PETITE-DTG Study Protocol

Study Title:

Open label, single arm, two-stage trial to evaluate the single and multi-dose pharmacokinetics and safety of the paediatric dolutegravir (10 mg, scored) dispersible tablet in HIV-exposed neonates

Short Title:

PharmacokinEtics and safeTy of DolutegravIr in neonaTEs: PETITE-DTG Study

Protocol version	1.0
Date	30/05/2022
Sponsor	Stellenbosch University, Tygerberg Hospital, Cape Town, South Africa
Funder	Unitaid

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1. Contact Information

1.1. Principal Investigators

The principal investigators were responsible for developing the protocol and the analysis plan. They will participate in the organization of trainings for the teams involved in the study. The principal investigators will also inform the necessary regulatory authorities in South Africa of the progress of the study.

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16 January 2022

Date of Signature (dd/mmm/yyyy)

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1.7. Investigator Signature Page

I have read and understood the PETITE-DTG study protocol and agree to conduct the study per protocol and in accordance with the Declaration of Helsinki, International Conference on Harmonisation – Good Clinical Practice (ICH-GCP) and local regulations. I will be responsible for supervising the conduct of study at my site and ensuring that all staff members involved with the study comply with protocol requirements. I agree that the sponsor and its representatives can have access to any source documents from which case report form information may have been generated.

Principal Investigator at Tygerberg Hospital:

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Investigator Signature

16 January 2022

Date of Signature (dd/mmm/yyyy)

Contents

2. Abbreviations and Definitions

Abbreviation	Definition
3TC	Lamivudine
ABC	Abacavir
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine transaminase
ART	Antiretroviral therapy
ARV	Antiretroviral
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
BID	Twice a day
CI	Confidence Interval
CL/F	Apparent oral clearance
C_{max}	Maximum plasma concentration during the dosing interval
C_{min}	Minimum plasma concentration during the dosing interval
C_{24}	Concentration 24 hours post-dose
CRF	Case report form
CYP	Cytochrome P450
DAIDS	Division of AIDS
DSMB	Data and Safety Monitoring Board
DTG	Dolutegravir
EAE	Expedited adverse event
FAMCRU	Family Centre for Research with Ubuntu
FDA	United States Food and Drug Administration
FDC	Fixed dose combination
GCP	Good clinical practice
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Conferences on Harmonisation
INSTIs	Integrase Strand Transfer Inhibitors
IQR	Interquartile range
LPV	Lopinavir
LPV/r	Lopinavir/ritonavir (ritonavir-boosted lopinavir)
MedDRA	Medical Dictionary for Regulatory Activities
NAT	Nucleic acid test
NIH	National Institutes of Health

NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
NVP	Nevirapine
OD	Once daily
PHPT	Program for HIV Prevention and Treatment
PI	Principal Investigator
PID	Participant identification number
PK	Pharmacokinetic(s)
PMTCT	Prevention of mother-to-child HIV transmission
RTV	Ritonavir
SAE	Serious adverse event
SOP	Standard operating procedure
$T_{1/2}$	Half life
T_{max}	Time of maximum of concentration
UGT	Uridine 5'-diphospho-glucuronosyltransferase
V/F	Apparent volume of distribution
VL	Viral Load
WHO	World Health Organization
ZDV	Zidovudine

3. Synopsis

Full study title	Open label, single arm, two-stage trial to evaluate the single and multi-dose pharmacokinetics and safety of the paediatric dolutegravir (10 mg, scored) dispersible tablet in HIV-exposed neonates
Short study title	**P**harmacokin**E**tics and safe**T**y of the dolutegrav**I**r in neona**TE**s: **PETITE-DTG**
Sponsor	Stellenbosch University, South Africa
Funder	Unitaid
Protocol version	1.0
Date	30/05/2022
Amendments	NA
Study site	Family Centre for Research with Ubuntu (FAMCRU) at Tygerberg Hospital, Stellenbosch University, Cape Town, South Africa
Type of study	Phase I/II, open-label, single arm, two-stage pharmacokinetic and safety study
Name of product & manufacturer	Dolutegravir (MYLTEGA DT): 10 mg, scored dispersible tablet, Mylan (Viatris) Ltd
Study Objectives	Primary Objectives • To evaluate the pharmacokinetics of dolutegravir (DTG) during the first 28 days of life in HIV-exposed term neonates (born to a mother with HIV) following administration of DTG dispersible tablet • To determine the safety of DTG during the first 28 days of life in HIV-exposed term neonates following administration of DTG dispersible tablet Secondary Objective • To assess the acceptability of DTG dispersible tablet for the neonate and the caregiver
Study Endpoints	Primary Endpoints • DTG plasma pharmacokinetics parameters: area under the concentration time curve (AUC); maximum plasma concentration (C_{max}), apparent clearance (CL/F), and trough concentration (C_{trough}) • Occurrence of the following events: adverse events of Grade 3 or higher; treatment-related adverse events of Grade 3 or higher; any adverse events

	Secondary Endpoint • Acceptability to caregivers and neonates of using DTG dispersible tablet will be measured by means of a questionnaire
Study population	HIV-exposed term neonates born to mothers with HIV on a DTG-based antiretroviral therapy, with a birth weight ≥2000 g, and who are on antiretroviral (ARV) postnatal prophylaxis.
Study design	Participants will be enrolled in two stages: • **Stage 1** will assess a single dose of the DTG dispersible tablet in two sequential cohorts: Cohort 1A (n=8) and Cohort 1B (n=8). • **Stage 2** will assess multiple-doses of the DTG dispersible tablet in a single cohort: Cohort 2 (n=24). Per national guidelines, all infants receive a birth HIV nucleic acid test (NAT). HIV NAT test results for the infant may or may not be available (HIV pending) at the time of study entry. If an HIV NAT result comes back positive whilst the neonate is on study, the neonate will not receive any further DTG doses, revert to standard of care antiretroviral therapy (ART), and be followed for safety for the duration of the study. **Stage 1: Single Dose of the DTG dispersible tablet** Eligible HIV-exposed term neonates (pending HIV status) on standard of care ARV prophylaxis will be enrolled. Cohort 1A will open first. • Cohort 1A: 8 neonates weighing ≥2000 g at birth, born to women on DTG-based ART, will receive a single 5 mg dose of the DTG dispersible tablet at *≥14 days and <28 days of life*. Following DTG administration, blood samples (0.4 mL/time point) will be drawn at 1, 2, 3, 4, 6, 24-30 hours post-dose. All neonates will be followed for safety until 2 weeks after the pharmacokinetic evaluation visit. DTG will be administered in addition to standard of care ARV prophylaxis. After Cohort 1A is complete, and if no safety concerns are noted, accrual will continue into Cohort 1B. However, if safety hold criteria are met in Cohort 1A (Section 11.1.1), the study will be paused and data reviewed by the Data and Safety Monitoring Board (DSMB). • Cohort 1B: 8 neonates weighing ≥2000 g at birth, born to women on DTG-based ART, will receive a single 5 mg dose of the DTG dispersible tablet at *<14 days of life*. Following DTG administration, blood samples (0.4 mL/time point) will be drawn pre-dose, and at 1, 2, 4, 6, and 24-72 hours post-dose. All neonates will be followed for safety until 2 weeks after the PK visit. DTG will be administered in addition to standard of care ARV prophylaxis.

If safety hold criteria are met during Cohort 1B (Section 11.1.2), the study will be paused and data reviewed by the DSMB. After Cohort 1B is complete, all safety and PK data for Stage 1 will be reviewed. Pharmacokinetic modelling and simulation analyses using Stage 1 PK data will be performed to select the multi-dose schedule of the DTG dispersible tablet for Cohort 2. All findings will be presented to the DSMB and agreed upon before opening Cohort 2.

Stage 2: Multi-Dose of the DTG dispersible tablet

Eligible low risk* HIV-exposed term neonates (pending HIV status) born to virologically suppressed mothers with HIV on DTG-based antiretroviral therapy with a birth weight ≥2000 g on ARV postnatal prophylaxis will be enrolled.

In South Africa, women with HIV routinely receive an HIV-1 RNA viral load (VL) at delivery and their infants receives a minimum of 6 weeks of ARV postnatal prophylaxis, dependent on the risk of HIV acquisition in the infant. HIV-exposed neonates start a prophylaxis regimen of nevirapine (NVP) and zidovudine (ZDV) at birth, and continue this until the maternal VL result at delivery is available. If the maternal VL is ≥1000 copies/mL the infant is classified as 'high-risk' and continues both ARVs, else infants are deemed 'low'-risk, and ZDV is stopped and NVP continued (1). However, NVP is an inducer of CYP3A, one of the metabolic enzymes of DTG, therefore concomitant use may impact DTG pharmacokinetics. Thus, to minimize this impact, the low-risk* neonates enrolled in Stage 2 will receive ZDV prophylaxis, instead of NVP, for the duration of the study.

- Cohort 2: 24 neonates weighing ≥2000 g at birth, born to women on DTG-based ART, will receive 5 mg of DTG (half of the dispersible tablet) *using the dosing strategy developed through modelling of the Cohort 1 data*. DTG administration will begin within 7 days of life and continue until day 28 of life. DTG will be administered in addition to ZDV prophylaxis.

Multiple DTG dosing schedules during the first 28 days of life are foreseen and several potential options are shown in the table below:

	Neonate - Week of Life			
Dosing Schedule*	**Week 1**	**Week 2**	**Week 3**	**Week 4**
1	Q72 hrs		Q48 hrs	
2	Q72 hrs		Q24 hrs	
3	Q48 hrs		Q48 hrs	
4	Q48 hrs		Q24 hrs	
5	Q24 hrs		Q24 hrs	

**A DTG dosing schedule not listed in the table may be selected based on the PK analysis from Stage 1, and may differ by birth weight (e.g., above and below 3000 g)*

***Low risk**: Neonate born to a mother with a plasma HIV-1 RNA result **<50 copies/mL** in the 4 weeks prior to delivery or between delivery and infant study entry*

	All infants in Stage 2 will be followed for safety until 2 weeks after the last PK visit. An interim PK and safety analysis will be performed after 50% of participants have completed Cohort 2 to assess if a dose adjustment is required and to review safety data. Following the DSMB interim review, a different DTG dosing schedule may be selected based on the data available for the remainder of Cohort 2 infants. Any change to the DTG dosing schedule will be agreed upon by the DSMB before implementation.
Inclusion criteria	Written informed consent to participate in the study must be obtained for all participants from the parent(s) or legal guardian. **Stage 1** • HIV-exposed term neonate (pending HIV status) born to a woman within HIV on DTG-based ART • Birth weight of ≥2000 g and on standard of care ARV prophylaxis **Cohort Specific Inclusion Criteria must be met at Study Entry:** ***Cohort 1A***: Infant <14 days of life ***Cohort 1B***: Infant ≤3 days of life **Stage 2** • Low risk* HIV-exposed term neonate (pending HIV status) born to a virologically suppressed woman on DTG-based ART **Neonate born to a woman with a documented plasma HIV-1 RNA result <50 copies/mL in the 4 weeks prior to delivery or between delivery and infant study entry* • Birth weight of ≥2000 g and on standard of care ARV prophylaxis **Cohort Specific Inclusion Criteria must be met at Study Entry:** ***Cohort 2***: Infant <7 days of life
Exclusion criteria	The presence of any of the following at entry will exclude a participant from study enrolment: • Less than 37 weeks gestational age at birth • Known blood group incompatibilities which can result in hemolytic disease of the newborn (e.g., Rh-negative mother, presence of antibodies on neonatal red blood cells, etc.) • Total bilirubin values approaching an exchange transfusion as defined by local guidelines (Section 18.2) • Haemoglobin value of <13.0 g/dL • Platelet count of less than 50,000 cells/mm^3 • Decreased total white blood cell count (Grade 3 and above) • Creatinine value more than 1.3 the upper limit of normal (ULN) for gestational age and postnatal age (Grade 2 and above)

	• AST or ALT of more than 2.5 the ULN (Grade 2 and above) • Any other current Grade ≥3 event on the DAIDS toxicity table • Severe congenital abnormalities or critically ill neonates at discretion of the examining clinician • Receiving medicine(s) that can impact DTG pharmacokinetics (Section 8.7) • Participation in another clinical trial • HIV-infected neonates For Cohort 1A and 1B, laboratory tests may be repeated during the screening period, with the latest results used to determine exclusion of participant from study enrolment. For Cohort 2, no screening bloods will be repeated due to blood draw limitations (Section 13.7). Laboratory results already available as standard of care may be used to assess eligibility and will not be required to be repeated.
Study duration	1st enrolment to last follow-up will be 27 months for the duration of the whole trial.
Sample size	40 evaluable neonates across the two stages: Stage 1, two sequential cohorts - Cohort 1A (n=8) and Cohort 1B (n=8); and Stage 2, Cohort 2 (n=24). In each cohort, we aim to achieve at least 80% power to obtain a 95% CI for DTG CL/F that lies within 60% and 140% of the geometric mean estimate of DTG CL/F in a prior relevant study, per FDA guidance for paediatric PK studies (2). In children in the lowest weight band (3 to <6 kg) of the ODYSSEY trial, DTG CL/F geometric coefficient of variation (CV%) with the 5 mg dispersible DTG tablet was 33%. In neonates, the variability is expected to be higher, with CV% within the range of 30% to 50%. Conservatively assuming that, in each cohort, CV% will be 50%, a sample size of 8 evaluable neonates in Cohort 1A, 8 in Cohort 1B and 24 in Cohort 2 will achieve 86%, 86% and >99% power, respectively, to obtain a 95% CI that lies within 60% and 140% of the geometric mean estimate. These power calculations are based on the Student's t-distribution and apply the methodology described by Wang *et al*.(3) for rich PK sampling.
Statistical section	*PK analysis:* In Stage 1 (Cohort 1A and IB), a non-compartmental pharmacokinetic analysis will be performed to calculate the subject PK parameters using Phoenix WinNonLin (Certara, USA). Maximum concentration (C_{max}), time to maximum concentration (T_{max}) and last post-dose concentration (C_{last}) will be taken directly from the observed concentration-time data. $AUC_{0\text{-}all}$ and $AUC_{0\text{-}inf}$ will be determined using the linear-up log-down-trapezoidal method. Cohort 1A and 1B PK data will be pooled for pharmacokinetic modelling and simulation analyses to select the multi-dose schedule for Cohort 2. In Cohort 2, a non-compartmental PK analysis will be performed to calculate the individual subject PK parameters. In addition, a population PK analysis will be performed using all the plasma

	concentration data generated during the study. Population means and variances of PK parameters for DTG will be estimated using nonlinear mixed-effects regression models. Subject covariates will be assessed to explain sources of inter-subject PK variability. Changes in DTG drug exposures and trough concentrations in neonates following multi-doses of DTG during the first 28 days of life will be estimated using the final model. *Safety analysis:* Safety and tolerance of DTG will be evaluated through the numbers and proportions of participants experiencing events, such as AEs of Grade 3 or higher. Clopper-Pearson 95% confidence intervals for these proportions will be calculated. *Acceptability analysis:* Categorical variables of the acceptability questionnaire will be tabulated with counts and percentages, and continuous variables will be summarized using descriptive statistics. All individual answers (including text fields) to the acceptability questionnaire for all participants will be provided in a listing.

4. Introduction

4.1. Background

In 2020, an estimated 150,000 children acquired HIV through vertical and breastmilk transmission, despite 85% of pregnant women living with HIV accessing antiretrovirals (ARVs) (4). The proportion of children living with HIV receiving antiretroviral therapy (ART) lags behind that in adults, at 54% compared with 73%, and 120,000 children and adolescents died of AIDS-related causes in 2020 (4, 5). Whilst the benefits of early treatment for both adults and children living with HIV are now well-established, formulations that are safe, effective, and acceptable for children across the age spectrum are lacking. This agenda has been taken forward in a number of initiatives gathered under the umbrella of the World Health Organization (WHO) hosted Global Accelerator for Paediatric formulations (6). Evidence-based guidance on priority formulations most needed for low- and middle-income countries is also provided by the WHO-convened Paediatric Antiretroviral Drug Optimization (PADO) (7).

Early infant diagnosis with rapid initiation of ART from birth is increasingly recommended for treatment of neonates (≤ 28 days of life) who are HIV-infected (8, 9). Early ART may also be advantageous for HIV-exposed neonates (born to mothers who are HIV-infected) at high risk of HIV acquisition, as a triple drug regimen could minimize the risk of mother-to-child HIV transmission and simultaneously provide presumptive treatment for a neonate subsequently confirmed as HIV-infected. However, initiating such strategies in neonates is challenging because of the limited number of neonatal formulations and lack of dosing information.

