Investigating the Effectiveness of Antiretroviral Therapy in sub-Saharan Africa

Thesis was submitted in accordance with the requirement of the University of Liverpool for the degree of Doctor of Philosophy by

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This thesis is the product of my original research work conducted while registered as a doctoral student at the University of Liverpool. I declare that all work reported has not been presented in part or wholly for the award of any other degree or qualification

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LIST OF ABBREVIATIONS

µg	Microgram (s)
µL	Microlitre(s)
β	Beta
3TC	Lamivudine
95%CI	95% Confidence interval
ABC	Abacavir
ABCB	ATP binding cassette B
ABCC	ATP binding cassette C
ABCG	ATP binding cassette G
ACT	Artemisinin-Combination Therapies
AEs	Adverse events
AIDS	Acquired immunodeficiency syndrome
AL	Artemether lumefantrine
ALT	Alanine aminotransferase
ANC	Antenatal care
ANOVA	Analysis of variance
AQ	Amodiaquine
ARM	Artemether

ART	Antiretroviral therapy
ARV	Antiretroviral
AS	Artesunate
AST	Aspartate transaminase
ATP	Adenosine triphosphate
ATV	Atazanavir
AUC	Area under the concentration curve
AZT	Zidovudine
BAF	Bioanalytical facility
BCRP	Breast Cancer Resistance Protein
BIC	Bictegravir
BM	Breast milk
BMI	Body mass index
C_{24}	Concentration 24 hours post dose
cART	Combination antiretroviral therapy
CBZ	Carbamazepine
CCR5	Chemokine receptor type 5
CD	Cord blood
CD4	cluster of differentiation 4

CDC	Centre for disease control
CFTR Gene	Cystic fibrosis transmembrane conductance regulator geneCI
	Confidence interval
CL	Clearance
Cmax	Maximum concentration
Cmin	Minimum concentration
CNS	Central Nervous System
CNT	Concentrative
CROI	Conference on retroviruses and opportunistic infections
CSF	Cerebrospinal fluid
Ct	trough concentration
CV	Coefficient of variance
CXCR4	Chemokine receptor type X4
CYP	Cytocrome P 450
DAA	Direct acting antivirals
DAIDs	Division of AIDS
DAPCI	Desorption atmospheric pressure chemical ionisation
DBLF	Desbutyl-lumefantrine
DBMS	Dried breast milk spots

DBS	Dried blood spots
DEAQ	Desethyl-amodiaquine
DHA	Dihydroartemisinin
dL	Decilitre
DMSO	dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOLACT	Dolutegravir /Artemisinin Combination therapies
DOLPHIN	Safety and Pharmacokinetics of Dolutegravir in Pregnant HIV Mothers and Their Neonates
DRV	Darunavir
DTG	Dolutegravir
DTG-d5	Dolutegravir d5
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EFV	Efavirenz
eGFR	estimated Glomerular filtration rate
ELV	Elvitegravir
EMA	European Medicines Agency

ENCORE	Efficacy of 400 mg efavirenz versus standard 600 mg dose in HIV-infected, antiretroviral-naive adults
Env	Envelop
ESI	Electrospray ionisation
F	Bioavailability
FDA	U S Food and Drug Administration
FTC	Emtricitabine
GCP	Good clinical practice
GM	Geometric mean
GMR	Geometric mean ratio
H	hour
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIC	High income countries
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
HLA-B*5701	Human leucocyte antigen-B*5701
HPLC	High performance liquid chromatography

HQC	High quality control
IC90	90% Inhibitory concentration
IC50	50% Inhibitory concentration
ICH-GCP	International Conference on Harmonisation-Good Clinical Practice (Food and Drug Administration guideline)
IMPAACT	International Maternal Paediatrics Adolescent AIDS Clinical Trials Network
INST	Integrase strand transfer inhibitor
IP	Infant plasma
IQR	Interquatile range
IRIS	Immune reconstitution inflammatory syndrome
IS	Internal standard
IU	International unit
Kg	Kilogram
L	Litre
LCL	Lower confidence level
LC-MS	Liquid Chromatography Mass Spectrometry
LF	Lumefantrine
LLOQ	Lower limit of quantification

