CLINICAL TRIAL PROTOCOL

PROTOCOL TITLE

An open-label, sequential non-randomised pharmacokinetics study of dolutegravir (DTG) plasma exposure when given as twice or once daily DTG in the presence of rifampicin in children with Human Immune Deficiency Virus (HIV) and tuberculosis (TB) between 3-35kg in South Africa (ORCHID) (Stage 1 **using twice daily in accordance with the standard of care dosing**)

Short title: Pharmacokinetics of twice or once daily DTG (50mg FCT/10mg DT) in children with HIV and TB

Name of product(s)/ Project code	Antiretroviral treatment: Dolutegravir (DTG) – 50mg film coated tablet (FCT)/10mg dispersible tablet (DT) DTG is part of a combination therapy that includes two reverse Nucleoside Reverse Transcriptase Inhibitors (NRTIs), abacavir (ABC) or zidovudine (AZT) plus lamivudine (3TC). Anti-tuberculosis treatment: rifampicin (RIF) is part of a multidrug regimen for 6 to 9 months, in combination with other anti-tuberculosis treatments, e.g. isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA).
Drug Class	Dolutegravir (Integrase Strand Transfer Inhibitor-InSTI),
Phase	Phase IV pharmacokinetic drug-drug interaction study
Indication	Co-infection with HIV and TB
Protocol Number	CAPRISA258 (CAP258)
Sponsor	National Institutes of Health (NIH)
Co-sponsor	South African Medical Research Council (SA MRC)

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Protocol Version / Date	V1.0 dated 07 April 2020
Protocol Amendment Number / Date	V1.1 dated 09 July 2020 V1.2 dated 19 January 2021 V1.3 dated 18 April 2022

SIGNATURE PAGE

Medically Qualified Officer

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I have read this protocol and agree that it contains all the necessary details for carrying out this trial. I will conduct the trial as outlined herein and will complete the trial within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of this trial. I will discuss this material with them to ensure they are fully informed regarding the drug and the conduct of the trial.

I will use only the informed consent form approved by the sponsor or its representative and will fulfil all responsibilities for submitting pertinent information to the Institutional Review Board/Independent Ethics Committee (IRB/IEC) responsible for this trial.

I agree that the sponsor or its representatives shall have access to any source documents from which case report form information may have been generated.

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ABBREVIATIONS – GLOSSARY OF TERMS

3TC	Lamivudine	GI	Gastrointestinal
ABC	Abacavir	GMR	Geometric Mean Ratio
AE	Adverse event	HCT	Haematocrit
AFB	Acid-fast bacilli	ART	Active Antiretroviral Therapy
AIDS	Acquired Immunodeficiency Syndrome	HIV	Human Immunodeficiency Virus
ALP	Alkaline Phosphatase	ICH	International Conferences on Harmonisation
ALT	Alanine aminotransferase (SGPT)	IEC	Independent Ethics Committee
ART	Antiretroviral therapy	INH	Isoniazid
ARV	Antiretrovirals	IRIS	Immune Reconstitution Inflammatory Syndrome
AST	Aspartate aminotransferase	IV	Intravenous
BID/ bd	Bis in Die (Twice-daily)	mL	Millilitre
BMI	Body Mass Index	MUAC	Middle-upper arm circumference
BSA	Body Surface Area	NLMEM	Nonlinear mixed-effects models
		NRTIs	Nucleoside Reverse Transcriptase Inhibitors
		NNRTIs	Non-Nucleoside Reverse Transcriptase Inhibitors
CBC	Complete blood count	PIs	Protease inhibitors
CI	Confidence Interval	PO/ po	Per os (orally)
CK	Creatine kinase	PK	pharmacokinetics
C_{max}	Maximum observed plasma concentration	PZA	Pyrazinamide
C_{trough}	Minimum observed plasma concentration	QT_c	Corrected QT interval
CRO	Clinical Research Organisation	QT_cF	Corrected QT interval using Fridericia's formula
CRF	Case report form	RH	Rifampicin-Isoniazid combination
CTU	Clinical Trials Unit	RIF	Rifampicin
CYP	Cytochrome	SA	South Africa/ South African

dly	Daily	SAE	Serious adverse event
DAIDS	Division of AIDS, NIH (US)	Sol.	Solution
DT	Dispersible tablet	FCT	Film Coated Tablet
DTG	Dolutegravir	TB	Tuberculosis
DSMB	Data and Safety Monitoring Board	ULN	Upper limit of normal
EAE	Expedited Adverse Event	VL	HIV-1 RNA Viral Load
ECG	Electrocardiogram	WBC	White blood cell
EFV	Efavirenz	WHO	World Health Organization
EMB	Ethambutol	WNL	Within normal limits
FDA	Food and Drug Administration		
FTC	Emtricitabine		
GCP	Good Clinical Practice		

PROTOCOL SYNOPSIS

Protocol Title	**Pharmacokinetics of twice or once daily DTG in children co-infected with HIV and tuberculosis (Stage 1 using twice daily in accordance with the standard of care dosing)** An open-label, sequential non-randomised pharmacokinetics study of DTG plasma exposure when given as twice-daily DTG in the presence of rifampicin-containing TB treatment in South African children with Human Immune Deficiency Virus (HIV) and tuberculosis (TB) who weigh between 3-35kg
Indication	Co-infection with HIV and Tuberculosis
Protocol Number	CAPRISA258 (CAP258)
Background Information and Trial Rationale	Currently, approximately 40 million people are living with HIV (PLWHIV) worldwide, and about half of PLWHIV live in Eastern and Southern Africa. [1,2] Approximately 1.8 million children are living with HIV (with ~160K new infections annually), almost all as a result of mother-to-child transmission (MTCT). Only 52% are on ART, and 62% on ART are still on a non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimen (largely nevirapine) despite reports of >60% resistance to NNRTI among ART-naïve children (<18 months) with HIV. [3,4] Co-treatment of HIV and tuberculosis (TB) in infants and young children is virtually unavoidable in high endemic areas of Africa. These concomitant treatments not only increase the number of medicines taken concurrently but involve drugs that have interactions with antiretroviral drugs. The use of rifampicin (RIF) – a strong inducer of cytochrome P450 enzymes-- leads to major drug-drug interactions with antiretrovirals using the same metabolic pathway. In adults with concomitant administration of RIF, dolutegravir $AUC_{0\text{-}24h}$, C_{max} and C_{trough} are decreased by 54%, 43% and 72%%, respectively. [5] Increasing the dose of DTG by using 50mg FCT twice daily (double dose DTG) ensures adequate exposure to DTG in adults co-treated with RIF; however, there are limited data for children.

The available data describing the pharmacokinetics, safety and efficacy of double dose DTG in children (3-35kg) taking TB treatment is limited to 13 children (5 children on 25mg DTG twice-daily and eight children on 50mg FCT DTG twice daily while there is no data in children on the DTG DT. Children receiving the higher dose tended to have higher DTG exposures both during and after TB treatment. The study will be conducted in two phases. Stage one will evaluate the pharmacokinetics, safety and efficacy of double dose DTG in children (weighing 3-35kg). Based on modelling using data from stage one of the study and other available data, stage two will evaluate the use of once-daily dosing of 50mg/10mg DT DTG in children (3-35kg). The stage 2 protocol will be submitted as a separate protocol at a later stage. [6]

Stage 1 proposed study will provide evidence to support the use of a twice-daily dose of 50mg FCT/10mg DT DTG in children (3-35kg) co-treated with RIF.

Trial Objectives

Primary Objective:

- To determine the pharmacokinetics (C_{trough}, C_{max} and AUC_{0-24h}) of DTG twice daily in children (3-35kg) who are taking a RIF-based regimen for the treatment of TB.

Secondary objectives

- To assess the safety and tolerability of twice-daily DTG in HIV-infected children with concomitant RIF-based anti-TB treatment.
- Compare the model-based estimates of the DTG PK measures of exposure (C_{trough}, C_{max} and AUC_{0-24h}) during anti-TB treatment during treatment with DTG-based ART one month after completing TB therapy.
- To explore the effects of age, weight, sex, initial severity of TB and anthropometric measurements on DTG pharmacokinetics, i.e. exposure, C_{trough}, Cmax and AUC0-24h.
- To assess adherence to therapy (Pillcount CRFs, drug accountability, and drug levels in hair)
- To describe viral load evolution before, during and after co-treatment and monitor resistance in children failing therapy

- To explore the effect of pharmacogenetic variants on the levels of anti-TB drugs and ARVs during co-treatment.

Main Endpoints

Primary endpoint:

- Pharmacokinetics (C_{trough}, C_{max} and AUC_{0-24h}) of DTG FCT 50mg/10mg DT twice daily in children (3-35kg) who are taking a RIF-based regimen for the treatment of TB. Population plasma PK parameters of DTG in the presence or absence of RIF-based TB treatment, including absorption rate constant (k_a), the volume of distribution (Vd), and oral clearance (Cl/F) and between-subject variability terms; post-hoc Bayesian predictions of secondary PK parameters of DTG including AUC and C_{min} with HRZE dosing

Secondary endpoints:

- Grade 3 or higher adverse events
- HIV-1 RNA viral load
- Dose options for DTG given with RIF-containing TB treatment among children 3-35kg, derived by simulation using nonlinear mixed-effects models to support once-daily DTG.

Trial Design

Stage one of this is a single centre, open-label, non-randomised, prospective study to evaluate the pharmacokinetics of twice-daily DTG and concurrent RIF treatment and to assess the safety, tolerability, and virological effect of twice-daily DTG in HIV-TB co-infected children weighing >3kg and ≤35kg.

DTG will be administered as a solid formulation tablet - 50mg FCT and dispersible tablet - 10mg DT during and after anti-TB treatment. Actual doses for companion antiretrovirals and anti-TB drugs will be based on the South African (SA) weight band dosing recommendations and provided as per the SA National ART and TB treatment guidelines standard of care.

Main Entry Criteria

Inclusion

Exclusion

Inclusion criteria

- Children <18 years and >4 weeks with confirmed HIV-1 infection weighing 3-35kg (lower thresholds for this weight-band may be included in accordance with evolving guidelines), ART-naive or experienced, with

plans to use DTG for HIV treatment

- Diagnosis of TB disease with clinician initiating rifampicin-containing first-line therapy
- Parents/legal guardians/caregivers and children give informed written consent (or assent, where applicable) to be in the study
- Girls who have reached menses must have a negative pregnancy test at screening and be willing to adhere to two effective methods of contraception (barrier and a non-barrier form of contraception during the study, starting at least 14 days prior to enrolment) if sexually active. The parents/caregivers will be counselled together with the child if the child tests positive to reduce any social harm that may arise.

