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The feasibility of retention of subsequent pregnancies in clinical trials:

The incidence of subsequent pregnancies in women enrolled in the DolPHIN–2 studies.

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Dissertation submitted to the University of Cape Town in partial fulfilment of

the requirements for the MASTER OF PUBLIC HEALTH degree

in the

School of Public Health and Family Medicine

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February 2024

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A. Preamble

1. Declaration

I, Amanda Adelheid Marshall (BRCAMA002), hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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1. Acknowledgements

To my supervisor, Dr Thokozile Malaba, thank you for your unwavering support, guidance, and time investment in my journey as a researcher. Your patience and willingness to guide me post long flights or even while on vacation is an illustration of what determination and work ethic manifests as.

To Mr Jim Read who helped compile my data frame from over 24 source entry forms and emails with wordy requests.

To my husband, Michael Marshall, who took on extra bedtime routines, and extra bath times and who fed me throughout the last three months until dissertation submission. Without you, this would not have been possible.

To Zac Marshall, who at a tender 2 months old, gave me the courage and apply, the determination to succeed and the inspiration to endure. I did this for you, my baby.

To my parents, Dorothy, and Alex Brackmann, who continue to be my biggest cheerleaders, who always demonstrate their pride and are unashamed to display love. You gave me the gumption to try.

To my in-laws, Louise, and Alan Marshall, who baby sat for endless nights, looked after Zac when sick and allowed me some weekends to complete assignments, I'd be lost without your support.

To my study group, Tarien Conradie, Zeena Morar and Jess Davies who just got me and supported me throughout the MPH journey.

And last but not least to Jehovah, who strengthened me through hospitalizations, buying of houses, starting of businesses and raising an incredible child through the most tiresome pandemic.

2. List of Abbreviations:

3TC	Lamivudine
APGAR	Appearance, pulse, grimace, activity, and respiration
ART	Antiretroviral treatment
CRF	Case report forms
DoLPHIN2	Dolutegravir in Pregnant HIV mothers and Neonates
DTG	Dolutegravir
EFV	Efavirenz
FDA	US Food and Drug Administration
FDC	Fixed dose combination
FTC	Emtricitabine
HCG	Human chorionic gonadotrophin
HS	HAART Standard
IMPAACT	International Maternal Paediatric, Adolescent AIDS Clinical Trials Network
IQR	Interquartile range
ITT	Intention to treat
LMIC	Low- or middle-income countries
MTCT	Mother-to-child transmission
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitors
NTD	Neural tube defect
OHRP	Office for Human Research Protection
PMTCT	Prevention of Mother-to-child transmission
PMTCT	Prevention of mother-to-child transmission
PROMISE	Promoting Maternal and Infant Survival Everywhere
SoC	Standard of care
TDF	Tenofovir
UCT-HREC	University of Cape Town Human Research Ethics Committee
VESTED	Virologic Efficacy and Safety of ART, Combinations with TAF/TDF, EFV, and DTG
WHO	World Health Organization
WLHIV	Women living with HIV
ZDV	Zidovudine

B. Manuscript

3. Abstract

Background

Inadequate safety and efficacy data for medications during pregnancy, particularly for antiretroviral therapy (ART) regimens, stems from the exclusion of pregnant and postpartum women from clinical trials. Despite global efforts, challenges persist in enrolling this population. In the context of antiretroviral therapy, (ART) the shift from efavirenz (EFV) to dolutegravir (DTG) as the World Health Organization-recommended (first-line regimen) occurred with limited pregnancy data. In some clinical trials, becoming pregnant resulted from withdrawal from the trial, emphasizing the ongoing challenges in including pregnant women in trials. Furthermore, longer term follow-up beyond the duration of trials has been lacking. The objective of this secondary analysis is to provide an interim pathway, of retaining women who subsequently become pregnant and obtaining comprehensive pregnancy data, allowing pregnant women to continue participating in clinical trials. However, evidence on the incidence of subsequent pregnancies among women in such trials is limited and thus we aim to describe it...

Methodology

This secondary analysis included data from the 250 women (South Africa n=114, Uganda n=135) who met the intention to treat (ITT) criteria in the DolPHIN-2 study and were actively followed up over time. This study focuses on describing the incidence of subsequent pregnancies in a cohort of women enrolled in the DoLPHIN-2 randomized trial and its observational extension study D2 TRIO. The aim of this trial was to explore the safety and efficacy of DTG in pregnant women aged ≥18 years of age, presenting late (≥28 weeks gestational age) for antenatal care. Women were randomised 1:1 to receive DTG or EFV. Data from South Africa and Uganda collected between January 22, 2018, and September 5, 2023, were analysed descriptively.

Results

This secondary analysis included data from the 250 women (South Africa n=114, Uganda n=135) who met the intention to treat (ITT) criteria in the DolPHIN-2 study and were actively followed up over time. Within this cohort of women, 21% (n=53) experienced at least one subsequent pregnancy, with 7% (n=18) during the clinical trial and 14% (n=34) during the observational extension study. A higher proportion of subsequent pregnancies occurred in the EFV arm (57%, n=30) and at the Uganda site (58%, n=31) compared to the South African site (42 %, n=22). Characteristics of women with subsequent pregnancies revealed a majority being multigravida (87%, n=46), unmarried (90%, n=40), with a high school education (70%, n=33), and unemployed

(57%, n=25). Among women with outcome data, 66% (n=23) had a live birth, 11% (n=4) had a termination of pregnancy, and most infants had a normal birth weight (90%, n=19).

Conclusion

This secondary analysis demonstrated that a high proportion of women had a subsequent pregnancy during the study period. There are calls for pregnant women to be involved in more safety and efficacy medications studies to broaden medication availability to this population. In the interim, instead of excluding women who because subsequently pregnant from clinical trials we recommend that they are retained. This study demonstrates that there may be sufficient numbers who have subsequent pregnancies, who typically would have been excluded, who can shed light on valuable early pregnancy information we would not otherwise have access too.

Keywords: Subsequent pregnancy, EFV, HIV, DTG, safety, enrolment, retain.

4. Background

Pregnancy is a normal physiological process however it is often associated with pathological processes that necessitate the use of medications. Given the unique and complex physiological state of pregnancy, special attention is necessary for medication use, considering the various pharmacokinetic and physiological changes experienced by pregnant women(1).

Although a substantial number of pregnant women are on medication, there is limited data on the safety and efficacy of these therapies during pregnancy. Nevertheless, ethical considerations often limit the inclusion of women in adequate numbers in randomised controlled trials during drug development, citing concerns for both mother and the developing foetus (1, 2). Consequently, medication prescriptions are based primarily on data from non-pregnant or lactating adults so the implications of their use in pregnancy are unknown (3). Of particular concern is that >80% of pregnant women receive medications not studied in pregnancy (4).

An important type of medication used during pregnancy is antiretroviral therapy (ART). This intervention represents an important achievement in response to the HIV epidemic, specifically in reducing mother-to-child transmission (MTCT) and improving maternal and infant health. Approximately 1.5 million women living with HIV (WLHIV) become pregnant worldwide yearly, with the majority residing in low- or middle-income countries (LMIC)(5). In 2019, WHO recommended a transition from the previously first-line efavirenz (EFV) -based regimens to dolutegravir (DTG)-based regimens (6). DTG has been shown to be advantageous over EFV, including fewer side effects, fewer medication interactions (7) and a higher genetic barrier to resistance (8).

Despite the ongoing global efforts to enrol pregnant women in clinical trials, progress is slow, so *retaining* women who become pregnant during a study could be a solution to obtaining valuable data on pregnancy. Understanding the proportion of women who conceive during their participation in clinical trials would be valuable in advancing efforts to include and retain pregnant women in research and obtain pregnancy safety data during the early stages of medication development, instead of excluding them. Although this is recommended there is little evidence on the incidence of subsequent pregnancies and the feasibility of this strategy. The cohort of WLHIV in our study, who were exposed to DTG and enrolled in trials while becoming pregnant, presents a unique opportunity to describe the incidence of subsequent pregnancies within a clinical trial context. The aim of this study is to describe the incidence of subsequent pregnancies in women enrolled in the DolPHIN-2 clinical trial and the D2 TRIO extension observational study. The specific objectives are (1) To describe the clinical and demographic characteristics of women enrolled In the DolPHIN-2 studies who had a subsequent pregnancy following the index pregnancy. (2) To describe the incidence of subsequent

pregnancies overall and through different study designs and (3) To describe outcomes of subsequent pregnancies.

5. Methods

This study is a secondary descriptive analysis of an open-label, multi-centre randomised controlled trial Dolutegravir in Pregnant HIV Mothers and their Neonates (DolPHIN-2) (https://clinicaltrials.gov/ct2/show/NCT03249181) and its observational study D2 TRIO (referred to collectively as the D2 studies). The DolPHIN-2 trial has been described in full elsewhere(9). Briefly, the aim of this trial was to explore the safety and efficacy of DTG in pregnant women aged ≥18 years of age, presenting late (≥28 weeks gestational age) for antenatal care. The rationale for the parent study including women presenting late for antenatal care (ANC) is that women may not access prenatal care early in pregnancy for various reasons, including socio-economic barriers, lack of awareness, or health system issues. Studying the outcomes for women presenting late in pregnancy provided valuable insights into the effectiveness of DTG in real-world scenarios where late presentation is common. Additionally, by enrolling women late in pregnancy, the study sought to determine the effectiveness of dolutegravir (DTG) in rapidly reducing viral load to minimise the risk of transmission during delivery. The parent study did not enrol any minors, given that the parent study was one of the first studies to investigate DTG in pregnancy the investigators of the D2 studies did not feel justified in including minors in this study as they are particularly vulnerable. Women were randomised 1:1 to receive DTG (50mg once daily) + 2 nucleoside reverse transcriptase inhibitors (NRTIs) or EFV + 2 NRTIs (SoC) Tenofovir (TDF) and Lamivudine (3TC) (this is in fact the standard of care (SoC) NRTI backbone in both Uganda and South Africa). Outcomes were assessed at delivery and at 72 weeks postpartum. The D2 TRIO study involved DolPHIN-2 women who were passively followed up in an observational manner to assess longer-term safety and efficacy of DTG up until 192 weeks postpartum. Study participants were recruited from two high HIV prevalence settings, South Africa, and Uganda. Ethical approval for the parent studies were obtained in South Africa (University of Cape Town Human Research Ethics Committee (UCT-HREC)), Uganda (Joint Clinical Research Centre Research Ethics Committee), and the United Kingdom (Central University Research Ethics Committee for Physical Interventions); with approval for this analysis also obtained from UCT-HREC. Written informed consent was obtained from all participants.