LLQ	Lower limit of quantification
LMIC	Low and middle income countries
Log10	Logarithm to base 10
LPV	Lopinavir
LQC	Low quality control
MAF	Minor allele frequency
MATE1	multidrug and toxin extrusion transporter 1
MEC	Minimum effective concentration
mg	Milligram
Min	Minute
mL	Millilitre
mm	millimetre
MORU	Mahidol Oxford Tropical Medicine Research Unit
MP	Maternal plasma
MQC	Medium quality control
MRP	Multidrug resistance-associated protein
MSM	Men who have sex with men
MTCT	Mother to child transmission
MVC	Maraviroc

ng	Nanogram
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institute of Health
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitor
NUC	National Universities Commission
NVP	Nevirapine
OAT	organic anion transporter
OATP	Organic-anion-transporting polypeptide
OCT	Organic cation transporters
PANNA	Pharmacokinetics of newly developed ANtiretroviral agents in HIV-infected pregNAnt women
PCR	Polymerase chain reaction
PD	Pharmacodynamics
PEP	Post exposure prophylaxis
PGx	Pharmacogenetics
PI	Protease inhibitors
PK	Pharmacokinetics
PLWH	People living with HIV

PMTCT	Prevention of mother to child transmission of HIV
PrEP	Pre-Exposure Prophylaxis
PROUD	Pre-exposure Option for reducing HIV in the UK: immediate or Deferred
QC	Quality control
RAL	Ribonucleic acid
RANTES	Regulated on Activation, Normal T Expressed and Secreted
RNA	Ribonucleic acid
RPV	Rilpivirine
RTV	Ritonavir
S	Seconds
SAEs	Severe adverse events
SD	Standard deviation
SJS	Stevens-Johnson syndrome
SLC	Solute carrier transporters
SNP	Single nucleotide polymorphism
SoC	Standard of Care
SRM	Selective reaction monitoring
SSA	sub-Saharan Africa

$t_{1/2}$	half-life
TAF	Tenofovir Alafenamide
TB	Tuberculosis
TBME	Tert-Butyl-Methyl-Ether
TETFund	Tertiary Education trust Fund
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir
Tmax	Maximum time
UCL	Upper confidence level
UGT	Uridine 5'-Diphospho-Glucuronosyl Transferase
UK	United Kingdom
ULN	Upper limit of Normal
UNAIDS	United Nations Programme on HIV and AIDS
Vd	Volume of distribution
VESTED	Evaluating the Efficacy and Safety of Dolutegravir-Containing Versus Efavirenz-Containing Antiretroviral Therapy Regimens in HIV-1-Infected Pregnant Women and Their Infants
Vd	Volume of distribution
WHO	World Health Organisation

ZDV	Zidovudine

PUBLICATIONS

Journal Articles

Gini J, Penchala SD, Amara A, Challenger E, Egan D, Waitt C, Lamorde M, Orrell C, Myer L, Khoo S, Else LJ. Validation and clinical application of a novel LC–MS method for quantification of dolutegravir in breast milk. *Bioanalysis*. 19 Nov 2018 https://doi.org/10.4155/bio-2018-0085

Walimbwa S, Lamorde M, Waitt C, Kaboggoza J, Else L, Byakika-Kibwika P, Amara A, **Gini J**, Winterberg M, Chiong J, Tarning J. and Khoo S. Drug Interactions Between Dolutegravir and Artemether-Lumefantrine or Artesunate-Amodiaquine. *Antimicrob Agents Chemother.* 2018 Nov 12. pii: AAC.01310-18. doi: 10.1128/AAC.01310-18.

Gini J, Amara A, Penchala SD, Back DJ, Else L, Egan D, Chiong J, Harri BI, Kabilis ED, Pama PP, Stephen M and Saye Khoo. Widespread use of herbal medicines by people living with human immunodeficiency virus and contamination of herbal medicines with antiretrovirals in Nigeria. *International Journal of STD & AIDS*. 2018 Nov 30: https://doi.org/10.1177/0956462418809749.

