 1 and 2 trials), showing that even if TDF is not active, the regimen is efficacious (20). Post-hoc analysis of the SAILING study found that in patients with resistance to at least two classes, those receiving DTG with two NRTIs did not develop DTG resistance, even when both NRTIs were inactive (27).

Multiple small studies have also supported this finding: the DOLULAM switch trial found that none of

the 27 patients switched to DTG/3TC dual therapy developed virological failure by 96 weeks, despite 37% having the M184V mutation (28). A French study that switched 239 virally suppressed patients to a DTG-based regimen, found that regardless of the genotypic susceptibility score, only one patient (who had a history of raltegravir use) developed virological failure (29,30). This suggests that the crippling effect of the NRTIs translates to protection from the development of second-line resistance in vivo and that a second-line TLD regimen could be effective.

There are two ongoing studies investigating the recycling of the TDF/XTC backbone. The DTG and Darunavir Evaluation in Adults Failing Therapy (D²EFT) is an ongoing non-inferiority trial to compare TDF/XTC/DTG, DTG/DRV/r and the WHO standard of care NRTI/PI (DRV/r) combination in HIV-1 patients failing first-line ARVs in predominantly LMICs. The backbone in the standard of care arm will be based on clinical judgement and may be guided by resistance testing if available, whereas the backbone in the experimental NRTI-DTG arm is pre-specified as TDF/XTC (31). This study is expected to be completed in December 2021 (31). The NADIA study is a Phase III trial to investigate DTG and DRV/r with TDF/XTC compared to an AZT backbone as a second-line option for 420 patients failing EFV-based first-line treatment. While D²EFT and NADIA are conducted, our study aims to investigate the recycling of the TDF/XTC backbone with DTG (TLD) and provide evidence in the interim to address this important public health question.

1.3 Dosing

EFV induces enzymes (UGT1A1 and CYP3A4) and transporters (P-glycoprotein and breast cancer resistance protein) involved with DTG absorption and metabolism, which reduce plasma DTG concentrations at the end of the dosing interval up to 75% (33, 34). In a study assessing a DTG/EFV combination, DTG required twice daily dosing when co-administered with EFV (33). However, simulations of switching from EFV to DTG based regimens done by Generaux et al. estimated that EFV concentrations remains above the minimum effective concentration (MEC) up to 3 days after discontinuation, and DTG trough concentrations reach MEC 3 days after switch. In *CYP2B6* slow metaboliser genotypes, this is extended for DTG to 6 days post switch, however the EFV concentrations take 8 days to drop below the MEC (34).

Generaux et al. thus predict that no DTG dose adjustment is required when switching from an EFV-based regimen in virologically suppressed patients. A pharmacokinetic sub-study of the STRIIVING study confirmed this in patients, finding that DTG concentrations were maintained above the IC90 (concentration required for 90% inhibition) at all times after switch from an EFV-based regimen, and that there was no point post-switch at which both EFV and DTG concentrations were below their respective IC90 concentrations (35).

However, our study population will be switched with a raised VL and a high likelihood of EFV and NRTI resistance mutations; in this setting exposure to sub-therapeutic concentrations of DTG could drive the development of InSTI resistance mutations. Thus in Stage 1, patients are initiated on a supplemented regimen of TLD with 50 mg DTG taken 12 hours later for the first 14 days, after which they receive TLD once daily for the duration of the study (48 weeks).

1.4 Rationale for protocol amendment

Between August 08, 2019 and June 17, 2020, 61 participants were enrolled in Stage 1. The primary efficacy outcome was defined as the proportion of participants achieving plasma HIV VL <50 copies/mL at 24 weeks. Our interim findings suggest excellent virological suppression with TLD in second-line with a lead-in dose of DTG 50 mg twice daily for the first 14 days, showing 33 (87%) of 38 participants achieving HIV VL <50 copies/mL at 24 weeks. 37 (97%) participants achieved HIV VL <100 copies/mL at 24 weeks. Secondary outcomes were the proportions of participants achieving virological suppression at 12 and 48 weeks. Results at week 12 were consistent with those at week 24, at which point virological suppression was achieved in 41 (82%) of 50 participants. The higher dose of DTG at 50 mg twice daily for the first 14 days was well tolerated. No drug-related grade 3 or 4 adverse events occurred. Virological failure (defined as 2 consecutive VL >1000 copies/mL after 12 weeks) on TLD as second-line was not observed, despite high levels of baseline NRTI drug resistance, with 65% of participants having no fully active NRTI. Review of Stage 1 preliminary findings led us to modify the study design for Stage 2 to a Phase II, randomised, double-blind, placebo-controlled trial of TLD fixed dose combination daily with a lead-in supplemental 50 mg dose of DTG versus matching placebo taken 12 hours later for the first 14 days in patients failing a first-line TXE regimen.

This strategy of giving a lead-in supplemental dose of DTG is in view of the inducing effect of EFV on DTG metabolism and transport that persists for 2 weeks after EFV is stopped. It is safe to switch patients with undetectable VL from EFV to DTG without the need for dose adjustment. Additionally, pharmacokinetic studies show that as plasma concentrations of EFV fall, there are gradual rises in DTG plasma concentrations so that at no time point are both drugs at sub-therapeutic concentrations. However, DTG plasma concentrations are slightly below normal concentrations for the first 2 weeks of dosing and this might be an issue for those that already harbour NRTI and EFV drug resistance, which is common in patients failing first-line regimens. Previous TLD efficacy without a lead-in supplemental dose of DTG has also only been proven in virologically suppressed patients switching from TXE to TLD. In patients with virological failure on a first line TXE regimen switching to TLD, we will investigate whether DTG dose adjustment to achieve effective plasma concentrations during the period that induction wanes is required to avoid InSTI resistance and thus virological failure.

We decided to drop the ALD arm as there are two ongoing studies investigating the recycling of the TDF/XTC backbone with DTG in second line. Both D²EFT and NADIA will address the question of whether patients with virological failure on TDF-based first line regimen must switch to AZT, or can remain on TDF. However, neither of the ongoing large trials investigating TLD as second-line is using the lead-in supplemental dose strategy. Our proposed new Stage 2 would thus address a knowledge gap that these two trials are not addressing.

The additional dose of DTG may be challenging to implement in high burden settings. The need for DTG as single tablets increases cost, pill burden and the risk of stock outs. However, DTG single tablets need to be made available for second-line treatment as part of the ALD combination. If the TLD combination with lead-in supplemental dose strategy is proven to be effective, it would reduce the pressure on DTG demand by requiring only a 2 week supply of DTG rather than an ongoing supply as would be required for ALD as second-line.

DTG 50 mg twice daily was well tolerated in an InSTI experienced population in the VIKING study, with similar safety profile to that observed for DTG 50 mg once daily. No association was found between DTG exposure and relevant safety parameters (48). Nevertheless, a variety of safety measures will be put in place to ensure that no harm will come to the participants.