Drug pharmacokinetics (PK) in neonates can differ substantially from older children due to both body size and maturation of the physiological processes underlying drug absorption, distribution, metabolism and excretion (10). Body size has a non-linear but mostly predictable relationship with drug elimination and can be taken into account using the allometric scaling theory (11). On the other hand, the effects of maturation are variable and dependent on the organs and metabolic pathways involved. Each drug must therefore be studied independently in neonates. When selecting doses for very young children, extrapolating from older children can result in under- or over-dosing, and incomplete viral suppression or toxicity. As a further complication, in the case of fixed-dose combination (FDC) formulations, doses for individual drugs cannot be adjusted separately, and the drug ratio selected for older children may not be optimal for neonates (12).

There are currently only five ARVs with appropriate formulations and sufficient data on dosing and safety to be recommended for use in term neonates from birth: zidovudine (ZDV), lamivudine (3TC), emtricitabine, nevirapine (NVP), and raltegravir (RAL) (13). Currently available ART for neonates in low- and middle-income countries is based on NVP, a non-nucleoside reverse transcriptase inhibitor (NNRTI), and two nucleoside reverse transcriptase inhibitors (NRTIs). Although NVP dosing guidelines are available for term infants, continued use of this formulation is of concern due to growing levels of drug resistance to NNRTIs in Africa, including the transmission of NNRTI drug resistant viruses from HIV-infected mothers to their infants (7, 14).

The HIV protease inhibitor lopinavir, boosted with ritonavir (LPV/r), has been widely used for the treatment of paediatric HIV. LPV/r-based treatment is recommended by the WHO and remains one of the most commonly used drugs for children <3 years of age in many settings, including South Africa (15). There are currently no LPV/r-based formulations approved for

neonates younger than 2 weeks of age and <42 weeks corrected gestational age. Generic paediatric FDC granule formulations of LPV/r have been developed and are being studied for their applicability for use in neonates. However, initial data from the PETITE study assessing the "4-in-1" FDC granule formulation of ABC/3TC/LPV/r in neonates showed that LPV/r levels were extremely low, preventing the use of this FDC formulation in this population (16). The PETITE study is now assessing a separate solid formulation of LPV/r (40/10 mg) granules (Viatris Ltd) with an ABC/3TC dispersible tablet, which allows more flexibility in terms of doses.

Integrase strand transfer inhibitors (INSTIs)-based regimens are however preferred for HIV treatment in children and adults, but there are challenges in using these regimens in neonates. Although a granule formulation of RAL is approved for use in neonates, it has a low resistance barrier and is not widely available in sub-Saharan Africa. In 2020, a paediatric 5 mg dispersible tablet of the INSTI dolutegravir (DTG), manufactured by ViiV Healthcare, USA, was approved by the US FDA for use in infants from 4 weeks of age weighing at least 3 kg, and this is now part of the preferred 1st-line ART regimen for infants and children (9). To allow rapid access of DTG for children with HIV living in high burden settings, a 10 mg scored generic DTG dispersible tablet (17) was recently developed by Unitaid, Clinton Health Access Initiative (CHAI) and ViiV Healthcare, together with generic suppliers, which reduced the cost of HIV treatment by 75% for children in low- and middle-income countries (18). Infants and children will now be able to benefit from this more affordable, child friendly generic DTG formulation, however, no dosing and safety data are available for neonates.

At the most recent WHO convened PADO-5 meeting in 2021, it was highlighted that PK and safety data of DTG in neonates remains a high priority research question. Given the lack of formulations for prevention and treatment of neonatal HIV, there is an urgent need for information on the PK and safety of novel, inexpensive, solid based ARV formulations suitable for neonates, along with their acceptability to caregivers and neonates. There is strong scientific and strategic value of studying this accessible DTG product in neonates with the PETITE-DTG study, which if proven to be safe and effective, will rapidly inform WHO policies and clinical practice for HIV prevention and treatment in this underserved population.

4.2. Dolutegravir Adult and Paediatric Solid Formulations (Original)

Dolutegravir (TIVICAY) is an INSTI first approved by the US FDA in 2013 for use in combination with other antiretroviral agents for the treatment of HIV-1 infection (19). The initial DTG formulation approved was a 50 mg film-coated tablet (FCT). The recommended DTG dose is 50 mg once daily for treatment-naïve or treatment-experienced INSTI-naïve adults. This dose was also approved for children aged 12 years and older weighing at least 40 kg. A higher DTG dose of 50 mg twice daily was approved for individuals with INSTI-associated resistance mutations or when co-administered with drugs that induce enzymes involved in DTG metabolism (e.g., UGT1A or CYP3A). In 2016, 10 and 25 mg DTG-FCT were approved by the US FDA for use in paediatric patients weighting at least 30 kg, with a recommended dose of 35 mg once daily for children weighing 30 to <40 kg.

In 2020, the DTG paediatric dosing information in the package insert was revised to include guidelines for use of a new 5 mg DTG dispersible tablet (DTG-DT) for oral suspension. This DTG dosing update was based on data generated by the IMPAACT P1093 and ODYSSEY trials (20). Weight band dosing was approved for children ≥4 weeks of age and weighing between ≥3kg and <20 kg. Paediatric patients >20 kg could use either the adult dose of 50 mg DTG-FCT once

daily or use an equivalent dose with the DTG-DT [i.e., 30 mg once daily (6x 5 mg dispersible tablet) as the bioavailability of the 5 mg DTG-DT is approximately 1.6-fold that of DTG-FCT]. Of note, the paediatric DTG dosing approved by the European Medicines Agency (EMA) is slightly different from the US FDA with the EMA opting to have different doses for children in the 6 to <10 kg weight band who are less than or above 6 months age (21). The EMA recommends children weighing 6 to <10 kg who are <6 months of age to receive 10 mg DTG once daily and children who are >6 months of age to receive 15 mg once daily, while the FDA recommends all children in this weight band to receive 15 mg once daily independent of age.

4.3. Dolutegravir 10 mg Scored Dispersible Tablet (Generic)

In 2020, Mylan Laboratories Limited (note: Mylan merged with Upjohn in November 2020 to form a new company called Viatris Ltd) received tentative approval from the US FDA for its New Drug Applications for DTG-DT (10 mg, scored) for the treatment of HIV-1 infection in paediatric patients >4 weeks of age. This is the first generic version of DTG with scoring to allow for dose adjustment across the WHO weight bands. Generic paediatric 10 mg scored DTG-DT is a key component of the President's Emergency Plan for AIDS Relief (PEPFAR) to ensure infants and children living with HIV can rapidly access DTG (22).

4.4. Study Rationale

There is an increasing consensus that the provision of ART to HIV-exposed neonates at high-risk of HIV acquisition is beneficial, either to minimize the risk of vertical HIV transmission or to provide empiric therapy for a neonate subsequently confirmed HIV-infected. However, a major hurdle to providing ART to neonates is the lack of dosing information and child friendly formulations, i.e., only individual oral solutions so far, and no solid paediatric FDCs proven to be applicable for neonates.

The WHO-led expert group responsible for prioritizing paediatric antiretroviral drugs and formulations (PADO) has indicated the need to assess the use of DTG in HIV-exposed and infected neonates as a high priority considering the limited ARV drug options available for neonates (7). In response, the PETITE-DTG study has been developed to directly address this research gap **with a focus on** the generic paediatric DTG formulations that will be available in high burden HIV settings. The PETITE-DTG study has been designed to assess the safety and PK of DTG when administering the **generic 10 mg scored DTG-DT** (supplied by Viatris Ltd).

Given no PK data on DTG in neonates, the study design has been developed into two stages. Neonates enrolled in Stage 1 (Cohorts 1A/1B) will receive a single dose of DTG, while neonates enrolled in Stage 2 will receive multi-doses of DTG. Taking into consideration the fixed 5 mg dose and the anticipated slower metabolism of DTG during the first month of life, it was deemed safest to perform initial assessments with a single dose in 'older' neonates (Cohort 1A) before moving to 'younger' neonates (Cohort1B). After Stage 1 (Cohort 1A and 1B) is complete a Data Safety and Monitoring Board (DSMB) meeting will meet to review the Cohort 1 safety and PK data of the DTG-DT and review the proposed multi-dose strategy to be assessed in Cohort 2, which and may differ by birth weight (e.g., above and below 3000 g). An interim PK and safety analysis will also be performed after 50% of participants have completed Cohort 2 to review safety data and assess if any dose adjustment is required. Following the DSMB interim review, a different DTG dosing schedule may be selected based on the data available for the remainder of Cohort 2 infants. Any change to the DTG dosing schedule will be agreed upon by the DSMB before implementation.

It is expected that the safety and PK data generated in the PETITE-DTG study will complement other ongoing studies of paediatric solid formulations of DTG in neonates (e.g., IMPAACT 2023), and/or older children with HIV. Taken together, these studies will provide robust safety and PK data for DTG from birth, thereby allowing neonates to access this much needed treatment option and providing treatment continuity from birth.

5. Study Objectives and Endpoints

5.1. Primary Objectives

- To evaluate the pharmacokinetics of dolutegravir (DTG) during the first 28 days of life in HIV-exposed term neonates (born to a mother with HIV) following administration of DTG dispersible tablet
- To determine the safety of DTG during the first 28 days of life in HIV-exposed term neonates following administration of DTG dispersible tablet

5.2. Secondary Objectives

- To assess the acceptability of DTG dispersible tablet for the neonate and the caregiver

5.3. Endpoints

5.3.1. Primary endpoints

- DTG plasma pharmacokinetics parameters: area under the concentration time curve (AUC); maximum plasma concentration (C_{max}), apparent clearance (CL/F), and trough concentration (C_{trough})
- Occurrence of the following events: adverse events of Grade 3 or higher; treatment-related adverse events of Grade 3 or higher; any adverse events

5.3.2. Secondary endpoints

- Acceptability to caregivers and neonates of using DTG-DT will be measured by means of a questionnaire

6. Study Design and Setting

6.1. Study Design

A Phase I/II, open-label, single arm, two-stage trial to evaluate the single and multi-dose PK and safety of DTG in HIV-exposed neonates on ARV prophylaxis. HIV-exposed term neonates born mothers with HIV on DTG-based antiretroviral therapy with a birth weight ≥2000 g who are on ARV postnatal prophylaxis will be enrolled.

Subject will be enrolled in two stages:

- **Stage 1** will assess a single 5 mg dose of the DTG dispersible tablet in two sequential cohorts: Cohort 1A (n=8) and Cohort 1B (n=8).

- **Stage 2** will assess multiple 5 mg doses of the DTG dispersible tablet in a single cohort: Cohort 2 (n=24).

Per national guidelines, all infants receive a birth HIV nucleic acid test (NAT). HIV NAT test results for the infant may or may not be available (HIV pending) at the time of study entry. HIV NAT results are typically available within 72 hours of the blood sample being taken and are checked and acted upon by the hospital HIV PMTCT service, as part of standard of care (Section 9.1). If an HIV NAT result comes back positive whilst the neonate is on study, the neonate will not receive any further DTG doses, revert to standard of care antiretroviral therapy (ART), and be followed for safety for the duration of the study.

6.1.1. Stage 1: Single Dose of the DTG generic dispersible tablet

Cohort 1A will open to accrual first (Schema, Section 6.2, **Figure 1**).

- Cohort 1A: 8 neonates weighing ≥2000 g at birth will receive a single dose of 5 mg DTG at ≥14 days and <28 days of life.

DTG will be administered in addition to standard of care ARV prophylaxis. After Cohort 1A is complete, and if no safety concerns are noted, accrual will continue into Cohort 1B. However, if safety hold criteria are met in Cohort 1A (Section 11.1.1), the study will be paused and data reviewed by the DSMB.

- Cohort 1B: 8 neonates weighing ≥ 2000 g at birth will receive a single dose of 5 mg of DTG at <14 days of life.

If safety hold criteria are met during Cohort 1B (Section 11.1.2), the study will be paused and data reviewed by the DSMB. After Cohort 1B is complete, all safety and PK data for Stage 1 will be reviewed. Pharmacokinetic modelling and simulation analyses using Stage 1 PK data will be performed to select the multi-dose schedule of the DTG dispersible tablet for Cohort 2 (Section 11.4). All findings will be presented to the DSMB and agreed upon before opening Cohort 2.

6.1.2. Stage 2: Multi-Doses of the DTG generic dispersible tablet

- Cohort 2: 24 neonates weighing ≥ 2000 g at birth will receive multiple 5 mg doses of DTG starting within 7 days of life until day 28.

Low-risk* term neonates born to virologically suppressed mothers enrolled in Stage 2 will receive ZDV prophylaxis, instead of NVP, for the duration of the study. An interim PK and safety analysis will be performed after 50% of participants have completed Cohort 2 to assess if a dose adjustment is required and to review safety data. If no major safety or PK concerns are noted, enrolment into Cohort 2 will continue. If needed, a different DTG dosing schedule may be selected based on the data available for the remainder of Cohort 2 infants. Any change to the DTG dosing schedule will be agreed upon by the DSMB before implementation.

*[*Low risk: Neonate born to a mother with a plasma HIV-1 RNA result **<50 copies/mL** in the 4 weeks prior to delivery or between delivery and infant study entry]*

6.2. Study Schema

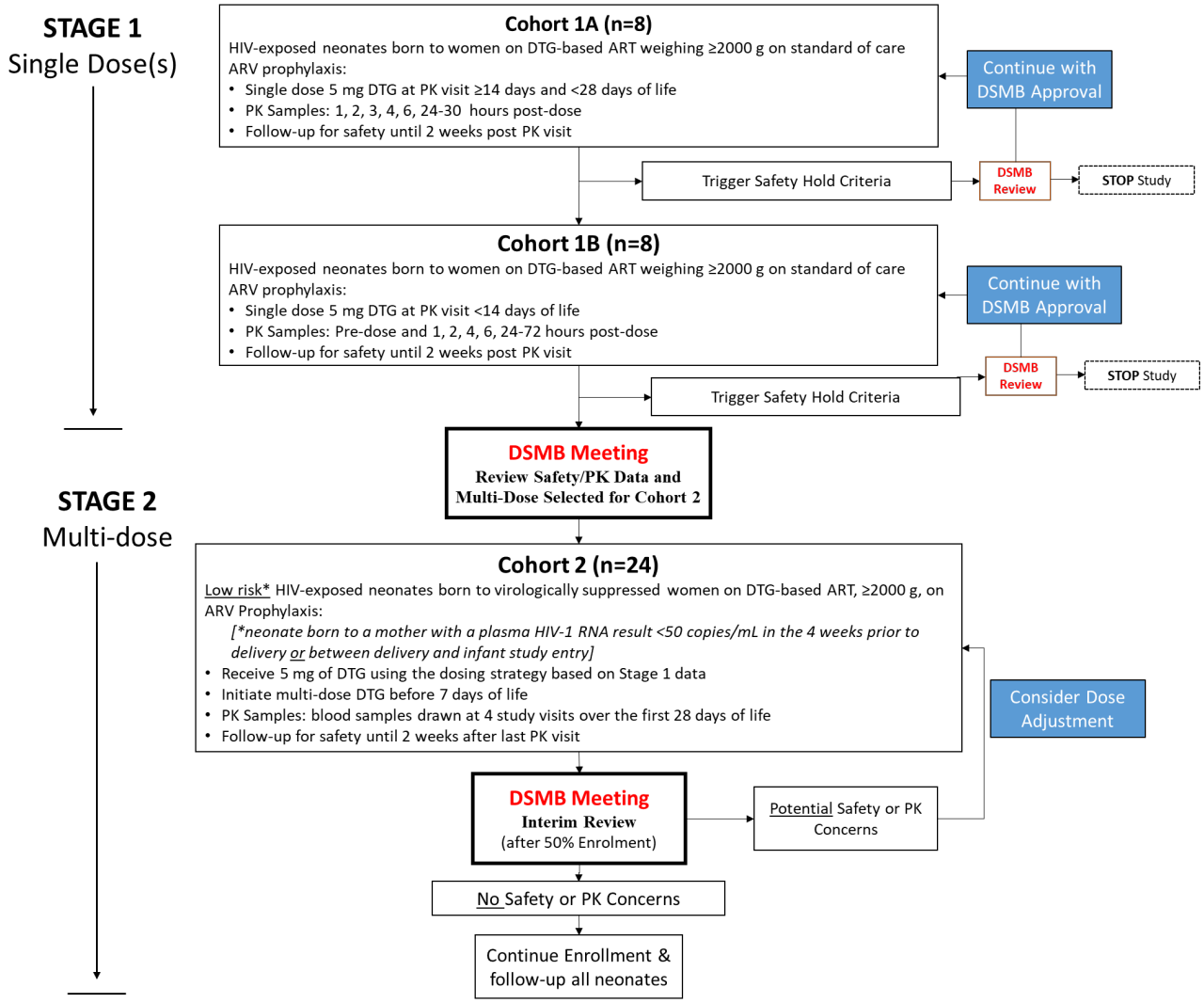


Figure 1: PETITE-DTG Study Schema

6.3. Study Setting

This single site study will be implemented at the Family Centre for Research with Ubuntu (FAMCRU), an academic research centre in the Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences (FMHS), Stellenbosch University (SU). FAMCRU is located within the associated Tygerberg Hospital (TBH), a large secondary and tertiary referral centre situated in the Western Cape Province, South Africa.