Joshua Gini *, Adeniyi Olagunju, Laura Dickinson, Catriona Waitt, Megan Neary, Laura J Else, Marco Siccardi and Saye Khoo. Impact of Pharmacogenetics and Pregnancy on Tenofovir and Emtricitabine Pharmacokinetics. *Pharmacogenomics*. 28-Nov-2018

Conference Abstracts (Poster)

Joshua Gini, Bala I. Harri, Elkanah D. Kabilis, Mark Stevens, Paul P. Pama, Alieu Amara, Dilly P. Sujan, Justin Chiong, Saye Khoo. Contamination of herbal medicines with ARVs and widespread use by PLWH in Nigeria [Abstract # 434]. *Conference on Retroviruses and Opportunistic Infections (CROI 2016),* Hynes Convention Centre, Boston, MA, USA, February 22-25 2016.

Stephen I. Walimbwa, Mohammed Lamorde, Catriona Waitt, Julian P. Kaboggoza, Laura Else, Pauline Byakika-Kibwika, Alieu Amara, **Joshua Gini**, Markus Winterberg, Joel Tarning, Saye Khoo. Dolutegravir interactions with artemether-lumefantrine and amodiaquine-artesunate [Abstract # 459]. *Conference on Retroviruses and Opportunistic Infections (CROI 2018),* Hynes Convention Centre, Boston, MA, USA, March 04-07 2018

Waitt CJ, Walimbwa SI , Orrell C , Lamorde M , Heiberg C, Mehta U , Kabogozza JP , Coombs JA , Byamugisha J , **Gini J** , Kintu K , Amara A , Malaba T , Else LJ . DolPHIN-1: Dolutegravir vs Efavirenz when Initiating Treatment in Late Pregnancy – An Interim Analysis [Abstract # 807]. *Conference on Retroviruses and Opportunistic Infections (CROI 2018).* Hynes Convention Centre, Boston, Massachusetts March 4-7, 2018

GENERAL ABSTRACT

Antiretroviral therapy (ART) has revolutionised outcomes of HIV infection among people living with HIV (PLWH). But despite these great strides, ongoing concerns of drug-drug interactions, widespread use of herbal medication while using ART, safety in pregnancy and breastfeeding are challenges of HIV treatment in PLWH. Potent new ART are not widely used in most low and middle income countries (LMIC) due to insufficient safety data and fear of adverse events. This Thesis aimed at evaluating the pharmacokinetic (PK) safety of DTG in pregnant and breastfeeding women, drug drug interaction of DTG and Artemisinin Combination Therapy (ACT), and evaluate the factors that affect ART in PLWH such as use of herbal medication among PLWH. Therefore the thesis evaluated the following:

Chapter 2 reviewed the clinical PK and pharmacogenetic, drug-drug interactions and safety of DTG in diverse populations from different studies, and concluded that DTG has a variable PK influenced by factors such as food, research conditions and population variability.

Chapter 4 evaluated the PK of DTG in 3rd trimester of pregnancy and postpartum women and found no clinically significant difference in DTG PK between pregnancy and postpartum period in women. Safety and efficiency of HIV control was also evaluated and concluded that DTG efficiently control viral load within a short period of time even when women present late in pregnancy.

Chapter 5 evaluated the drug-drug interaction of DTG and ACT, and concluded that DTG can be co-administered with ACT in treatment of malaria among PLWH on DTG.

Chapter 6 evaluated the impact of pharmacogenetics and pregnancy on tenofovir and emtricitabine Pharmacokinetics. An estimated 1-2-fold increase in FTC blood concentration was observed in pregnant and postpartum women with *ABCC2* 12:g.154962860T>C T allele allele compared to women with CT and CC allele. Sample size was small and was recognise as a limitation. Therefore require verification with larger clinical studies and result should be interpreted with caution.

Chapter 7 evaluated the widespread use of herbal medicines amongst PLWH and contamination of herbal medicines with ART in Nigeria as a recognised challenge of ART in sub-Saharan Africa.

In conclusion, approximately 2-4% of maternal plasma DTG concentration was excreted in BM, and DTG PK changes were not clinically significant in both pregnancy and postpartum period. DTG C_{trough} decreased by 37%, when DTG was administered with AL, 42% when administered with AS-AQ and 24% decrease in AUC_{0-24} when administered with AS-AQ, but were all above the protein adjusted IC_{90} for the C_{trough} and does not warrant dose adjustment. An estimated 41.8% use of herbal medication was recorded amongst 742 PLWH attending HIV clinics. Herbal use preceded HIV therapy in 38.4% while 14.5% were yet to commence ART. A total of 3 (2%) out of 138 herbal samples evaluated, were contaminated with detectable levels of tenofovir and emtricitabine which is a concern, though implication is unknown.