One major risk to participants is the development of ARV resistant mutations. We will monitor for virological failure at frequent intervals. Virological suppression will be monitored by a drug safety monitoring board (DSMB) who will convene at two monthly intervals. Resistance testing will be performed on those participants who develop virological failure and an alternative ART regimen will be available should DTG resistance develops. During follow up visits, any adverse events reported by participants or observed by the investigators will be recorded in the source documents. Adverse events will be graded for severity according to the Division of AIDS table, and any serious adverse events (SAE) will be reported to the principal investigator immediately. SAEs and all unexpected adverse events suspected to be related to the study drugs will be reported to UCT's HREC according to the current guidelines.

1.4 Importance

There are already 500 000 people using DTG worldwide, with South Africa lagging behind Botswana, Kenya and Brazil who have already started using DTG as the first-choice first-line (12). Many of these countries are switching patients on a TXE blindly to TLD in a setting where VL monitoring is not readily available (<50% of patients in LMICs have access to regular VL testing (23)). As of July 2018, there is no clinical data to support switching patients from TXE directly to TLD without a known, suppressed VL (23). Evidence on viral suppression and the development of DTG resistance mutations in the presence or absence of resistance to the backbone drugs would be invaluable in informing this strategy further and in interpreting patient outcomes. The ongoing studies, such as D²EFT and NADIA will have results available from 2021, thus interim answers to the question of a TDF/XTC backbone with DTG in second-line are urgently needed.

Furthermore, patients who struggle with adherence and engagement are a vulnerable group of patients who need better second-line options as part of a strategy to support their care. DTG has already proven an attractive second-line option, and a regimen with a recycled TDF/XTC backbone addresses many of the problems with the current second-line options, affording a unique opportunity to test a second-line regimen that could potentially be better than our current first-line option.

Finally, our hypothesis is that a lead-in supplemental dose of DTG may reduce the risk for the development of resistant viral strains and thus treatment failure. The alternative hypothesis is that the lead-in dose is not required and virological suppression and protection from emergence of resistance will be comparable in both arms. To our knowledge, this is the first study to evaluate the strategy of a lead-in supplemental DTG dose in patients with virological failure switching from an EFV-based to DTG-based regimen. If the proportion of patients achieving virological suppression is acceptable in both arms, this study together with other studies being conducted on TLD in second-line could pave the way for a Phase III trial to address this question or could directly influence clinical policy.

2. Problem statement

In order to increase retention on second-line regimens as well as increase the availability of treatment to a growing population in need of second-line options, more efficacious, cheaper regimens with better tolerability and a lower pill burden are required. DTG fulfils the WHO recommended criteria and overcomes most of the issues associated with current second-line options.

The question of which backbone to use is still open and results of the D²EFT and NADIA studies will not be available until 2021 at least. We hypothesise that when DTG is used with TDF and 3TC, even if both are compromised by resistance mutations, the residual activity and crippling will result in an effective second-line regimen that has good tolerability, a low pill burden, low monitoring requirements and low costs.

As TDF/XTC in second-line is not an established second-line NRTI backbone, we will do frequent VL testing as well as genotypes (at baseline and in the event of failure) to clarify the impact that the combination has on outcomes in the presence or absence of K65R and M184I/V mutations. An assessment of impact on viral suppression and retention in care of a TLD regimen in second-line patients who have failed a first-line TXE regimen, can only be undertaken as research with close monitoring.

Our interim findings from Stage 1 showed excellent virological suppression with TLD in second-line with a lead-in dose of DTG 50 mg twice daily for the first 14 days. In the new Stage 2, we will evaluate virological suppression with and without this lead-in dose.

3. Research aims and objectives

3.1. Research aims

The aim of this study is to determine the proportion of patients achieving virological suppression when recycling the TDF/XTC backbone with DTG (TLD combination) as a second-line with and without a lead-in supplemental dose of DTG, in patients failing a TXE first-line regimen.

3.2. Research objectives

Primary objectives

1. Stage 1: to describe the proportion of patients achieving HIV VL <50 copies/mL at 24 weeks on TLD (with a lead-in supplemental DTG dose for 14 days) as a second-line regimen in patients who have failed a TXE first-line regimen
    a. Overall
    b. Stratified by the presence or absence of resistance to both TDF and 3TC on initiation of the second-line regimen
2. Stage 2: to describe the proportion of patients achieving HIV VL <50 copies/mL at 24 weeks on TLD with and without a lead-in supplemental DTG dose for 14 days, as a second-line regimen in

patients who have failed a TXE first-line regimen

a. Overall
b. Stratified by the presence or absence of resistance to both TDF and 3TC on initiation of the second-line regimen

Secondary objectives

1. To describe the proportion of patients achieving HIV VL <50 copies/mL at other time points (12 and 48 weeks) and by per protocol analysis, on TLD as a second-line regimen in patients who have failed a TXE first-line regimen in Stage 1 and 2
    a. Overall
    b. Stratified by the presence or absence of resistance to both TDF and 3TC on initiation of the second-line regimen
2. To determine the proportion of patients who develop DTG resistance mutations and new NRTI mutations on second-line TLD
    a. To determine whether the development of DTG resistance mutations is associated with the presence or absence of resistance to both TDF and 3TC on initiation of the second-line regimen
3. To describe the resistance profile of patients failing a TXE first-line regimen in this setting
4. To evaluate the trough concentrations of DTG when switching from an EFV based first-line regimen, and to assess the pharmacokinetic requirement for a lead-in supplemental dose of DTG
5. To describe markers of adherence between those failing TLD at 24 and 48 weeks and to compare to a group of matched controls
6. To describe other clinical characteristics, adverse events and all-cause mortality among the study cohort

4. Methodology

5.1. Study design

This study will be a Phase II, randomised, double-blind, placebo-controlled trial of TLD fixed dose combination daily with a lead-in supplemental 50 mg dose of DTG versus matching placebo taken 12 hours later for the first 14 days in patients failing a first-line TXE regimen. Given that these patients will all have elevated VL, the high baseline risk of NRTI and EFV resistance these patients are at and the inducing effect of EFV on DTG metabolism that persists for 2 weeks after EFV is stopped, we propose two stages:

- Stage 1: patients initiated on TLD with a lead-in supplemental dose of 50 mg DTG taken 12 hours later for 14 days (to make the dose 50 mg twice daily), with continuation of TLD for the duration of the study (48 weeks)

- Stage 2: will describe VL suppression in two parallel arms, but will not be powered for a formal

non-inferiority comparison between the two:

- **Arm 1:** patients initiated on DTG at 50 mg once a day as part of a fixed dose combination of TLD, with a lead-in supplemental 50 mg dose of DTG taken 12 hours later for 14 days, with continuation of TLD for the duration of the study (48 weeks)

- **Arm 2:** patients initiated on DTG at 50 mg once a day as part of a fixed dose combination of TLD, with a matching placebo taken 12 hours later for 14 days, with continuation of TLD for the duration of the study (48 weeks)

A pharmacokinetic sub-study will be conducted on 12 participants in Stage 1 and in 24 participants in Stage 2 (the first 36 participants in both stages who consent and is logistically feasible for enrolment into the pharmacokinetic sub-study), to assess the trough concentrations of DTG and off-treatment concentrations of EFV at day 3, 7, 14, and 28. Eligibility criteria are not different from eligibility for the parent study, with the only additional inclusion criterion being willingness to participate.