The analysis for this study draws on data collected In the D2 studies between 22nd of January 2018 and 05th of September 2023. During DolPHIN-2 study visit 1, enrolment occurred during the antenatal period where the randomised ART was offered on initial diagnosis as per National Health protocols in both countries, till laboratory tests confirmed clinical eligibility. Visit 2 occurred approx. 7 days later where clinical eligibility was either confirmed or the participant was withdrawn and placed on the National SoC regimen. Visit 3 occurred on day 28 (tolerance ± 14 days), visit 4 (if required) at 36 weeks gestation (tolerance ± 14 days), visit 5 typically

on delivery (tolerance for blood sampling + 14 days), visit 6 occurred at 6 weeks postpartum (tolerance ± 14 days), visit 7, took place at 12 weeks postpartum (tolerance ± 14 days), visit 8 – 10 took place every 24 weeks till 72 weeks postpartum which marked the end of the clinical trial. During the D2 TRIO observational study there were 5 study visits with approx. 24 weeks spacing between where the cohort were followed up for a total of 192 weeks which marked the end of the observational study. The study procedures (including counselling) were the same in Uganda and South Africa as per protocols of the DolPHIN-2 and D2 TRIO studies.

It should be noted that in this secondary analysis of subsequent pregnancies, not the index pregnancy, the timing of presentation to ANC was not known to this study as the participants had already been enrolled. This study did not have ethical approval for collection of data through antenatal care records. Consequently, data was only obtained through standardised questionnaires, laboratory results, case report forms, and anthropometric measures as part of the study.

For the clinical trial, before DTG safety was confirmed, as part of the protocol of parent study, women who fell pregnant were switched from DTG to EFV the current SoC. Therefore, there was a need to perform a pregnancy test at follow up visit. Once DTG was recommended as first line of medication by the WHO, there was no need to switch the DTG, as it was now SoC, therefore the study stopped performing pregnancy tests. Additionally, retaining these women with subsequent pregnancy did not impact on primary and secondary outcomes of the trial. During the observation extension D2 TRIO, there were long periods between study visits, women fell pregnant between these visits and some attended study visits clearly pregnant. At study visits pregnancy was identified through a clinical examination, urine sample at the clinic as part of DolPHIN-2, self-reported blood test, or confirmatory ultrasound. Of the pregnancy tests that were confirmed by clinical trial staff at study visit 'Orient Gene: Rapid Diagnostic tests for in vitro diagnostic use only, one step pregnancy test strip (urine), were used to confirm pregnancy. Product information on blood results, clinical examination notes and or ultrasound that confirmed pregnancy were reported to the study investigators via self-report only, as during the secondary analysis ethical approval was not granted for folder review. Women who underwent some form of pregnancy ascertainment, were those who reported having sexual partners or not being on contraception.

Women who became pregnant during follow-up, including women with >1 subsequent pregnancy, were retained in the D2 studies and the birth outcomes were recorded where possible. For this analysis a subsequent pregnancy was defined as any pregnancy that occurred after the index pregnancy (DolPHIN-2 enrolment), with inclusion of any subsequent pregnancy following the index pregnancy. Across both studies contraceptive advice was given. In DolPHIN-2, however women in the DTG arm were specifically counselled around the potential risk and benefit of conceiving while on the medication due to current concerns at the

time (preliminary results of the Tsepamo study and concerns related to neural tube defects (22) before WHO recommended DTG as first line ARV. That conversation in itself may have acted as a deterrent to conceive while on the medication. Additionally, certain contraceptive forms were offered only to women on the DTG arm such as contraceptive subdermal implant, combined oestrogen and progesterone oral contraceptive and percutaneous contraceptive patches due to their interactions with the EFV. Women on DTG who declined effective contraception were switched from DTG to SoC.

Given the passive nature of follow-up of subsequently pregnant women particularly during the D2 TRIO study, information on the birth outcomes (pregnancy loss, live birth, and stillbirth) was collected when possible and or available. The passive nature of data collection is only in reference to subsequent pregnancy data, as this was not an outcome of interest for the parent study. Therefore, if data was not available at study visits it was not actively pursued through folder reviews due to a lack of ethical approval. Birth outcomes were described, however not all outcomes were available as a number of women were still pregnant at the end of the study, however the outcomes that were available were described. Pregnancy losses were classified according to Induced abortion/ terminations (<28 weeks gestation), Stillborn/macerated stillbirths include late foetal deaths (weighing more than 1000g or >28 weeks gestation). Gestational age at pregnancy confirmation was calculated based on last normal menstrual period (LNMP), fundal height or ultrasound because few women had all three LNMP, and ultrasound dating completed. Gestation age at pregnancy confirmation was classified as first trimester (0-12w), second trimester (13-27w) or third trimester(28-40w). Additional birth-related outcomes of interest included infant sex, if there were complications, delivery mode classified as elective caesarean, emergency caesarean and normal vaginal birth, infant birth weight where normal (>2500g), low Birth weight (1500- 2500g) and very low birth weight (<1500g). Infant feeding was obtained via self-report from participants at study visits. Unfortunately, infant date of birth was not consistently available therefore gestational age at delivery was not calculated. As this study involved secondary analysis, we did not have ethical clearance to access the Road To Health Care (RTHC) or maternity case record booklets of the maternal participants. Additionally, due to the long follow-up periods between visits, many subsequent pregnancies were identified or known to the study team later in pregnancy. Consequently, gestational age data was not measured in many of the pregnancies. While the date of pregnancy outcome or the date of birth of the infant was known through self-report, the absence of gestational age measurements during pregnancy prevented us from determining the proportion of subsequent pregnancies that experienced outcomes dependent on gestational age, such as preterm delivery or size for gestational age.

6. Statistical methods

Descriptive statistics were used to summarise baseline socio-demographic and clinical characteristics of the women as well as infant outcomes. Continuous variables were summarised using the median and the interquartile range (IQR). Categorical variables were described using proportions and frequencies. All statistical analyses were conducted using R version 4.0.4 (2021-02-15) software developed in collaboration by the University of Auckland, New Zealand. All birth outcomes were calculated as a proportion of total subsequent pregnancies. Medians and interquartile ranges (IQR) were used to summarise the distribution of age, viral load, gravidity, and parity. To determine if there was a significant difference between women with subsequent pregnancy and those who were not pregnant, we conducted several statistical tests. For numerical variables, normality of the two independent samples was assessed using the Shapiro-Wilk test. If the p-value was not significant (>0.05) and the variances were dissimilar, we employed the Mann-Whitney U-test for non-parametric data and reported the corresponding p-values. Continuous data were summarized using medians and interquartile ranges and where this was available this was reported on rather than when described in categories. For categorical variables, we performed chi-square analysis, with p-values presented for these comparisons. Pregnancy outcomes for women who were still pregnant by the end of the study were defined as ‘not yet available’ and treated in the analysis as missing.

7. Results

8.1 Baseline Demographics

Of the 268 women randomised in the DolPHIN-2 clinical trial, 250 met the intention to treat (ITT) criteria and were actively followed up over time (and met the inclusion criteria following post-enrolment laboratory examinations i.e., were clinically eligible). A detailed description of study population groups (safety and ITT populations) can be found elsewhere (9).

Out of the 250 randomised, 66% (n=166) had reason to believe that they may have been pregnant in order to attain pregnancy status these women either took a pregnancy tests, had ultrasound, or underwent a clinical examination. The other 34% where either on long-acting contraceptives or reported having no sexual partners and therefore had no reason to suspect they were pregnant. Out of 250 women, the overall study incidence of subsequent pregnancy was 21% (n=53). Three women experienced more than one subsequent pregnancy during the course of both studies, therefore this study reports 53 women with subsequent pregnancies but later describe 56 birth outcomes to include the multiple subsequent pregnancies. Of the n=53 women with subsequent pregnancies reported, 34% (n=18) occurred during the DolPHIN-2 component, while 66% the (n=35) occurred during D2 TRIO observational extension component. When looking at the proportion of subsequent pregnancies compared to the entire participant population (n=250), 7% of subsequent pregnancies occurred during DolPHIN-2, whereas 18% of subsequent pregnancies occurred during the observational extension D2 TRIO. A higher proportion of women experiencing subsequent pregnancies were in the EFV arm (57%, n=30) and were from the Uganda (58%, n=31) site.

At the time of DolPHIN-2enrolment, the median age of women with a subsequent pregnancy was 28 years (interquartile range [IQR], 25-30) while those without a subsequent pregnancy median age was 28 (IQR, 26-32). The majority of women (subsequent pregnancy 43%, n=19, no subsequent pregnancy 36%, n=47) were aged between 25-29 years. The majority of women experiencing a subsequent pregnancy were multigravida (87%, n=46), not married (91%, n=40), had high school as their highest level of education (70%, n=33) and were unemployed (57%, n=25). Whereas women without a subsequent pregnancy were multigravida (89%, n=175), not married (83%, n=110), had high school as their highest level of education (67%, n=85) and were unemployed (64%, n=84). Pertaining to abortion, spontaneous abortion and/ or induced abortion, these rates were similar between women who reported a subsequent pregnancy (21%, n=11) and those not pregnant (20%, n=39). Based on supplementary table 1 (found in the appendix), women who did not suspect a pregnancy and were not pregnant, compared to those who suspected a pregnancy and were not pregnant, compared to those with a confirmed subsequent pregnancy displayed relatively similar characteristics such as age, marital status, level of education, gravidity, and contraceptive type. A notable difference was those

who did not suspect a pregnancy and were not pregnant had a higher proportion of unemployment (70%, n=32) compared to those who suspected a pregnancy but tested negative (60%, n=52) and those with a confirmed subsequent pregnancy (57%, n=25).

Significance testing using either the Mann-Whitney U-test or Chi-Squared test all yielded insignificant p-values. This was anticipated due to the small sample size where the study was not powered enough to yield significant results. However, we do feel the findings warrant careful consideration and shed light on some important data otherwise unavailable.

Women with subsequent pregnancies self-reported use of the following contraception following delivery of the index pregnancy: injectable progesterone (47%, n=34), implant (22%, n=16), barrier methods (8%, n=6), intrauterine devices (4%, n=3); while 12% (n=9) had reported that they were practicing abstinence. The parent study had excluded a total of 22 women from their analysis: 12 participants due to Loss to follow up, 1 participant gave birth early, 3 withdrew consent, 1 was receiving an ART regimen at enrolment and 5 did not attend the clinic. Therefore 103 participants of the 250 were followed for the full 72 weeks. Data from the D2 TRIO observational study is yet to produce data on their Loss to follow up numbers as analysis was not yet complete at the time of this study. Given that pregnancy data being unavailable was based on the timing of the pregnancy rather than any other characteristics, there were no differences between the women who we had data on compared to those who had not yet delivered, therefore there was no loss to follow up in this secondary analysis. Participant characteristics (sociodemographic and obstetric history) at the time of DolPHIN-2enrolment are summarised in Table 1.

INSERT TABLE 1

8.2 Birth outcomes

Table 2 describes the birth outcomes for the subsequent pregnancies with outcome data.

Out of the 56 pregnancies, 38% of the delivery outcome data was unavailable. The data on infant birth weight was 60% incomplete. Additionally, the data on infant sex was 59% incomplete, and the data on delivery mode was 70% incomplete. The data from participants exhibited patterns of missingness that were not confined to specific individuals but occurred sporadically across the entire sample. This was due to a combination of women who were still pregnant at the last study visit (n=8) and missing data not being actively pursued by study staff as subsequent pregnancy was not an outcome of interest. The majority of subsequent pregnancies were confirmed in their first trimester of pregnancy (51%, n=27) (p=0.03), with most identifying gestational

age at confirmation by calculating LNMP (58%, n=31) (p<0.05). Among the women with outcome data, 66% (n=23) had a live birth, while 11%(n=4) had a termination of pregnancy (p<0.05).