In South Africa, 7,800,000 people were living with HIV in 2020, with an HIV prevalence among adults (15-49 years) of 19.1%. Newly infected people with HIV were 230,000, and 83,000 people died from an AIDS-related illness. Women are disproportionally affected by HIV in South Africa. Of the 7,800,000 adults living with HIV, 4,800,000 (61.5%) were women. New HIV infections among young women aged 15 to 24 years were more than double those among young men: 140,000 new infections among young women, compared to 77,000 among young

men. ARVs to prevent vertical HIV transmission was accessed by 97 (60->98) % of pregnant women living with HIV, preventing 52,000 new HIV infections among newborns (23).

The national maternal HIV prevalence in South Africa remains high at around 30 %, but vertical transmission rates in the first 2 months of life have decreased life to 0.7% in 2019, with the remainder of childhood transmissions occurring during the breastfeeding period (24). Prevention of mother-to-child transmission (PMTCT) services are well established, with all pregnant women qualifying for combination ART. In June 2021, the South African National Department of Health released a circular (Reference: 2021/06/29/EDP/01) updating guidance for the use of DTG in pregnancy, now supporting the use of a DTG preferred first-line ART regimen for all adults living with HIV, including pregnant women and women of child-bearing age (25). A statement from the South African Clinicians' Society followed soon afterwards recommending that all women, including pregnant women, preferentially be started on tenofovir/lamivudine/dolutegravir (TLD) (26), which has been the clinical practice since.

In South Africa, women with HIV routinely receive an HIV-1 RNA viral load (VL) at delivery and their infants receives a minimum of 6 weeks of ARV postnatal prophylaxis, dependent on the risk of HIV acquisition in the infant. HIV-exposed neonates start a prophylaxis regimen of nevirapine (NVP) and zidovudine (ZDV) at birth and continue this until the maternal VL result at delivery is available. If the maternal VL is ≥1000 copies/mL the infant is classified as 'high-risk' and continues both ARVs, else infants are deemed 'low'-risk and ZDV is stopped and NVP continued. All HIV-exposed neonates receive an HIV NAT test at birth, 10 weeks, 6 months, and in those who are breastfeed, at 4 weeks post-cessation of breastfeeding (1).

7. Selection of Participants

All relevant medical and non-medical conditions must be taken into consideration when deciding whether this protocol is suitable for a participant. Any questions regarding a participant's eligibility should be discussed with the Principal Investigator (PI) prior to the participant's enrolment.

Prior to screening and enrolment for the study the parent(s) or legal guardian must give written consent for the neonate to participate in the study.

7.1. Inclusion Criteria

Written informed consent to participate in the study must be obtained for all participants from the parent(s) or legal guardian.

Stage 1: Inclusion Criteria

- HIV-exposed neonate (pending HIV status) born to a woman within HIV on DTG-based ART
- Birth weight of ≥2000 g and on standard of care ARV prophylaxis

Cohort Specific Inclusion Criteria in Stage 1 must be met at **Study Entry:**

Cohort 1A: Infant <14 days of life
Cohort 1B: Infant ≤3 days of life

Stage 2: Inclusion Criteria

- Low risk* HIV-exposed neonate (pending HIV status) born to a virologically suppressed woman on DTG-based ART

  **Neonate born to a woman with a documented plasma HIV-1 RNA result <50 copies/mL in the 4 weeks prior to delivery or between delivery and infant study entry*

- Birth weight of ≥2000 g and on standard of care ARV prophylaxis

Cohort Specific Inclusion Criteria in Stage 2 must be met at **Study Entry:**

Cohort 2: Infant <7 days of life

7.2. Exclusion Criteria

The presence of any of the following at entry, will exclude a subject from study enrolment:

- Less than 37 weeks gestational age at birth
- Known blood group incompatibilities which can result in hemolytic disease of the newborn (e.g., Rh-negative mother, presence of antibodies on neonatal red blood cells, etc.)
- Total bilirubin values approaching an exchange transfusion as defined by local guidelines (Section 18.2)
- Haemoglobin value of <13.0 g/dL
- Platelet count of less than 50,000 cells/mm^3)
- Decreased total white blood cell count (Grade 3 and above)
- Creatinine value more than 1.3 the upper limit of normal (ULN) for gestational age and postnatal age (Grade 2 and above)
- AST or ALT of more than 2.5 the ULN (Grade 2 and above)
- Any other current Grade ≥3 event on the DAIDS toxicity table
- Severe congenital abnormalities or critically ill neonates at discretion of the examining clinician
- Receiving medicine(s) that can impact DTG pharmacokinetics (Section 8.7)
- Participation in another clinical trial
- HIV-infected neonates

8. Study Treatment

The study treatment is a 10 mg scored dispersible tablet of DTG for oral suspension, supplied by Mylan (Viatris) Ltd.

To administer 5 mg of a DTG dose, the 10 mg DTG-DT will be broken in half along the score and one half of the tablet (5 mg) will be dispersed in 5 mL of water. The entire volume will then be administered to the neonate. Partial volumes of the oral suspension are not allowed as the drug does not disperse evenly.

8.1. General Pharmacokinetics of Dolutegravir

DTG is rapidly absorbed following oral administration with maximum plasma concentrations (C_{max}) 2 to 3 hours post-dose. DTG is primarily metabolized via UDP-glucuronosyltransferases (UGT)-1A1 with some contribution from cytochrome (CYP)-3A and these metabolites are excreted in the urine. Renal elimination of unchanged DTG is <1% of the dose. Small, reversible increases in serum creatinine with DTG use have been shown due to inhibition of tubular secretion of creatinine (via inhibition of OCT2), but without affecting renal glomerular function (27).

The terminal plasma half-life of DTG after oral intake is approximately 14 hours. Steady-state DTG pharmacokinetics are achieved within ~5 days with once daily oral dosing and average accumulation ratios for AUC, C_{max}, and C_{24} range from 1.2 to 1.5 in adults. A summary of the steady-state PK parameters for DTG in adults with HIV receiving 50 mg once and twice daily are shown in **Table 1** (19).

Table 1. Geometric Mean (%CV) Steady-State PK Parameters of DTG in Adults Living with HIV

PK Parameters	50 mg Once Daily	50 mg Twice Daily
AUC_{0-24} (µgxh/mL)	53.6 (27)	75.1 (35)
C_{max} (µg/mL)	3.67 (20)	4.15 (29)
C_{min} (µg/mL)	1.11 (46)	2.12 (47)

Dolutegravir is >98% bound to human plasma proteins. A population PK analysis estimated the apparent volume of distribution (V/F) and apparent clearance (CL/F) following 50 mg once daily to be 17.4 Litres (L) and 1.0 L/hour, respectively. DTG concentrations have also been detected in the cerebrospinal fluid indicating it crosses the blood–brain barrier. Of note, DTG plasma concentrations increase in a less than dose-proportional manner at doses over 50 mg.

Both film-coated and dispersible tablets of DTG can be taken with or without regard to food; however, intake of DTG with food increases its absorption and slows the rate of absorption. For example, with a high fat meat the DTG AUC_{0-inf} and C_{max} increase by ~65% and the Tmax increases from 2 to 5 hours. Dolutegravir has been shown to be a substrate of UGT1A3, UGT1A9, BCRP, and P-glycoprotein *in-vitro*, so concomitant medications that induce or inhibit these enzymes and/or transporters may impact the pharmacokinetic profile of DTG.

Multiple drug-drug interactions have been reported and, in some cases, DTG dose adjustment from once to twice daily is necessary (e.g., carbamazepine, rifampicin), or changes to the timing of DTG administration relative to concomitant drugs (e.g., medications containing polyvalent cations, oral iron and/or calcium supplements) is needed. Refer to Section 8.7 – Concomitant Medications for more information.

8.2. Dolutegravir Dosing in Paediatrics

Several formulations of DTG have been approved by the US FDA for use in children: 10, 25 and 50 mg FCT for oral use and a 5 mg DT for oral suspension. The pharmacokinetics of these formulations of DTG have been evaluated in the IMPAACT P1093 trial [ClinicalTrials.gov Identifier: NCT01302847] and in 2 weight-band-based PK substudies from the ODYSSEY trial [NCT02259127, (28, 29)]. Of note, the bioavailability of the DTG-DT is ~1.6-fold that of DTG-FCT (19). Steady-state plasma PK of DTG at doses by weight bands are summarized in **Table 2**.

Table 2. Summary of DTG PK Parameters in children living with HIV (analyses from IMPAACT P1093 and ODYSSEY trials (19))

			Pharmacokinetic Parameter Geometric Mean (%CV)		
Weight Band	**DTG Dose**	**n**	**C_{max} (µg/mL)**	**AUC_{0-24} (µgxh/mL)**	**C_{24} (µg/mL)**
3 kg to <6 kg	5 mg DT OD	8	3.80 (34)	49.37 (49)	0.962 (98)
6 kg to <10 kg	15 mg DT OD	17	5.27 (50)	57.17 (76)	0.706 (177)
10 kg to <14 kg	20 mg DT OD	13	5.99 (33)	68.75 (48)	0.977 (100)
14 kg to <20 kg	25 mg DT OD	19	5.97 (42)	58.97 (44)	0.725 (75)
20 kg to <25 kg	30 mg DT OD	9	7.16 (26)	71.53 (26)	0.759 (73)
≥20 kg	50 mg FCT OD	49	4.92 (40)	54.98 (43)	0.778 (62)

DT: Dispersible tablet; FTC: Film-coated tablet; OD: Once daily

Mean steady-state DTG exposures (AUC_{0-24h}) and C_{24h} in paediatric participants with HIV were comparable to those in adults receiving 50 mg once or twice daily. Mean DTG C_{max} was generally higher with once daily dosing in children than adults (**Table 1**), but the increase was not considered clinically significant as the safety profiles were similar in both populations.

The paediatric DTG dosing recommendations were derived from a population PK analysis combining IMPAACT P1093 and ODYSSEY data (20). The US FDA licensed weight-band dosing of the DTG-DT is now recommended for infants and children in the WHO dosing guidelines (9). **Table 3** shows the 2021 WHO weight-based dosage of the scored 10 mg DTG-DT for infants and children 4 weeks and older and weighing at least 3 kg.

Table 3: 2021 WHO weight-band dosing for DTG 10 mg scored dispersible tablet in infants 4 weeks and older & weighing at least 3 kg

	Dolutegravir Tablets for Oral Suspension	
Weight Band	**Daily DTG Dose**	**Number of 10-mg Tablets**
3 kg to <6 kg	5 mg OD	½ (one-half)
6 kg to <10 kg	15 mg OD	1 ½ (one and one-half)
10 kg to <14 kg	20 mg OD	2 (two)
14 kg to <20 kg	25 mg OD	2 ½ (two and one-half)
20 kg or greater	30 mg OD	3 (three)

OD: Once daily

The weight-based DTG dose is recommended to be increased for children receiving rifampicin-containing TB treatment to twice daily.

8.2.1. Dolutegravir Dosing in Neonates

No specific DTG dosing guidelines are available for neonates. Based on the WHO weight band dosing guidelines the DTG dose range is between 0.85 to 1.67 mg/kg for children >4 weeks and weighing between 3 and 5.9 kg. In Cohort 1A, 5 mg of DTG, i.e., half of the scored 10 mg DTG-DT, will be administered to neonates who are ≥14 days and <28 days of life. For a 3 kg neonate, this translates to a 1.67 mg/kg dose, but higher drug exposures are anticipated due to the expected slower metabolism in these younger infants. If the drug exposures following

this single dose are found to be safe, it is planned to administer a single 5 mg dose of DTG to neonates ≤14 days (Cohort 1B).

It is unknown the extent to which the DTG exposure will be increased as the enzyme pathways involved in DTG metabolism (UGT1A1 and CYP3A) mature at different rates during early life (30, 31). As expected, the washout elimination of DTG in neonates after *in utero* exposure was slow, with a median elimination half-life of 32.8 hours compared to 12-14 hours in children and adults. In this context, physiologically based pharmacokinetic (PBPK) modelling (32) has been utilized to predict DTG PK and dose optimization in neonates (33). This PBPK model combined existing knowledge on the anatomical, physiological, and metabolic changes (i.e., ontogeny of CYP3A4 and UGT1A1) in neonates with available physicochemical and *in-vitro* data of DTG. This PBPK model was validated using observed clinical data for RAL and midazolam in neonates. Using the final PBPK model, multiple DTG dose strategies were simulated in 100 healthy term virtual neonates (weight ranging from 3.0 to 4.5 kg) with the goal of achieving therapeutic DTG plasma $AUC_{0\text{-}24}$ (50.1 μgxh/mL) and trough concentration (0.99 μg/mL) observed in children. **The model predictions indicated that a 5 mg DTG dose may be suitable for term neonates, but any regimen would likely require increased dosing intervals during early life compared to older infants (e.g., every 48 or 72 hours compared to every 24 hours)**. The authors' preferred regimen was 5 mg DTG doses every 48 hours from Day 1–20 of life, increasing to 5 mg once daily on Day 21. An important caveat of this PBPK model was the absence of maternal transfer of DTG through the placenta or breast milk. It has been shown that DTG readily crosses the placenta with a reported cord blood to maternal plasma ratio of 1.25 (1.07–1.40) (34). Breastfeeding has been reported to contributed relatively little to infant plasma exposure in the DolPHIN-1 study. The relative infant dose to that of the mother was estimated to be 0.27% (range, 0.13%–0.71%; n = 26) (35).

The 2021 WHO guidelines recommend ART to be initiated urgently among all pregnant and breastfeeding women living with HIV and the preferred 1st-line regimen is DTG in combination with an NRTI backbone (9). Most high burden counties have adopted DTG in their national guidelines, including South Africa. The introduction of the generic FDC of TDF/3TC/DTG or "TLD" across Africa is facilitating the rapid transition of adults to DTG, with the majority of adults now receiving DTG based-ART in many counties (36). The South African National Department of Health supports the guidance that all newly diagnosed pregnant women should be initiated on TLD (26). For this reason, it was decided to focus on HIV-exposed neonates born to mothers on DTG-based regimens, so the PETITE-DTG study results are applicable to South Africa and similar settings. Thus, it is important to consider transplacental transfer of DTG and any potential impact it may have on neonatal DTG dosing and exposure.

A recent population PK modelling and simulation study using data from the IMPAACT P1026s trial was performed to assess DTG dosing requirements during the first days of life as a function of maternal DTG dosing history (37). Maternal and infant population PK models were developed to describe infant DTG concentrations at delivery (i.e., estimate the *in-utero* dose of DTG) and transplacental washout. Of note, the neonate PK model incorporated a maturation function for UGT1A1-mediated DTG clearance, and the Ka was fixed to the adult value. Using these PK models, Monte Carlo simulations of virtual infants were performed to simulate neonatal concentrations following two doses of DTG after birth for infants born to mothers receiving DTG prior to delivery. The lower and upper ranges of the PK targets selected was 0.256 μg/mL ($4xIC_{90}$) and 7.34 μg/mL (2-fold above the adult C_{max} value), respectively.

Simulations revealed that among neonates whose mother had taken DTG between 6 hours to 24 hours prior to delivery, a 5 mg DTG dose at 24 to 72 hours after birth maintained median C_{max} concentrations below the target. These data are also supportive that a single 5 mg DTG dose at >3 days of life proposed in Stage 1 of the PETITE-DTG study will not lead to excess DTG concentrations. **However, it is clear that the timing of the second DTG dose is very important.** For example, if the first dose was administered 48 hours after birth, and a second dose 48 hours later, median C_{max} were within the target; but administering the second dose 24 hours after the first dose led to a C_{max} above the target. The optimal DTG dosing strategy for neonates remains to be determined and the data generated in Stage 1 of the PETITE-DTG study will guide the timing of DTG doses for the multi-dose stage in Cohort 2.