Progression from Stage 1 to Stage 2 will be based on the lower bound of the 95% confidence interval (CI) for the primary endpoint (VL suppression at 24 weeks) in a subset of the first 23 participants being ≥65.0%. This proportion will be determined in participants who have an HIV VL done at 24 weeks, have had a pharmacy refill collected at the prior visit and who are still on TLD (i.e. per protocol analysis to decide on progression to Stage 2).

If the lower bound of the 95% CI for the primary endpoint of the first 23 participants is <65.0%, enrolment for Stage 2 will not commence but the follow up of participants in Stage 1 will continue to 24 weeks. If it is ≥65.0%, the enrolment of participants for Stage 2 will commence alongside the follow-up of Stage 1 participants.

Whether Stage 2 enrolment has commenced or not, the results will be re-evaluated after the full cohort of 65 participants reaches 24 weeks.

1. If the lower bound of the 95% CI is ≥65.0%, enrolment for Stage 2 can proceed if it has not already after the interim analysis of 23 participants.

2. If the lower bound remains below 65.0% or is now <65.0% after enrolment for Stage 2 has already started, we will refer to the DSMB for a decision on whether to not progress to Stage 2 or continue Stage 2 enrolment and on the further follow up of Stage 1 patients, with the option of discontinuing the trial and transferring patients back into clinic care (as outlined in the safety section of the protocol).

This study is not powered for formal statistical comparison between the two arms in Stage 2 yet informal comparison of virologic suppression (point estimates and 95% CI) achieved in the two arms will be possible. There are precedents for conducting Phase II non-comparative trials with active controls arms to evaluate virologic suppression endpoints with InSTI-based ART regimens. The INSPIRING study was also a non-comparative, active-control, randomised, open-label study with two arms randomising patients on tuberculosis treatment to DTG or EFV-based treatment. It was designed to describe the primary

outcomes at 48 weeks with 95% CI without making claims on the statistical significance of comparisons (36). The REFLATE (Raltegravir for the treatment of patients co-infected with HIV and tuberculosis) study randomised patients on tuberculosis treatment to three non-comparative ART arms and describes the virological suppression at 24 weeks in each arm without statistically comparing them (37). In the same way, our study will describe the outcomes in each arm without making statistical claims of significance in a comparison between the two arms.

5.2. Study area and setting

The study will be conducted through the ART clinic, Welcome Service and Risk of Treatment Failure (ROTF) programmes at Michael Mapongwana Community Health Centre (CHC) and Ubuntu Antiretroviral Clinic at Site B CHC in Khayelitsha.

Khayelitsha is a large, peri-urban informal settlement outside of Cape Town, South Africa. It is home to approximately 500 000 people, most of whom speak isiXhosa as their first language. It has a large population of people living with HIV (43 281 patients on antiretrovirals in 2018).

The Welcome Service is a Médecins Sans Frontières (MSF) intervention for patients who have disengaged with care or who are intermittently engaging with services. It links these patients to the clinic and provides a package of medical and psychosocial support over nine visits, with the aim of improving retention in care and preventing treatment failure. It is suspected that many of these patients already harbour resistant mutations and will require switch to second-line treatment. It is provided at Michael Mapongwana CHC and Ubuntu Antiretroviral Clinic at Site B CHC currently.

The ROTF programme is a four-visit series of nurse-led enhanced adherence settings for patients identified with a high VL. It was implemented by MSF and is now run by the clinic staff and supported by other NGOs. We also suspect that this population already harbour resistant mutations as many patients do not suppress even after the enhanced adherence support and require switch to second-line. This service is provided at Michael Mapongwana CHC and Ubuntu Clinic.

Patients will be enrolled at both sites through the ART clinics, ROTF and the Welcome Service.

5.3. Study population and sampling

5.3.1 Eligibility

5.3.1.1 Inclusion criteria

HIV positive patients over 18 years old, who have failed their first-line ART regimen of TXE, are able to attend the study clinic for one year of scheduled visits and who have given written, informed consent will be enrolled in this study. In female patients of child-bearing potential, those willing to use effective and

reliable contraception for the duration of the study will be eligible (see 'Management of women of child bearing age in both phases')

Failure of a first-line regimen is defined as:

- A VL of >1000 copies/mL (within the previous two months) and an immediately prior VL >1000 copies/mL, taken 2-24 months prior (based on data captured by National Health Laboratory Service(NHLS))

5.3.1.2 Exclusion criteria

- If the patient has two VLs 2-3 months apart: >2 log drop in VLs between the most recent VL (within the previous two months) and the immediately prior VL (taken 2-3 months prior)
- CD4 count <100 cells/µl
- Renal impairment (estimated Cr Cl <50 ml/min using the MDRD formula)
- ALT >100 U/L or total bilirubin >twice the upper limit of normal
- Pregnant or desire to become pregnant during the study period (48 weeks)
- Breastfeeding
- Being treated for active tuberculosis or concern that patient has undiagnosed active tuberculosis (based on symptom screening) as rifampicin reduces the concentrations of DTG and thus requires dose adjustments (10)
- Any current diagnosis of malignancy
- Allergy or intolerance to one of the drugs in regimen
- Active, severe psychiatric disease judged likely to impact adherence
- Current substance abuse judged to be likely to impact adherence
- On treatment for AIDS-defining condition (not including secondary prophylaxis maintenance therapy)
- Any other clinical condition that in the opinion of an investigator puts the patient at increased risk if participating in the study

5.3.3. Management of women of child bearing age in both phases

Women of child-bearing age will not be excluded from this study, but their reproductive health needs will be actively managed as pregnancy and the intention to become pregnant within the 48 weeks of the study are exclusion criteria. This is because of an identified signal indicating a potential increase in risk of neural tube defects in infants born to women taking DTG at conception (38). Since August 2014, the Botswana Harvard AIDS Institute Partnership has monitored birth outcomes at eight government hospitals in Botswana to evaluate the prevalence of neural-tube defects associated with ART exposure at the time of conception, alongside Botswana's implementation of DTG as the first-option first-line ART across the country from 2016. As of May 2018, four infants born to women taking DTG at the time of conception had been found to have a neural-tube defect, equating to a 0.94% prevalence compared to 0.12% in the infants born to mothers taking a non-DTG ART at conception (38). Ongoing birth-outcome surveillance in Botswana showed decreasing prevalence of neural tube defects associated with DTG exposure at conception, however it is still slightly higher than with exposure to non-DTG ART at conception (3 per 1000 deliveries vs. 1 per 1000 deliveries) (47).