Investigating the Effectiveness of Antiretroviral Therapy in sub-Saharan Africa

CHAPTER 1

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Chapter 1

Investigating the Effectiveness of Antiretroviral therapy in sub-Saharan Africa

1.1 Introduction

Antiretroviral therapy (ART) against HIV is efficacious in reducing mortality and improving life expectancy in all geographical locations (1). It results in viral load suppression, immunological recovery and improves quality and quantity of life of people living with HIV (PLWH) (2). ART has revolutionised HIV outcomes in both high income countries (HIC) and low and middle income countries (LMIC), but more needs to be done in the latter. Though ART is beneficial to PLWH, access to care is variable. In LMIC, less than 50% of PLWH have access to ART while > 90% have access in HIC (3). These regional differences in access and quality of treatment across the globe impacts significantly on outcomes (4, 5). Less impact has been seen in sub-Saharan Africa (SSA) compared to HIC of Europe and America. Mortality and morbidity are still high despite great strides to intensify ART access (3). HIV burden still remains a great problem in SSA. It accounted for 67% (25.5 million out of the 36.7 million PLWH globally) of global burden in 2016 making it an important target region in the quest to end HIV epidemic (6). Mortality, transmission rates and morbidity remain high in SSA. Over 73% (730,000 out of 1 million) AIDS related death and 64.4% (1.160 million out of the 1.8 million) new infections were recorded in SSA in 2016 (3). Despite the high disease burden and deaths in the region, only 61% of adults and 51% of children were receiving ART in East and Southern Africa and 36% of adults and 22% of children in west and central Africa compared to >95% of women and children and 75-95% of

adults in France, United Kingdom, United State, Australia and Canada (3). A total of 20.9 million people have access to ART globally, but only an estimated 11 million (approximately 1.1 million from West and central Africa and about 10 million from South and Eastern Africa) are from SSA despite accounting for 69.5% (25.5 million) of the global burden of infection (3, 7). Over 50% of PLWH in SSA are women of reproductive age. A large proportion of future mothers are likely to expose their babies to HIV, risk of vertical transmission and increased burden of paediatric HIV infections is likely (3). Disproportionate access to ART (approximately 28% in West and Central Africa) is likely to deterrent to the success of the proposed efforts to end HIV epidemic by 2030 (3, 8). It is necessary to critically evaluate the effectiveness of ART programmes as we aim at improving access to ART. Drug safety, appropriate use of drugs and adherence will need to be ensured for these targets to be met. Limited access to ART promotes herbal/traditional medicine use, poor adherence, increased transmission rates and mortality from complications of AIDS (9, 10).

1.2 Pregnancy and Breastfeeding

An important component of ending the HIV epidemic is intensifying treatment among women of reproductive age (>15 years) who account for 48.5% of global, and 51% of SSA HIV burden (3). In 2016, women of reproductive age (15-24years) accounted for 26% of new infection in East and Southern Africa and 22% in West and Central Africa (3). New infection was largely promoted by human right abuses, child marriages, transactional relationships between young girls and older men as a means of affording their basic needs, conflicts and lawless societies which make more young women vulnerable to an imbalance of power and gender-related abuses (3). New HIV infection during pregnancy is also a concern, as most women are likely to be

diagnosed late, exposing the baby to high risk of infection. This high prevalence of HIV infection among women of reproductive age has a direct association with increases in the risk of vertical transmission, and incidence of paediatric HIV infections. Therefore, a consistent rise in the incidence of new infections in women of reproductive age is detrimental to the success of ending vertical transmission of HIV to neonates. Increased education, HIV testing, safe sex practices, prevention of unwanted pregnancies, treatment of all diagnosed cases and high standard antenatal care is required during pregnancy to ensure healthy babies and no compromise to maternal health. Embarking in practices that will improve access to ART, optimise adherence and ensure drug safety in pregnancy and breastfeeding are essential in eliminating vertical transmission. Apart from optimum highly active antiretroviral (HAART) treatment, taking all recommended safety precautions to prevent transmission during breastfeeding is important in protecting babies from HIV infection. Exclusive breastfeeding for six months, prompt cessation of breastfeeding in case of nipple cracks, ulcers, infection or abscess are all important precautions to protect babies from infection. Failure to have efficient system for preventing vertical transmission will increase the rate of HIV infection in children, as observed in many SSA countries where care and access to ART is poor (11).