Exclusion criteria:

- History or presence of known allergy or contraindications to DTG
- Alanine aminotransferase (ALT) ≥5 times the upper limit of normal (ULN), OR ALT ≥3xULN, and bilirubin ≥2xULN
- Severe hepatic impairment or unstable liver disease (as defined by the presence of ascites, encephalopathy, coagulopathy, hypoalbuminaemia, oesophageal or gastric varices, or persistent jaundice), known biliary abnormalities (except for Gilbert's syndrome or asymptomatic gallstones)
- Pregnancy or breastfeeding
- Concurrent illnesses that could influence drug PK, i.e. severe diarrhoea, vomiting, renal or liver disease
- Treatment with concomitant medications known to have interactions with DTG
- Participants that are eligible for the study but refuse to give consent and/or assent.

Study Duration

It is expected that Stage1 enrolment will take approximately one year and the follow-up of most children will be nine months (6 months anti-TB therapy for children already on antiretrovirals at TB therapy initiation, and three months follow-up). Children expected to be on TB treatment for longer than nine months at the start of the study will be excluded. However, children deemed to need continuation of their TB treatment once enrolled in the study will be followed up until three months following completion of the anti-TB

treatment.

Study Drugs

DTG 50mg film-coated tablet (FCT) and 10mg dispersible tablet (DT) twice daily and RIF (weight band dosed according to South African National Guidelines are the primary study drugs).

Statistics

Sample size

Randomisation

Summary of analysis

Sample size

Based on a within-subject variability (CV%) of 33% for DTG C_t estimated from previous PK studies involving DTG, an expected withdrawal rate of 20%, and a goal to detect a difference of at least 25% in exposures (that or higher would be clinically relevant), it is estimated that a sample size of 12-15 subjects to achieve at least ten evaluable subjects will provide precision for half the width of the 90% confidence interval (CI) on the log scale for the treatment difference that would be within 26% of the point estimate for AUC, C_{max}, and C_t (comparing paediatric PK to historical PK values in adults with HIV taking DTG).[7] At least 12-15 children will be included in the study of the DTG 50 mg FCT and an additional 20 children using the DTG 10mg DT. However, this number may need to be increased if inter-subject variability of DTG PK parameters in children appears to be larger than the 33% reported in adults, we have fewer children with evaluable PK data, or if the threshold for the weight-bands under study are changed based on evolving WHO guidelines on DTG dosing.

1. BACKGROUND AND STUDY RATIONALE

The HIV epidemic

Currently, approximately 40 million people are living with HIV (PLWHIV) worldwide, and about half of PLWHIV live in Eastern and Southern Africa.[2][1] By the end of 2017, an estimated 21.7 million people were accessing antiretroviral treatment (ART) globally. [2] South Africa has the largest HIV epidemic in the world with more than 7 million PLWHIV and the largest treatment programme with 4.4 million people on ART; >25% live in KwaZulu-Natal. [2] Approximately 1.8 million children are living with HIV (with ~160K new infections annually), almost all as a result of mother-to-child transmission (MTCT). Only 52% are on ART, and 62% on ART are still on a non-nucleoside reverse transcriptase inhibitor (NNRTI) based regimen (largely nevirapine) despite reports of >60% resistance to NNRTI among ART-naïve young children (<18 months) with HIV. [4] South Africa has one of the largest public access paediatric ART programs in the world, with over 50,000 children on antiretrovirals (ARVs) in the province of KwaZulu-Natal alone. [1-4,7-11] The World Health Organisation (WHO) currently recommends the Integrase Strand Transfer Inhibitor (InSTI), Dolutegravir (DTG) as the preferred backbone of first-line ART regimens for the treatment of HIV-1 infection in adults, adolescents and children, particularly in Low and Middle-Income Countries (LMIC) regions such as Southern and Eastern Africa), where pre-treatment drug resistance to NNRTI reaches 10%. [3]

Antiretroviral therapy of HIV infected children should be initiated as soon as possible in infancy [12]; it has transformed the prognosis of HIV infection and turned HIV in children, as in adults, into a manageable chronic infection. *Mycobacterium tuberculosis* (*M. tuberculosis*) is a common infection in HIV infected African adults and children. Nine countries (South Africa, Swaziland, Lesotho, Namibia, Botswana, Mozambique, Zambia, Zimbabwe and Malawi) account for nearly 50% of the global TB/HIV burden. HIV infected infants have a 20 fold increase in the risk of developing TB compared to uninfected infants, and 23.4 cases of active TB per 100 observation years are documented among HIV-infected children. [13]

HIV therapy

Only about half of children with HIV are on ART, and most are treated with NNRTI-containing regimens. However, 50-60% of children (<18 months) have HIV that is resistant to NNRTI. [3] Viral suppression remains poor, particularly in young children. Available drug formulations are unpalatable and difficult to dose. The lack of safe, potent ART in age-appropriate formulations, including FDC that may be used in different sub-populations (e.g. tuberculosis co-infection), is a critical barrier to the scale-up of ART treatment in paediatrics globally. [4,8] WHO currently

recommends the use of DTG 50mg/10mg DT FCT in patients >3kg and 4 weeks of age. Based on data (from PENTA 20-Odyssey and IMPAACT) shared with WHO, that same dose of 50mg once daily was also provisionally recommended for children 20-25kg [11,15,16] and has been included in the updated 2019 WHO ART treatment guideline released at the 2019 International AIDS Society meeting in Mexico. [16,17] **Table 1** [14,18] shows PK data for (AUC, C_{trough} and C_{max}) DTG 50mg FCT compared to 30mg dispersible tablets (DT) tablets in children living with HIV weighing 20 to <25 kg. The table includes reference PK from adults. [14,18] DTG 50mg/10mg DT FCT and 30mg DT showed similar C_{trough} for children weighing 20 to <25kg, but the maximum concentration (C_{max}) exceeded reference values for approved adult DTG dosing (**Table 1**).

Table 1. Participant demographics and PK parameters by dose and formulation in children 20 to <25kg and adult reference populations

Table 1: Participant demographics and PK parameters by dose and formulation in children 20 to <25kg and adult reference populations.

	ODYSSEY		Ref. ODYSSEY[2]	Ref. Adults [4,5]	
WHO weight band	20 to <25 kg		20 to <25 kg	≥ 40kg	
Dose (mg) and formulation	30 DT	50 FCT	25 FCT	50 FCT	50 FCT BID
N	8#	7#	14#	10[a]	24[b]
Sex male, n (%)	4 (50%)	4 (57%)	7 (50%)	10 (100%)	18 (75%)
Age (years)	8.6 (6.8-11.3)	9.7 (8.1-11.7)	9.3 (7.1-11.3)	34 (22-53)	47 (33-68)
Weight (kg)	21.8 (20.3-22.7)	22.4 (20.5-24.5)	23.4 (20.2-24.3)	-	-
Dose (mg/kg)	1.4 (1.3-1.5)	2.2 (2.0-2.4)	1.1 (1.0-1.2)	--	-
C_{trough} (mg/L)	0.71 (74)[c]	0.77 (51)	0.32 (94)[d]	0.83 (26)	2.72 (70)
AUC_{0-24h} (mg*h/L)	71.8 (28)	62.8 (30)	30.1 (41)	43.4 (20)	93.4 (50)
C_{max} (mg/L)	7.42 (25)	6.07 (29)	3.20 (40)	3.34 (16)	5.41 (40)

PK parameters are geometric means with coefficient of variation (%). Other data are mean (range) for age, dose mg/kg, and weight, unless otherwise indicated. [a]Fasted HIV-positive adults. [b]HIV-positive treatment-experienced adults, fed state not specified. [c]One participant had a C_{trough} of 0.30mg/L which is below the EC_{90} for DTG of 0.32mg/L. [d]Ten participants had C_{trough} below 0.32 mg/L (EC_{90}). #Two participants on 30mg DT and four participants on 50mg FCT participated also in the ODYSSEY PK substudy on 25mg FCT.

Although short-term safety data were reassuring, ongoing longer-term safety needs to be assessed before these results can support the use of either 50mg FCT or DTG 30mg DT in this weight band definitively. Adult DTG 50mg could offer a practical and accessible dosing strategy for children 20 to <25kg, allowing rapid alignment of WHO-preferred ART regimens for adults and children ≥20kg in LMIC.[10][3] Although the 25mg DTG tablet has been shown to be safe in 14-25kg C_t, concentrations were found to be considerably lower than historical PK parameters in adults on DTG 50mg once daily. [18,19]

Additional PK data further supports the WHO recommendation of DTG as the preferred first line ART choice in all children >3kg and four weeks of age and the inclusion of the DTG 10mg DT in the dosing recommendations. [20]

HIV/TB co-infection

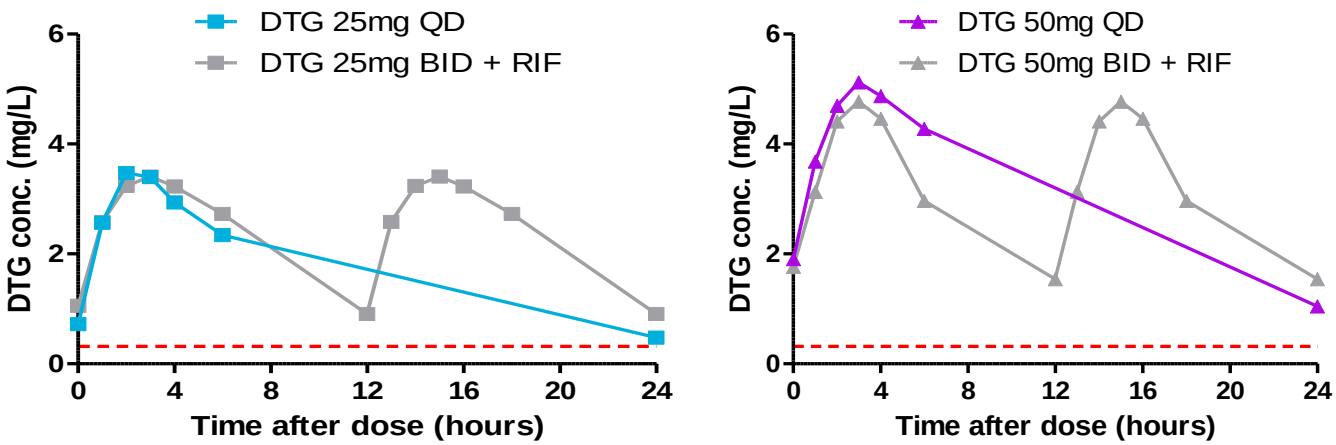
ART is further complicated by the high prevalence of co-morbidities such as malnutrition, anaemia, and co-infections, such as TB and malaria, which require concomitant treatment. [7,12,14] These concomitant treatments not only increase the number of medicines to be taken but use drugs having interactions with antiretroviral drugs (PIs and NNRTIs). In addition, as a preventive measure, HIV-infected infants need to receive cotrimoxazole for the prophylaxis of *Pneumocystis jirovecii* pneumonia, and if exposed to TB, isoniazid (INH) prophylaxis.