The WHO recommends a woman-centred approach to the management of this risk, empowering women to make informed decisions about their health. There are no reported or expected drug-drug interactions between DTG and hormonal contraception, thus the WHO recommends providing women of childbearing potential with reliable contraception alongside DTG (12).

Women of child-bearing potential consenting to take part in this study will be required to commit to the use of effective and reliable contraception, including IUCDs, injectable, implantable or oral hormonal contraception, for the duration of the study. The study will facilitate their access to these through government services and study doctors will be required to frequently check that this is maintained. We will also implement additional counselling about the potential risks associated with the use of dolutegravir and conception so that fully informed consent to participate is obtained.

Pregnancy tests will be done at baseline and regularly throughout the study. If a woman is found to be pregnant on the DTG arm during the study or expresses the wish to become pregnant, she will be switched to a standard PI-based second-line. If a woman becomes pregnant during the study period, she will be followed up throughout the pregnancy. We will refer the mother and infant pair to a paediatric colleague at Red Cross Childrens Hospital for a formal assessment for foetal anomalies in the first 4 weeks of the post-partum period. Outcomes will be reported to the ethics committees and DSMB. Any pregnancy complication that results in a non-viable pregnancy outcome (eg. miscarriage) will be investigated in partnership with gynaecology and obstetric colleagues and reported to the ethics committees and DSMB.

5.3.4. Sample size

Assuming VL suppression (HIV VL <50 copies/mL at week 24) of 82% (as achieved by the DTG arm at 24 weeks in the DAWNING trial (16)) is achieved at the modified intention to treat (mITT) analysis at 24 weeks, a sample size of 57 will produce a 95% CI of 72 - 92%. To account for participants discontinuing the regimen or dropping out (e.g. stopping contraception), 65 patients will be enrolled into Stage 1 and into each arm of Stage 2 (total of 195 participants).

Sample size for the Pharmacokinetic study

We used SAS Programmer 9.4 (SAS Institute, Cary, NC, USA) to calculate the sample size. In Stage 1 it is unclear what the geometric mean ratio (GMR) of TDG trough concentrations between an EFV-induced and non-induced state will be for 50 mg DTG 12 hourly, but it is likely that GMR will exceed 1. A GMR of 1.22 was reported in a study of 12 participants that assessed the interaction between rifampicin and DTG – 14 days on rifampicin with 50 mg DTG 12 hourly versus 7 days on only 50 mg DTG daily (40). Given that EFV is a weaker inducer than rifampicin it is not unreasonable to assume that the GMR will be higher than 1.22 making 12 enrolled participants in Stage 1 adequate.

Based on a pharmacokinetic study of patients switching from EFV to DTG, we expect the GMR of DTG trough concentrations on day 7 versus day 28 to be 0.66 for participants receiving 50 mg DTG daily (39). With an expected coefficient of variance of 55%, power of 80%, and 10% level of significance a sample of 10 participants would be sufficient to determine a difference of this magnitude in the GMR for participants not receiving a lead-in supplemental DTG dose. Based on an assumed withdrawal rate of 20% we will need to enrol 12 participants. In order to compensate for the blinding in Stage 2 the sample size will be doubled to 24 participants. Therefore a total of at least 36 participants will be enrolled in the

pharmacokinetic sub-study.

Sample size for the analysis to progress from stage 1 to stage 2

Assuming a VL suppression (HIV VL <50 copies/ml at week 24) rate of 82% is achieved at the mITT analysis at 24 weeks, a sample size of 23 will produce a 95% CI of 66 - 98%. Thus the decision to progress from Stage 1 to Stage 2 will be taken after 23 participants have completed 24 week assessment and who have an HIV VL done at 24 week who have had a pharmacy refill collected at the prior visit and who are still on TLD.

5.4 Study procedure

5.4.1. Recruitment & pre-screening

Patients will be recruited from the clinics, the Welcome Services and ROTF at both study sites. The clinician at their routine visit will identify eligible patients from their routine VL, explain the purpose of the study and make clear that the patient's routine care is in no way contingent on participation in the study. If the patient agrees, they will be referred to the study for screening and enrolment.

MSF has played a key role in the Ubuntu Site B and Michael Mapongwana ART clinics since 2002. MSF initially established these clinics in partnership with the provincial government and after handing over the running of these clinics to the province, has continued to play a strategic role in supporting key initiatives aimed at enhancing quality of care and outcomes of patients on ART with a focus on patients with specific challenges. Presently, MSF is supporting initiatives aimed at enhancing retention in care and reducing delays in switches to second line ART in clinics in Khayelitsha. As part of this MSF has permission to access electronic data systems which flag patients who have had repeated high HIV VLs and are eligible for switch to second line. We propose that MSF staff will work with provincial staff at the clinic to flag such patients and ask them to attend the clinic for a discussion regarding switching to second line with clinic staff. As part of this discussion the option of enrolling in the ARTIST trial will be discussed. If they are interested they will be referred to the study staff. If patients do not wish to enrol in the ARTIST trial but are eligible for second line switch this will be done within the ART clinic according to state protocols and separate from the trial.

5.4.2. Informed consent

After referral from the Welcome Service/ROTF clinician, the patient will be contacted by the study staff. They will be taken through the process of informed consent and will have the study explained in detail by the counsellor, with the study nurse and doctor available to answer any questions. If they agree, informed consent will be taken.

5.4.3. Screening & enrolment

Screening

The patients will be seen by the study nurse and doctor who will perform an assessment for eligibility, perform all blood tests and manage the collected specimens.

The bloods taken on the screening visit will be sent for a CD4 count, creatinine, total bilirubin and ALT. A urine dipstick pregnancy test will be performed if the patient is female, unless the patient is post-menopausal.

Clinical status (history and examination), mental health status (history), substance abuse, tuberculosis status (symptom screening and history) and the patient's desire to fall pregnant will also be assessed.

Enrolment

Enrolment (week 0 visit) will take place within 8 weeks of the screening visit. If a patient returns after this time they will require re-screening.

On enrolment, the first 65 participants will receive TLD with supplemental DTG 50 mg for the first 14 days. The subsequent 130 participants will be randomised 1:1 to either TLD daily with a lead-in supplemental 50 mg dose of DTG or matching placebo taken 12 hours later for 14 days and started on treatment at the week 0 visit. Baseline VLs (and urine pregnancy tests in female participants) will be performed on week 0. Participants will be examined and contraception use and desire to fall pregnant checked.