Another consideration is the background NVP ARV infant prophylaxis. Nevirapine is an inducer of CYP3A which is one of the metabolic pathways of DTG and therefore concomitant use could impact DTG concentrations. Indeed, a drug-drug interaction study of DTG and NVP in adults revealed that DTG C_{max}, AUC_{0-24}, C_{24} were reduced by an average of 8%, 19% and 34%, respectively (38). The oral clearance of DTG was 23% faster with concomitant NVP use (1.12 L/r vs 0.91 L/hr), with the plasma half-life 15% faster. Maximum induction of CYP3A by NVP occurs 2 to 4 weeks after initiation (39, 40). Based on this data it is anticipated that concomitant NVP use could decrease DTG concentrations in neonates. As the future clinical application of DTG in neonates will be without concomitant NVP, determining optimal DTG dosing in neonates in the absence of NVP will provide the most informative data. For this reason, neonates who receive multiple doses of DTG in Stage 2 of the PETITE-DTG study will receive ZDV prophylaxis, instead of NVP, for the duration of the study. Only infants born to virologically suppressed mothers will be enrolled in Stage 2.

Overall, these data are encouraging and support the assessment of the 5 mg DTG-DT in neonates; however, to be cautious, a multi-step, two-stage study design was selected for the PETITE-DTG trial to maximise the safety of study participants. Only if no safety concerns are observed in Stage 1, and an appropriate DTG dosing regimen is identified, will the study proceed to assess multi-doses of the DTG-DT in Stage 2.

8.3. Drug Administration

The DTG-DT will be administered with water and drug administration for neonates will adapted from the package insert instructions (19).

To administer 5 mg of DTG to neonates:

- Break the 10 mg scored DTG-DT in half along the score.
- Mix one half (5 mg) of the DTG-DT for oral suspension in 5 mL (one teaspoonful) of drinking water
- Swirl the suspension so that no lumps remain
- After completely mixed draw up all the prepared medicine into a 5 mL syringe
- Place the tip of the syringe against the inside of the neonate’s cheek and give the dose slowly
- Swirl a further 5 mL of drinking water into the dosing cup, draw it into the syringe, and give it all to the neonate. (Do not use any other drink to prepare the dose)
- Give the oral suspension to the child within 30 minutes of mixing

8.4. Direct Observed Treatment

The study nurse will prepare and administer a single 5 mg dose (half of a 10 mg scored DTG-DT) in Stage 1 (Cohorts 1A and 1B). Direct observation of treatment administration (DOT) will be performed for all neonates at the PK visits in Stage 2. For first administration in Stage 2 (Cohort 2), the investigator will explain drug administration to the caregiver and then observe as the caregiver administer the drug. Observed administration will provide an opportunity for the caregivers to have a first experience with administration of DTG in the presence of the investigator, hence the opportunity to address any difficulties. Prior to Visit 2 in Cohort 2, mothers will be contacted to ensure the DTG doses were administered per dosing schedule.

8.5. Study Treatments Labelling, Packaging and Storage

This is an open label study. Following study approval from Stellenbosch University (SU) Health Research Ethics Committee (HREC), South African Health Products Regulatory Authority (SAHPRA), and the WHO Ethics Committee, Viatris Ltd will supply the study drugs under controlled temperature conditions and the labels will follow country requirements (in particular: product name, composition, batch number/Lot No., expiry date and storage instructions).

The drugs will be shipped under controlled temperature to the pharmacy at the FAMCRU site, which is managed by the study pharmacist. After receipt, the pharmacist will verify the drug status for sealed condition of bottles, and the quantities received against the information provided by Viatris Ltd for the shipped drugs. All study medications must be kept in the pharmacy and access limited only to the appropriate study personnel and stored per recommendations. Storage temperature monitoring records will be maintained by the study pharmacist and kept at the pharmacy.

8.6. Study Drug Accountability

Study-specific forms will be used for accountability of the study medication. Appropriate records concerning receipt, use, returns, loss and any other disposition of the study medication will be maintained by investigators or their delegates on site, under the supervision of the Principal Investigator. Study monitors will check and document accountability and expiry details of the study medication during on-site monitoring visits.

The investigational medicinal product cannot be used for purposes other than this study. Under no circumstances may the investigator or site staff allow the medications to be used other than as directed by this protocol. Adequate records on receipt, use, return, loss, or other disposition of medication must be maintained.

In Cohort 2, parents will bring back remaining drugs for purposes of pill count and calculation of adherence by site staff. Adherence will be documented in the Case Report Form (CRF).

8.7. Concomitant Treatments

A complete list of current medications will be taken at the screening/entry visit and at all other visits, including on the day of PK sampling. Any concomitant medications will be evaluated for known interactions with the investigational products and will be checked with the package insert and on the www.hiv-druginteractions.org website to see whether the concomitant treatment may be problematic.

DTG is contraindicated in patients with previous hypersensitivity reaction to dolutegravir. Dofetilide is the only contraindicated medication listed in the DTG package and is not allowed. Potential participants who are receiving medications that require a DTG dose adjustment to twice daily (e.g., rifampicin, phenobarbitone) will be excluded from entry in this study.

Medications containing polyvalent cations, oral iron and/or calcium supplements reduce DTG exposures, and changes to the timing of DTG administration relative to concomitant drugs are necessary to overcome this effect. Any participants receiving such medications will have DTG administered at least 2 hours before or 6 hours after taking the medication/supplements containing polyvalent cations, calcium or iron.

During the study, should there be a clinical indication for any additional medication including medication given to treat an adverse event after study drug(s) administration, the name of the drug(s), the dosage, and the date and time of administration must be recorded on the CRF.

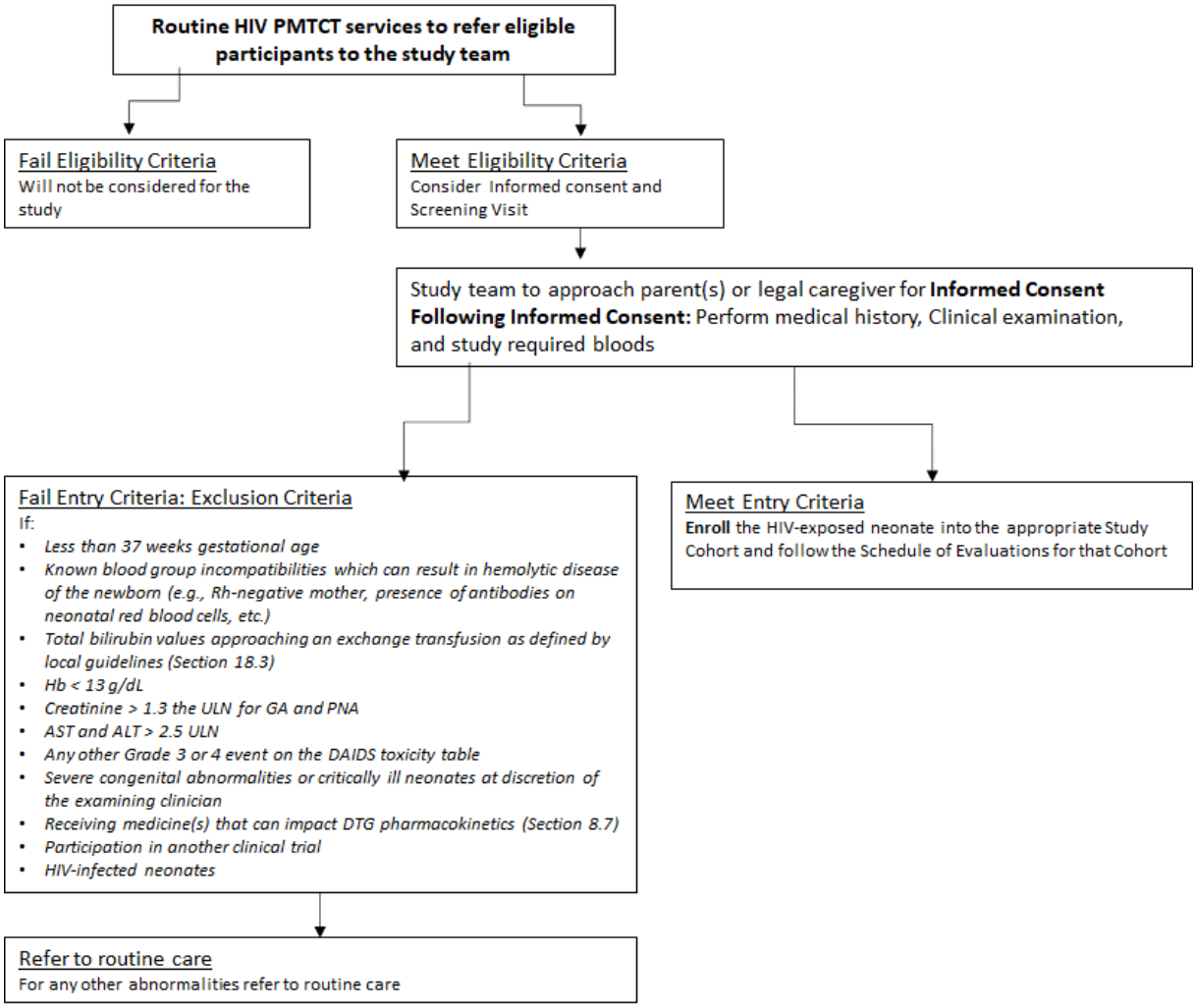
9. Study Procedures and Assessments

9.1. Screening and Enrolment Procedures

Tygerberg Hospital has an active HIV PMTCT service as part of standard of care. The role of this routine PMTCT service is to actively identify, care for and collect real-time information on mothers with HIV (and their neonates) delivering at Tygerberg Hospital. Data generated are submitted to the Department of Health for statistical analysis. The PMTCT nurses ensure that mothers with HIV and their neonates receive the appropriate ARV treatment/prophylaxis, and are linked back to HIV care, if needed. The PETITE-DTG study team will not directly contact potential participants within the routine PMTCT service, rather the medical staff working in the routine PMTCT services will refer potential study participants to the study team for consideration. All mothers referred will first be consulted by medical staff from the routine PMTCT services in order to determine whether they agree to be in contact with the PETITE-DTG research team.

Figure 2 illustrates the procedure for the initial assessment of study eligibility, i.e., the routine PMTCT services identifying HIV-exposed neonates who meet the Stage and Cohort-specific inclusion criteria. The PMTCT nurses will then inform the study team of a potential participant. Postpartum women with HIV receiving DTG-based ART will be allowed to breastfeed or formula feed depending on the mothers' choice. Documentation of maternal HIV-1 RNA result (performed per standard of care) required as part of the inclusion criteria for neonates in Stage 2 will be abstracted from the medical records. If an HIV-exposed neonate is identified as eligible, the parent(s) will be approached to undergo the informed consent process. Following written informed consent from the parent(s) or legal guardian, the participant will be screened for eligibility. A medical history, clinical examination and study required bloods (haematology, chemistry and HIV NAT samples) will be carried out by the study team. If the study required bloods were taken as part of clinical care, results will be documented, and no additional blood draws performed. If the study required bloods (haematology and chemistry) are abnormal (Section 7.2), the neonate will be referred to routine care. Only if the study required bloods (haematology and chemistry) are within the range specified for eligibility will the neonate be allocated to either Cohort 1A, Cohort 1B or Cohort 2 and follow the SOE for that respective Cohort (Section 9.3). Laboratory tests may be repeated during the screening period, with the latest results used to determine exclusion of participant from study enrolment.

Figure 2: Summary of procedure for the initial assessment of study eligibility



9.2. Description of Study Procedures and Assessments

9.2.1. Stage 1, Cohort 1A: Screening/Entry Visit

The screening and entry visit(s) will take place when the neonate is <14 days of life. Screening and entry procedures may be performed as one or 2 separate visit(s). HIV-exposed neonates enrolled in Cohort 1A will continue to receive ARV prophylaxis as per local guidelines for the duration of the study.

If an HIV NAT test result comes back positive for a neonate at any time during the study, the neonate will come off study and will be treated as per standard of care (any prior PK samples will be included in the analysis).

At the end of study follow-up, HIV negative infants will continue standard of care HIV prophylaxis, if indicated, as per local guidelines.

9.2.1.1. Cohort 1A: PK Visit 1a/1b

After study entry, PK Visit 1a and 1b for the neonate will be scheduled at **≥14 days and <28 days of life**. On the morning of the PK visit 1a, study nurses and physicians will take a medical history and examine the neonate. Afterwards, a single 5 mg dose of DTG (half of the 10 mg DTG-DT) will be administered. The study team will support the preparation and administration of the DTG-DT mixed with water with the parent(s) or caregiver/legal guardian and document the exact time of intake used for administration. Acceptability testing of the DTG-DT will be performed.

Following an observed dose, blood will be drawn at 1, 2, 3, 4, 6 (PK Visit 1a) and 24-30-hours (PK Visit 1b) post-dose. A blood draw window of +/- 15 minutes is acceptable for samples up to 6 hours post-dose. The last samples can be drawn anytime between 24 and 30-hours post-dose. It is planned to draw 0.4 mL per time point - a total blood volume of 2.4 mL for PK assessment. A short intravenous catheter (heparin lock) can be used to reduce repeated skin puncture and ease the discomfort of blood sampling.

9.2.1.2. Cohort 1A: Safety Visits

One week after PK visit 1a (+/-4 days), we will conduct a clinical and laboratory safety visit. Medical staff will take a recent medical history from the parent(s) or caregiver/legal guardian and examine the neonate. Study required bloods will be drawn, and an HIV NAT will be performed. If any tests yield abnormal results, as specified by protocol specific safety criteria (Section 10), we will consult paediatricians for appropriate management of the neonate. If the clinical examination and all tests are normal, a telephonic safety visit will be conducted 2 weeks after PK Visit 1a (+/-4 days). Medical staff will once again take a recent medical history from the parent(s). If no issues are reported, the neonate will exit the study at the conclusion of this visit. If any concerns are noted, the neonate will be brought back for clinical and special investigations as needed. The total study duration for each individual will depend on the age at enrolment and can range from 2 to 6 weeks.

If study safety hold criteria (Section 11.1.1) are met at any time during Cohort 1A the study will be paused, safety data reviewed, and a DSMB meeting held to determine if the study should continue (Section 6.2, **Figure 1**). After Cohort 1A is complete, if no major safety concerns are noted, accrual will continue into Cohort 1B.

9.2.2. Stage 1: Cohort 1B

The screening and entry visit(s) will take place within ≤3 days of life. Screening and entry procedures may be performed as one or 2 separate visit(s). HIV-exposed neonates enrolled in Cohort 1B will continue to receive ARV prophylaxis as per local guidelines for the duration of the study.

If an HIV NAT test result comes back positive for a neonate at any time during the study, the neonate will not receive any further study drugs, come off study and will be treated as per standard of care (any prior PK samples will be included in the analysis).

At the end of study follow-up, HIV negative infants will continue standard of care HIV prophylaxis, if indicated, as per local guidelines.

9.2.2.1. Cohort 1B: PK Visit 1

After study entry, PK visit 1a and 1b for the neonate will be scheduled at **< 14 days of life**. On the morning of the PK visit, study nurses and physicians will take a medical history and examine the neonate. Afterwards, a single 5 mg dose of DTG (half of the 10 mg DTG-DT) will be administered. The study team will support the preparation and administration of the DTG-DT mixed with water with the mother and document the exact time of intake used for administration. Acceptability testing of the DTG-DT will be performed.

Blood will be drawn pre-dose, and then following an observed dose, at 1, 2, 4, and 6 (PK Visit 1a), and 24-72 hours (PK Visit 1b) post-dose. A blood draw window of +/- 15 minutes is acceptable for samples up to 6 hours post-dose. The last sample can be drawn anytime between 24 and 72-hours post-dose. It is planned to draw 0.4 mL per time point - a total blood volume of 2.4 mL for PK assessment. A short intravenous catheter (heparin lock) can be used to reduce repeated skin puncture and ease the discomfort of blood sampling.

9.2.2.2. Cohort 1B: Safety Visits

One week after PK Visit 1a (+/-4 days), we will conduct a clinical and laboratory safety visit. Medical staff will take a recent medical history from the parent(s) and examine the neonate. Study required bloods will be drawn, and an HIV NAT will be performed. If any tests yield abnormal results, as specified by protocol specific safety criteria (Section 10), we will consult with paediatricians for appropriate management of the neonate. If all tests are normal, a telephonic safety visit will be conducted 2 weeks after PK Visit 1a (+/-4 days). Medical staff will once again take a recent medical history from the parent(s). If no issues are reported, the neonate will exit the study at the conclusion of this visit. If any concerns are noted, the neonate will be brought back for clinical and special investigations as needed. The total study duration for each individual will depend on the age at enrolment and can range from 3 to 4 weeks.