The median birth weight of the infants was 3200g (IQR2900-3500g), with the overwhelming majority of infants classified as having a normal birthweight (90%, n=19), with only 10% (n=2) classified as low birth weight (<2500g) (p<0.05). Majority of women gave birth via natural birth (82%, n=14) (p<0.05) and employed mixed feeding as their infant feeding strategy (96%, n=52) (p<0.05).

INSERT TABLE 2

8. Discussion

The D2 studies assessing the safety and efficacy of DTG in late-presenting women in South Africa and Uganda presented an opportunity to examine the incidence of subsequent pregnancy in a cohort enrolled in a clinical trial. Our data highlights the importance of retaining pregnant women engaged in clinical trials to gather pregnancy-related data. This is particularly important as 21% of women overall enrolled in the D2 studies experienced a subsequent pregnancy during the follow-up period. Retaining pregnant women in trials can offer valuable insights into early pregnancy and medication safety.

The unique design of the D2 studies allowed us to observe subsequent pregnancies across two distinct study designs, which had different procedures that could potentially impact participant behaviour regarding pregnancy intentions. The incidence of subsequent pregnancies in the DolPHIN-2component was 7% (n=250) compared to 14% within the D2 TRIO observational extension. DolPHIN-2 was primarily used when comparing its findings with other trials, because the trials described below were designed similarly around inclusion criteria and follow up visit spacing. The aim of the secondary analysis was to compare pregnant women who were under trial conditions and within the same time frame (approx.72 weeks), comparing the D2 TRIO observational component would have had a higher incidence purely because women were followed up for longer (an additional 120 weeks).

When comparing the DolPHIN-2 component findings to other clinical trials of a similar nature, such as the 'HAART Standard' component of the PROMISE study (PROMISE HS) clinical trial (10), which evaluated adverse pregnancy outcomes among women who conceive on ART (regimen was lopinavir/ritonavir plus fixed-dose combination emtricitabine (FTC)/ tenofovir disoproxil fumarate (TDF)[1]. The DolPHIN-2 study had a lower incidence of subsequent pregnancies compared to 17% of the women in PROMISE HS (10). As part of the

[1] Additional PROMISE HS study-supplied ART included lamivudine (3TC), zidovudine (ZDV), fixed-dose combination 3TC/ZDV, TDF, fixed-dose combination FTC/TDF/rilpivirine, atazanavir, raltegravir, and ritonavir) [(regimen was lopinavir/ritonavir plus fixed-dose combination emtricitabine (FTC)/ tenofovir disoproxil fumarate (TDF) (10)

VESTED trial (11), which was similar in design (evaluated DTG based regimens vs EFV based) found that only 3% of women had subsequent pregnancies. The lower incidences observed in the D2, and VESTED trials may be linked to the neural tube defect (NTD) concerns around DTG that emerged around the time these studies were following up women (6, 11, 12, 13). Although all trials warn about the potential risks of conceiving while on study drugs, we hypothesize that the circumstances surrounding DTG at the time was different because there was specific data coming out from the Tsepamo study that raised concerns globally (22). As a precaution, as part of study procedures women in the DTG arms were strongly counselled about the potential risks of conceiving while on DTG, and thus received thorough contraceptive guidance and recommendations. We hypothesise that these study procedures could have contributed to the lower incidences observed in these two studies. Additionally, linked to this, we observed differences in subsequent pregnancy incidence by regimen group with a higher proportion in the EFV arm (57%) compared to the DTG arm likely due to the aforementioned study procedures. Similarly, VESTED had more women on the EFV ARV regimen conceived compared to the DTG regimen (11, 12). The lower incidence in the VESTED compared to the DolPHIN-2 may be attributed to a difference in protocol, the VESTED study asked specific questions pertaining to fertility intention which may have impacted on behaviour of study participants and led to a lower incidence in the VESTED study (11). It is speculated that the 34% of women who did not take the pregnancy test had done so either because they were visibly pregnant when attending the study visit or reported having no sexual partners at the time of the visit.

The observational extension component had a higher proportion of the overall subsequent pregnancies seen in the D2 studies. By study design, an observational study is less prescriptive and allows for people to be observed in a more natural context. The majority of subsequent pregnancies occurred during the D2 TRIO observational study, which was expected, given the counselling with regard to contraceptive use, which may have lost its momentum following longitudinal data availability from the Tsepamo study (22). Visits were less frequent and widely spaced during the observation period. We hypothesise that counsel around contraception lessened during the observational study as researchers have less control over intervention outcomes and aim to study women in as natural capacity as possible. While the data in this study originated from the follow-up of individuals involved in a randomised trial, they could be perceived as more observational (13). The opportunity to observe participants over such a long follow up was extremely valuable, as other studies don't have such an extended follow up period as the D2 studies. For instance, the clinical trial arm, DolPHIN-2 study, was observed from index pregnancy to 72 weeks post-partum, during the observational extension D2 TRIO women were followed for up to 192 weeks, whereas traditionally clinical trials only follow pregnant women up until 72 weeks postpartum. Traditionally pregnancy medication trials end after 72 weeks, the additional time afforded by the observation study allowed for more women to have subsequent pregnancies allowing us a larger sample to analyse. Theoretically, it demonstrates the

importance of extending following up of clinical trials as it gives an opportunity for more data to be collected. There is then opportunity in designing studies around this to allow for additional information. The additional time allows these women of childbearing age to have additional pregnancies, which would allow studies to collect earlier safety information pertaining to peri-natal data, as typically studies enrol women in their second trimester of pregnancy. If the subsequent infants are also consented to the study this would allow us to get data related to early child development along with the index pregnancy information. However, with the subsequent pregnancies we would have data from conception. he differences in incidence of subsequent pregnancies between the clinical and observational design arms could be a result of either time and or protocol questionnaire content.

The pregnancy incidence rates observed in the study could be partly explained by the varying underlying reproductive rates across different countries and settings. The birth rate in South Africa in 2023 was 18,994 births per 1000 people (14) compared to 35,135 births per 1000 people in Uganda (15). As found in our study Uganda has a higher birth rate than South Africa. Additionally, it is observed that most of the women who had subsequent pregnancies were on some form of contraceptive, this could be a result of either method failure or social desirability where women are inaccurately reporting contraceptive use. These subsequent pregnancies represent combination of factors (1) Women who intended to become pregnant- these women may have reported contraceptive use (despite the lack of its use) due social desirability, given the active counselling on contraceptive use by trial investigators– they didn't want to disappoint trial staff (2) Among those who did not want to become pregnant, and became pregnant unintentionally, there may be some evidence of contraceptive failure. The DolPHIN-2 protocol had certain contraceptive methods that the investigators had concerns re interactions between hormonal contraceptives and EFV, these contraceptives were only offered to women on the DTG arm. Our secondary analysis noted that injectable progesterone and implants were the most common contraception used despite this, women fell pregnant while on some form of contraceptive. Hormonal contraception, especially injectable or implant forms, is routinely provided after infant delivery (and often during the third stage of labour in South Africa) as part of a comprehensive public health and family planning strategy. Studies have found implant failures among women taking EFV based ART (30). Other evidence has also found injectable contraceptives have reduced due to hormonal interactions with ART, especially EFV (31). This may be the reason why our findings demonstrate a higher proportion of women on the EFV arm having subsequent pregnancies. Women who had subsequent pregnancies demonstrated similar attributes. Within the D2 studies women between the ages of 25-29 years had more subsequent pregnancies. It is unsurprising to find that women between these had more subsequent pregnancies as this is the life stage at which most women are of childbearing age and is consistent with other literature (15). Pregnancy intention in the DolPHIN-2 was, not directly asked but given the life stage of the participants it is to be expected. Women of this age group also tend to have shorter spacing between

children. This is however important to bear in mind when considering participant characteristics when enrolling into a clinical trial. When enrolling women into clinical trials where we aim to glean as much information on safety in pregnancy, this age bracket would be ideal, as women are likely to have more children, as some participants had up to three children during the course of the D2 studies. This is also in-line with public health outcomes were studies found that women with subsequent pregnancies who were diagnosed with HIV in their index pregnancy showed better health outcomes with subsequent pregnancy (10, 11). Literature also supports the notion that there is an increase in pregnancy incidence while pregnant as public health strategies of PMTCT improves (10, 15). With participants index pregnancy babies being older and with more time to space the infants, participants were in a position to have a subsequent pregnancy. However, there could also be an alternative reason behind the difference, during the clinical trial after the index pregnancy as well as after every study visit during the 72 weeks women were reminded about contraception, as per the protocol. The leading questions on contraceptive use that was more strictly adhered to in the clinical trial may have indirectly impacted on women's behaviour(16). During the clinical trial researchers were actively asking women about contraceptive use, which would provide conscious or unconscious pressure to use contraceptives, but despite being asked there were still women falling pregnant during the clinical trial. The women enrolled in this trial were of traditional childbearing age and thus are likely to have had pregnancy intent leading to subsequent pregnancies. Whereas during the observational study, the incidence of subsequent pregnancies increased, either because more time passed allowing for the likelihood of additional pregnancies or because there was no active surveillance on contraceptive use. Suggesting that, by removing questions on contraception use or contraception prompts from questionnaires and protocols, could increase the natural incidence of these pregnancies. Questionnaires that probe pregnancy intention could also allow us to follow women who intend on having a subsequent pregnancy, this will be more cost-effective as we follow a smaller cohort who are more likely to have the outcome of interest.

Outcomes of subsequent pregnancies in various trials revealed noteworthy differences, reflecting distinct patterns in live births and terminations, shedding light on the varied reproductive outcomes observed across these studies. D2 studies' birth outcomes had a similar alignment to other studies. 66% of the infants that had outcome data in the D2 studies had live births which was similar to findings from VESTED which had (60%) but was less than what was found in PROMISE (75%) (10, 12). D2 studies had a similar distribution of terminations at (11%) with PROMISE HS at (15%) and VESTED at (5%) (10, 12). There were no reported stillbirths or miscarriages, however, given that VESTED found a small proportion of women who experienced these outcomes suggests that the D2 findings are likely due to the fact that women experiencing these outcomes, particularly miscarriages, did not inform the study of their pregnancy and its outcome (11). This is likely an artifact of the study design where surveillance of subsequent pregnancies was passive with no active processes to detect subsequent pregnancies. Therefore, it is likely that D2 subsequent pregnancies are an

underestimate of the true incidence, as we lacked access to all data, a woman may have had a miscarriage before we could discover she was pregnant. A factor that contributed to lower outcome ascertainment was that some women were still pregnant at study termination (23%, n=8). Additionally, unsurprisingly the length of the follow up period meant there was some inevitable loss to follow-up as some participants who had undesired outcomes may not have returned to the study. Moreover, descriptions pertaining to terminations created challenges, especially in Uganda, due to legal implications. In respect of cultural sensitivities, the research team did not demand differentiation on the nature of the recorded abortions. Which may have led to an underreporting of abortions or terminations. Within the D2 studies, subsequent pregnancy was not an outcome of interest and therefore data on subsequent pregnancy and infant outcomes were not actively pursued, but rather passively recorded leaving large gaps in the data, data that could have provided a rich understanding of pregnancy safety information. An exploration of the characteristics of women within the context of enrolment in a clinical trial who experienced a subsequent pregnancy is of value for potentially informing future study designs.