Neuropsychiatric testing, neurocognitive testing and sleep assessments will be performed by a trained doctor or nurse at baseline (enrolment visit). The following neuropsychiatric and neurocognitive tests will be performed:

- Brief Symptoms Inventory – 18, Anxiety Subscale (BSA)
- Centre for Epidemiologic Studies Depression Scale (CESD)
- Simioni Neurocognitive Symptom Questions
- Cognitive Assessment Tool – Rapid Version 2.0 (CAT-rapid V2)

Blood for genotypic resistance testing will be taken and stored for testing at the end of the study. In Stage 1 and Stage 2, dried blood spots (DBS) will be taken for storage to test concentrations of tenofovir-diphosphate (TDF-DP) after analysis of VL suppression, in order to assess adherence. Samples will be securely stored under appropriate conditions at the CIDRI-Africa lab until analysed.

An extra blood sample will be taken at enrolment for DNA and stored for genetic testing at the end of the study, to determine *CYP2B6* metaboliser genotype. The following single nucleotide polymorphisms (SNP) will allow categorisation of participants into slow, intermediate, and extensive metabolisers: *CYP2B6* 516G→T (rs3745274), 983T→C (rs28399499), 15582C→T (rs4803419), and *CYP2A6* 48A→C (rs28399433) (42). We will also store DNA for future genetic studies that are related to HIV and its treatment, such as evaluation for genetic polymorphisms associated with DTG metabolism. We would require HREC approval of any such future study. A separate informed consent for genetic testing will be performed at screening visits. For participants already enrolled in Stage 1, informed consent for genetic testing will be performed at the next study visits and consenting participants will have samples for storage collected at the same visits. We intend to approach all participants in Stage 1 and Stage 2 to participate in the genetic sub-study (n=195).

Randomisation

The participants enrolled in Stage 1 will not be randomised. Randomisation will occur in Stage 2 to either

TLD daily with a lead-in supplemental 50 mg dose of DTG or matching placebo. The purpose of the Stage 1 is to provide initial data regarding the safety of the strategy with the lead-in DTG dose. The purpose of Stage 2 is to evaluate the strategy with and without the lead-in supplemental dose of DTG and allow informal comparison between the two arms, and thereby provide preliminary Phase II data as to the requirement for a lead-in dose. After the initial 65 participants are enrolled in Stage 1, subsequent participants in Stage 2 will be randomized in a 1:1 ratio to receive either TLD daily with a lead-in supplemental 50 mg dose of DTG or matching placebo. A randomisation sequence will be prepared by an independent pharmacist before Stage 2 commences using block randomisation utilizing a block size of 6. The independent pharmacist will prepare opaque sealed envelopes labelled 1 to 130 containing the allocation assignment for the sequentially enrolled participants. The independent pharmacist will share the randomisation sequence with the study pharmacist and study statistician. The trial will be double-blind. Only the independent and study pharmacists and study statistician will have access to the randomisation code.

5.4.4. Follow-up Visits

Patients will be followed up at weeks 2, 4, 8, 12, 16, 20, 24, 36, 48 and 52 weeks after enrolment.

A doctor or nurse will perform the clinical consultation, ARV prescription and subsequent follow-up assessment. Sleep assessment will be done at every visit. Neuropsychiatric and neurocognitive testing will be done at weeks 0, 2, 4, 12, 24, and 48. The study nurse will perform all blood tests and manage the collected specimens, as well as provide standardised enhanced adherence counselling along with the counsellor for all patients in the study. This will be based on the enhanced adherence counselling that was developed for patients in the Welcome Service. Contraception use and pregnancy wishes will be checked at each visit (for female participants), as well as the physical status of the patient. Self-reported adherence will also be determined by the study nurse.

Participants will be given a window period of 16 days to attend after the scheduled appointment, and may also attend up to 16 days before the scheduled visit. A text message will be sent to participants the day before the appointment to remind them. If participants miss an appointment a text message will be sent the next day to follow up, and if they do not return or make contact by day 3 after the scheduled appointment date, they will be called. Consent for text messaging and calls will be specifically obtained.

If participants miss three consecutive visits they will be considered lost to follow up (LTFU) by the study definition and traced by routine clinic procedures (counsellor phone calls and community care worker home visits) in order to assess their clinical status and adverse events and to encourage their return to care. This is both to ensure complete data collection and ensure continuity of care for the patient. If they return to care, the patient will continue to be seen by the study staff for the remainder of their 48 weeks after enrolment to ensure full follow up. If the LTFU was triggered by an adverse event or other outcome which may have implications for the research, as judged by the PI, this will be reported to the DSMB and ethics committees. This information will be gathered either by phone or at home visit or when they return to care.

5.4.5. Monitoring

HIV VL will be performed at baseline and at weeks 4, 8, 12, 16, 20, 24, 36, and 48. If any of the VL after

week 12 are >50 copies/mL, or if there is <1 log decline in HIV VL from baseline value at any visit from week 4 onward, or if VL was suppressed at any time during treatment and subsequently rebound to >50 copies/mL, enhanced adherence support will be given and the VL repeated 2 weeks after enhanced adherence counselling. If there is no decline and the repeat VL is >500 copies/mL, genotypic resistance testing will be performed on a new as well as the baseline stored sample, and the case reviewed by the trial steering committee to decide on further management. If the repeat VL is 50-500 copies/mL, enhanced adherence counselling and routine VL monitoring will be continued. We define the endpoint of virological failure as having 2 consecutive VL >1000 copies/mL after week 12.

Participants will have a repeat CD4 count at weeks 24 and 48, and a repeat creatinine at weeks 4, 16 and 48. If the eGFR declines to <50 mL/min then TDF will be discontinued and replaced with AZT as per national guidelines. TDF may be reintroduced if there is an alternative explanation for the decline in renal function once the eGFR has increased to >60 mL/min.

Urine pregnancy tests will be performed in female participants at every visit, unless postmenopausal. In Stage one and Stage two, DBS will be taken and stored for TDF-DP concentrations at weeks 0, 12, 24, 36 and 48 in order to assess adherence in those who do not virologically suppress. These will be analysed in a case control sub-study (cases will be participants who do not achieve virologic suppression at weeks 24 and 48). In Stage 2, DTG trough concentrations will be taken at weeks 2, 12 and 24 weeks.

Blood samples will be taken by the study nurse and sent to the NHLS laboratory as study specimens.

5.4.5.1 Pharmacokinetic component

Twelve participants from Stage 1 and 24 from Stage 2 will be invited to participate in the pharmacokinetic sub-study. DTG trough concentrations (prior to a DTG dose) will be taken on days 3, 7, 14, and 28. Residual EFV plasma concentrations will be taken at enrolment and at the same time points as the DTG trough concentrations on day 3, 7, 14, and 28.

We will perform genetic testing on all participants to determine *CYP2B6* metaboliser genotype as it has been shown that the induction effect of EFV is greater in slow metabolizers (41), which would result in lower DTG trough concentrations when switching from EFV. The following SNPs will allow categorisation of participants into slow, intermediate, and extensive metabolisers: *CYP2B6* 516G→T (rs3745274), 983T→C (rs28399499), 15582C→T (rs4803419), and *CYP2A6* 48A→C (rs28399433) (42).