1.3 Children and vertical transmission

SSA alone accounts for approximately 90% of vertical transmission, paediatric HIV and AIDS related deaths (3). In 2016, UNAIDs estimated 2.1 million children < 15 years living with HIV globally and majority are from sub-Saharan Africa (3). It was the leading cause of death among children and adolescent accounting for 120,000 deaths globally. Although there is a decline in the rate of transmission since intensifying

PMTCT programs, ART only reduces the risk of transmission, but does not eliminate transmission risk. Therefore, it is highly dependent on the effectiveness of the system. Vertical transmission in SSA still remains relatively high compare to other regions of the world (3, 12). This reflects the effectiveness of PMTCT program and need of system strengthening in ensuring program success in the region. Clinical studies that aim at identifying factors mitigating against eliminating paediatric HIV require attention for maximising prevention of vertical transmission. Though successes have been significant, early testing and diagnosis of HIV in paediatric SSA population has been poor, resulting in many undiagnosed cases and poor disease outcomes (3, 13, 14). In 2016, only 43% of HIV exposed children were tested for HIV and few HIV positive children were treated (3, 15). In 2015 West Africa alone accounted for 45% of new HIV infection in children (3) and Nigeria alone accounted for an estimated 41,000 new cases of paediatric HIV (15). In 2017, only 59% of children in East and Southern African and 26% in Central and Western Africa were receiving ART compare to over 90% of children in most HIC (16). Although priority is given for prompt treatment of diagnosed HIV in children, 57% (1.2 million) of children living with HIV are not on ART. Most of these are from SSA, and this is due to low rates of HIV testing. This low rate of testing was attributed to lack of expertise, polymerase chain reaction (PCR) machines and other peculiar logistics in the region such as unstable of power (17, 18) .

1.4 Prevention of Mother to Child Transmission of HIV (PMTCT)

1.4.1 Scale of the problem:

Women of reproductive age have the highest burden of HIV in SSA with risk of transmission to their babies (3). Poor access to HIV services, late presentation and

delayed diagnosis during the antenatal period results in increased risk of vertical transmission in LMIC. Therefore, mitigating against achieving the desired UNAIDS targets (8, 19). Prior to 2016, WHO recommended ARVs to all HIV positive pregnant women during pregnancy, and discontinuation after delivery or breast feeding (20-22). The practice was associated with poor maternal health and risk of resistance to exposed drugs and reduced chances of successful treatment with the same drugs in future (23). However, since changes in 2016, all diagnosed PLWH are required to have ART commenced immediately and maintained for life (24). Current treatment does not just aim at treating disease in infected persons, but using treatment as a prevention strategy (25, 26). Integrating HIV testing and treatment as part of antenatal care significantly reduced HIV burden in children, but without adequate treatment vertical transmission still occur more frequently than expected (27). In 2012, over 90% of paediatric new infections were due to vertical transmission, despite UNAID report of 2017 revealing decline in paediatric infections. This means the current progress is far off the expected pace needed for 2020 targets (8, 19). Prevention of infection in women of reproductive age and unintended pregnancies among infected women is significant in preventing paediatric HIV infections (20). Pre-exposure prophylaxis among high risk women of reproductive age is an important strategy in reducing the burden of HIV among women of reproductive age (28).

Mother to child transmission risk is increased in the following: New HIV infection acquired during pregnancy, maternal high viral load at delivery, treatment failure due to resistant viruses, poor adherence to ART and late presentation for antenatal and ART during pregnancy. Therefore adequate treatment of pregnant women with ART that lower the viral load as fast as possible is required when women present late (24).

1.4.2 Difference between sub-Saharan Africa and Europe

There is no difference in treatment outcomes of HIV between SSA, Europe or America when ART is deployed for treatment of HIV. But when factors such as access to ART, quality of care, inappropriate use of drugs, poor monitoring, adverse events and drug drug interactions are taken into account, obvious differences are observed in the outcomes of HIV infections between SSA and Europe. Linkage to HIV care following diagnosis of HIV is high in Europe as reported by a systematic review that evaluated linkage to care following diagnosis in European population (29), while it is poor in SSA. Reports from SSA population revealed that retention of diagnosed HIV patients and ART initiation is poor (30), and loss to follow up after testing is high. Access to ART is difficult in some cases and drug options for treatment are limited. Diagnostic tools are lacking, and these factors significantly impacts outcome of HIV treatment. Other differences of HIV care between Europe and SSA include: Lack of adequately trained physicians and other health care workers to offer best clinical HIV services to patients, lack of hospital HIV treatment policies, protocols and adequate laboratory services for monitoring in SSA compare to Europe (31).