If study safety hold criteria (Section 11.1.2) are met at any time during Cohort 1B the study will be paused, safety data reviewed, and a DSMB meeting held to determine if the study should continue (Section 6.2, **Figure 1**). After Cohort 1B is complete, all safety and PK data for Stage 1 will be reviewed. Pharmacokinetic modelling and simulation analyses using Stage 1 PK data will be performed to select the multi-dose schedule of 5 mg DTG for Cohort 2. All findings will be presented to the DSMB before opening Cohort 2 (Section 11.4).

9.2.3. Stage 2: Cohort 2

Low-risk HIV-exposed neonates in Cohort 2 will receive multiple 5 mg doses of DTG.

DTG administration will begin within 7 days of life and continue until day 28 of life on top of ARV postnatal prophylaxis. Due to the potential drug-drug interaction between NVP and DTG, the low-risk neonates enrolled in Stage 2 will receive ZDV prophylaxis, instead of NVP, for the duration of the study (Section 8.2.1 and Section 13.6).

Due to the fixed 5 mg DTG dose, and the anticipated slow metabolism in neonates, the multiple DTG dosing schedule will likely require longer dosing intervals during early life compared to older infants (e.g., every 48 or 72 hours compared to every 24 hours (Section 8.2.1). Possible DTG dosing schedules for 5 mg of the dispersible tablet during the first 28 days of life are shown in **Table 4**. The pharmacokinetic modelling and simulation analyses of Stage 1 PK data will support the multi-dose schedule of 5 mg DTG for Cohort 2.

Table 4: Potential multi-dose schedules of 5 mg DTG for neonates in Cohort 2 during the first 4 weeks of life.

	Neonate - Week of Life			
DTG 5 mg Dosing Schedule	**1**	**2**	**3**	**4**
1	Q72 hrs		Q48 hrs	
2	Q72 hrs		Q24 hrs	
3	Q48 hrs		Q48 hrs	
4	Q48 hrs		Q24 hrs	
5	Q24 hrs		Q24 hrs	

*Note: *A DTG dosing schedule not listed in the table may be selected based on the PK analysis from Stage 1, and may differ by birth weight (e.g., above and below 3000 g)*

If an HIV NAT test result comes back positive for a neonate at any time during the study, the neonate will not receive any further study drugs, come off study and will be treated as per standard of care (any prior PK samples will be included in the analysis).

At the end of study follow-up, HIV negative infants will continue standard of care HIV prophylaxis, if indicated, as per local guidelines.

An interim PK and safety analysis will be performed after 50% of participants have completed Cohort 2 to assess if a dose adjustment is required and to review safety data. Following the DSMB interim review, a different DTG dosing schedule may be selected based on the data available for the remainder of Cohort 2 infants. Any change to the DTG dosing schedule will be agreed upon by the DSMB before implementation.

9.2.3.1. Cohort 2: Screening and Entry visit(s)

The screening and entry visit(s) will take place when the neonate is <7 days of life (Section 9.1). Screening and entry procedures may be performed as one or 2 separate visit(s). At the Entry visit, ZDV prophylaxis will be initiated instead of NVP and continue until DTG is stopped at Day 28 of life. Thereafter, the infant will switch back to NVP from ZDV and continue prophylaxis per national guidelines.

The study team will support the preparation and administration of the 1st dose of the DTG-DT (half-tablet) mixed with water with the parent(s) or caregiver/legal guardian. The exact time of intake will be recorded. The mother will be explained how to prepare and administer DTG at home. Acceptability testing of the DTG-DT will be performed.

Two blood samples will be drawn for PK assessment, one sample immediately prior to administering the 1st DTG dose and another sample at 1-3 hrs post-dose (**Table 5**). It is planned to draw 0.4 mL of blood per time point.

9.2.3.2. Cohort 2: Study Visits 2 and Visit 3

It is anticipated that DTG could be dosed every 24, every 48 or every 72 hours, or some other combination, that is expected to achieve target DTG concentrations throughout the first 4 weeks of life. The PK sampling schedule has been designed to cover these different possible dosing scenarios.

After the Entry, Visit 2 can be scheduled at **any time from the 3rd dose of DTG** but before Week 4 of life. The mother will be instructed not to administer the morning DTG dose prior to attending this study visit. On the morning of Visit 2, clinic study nurses and physicians will take a medical history, examine the neonate, and assess adherence. Assessments will include dispensing diary cards to caregivers, reviewing or collecting diary cards and establishing study drug adherence (pill count). The PK assessment should be deferred if the mother/caregiver reports their baby missing any of the previous 3 scheduled doses prior to the PK sampling visit (Section 9.4).

Prior to the morning DTG dose, a blood sample will be taken for pre-dose PK evaluation and then the protocol-specified DTG dose will be administered. The exact time of intake will be recorded. Acceptability testing of the DTG-DT will be performed.

Following an observed dose, blood will be drawn pre-dose, and at 1, 2, 4, and 6 (Visit 2), and 24-72 hours (Visit 3) post-dose (**Table 5**). A blood draw window of +/- 15 minutes is acceptable for samples up to 6 hours post-dose. The last sample can be drawn anytime between 24 and 72-hours post-dose. It is planned to draw 0.4 mL per time point - a total blood volume of 2.4 mL for PK assessment. A short intravenous catheter (heparin lock) can be used to reduce repeated skin puncture and ease the discomfort of blood sampling.

9.2.3.3. Cohort 2: Visit 4

At least 1 week after Visit 2 (+/-4 days), we will conduct a clinical and laboratory safety visit. Medical staff will take a recent medical history from the parent(s) and examine the neonate. Study required bloods will be drawn. If any tests yield abnormal results, as specified by protocol specific safety criteria (Section 10), we will consult with paediatricians for appropriate management of the neonate. Adherence assessments will include dispensing diary cards to caregivers, reviewing or collecting diary cards and establishing study drug adherence (pill count). Acceptability testing of the DTG-DT will also be performed. *Note:* Visit 4 can be conducted on the same day as Visit 5, but only if both fall within week 4 of life.

9.2.3.4. Cohort 2: Visit 5 and Safety Phone call

Study Visit 5 will be performed in Week 4 of life (Day 22 to 28 of life). The mother will be instructed not to administer the morning DTG dose prior to attending this study visit. Medical staff will take a recent medical history from the parent(s) perform a clinical and laboratory safety visit. Adherence assessment and acceptability testing of DTG will be performed. Study required bloods will be drawn at visit 5, including an HIV NAT test. If any tests yield abnormal results, as specified by protocol specific safety criteria (Section 10), we will consult with paediatricians for appropriate management of the neonate.

Two blood samples will be drawn for PK assessment, one sample immediately prior to administering the morning DTG dose and another sample at 1-3 hrs post-dose (**Table 5**). It is planned to draw 0.4 mL of blood per time point.

The administration of DTG will be stopped by day 28 of life, after which local ARV prophylaxis will be continued as per standard of care.

A telephonic safety visit will be conducted 2 weeks after Visit 5 (+/-4 days). Medical staff will once again take a recent medical history from the parent(s). If no issues are reported, the neonate will exit the study at the conclusion of this visit. If any concerns are noted,

the neonate will be brought back for clinical and special investigations as needed. The total study duration for each individual will depend on the age at enrolment and can range from 5 to 6 weeks.

9.2.3.5. Cohort 2: Summary of PK Sampling

A summary of the PK sampling scheduled in Cohort 2 is shown in **Table 5** below:

Table 5: Blood sampling schedule for pharmacokinetic assessments for Cohort 2

Study Visit	Dose of DTG	PK Sampling
Entry	1st DTG dose (<7 days of life)	pre-, 1-3 hrs post-dose
Visit 2/3	Any time from 3rd DTG dose but before Week 4 of life	pre-, 1, 2, 4, 6, 24-72* hrs post-dose
Visit 5	Any DTG dose in Week 4 of life	pre-dose, 1-3 hrs post-dose

**Depends on DTG dosing schedule*

The blood sampling has been designed to ensure blood volume limitation are in alignment with the SU HREC guideline for paediatric blood volumes for research purposes (Section 13.7)

9.3. Schedule of Evaluations

Table 6. Schedule of evaluations for Stage 1 Cohort 1A (n=8): Single dose of DTG in neonates in week 3 and 4 of life

Evaluation	Screening and Entry Visit(s)[a]	PK Visit 1a	PK Visit 1b	Safety Visit 1	Safety Phone Call
Visit windows	< 14 days of life	≥ 14 days and less than 28 days of life	24-30 hours post single DTG dose	1 week after PK Visit 1a (+/-4 days)	2 weeks after PK Visit 1a (+/-4 days)
Clinical					
Informed Consent	X				
History	X	X	X	X	X
Physical examination [b]	X	X		X	
Dispense DTG		X			
Acceptability questionnaire		X			
Laboratory					
Haematology [c]	X (0.5 mL)			X (0.5 mL)	
Chemistry [d]	X (0.8 mL)			X (0.8 mL)	
HIV-1 NAT Test [e]	X (0.8 mL)			X (0.8 mL)	
Pharmacology					
PK Blood Sampling [f]		X (2mL)	X (0.4mL)		
Total blood volume	**2.1 mL**	**2 mL**	**0.4 mL**	**2.1 mL**	

[a] Screening and entry procedures may be performed as one or 2 separate visit(s). All study required results should be available before entry with the exception of the HIV-1 NAT result which may be pending at time of study entry; [b] Physical and medical assessment includes length, weight, head measurements and monitoring of vital signs; [c] Haemoglobin, white blood cell count and differential, and platelets; [d] Creatinine, ALT, AST, total and direct bilirubin; [e] If HIV-1 NAT Test comes back positive, the HIV-infected neonate will be taken off study and initiated on standard of care HIV-treatment, as per local guidelines; [f] PK blood sampling at PK Visit 1a/b will occur at 1, 2, 3, 4, 6 and 24-30 hours post DTG single dose. Mothers and their neonates will return home after PK Visit 1a sampling time-points (1, 2, 3, 4 and 6 hours) and attend the following day for PK Visit 1b to complete the 24-30 hour PK sampling procedure. Laboratory tests may be repeated during the screening period dependent on specified blood draw limits.

Table 7. Schedule of evaluations for Stage 1 Cohort 1B (n=8): Single dose of DTG in neonates in week 1 and 2 of life

Evaluation	**Screening and Entry Visit(s)[a]**	**PK Visit 1a**	**PK Visit 1b**	**Safety Visit 1**	**Safety Phone Call**
Visit windows	≤3 days of life	<14 days of life	24-72 hours post single DTG dose	1 week after PK Visit 1a (+/-4 days)	2 weeks after PK Visit 1a (+/-4 days)
Clinical					
Informed Consent	X				
History	X	X	X	X	X
Physical examination [b]	X	X		X	
Dispense Study Drug		X			
Acceptability questionnaire		X			
Laboratory					
Haematology [c]	X (0.5 mL)			X (0.5 mL)	
Chemistry [d]	X (0.8 mL)			X (0.8 mL)	
HIV-1 NAT Test [e]	X (0.8 mL)			X (0.8 mL)	
Pharmacology					
PK Blood Sampling [f]		X (2 mL)	X (0.4 mL)		
Total blood volume	**2.1 mL**	**2 mL**	**0.4 mL**	**2.1 mL**	

[a] Screening and entry procedures may be performed as one or 2 separate visit(s). All study required results should be available before entry with the exception of the HIV-1 NAT result which may be pending at time of study entry; [b] Physical and medical assessment includes length, weight, head measurements and monitoring of vital signs; [c] Haemoglobin, white blood cell count and differential, and platelets; [d] Creatinine, ALT, AST, total and direct bilirubin; [e] If HIV-1 NAT Test comes back positive, the HIV-infected neonate will be taken off study and initiated on standard of care HIV-treatment, as per local guidelines; [f] PK blood sampling will occur at the following time points: pre-dose and 1, 2, 4, 6 and 24-72 hours post dose. Mothers and their neonates will return home after PK Visit 1a and attend 24–72 hours later (as applicable) for PK Visit 1b. Laboratory tests **may** be repeated during the screening period dependent on specified blood draw limits.

Table 8. Schedule of evaluations for Stage 2 Cohort 2 (n=24): Multiple doses of DTG in neonates commencing within 72 hours of life

Evaluation	**Screening and Entry Visit(s)[a]**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Safety Phone Call**
Visit windows	< 7 days of life	Any time from 3rd DTG dose but before Week 4 of life[b]	24 to 72 hours post Visit 2 DTG dose	1 week after Visit 2 (+/- 4 days)[c]	Week 4 of life (Day 22 – 28 of life)	2 weeks after Visit 5 (+/-4 days)
Clinical						
Informed Consent	X					
History	X	X	X	X	X	X
Physical examination [d]	X	X		X	X	
Dispense Study Drug [e]	X	X	X	X	X	
Acceptability questionnaire	X	X		X	X	
Adherence assessments		X		X	X	
Laboratory						
Haematology [f]	X (0.5 mL)			X (0.5 mL)		
Chemistry [g]	X (0.8 mL)	X (0.8 mL)		X (0.8 mL)	X (0.8mL)	
HIV-1 NAT Test [f]	X (0.8 mL)				X (0.8 mL)	
Pharmacology						
PK Sampling [i]	X (0.8 mL)	X (2.0 mL)	X (0.4 mL)		X (0.8mL)	
Total blood volume	**2.9 mL**	**2.8 mL**	**0.4 mL**	**1.3 mL**	**2.4 mL**	

[a] Screening and entry procedures may be performed as one or 2 separate visit(s). All study required results should be available before entry with the exception of the HIV-1 NAT result which may be pending at time of study entry; [b] Visit 2 and 3 should be scheduled any time from 3rd DTG dose but before week 4 of life; [c] Visit 4 can be conducted on the same day as Visit 5 but only if both falls within week 4 of life; [d] Physical and medical assessment includes length, weight, head measurements and monitoring of vital signs vital signs; [e] 1st DTG dose administered <7 days. The timing of the next dose of DTG and subsequent DTG doses will be determined using data from Cohort 1A and 1B. DTG will be administered until the neonate is 28 days old; [f] Haemoglobin, white blood cell count and differential, and platelets; [g] Creatinine, ALT, AST, total and direct bilirubin; [h] If HIV-1 NAT Test comes back positive, the HIV-infected neonate will be taken off study and initiated on standard of care HIV-treatment, as per local guidelines; [i] For the 1st DTG dose (PK: pre-dose sample and 1 more sample 1-3 hours post-dose); Any time from 3rd DTG dose but before week 4 of life (PK: pre-dose and 1, 2, 4, 6 and 24-72 hours post dose); For any DTG dose in Week 4 of life (PK: pre-dose sample and another sample 1-3 hours post-dose). Laboratory tests **may not** be repeated during the screening period as this will exceed blood draw limits.

9.4. Rescheduling, Deferment or Termination of PK Sampling

Stage 1: Cohorts 1A and 1B

The PK blood sampling will be terminated if:

- Emesis occurs within 30 minutes of study drug administration for which the study team deem that the study drug administration has not been completely retained.
- If either the first or second PK sample at 1 or 2 hours post-dose are missed.

If the PK sampling is terminated the child will continue in the study for safety assessment but repeat PK sampling will not be performed. Participants who do not complete the PK visit in Stage 1 will be replaced.

Cohort 2

The PK visit will be deferred at Visit 2 if the mother/caregiver reports their baby missing any of the previous 3 scheduled doses. In this event, the subject will be asked to continue on their current dosing, and if adherence is confirmed for 3 consecutive doses the PK visit can be performed.

Once started, the PK blood sampling at Visit 2 will be terminated if:

- Emesis occurs within 30 minutes of study drug administration for which the study team deem that the study drug administration has not been completely retained.

  The PK blood sampling at Visit 2 may be rescheduled if it remains within the study visit window and the maximum blood volume drawn does not exceed the amount allowed. If it is not possible to complete the PK sampling at visit 2, the child will continue in the study and complete the remaining visits per protocol. Participants who do not complete the PK sampling at Visit 2 will be replaced.

9.5. Acceptability

Acceptability generally refers to '*The overall ability and willingness of the patient to use and its caregiver to administer the medicine as intended*' (41). In this definition, the word "medicine" refers here to the therapeutic entity as it is to be delivered to the end user and includes: the type of dosage form, its formulation i.e., its composition and appearance, the dose of active substances, the dosing frequency, and the primary packaging. In this specific case, the product is a single scored dispersible tablet of DTG for suspension in water to be administered to HIV-exposed neonates.