Pharmacokinetic blood samples will be taken by the study nurse. The participant will not need to see the clinician for a consultation, but has the opportunity to ask questions or bring up issues, upon which the nurse taking the blood will assess whether the patient requires an ad hoc consultation that day.

The drug assays will be done at the Division of Clinical Pharmacology laboratory, University of Cape Town (UCT), which has developed validated assays for DTG and EFV in plasma and TFV-DP on DBS using liquid chromatography-tandem mass spectrometry. Whole blood will be used for DNA extraction by Qiasymphony® according to manufacturer's instructions. Targeted genotyping will be done with

Taqman® assays for selected SNPs that determine *CYP2B6* metaboliser status.

5.4.6. Post-trial access to the study drug

At the end of 48 weeks, participants will exit the study and resume per guideline follow up in the government ART clinic. On their week 48 visit an appointment will be made for the following month in the ART clinic for the participant to transition back to provincial care. The decision on further treatment will made according to the findings of the study, the availability of the study drug in the public sector at that point and the discretion of the treating clinician. Available options include ALD, the WHO recommended second line (10,12) and AZT/3TC/LPV/r (6). If the participant has virologically failed at the end of the study, darunavir, rilpivirine or raltegravir could also be incorporated into the regimen depending on genotypic resistance results. The participants' results and progress will be communicated in writing to their chosen clinic.

Figure 1. Study Procedure

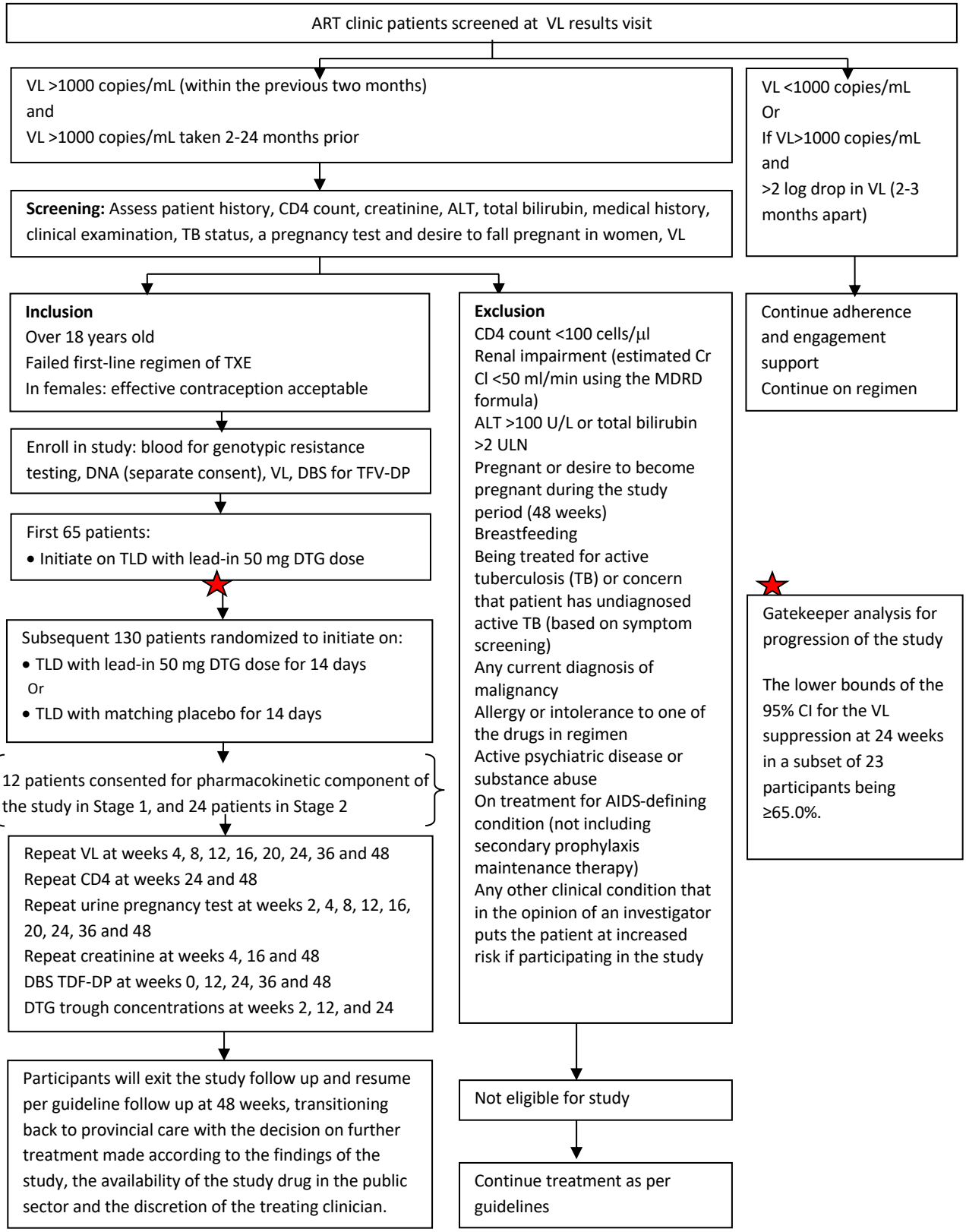

Table 1. Schedule of events

Visit	Screening	Week										
		0	2	4	8	12	16	20	24	36	48	52
Informed consent	X											
Inclusions & exclusions	X	X										
Medical history	X	X	X	X	X	X	X	X	X	X	X	X
Confirm use of acceptable contraception	X	X	X	X	X	X	X	X	X	X	X	X
Physical exam if symptoms	X	X	X	X	X	X	X	X	X	X	X	X
Adverse event screening		X (Baseline)	X	X	X	X	X	X	X	X	X	X
Sleep assessment		X	X	X	X	X	X	X	X	X	X	X
Neuropsychiatric and neurocognitive testing		X	X	X		X			X		X	
Pregnancy test	X	X	X	X	X	X	X	X	X	X	X	X
Creatinine	X			X			X				X	
ALT	X											
Total bilirubin	X											
HIV VL	X			X	X	X	X	X	X	X	X	
CD4 count	X								X		X	
Genotype		X										
Stored blood for DNA		X										
DTG trough and EFV concentrations (PK sub-study)		Day 0, 3, 7	X	X								
DTG trough concentrations			X			X			X			
TDF-DP DBS		X				X			X	X	X	

6. Data management and analysis

6.1 Data collection

6.1.1 Data to be collected

Demographics

- Age
- Gender
- Address

Baseline clinical information

- Medical history
    - VL history
    - CD4 history
    - ART history: ART regimens and start dates, periods of interruptions >3 months and other pertinent aspects of the ART history
    - Encounter history
    - Appointment/engagement history
    - Tuberculosis history
    - Obstetric history
    - Mental health history
    - Substance use screening
    - Other comorbidities
- Clinical examination findings