In South Africa, a large proportion of HIV-infected infants and children develop TB. Standard treatment of pulmonary TB uses a four-drug combination of INH, RIF, pyrazinamide (PZA) and ethambutol (EMB) for the two months of the intensive phase, followed by four months of INH/RIF for the continuation phase. [15,17] Like ART, the treatment of TB utilises weight banded dosing with adjustment to doses made through the course of therapy. It also requires clinical monitoring, notably hepatotoxicity. Adherence is the main concern, and directly observed treatment has been a key strategy to minimise resistance and relapse.

Aside from the numerous drugs, the treatment of HIV-TB co-infected children is complicated in many ways. In particular, initiating ART in an HIV-TB co-infected patient may lead to paradoxical clinical and radiological worsening due to the immune reconstitution inflammatory syndrome (IRIS).

Rationale for DTG 50mg FCT twice daily during HIV/TB co-treatment

WHO recommends using DTG 50mg FCT/10mg DT dosed twice daily in patients with HIV and TB while on rifampicin-based treatment for TB, based on data from the INSPIRING study done in adults. [21,22] However, no data exist for children with HIV-associated TB. Data for six children aged 6-12 and six children aged 12-18 who developed TB while on DTG-based ART in the Odyssey study and were transitioned to 50mg twice-daily were reported at the Conference on Retroviruses and Opportunistic Infections (CROI) in Mar 2020. Modelling data from these results support the use of a daily dose of 50mg DTG in children (20-35kg) using the new RIF doses. [6] No published data exist for younger children. We, therefore, propose to determine the PK, safety and efficacy of twice-daily DTG 50mg FCT/10mg DT in South African children 3-35kg. While the ontogeny of UGT1A1 has been explored (protein abundance and in vitro activity increase 8-fold from neonate to adult), [23,24] the effect of age on interactions between a potent inducer, like rifampicin, and a UGT1A1 (and CYP3A) substrate like DTG have not.



Parameter		DTG 25mg BID + RIF (n=5)	DTG 50mg BID + RIF (n=7)	Adult reference DTG 50mg QD[1] (N=16)
C_{trough} (mg/L)	GM (CV%)	0.90 (16)	1.11 (99)	0.83 (26)
AUC_{0-24h} (h*mg/L)	GM (CV%)	53.4 (21)	60.3 (63)	43.4 (20)
C_{max} (mg/L)	GM (CV%)	3.62 (24)	4.50 (47)	3.3 (16)

Figure 1. Trial Rational: Daily DTG 50mg FCT/10mg DT can overcome negative interactions with RIF [5]

Rational for DTG 10mg DT dose selection during HIV/TB co-treatment

A published population pharmacokinetic in adults with TB was scaled down allometrically by body weight to the pediatric population (in-house model). Since the maturation of the pathways involved in the dolutegravir elimination is not well known, the effect of age on clearance was included based on the literature values referring to related pathways such as CYP3A4. [25] The effect of formulation on absorption parameters (bioavailability and rate of drug absorption) was obtained and was assumed to be 80% higher bioavailable and faster absorption. For the effect of rifampicin on enzyme induction, we assumed an increase of 63% in DTG clearance based on the Odyssey trial. [6] The stochastic simulation and estimation (SSE) procedure in PsN [26] was used to perform a Monte-Carlo simulation of 1000 replicates of a clinical study including 32 patients, as outlined in **Table 2**. For each replicate, the model was fit to the simulated data, thus re-estimating the parameters and producing confidence intervals based on the 1000 replicates.

Results

The analysis revealed that this design is expected able to yield a precision of <30% RSE for all typical values of the PK model parameters. Due to the limited number of patients, especially in the lower weight bands, prior information from literature was used to guide the estimation of

maturation parameters. In particular, the overall uncertainty in the maturation with age is depicted in Figure 1

Table 2. Stochastic simulation and estimation (SSE) procedure in PsN used to perform a Monte-Carlo simulation of 1000 replicates of a clinical study

			Total daily Rif mg	
Weight (kg)	DTG (10mg DT)*	DTG (50mg F.C.T)	With rifampicin	Without rifampicin
3-5.9 kg	1 x 10mg DT		20 mg	10 mg
6-9.9 kg	1.5 x 10mg DT		30 mg	15 mg
10-14.9 kg	2 x 10mg DT		40 mg	20 mg
15-19.9 kg	2.5 x 10mg DT		50 mg	25 mg
>= 20 kg		1 x 50mg FCT	100 mg	50 mg
*DTG is dosed twice daily with rifampicin then once daily after rifampicin is stopped N = 32 children: 12 children with 20 - 35 kg on 50 F.C.T. 20 children on 10 disp tablet 5 children in each weight band. Number of visits: 2 visits. With and without rifampicin Sampling schedule: pre and 1, 2 ,3 ,4 6 and 12 or 24 (in case of no-rifampicin visit)				

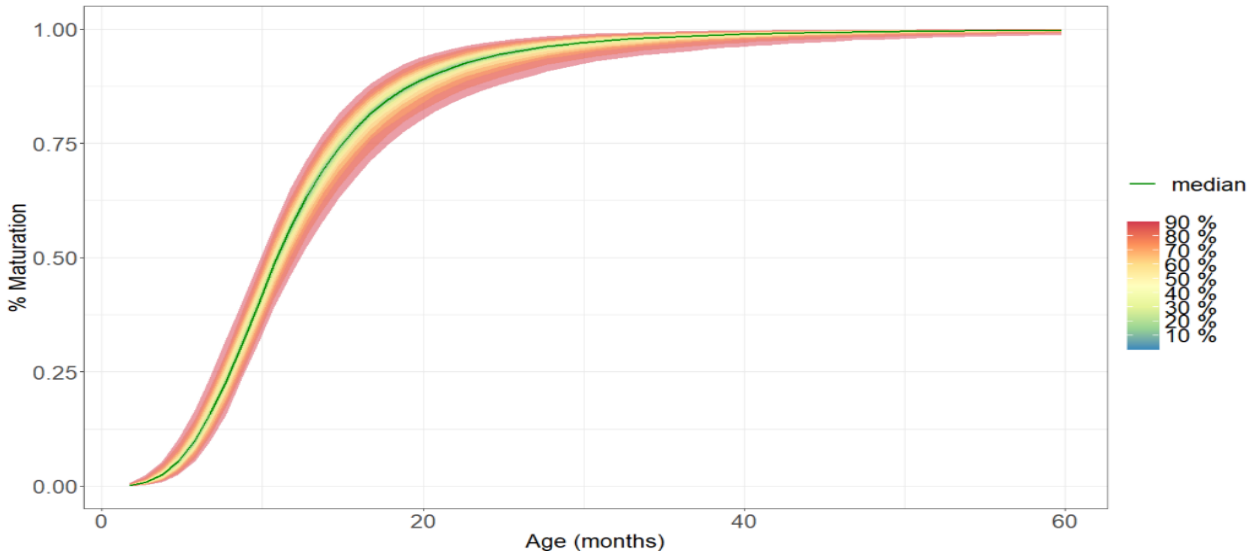


Figure 2. Overall uncertainty in maturation vs age

*Shaded regions represent different confidence intervals. The solid line is estimated median.

Tuberculosis therapy

The treatment of drug-susceptible TB in children follows the same principles as in adults (2 months INH/RIF/PZA/EMB followed by four months INH/RIF). Until 2009, doses for children had been

extrapolated from adult pharmacokinetic studies. However, data showed that the RIF, INH, PZA, and EMB doses recommended for children prior to 2009 did not achieve adequate serum concentrations, leading to a change in WHO dosage for these drugs in that year [19,21,23,27,28]. As a result, multiple combinations of tablets are now required to treat children with TB because the fixed-dose combinations (FDCs) currently available for children, while child-friendly and dispersible, have not been tailored to deliver the newly recommended doses. [29] Many reasons discourage manufacturers from producing FDCs corresponding to these new dosage requirements, including formulation difficulties, lack of clear direction from WHO on the recommended new composition of FDC corresponding to new dosages, and regulatory issues regarding lack of data in children. In addition, there is a lack of PK data in young children. However, a recent study published PK data for 20 children below the age of 2, comparing the new and old dosages of INH, RIF and PZA [30], showing that target concentrations were more likely to be achieved with the revised WHO dosage recommendations.

HIV/TB concomitant therapy

RIF is a strong inducer of cytochrome P450 enzymes, particularly CYP3A4, and also, to a lesser extent CYP2B6. P-glycoprotein, an important transmembrane efflux protein and product of the MDR-1 gene, is also induced [31], leading to major drug-drug interactions with antiretrovirals using the same metabolic pathway (PIs, NVP). RIF is also a substrate of the p-glycoprotein pump. Complex interactions between DTG-based Antiretroviral Therapy (ART) and rifampicin are, therefore, likely at the cellular level. There are no clinically significant interactions expected with the other anti-TB drugs. With concomitant administration of RIF, dolutegravir $AUC_{0\text{-}24h}$, C_{max} and C_{trough} are decreased by 54%, 43% and 72% [5], respectively, LPV exposure is decreased by up to 90% [32-34], and nevirapine exposure is also significantly decreased. [35] NRTIs, on the other hand, are not meaningfully affected by RIF administration. Potent enzymatic inducers such as rifampicin, phenobarbital and phenytoin may, via their action on UDP-glucuronyltransferases, slightly decrease the plasma concentrations of abacavir. [37] The ABC/AZT/3TC combination is considered an alternative ARV treatment option for HIV-infected patients on anti-TB therapy. However, this combination has inferior efficacy when viral load is high. [38,39] Efavirenz (EFV) has limited drug interactions with RIF. Still, it has not been approved for use in young children because of the high variability of blood levels below three years of age (impact of genetic polymorphism on drug levels). For children >3 years old and weighing <10kg, accurate dosing is not possible.

Finally, the replacement of RIF by rifabutin, an anti-TB drug with fewer PI drug interactions and successfully used in adults, is being evaluated in children in South Africa. [38] However, it is

important to note that there are no TB drugs FDC containing rifabutin and no paediatric rifabutin formulation; the drug is expensive and using it specifically for HIV infected children and or adults may complicate the TB program.

In summary: Co-treatment of HIV and TB in children is virtually unavoidable in high endemic areas of Africa, yet options for this vulnerable population remain extremely limited. A DTG based regimen in children (>3kg), adolescents and adults is now standard-of-care first-line therapy for HIV as recommended by the WHO, so it is critically important to know how to dose children who also have TB. While double dose DTG is the only option currently available for children who require co-treatment of HIV and TB, recent PK modelling supports the continuation of daily DTG. This approach needs further clinical evaluation.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

- To determine the pharmacokinetics (C_{trough}, C_{max} and AUC_{0-24h}) of DTG 50mg FCT/10mg DT twice-daily in children (3-35kg) who are taking rifampicin-based regimen for the treatment of tuberculosis.