Additional characteristics of the women who had subsequent pregnancies include more unmarried women than married women who became subsequently pregnant. Regarding marital status, a study found that women who are married are less likely to experience unmet needs for family planning compared to women who have never been married (17). Unmarried women are likely met with less resistance from husbands or partners and may be less exposed to family planning as a result (17). The D2 studies demonstrated that more unemployed women had subsequent pregnancies. Based on this data it is hypothesized that women who were working incurred time restraints and simply didn't have the time to have additional children as this may impact their future employment potential. Notably however women who did not suspect a pregnancy and were not pregnant had a higher proportion of unemployment (70%) perhaps suggesting that socio-economic reasons may have impacted decisions around pregnancy intention. Usually, a rise in unemployment leads to a decline in fertility rate (18), however, evidence in the D2 studies shows the reverse, where more unemployed women, who had a high school level of education, had subsequent pregnancies. It is likely women who are employed, or in tertiary-level education would prefer to space their children more and thus not have as many children within the short space of time observed in this study(17).

Adapting future clinical trial protocols to actively retain subsequent pregnancies as an outcome of interest is an important strategy that will improve the quantity and quality of data being collected about these pregnancies. By deeming subsequent pregnancy an outcome of interest, this would allow for more active and thorough data collection, possibly through a sub-study, with subsequent cohorts, designed around subsequent pregnancies. A subsequent cohort could allow for long-term follow-up, infant follow up as well as possible comparisons, which could provide a solution to obtaining early and meaningful safety information

on medication impact during preconception, providing insights otherwise inaccessible. This was evident in the PROMISE HS trial (10), one of the first of its kind studies, where women who became subsequently pregnant were actively retained and safety data derived on medication use. Theron et al (19) provided an opportunity to examine the impact of ART at conception on pregnancy outcomes with subsequent pregnancies by demonstrating what can be achieved as a result of active data collection, where there was a very deliberate and intentional effort to collect data. This additional data allowed them to calculate time to event analysis as well as allow for comparison between index pregnancies and subsequent pregnancies to rule out clustering of adverse incidents. These types of studies traditionally end after 72 weeks. This gives us an opportunity to glean more safety data for the parent study and in this study to see what the incidence of subsequent pregnancies could be given additional time. There is then opportunity in designing studies around this to allow for additional information.

When considering what the benefits could be with regard to research, the needs of the pregnant women need to be considered. A study that looked at factors influencing clinical drug trial participation by pregnant women and their spouses found that 95% of pregnant women thought that there was a need for the development of drugs during pregnancy (20). The study found that the usage of untested or sub-therapeutic drug regimens in clinical practice paradoxically increases the risk for foetuses (20). When recruiting pregnant volunteers for clinical drug trials, researchers should conduct in-depth consultations and comprehensively inform the pregnant women and their families of the pros and cons of their involvement (20).

The D2 studies strengths lay in the fact that there was access to longer-term data allowing us to follow women through time, most studies have time frames limiting to up till 72 weeks post-partum, whereas the D2 studies allowed us the opportunity to follow women for 192 weeks, this additional time provided enabled us to analyse additional subsequent pregnancies in the observational cohort. However, a limitation is that the study did not now allow us to use this additional time to gain further understandings of time to event analysis or associations, as subsequent pregnancy was not an outcome of interest. Although we had a small sample size, information derived from this analysis has been useful in providing a broad overview of characteristics of the women who could potentially enrol or be retained in future clinical trials, as women with these characteristics are likely to have subsequent pregnancies. However, the small sample size led to the study not being powered enough to detect differences in birth outcomes. The main limitation of this analysis is that because subsequent pregnancies and their outcomes were not outcomes of interest in the D2 studies the data for these were passively (treated as nice to have information) collected and not active (treated as an outcome of interest where data is strictly recorded). Additionally, some women were still pregnant at the end of the study leading to additional missing information. Linked to this some important information from the subsequent pregnancy such as infant HIV test information, as well as gestational age at delivery was not

available. This missing information affects our findings by limiting the amount of birth outcome data we can analyse and present. The lack of data on date of birth meant we were not able to determine gestational age-based outcomes which could have been of interest given these women were exposed to DTG earlier in pregnancy. Missing data on birthweight may have given us information regarding any associations between early exposure to DTG and infant health. Subsequent infants were not consented into the study and therefore data on viral load, mother to child transmission and HIV status was not known to the study which limited critical birth outcome information. Another limitation is that demographic information of these women at the time of conception was not available thus in this analysis only enrolment demographic information could be provided. Although long-term follow-up is considered a strength of this analysis the passive approach and time frame distance between some study visits meant that some pregnancies were only detected by the study well into the pregnancy. Given some of the cultural sensitivities outlined above there are likely to be quite a few pregnancies that were not detected in women who were lost to follow-up. The inclusion and exclusion criteria of the parent studies may have introduced some bias due to selection of study participants into the results of this study as, the exclusion of younger participants and women who presented early may have impacted the findings. By excluding younger participants, we have reported fewer people who could have had subsequent pregnancies due to their age, especially after considering the benefits of longer term follow up and the higher frequency of additional subsequent pregnancies. Due to their age, these women may have a higher pregnancy intension and thus dilute the incidence of subsequent pregnancy. By only including older women there may be an overrepresentation of factors related to subsequent pregnancy in this secondary analysis. Additionally, by excluding early pregnancy presenting women we lack additional insights on the factors leading to subsequent pregnancy as these women may differ from late presenting women in terms of health seeking behaviour and risk of adverse pregnancy outcomes.

9. CONCLUSION

The D2 studies demonstrated that one in five women had a subsequent pregnancy. This outlines the value of retaining women who fall pregnant during clinical trials as a way of collecting medication safety and efficacy data (10, 13, 15). It also demonstrates the value of following up with women over a long term. This approach for obtaining pregnancy-related data will require adapting future clinical trial protocols to actively include subsequent pregnancies as an outcome of interest, offering an interim solution to the complexities of enrolment. This methodology could provide insights otherwise inaccessible, contributing to improved healthcare for pregnant and postpartum women.

10. List of abbreviations:

3TC	Lamivudine
APGAR	Appearance, pulse, grimace, activity, and respiration
ART	Antiretroviral treatment
CRF	Case report forms
DoLPHIN2	Dolutegravir in Pregnant HIV mothers and Neonates
DTG	Dolutegravir
EFV	Efavirenz
FDA	US Food and Drug Administration
FDC	Fixed dose combination
FTC	Emtricitabine
HCG	Human chorionic gonadotrophin
HS	HAART Standard
IMPAACT	International Maternal Paediatric, Adolescent AIDS Clinical Trials Network
IQR	Interquartile range
ITT	Intention to treat
LMIC	Low- or middle-income countries
MTCT	Mother-to-child transmission
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitors
NTD	Neural tube defect
OHRP	Office for Human Research Protection
PMTCT	Prevention of Mother-to-child transmission
PMTCT	Prevention of mother-to-child transmission
PROMISE	Promoting Maternal and Infant Survival Everywhere
SoC	Standard of care
TDF	Tenofovir
UCT-HREC	University of Cape Town Human Research Ethics Committee
VESTED	Virologic Efficacy and Safety of ART, Combinations with TAF/TDF, EFV, and DTG
WHO	World Health Organization
WLHIV	Women living with HIV
ZDV	Zidovudine

11. Declarations

- Ethical approval and consent to participate: Ethical approval was granted for this analysis by the Human Research Ethics Committee at the University of Cape Town (UCT HREC REF: 707/2023) and for the parent study from were obtained in South Africa (University of Cape Town Human Research Ethics Committee (UCT-HREC)), Uganda (Joint Clinical Research Centre Research Ethics Committee), and the United Kingdom (Central University Research Ethics Committee for Physical Interventions).

- Availability of data and materials. The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

- Competing interests. The authors declare that they have no competing interests.

- Funding. None

- Author contributions: Data management and database collation was conducted by Read J, Marshall AA performed the data analysis and writing and Malaba TR, provided editing, literature review suggestions and article cohesion, Waitt C, provided concept conception. All authors read and approved the final manuscript.
- Acknowledgements: To the D2 team for trusting me with this valuable information. To Dr T Malaba for her contribution and guidance and Mr J Read for his assistance with data management.

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13. References

1. Sheffield JS, Siegel D, Mirochnick M, Heine RP, Nguyen C, Bergman KL, et al. Designing drug trials: considerations for pregnant women. Clin Infect Dis. 2014;59 (7 Suppl):S437-44.
2. Theron G, Fairlie L, Pinilla M, McCarthy K, Owor M, Chinula M, et al. Pregnancy Outcomes of Women Conceiving on Antiretroviral Therapy (ART) Compared to Those Commenced on ART During Pregnancy. Clin Infect Dis. 2021;73:312-20.
3. Univesity of Birmingham. Safe and effective medicines for use in pregnancy: a call to action [Pregnancy and Maternal Health]. Edgbaston, Birmingham, B15 2TT, United Kingdom.2021.[cited 2021 January]. Available from: https:// www. birmi ngham. ac. uk/ docum ents/ colle ge- mds/ centr es/ bctu/ 21560policy- commi ssion- mater nal- health- report. Pdf.
4. David AL, Ahmadzia H, Ashcroft R, Bucci-Rechtweg C, Spencer RN, Thornton S. Improving Development of Drug Treatments for Pregnant Women and the Fetus. Ther Innov Regul Sci. 2022;56(6):976-90.
5. Waitt C, Orrell C, Walimbwa S, Singh Y, Kintu K, Simmons B, et al. Safety and pharmacokinetics of dolutegravir in pregnant mothers with HIV infection and their neonates: A randomised trial (DolPHIN-1 study). PLoS Med. 2019;16(9):e1002895.
6. World Health Organisation. Update of recommendations on first and second-line antiretroviral regimens 2019. [cited 2019 July]. Available from: https://iris.who.int/bitstream/handle/10665/325892/WHO-CDS-HIV-19.15-eng.pdf?sequence=1
7. Cottrell ML, Hadzic T, Kashuba AD. Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir. Clin Pharmacokinet. 2013;52(11):981-94.
8. Llibre JM, García F, García Deltoro M, Blanco JL and Delgado R. Genetic Barrier to Resistance for Dolutegravir. AIDS Rev. 2015;17:59-68.
9. Kintu K, Malaba TR, Nakibuka J, Papamichael C, Colbers A, Byrne K, et al. Dolutegravir versus efavirenz in women starting HIV therapy in late pregnancy (DolPHIN-2): an open-label, randomised controlled trial. Lancet HIV. 2020;7(5):e332-e9.
10. Hoffman RM, Brummel SS, Britto P, Pilotto JH, Masheto G, Aurpibul L, et al. Adverse Pregnancy Outcomes Among Women Who Conceive on Antiretroviral Therapy. Clin Infect Dis. 2018;68(2):273-9.
11. Lockman S, Brummel SS, Ziemba L, Stranix-Chibanda L, McCarthy K, Coletti A, et al. Efficacy and safety of dolutegravir with emtricitabine and tenofovir alafenamide fumarate or tenofovir disoproxil fumarate, and efavirenz, emtricitabine, and tenofovir disoproxil fumarate HIV antiretroviral therapy regimens started in pregnancy (IMPAACT 2010/VESTED): a multicentre, open-label, randomised, controlled, phase 3 trial. Lancet. 2021;397(10281):1276-92.
12. Fairlie L, Ziemba L, Coletti A, Chinula L, Shapiro R, Stringer J, et al. Adverse Outcomes in Subsequent Pregnancies in the IMPAACT 2010 Trial. 2022. Conference on Retroviruses and Opportunistic Infections (CROI)2022.[cited 2022 February 12-16]. Available from: https://www.impaactnetwork.org/sites/default/files/inline-files/IMPAACT2010_Fairlie_Subsequent-pregnancies_CROI2022-poster_0.pdf
13. Lockman S, De Gruttola V. Outcomes Following Pregnancy Conception on Antiretroviral Therapy: A Call for More Data. Clin Infect Dis. 2018;68(2):280-1.
14. Myer L, Carter RJ, Katyal M, Toro P, El-Sadr WM, Abrams EJ. Impact of antiretroviral therapy on incidence of pregnancy among HIV-infected women in Sub-Saharan Africa: a cohort study. PLoS medicine. 2010;7(2):e1000229.
15. Committee Opinion. Ethical Considerations for Including Women as Research Participants. The American College of Obstetricians and Gynecologists. 2015;Number 646 [cited 2015 November]. Available from: https://www.acog.org/-/media/project/acog/acogorg/clinical/files/committee-opinion/articles/2015/11/ethical-considerations-for-including-women-as-research-participants.pdf
16. Oginni AB, Adebajo S. Trend and Determinants of Unmet Need for Family Planning Services among Currently Married Women and Sexually Active Unmarried Women Aged 15-49 in Nigeria (2003—2013). Afr. pop. stud. 2015;29(1):1483-99.