The precise contribution of acceptability to adherence is difficult to establish (42, 43) but acceptability may have a direct impact on the efficacy and safety of a medication, while the level of adherence may impact efficacy and safety secondarily.

Our aim is to identify all obstacles that may prevent HIV-exposed neonates "from using and caregivers from administering the medicine as intended". Assessment will take into account and document the relevant characteristics of the children and/or their caregivers:

- Age
- Inherent ability of the child to take the medicine; dependence on caregiver to prepare and administer it.

And measure key characteristics of the medicinal product:

- Palatability
- Swallowability (related to the volume of the intake and the texture of the suspension after reconstitution, depending on the vehicle) as well as the device used to administer the dose.
- The complexity for the caregiver to prepare the dose correctly prior to administration, if required (determination of the dose, weight band width and frequency of dose adjustment)
- Required dose (e.g., the dosing volume after product reconstitution).
- Need for a vehicle (e.g., water, formula milk, breastmilk)
- Dosing frequency and duration of treatment (once or twice daily)
- Selected administration devices (e.g., syringe, or cup) (44)
- Primary container closure system
- Actual mode of administration that reflects understanding of user instructions and feasibility of following them

In this study, acceptability will be recorded for all cohorts during each PK visit by focusing on several observation items:

- Attitude of the child when presented with the formulation: facial expression, crying or smiling, reaction to drug intake, fighting drug intake, incident e.g., spitting out the suspension, etc.
- Swallowability, i.e., ability to take the full dose, in one or two steps
- The way the caregiver prepares the dose (e.g., breaking the scored tablet, mixing with the appropriate volume of water, using an appropriate dosing device, calculating the right dose, etc)

In between visits, the experience of the caregiver preparing the medicine and administering it will be assessed as well as how the child reacted to medicine intake, ability to swallow the full dose, and incidents/accidents.

10. Safety Reporting

10.1. Adverse Event Definition

An adverse event (AE) is 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 study drug, which may or may not be causally related to the study drug. Abnormal laboratory (haematology and biochemistry) results will be reported as AEs if the abnormality newly occurs or worsens after institution of the study treatment. Any untoward medical occurrence identified from birth to the time of study treatment will be considered a pre-existing condition.

10.2. Serious Adverse Event Definition

An AE is defined as serious if it meets at least one of the following criteria below:

- fatal
- life-threatening
- requires or prolongs hospitalization
- results in persistent or significant disability
- any important medical event

Additional serious adverse events (SAEs) for this study protocol are:

- any Grade 3 or higher adverse event on the DAIDS toxicity table
- for abnormal renal function and high bilirubin levels, any Grade 3 or higher adverse event as classified by the **protocol specific safety criteria** (refer to **Table 9**)

10.3. Adverse Event Notification Procedure

The investigator is required to report all directly observed AEs and all AEs reported by the parents using concise medical terminology. The adverse events reporting period for this trial begins with participant informed consent until the study ends. Each AE must be evaluated by a physician directly involved in the clinical evaluation of the research participants. The investigator must classify all AEs as serious or non-serious using the definitions above *and* determine relation to study drug. The AEs should be reported on study CRFs, regardless of their relationship to study drug. This classification, as well as the evaluation of severity and causal relationship, will determine the reporting procedure for the AE.

Non-serious adverse events are to be reported on the AE CRFs only and will be submitted to the Stellenbosch University Human Research Ethics Committee (SU-HREC). All events will be reviewed by the Serious Adverse Event Evaluation Group. On the CRF, a given AE 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.

All serious adverse events will be reported in an *expedited manner*, irrespective of their relationship to study drug. All SAEs (as defined in Section 10.2) will be completed on a standard SAE report form and submitted to the SU-HREC ***immediately, but no later than 3 reporting days*** after the site becomes aware of an event that meets criteria for expedited reporting. The time frame for expedited reporting of individual SAEs begins when the clinical research site recognizes that an event fulfils the criteria for expedited reporting. Immediate clinical management of all adverse events will be the responsibility of the local study team. All SAEs will be followed and reported until resolution or stable state. On a monthly basis, all SAEs will also be reviewed by a Serious Adverse Event Evaluation Group (Section 14.2).

10.4. Adverse Event Severity Grading

AEs and toxicities will be graded for severity according to the DAIDS Table for Grading the Severity of Adult and Paediatric Adverse Events (Version 2.1, July 2017; available at: https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf. It is to be noted there exists a distinction between severity (grade, reflecting degree of abnormality of the event) and seriousness (reflecting the immediate consequences) of adverse events.

In case of AEs not described in the DAIDS Table, the investigator will use the terminology “mild”, “moderate”, “severe” or “life-threatening” or “death” to describe the maximum severity of the adverse event.

These severity grades are defined as follows:

MILD	Does not interfere with patient's usual functions
MODERATE	Interferes to some extent with patient's usual functions
SEVERE	Interferes significantly with patient's usual functions
LIFE-THREATENING	The patient is at risk of death at the time of the AE: it does not refer to an AE that hypothetically might have caused death if more severe
DEATH	Death related to AE

Protocol specific safety criteria (Table 9) will be used to evaluate renal function and high bilirubin levels and replace the DAIDS toxicity table for these parameters.

Table 9. Protocol Specific Safety criteria

Parameters	Grade 1	Grade 2	Grade 3	Grade 4
Creatinine, High (μmol/L)	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN	> 1.8 to < 3.5 x ULN	≥ 3.5 x ULN
Bilirubin, High	Mild jaundice, no treatment	Phototherapy	Exchange transfusion	Acute bilirubin encephalopathy

Creatinine will be graded according to the upper limits of 'normal' for a term neonate (**Table 9**), which will serve as a proxy for evaluating abnormal renal function. Study investigators will make use of protocol specific creatinine laboratory reference ranges (Appendix, **Table 18.1**) representing standardised accepted 'normal' creatinine levels for term neonates (45). At birth, creatinine values are elevated and reflect maternal renal function. Over the first 24 to 36 hours, a transient raise in creatinine levels occur, after which it falls quickly (day 5- 7 of life), and then creatinine levels stabilize at around 9 days (46). Given these renal physiological adaptations, a change from baseline creatinine and/ a change in creatinine clearance as per the DAIDS toxicity grading table will *not be used*, as these grading developed for children may be inaccurate in neonates.

A clinical grading system was developed for reporting hyperbilirubinemia making use of both clinical findings and intervention levels required to manage jaundice in the neonate (**Table 9**). Hyperbilirubinemia values will be captured and jaundiced will be managed according to the 2006 Western Cape Consensus Guidelines (Appendix, Section 18.2 (47)), as per standard of care. Neonates on phototherapy will be allowed to continue with study drug. Dolutegravir will be permanently discontinued in any neonate who requires an exchange transfusion.

10.5. Adverse Event Causality Assessment

For both non-serious and serious adverse events, the investigator is required to assess the possible relationship between the adverse event and the study drug, i.e., determine whether there exists a reasonable possibility that the study drug caused or contributed to the AE.

Relatedness of these events should be reported as follow:

- Related. There is a reasonable possibility that the adverse event may be related to the administration of study agent.
- Not related. There is no reasonable possibility that the adverse event may be related to the administration of study agent.

10.6. Hypersensitivity Reaction to Dolutegravir

Hypersensitivity reactions to DTG have been reported in less than 1% of participants in Phase 3 clinical trials and were characterized by rash, constitutional findings, and sometimes organ dysfunction, including liver injury. The DAIDS Toxicity Table will capture symptoms and signs of possible DTG hypersensitivity, and if suspected, will be managed as a SAE. Hypersensitivity to DTG is a multi-organ clinical syndrome usually characterized by fever, rash, general malaise, blisters or peeling of the skin, oral blisters or lesions, conjunctivitis, facial oedema, hepatitis, eosinophilia, angioedema, and difficulty in breathing. Clinical status, including liver aminotransferases, should be monitored and appropriate therapy initiated. Discontinue DTG as soon as a hypersensitivity reaction is suspected and if the diagnosis of DTG hypersensitivity is made DTG should never be re-started (17).

10.7. Risk of Elevated Bilirubin Levels in in neonates

10.7.1. Bilirubin Metabolism in Neonates

Between 25-50% of all term neonates develop clinical jaundice characterized by high levels of unconjugated/free bilirubin. The serum bilirubin of term neonates rises to a peak of 103 to 137 μmol/L by 3 days of life and then falls. A rise to 205 μmol/L is still within the normal physiological range and may be attributed to an increase in bilirubin production, reduced uptake of bilirubin from plasma, reduced conjugation (immature UGT1A1 enzyme activity) and decreased hepatic excretion of bilirubin (46). Bilirubin values exceeding 342 μmol/L have been considered levels at which kernicterus can occur (48, 49). Phototherapy are the mainstay of treatment for unconjugated hyperbilirubinemia resulting in less free bilirubin available to cross the blood-brain barrier, and thereby protecting against kernicterus and associated brain damage. In rare cases, exchange transfusions are performed to rapidly decrease bilirubin values.

Normally, newborn produce more bilirubin compared to adults. After birth, fetal haemoglobin is broken down and replaced with adult haemoglobin. Heme-containing proteins from old red cells are converted to insoluble bilirubin which is then bound to albumin and transported to the liver. Once in the liver, the enzyme UGT1A1 converts the unconjugated bilirubin to water-soluble conjugated bilirubin. End-products of this process, i.e., bile, is then secreted into the digested tract with bilirubin metabolites leaving the body through faeces (50).

The bilirubin-conjugating capacity of infants is dependent on UGT1A1 enzyme activity, which is typically slow at birth but rapidly increases in the first months of life. UGT1A1 is expressed at 1% of adult levels between 30- and 40-weeks' gestation, reaching adult levels by 14 weeks of postnatal life (51). Immature UGT1A1 early in life may lead to less conjugation and contribute to elevated free bilirubin levels leading to the jaundice in the newborn.

10.7.2. Dolutegravir, Protein Binding and Hyperbilirubinemia

Dolutegravir is a well-tolerated potent ARV with minimal side effects. The drug is highly bound to plasma proteins and can displace bilirubin from albumin. DTG poses a theoretical risk of increased free bilirubin concentrations in the neonate at high DTG concentrations and in the presence of low serum albumin. However, DTG concentrations achieved in adults at approved DTG dosing are much lower than concentrations expected to result in the displacement of bilirubin from albumin (52).

An *in-vitro* experiment performed in the presence of low albumin concentrations has shown DTG to displace bilirubin from albumin to an equivalent extent as sulfisoxazole, raising concerns of increased free bilirubin and risk of bilirubin neurotoxicity. The clinical relevance of these experimental findings are however unclear as normal albumin concentrations (500-700 µmol/L) in adults and children typically exceeds DTG plasma concentrations by around 100-fold. The authors conducting these *in-vitro* studies conclude that unless the DTG concentrations are very high in a neonate, it is unlikely to have a significant impact on bilirubin to albumin binding because of the low DTG concentrations relative to the amount of albumin in the body (52).

To minimize the risk of bilirubin toxicity, we will exclude neonates born from mothers with known blood group incompatibility that can result in haemolytic disease of the newborn. We will not enroll neonates at high risk of bilirubin toxicity, which include preterm neonates, unwell neonates with a history of birth trauma, those with hypothermia, or suspected sepsis (53). Neonates with very high bilirubin values close to exchange, as defined by local guidelines (47), will also not be eligible for the study. Total and direct bilirubin will be drawn at baseline and monitored throughout the study. For safety purposes, a single dose of DTG will first be administered to older neonates (>2 weeks of age) in Cohort 1A, before moving to a single dose in younger neonates (<2 weeks of age) in Cohort 1B. Only if no safety concerns are observed will a multi-dose DTG strategy be considered, and neonates enrolled into Cohort 2.

10.8. Participant Study Drug Discontinuation Criteria

For all AEs/toxicity, the management and temporary/permanent discontinuation of the study drug should generally follow the criteria below, but clinicians should use their clinical judgment as to the best management for an individual participant.

- Grade 1 and Grade 2 AEs/toxicity:
  Continue the study drug. Manage using symptomatic measures and other concomitant medication, if appropriate.
- Grade 3 AEs/toxicity:
  Request laboratory results and repeat confirmatory laboratory results within 72 hours if relevant. Continue study drug pending receipt of the confirmatory laboratory tests/repeat observations, unless there is an immediate need to substitute ART. Continue to work-up to exclude other causes. Following confirmation of toxicity, and lack of other cause data (i.e., possibly, probably, or definitely related to the study drug) hold the treatment and follow abnormal laboratory values until they are Grade 2 or less.
- Grade 4 Potentially Life-Threatening or Fatal AEs/toxicity:
  Request laboratory results and repeat confirmatory laboratory results within 72 hours if relevant. Withhold the study drug at the discretion of the site investigator/ health care provider for clinical events, while awaiting a repeat assessment/ confirmation of an abnormal laboratory test. Continue to investigate to exclude other causes and make an assessment on relation to study drug or not. Following confirmation of toxicity and lack of other cause data: (i.e., possibly, probably, or definitely related to the study drug) the study drug should be discontinued immediately. Participants who permanently discontinue study treatment due to an adverse event related to the study treatment will continue to be followed in the study for safety monitoring at intervals to be determined by the investigator in consultation with the study team.

Study drug will be **permanently discontinued in participants** with the following condition:

- any grade 4 event on the DAIDS toxicity table
- a bilirubin at exchange value

In addition, study drugs will not be administered to any participant if the HIV NAT result comes back as positive at any time during the study.

10.9. Early Study Termination Criteria

The study can be terminated or suspended early by the Sponsor. Reasons for termination of the study may include, but are not limited to, the following:

- The investigator or study site exhibit serious and/or persistent non-adherence to the clinical study Protocol, the Declaration of Helsinki, ICH–GCP, and/or applicable regulatory requirements
- A DSMB recommendation to terminate the study

11. Study Safety and PK Exposures Criteria for Interim Analyses

11.1. Study Safety Hold Criteria

The following **safety hold criteria will lead to a pause/temporarily hold** in the study, until such time as the Serious Adverse Event Evaluation Group (Section 14.2) have reviewed the SAEs, determined causality, and informed the DSMB. The DSMB will assess causality and advise on study continuation or termination.

11.1.1. Cohort 1A: Safety Hold Criteria

Accrual of neonates into Cohort 1A (n=8) will be paused if:

- 1 participant has a fatal or life-threatening adverse event assessed as related to study drug, or
- 2 participants experience the same related grade 3 or higher adverse event, or
- 3 participants experience different related grade 3 or higher adverse events

11.1.2. Cohort 1B: Safety Hold Criteria

Accrual of neonates in Cohort 1B (n=8) will be paused if:

- 1 participant has a fatal or life-threatening adverse event assessed as related to study drug, or
- 2 participants experience the same related grade 3 or higher adverse event, or
- 3 participants experience different related grade 3 or higher adverse events

11.1.3. Cohort 2: Safety Hold Criteria

Accrual of neonates in Cohort 2 (n=24) will be paused if:

- 1 participant has a fatal or life-threatening adverse event assessed as related to study drug, or
- 25% of participants (3 of the first 12; or 6 of 24 neonates) experience the same related grade 3 or higher adverse event, or
- Any related AE leading to permanent discontinuation of the study drug

11.2. Assessment of DTG Plasma Drug Concentrations

11.2.1. PK Samples: Collection, Storage and Shipping

Blood samples collected for plasma drug measurement will be centrifuged and the plasma frozen at -70°C or lower. Samples will be shipped to the BioAnalytical Laboratory at the Faculty of Associated Medical Sciences (AMS-BAT), Chiang Mai University, in Chiang Mai, Thailand on dry ice following IATA shipping guidelines for analysis. No clinical samples will be stored for future testing and no genetic testing will be performed on samples collected within the study. Remaining samples will be kept in case repeat testing is required but will be destroyed within 6 months of study closure.

11.3. Quantification of Antiretroviral Drug concentrations

All antiretroviral plasma concentrations of DTG will be measured using a validated liquid chromatography-triple quadrupole mass spectrometry assay (LC-MS/MS). The method has been validated based on US-FDA guidelines. The laboratory facility participates in the international external quality control (QC) program of the Clinical Pharmacology Quality Control (Precision Testing) program (University at Buffalo, NY, USA) funded by the National Institutes of Health, USA.