Follow up visit information

- Symptoms and clinical examination findings
- Barriers to adherence as recorded in the counselling notes
- Time and date of last treatment dose
- Neuropsychiatric and neurocognitive testing at weeks 0, 2, 4, 12, 24, and 48 using the following battery
    - Brief Symptoms Inventory – 18, Anxiety Subscale (BSA)
    - Centre for Epidemiologic Studies Depression Scale (CESD)
    - Simioni Neurocognitive Symptom Questions
    - Cognitive Assessment Tool – Rapid Version 2.0 (CAT-rapid V2)
- Sleep assessment at weeks 0, 2, 4, 8, 12, 16, 20, 24, 36 and 48

Laboratory values

- VL at baseline and weeks 4, 8, 12, 16, 20, 24, 36 and 48

- CD4 at baseline and weeks 24 and 48
- Residual EFV concentrations in 12 participants in Stage 1, and 24 participants in Stage 2
- DTG trough concentrations in 12 participants in Stage 1, and 24 participants in Stage 2
- EFV metaboliser genotype
- Stored blood for DNA
- Genotypic resistance results
    - Baseline
    - Repeat results in those with VL >500 copies/mL after enhanced adherence support
- Creatinine at baseline and weeks 4, 16 and 48
- Urine pregnancy test results at baseline and weeks 0, 2, 4, 8, 12, 16, 20, 24, 36, 48 and 52
- ALT and total bilirubin at baseline
- DBS for TDF-DP concentrations at weeks 0, 12, 24, 36 and 48 (case-control)
- DTG trough concentrations at weeks 2, 12 and 24 in stage 2
- Other blood results ordered as needed

Clinical Outcomes

- Adverse events
    - Grade 3 or 4
    - Serious
    - Requiring discontinuation of any of the ARVs in the regimen
    - Neuropsychiatric adverse events
- Mortality (all-cause) with causality assessment
- Appointments and attendance

6.1.2 Method of collection

All data will be recorded by the clinicians, nurses and counsellors on the case report forms (CRFs). Data will be captured from the CRFs on a weekly basis and uploaded into a study database (RedCap) by dual capturing.

Background information will be collected from provincial Single Patient Viewer.

6.2 Storage

Patient data will be stored on a password-encrypted hard drive, kept off the clinic premises at UCT.

6.3 Analysis

Analysis will be performed in Stata Version 14 (43). Descriptive statistics will be presented with 95% CI. The detailed analysis plan will be described in a Statistical Analysis Plan which will be written before the first participant is enrolled.

Unless otherwise specified, we will use the modified intention to treat analysis (mITT) according to the FDA snapshot analysis algorithm for analysing virological failure. The FDA snapshot algorithm regards

those with measured HIV VL ≥50 copies/mL, those with missing HIV VL 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 (44). 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.

6.3.1 Primary outcome:

VL suppression of HIV VL <50 copies/mL at 24 weeks is the primary end point. The proportion (with 95% CI) of participants achieving HIV VL <50 copies/mL at 24 weeks will be assessed, in Stage 1 and in each arm in Stage 2.

a. Overall
b. Stratified by the presence or absence of resistance to both TDF and 3TC on initiation of the second-line regimen

6.3.2 Secondary outcomes:

1. Resistance
    a. Resistance profile at enrolment (NRTI and EFV resistance)
    b. DTG and NRTI resistance in those who experience virologic failure
2. Pharmacokinetics
    a. The median (and IQR) residual EFV concentrations in the first 28 days will be described.
    b. The GMR (with 90% confidence intervals) of DTG trough concentrations for both supplemented and non-supplemented participants will be analysed as day 3 versus day 28, day 7 versus day 28, and day 14 versus day 28.
    c. The proportion of participants with DTG trough concentrations above the PA-IC_{90} at all pharmacokinetic time points will be described.
    d. All metrics will be stratified by *CYP2B6* metaboliser genotype.
    e. Adherence to treatment (as indicated by self-reporting, DBS TDF-DP concentrations at weeks 0, 12, 24 and 48 in stage one and stage two) in those who experience virological failure and matched controls from among those who are suppressed at 24 and 48 weeks
3. Other clinical outcomes
    a. Number of adverse events:
        i. Grade 3 or 4
        ii. Serious
        iii. Requiring discontinuation of any of the ARVs in the regimen
        iv. Mortality (all-cause)
    b. CD4 counts: median (IQR) at 24 and 48 weeks, and change from week 0
    c. Virological suppression
        i. Modified intention to treat at 12 and 48 weeks
        ii. Per protocol at 12, 24 and 48 weeks
            1. In addition, a sensitivity analysis will be performed where those patients who met the definition of failure at 24 and 48 weeks but who had no

TDF-DP concentrations at 24 and 48 weeks, indicating poor adherence, will be removed from analysis

2. Virological failure is defined as having 2 consecutive VL >1000 copies/mL after 12 weeks

iii. Overall and stratified by baseline resistance with both K65R and M184I/V mutations

6. Ethical considerations

7.1 Confidentiality

All participants interactions will maintain strict confidentiality and names will be removed from the datasets for analysis. Participant identifiers (including study numbers linked to patient name) will be stored in one participant log file that will be locked in a cabinet. All other study documents will use the study number only.

7.2 Autonomy

Informed consent will be obtained from every participant for screening and for the study in advance of enrolment into the study. Participants will be assessed by the counsellor or study nurse taking consent for their capacity to consent. All participants providing consent will be >18 years old, with no clinical reason to suspect that they are not of sufficient capacity to consent.

Additional consent will also be taken for pharmacogenomics analyses. Consenting participants will have an additional blood sample taken and stored for future genetic studies that are related to HIV and its treatment. A separate informed consent for genetic testing will be obtained from every participant at screening in both stages. For participants that have already enrolled in Stage 1, informed consent for genetic testing will be obtained at the next study visit. Consenting participants will have stored blood sample taken at the same study visit. Unwillingness to participate in genetic testing will not exclude participants from the main study.

Consent will be explained verbally and in a written form. isiXhosa speaking patients will have the option of a translated consent form in isiXhosa, with explanation by an isiXhosa-speaking healthcare worker. The explanation will include information to inform the participant of:

1. The nature of the research study
2. The voluntary nature of their participation
3. The aims of the study
4. The duration of the participant's involvement
5. The expected benefits to the participant and to others
6. The expected nature of the drugs being tested
7. The procedures involved in participation, including text messaging and calls to remind the patient of their appointment
8. The alternative standard of care medical therapy

9. The risks, inconvenience, discomfort and distress that may reasonably be anticipated by participating in the study
10. The potential for unforeseen risks
11. Their ability to refuse to participate and withdraw their consent at any time without reason, and that this will not affect their care
12. That the participant may be withdrawn from the study if the investigating physician considers this is necessary in the best interests of the participant
13. That personal information may be scrutinised during audit by competent authorities and properly authorised people, but all personal information will be treated as strictly confidential and will not be made publicly available
14. That information generated by the study may be published but that no details will be divulged from which the participant could be identified
15. That their samples will be stored and kept secure and confidential, but that they may be used for further testing for future studies outside the scope of this study
16. That study information will be retained for a period after the end of the trial
17. The compensation arrangements that are available
18. Contact details for emergencies

7.3 Beneficence

Depending on the results of this study together with other studies being conducted, the evidence could be used to advocate for the use of fixed-dose TLD formulations as second-line, which could benefit future patients by providing them with an efficacious regimen that is more tolerable, has low monitoring requirements and has a low pill burden.