17. Currie J, Schwandt H. Short- and long-term effects of unemployment on fertility. Proc Natl Acad Sci U S A. 2014;111(41):14734-9.
18. Theron G, Brummel S, Fairlie L, Pinilla M, McCarthy K, Owor M, et al. Pregnancy Outcomes of Women Conceiving on Antiretroviral Therapy (ART) Compared to Those Commenced on ART During Pregnancy. Clin Infect Dis. 2020;73(2):e312-e20.
19. Zhao Y, Zhang L, Geng Y. Clinical Drug Trial Participation: Perspectives of Pregnant Women and Their Spouses. Patient Prefer Adherence. 2021;15:2343-52.
20. Malaba TR, Nakatudde I, Kintu K, Colbers A, Chen T, Reynolds H, et al. 72 weeks post-partum follow-up of dolutegravir versus efavirenz initiated in late pregnancy (DolPHIN-2): an open-label, randomised controlled study. Lancet HIV. 2022;9(8):e534-e43.
21. Abrams EJ, Pozniak A, Lockman S, Colbers A, Belew Y, Clayden P, et al. Enhanced and Timely Investigation of ARVs for Use in Pregnant Women. J Acquir Immune Defic Syndr. 2021;86:607-15.
22. Zash R, Diseko M, Jacobson DL, Mayondi GK Mabuta J, Jackson-Gibson M, Mmalane M, et al. Update on neural tube defects with antiretroviral exposure in the Tsepamo Study, Botswana 2021.[cited 2022 July 29 to August 2]. Available from: https://programme.aids2022.org/PAGMaterial/PPT/3726_4873/AIDS2022_TsepamoUpdate.pdf
23. World Health Organisation. Consolidated Guidelines on HIV prevention, testing, treatment, service delivery and monitoring: Recommendations for public health approach. 2021. [cited 2021 July] Available from: https://www.who.int/publications/i/item/9789240031593
24. Zash R, Makhema J, Shapiro RL. Neural-Tube Defects with Dolutegravir Treatment from the Time of Conception. N Engl J Med. 2018;379(10):979-81.
25. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. Lancet Glob Health. 2018;6(7):e804-e10.
26. Ayalew HG, Liyew AM, Tessema ZT, Worku MG, Tesema GA, Alamneh TS, et al. Prevalence and factors associated with unintended pregnancy among adolescent girls and young women in sub-Saharan Africa, a multilevel analysis. BMC Women's Health. 2022;22(1).
27. Kaye DK. The moral imperative to approve pregnant women's participation in randomized clinical trials for pregnancy and newborn complications. Philos Ethics Humanit Med. 2019;14(1):11.
28. Taylor MM, Kobeissi L, Kim C, Amin A, Thorson AE, Bellare NB, et al. Inclusion of pregnant women in COVID-19 treatment trials: a review and global call to action. Lancet Glob. Health. 2021;9(3):e366-e71.
29. Manningham-Buller E, Abbas-Hanif A, David A, Ekechi C, Green M, et al. Healthy mum, healthy baby, healthy future. University of Birmingham; 2022. [cited 2022 May]. Available from: https://www.birminghamhealthpartners.co.uk/wp-content/uploads/2022/05/Final-Healthy-Mum-Healthy-Baby-Healthy-Future-Report-AW_Accessible-PDF-REDUCED-FILE-SIZE.pdf
30 Pyra M, Heffron R, Mugo NR, Nanda K, Thomas KK, Celum C, et al. Effectiveness of hormonal contraception in HIV-infected women using antiretroviral therapy. AIDS. 2015;29(17):2353-9.
31 Jacobstein R, Polis CB. Progestin-only contraception: injectables and implants. Best Pract Res Clin Obstet Gynaecol. 2014;28(6):795-806.

C. Manuscript

Appendices

I. Insert

A figure 1

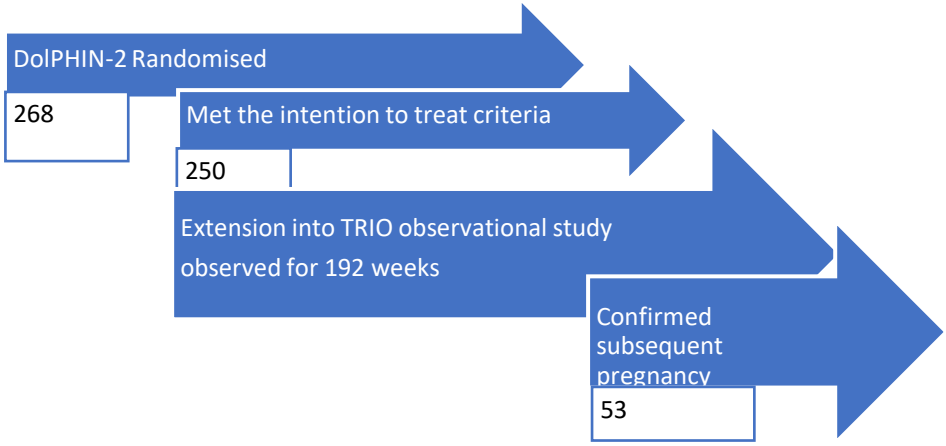

Figure 1: Flow diagram demonstrating the sample size of participants from the DolPHIN-2 clinical trial who were randomised, to those meeting the intention to treat criteria, followed through the observational extension D2 TRIO, to those with a confirmed subsequent pregnancy.

Table 1: Baseline characteristics of WLHIV randomised in a DolPHIN-2 Clinical Trial to a EFV or DTG arm who had subsequent pregnancies in Uganda and South Africa.

	N= 250		
Maternal characteristics at baseline N (%)	No subsequent pregnancy N= 197 (79)	Confirmed Subsequent pregnancy. N= 53 (21)	P value
Country			0,5834
South Africa	92 (47)	22 (42)	
Uganda	104 (53)	31 (58)	
Number of Subsequent pregnancies per design arm			0.0195
DolPHIN- 2 clinical trial		18 (34)	
D2 TRIO observation study		35 (66)	
Age (years)			
<24	25(19)	10 (23)	
25-29	47 (36)	19 (43)	
>30	58(45)	15 (34)	
Median (IQR)	29 (26-32)	28 (25-30)	0.1796
Married			0,3265
Yes	22 (17)	4 (9)	
No	110 (83)	40 (91)	
Employment status			0.5304
Employed	48 (36)	19 (43)	
Unemployed	84 (64)	25 (57)	
Level of education			0.1012
Primary	33 (26)	5 (12)	
Secondary	85 (67)	33 (77)	
University/Tertiary/Vocational	8 (7)	5 (11)	
Gravidity			
1	21 (11)	7 (13)	
2	55 (28)	15 (28)	
≥3	120 (61)	31 (59)	
Median (IQR)	3 (2-4)	3 (2-3)	0.3849
Parity			
1	94 (48)	27 (51)	
2	48 (24)	15 (28)	
3	34 (18)	10 (19)	
≥4	20 (10)	1 (2)	
Median (IQR)	2 (1-3)	1 (1-2)	0.2628
Previous pregnancies Abortions			1
No	157 (80)	42 (79)	
Yes	39 (20)	11 (21)	
Contraceptive type [1]			0.09914
Injectable progesterone	110 (44)	34 (47)	
Implant	44(18)	16 (22)	
Abstinence	33 (13)	9 (12)	
Barrier	24 (10)	6 (8)	
Intrauterine	33 (13)	3 (4)	
Other	5 (2)	5 (7)	
ART regimen at randomization			0,3703
DTG	101 (52)	23 (43)	
EFV	95 (48)	30 (57)	
Viral load[2] at randomisation (copies/mL) Median (IQR)	28093 (6456 - 58451)	25954(4028-77227)	0.5799
[1] Some women employed more than one contraceptive type. [2] Viral load was collected on all participants, so the sample size is the sample size of the entire column, n=197 for those who did not have a subsequent pregnancy and n=53 for those who did have a subsequent pregnancy. Acronyms are as follows: DolPHIN- 2 Dolutegravir in Pregnant HIV Mothers and their Neonates, DTG Dolutegravir, EFV Efavirenz, ART Antiretrovirals, IQR interquartile range.			

Table 2: DolPHIN-2 clinical trial and observational follow up D2 TRIO subsequent birth outcomes.