11.4. Interim PK Analysis: Cohorts 1A and 1B

For the treatment of HIV-infection it is accepted that a drug will provide an effective response in the paediatric population if comparable drug exposures are achieved to those demonstrated to be safe and effective in adults. From a PK perspective, the planned interim PK analysis after Cohort 1A/1B is to select the appropriate multi-dose schedule to be assessed in Cohort 2. The goal is to ensure that neonates are not exposed to sub- or supratherapeutic drug exposures following multi-doses of DTG. The primary focus will be to identify a multi-dose schedule for Cohort 2 that will achieve DTG plasma trough and maximum concentrations comparable to those observed in adults and children.

11.4.1. Pharmacokinetic Criteria for DTG Dose Selection for Cohort 2

Pharmacokinetic modelling and simulation analyses using Stage 1 concentration data will inform the multi-dose schedule of the DTG-DT for Cohort 2. The PK targets for the PETITE DTG study are based on published data and aim to be in agreement with the clinical studies conducted as part of the regulatory approvals for various DTG formulations. At steady-state, the geometric mean (%CV) DTG trough concentrations were 1.11 (46) µg/mL and 2.12 (47) µg/mL in adults receiving 50 mg once daily and twice daily, respectively (Table 1). The ongoing IMPAACT 2019 study is assessing the PK, safety, and tolerability of a novel pediatric fixed-dose dispersible tablet of ABC/3TC/DTG in children living with HIV less than 12 years of age [US FDA Investigational New Drug (IND) #141,131]. The IMPAACT 2019 study is being conducted in collaboration with ViiV Healthcare Ltd, the originator pharmaceutical company, which has been responsible for the development and licensing of DTG (54). Weight band dosing is under investigation in the IMPAACT 2019 trial and PK targets used for dose confirmation were established based on adult data: a lower PK target for DTG C_{24} was set as 60% of the mean concentrations observed in adults receiving 50 mg once daily (e.g., 1.11 x 0.6 = 0.67 µg/mL) and an upper target set as 140% of the mean concentration observed in adults receiving 50 mg twice-daily (e.g., 2.12 x 1.4 = 2.97 µg/mL). We will apply the same lower DTG C_{24} target of

0.67 µg/mL for the PETITE-DTG study. This C_{24} target is based on DTG data generated during the developed of DTG by ViiV Healthcare. This PK target is also comparable to the observed C_{24} values in children in the IMPAACT P1093 and ODYSSEY trials. The DTG C_{24} in children using the DTG-DT dosed per WHO weight bands ranged from 0.71 to 0.98 µg/mL (**Table 2**).

Extremely high DTG plasma concentrations should be avoided in neonates due to the risk of DTG displacing unconjugated bilirubin from albumin as such elevated unconjugated bilirubin levels could lead to neonatal brain damage (Section 10.7.2). The geometric mean (%CV) DTG steady-state C_{max} were 3.67 (20) µg/mL and 4.15 (29) µg/mL in adults receiving 50 mg once daily and twice daily, respectively (**Table 1**). The geometric mean (%CV) DTG C_{max} in children using the DTG-DT ranged from 3.08 (34) to 7.16 (26) µg/mL (**Table 2**). Individual subject DTG C_{max} values between 12 to 14 µg/mL were observed in children <14 kg receiving DTG-DT and no associated drug toxicity was reported (29). Using the highest C_{max} and associated variability observed in children, it is estimated that upper 95% CI of C_{max} values would be 8.5 µg/mL. No clear toxicity cut-off is defined for DTG C_{max} but we have set an upper C_{max} limit of 17.0 µg/mL (2-fold above the values observed in children). The choice of this C_{max} target is based on historical plasma data in adults and children and their associated safety profiles and remains below those shown *in-vitro* to displace unconjugated bilirubin from albumin with normal albumin concentrations. A similar C_{max} upper target of 18.35 µg/mL is being used in the IMPAACT 2023 trial assessing a novel liquid DTG formulation in neonates developed by ViiV Healthcare (55). As new data become available on DTG in young children and neonates the C_{max} target may be adjusted lower.

A multi-dose DTG regimen will be selected that maintains DTG C_{24} above 0.67 µg/mL and the C_{max} below 17.0 µg/mL for the majority of neonates. The multi-dose strategy selected for the paediatric solid formulations of DTG after analysis of Cohort 1A/1B will be presented to and agreed upon by the DSMB prior to being studied in Cohort 2.

Of note, the DTG dosing schedule selected in Cohort 2 could differ by birth weight (e.g., above and below 3000 g).

While not a specific requirement for the selection of the multi-dose strategy the DTG AUC_{0-24} with the proposed dosing regimen will also be calculated and compared to the target of 35.1 to 134 (µgxh/mL) used in the IMPAACT 2019 study. This exposure range represents the lower 90% CI of the AUC_{0-24} observed in adults receiving 50 mg once daily and the upper 90% CI for observed in adults receiving 50 mg twice-daily.

11.5. Interim PK Analysis after Completion of 50% Enrolment in Cohort 2

An interim PK analysis is planned after 50% of participants have completed the follow-up in Cohort 2. The aim of a multi-dose strategy with paediatric solid formulations is to achieve DTG exposures comparable to those observed in adults and children, i.e., a DTG C_{24} above 0.67 µg/mL and a C_{max} below 17.0 µg/mL for the majority of neonates. The proportion of participants with an C_{24} < 0.5 µg/mL (EC_{95}) will also be calculated. The DSMB will review the interim PK data in relation to the observed C_{24} and C_{max} values and determine if a dose adjustment is required. A different DTG dosing schedule may be selected based on the data available for the remainder of Cohort 2 infants. Any change to the DTG dosing schedule will be agreed upon by the DSMB before implementation.

12. Statistical Considerations

12.1. Number of Participants

Forty evaluable neonates: Stage 1, two sequential cohorts - Cohort 1A (n=8) and Cohort 1B (n=8); Stage 2, Cohort 2 (n=24).

One of the primary objectives of this study is to assess the pharmacokinetics of DTG following administration of 5 mg (half of the 10 mg DTG-DT). In each cohort, we aim to achieve at least 80% power to obtain a 95% CI for DTG CL/F that lies within 60% and 140% of the geometric mean estimate of DTG CL/F in a prior relevant study, per FDA guidance for paediatric PK studies (2). In children in the lowest weight band (3 to <6 kg) of the ODYSSEY trial, DTG CL/F geometric coefficient of variation (CV%) with the 5 mg dispersible DTG tablet was 33%. In neonates, the variability is expected to be higher, with CV% within the range of 30% to 50%. Conservatively assuming that, in each cohort, CV% will be 50%, a sample size of 8 evaluable neonates in Cohort 1A, 8 in Cohort 1B and 24 in Cohort 2 will achieve 86%, 86% and >99% power, respectively, to obtain a 95% CI that lies within 60% and 140% of the geometric mean estimate. These power calculations are based on the Student's t-distribution and apply the methodology described by Wang et al. for rich PK sampling (3).

The other primary objective of this study is to evaluate the safety of DTG in neonates. This will be assessed through proportions of participants with events, such as AEs of Grade 3 or higher.

Table 10 provides binomial probabilities of having at least one subject with an event for a range of sample sizes and true proportions of participants with an event. For example, if the true proportion of participants experiencing an AE of Grade 3 or higher is 5%, the probability of having at least one participant experiencing an AE of Grade 3 or higher out of 40 participants is 0.87.

Table 10. Binomial probabilities of having at least one participant with an event for a range of sample sizes and true proportions of participants with an event.

True proportion of participants with an event	Sample Size			
	N=8	N=16	N=24	N=40
1%	0.08	0.15	0.21	0.33
2%	0.15	0.28	0.38	0.55
3%	0.22	0.39	0.52	0.70
4%	0.28	0.48	0.62	0.80
5%	0.34	0.56	0.71	0.87
6%	0.39	0.63	0.77	0.92
7%	0.44	0.69	0.82	0.95
8%	0.49	0.74	0.86	0.96
9%	0.53	0.78	0.90	0.98
10%	0.57	0.81	0.92	0.99

The precision of the observed proportions of participants with an event will be measured using 95% Clopper-Pearson confidence intervals (CI). For example, if 3 (8%) out of 40 participants experience an AE of Grade 3 or higher, the 95% CI would be 2% to 20%.

12.2. Statistical Analysis Plan

12.2.1. Planned Analyses

Planned interim analyses for futility based on safety and/or PK data will be conducted after the last participant completes follow up in Cohorts 1A and 1B, respectively, and after 50% of participants have completed follow up in Cohort 2; or early termination of the study. The final analysis will be conducted after all participants complete the study; or early termination of the study.

Analysis Populations

The following analysis populations will be used in this study:

- Screened: all infants who were screened for eligibility.
- Enrolled: all infants who passed screening and entered the study.
- Safety: all enrolled infants who received at least one dose of study treatment.
- PK: all infants in the Safety population who had at least one PK assessment.

12.2.2. General Considerations for Data Analyses

Variable description: Unless otherwise specified, categorical variables will be tabulated with counts and percentages and continuous variables will be summarized with descriptive statistics (e.g. mean, standard deviation, median, first quartile, third quartile, minimum, maximum).

12.2.3. Safety Analysis

Adverse events reported during the study period will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and tabulated by severity using the DAIDS AE grading table (version 2.1 July 2017). Causality of AEs with each study drug will also be tabulated. Safety and tolerance of the study drugs will be evaluated through the number and proportion of participants experiencing a safety event, in particular an AE of Grade 3 or higher or a treatment-related AE of Grade 3 or higher. Clopper-Pearson 95% confidence intervals for these proportions will also be calculated.

12.2.4. Pharmacokinetic Analysis

In Cohort 1, a non-compartmental pharmacokinetic analysis will be performed to calculate the PK parameters using Phoenix WinNonLin (Certara, USA). The following PK parameters will be determined: C_{max}, T_{max}, C_{24}, $AUC_{0\text{-}24}$, $AUC_{0\text{-}infinity}$, and Ratio $AUC_{0\text{-}24}$ /$AUC_{0\text{-}infinity}$. C_{max}, C_{last} and T_{max} will be taken directly from the observed concentration-time data. $AUC_{0\text{-}all}$ and $AUC_{0\text{-}inf}$ will be determined using the linear-up log-down trapezoidal method. Total body clearance for extravascular administration will be calculated using Dose/ $AUC_{0\text{-}infinity}$. Median (range), means (standard deviations), and geometric means with 95%CI for each PK parameter will be calculated separately for Cohorts 1A and 1B, as well as for Cohort 1A/1B together.

Pharmacokinetic modelling and simulation analyses using all Stage 1 PK data will be performed to select the multi-dose schedule of 5 mg DTG for Cohort 2. Using appropriate PK models the DTG concentration time profiles will be simulated for different DTG dosing schedules (**Table 4**) and the C_{24} and C_{max} estimated. The DTG dosing schedule which best fits the PK target criteria (Section 11.4.1) will be proposed to the DSMB for assessment in Stage 2.

In Cohort 2, both non-compartmental and population PK analyses will be performed using the plasma concentration data generated. Population means and variances of PK parameters for DTG will be estimated using nonlinear mixed-effects regression models (e.g., using NONMEM VII software). Subject covariates will be assessed to explain sources of inter-subject PK variability. Changes in DTG drug exposures and trough concentrations in neonates following multi-doses of DTG during the first 28 days of life will be estimated using the final model.

If necessary, pooling concentration data available in other studies of DTG in children living with HIV, particularly the IMPAACT P1093, 2019, 2036 and/or ODYSSEY trials, will be considered for model development with agreement from the corresponding study teams.

12.2.5. Acceptability analysis

Categorical variables of the acceptability questionnaire will be tabulated with counts and percentages, and continuous variables will be summarized using descriptive statistics. All individual answers (including text fields) to the acceptability questionnaire for all participants will be provided in a listing.

12.2.6. Other Analyses

Analyses of participant disposition, protocol deviations, demographic and baseline characteristics, concomitant medications and treatment exposure and compliance will also be performed.

A detailed description of all planned analyses and data presentations for this study will be provided in a separate Statistical Analysis Plan.

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. These documents include Investigator's Site File, patient clinical source documents, laboratory and diagnostic reports and enrolment logs. The Investigator's Site File will contain the protocol/protocol amendments, CRFs, regulatory approval with correspondence, sample informed consent, drug accountability records and other relevant documents/ correspondence etc.

13.2. Data Collection and Management

At the site, all data required in the protocol should be recorded in the participant's source documents. Source documents include laboratory, diagnostic and consultation reports, history and physical examination reports etc., for possible review and/or audit by the Regulatory Authorities.

Data from source documents should be reported on the provided CRF. The information on the CRF will be entered into the clinical trial database by the site or CRFs may be scanned and emailed using secure encrypted systems to the data centre for data entry. The trial data will be stored in a computer database maintaining confidentiality in accordance with regulatory practice. It is the investigator's responsibility to ensure the accuracy, completeness, and timeliness of the study data reported on CRFs. Study data reported on the database will be

checked for consistency with CRF/source documents by clinical monitors, and any discrepancies found will be notified to the investigator who is responsible for resolving the data discrepancies, reporting data changes and maintaining accurate and consistent data in source documents, CRF and clinical database. For record retention, the investigator must keep all study documents on file for at least 5 years (maximum 10 years) after completion or discontinuation of the study. After that period the documents may be destroyed with prior permission from the Sponsor subject to local regulations.

13.3. Monitoring, Audits and Inspections

Monitoring visits to the trial site will be made periodically by designated external clinical monitors, and at some visits with members of Viatris's Operations team, in accordance with ICH-GCP Guidelines. The trial site may also be subject to quality assurance audits by Viatris Ltd, or other designated representatives and/or to inspection by regulatory authorities.

The investigators will permit designated clinical monitors to inspect all CRFs and SAE report form(s), medical records, and laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at any time during the study. The corresponding source documents for each patient will be made available, provided patient confidentiality is maintained in accord with local regulations.

Clinical monitors will conduct all monitoring activities in accordance with a monitoring guideline agreed with the Sponsor. A site initiation visit with staff members from the clinical site, pharmacy and laboratory if applicable, will be performed before screening.

The first monitoring visit will be planned and held shortly after the first patient has been enrolled.

Additional visits will be performed during the trial according to the monitoring plan.

The objectives of monitoring visits are to check that the trial is conducted according to the study protocol and the principles of Good Clinical Practice, in particular that:

- Informed consents have been dated and signed by the parent(s)/legal guardian and by the investigator, before any intervention induced by the protocol;
- All serious adverse events were declared; and
- Data collected on the CRFs conform to source documents.

Monitors will also:

- Verify the content of the Investigator Site File, investigational medicinal product accountability; and
- Ensure compliance with the protocol and relevant regulations.

Medical data will be reviewed in strict respect of confidentiality. Source data verification will be specified in the monitoring plan agreed by the Sponsor.

13.4. Regulatory and Ethical Considerations

This study will be conducted according to the principles of Good Clinical Practice and taking into account the Declaration of Helsinki and the International Conference on Harmonisation guidelines for clinical research. The study protocol and informed consent documents will be submitted to the Human Research Ethics Committee (HREC) of Stellenbosch University, the South African Health Products Regulatory Authority (SAHPRA), and the WHO Research Ethics

Review Committee. Subsequent modifications will be submitted for approval before implementation.

An agreement will be in place between Stellenbosch University (FAMCRU), the Faculty of Associated Medical Sciences, Chiang Mai University (AMS-CMU), and Viatris Ltd, setting out respective roles and responsibilities. The trial will also be registered with the Pan African Clinical Trials Registry (PACTR).

13.5. Informed Consent

Written informed consent will be obtained from the parent(s) or legal guardian of eligible neonates before any procedures are performed. The informed consent process will include information exchange, detailed discussion, and assessment of understanding of all required elements of informed consent, including the potential risks, benefits, and alternatives to study participation. A copy of the informed consent containing basic information about the study will be provided to the parent(s) or legal guardian in the language of their choice (Xhosa, English or Afrikaans).

13.6. Potential Benefits and Risks of the Research to the Subject

The generic DTG 10 mg scored dispersible tablet from Viatris Ltd has received tentative approval from the U.S. FDA for use in infants and children 4 weeks and older and weighing at least 3 kg, and is currently under review with SAHPRA for regulatory approval in South Africa. Regulatory approval has not been requested for neonates due to lack of PK and safety data in this population. The PETITE-DTG study will generate this data for DTG in neonates, which will inform future ARV dosing and DTG use in neonates for HIV prevention and treatment.

We are proposing a study that requires the collection of personal and clinical data, as well as blood specimens, from neonates born to mothers living with HIV. Data will be entered into the clinical trial database by the site or CRFs may be scanned and emailed using secure encrypted systems to the data centre for data entry. All data will be de-identified and anonymised as to protect participant confidentiality. The PETITE-DTG study will comply with International General Data Protection Regulation (GDPR) and South African POPIA principles (Section 13.8). Most study procedures are routine medical procedures that are associated with minimal to no risk in participants. Blood collection may cause some discomfort and some bleeding or bruising may occur where the needle enters the skin. Well-trained and experienced study staff will perform the phlebotomy to minimize risk.