2.1.2 Secondary Objectives

- To assess the safety and tolerability of twice-daily DTG in HIV-infected children with concomitant RIF-based anti-TB treatment.
- Compare the model-based estimates of the DTG PK measures of exposure (C_{trough}, C_{max} and AUC_{0-24h}) during anti-TB treatment during treatment with standard DTG doses one month after completing TB therapy.
- To explore the effects of age, weight, sex, initial severity of tuberculosis and anthropometric measurements on DTG pharmacokinetics, *i.e.* exposure, C_{trough}, C_{max} and AUC_{0-24h} concentrations with/without concomitant anti-TB treatment.
- To assess adherence to therapy (**Pillcount CRF**, drug accountability, and drug levels in hair)
- To describe viral load evolution before, during and after co-treatment and monitor resistance in children failing therapy
- To explore the effect of pharmacogenetic variants on the levels of anti-TB drugs and ARVs during co-treatment.

2.2 Study Endpoints

2.2.1 Primary Endpoint

- Description of the pharmacokinetics (C_{trough}, C_{max} and AUC_{0-24h}) of DTG 50mg FCT/10mg DT twice daily in children (3-35kg) who are taking a rifampicin-based regimen for the treatment of tuberculosis. Population plasma PK parameters of DTG in the presence or absence of RIF-based TB treatment, including absorption rate constant (k_a), the volume of distribution (Vd), and oral clearance (Cl/F) and between-subject variability terms; post-hoc Bayesian predictions of secondary PK parameters of DTG including AUC and C_{min} with HRZE dosing

2.2.2 Secondary Endpoints

- **Pharmacokinetics of DTG**

Nonlinear mixed-effects models (NLMEM) will be used to describe the PK of DTG in an integrated model which will be used to estimate the primary PK parameters of DTG including ka, Vd, and CL. The effect of concomitant TB treatment, as well as other covariates on these parameters, will be evaluated in the model. The model can also be used to derive individual estimates of traditional (secondary) DTG PK measures such as Area Under the Curve (AUC), C_{max}, C_{trough}, t_{max}, half-life ($t_{1/2}$).

- **Safety**

Subjects will be monitored clinically and biologically for safety. Biological monitoring will focus on liver function. Adverse Events and Serious Adverse Events will be graded following DAIDS grading tables (see Appendix B).

- **Efficacy**

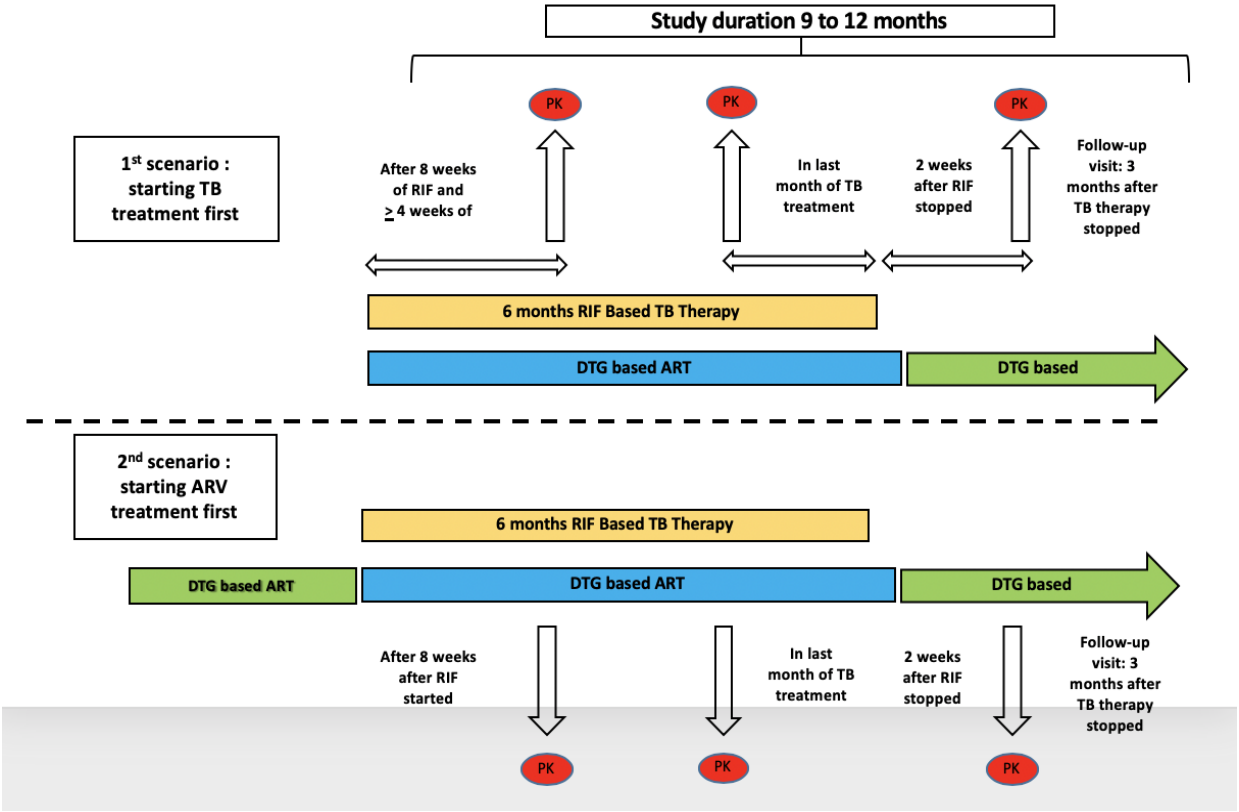
Antiretroviral efficacy is based on HIV viral suppression. The cut-off value related to virological failure is defined as a viral load superior to 400 copies per mL, confirmed within a month. Viral load will be assessed as per the SOE (**Table 2**)

The clinical outcome of the anti-TB treatment will be assessed retrospectively by a central panel that will review all cases for diagnostic accuracy and response to therapy.

- **Enzyme polymorphisms**

Enzyme polymorphism analysis will be conducted at a later stage from stored samples to determine the importance of polymorphisms in genes regulating anti-TB drug and antiretroviral concentrations and effects in the study population.

3 STUDY DESIGN AND STUDY DESIGN RATIONALE



3.1 Study design

This is a single centre, open-label, non-randomised, prospective study evaluating the steady-state pharmacokinetics of twice-daily dose DTG administered during concurrent RIF treatment and assessing safety and tolerance in HIV-TB co-infected children weighing 3 to 35 kg.

DTG will be administered as a twice-daily dose of 50mg FCT/10mg DT tablet formulation both before starting and after completion of the standard six-month RIF-based anti-TB treatment. The NRTI background and anti-TB drugs will be prescribed following the national weight band dosing guidelines.

Those initially diagnosed with TB are likely to be sicker, and the recommendation is to start anti-TB treatment first and follow with ART two weeks later.

3.2 Study duration and duration of subject participation

The study duration will be a maximum of 48 weeks. Eligible children whose parents/guardians have provided informed consent will be enrolled. PK sampling will be done during TB treatment at Week 8 and in the last month before rifampicin is stopped ~ **(approximately)** week 24 (+/-2 weeks). This

may vary, depending on TB treatment duration. At least two weeks after stopping RIF, PK sampling will be done at ~week 32 +/- 2 weeks. Intensive PK sampling will be done at Weeks 8 and 32 **(This may vary, depending on TB treatment duration)**. Children will be given the option to either be admitted to the Paediatric unit overnight or arrive early in the morning for the PK. Medication adherence during the last three days prior to the PK day will be confirmed, ensuring that none of the doses was missed. DTG and rifampicin (observed doses the latter as part of full TB treatment)) will be given at the same time. PK blood samples (intensive) will be taken via i.v. cannula at t=0 (pre-dose), and at t=1, 2, 3, 4, 6, 12 or 24h (after the dose) to measure DTG concentrations. A double trough sample (both 11 and 12 hours post-dose - sparse sampling) will be done at ~ **(approximately)** the week 24 visit.

All subjects will be followed up for at least three months following the completion of TB treatment up to a maximum of 48 weeks.

4. SUBJECT POPULATION

HIV-TB co-infected children weighing between 3 and 35 kg receiving, or with an indication for DTG based combination therapy and concurrent RIF-based anti-tuberculosis treatment at the study sites.

Subjects must meet **all** of the following inclusion criteria to be eligible for enrolment into the study:

4.1 Inclusion criteria

- Children <18 years and >4 weeks with confirmed HIV-1 infection weighing 3-35kg ART-naive or experienced, with plans to use DTG for HIV treatment
- Diagnosis of TB disease with clinician initiating rifampicin-containing first-line therapy
- Parents/legal guardians/caregivers and children give informed written consent (or assent, where applicable) to be in the study
- Girls who have reached menses must have a negative pregnancy test at screening and be willing to adhere to two effective methods of contraception (barrier and a non-barrier form of contraception during the study, starting at least 14 days prior to enrolment) if sexually active. The parents/caregivers will be counselled together with the child if the child tests positive in order to reduce any social harm which may arise.

4.2 Exclusion criteria:

- History or presence of known allergy or contraindications to DTG
- Alanine aminotransferase (ALT) ≥5 times the upper limit of normal (ULN), OR ALT ≥3xULN and bilirubin ≥2xULN
- Severe hepatic impairment or unstable liver disease (as defined by the presence of ascites, encephalopathy, coagulopathy, hypoalbuminaemia, oesophageal or gastric varices, or persistent jaundice), known biliary abnormalities (except for Gilbert's syndrome or asymptomatic gallstones)
- Pregnancy or breastfeeding
- A concurrent illness that could influence drug PK, i.e. severe diarrhoea, vomiting, renal or liver disease
- Treatment with concomitant medications known to have interactions with DTG
- Participants that are eligible for the study but refuse to give consent and/or assent

4.3 Co-enrolment criteria

- The Trial management will consider Co-enrolment into other studies should they not compromise the study.

5. ASSESSMENTS, SCHEDULE OF EVENTS AND MANAGEMENT OF SUBJECTS

All subjects will be followed up for at least three months following the completion of TB treatment up to a maximum of 48 weeks.

5.1 Screening for eligibility

Screening and enrolment do not require any study-specific evaluation. HIV and TB evaluations, as well as initial therapeutic decisions, are all part of the routine standard of care. The enrolment form will confirm eligibility, i.e. that inclusion criteria are met and that no exclusion criteria are present. Informed consent may be signed immediately, and subjects enrolled as soon as they are ready. In the rare event where the subject would be subsequently found unable to participate in the study, the **Termination CRF** would be filled, and no further data would be recorded. Management and follow-up of the child would continue following the standard of care at site.