N (%)	N= 56	P values
Birth Site		0.1088
South Africa	22 (39)	
Uganda	34 (61)	
How gestational age was determined		<0.05
Determined by LNMP	31 (58)	
Fundal height	1 (2)	
Ultrasound	21 (40)	
Gestational age at pregnancy confirmation (Trimester- weeks)		0.0032
First trimester (0-12)	27 (51)	
Second trimester (13-27)	19 (36)	
Third trimester (28-40)	7 (13)	
Median (IQR)	13 (8-23)	
Delivery outcome[1] 38% of infant data missing		
Alive	23 (66)	<0.05
Induced abortion/ terminations (<28w)	4 (11)	
Pregnancy ongoing at last contact	8 (23)	
Stillborn/ macerated	0	
Birthweight (g) [2] 60% of infant data missing		
Normal (>2500)	19 (90)	<0.05
Low Birth weight (1500- 2500)	2 (10)	
Very low birth weight (<1500)	0	
Median (IQR)	3200 (2900-3500)	
Infant sex [3] 59% of infant data missing		0.1444
Male	15 (65)	
Female	8 (35)	
No infants with complications	1 (2)	
Delivery mode [4] 70% of infant data missing		<0.05
Elective caesarean	2 (12)	
Emergency caesarean	1 (6)	
Normal vaginal birth	14 (82)	
Feeding method		<0.05
Breastfed	2 (4)	
Mixed feeding	52 (96)	
.. [2]. [3].[4]. Three women experienced more than one subsequent pregnancy during the course of both studies, therefore this study reports 53 women with subsequent pregnancies but later describe 56 birth outcomes to include the multiple subsequent pregnancies		

Figure 2:

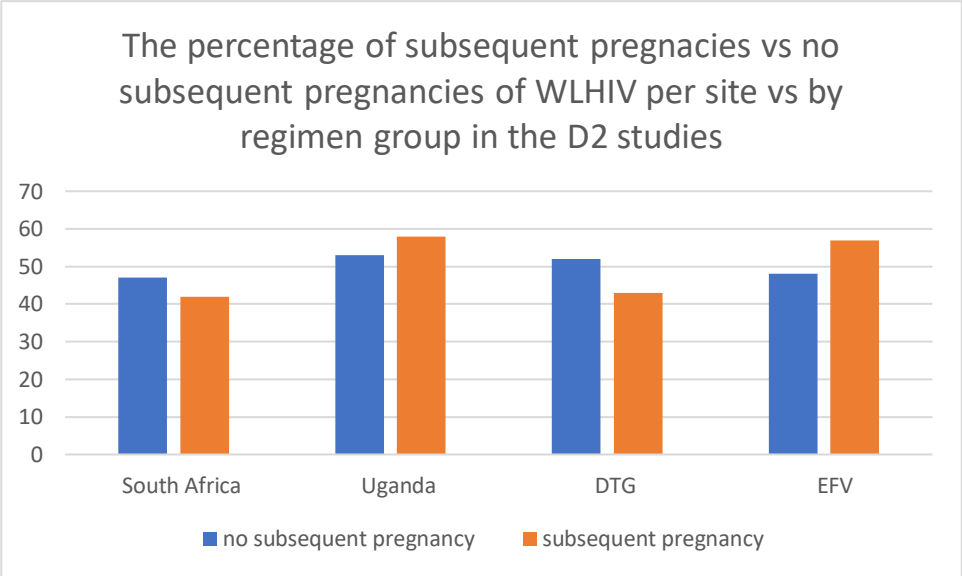

Figure 2: Shows a bar graph of the percentage of those with a subsequent pregnancy compared to those without a subsequent pregnancy by site and by group regimen in the DolPHIN -2 study

Table 3: Supplementary baseline characteristics of WLHIV randomised in a DolPHIN-2 Clinical Trial to a EFV or DTG who had subsequent pregnancies in Uganda and South Africa.

Maternal characteristics at baseline N (%)	N=250		
	N= 83	N=166 (66)	
	No subsequent pregnancy N= 83 (33)	Subsequent pregnancy suspected but not confirmed. N= 113 (45)	Subsequent pregnancy confirmed. N= 53 (21)
Country			
South Africa	41 (49)	51 (45)	22 (42)
Uganda	42 (51)	62 (55)	31 (58)
Number of Subsequent pregnancies per design arm			
DolPHIN- 2 clinical trial			18 (34)
D2 TRIO observation study			35 (66)
Age (years)			
<24	10 (22)	15 (17)	10 (23)
25-29	15 (33)	32 (38)	19 (43)
>30	20 (45)	38 (45)	15 (34)
Married			
Yes	5 (11)	17 (20)	4 (9)
No	41 (89)	69 (80)	40 (91)
Employment status			
Employed	14 (30)	34 (40)	19 (43)
Unemployed	32 (70)	52 (60)	25 (57)
Level of education			
Primary	11 (26)	22 (26)	5 (12)
Secondary	28 (65)	57 (69)	33 (77)
University/Tertiary/Vocational	4 (9)	4 (5)	5 (11)
Gravidity			
2	32 (39)	23 (20)	15 (28)
3	23 (28)	37 (33)	18 (34)
>3	22 (27)	38 (34)	13 (25)
Parity			
1	45 (54)	49 (43)	27 (51)
2	19 (23)	29 (26)	15 (28)
3	10 (12)	24 (21)	10 (19)
≥4	9 (11)	11 (10)	1 (2)
Previous Abortions			
No	71 (86)	86 (76)	42 (79)
Yes	12 (14)	27 (24)	11 (21)
Contraceptive type [1]			
Injectable progesterone	36 (50)	74 (42)	34 (47)
Implant	12 (16)	32 (18)	16 (22)
Abstinence	7 (10)	26 (15)	9 (12)
Barrier	8 (11)	16 (9)	6 (8)
Intrauterine	9 (12)	24 (14)	3 (4)
Other	1 (1)	4 (2)	5 (7)
ART regimen at randomization			
DTG	40 (48)	61 (54)	23 (43)
EFV	43 (52)	52 (46)	30 (57)
Viral load at randomisation (copies/mL) Median (IQR)	27957 (7524-57682)	288229(5772-58417)	25954(4028-77227)

[1]Contraceptive types may be larger than population sizes as participants may have employed more than one type of contraceptive e.g., barrier and abstinence etc.

D. Protocol

PROTOCOL

14.1 Background

Approximately 1.5 million women living with HIV (WLHIV) become pregnant worldwide each year, with the majority residing in low- or middle-income countries (LMIC)(5). Antiretroviral therapy (ART) as an intervention represents an important achievement in response to the HIV epidemic, specifically in reducing mother-to-child transmission (MTCT) and improving maternal and infant health. Randomised trials in pregnant women initiating ART comparing the previously recommended first line efavirenz (EFV) -based regimens with dolutegravir (DTG)-based regimens, from the DolPHIN-2 (21), and VESTED trial (11); are in part some of the reasons the World Health Organisation (WHO) in 2019 policy brief recommend a transition from EFV to DTG based regimens (21). DTG offers several advantages over EFV, including fewer side effects, fewer medication interactions (7), a higher genetic barrier to developing medication resistance (8), and a fixed-dose combination, enhancing tolerability and treatment adherence. Consequently, there was a global shift towards using DTG as the first-line antiretroviral ART regimen. There was, however, limited safety information available for the use of DTG in pregnancy at the time of this transition (21).

Concerns persist due to the inadequate safety and efficacy data for many ART regimens in pregnant and postpartum women, resulting from their exclusion from trials. Safety information for medication use during pregnancy typically becomes available approximately 6 years later, owing to the potential risk to both the mother and child (22). A major area of concern is that between 1920 and 2010, 91% of medication approved by the US Food and Drug Administration (FDA) lacked data on safety and/or efficacy in pregnancy (22). While ethical concerns prevent the inclusion of pregnant women in trials during medication development, valuable safety data on conception can be obtained from women enrolled in medication trials who *subsequently* become pregnant. There is an international movement towards including pregnant women in trials, as this will enable careful monitoring and observation to assess pregnancy outcomes (19). Although the number of women involved may not be sufficient to establish causality, this data provides valuable insights that would not ordinarily be accessible, guiding future research directions.

In 2019, the WHO released a policy update based on concerns from early findings from Tsepamo study in Botswana (23), where women on DTG initially appeared to have an increased association of neural tube defects. However, as more time passed, it was noted that the risk was lower than initially observed, and DTG was deemed safe for women of childbearing age (23, 24, 25, 26). Despite this, these results led to guideline changes by health directorates, resulting in suboptimal care for this vulnerable group of women due to the lack of safety information available on DTG at the time. The example of the fallout from the early Tsepamo

study highlights the importance of increasing the evidence around medication use in pregnancy, specifically describing the outcomes that could be potentially related to the medication use.

This is particularly of importance given the high rate of unplanned pregnancies in sub- Saharan Africa where the overall prevalence is 30% with the highest magnitude observed in Southern Africa at 60%(27). Unplanned pregnancies place women at risk to a number of risk factors which include vertical transmission of HIV(27). These women are unaware of the risk they place on their unborn child especially regarding late initiation on ARV treatment and the potential to reach viral load suppression prior to birth(21).

Considering the high proportion of WLHIV of childbearing age in sub-Saharan African countries, the widespread adoption of DTG as the recommended first line ART regimen, and the high prevalence of subsequent and unplanned pregnancies this population experiences, accurately describing the occurrence of subsequent pregnancies becomes paramount.

14.2 Rationale

Pregnancy is a unique and complex physiological state that requires special attention when it comes to medication use. The well-being of both the expectant mother and the developing foetus is of utmost importance, and understanding the implications of medication use during pregnancy is crucial for ensuring a healthy and safe pregnancy. Many medications are prescribed based on data from non-pregnant or lactating adults, and this data may not fully capture the specific implications of medication use during pregnancy (3). The lack of comprehensive research in this area creates a research gap and leaves healthcare providers and pregnant women with limited information to make informed decisions (4, 28). Additionally, some medications can pose potential risks to the developing foetus and may need to be avoided or closely monitored.

Despite these concerns, there are currently no legal or regulatory requirement for new medication to be tested during pregnancy, resulting in >80% of pregnant women receiving treatment that have not been studied in pregnancy (4). This practice essentially leaves pregnant women to be reliant on medications with little safety and efficacy data, to use sub-optimal older therapies which are often not as effective as newer therapies or worse they are deprived of therapies completely (28). The latter scenario was in evidence during the COVID- 19 pandemic where vaccinations were initially not offered to pregnant women due to a lack of safety information(29). The impact of this was that unvaccinated pregnant women had more COVID-19 related adverse incidents than vaccinated pregnant women(4, 29). This decision was detrimental to many

women who were deprived of vaccination as vaccination not only protected themselves but also their unborn child through the transmission of antibodies (4).

Despite the ongoing global efforts to enrol pregnant women in clinical trials, this process is slow, by simply retaining women who become pregnant during a study, we gain insights into their pregnancy outcomes and the effects of medications or interventions used before conception. By including pregnant women in clinical trials or ensuring that women who become pregnant during trials are retained and appropriately monitored, several important objectives can be achieved. Firstly, it allows for the provision of accurate information and evidence-based guidance on the use of effective treatments during pregnancy (4, 30). This ensures that pregnant women have access to the most appropriate and safe interventions, promoting their own health as well as the well-being of their developing foetus (4, 30). This approach allows us to gather real-world data specific to pregnant women, which is often difficult to achieve through traditional clinical trials that may exclude pregnant individuals. Secondly, incorporating pregnant women in clinical trials helps to identify any potential risks or adverse effects of treatments specifically in this population (4, 30). This knowledge is crucial for minimizing avoidable harm and improving the quality of care provided to pregnant women (4, 28). Thirdly, actively involving pregnant women in research enhances equitable access to the potential benefits of participation (4, 30). It ensures that the unique healthcare needs of pregnant women are taken into consideration and that they can contribute to scientific advancements (4). The data gleaned from pregnant women remaining in trials enables access to earlier data on safety and efficacy that researchers would not traditionally have access to. By utilizing this opportunity, we can gain a deeper understanding of the impact of treatments on pregnancy and potentially improve maternal and foetal health outcomes. Although there is a global push to *enrol* pregnant women in trials, what this study proposes is, by just *retaining* women who subsequently become pregnant, would give us access to valuable information we would not ordinarily have acquired. This study's approach presents a valuable and practical avenue to address the research gap in pregnancy-related medication and intervention safety.