7.4 Non-maleficence

The potential for the development of DTG resistance is considered low (2, 9, 24, 27–29, 31) but we will monitor for virologic failure at frequent intervals (0, 4, 8, 12, 16, 20, 24, 36, and 48 weeks). Virologic suppression will be monitored by the DSMB who will convene at two monthly intervals during the study to review data with a pre-defined stopping rule based on failure to achieve a threshold of virologic suppression. Resistance testing will be performed on those participants who has a HIV VL >500 copies/mL after enhanced adherence support and an alternative ART regimen will be available (through public sector access that would include a boosted PI) should DTG resistance develop.

In order to address the potential risk for neural tube defects if taking DTG peri-conception, women of child-bearing potential will be offered reliable and consistent contraception as recommended by the WHO (12) and only those for whom this is an acceptable option will be eligible for the study.

Fair monetary compensation will be provided to participants for attending study visits, in line with the National Health Research Ethics Council (NHREC) guidance in order to compensate them for their time and inconvenience and to reimburse them for their expenses (45).

7.5 Justice

This study has potential applicability to other populations

1. In the coming years DTG will become widely available in lower resource settings and 70 low and middle income countries have already included or are planning to include DTG in national guidelines (12). The knowledge gap on the safety of transitioning patients from EFV to DTG if they are not known to be virologically suppressed forms a barrier to expanding DTG access in countries where VL monitoring is not routinely available. VL testing is currently only available on a national level in South Africa, Namibia and Botswana, covering less than 50% of patients in LMICs (23). There is a need for clinical data to support switching patients from TXE to TLD with a detectable or unknown VL (23). If the TLD regimen proves robust in the face of NRTI resistance mutations, there is potential for mass transfer from TXE to TLD without the requirement for a VL to guide the decision. Maintaining the same backbone and removing the need to perform a VL would simplify implementation and reduce the cost of transition. TLD in a generic fixed dose combination will also already be procured for first-line ART programmes so would be readily available at a low cost. This study could contribute evidence to inform this strategy and facilitate this programmatic transition.

2. Children have even fewer second-line options than adults, especially if they were initiated on a PI-based regimen. The use of DTG is supported by the FDA for children over six years of age and >30 kg and the WHO recommends its use for all HIV positive individuals older than 6 years and >15 kg (12). Thus there is the potential for expansion of TLD to younger populations in whom TDF is already recommended (>15 years and ≥40 kg (6)).

3. Evidence on the use of DTG in second-line regimens can help to advocate for access to DTG as a valuable second-line option that is efficacious, tolerable and has a low pill burden.

8. Safety

1. Intense monitoring of HIV VL (0, 4, 8, 12, 16, 20, 24, 36, and 48 weeks) allows for a change in regimen if virologic suppression is not achieved or maintained.
    a. If a participant has a VL >500 copies/mL despite enhanced adherence support, a second genotype will be performed and the resistance results of the genotype on initiation and on failure will be discussed by the trial steering committee and used to inform the design of a new regimen that could include standard of care PIs, darunavir, rilpivirine and/or raltegravir.
    b. We anticipate that such events, if they do occur, will be infrequent (around 5% of participants in DTG monotherapy studies, and likely less with two NRTIs added). Furthermore, we anticipate that because no patients will be PI-experienced, a PI-based alternative suppressive regimen will be available in all cases where DTG resistance develops. We will communicate a detailed written ART plan after study completion to the clinic who is taking over care of such a patient and Prof Meintjes will be available to advise on on-going care of such patients after the trial – he has provided patient advice to clinicians in Khayelitsha since 2003. We anticipate that it is very unlikely such mutants would result in onward transmission. The availability of suppressive alternative regimens make this unlikely and the signature DTG mutation (R263K) severely compromises the

virus in terms of replicative capacity meaning that viruses carrying the mutation disappear rapidly from the circulating population of viruses when DTG drug pressure is removed.

2. If a participant experiences any new symptom or is concerned, they will be encouraged to make telephonic contact with the clinic regarding any new symptoms by sending a "Please Call Me Message" which is free of charge. We will phone them. If they have symptoms of an SAE or unexpected event they will then be asked to attend the clinic and be reimbursed.
3. An independent DSMB will be established consisting of four independent, experienced HIV clinical researchers (quorum will be two HIV clinical researchers). The study statistician will prepare the report for the DSMB but will not be a voting member of the DSMB. Two-monthly teleconferences will take place after the first participant enrolled reaches 12 weeks in the study, to monitor VL data.
    a. Stopping rule: if <65% of participants fail to achieve HIV VL <50 copies/mL by week 16 (after a minimum of 10 participants have reached 16 weeks, excluding those who have been transferred out or died from non-HIV related causes). This rule may be modified by the DSMB prior to the trial starting when the Charter is agreed.
    b. Adverse events or factors with implications for the research or that trigger a participant to become LTFU will be reported to the DSMB.
4. If a participant falls pregnant or expresses the wish to fall pregnant during the trial she will be immediately switched to a PI-based regimen.

5. All ARVs will be sourced from the Western Cape Department of Health. Until the ARVs are available in state, they will be purchased from quality assured sources and will be WHO pre-qualified and registered for use in South Africa by SAHPRA (South African Health Products Regulatory Authority – SA regulator).

9. Human resources

A nurse study coordinator, two medical officers, two nurses, three counsellors, a pharmacist, a data capturer and a driver will be employed for the specific purpose of implementing this trial and the RADIANT TB trial (MSF ERB approval reference 18109, received 15 February 2019). All research staff will undergo Good Clinical Practice (GCP) training. Study staff will undergo appropriate training (GCP, BLS, ACLS where appropriate and on SOPs) before commencing patient enrolment and receive adequate supervision from the PIs. Staff training will include a 2 day start-up meeting covering all SOPs for both this trial and the other trial (RADIANT TB) which will be supported by the same staff. The external study monitor and PIs will be present at both days of this start-up meeting.

10. Timeline

	2018	2019				2020				2021			
Quarter:	4	1	2	3	4	1	2	3	4	1	2	3	4
Protocol &ethics submission	X	X											
Preparation of CRFs	X	X											
Staff recruitment		X	X										

Enrolment			X	X	X	X	X	X	X				
Follow up			X	X	X	X	X	X	X	X	X	X	X
Analysis						Progress to stage 2	Interim analysis					Complete analysis	
Write up									X (stage 1)				X
Dissemination													X

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