In the enrolment process clinicians will be asked to confirm the following for all children:

- Documentation of a confirmed diagnosis of HIV-1 infection following SA clinical guidelines are available

- That the child weighs >3kg ≤35 kg
- That rifampicin based TB therapy and DTG co-therapy is needed
- That the parent or legal guardian is able and willing to provide written informed consent and able to attend study visits
- That there is no concomitant/chronic treatment with potent enzyme-inducing/inhibiting drugs other than those in the study treatments except minor inducers/inhibitors and drugs used in the short term as part of the management of the condition
- The clinician anticipates that anti-TB treatment duration will be shorter than nine months
- That the child does not suffer from a condition that, in the investigator's opinion, would compromise the child's participation in this study
- That the child received no treatment with experimental drugs for any indication within 30 days prior to study entry

Where childreninitiated TB therapy prior to the initiation of antiretroviral therapy, the clinician will be asked to confirm that:

- The rifampicin-based TB therapy is at the dose suggested in the protocol.
- The patient will require DTG (and that dosing can be done at the dose suggested in the protocol).
- That at the projected time of the PK, the child will have been on rifampicin for 1-2 months AND on DTG for at least seven days. This will be recorded on the **SOURCE DOC.**

Where children already on antiretroviral therapy require TB therapy, the clinician will be asked to confirm on the **SOURCE DOC** that:

- The current DTG dose is at or can be changed to the dose suggested in the protocol
- Rifampicin based TB at the dose and regimen suggested in the protocol will be started

Where co-therapy was already initiated at the time of screening, the clinician will be asked to confirm that:

- The child received rifampicin and other TB medications at the dose suggested in the protocol
- The child received DTG at the dose suggested in the protocol or can be changed to the dose suggested in the protocol
- The child received the DTG dose at the dose suggested in the protocol or as per SOC **AND** from the time rifampicin was initiated

- At the projected time of the PK, the child will have been on rifampicin for 1-2 months **AND** on DTG for at least seven days

None of these eligibility criteria requires study-specific clinical or biochemical evaluations. All safety evaluations are in accordance with the standard of care guidelines. Therefore, if all are met, parents/guardians can be approached, the study explained to them and participation offered for their child.

5.2 Informed consent and enrolment

Eligible children whose parents/guardians have provided written informed consent and meet inclusion/exclusion criteria can be immediately enrolled. Informed consent forms, as well as identification and contacts, will be kept confidentially in a locked cabinet at the site. All case report forms will be identified by a Patient Identification Number to protect confidentiality.

The investigator or designated study team member will fully explain the nature, conditions and consequences of the study to the responsible parent or legal guardian and the child if he/she is able to understand the nature of the study. For older children who understand their clinical condition, assent will be obtained. A competent translator familiar with the study will assist in the informed consent process when necessary. Subject information and consent documents will be available in isiZulu and English.

A witness independent of the study will countersign the consent documents of illiterate parents or legal guardians. Each parent or legal guardian will be given a copy of the signed consent document. No patient will be enrolled prior to or without the written informed consent of a parent or legal guardian.

Medical history, baseline clinical status, HIV WHO staging, TB evaluation, virology, immunology and safety/toxicity haematology and chemistry evaluations will be obtained at screening and enrolment. Prior results will be used when appropriate: viral load and CD4 count within three months of enrolment, all other biological evaluations within one month of enrolment. Data from any additional blood cultures, chest X-rays (CXR), blood tests or procedures performed during the study period will be collected as part of the study.

HIV status: This one-page form documents how the subject's HIV infection was diagnosed and confirmed. This form is filled only once.

The one-page **Contact form** ensures that addresses and telephone numbers are obtained so that the risk of loss to follow-up is minimised. This form is for the site study team only; it always remains at the site and is updated at each visit. This form is filled in only once.

Socio-demographic data CRF: this one-page form records basic information on the child and the caregivers. This form is filled in only once.

The **Medical History CRF** records significant illnesses and allergies, while prescribed treatments are reflected on the **Concomitant Medications CRF**.

HIV viral load is recorded on the **HIV Monitoring-Viral load CRF,** and CD4 evaluations are recorded on the **HIV Monitoring-CD4 CRF**.

WHO staging is recorded on the **Physical Examination CRF.** This one-page form records basic clinical data such as anthropometry, vital signs and any new/persisting signs. This form will be filled at all visits. These are fully described in the **Adverse Event CRF**.

The **Baseline TB Severity CRF** records the type of TB.

Laboratory Microbiology Smear Results, Laboratory Microbiology Culture Results and Laboratory Microbiology Antibiotic Susceptibility CRFs are used to assess the certainty of the TB diagnosis as well as decide on its severity. However, the study will not interfere with the management at the site regardless of the outcome of this assessment.

Chest X-ray: This document is complementary to the Tuberculosis assessment CRF. It records the clinician's assessment of the X-ray made at the site. A good quality digital photo of the radiography will be joined to the CRF for documentation. Where follow-up CXRs are performed because they are clinically indicated, this data will be captured and images stored.

Chemistry, **Urine analysis**, **Haematology**, **immunology** and other **Lab results forms**: Lab test requests will use the site standard forms. If this is impossible, the corresponding lab results CRFs may be filled while the original results kept in the hospital file for verification. Detailed identification of the scanned lab slip file is very important as the slip may not be transmitted with the corresponding CRF.

Adverse events, Serious Adverse Events, and concomitant treatment forms may be filled as needed at any visit.

5.3 Monthly drug dispensation visit

At the monthly routine drug dispensation visit, the contact form will be updated, basic clinical evaluation performed and concurrent diseases/adverse events, concomitant treatments documented, and the drug dispensation/adherence form filled. Since the main toxicities of DTG involve gastrointestinal (GI) side effects (especially nausea, vomiting, and diarrhoea), as well as liver toxicity, visits to evaluate safety and toxicity will include questioning for the presence of GI side effects and blood sampling for liver function tests (LFTs). In addition, a neuropsychiatric and suicidal ideation evaluation is addressed in the **Acceptability, Mood and Sleep CRF**. The three-page questionnaire will also address the acceptability of the medications to the parents in terms of storage, palatability, side-effects, etc. **Adverse events, Serious Adverse Events, Concomitant Medication, and Termination CRFs (including Cause of Death)** may be filled as needed. The ARV and TB treatments will be dispensed according to the South African guidelines. The routine measures will be employed to support treatment adherence.

5.4 Pharmacokinetic evaluation visits

PK sampling will be done during TB treatment at Week 8 and approximately two weeks before rifampicin is stopped ~ (**approximately**) week **24** (+/-2 weeks). This may vary, depending on TB treatment duration. Children will continue on DTG twice-daily dosing for two weeks after rifampicin is stopped. After **at least** two weeks on DTG once-daily dosing, PK sampling will be done at ~ **(approximately**) week 32 (+/-2 weeks), **and this timepoint may vary due to the variable duration of TB treatment in children.** Intensive PK sampling will be done at **(approximately)** Weeks 8 and 32. Children will either be admitted to the Paediatric unit overnight or have transport organised for travel to and from the PK visits. Medication adherence during the last three days prior to the PK day will be confirmed, ensuring that none of the doses was missed. DTG and rifampicin (observed doses) will be given at the same time. PK blood samples (intensive) will be taken via an I.V. cannula at t=0 (pre-dose) and at t=1, 2, 3, 4, 6, 12 or 24h (after the dose) to measure DTG concentrations. A double trough sample (both 11 and 12 hours post-dose) will be done at ~ **(approximately)** week 24 (2 weeks before rifampicin is stopped).

Methods for PK analysis:

In order to participate in the PK evaluation, the subject must have been taking the prescribed dose of DTG for at least one week and the RIF for at least four weeks. If the participant vomited after

taking his/her ARV treatment the previous evening or has acute diarrhoea, or if the subject has been less than 100% adherent to treatment during the previous three days (as assessed using the standardised treatment adherence questionnaire), the PK sampling day will be deferred to a time when the problem has resolved (up to 4 weeks). However, the first PK evaluation should be performed when the subject is in the intensive phase. Parents or caregivers will be asked to record the exact time of the DTG dose the previous day before the PK day. On each PK sampling day, a standardised questionnaire will be administered to evaluate adherence.

Parents or caregivers will also be asked to ensure that the children do not eat for 1 hour before dosing and for 1 hour after dosing (fasting for 2 hours) on the morning of the PK evaluation.

Children will have 12-hour PK visits, during which time six blood samples for PK analysis will be taken. Children will be given the option to either be admitted to the Paediatric unit overnight or be provided with transport home. Medication adherence during the last three days prior to the PK day will be confirmed, ensuring that none of the doses was missed. DTG and rifampicin (observed doses) will be given at the same time. PK blood samples (intensive PK sampling) will be taken via i.v. cannula at t=0 (pre-dose), and at t= 1, 2, 3, 4, 6, 12 or 24h (after the dose) to measure DTG concentrations. A double trough sample (both 11 and 12 hours post-dose) will be done at the sparse PK visit ~ week 22-24 (2 weeks before rifampicin is stopped).

Blood samples will be spun down immediately and stored at -80°C as soon as possible at the CAPRISA laboratory. DTG concentrations in the plasma will be measured using HPLC/MS-MS in the University of Cape Town pharmacology laboratories according to validated methods. Optional hair samples will be collected, and analysis will be conducted similar to a published method for DTG at the University of California, San Francisco as described above.

During the visit, the **Contact** form is updated, the **Physical Examination** performed and recorded, and the **Treatment and Pill Count CRFs** are filled.

The **ART PK level CRF** (2 pages) **Intensive PK level CRF** (5 pages) records the dose, date and time of intake of each of the antiretroviral and anti-tuberculosis drugs taken the day before and on the morning of the PK evaluations. Any vomiting/spitting out of medication between intake and PK evaluations is recorded.

Adverse events, Serious Adverse Events, Concomitant medication and Termination CRFs may be filled as needed.

5.5. End of the study visit

The last study visit will take place at week 48. Appropriate laboratory, clinical and other evaluations will be performed in accordance with SoE. CXR will only be repeated if deemed necessary by the treating physician.

At the end of the study, the **Physical Examination** and **TB** assessment is performed and recorded, and the **Treatment** and **Pill Count** form is filled.

Adverse events, Serious Adverse Events, Concomitant Treatment, and **Termination CRFs** may be filled as needed.

5.6 Management of patients with adverse events/ toxicities and serious adverse events (all visits)

In patients with symptoms of TB at the initiation of antiretroviral and TB therapy, clinical improvement is expected (defervescence, improvement of respiratory symptoms, and decrease in size of lymph nodes). However, a paradoxical flare-up of TB symptoms during the first months of antiretroviral therapy is not uncommon. Symptoms/ manifestations suggestive of TB-IRIS (and other opportunistic infection) will be monitored. These symptoms are related to neither ARVs nor anti-TB drugs but rather to the abrupt improvement of the patient's immune status. They are well known to clinicians, and managing them is part of the standard clinical care of HIV.