Gaining an understanding of the proportion of women who conceive during their participation in clinical trials would be valuable in advancing efforts to include and retain pregnant women in research and obtain pregnancy safety data during the early stages of medication development. The cohort of women in our study, who were exposed to DTG and enrolled in trials while becoming pregnant, presents a unique opportunity for us to describe the incidence of subsequent pregnancies.

14.3 Aim and objectives of study

The aim of this proposed study is to describe the incidence of subsequent pregnancies in women enrolled in the DolPHIN-2 clinical trial and the DolPHIN-2 TRIO extension observational study. The specific objectives are:

- To describe the clinical and demographic characteristics of women enrolled In the DolPHIN-2 studies who had a subsequent pregnancy following the index pregnancy.
- To describe the incidence of subsequent pregnancies overall and by treatment regimen (EFV vs DTG)
- To describe outcomes of subsequent pregnancies

15 Methodology

15.1 Study Design

The proposed study is a secondary analysis of an open-label, multi-centre randomised controlled trial *Dolutegravir in Pregnant HIV Mothers and their Neonates* (DolPHIN-2) (https://clinicaltrials.gov/ct2/show/NCT03249181). DolPHIN -2 trial has been described in full elsewhere [13]; however briefly; the aim was to explore the safety and efficacy of DTG in late presenting pregnant women with outcomes assessed at delivery and at 72 weeks postpartum. Additionally, this proposed study will also include an analysis of the observational extension of the study (TRIO) where DolPHIN-2 women were transitioned into, to assess longer-term safety and efficacy of DTG up until 192 weeks postpartum. The analysis for this proposed study will draw on data collected between 22nd of January 2018 and 05th of September 2023.

15.2 Study Setting

The parent studies took place in two high HIV prevalence settings, South Africa and Uganda. In South Africa, eligible women were recruited from eight primary antenatal facilities in Cape Town and were enrolled at Gugulethu Midwives obstetric unit, housed in a peri urban suburb. In Uganda, eligible women were recruited from eight primary antenatal facilities in Kampala and Wakiso District, and they were enrolled at Kawempe Hospital.

15.3 Study population and sampling

Among the study population, all the women enrolled in DolPHIN-2 and TRIO and followed up prospectively for 192 weeks will be included. Sampling methods have been described elsewhere [13]

Inclusion criteria:

- Aged 18 years or older.
- Enrolled into parent studies.
- Evidence of informed consent

15.4 Data collection

Data collected during the parent studies will be used for this proposed study. Data was collected via standardised enrolment questionnaires, anthropometric material, and infant measures as well as Case report forms (CRF) (Appendix1) and laboratory records.

15.4.1 Standardised enrolment questionnaires

In the parent study, the questionnaires completed by participants which included demographic information and medical history.

15.4.2 Laboratory results

Data collected during the parent study during antenatal and postpartum follow ups to 192 weeks postpartum. Date and result of urine pregnancy test, date, and result of HCG test (if performed), estimated gestational age (ultrasound, determined by LNMP, son, and or fundal height)

15.4.3 Case Report Form information

Collected by trained data collectors on sight during each follow up review. The data collected via the CRF will include:

- Date aware of pregnancy,
- Date and result of urine pregnancy test,

- Date and result of HCG test (if performed)
- Estimated gestational age.
- Expected date of delivery,
- How was the pregnancy identified, if an ultrasound was completed and date, estimated deliver date, pregnancy outcome, date of pregnancy outcome, gestational age and Infant details such as gender, birthweight, head circumference, length, congenital anomalies noted and apgar score.

15.4.4 Anthropometry results

Any demographic information gathered during data collection pertaining to the outcome of interest.

15.5 Outcomes of Interest

The main outcome of interest is the event of a subsequent pregnancy regardless of the pregnancy outcome. This will be described in proportions and compared via treatment arm.

15.5.1 Variable definitions:

Primary Outcome:

- Subsequent pregnancies: Any pregnancy described subsequent to the index pregnancy (at study enrolment) regardless of the outcome of the pregnancy (this will include any pregnancy loss, miscarriage, abortion/termination, or still birth), determined by ultrasound (gold standard) or urine test.

Secondary outcomes:

- Pregnancy outcome: will be categorised as a
    - pregnancy loss
        - ectopic pregnancies identified by the research sonographer,
        - miscarriage will be defined as a pregnancy loss <28 weeks.
        - still birth defined as foetal death occurring before/ during labour and birth based on 1 minute APGAR score of 0)
    - live birth
- Gestational age at delivery: in terms of preterm (<37weeks) with gestational age based on the best available measures, ultrasound (gold standard measurement), if ultrasound is not available then measurement of symphysis fundal height (SFH) or using the first day of the last menstrual period (LMP).

15.6 Exposure of interest:

The exploratory exposures of interest will be the regimen arm (DTG or EFV).

Table 1 will summarise the variables to be included in analysis.

Table 1: Variables to be included in analysis.

Variable	Scale	Categories
Site	Categorical- Nominal	South Africa
		Uganda
Number of subsequent pregnancies	Numerical- Discrete	Median, IQR, percentages
Maternal characteristics		
Age (years)	Numerical – Continuous	Median, IQR, percentages
	Categorical	<24
		25-29
		>30
Education	Categorical – Binary	Finished High School
		Did not finish High school
Employment status	Categorical – Binary	Employed
		Unemployed
Socio-economic status	Categorical- Ordinal	Low
		Medium
		High
Marital status	Categorical- Nominal	Married
		Single
		Divorced
		Living together
Obstetric characteristics		
Gestation at pregnancy awareness (weeks)	Numerical continuous	Median, IQR, percentages
Mode of pregnancy confirmation	Categorical- Binary	HCG Urine test
		Ultrasound
Gravidity	Numerical- Discrete	Median, IQR, percentages
	Categorical- Ordinal	1
		2
		>3
Parity	Numerical – Discrete	Median, IQR, percentages
	Categorical- Ordinal	0
		1
		>2

Previous Miscarriage	Numerical- Discrete	Median, IQR, percentages
Previous Preterm	Numerical – Discrete	Median, IQR, percentages
Contraceptive	Categorical- Binary	Yes
		No
Contraceptive method chosen after index pregnancy	Categorical – Nominal	long-acting reversible contraception
		hormonal contraception
		barrier methods
		Other
Pregnancy outcome	Categorical- Nominal	Live birth
		Spontaneous abortion (<28w)
		Induced Abortion/ Termination (<28w)
		Still birth (28w+)
		Unknown
HIV		
ART regimen	Categorical- Nominal	SoC –TDF/XTC/EFV
		DTG
		Other
CD4 (cells/µL)	Numerical- Continuous	Median, IQR, percentages
	Categorical- Ordinal	<200
		201-350
		351-500
		>500
Viral load	Numerical continuous log (10)	Median, IQR, percentages
Obstetric outcomes		
How pregnancy was identified	Categorical- Nominal	Study team
		Patient self-reported
		Pregnancy test at study visit
Gestational age at delivery (weeks)	Categorical- Ordinal	Full Term (>37)
		Preterm (<37)
		Late Preterm (34-37)

		Moderately Preterm (32-34)
		Very Preterm (28-32)
Birthweight (g)	Numerical- Continuous	Median, IQR, percentages
	Categorical- Ordinal	Normal (>2500)
		Low Birth weight (1500- 2500)
		Very low birth weight (<1500)
Infant sex	Categorical – Nominal	Male
		Female
Infant Length (cm)	Numerical – Continuous	Median, IQR, percentages
Infant Head circumference (cm)	Numerical – Continuous	Median, IQR, percentages
Apgar Score	Numerical – Discrete	Median, IQR, percentages
Feeding method	Categorical – Nominal	Breastfed
		Formula fed
		Mixed feeding

15.7 Data management and Analysis Plan

15.7.1 Data Management

Data collected from the DolPHIN-2 trial and extension observational study (TRIO) was entered into a CRF. The CRFs developed for the studies were designed for use with the DataFax data management system. Datafax forms were transmitted to the DataFax unit at IDI and study data was entered into a study specific database by designated staff on a regular basis from completed CRFs, to ensure that it is up to date. The database is kept on a secure PC. Access to the database is given to authorised personnel only and a log of authorised personnel are stored in the trial master file. Data collected from the DolPHIN-2 studies were entered into a customized study Microsoft Access database, maintained in firewall protected servers with automated daily back-ups at the University of Cape Town and Makerere University. This data is protected by password encryption software known by the primary and secondary authors only. Backups are stored remotely. All study records include anonymous participant identification numbers, and no personal names or identifiers are documented. The data utilized for this analysis will be stored on a personal computer secured with password protection, ensuring access is restricted solely to the researcher.

15.7.2 Data Analysis

All statistical analyses will be conducted using R version 4.0.4 (2021-02-15) developed in collaboration by the University of Auckland, New Zealand. Continuous variables will be summarised using either the min, max, mean and standard deviation (SD) for normal distributed variables; or the median and the interquartile range (IQR) for non-normal distributed variables. Categorical variables will be described using proportions and frequencies. To ensure reliability of the data, missing data will be coded NA and be documented in the data diary for review. Missing data and implausible data entry points will be detected through data analysis and graphical evaluation, specific outliers will be traced, this data will be checked via the data manager of the parent study to determine if an error was made or if this is an influential data point that needs to be further evaluated. A data dictionary will be created as a reference to data coding, to enable data sharing, and as a reference to refer to for monitoring purposes. Each data entry is numbered for monitoring.

Analysis will focus on describing the subsequent pregnancies. Descriptive statistics will be used to describe the overall occurrence of the subsequent pregnancies as well as to describe any adverse birth and maternal outcomes.

16. Ethical considerations

The University of Cape Town Faculty of Health Sciences Research Ethics Committee (UCT-HREC; RECREF: 096/2017); the Uganda National Institution for Science and Technology and joint clinical research centre (HS106ES) and the Central University Research Ethics Committee for Physical Interventions at the University of Liverpool (reference 1506) approved the parent studies (See Appendix)

Ethical approval for this proposed study will be sought from the UCT-HREC. The researcher also completed Human Research Protection Training through the Office for Human Research Protection (OHRP).

16.1 Informed consent process

All women who met the eligibility criteria and who agreed to participate in the parent studies completed a written informed consent form (See Appendix). In consenting to participate in the studies, participants gave

permission for data collected throughout the duration of the studies and collected routinely to be analysed for future research projects. As part of this proposed study, pre-existing data collected from the enrolment questionnaires, CRF's including abstraction clinical and obstetric records will be accessed and utilised in accordance with the consent received from participants. Given that no direct contact with participants will be required for this study, we will not obtain direct informed consent from individual participants.