1.5 Pre-Exposure Prophylaxis (PrEP)

Prevention of HIV among high risk groups is one of the new approaches of preventing HIV infections. High risk groups who are HIV negative are commenced on ART with the aim of preventing them from getting infected by their partners who are HIV positive. Pilot studies such as PROUD provided encouraging result and currently recommended more frequently as a preventive measure for high risk group (32, 33). TFV and FTC combination are commonly used in PrEP (33, 34). It is well accepted by

high risk groups such as sex workers, men who have sex with men (MSM) and uptake is increasing (35, 36).

1.6 Breastfeeding

1.6.1 Breastfeeding and drug safety:

In many SSA countries, prevalence of breastfeeding among women is low, though higher than HIC. Exclusive breastfeeding is also not commonly practice among women who breastfeed their babies (37, 38). Mixed feeding is commonly practice and associated with increased infant mortality and diarrhoeal diseases among children (11, 39-41). WHO recommend exclusive breastfeeding by all mothers, including HIV positive mothers despite risk of HIV transmission because it is the acceptable, feasible, affordable, sustainable and more reliable safe method of feeding babies in most LMIC settings (42). However, due to risk of HIV transmission, all European guidelines universally recommend against breastfeeding (43-45). Breastmilk remains the best source of nutrition, but is complicated in HIV by risk of HIV transmission (46, 47). Fear of excretion of toxic concentration of drug in breast milk, concerns of transmission of multiclass resistant HIV virus during breastfeeding (48, 49) and poor pharmacovigilance of HIV transmission during breastfeeding are major research gaps that require attention to guarantee absolute prevention of breastfed infants of HIV positive mothers who breastfeed.

1.6.2 Mother to child transmission risk in breast feeding

Breastfeeding is associated with increased risk of MTCT especially in untreated HIV (11, 50-52). Despite risk and limited evidence, WHO still recommend exclusive breastfeeding as the preferred feeding method in HIV exposed infants (42). Use of ART to optimally suppress viral load in mothers is important in decreasing the risk of

transmission during breastfeeding (53), hence the importance of good PMTCT programs. Transmission risk is variable depending on breastfeeding method adopted by the mother (54). Exclusively breastfed babies have lower risk of HIV infection during breastfeeding compare to mixed fed babies (55). Risk of MTCT in exclusively breastfed babies may be lower than in mixed fed or predominantly breastfed babies during the postnatal period. In a study comparing the exclusively breastfed babies, mixed fed and predominantly breastfed babies followed up to 2 years, postnatal transmission risk was [4.03 (95% CI 0.98, 16.61), 3.79 (95% CI 1.40–10.29), and 2.60 (95% CI 1.21–5.55) greater risk at 6, 12, and 18 months, respectively when exclusively breastfed infants were compared with mixed fed babies, and predominantly breastfed babies were associated with a 2.63 (95% CI 0.59–11.67), 2.69 (95% CI 0.95–7.63) and 1.61 (95% CI 0.72–3.64) great risk when compared with exclusively breastfed babies (56, 57). Increased risk of MTCT is associated with CD4 cell count of <200 cells/μL, birthweight <2500g and high maternal VL (58). Maternal HIV Env-specific neutralizing and non-neutralizing responses also reduces the risk of MTCT during breastfeeding (59).

1.7 Monitoring PLWH

1.7.1 Relationship of immunological recovery and viral load suppression in ART treatment:

There is an inverse relationship between the CD4 cell count and HIV associated or opportunistic disease. Peripheral blood CD4 count and viral load changes are reliable surrogate markers of disease severity (2). In settings where routine viral load measurement is available, it is recommended at 6 months, 12 months and then after

every 12 months for monitoring ART response while CD4 count can be suspended in individuals who are virally suppressed, to be recommended only when clinically indicated (60). Access to viral load monitoring and CD4 cell count is poor in SSA, making follow-up ART monitoring and investigations difficult. Improving access to treatment with potent ART ensures adequate viral suppression, improve quality of life and reduce transmission rate (61).