A Serious Adverse Event (SAE) Evaluation Group will be established to help standardise evaluations and management. All SAEs, including liver toxicities Grade 3, will be reported within 24 hours and discussed with the SAE Evaluation Group. Alternatively, if toxicities are deemed related to anti-TB drugs, treatment may be halted, and drugs subsequently reintroduced one by one. The final decision regarding patient management will rest on the attending paediatrician and will not be mandated by the protocol or the protocol team. Where medications are altered from the expected course, data already collected will be retained, but where either rifampicin or DTG is stopped, further PK will not be performed.

Table 3. ORCHID Schedule of Evaluations

Description	Screening	Enrolment	Follow-up						End of Study
Trial Visit	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**	**Visit 9**
Day/Week	**D-28 to D-1**	**D0**	**D28**	**Week 8**	**Week 12**	**Week 24**	**Week 32**	**Week 40**	**Week 48**
Study Windows			**+/- 14 d**	**+/- 30 d**	**+/- 14 d**	**+/- 30 d**	**+/- 30 d**	**+/- 14 d**	**+/- 14 d**
Informed Consent	X	X							
Age verification	X								
HIV status verification	X								
Urine pregnancy test in females	X		X	X	X	X	X	X	X
Vitals including weight and height	X	X	X	X	X	X	X	X	X
Tanner scale	X					X			X
Targeted physical exam and clinical assessments	X		X	X	X	X	X	X	X
Safety bloods (Hb.Urea.Creatinine, e-GFR, ALT.Amylase), FBC as clinically indicated	X		X	X	X	X	X	X	X
Urine dipstick	X								X
Lipodystrophy assessment	X								X
DNQ Susceptible TB diagnosis/confirmation as part of SA DoH	X								
CD4+cell count	X					X			X
HIV RNA viral load[@]	X			X	X	X			X
ART administration		X	X	X	X	X	X	X	X
TB treatment^ (initiation and duration may vary)	X	X	X	X	X				
AEs/SAEs assessment			X	X	X	X	X	X	X
TB associated IRIS assessment			X	X	X	X	X	X	X
Concomitant medication review	X	X	X	X	X	X	X	X	X
PK blood draws				X[5]		~2 weeks before stopping RIF[6]	~2 weeks after stopping RIF[7]		
Optional pharmacogenetic sample		X							
Optional hair sample			X	X[4]	X[4]	X[4]			
Stored plasma sample for HIV-1 genotyping	X[1]					X[3]			X[3]
Acceptability and mood Questionnaire			X	X	X	X			X

[1] Children on ART for four months or more and with VL>50 copies/mL at baseline, repeat VL at three months and store additional 1mL for resistance testing; [2] Repeat at end of study if VL > 50 copies/mL; [3] To take 1mL blood for storage (resistance testing) if the last VL > 50 copies/mL; [4] Hair sampling for drug exposure/adherence evaluation; **[5], [6] and [7] The timepoint for the PK blood draw at Week 8, 24, and 32 may vary due to due to the variable timing of DTG/RIF start prior to enrolment and the duration of TB treatment in children and as described on page 25 (section 3.2 and page 32 (section 5.4)**

6 TREATMENTS

Drugs used in this study are those used as part of the standard treatment regimens for HIV and TB in South Africa or recommended by the WHO, according to the Department of Health or WHO guidelines, and their dosing will follow the weight bands used in South Africa or as recommended by the WHO.

6.1 Antiretroviral treatment

Dolutegravir (DTG)

DTG is part of a combination regimen that includes two NRTIs (ABC or AZT plus 3TC).

Table 4. DTG dosing chart [40]

Weight	DTG (10mg DT)	DTG (50mg FCT)
Frequency (with RIF)	Twice daily	Twice daily
Frequency (without RIF)	Once daily	Once daily
3 – 5.9 kg	1 x 10mg DT	
6 – 9.9 kg	1.5 x 10mg DT	
10 – 14.9 kg	2 x 10mg DT	
15 – 19.9 kg	2.5 x 10mg DT	
>20 kg		1 x 50mg FCT

Table 5. Antiretroviral drug dosing chart for children 2021 (Appendix F)

6.2 Anti-tuberculosis treatment

- RIF is part of a multidrug regimen in combination with isoniazid (INH) for 6 to 9 months, as well as ethambutol (EMB) and pyrazinamide (PZA) during the first two months of therapy.

Table 6. Anti-tuberculosis treatment dosing (preferred)

	Intensive Phase *					Continuation Phase	
	1	**2**	**3**	**4**	**5**	**1**	**2**
	RH 60/60mg	**PZA 500mg**	**RHPZA 75/50/150mg**	**RH/PZA/EMB 150/75/400/275mg**	**EMB 400mg or 400mg/8ml**	**RH 60/60**	**RH 75/50**
3–3.9 kg	¾ Tab 45/45mg	¼ Tab 125mg	¾ Tab 56/38/113mg		¾ Tab 75mg	¾ Tab 45/45mg	¾ Tab 56/38mg
4–5.9 kg	1 Tab 60/60mg	¼ Tab 125mg	1 Tab 75/50/150mg		¼ Tab 100mg	1 Tab 60/60mg	1 Tab 75/50mg
6–7.9 kg	1½ Tab 90/90mg	½ Tab (250mg	1 ½ Tab 113/75/225mg		¼ Tab 100mg	1½ Tab 90/90mg	1 ½ Tab 113/75mg
8–11.9 kg	2 Tab 120/120mg)	½ Tab 250mg	2 Tab 150/100/300mg		½ Tab 200mg	2 Tab 120/120mg	2 Tab 150/100mg
12–14.9 kg	3 Tab 180/180mg)	1 Tab 500mg	3 Tab 225/150/450mg		¾ Tab 300mg	3 Tab 180/180mg	3 Tab 225/150mg
15–19.9 kg	3½ Tablets 210/210mg)	1 Tab 500mg	4 Tab 300/200/600mg		¾ Tab 300mg	3½ Tablets 210/210mg	300/200mg
20–24.9 kg	4½ Tab 270/270mg)	1½ Tab 750mg			1 Tab 400mg	4½ Tab 270/270mg	
25–29.9 kg	5 Tab 300/300mg)	2 Tab 1000mg			1 ¼ Tab 500mg	5 Tab 300/300mg	
30–35.9 kg	6 Tab 360/360mg)	2.5 Tab 1250mg		2 Tab 300/150/800/550mg	1 ½ Tab 600mg	6 Tab 360/360mg	

6.3 Concomitant medications

Concomitant medications not allowed on this trial are:

- Long term treatment with potent enzyme-inducing or –inhibiting drugs other than those in the study treatments within four weeks of PK assessment days. (Study treatments include all anti-TB drugs and ARVs). This includes anti-fungal azoles, except where treatment has been discontinued for at least one week before PK evaluation.
- Chronic use of herbal medications is discouraged as a routine and parents/caregivers should be asked about these medicines during follow-up.

7. STUDY ASSESSMENTS

7.1 Timing of Assessments

Refer to the Schedule of events, Table 1, Section 5

7.2 PK Drug Assessment

LCMS: Validated liquid chromatography-mass spectroscopy (LC-MS) methods will be used to determine DTG concentrations in the samples prepared from plasma, in the Division of Pharmacology's laboratory at the University of Cape Town, which is ISO17025 accredited for this purpose.

Levels below the limits of quantification will be reported as such. Plasma (DTG): 0.5 mL blood samples will be centrifuged for 10 minutes at 3000 rpm, divided into 2, and stored at -80 °C immediately (within 30 minutes) after collection.

The blood samples should be kept in ice while awaiting centrifugation. From each sample, 2 x 0.125 mL aliquots of plasma will be transferred to into duplicate labelled polypropylene tubes (Eppendorf tubes suitable for storage at -80ºC). The plasma samples should be transferred immediately into a -80ºC freezer or stored in dry ice while awaiting transfer into a -80ºC freezer.

The label on each polypropylene storage tube will include the subject number, sampling occasion, and sampling time.

The duplicate plasma samples will be kept at -80ºC until analysed, up to 5 years after study completion.

7.3 Other Assessments

Blood samples will be stored for the following sub-studies, for which informed consent will be taken during the initial informed consent process.

- For subjects failing to suppress viral load, samples for possible HIV viral resistance testing will be stored.
- Hair samples (optional) will be taken at baseline from all children on ART. Hair samples will be taken from all children on week 4, 8, 12 and 24 follow-up visit for determination of drug levels to assess adherence to medication. This will be stored and measured at a later stage. (See Appendix D)
- A pharmacogenetic sub-study (optional) to evaluate the influence of specific pharmacogenetic variants on the levels of anti-TB drugs and ARVs during co-treatment will be conducted.

The Steering Committee (consisting of study investigators) will discuss and approve additional sub-studies apart from those mentioned above and will be submitted as separate protocols for ethical review.

7.4 Assessment of Safety

7.4.1 Laboratory examinations

Routine tests to monitor the safety and efficacy of treatment will be conducted according to the National Treatment Guidelines, with an additional focus on liver biochemistry. Subjects will be monitored clinically and biochemically for safety (see Table of Events, Table 1, Section 5). 0.5 mL samples each will be drawn for haematology and biochemistry assessments, with an emphasis on liver function. Liver function tests will be carefully reviewed for increases in ALT. Increases meeting the DAIDS criteria for Grade 3 or 4 events must be reported as SAEs. ALT increases meeting Grade 2 DAIDS criteria and higher should be repeated after 48 hours, and the child managed according to the Investigator's discretion.

All subjects will have monitoring of viral load and CD4 cell count in accordance with the SoE. Plasma will be stored for resistance testing at baseline and virological failure. Children whose viral load remains high will have more frequent monitoring of the viral load.

8. ADVERSE EVENT DEFINITIONS AND REPORTING

8.1 Adverse Event definition

An adverse event will be defined as any untoward medical occurrence (any unfavourable and unintended sign, symptom or disease, including an abnormal laboratory finding) in temporal association with the use of the investigational treatment and may or may not be causally related to it.

Abnormal laboratory (haematology and biochemistry) results will be reported as adverse events if the abnormality occurs or worsens after the institution of the study treatment and if they require clinical intervention or further investigation unless they are associated with an already reported clinical event.

8.2 Serious Adverse Event

An adverse event will be defined as serious if it is

- fatal
- life-threatening
- requires or prolongs hospitalisation
- results in persistent or significant disability

8.3 Eliciting Adverse Event information

The study clinician is required to report all adverse events and all adverse events using concise medical terminology.

8.4 Adverse Event reporting period

The adverse events reporting period for this trial begins upon subject enrolment in the trial (after the signature of informed consent) until the study ends.

All serious adverse events that occur must be reported to the PI and the SAE Evaluation Group.