16.2 Privacy and Confidentiality

Certain measures were put in place to reduce the potential for breaches of confidentiality in the parent study.:

- All personnel responsible for gathering and handling data received training on their ethical responsibilities to uphold participant confidentiality.
- Following standard protocol, participants were assigned anonymous identifiers on all study documents. Participant names were exclusively included on the informed consent and locator tracer documents, which were securely separated from the participant study documents.
- All databases were secured with password protection and hosted on a firewall protected UCT and at Makerere University server's. Throughout the study, no personal identifiers will be included in the data utilized for analysis. Instead, participants will be identified solely by the anonymous identifiers assigned during the parent study.
- Furthermore, the findings of the proposed study will maintain confidentiality by not disclosing information on individual patients.

16.3 Risks and benefits

A detailed list of the risk and benefits have already been outlined by the parent study (HREC REF: 451/2012).

16.3.1 Risk

Minimal risk is anticipated for the participants of this study. The only potential foreseeable risk is that of confidentiality being compromised. These risks have been mitigated via the data management procedures and privacy and confidentiality sections outlined above.

16.3.2 Benefit

The participants will not have direct benefit because of this proposed study. As the analysis is secondary in nature and the data has already been captured by the parent study. There will however be in-direct benefit

for pregnant women in the HIV community within sub-Saharan Africa. This description can shed light on periconception and the impact DTG had on subsequent pregnancies.

16.4 Reporting and implementation

Once the analysis is finalized, the findings of this study will be submitted to a suitable peer-reviewed journal as determined through collaboration with relevant stakeholders.

16.5 Logistics

Table 2 provides the time frame in which it is anticipated this proposed study will be conducted within.

Table 2: Study Time Frame

Task	Duration			
	September 23	October 23	November 23	December 23
Merge, clean and check data				
Analysis				
Prepare draft manuscript				
Prepare final manuscript and submit for publication				

16.6 Budget

Ms. Marshall will be performing this analysis as part of her MPH degree and will not be seeking any compensation.

17. References:

1. Sheffield JS, Siegel D, Mirochnick M, Heine RP, Nguyen C, Bergman KL, et al. Designing drug trials: considerations for pregnant women. Clin Infect Dis. 2014;59 Suppl 7(Suppl 7):S437-44.

2. Theron G BS, Fairlie L, Pinilla M, McCarthy K, Owor M, Chinula M, Makanani B, Violari A, Moodley D, Chakhtoura N,Browning R, Hoffman R, Fowle MJ. Pregnancy Outcomes of Women Conceiving on Antiretroviral Therapy (ART) Compared to Those Commenced on ART During Pregnancy. Clinical Infectious Diseases. 2021;73:312-20.

3. Birmingham Uo. Safe and effective medicines for use in pregnancy: a call to action [Pregnancy and Maternal Health]. Edgbaston, Birmingham, B15 2TT, United Kingdom2021. Available from: https:// www. birmi ngham. ac. uk/ docum ents/ colle ge- mds/ centr es/ bctu/ 21560policy- commi ssion- mater nal-health- report. Pdf.

4. David AL, Ahmadzia H, Ashcroft R, Bucci-Rechtweg C, Spencer RN, Thornton S. Improving Development of Drug Treatments for Pregnant Women and the Fetus. Ther Innov Regul Sci. 2022;56(6):976-90.

5. Waitt C, Orrell C, Walimbwa S, Singh Y, Kintu K, Simmons B, et al. Safety and pharmacokinetics of dolutegravir in pregnant mothers with HIV infection and their neonates: A randomised trial (DolPHIN-1 study). PLoS Med. 2019;16(9):e1002895.

6. WHO. Update of recommendations on first and second-line antiretroviral regimens 2019.

7. Cottrell ML, Hadzic T, Kashuba AD. Clinical pharmacokinetic, pharmacodynamic and drug-interaction profile of the integrase inhibitor dolutegravir. Clin Pharmacokinet. 2013;52(11):981-94.

8. Llibre JM PF, García F, García Deltoro M, Blanco JL and Delgado R. Genetic Barrier to Resistance for Dolutegravir. AIDS reviews. 2015;17:59-68.

9. Kintu K, Malaba TR, Nakibuka J, Papamichael C, Colbers A, Byrne K, et al. Dolutegravir versus efavirenz in women starting HIV therapy in late pregnancy (DolPHIN-2): an open-label, randomised controlled trial. Lancet HIV. 2020;7(5):e332-e9.

10. Hoffman RM, Brummel SS, Britto P, Pilotto JH, Masheto G, Aurpibul L, et al. Adverse Pregnancy Outcomes Among Women Who Conceive on Antiretroviral Therapy. Clinical Infectious Diseases. 2018;68(2):273-9.

11. Lockman S, Brummel SS, Ziemba L, Stranix-Chibanda L, McCarthy K, Coletti A, et al. Efficacy and safety of dolutegravir with emtricitabine and tenofovir alafenamide fumarate or tenofovir disoproxil fumarate, and efavirenz, emtricitabine, and tenofovir disoproxil fumarate HIV antiretroviral therapy regimens started in pregnancy (IMPAACT 2010/VESTED): a multicentre, open-label, randomised, controlled, phase 3 trial. Lancet. 2021;397(10281):1276-92.

12. Fairlie L BS, Ziemba L, Coletti A, Chinula L, Shapiro R, Stringer J, Browning R, Chakhtoura N, Mmbaga BT, Mhembere TP, Omoz-Oarhe A, Nagaddya B, Naidoo B, Lockman S. Adverse Outcomes in Subsequent Pregnancies in the IMPAACT 2010 Trial. 2022 Conference on Retroviruses

and Opportunistic Infections (CROI)2022.

13. Lockman S, De Gruttola V. Outcomes Following Pregnancy Conception on Antiretroviral Therapy: A Call for More Data. Clinical Infectious Diseases. 2018;68(2):280-1.

14. Pyra M, Heffron R, Mugo NR, Nanda K, Thomas KK, Celum C, et al. Effectiveness of hormonal contraception in HIV-infected women using antiretroviral therapy. AIDS. 2015;29(17):2353-9.

15. Myer L, Carter RJ, Katyal M, Toro P, El-Sadr WM, Abrams EJ. Impact of antiretroviral therapy on incidence of pregnancy among HIV-infected women in Sub-Saharan Africa: a cohort study. PLoS medicine. 2010;7(2):e1000229.

16. Opinion C. Ethical Considerations for Including Women as Research Participants. The American College of Obstetricians and Gynecologists. 2015;Number 646

17. Oginni AB AB, Adebajo S. . Trend and Determinants of Unmet Need for Family Planning Services among Currently Married Women and Sexually Active Unmarried Women Aged 15-49 in Nigeria (2003—2013). African Population Studies 2015;29(1):1483-99.

18. Currie J, Schwandt H. Short- and long-term effects of unemployment on fertility. Proc Natl Acad Sci U S A. 2014;111(41):14734-9.

19. Theron G, Brummel S, Fairlie L, Pinilla M, McCarthy K, Owor M, et al. Pregnancy Outcomes of Women Conceiving on Antiretroviral Therapy (ART) Compared to Those Commenced on ART During Pregnancy. Clinical Infectious Diseases. 2020;73(2):e312-e20.

20. Zhao Y, Zhang L, Geng Y. Clinical Drug Trial Participation: Perspectives of Pregnant Women and Their Spouses. Patient Prefer Adherence. 2021;15:2343-52.

21. Malaba TR, Nakatudde I, Kintu K, Colbers A, Chen T, Reynolds H, et al. 72 weeks post-partum follow-up of dolutegravir versus efavirenz initiated in late pregnancy (DolPHIN-2): an open-label, randomised controlled study. Lancet HIV. 2022;9(8):e534-e43.

22. Abrams EJ ML, Pozniak A, Lockman S, Colbers A, Belew Y, Clayden P, Mirochnick M, Siberry GK, MD, Ford N, Khoo S, Renaud F, Vitoria M, Venter WDF, Doherty M, Penazzato M. Enhanced and Timely Investigation of ARVs for Use in Pregnant Women. J Acquir Immune Defic Syndr. 2021;86:607-15.

23. Zash R HL, Diseko M, Jacobson DL, Mayondi GK Mabuta J, Jackson-Gibson M, Mmalane M, Gaolathe T, Lockman S, Makhema J and Shapiro R. Update on Neural Tube Defects with Antiret. 2021.

24. WHO. Consolidated Guidelines on HIV prevention, testing, treatment, service delivery and monitoring: Recommendations for public health approach. 2021.

25. Zash R, Makhema J, Shapiro RL. Neural-Tube Defects with Dolutegravir Treatment from the Time of Conception. N Engl J Med. 2018;379(10):979-81.

26. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. Lancet Glob Health. 2018;6(7):e804-e10.

27. Ayalew HG, Liyew AM, Tessema ZT, Worku MG, Tesema GA, Alamneh TS, et al. Prevalence and factors associated with unintended pregnancy among adolescent girls and young women in sub-Saharan Africa, a multilevel analysis. BMC Women's Health. 2022;22(1).

28. Kaye DK. The moral imperative to approve pregnant women's participation in randomized clinical trials for pregnancy and newborn complications. Philos Ethics Humanit Med. 2019;14(1):11.

29. Taylor MM, Kobeissi L, Kim C, Amin A, Thorson AE, Bellare NB, et al. Inclusion of pregnant women in COVID-19 treatment trials: a review and global call to action. The Lancet Global Health. 2021;9(3):e366-e71.

30. Manningham-Buller E BP, Abbas-Hanif A, David A, Ekechi C, Green M, et al. Healthy mum, healthy baby, healthy future. University of Birmingham; 2022.

E. Protocol Appendices

i. **BMC Pregnancy and Childbirth**

Author Guidelines

Research article

Criteria

Research articles should report on original primary research, or present a new experimental or computational method, test or procedure. Manuscripts reporting results of a clinical trial must conform to CONSORT 2010 guidelines. Authors of randomized controlled trials should submit a completed CONSORT checklist alongside their manuscript, available at www.consort-statement.org. Research articles may also report on systematic reviews of published research provided they adhere to the appropriate reporting guidelines which are detailed in our editorial policies. Please note that non-commissioned pooled analyses of selected published research and bibliometric analyses will not be considered. Studies reporting descriptive results from a single institution or region will only be considered if analogous data have not been previously published in a peer reviewed journal and the conclusions provide distinct insights that are of relevance to a regional or international audience.

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Title page

The title page should:

- present a title that includes, if appropriate, the study design e.g.:
    - "A versus B in the treatment of C: a randomized controlled trial", "X is a risk factor for Y: a case control study", "What is the impact of factor X on subject Y: A systematic review"
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Online database

Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.

Supplementary material/private homepage

Doe J. Title of supplementary material. 2000. http://www.privatehomepage.com. Accessed 22 Feb 2000.

University site

Doe, J: Title of preprint. http://www.uni-heidelberg.de/mydata.html (1999). Accessed 25 Dec 1999.

FTP site

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