The potential risk of participation in this study is associated with the neonatal administration of DTG. Thus, all participants enrolled will be healthy HIV-exposed neonates born to mothers with HIV on a DTG-based ARV regimen. All neonates will be of term gestation and have a minimum birth weight of ≥2000 grams as no DTG data are available for preterm neonates.

In South Africa, women with HIV routinely receive an HIV-1 VL at delivery and their infants receives a minimum of 6 weeks of ARV postnatal prophylaxis, dependent on the risk of HIV acquisition in the infant. All HIV-exposed neonates start a prophylaxis regimen of NVP and ZDV at birth and continue this until the maternal VL result is available. If the maternal VL at delivery is ≥1000 copies/mL the infant is classified as ‘high-risk’ and continues both ARVs, else infants are deemed ‘low’-risk and ZDV is stopped and NVP continued (1).

In Stage 1 (Cohort 1A and 1B), a single dose of DTG will be administered on top of standard of care ARV prophylaxis. The maternal VL result at delivery will not be required for enrollment in Stage 1 as there is no change to the standard ARV prophylaxis. Thus, the risk to participants in terms of HIV transmission is considered unchanged. However, in Stage 2 (Cohort 2), due to the anticipated drug-drug interaction between DTG and concomitant NVP postnatal prophylaxis neonates will receive ZDV prophylaxis, instead of NVP, for the duration of the study. For this reason, an extra inclusion criterion has been added so that only low risk infants born to virologically suppressed mothers will be enrolled in Stage 2. In this study, a 'low-risk' HIV-exposed neonate is defined as a neonate born to a mother with a plasma HIV-1 RNA result <50 copies/mL in the 4 weeks prior to delivery or between delivery and infant study entry. Our criteria to define a low risk infant is stricter that than current South African PMTCT guidelines that uses a maternal VL of <1000 copies/mL (1). Also, given that ZDV has been widely used for infant postnatal prophylaxis during the first 6 weeks of life for decades we believe the risk of risk of HIV transmission in these low risk breastfeeding infants is at least the same as those receiving standard of care prophylaxis. This is consistent with the current USA guidelines which recommends a 4-week neonatal ZDV prophylaxis regimen for newborns if the mother achieved viral suppression (defined as a confirmed HIV RNA level <50 copies/mL) on ART during pregnancy within 4 weeks of delivery (56).

Per national guidelines, all infants receive a birth HIV NAT, 10 weeks, 6 months, and in those who are breastfeed, at 4 weeks post-cessation of breastfeeding. The participants HIV NAT test result at birth may or may not be available at the time of study entry. However, if an HIV NAT result comes back positive whilst the neonate is on study, the neonate will not receive any further DTG doses, revert to standard of care ART, and be followed for safety for the duration of the study. Extra HIV NAT testing will be performed in all neonates during the follow-up.

There will likely be no direct benefits from DTG for HIV-exposed neonates in Cohort 1A and 1B receiving one single dose of the study drug, in addition to the standard of care prophylaxis. Low risk HIV-exposed neonates in Cohort 2, receiving multi-dose of DTG in addition to ZDV, may potentially benefit from receiving a combination of DTG and ZDV to protect against vertical HIV transmission.

All participants will be carefully monitored for any possible side effects. In general, DTG is well-tolerated with a very good safety profile and paediatric adverse event data are similar to those observed in adults. The most common adverse reactions reported in adults from 3 clinical trials were insomnia, fatigue, and headache; occurring in less than 3% of participants. Hypersensitivity reactions have been reported in less than 1% of participants on DTG and study drug should be stopped immediately if suspected. Hepatotoxicity can rarely develop and monitoring for this condition is recommended (19). DTG has also been shown *in-vitro* to displace bilirubin from albumin in the presence of low albumin concentrations. The clinical relevance of this has not been established but in theory, displacement of bilirubin from albumin by DTG can lead to elevated free bilirubin which if untreated may lead to kernicterus. Because of this potential risk for bilirubin toxicity with high DTG concentrations, neonates known to be at risk for very high bilirubin levels will not be enrolled in this study, i.e., mother Rh-negative, etc. Bilirubin will also be performed at entry and during the study. To minimise any potential risk, we will first study a single dose of the DTG-DT in neonates older than 2 weeks of life, followed by a single dose in neonates less than 2 weeks of life. Only once safety

criteria are met in Stage 1 (Cohort 1A and Cohort 1B), will we proceed to enrol participants in Stage 2 (Cohort 2) where we will be administering multiple DTG doses.

The 'PETITE' clinical trial platform at FAMCRU, SU has a proven track record to identify and enroll the required study participants rapidly within the specified timelines. While the COVID-19 pandemic in South Africa remains, the study team has been able to maintain the original timelines while adapting to meet government guidance. FAMCRU has a well-established standard operating procedure in place for managing COVID-19 risks. We do not foresee any changes related to COVID-19 impacting on timelines for the PETITE-DTG study. Risks associated with access to study product have been allayed with the generic manufacture (Viatris Ltd) already confirming their support

13.7. Blood Draw Limitations

The maximum amount of blood that may be drawn from a neonate for clinical and research purposes in one blood draw shall not exceed 5, 6 or 8 mL for a 2, 3 or 4 kg neonate, respectively (2.5% of the total blood volume), neither will the blood drawn in a 30-day period exceed 10, 12 or 16 mL for a 2, 3 or 4 kg neonate. This is in alignment with the SU HREC guideline for paediatric blood volumes for research purposes (57). Based on studies performed by our institution, the blood volumes required for this study have been feasible and acceptable.

Bloods drawn during the study period will be done for safety purposes, HIV diagnosis and PK assessments. There will be no long-term storage of any blood samples and no genetic testing will be performed.

13.8. Confidentiality

The study will enrol neonates born to women living with HIV. Every effort will be made to protect the confidentiality of the study participants. All site staff receive initial and ongoing training on human subject protection and confidentiality. Risk of social harm associated with participation in a study related to HIV will be explained to prospective enrollees during the consent process. To minimize this risk, study participants will not be identified by name on any study documents but will be identified by the provided patient identification number. All evaluation forms, laboratory specimens, reports and other records will be identified only by the patient identification number to maintain subject confidentiality. All records will be kept in a locked file cabinet. All computer entry and networking programs will be processed with patient identification number only. Clinical information will not be released without the written permission of the patient except when necessary for monitoring by the data centre or the Sponsor.

This study will comply with International General Data Protection Regulation (GDPR) and South African Protection of Personal Information Act 4 of 2013 (POPIA) principles. Collecting or accessing personal information for research: guidelines and resources Stellenbosch University is committed to protecting the privacy of our employees, collaborators, and research participants in line with POPIA as well as related South African legislation, global best practice, and our commitment to sound institutional governance. To help achieve this goal, SU has established a data privacy regulation to articulate the institutional stance on privacy and to clarify POPIA's principles within Stellenbosch University's institutional context and values (www.sun.ac.za/privacy).

13.9. Participant Reimbursement

Participants will not receive any payment for trial participation. Any medication that is required during the trial period will be provided free of charge to the parent(s)/legal guardian of the neonate. However, the parents/legal guardians would be compensated for expenses and loss of earnings directly related to participation in the trial. Parents/legal guardians will receive ZAR350 (which includes the cost for one meal) for each standard study visit (< 3 hours) and ZAR450 for each long PK visit (> 3 hours). In addition, long PK visits will include 3 meals OR an extra ZAR150 (ZAR50 per meal). FAMCRU has a fleet of vehicles which are used to transport participants (mother and baby) between their homes/local clinic and the clinical trial unit for all study visits, at no cost to the participant. All the aforementioned amounts are above the compensation recommendations by SAHPRA (ZAR300 per visit which *includes* money required for transport). Reimbursement at study visits will follow the guidance for '*Clinical Trial Participant Time, Inconvenience and Expense (TIE) Compensation Model*' by SAHPRA and the local ethics committees, considering time of visits, inconvenience of study procedures, and transport expenses.

13.10. Indemnity and Participant Compensation for Study-Related Activities

The study site 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. Immediate necessary care is available free of charge in the case of medical problems related to participation in this study and these costs will be covered through clinical trial insurance. Participants will be insured through clinical trial insurance, taken out by Stellenbosch University via Marsh Insurance Brokers. This insurance follows the Association of the British Pharmaceutical Industry (2014 ABPI) compensation guidelines for research related injury. These are regarded as the international gold standard. The insurance also follows Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa (SA GCP 2006). However, the study is not responsible for treatments unrelated to the study.

13.11. Participant's Withdrawal from the Study

If a participant withdraws from the study, the reason must be noted on the CRF. If a participant is withdrawn from the study because of a serious adverse event, thorough efforts should be made to clearly document the outcome.

A participant should be withdrawn from the trial if, in the opinion of the investigator, it is medically necessary and in the best interests of the participant, or if it is the wish of the participant/caregiver. If a participant does not return for a scheduled visit, every effort should be made to contact the participant.

If the participant withdraws consent, no further evaluations should be performed and no attempts should be made to collect additional data, with the exception of safety data, which should be collected if possible. Data collected prior to withdrawal will be included in study analysis.

13.12. Study Discontinuation

The study may be discontinued at any time by the ethics committee or other governmental or national regulatory agencies as part of their duties to ensure that research participants are protected.

13.13. Medical Care After Trial End

Following completion of the study, participants will return to their standard of care HIV prophylaxis as per local guidelines (1). Any unresolved adverse events related to study participation will be followed until resolution or the event is deemed chronic or stable and costs will be covered through clinical trial insurance. Participants will be insured through clinical trial insurance, taken out by Stellenbosch University via Marsh Insurance Brokers (Section 13.10). Evidence generated from this study will support the implementation of paediatric DTG formulations in South Africa and beyond.

14. Oversight and Trial Committees

14.1. Role of Study Sponsor

The Sponsor of the trial is Stellenbosch University. The management of the trial is delegated by the Sponsor to FAMCRU at Stellenbosch University, South Africa in collaboration with the Faculty of Associated Medical Sciences at Chiang Mai University in Thailand (AMS-CMU). FAMCRU is a clinical trial unit and academic research centre in the Department of Paediatrics and Child Health, Faculty of Medicine and Health Sciences, Stellenbosch University, located within the associated Tygerberg Academic Hospital complex, Cape Town, South Africa. FAMCRU will undertake the duties of the trial Sponsor and be responsible for the immediate management of SAEs and for the safety reporting to the research ethics committees, as appropriate. All SAEs will be reviewed by the Serious Adverse Event Evaluation Group (Section 14.2). An independent DSMB (see section 14.3) will review the safety and PK targets for the PETITE-DTG study and decide about study termination or continuation. Committees involved with the oversight of the trial are detailed in the following sections.

14.2. Serious Adverse Event Evaluation Group

A Serious Adverse Event Evaluation Group will be established to help monitor and standardize all SAE evaluations. The SAE Evaluation Group will be composed of key members from the study team. During study accrual, the SAE Evaluation Group will meet regularly to discuss all SAEs and determine causal relationship to study drug. At least 3 team members should participate in the meeting for quorum to be obtained. The SAE Evaluation Group will monitor the incidence and severity of all SAEs, and if appropriate, notify the DSMB of any serious or unexpected toxicity. If study safety hold criteria are met (Section 11), study enrolment will be paused, and the Serious Adverse Event Evaluation Group will meet to review any safety concerns, assign relationship to study drug, and notify the independent DSMB. The DSMB will then make recommendations about study termination or continuation.

14.3. Data Safety Monitoring Board

The study will be conducted under the supervision of an independent Data Safety Monitoring Board (DSMB). The DSMB will monitor safety and PK data, with the aim of protecting the

safety and interests of the trial participants and the scientific integrity of the research. The DSMB will review all safety data and specifically the SAEs that are reported during the course of the clinical trial. The DSMB will be composed of at least 3 experts in paediatric infectious diseases and/or pharmacology that will be independent of the Sponsor and the study team. All DSMB members must be free from any direct involvement with the trial. Any competing interests, both real and potential, must be declared. The DSMB will meet via teleconference at regular intervals determined by accrual rates and SAEs reported. At a minimum, the DSMB will convene prior to Cohort 2 enrollment, halfway through Cohort 2 enrollment, and if any study safety hold criteria (Section 11) are met. Details of the DSMB membership, meeting schedule and data review and analysis will be documented in the DSMB Charter.

15. Dissemination Plan

The investigators and organizations involved in this study have extensive experience in communicating and disseminating the results of their research, through multiple platforms, to the widest possible and most relevant audiences to maximize impact. Dissemination of relevant study results will occur at local, national and global levels. Dissemination activities will be targeted to engage the following stakeholders:

Local level:

- Study facility level (clinics and hospital where the trial is being conducted)
- Local health authorities (City Health and the Provincial Departments of Health)
- Study communities and families, including Community Advisory Boards

National level:

- South African National Department of Health
- South African HIV Clinicians Society (SAHCS)

Global level:

- Policy makers, including the World Health Organization (WHO, GAPf)
- The scientific research community (conference presentations, peer-reviewed publications)
- International advocacy groups (e.g., Treatment Action Group)

16. Scientific Communication

This study will be registered with a recognized clinical trial registry, e.g., the Pan African Clinical Trials Registry (PACTR). Before publication, all study results are considered confidential and shall not be made available to any third party by any member of the investigating team without an appropriate confidentiality agreement and/or written authorization of the sponsor. It is anticipated that the results of this trial will be of sufficient medical importance to warrant publication(s) in an international peer-reviewed journal, and/or presentations at scientific meetings. Authorship will be determined by mutual agreement.

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18. APPENDICES

18.1. Protocol Specific Laboratory Reference Ranges (45)

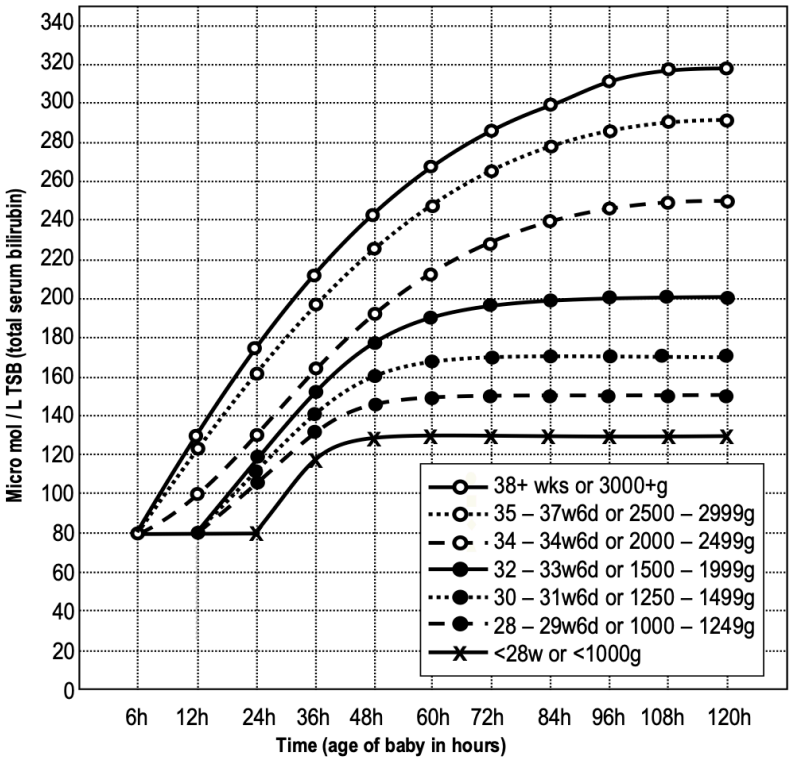
Creatinine (μmol/L)	**2 days of age**	**7 days of age**	**14 days of age**	**21 days of age**	**28 days of age**
Term Neonates	37 – 113	14 – 86	18 – 58	15 – 55	12 – 48

AST (U/L)	**1 – 7 days of age**	**8 – 30 days of age**
Term Neonates	24 - 100	20 - 72

ALT (U/L)	**1 – 30 days of age**
Term Neonates	6 - 40

18.2. Management of Hyperbilirubinemia: Western Cape Consensus Guidelines (47)

To start **phototherapy** when the total bilirubin value is at the threshold or above the line according to gestation or weight



To perform an **exchange transfusion** if the total bilirubin value is not expected to be below the threshold after 6 hours of intensive phototherapy