8.5 Adverse Event reporting requirements

All serious adverse events (SAE) are to be reported immediately (within 24 hours of awareness of SAE by the investigator) to the PI and the SAE Evaluation Group, using the SAE report form. This includes a description of the event, onset date and type, duration, severity, relationship to study drug, outcome, measures taken and all other relevant clinical and laboratory data. The initial report is to be followed by the submission of additional information (follow-up SAE form) as it becomes available. Any follow-up reports should be submitted as soon as possible, and if possible, within five working days.

Non-serious adverse events are to be reported on the CRF, which is to be submitted to the PI. In the CRF, a given adverse event will be recorded only one time per patient, and the severity recorded will be the maximum level reached. If several distinct episodes of the same condition occur, their number will be recorded in the CRF.

8.6 Grading of Adverse Event severity

Toxicities and adverse events will be graded according to the DAIDS grading scales found in Appendix B.

8.7 Adverse Event causality assessment For both serious and non-serious adverse events, the investigator is required to assess the possible relationship between the adverse event and the study drug or procedure, i.e. to determine whether there exists a reasonable possibility that the study drug caused or contributed to the adverse event, categorising the relatedness of these events as: definite, probable, possible, unlikely or unrelated.

The decision to adjust dosages, suspend and resume treatment, or to permanently interrupt treatment due to an adverse event, is that of study clinician.

9. WITHDRAWAL CRITERIA

If a subject withdraws from the study, the reason must be noted on the CRF. If a subject is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to document the outcome.

A subject should be withdrawn from the trial treatment if, in the opinion of the investigator, it is medically necessary or if it is the wish of the subject. If a subject does not return for a scheduled visit, every effort should be made to contact the subject. In any circumstance, every effort should be made to document the subject outcome.

10. DATA RECORDING AND REPORTING

All data will be recorded in the study-specific case record form of each participant. Double data entry will be used to transcribe the data for analysis into an electronic database.

11. DATA ANALYSIS AND STATISTICAL METHODS

11.1 Sample size determination

Based on a within-subject variability (CV%) of 33% for C_t estimated from previous PK studies involving DTG, an expected withdrawal rate of 20%, and an assumption that only a difference of at least 25% in exposures would be clinically relevant, it is estimated that a sample size of 12-15 subjects to achieve at least ten evaluable subjects will provide precision for half the width of the 90% confidence interval (CI) on the log scale for the treatment difference that would be within 26% of the point estimate for AUC, C_{max}, and C_t (comparing paediatric PK to historical PK values in adults with HIV taking DTG).[37] At least 12-15 children will be included in the study. However, this number may need to be increased if inter-subject variability of DTG PK parameters in children appears to be larger than the 33% reported in adults, we have fewer children with evaluable PK data, or if the threshold for the weight-bands under study are changed based on evolving WHO guidelines on DTG dosing.

11.2 Statistical Analysis

Analysis of enrolment and baseline characteristics of children will be descriptive.

The pharmacokinetic parameters $AUC_{0\text{-}10}$, C_{max}, $C_{trough,}$ t_{max} and $t_{1/2}$, - will be determined from the concentration-time profiles, using non-compartmental methods. Summary statistics will be provided by study day for the pharmacokinetic measures for DTG. The coefficient of variation (CV) will be calculated to express inter-individual variability in pharmacokinetic parameters [(SD/mean) x 100]. 90% confidence intervals (CI) will be constructed for the ratio of the geometric means (GMR) of the AUC, C_{max}, C_{trough} , which will be reported with 90% CIs.The main exposure parameter of interest will be C_{trough}, as it is the parameter which best correlates with virological suppression. Multivariate analyses will explore the relationship between children characteristics and C_{trough}.

Additional compartmental (and multivariate) pharmacokinetic modelling will be performed on DTG data using Nonlinear mixed-effects models.

To identify baseline factors (e.g. weight-for-age, height-for-age, BMI and MUAC at the time of each evaluation with dose/kg estimated based on current weight, as well as albumin concentrations, WHO disease stage and treatment sequence) possibly associated with the PK parameters, population analyses will be performed with NONMEM using the intensive PK data (and drug levels obtained on sparse samples if any) to determine compartmental PK parameters.

11.3 Efficacy Analysis

The rates of viral load suppression (<50 HIV RNA copies /mL) at the end of the study and clinical improvement/bacteriological TB cure and treatment completion three months after cessation of anti-tuberculosis will be determined. Logistic regression analyses will explore possible associations with baseline clinical, immunological and virological variables, as well as DTG exposure (at PK – approximately week 8 and week 24)

11.4 Safety Analysis

Safety and tolerance of DTG will be evaluated by summarising the incidence of documented Grade 3 or higher adverse events in children (judged to be at least possibly related to DTG), using the DAIDS Adverse Event Grading Scales (See Appendix B).

11.5 Interim analysis

No Interim analysis is planned for Stage 1 of the protocol,

12. OVERSIGHT OF THE TRIAL

12.1 Data Safety Monitoring Committee

As Stage 1 of the protocol will be studying the standard of care regimen for treatment, i.e. twice-daily dose DTG administered during concurrent RIF treatment, a Data Safety Monitoring Board (DSMB) will not be constituted for this stage. If the modelling data support once-daily, Stage 2 version of the protocol will be submitted, and a DSMB and SAHPRA approval will be sought.

12.2 Steering Committee

A Steering Committee will be composed of the overall Principal Investigator, the Co-Principal investigator, and co-investigators and a representative of CAPRISA. They will oversee the management of the trial, represent the site and identify and approve additional analysis that may be conducted using stored samples or data from this trial.

13 QUALITY ASSURANCE AND QUALITY CONTROL PROCEDURES

13.1 Investigator's file

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified according to GCP and Ethics Committee requirements.

13.2 Case report forms (CRFs)

Data will be collected by laboratory technicians, medical doctors, clinical officers and nurses authorised by the investigator. It will be supervised by the Investigator and signed by the investigator or by an authorised staff member. Study-specific information will be entered into the Case Report Form (CRF). Data that are derived should be consistent with the source documents, or the discrepancies should be explained. All CRF data should be kept anonymous, i.e. only identified by study patient number.

The investigator at each trial site should ensure the accuracy, completeness, legibility, and timeliness of all data reported to the sponsor in the CRFs and any other additional information that is required. The investigator is responsible for keeping all consent forms, screening forms, CRF and the completed subject identification code list in a secure location.

Data storage: A case record form will be compiled for each participant for collection of subject-specific details, study-related blood test results (apart from drug concentrations), drug dosing details and the exact times of PK sampling. The raw data relating to the drug concentrations will be kept in a study-specific document. All study-related documents will be stored for at least five years following study completion.

Electronic Data Capture (EDC): The paper-based CRFs will be captured and managed on a digital platform and stored directly in the central database. The database ensures compliance with medical data privacy, security, and Good Clinical Practice regulations. The Data Manager is responsible for the entire data management process.

13.3 Source documents

The verification of the CRF data must be by direct inspection of source documents. Source documents include subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, X-ray (photographs), pathology and special assessment reports, signed informed consent forms, consultant letters, and subject screening and enrolment logs.

The investigator must maintain source documents such as laboratory and consultation reports, history and physical examination reports, etc., for possible review and/or audit by CAPRISA and/or Regulatory Authorities. The Investigator/designee will record the date of each subject’s visit, together with a summary of their status and progress in the study.

13.4 Record Retention

The investigator must keep all study documents on file for at least 15 years after completion or discontinuation of the study. After that period the documents may be destroyed with prior permission from CAPRISA, subject to local regulations.

Should the investigator wish to assign the study records to another party or move them to another location, CAPRISA must be notified in advance.

13.5 Monitoring, audits and inspections

Monitoring visits to the trial site will be made periodically by CAPRISA Quality Assurance (QA) teams or designated clinical monitors to ensure that GCP and all aspects of the protocol are followed. Source documents will be reviewed for verification of consistency with data on CRFs. The investigator will ensure direct access to source documents by CAPRISA or designated representatives. It is important that the investigators and their relevant personnel are available during the monitoring visits.

The investigators will permit representatives of CAPRISA and/or designated clinical monitors to inspect all CRFs, medical records, laboratory worksheets and to assess the status of drug storage, dispensing and retrieval at any time during the study. The corresponding source documents for each subject will be made available provided that subject confidentiality is maintained in accord with local regulations. The inspections are for the purpose of verifying adherence to the protocol and ensuring the study is conducted according to GCP. It is important that the investigators and other trial site staff are available at these visits.

The monitoring visits provide CAPRISA with the opportunity to evaluate the progress of the study, verify the accuracy and completeness of CRFs, resolve any inconsistencies in the study records, as well as to ensure that all protocol requirements, applicable regulations, and investigator's obligations are being fulfilled. Four visit types are planned: pre-study, study start, during the study, and study end. Regulatory authorities may also perform visits.

It will be the clinical monitor’s responsibility to inspect the CRF at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered on them. The investigator agrees to cooperate with the clinical monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

13.6 Audits and inspections

The trial site may also be subject to quality assurance audits by CAPRISA QA team or designated representatives and/or to inspection by regulatory authorities or Independent Ethics Committees (IEC).

The investigators and their relevant personnel must be available for possible audits or inspections.

13.7 Data Management

Data management in the study will be done in accordance with CAPRISA data management processes and SOPS. After the CRF has been completed and monitored by the clinical monitor, CRFs will be collected, and data will be entered onto a database using double independent data entry or an Electronic Data Capture (EDC) format. The trial data will be stored in a computer database maintaining confidentiality in accordance with national data legislation and ICH guidelines.

In order to ensure data quality, a uniform hard copy CRF or CRF created on an EDC system will be designed for use at all the sites.

13.8 Confidentiality of trial documents and subjects records

The investigator must assure that subjects' anonymity will be maintained and that their identities are protected from unauthorized parties. On CRFs or other documents submitted to the sponsor, subjects should not be identified by their names, but exclusively by an identification code. The investigator should keep a subject enrolment list showing codes, names, and addresses. The investigator should maintain documents for submission to sponsor authorized representative, and the subject's signed written consent forms, in strict confidence.

14 PROTOCOL AMENDMENTS

The Principal investigator will ensure that the study protocol is strictly adhered to throughout and that all data are collected and recorded correctly on the CRF or EDC system.

All protocol modifications must be documented in writing. Any protocol amendment must be approved and signed by the sponsor and the Principal investigator/s and is to be submitted to the appropriate IEC for information and approval in accordance with local requirements, and to regulatory agencies if required. Approval by IEC (and Regulatory Authority, if applicable) must be awaited before any changes can be implemented, except for changes necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial [e.g. change in clinical monitor[s], change of telephone number[s].

The protocol amendment can be initiated by either sponsor or by any Principal investigator.

15 TERMINATION OF THE STUDY

Both the sponsor and the investigator reserve the right to terminate the study at any time prior to inclusion of the intended number of subjects, but they intend to exercise this right only for valid scientific or administrative reasons. Should this be necessary, both parties will arrange the procedures on an individual study basis after review and consultation. In terminating the study, the sponsor and the investigator will assure that adequate consideration is given to the protection of the subjects' interest.

Reasons for termination by the sponsor(s) may include but not be limited too:

- Too low enrolment rate;
- Protocol violations;
- Inaccurate or incomplete data;
- Unsafe or unethical practices;
- Questionable safety of the test article;
- Suspected lack of efficacy of the test article;
- Following the recommendation of the IEC; and
- Administrative decision.

Reasons for termination by the investigator may be:

- Insufficient time or resource to conduct the study
- Lack of eligible patients

If a study is terminated either by the sponsor or by the investigator, the investigator has to:

- Complete all CRFs to the greater extent possible
- Return all test articles, CRF, and related study materials to the sponsor who provided them
- Answer all questions of the sponsors or their representatives related to data of subjects enrolled at the site prior to study termination
- Ensure that subjects enrolled in the study who had not yet reached a follow-up time point are followed up with the necessary medical care.
- Provide in writing the reasons for his decision to the national health authority and the sponsor.

16 ETHICS

The experimental protocol for this study has been designed in accordance with the general ethical principles outlined in the Declaration of Helsinki and ICH guidelines for Good Clinical Practice (International Committee for Harmonization). CAPRISA assures that it will comply with all applicable state, local and foreign laws for protecting the rights and welfare of human subjects. This protocol and any protocol amendments will be reviewed/approved by an IEC before its implementation.

It is the responsibility of the Investigator to apply for review to the IEC of the country where the study takes place regarding local rules and regulations. Written approval from all involved IECs must be obtained before the implementation of any protocol-specified intervention/investigation provided to the subject [such as subject information sheets or descriptions of the study].

Any modifications made to the protocol after receipt of the IEC approval must also be submitted by the investigator in writing to the IEC in accordance with local procedures and regulatory requirements.

16.1 Informed consent process

Inclusion in the study will only occur if the parent/guardian gives written informed consent. It is the responsibility of the investigator/designee to obtain written informed consent from each individual participating in this study after the adequate presentation of the aims, methods,

anticipated benefits, and potential hazards of the study. The written informed consent document will be translated into the local languages, i.e. isiZulu. If needed, the person will be given time to discuss the information received with members of the community or family before deciding to consent. Included in the informed consent process will be a request to consent to store samples for later pharmacokinetic analysis. The parent/guardian will be asked to provide written and signed consent.

A separate signature page will be required to be filled in by the parent/guardian for storage of blood for enzyme polymorphism study and other future studies. Subjects can enrol for the PK study but decline to consent for the pharmacogenetic study.

If the subject is illiterate, a literate witness must sign (this person should have no connection to the research team, and, if possible, should be selected by the participant. If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All subjects (including those already being treated) should be informed of the new information, given a copy of the revised form and give their consent to continue in the study.

16.2 HIV status

All subjects included in this study will be HIV positive.

16.3 Patient costs

Patients and caregivers will be reimbursed an amount of R350 for travel to and from the study site and a meal but will not receive any payment for trial participation. During the Intensive PK Visits the patients and caregivers will be reimbursed as per the SAHPRA: Clinical Trial Participant Time, Inconvenience and Expense (TIE) Compensation Model (V1.0 May 2018). Any medication that is required during the trial period will be provided free of charge to the patient and caregiver/guardian. Food will be provided during the long pharmacokinetic study days, free of charge to the patient and the accompanying caregiver. This is seen as an essential part of the patient care plan bearing in mind the high prevalence of malnutrition and the poverty of these patients.

17 INSURANCE AND LIABILITY

The study sites will have insurance against any claim for damages brought by the research subject who suffers a research-related injury during the performance of the trial according to the protocol.

18 REPORTING AND PUBLICATION

Registration of clinical trials

Before a clinical trial is initiated, its details need to be registered in a publicly available, free to access, searchable clinical trial registry. All CAPRISA clinical trials should be registered with the South African National Clinical Trials Register, and all trials meeting the National Institutes of Health (NIH) definition of a clinical trial is also registered with Clinicaltrials.gov.

18.1 Publication policy

Public disclosure of results from clinical trials (including through journal publication) and dataset sharing

UKZN and CAPRISA have an established procedure to make their research data more broadly available. Information on the process for requesting and obtaining data is available on the CAPRISA website (www.caprisa.org). Datasets used for the analyses for a CAPRISA research article that has been published can be requested by any investigator through an online request lodged on the CAPRISA website. The request will be assessed by the CAPRISA Scientific Review Committee, and once approved, the dataset will be made available to the investigators making the request. In line with standard data access principles, CAPRISA will ensure that metadata on the datasets will be made available together with the study protocol and other relevant documents. Anonymization and other measures will be taken to protect individual and personally identifiable information in the datasets. In addition, summary results of the trial will also be made publicly available in a timely manner by posting to the results section of the clinical trial registry and papers will be made available through PubMed Central (for NIH studies) or through UKZN Research Space (for non-NIH studies) within 12 months of publication.

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20 APPENDICES

Appendix A: Concomitant Tuberculosis, South African Guidelines for Management of HIV in Children 2020, STG- EML

TB treatment

If the child is not yet on ART:

» Commence TB treatment first. Follow with ART, usually after 2-4 weeks. In children with TB meningitis, start ART at four weeks regardless of CD4 count to avoid IRIS.

» Check ALT before commencing ART. If the ALT is raised, discuss this with an expert as it may not be an absolute contraindication to treatment.

» Assess the child for possible disseminated TB disease.

» Be aware of the possibility of Immune Reconstitution Inflammatory Syndrome (IRIS).

If the child is already on ART:

» Commence TB treatment, taking into consideration possible drug interactions and the need for ART dosage adaptations. Do not stop ART.

If the child needs to take concomitant ART and rifampicin-containing treatment:

» Dolutegravir: use DTG twice daily

» Efavirenz: use the normal recommended dosage as per the dosing table.

» Abacavir and lamivudine: no adjustment of dosages

» Lopinavir/ritonavir: refer to the dosage table for the ritonavir boosting doses.

» Avoid using double-dose lopinavir/ritonavir solution in young children. If ritonavir powder is not available, consult an expert.

» Give pyridoxine (vitamin B_6) to all children on TB and ARV treatment due to shared toxicities of the regimens.

Appendix B: DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, Corrected Version 2.1

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]. Available from: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf

Appendix C: HIV testing in children, South African Guidelines for Management of HIV in Children 2020, STG-EML

All infants/children accessing care should have their HIV status determined.

- Patients with a previously positive HIV test and on ART should not be re-tested.
- Where mothers tested negative in pregnancy, maternal HIV status should be determined three monthly while breastfeeding.

Confirmation of HIV infection

Children < 18 months of age:

» **Birth:** Do HIV PCR at birth in all HIV exposed infants

» **10 Weeks:** Do HIV PCR at ten weeks of age in all HIV exposed infants.

» **6 Months:** Do HIV PCR at six months in all HIV exposed infants. HIV status of all infants not already known to be HIV-exposed should be established by offering the mother an HIV test.

» **Post cessation of breastfeeding:** If the child is breastfed and previous HIV PCRs are negative, repeat testing six weeks after complete cessation of breastfeeding. (If the child is 18 months or older, do an ELISA or rapid test).

» **Symptomatic child/infant:** The child should be tested for HIV infection if at any time the child has evidence suggesting HIV infection, even if the child has had a previous negative PCR test.

» If HIV PCR is positive at any time-point:
  - Confirm with a repeat HIV PCR test.
  - Initiate treatment while awaiting the second HIV PCR test result.

In children ≥ 18 months of age:

» Do Universal HIV rapid/ELISA test (HIV rapid test for ALL infants regardless of HIV exposure, except in those who previously tested HIV positive and are on ART)

» If 1st rapid test is positive, confirm the result with:
  - An HIV PCR test if an infant between 18-24 months
  - A second rapid test using a kit of a different manufacturer, and preferably on a different blood specimen if an infant is >24 months.

  Note:

  Rapid tests may be less reliable in children with advanced disease. If clinical findings suggest HIV infection, but the rapid test is negative, send a further specimen of blood to

the laboratory for formal ELISA testing. If test results are still equivocal, do an HIV PCR test.

Note:

» A child cannot be confirmed as HIV negative until at least six weeks after other potential HIV exposure (including cessation of breastfeeding). Exposure to ART through prophylaxis (NVP/AZT) or maternal ART may affect the sensitivity of HIV PCR tests.

» Manage children with discordant or indeterminate HIV test results as per National Department of Health Guidelines for the Prevention of Mother to Child Transmission of Communicable Infections (HIV, Hepatitis, Listeriosis, Malaria, Syphilis and TB).

Appendix D: Hair Sampling Procedures

Small samples of scalp hair used in these analyses for patients on HAART are collected in the following manner:

1. Clean the blades of a pair of scissors with an alcohol pad and allow blades to dry prior to use completely
2. Lift the top layer of hair from the occipital region of the scalp. Isolate a small thatch of hair (~20 fibres of hair) from underneath this top layer of hair from the occipital region (can use a hair clip to keep the top layer of hair away).
3. Place a small label with the patient's STUDY ID over the distal end of the hair thatch (the side furthest away from the scalp).
4. Cut the small hair sample off the patient's head as close to the scalp as possible
5. Unfold the piece of aluminium foil and place the cut thatch of hair inside the piece of foil. Refold the foil over to completely enclose the thatch of hair.
6. Place a STUDY ID label on the folded piece of foil
7. Place the folded piece of foil inside the plastic (e.g. Ziplock®) bag (each Ziplock bag will have a desiccant bag in it) and seal the bag
8. Hair samples should be kept at room temperature and in a dark place at each site prior to shipment (can be shipped without biohazard restrictions)

Appendix E: List of drugs contraindicated with DTG or affecting its concentrations

Concomitant Drug Class: Drug Name	***Effect on Concentration of DTG or Concomitant Drug***
Antacids (Maalox, Milk of Magnesia, others)	↓ DTG - Administer dolutegravir 2 hours before or 6 hours after antacids
Calcium carbonate, Ferrous fumarate (Iron) Multiple vitamins	↓ DTG - Administer dolutegravir 2 hours before or 6 hours after calcium/iron-containing supplements. Alternatively, administer dolutegravir simultaneously with supplements and food.
St. John's Wort (Hypericum perforatum)	↓ DTG – Do not co-administer

APPENDIX F:

Antiretroviral drug dosing chart for children 2021:

		3-3.9kg	4-5.9kg	6-9.9kg	10-13.9kg	14-19.9kg	20-35kg
No Rif	DTG 10mg DT	½ tab daily		1 ½ tab daily	2 tab daily	2 ½ tab daily	
	DTG 50mg FCT						1 tab daily
With Rif	DTG 10mg DT	½ tab twice daily		1 ½ tab twice daily	2 tab twice daily	2 ½ tab twice daily	
	DTG 50mg FCT						1 tab twice daily