1.8 HIV co infections

1.8.2 Malaria

The geographical overlap of malaria and HIV as highly endemic diseases in SSA impact significantly on the frequency and outcomes of disease (62). HIV associated immunosuppression increases the severity and incidence of malaria (62), while HIV coinfection with malaria increases mortality (63). Previous studies were unsure of the impact of HIV and immunosuppression on the severity of malaria (64), but more recent studies have related the use of antimalarial prophylaxis with improved outcomes of HIV. Intensified use of ARVs and malaria prophylaxis among HIV patients decreases severity of both malaria and HIV (65), but potential of drug-drug interaction should always be a consideration in treating HIV/malaria co infection to prevent recrudescence. Evaluating potential drug-drug interaction between ARVs and antimalarial drugs such as artemeter, lumefantrine, amodiaquine, mefloquine, primaquine, artesunate, sulphadoxine and pyrimethamine is important in this region before introduction of any new ARV even if reports have not raised safety concerns of ARVs and commonly prescribed antimalarial (66, 67).

1.9 Drug-drug interactions

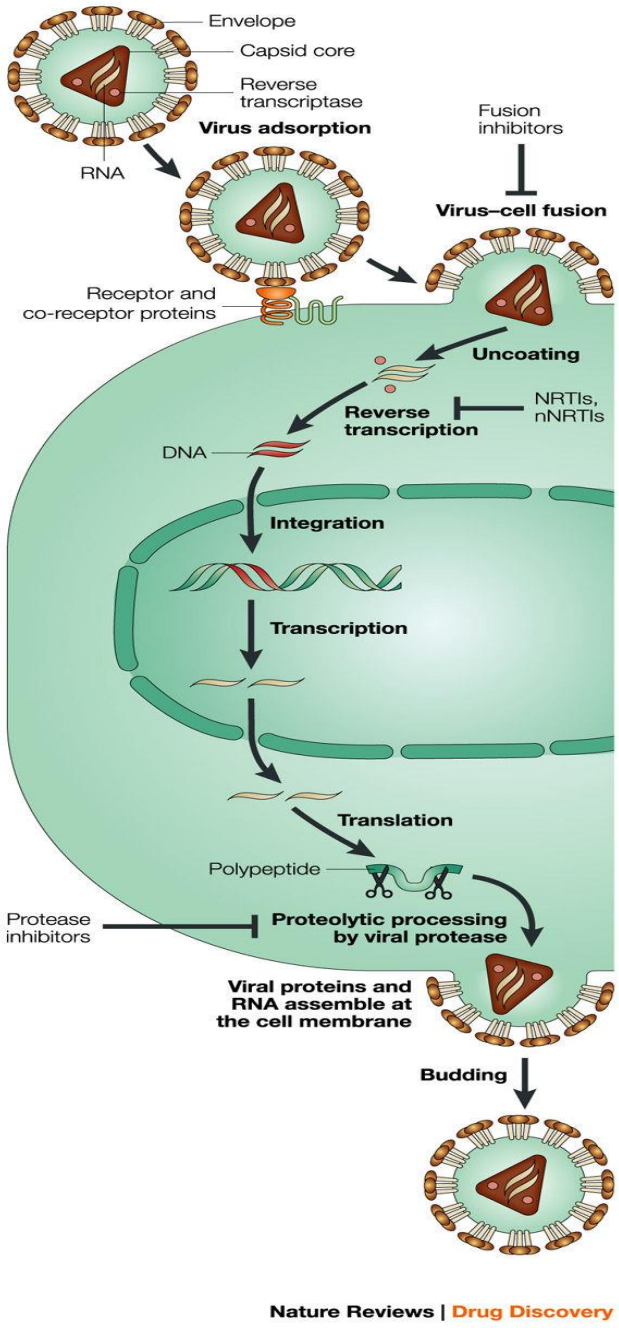
Polypharmacy is common in PLWH especially if there are associated coinfections or comorbidities. Treatment of HIV together with other diseases is associated with drug-drug interaction from shared metabolic pathways or inducing action of some drugs (68). Common inducers like rifampicin use in treatment of TB may induce CYP 2B6 (an important enzyme in metabolism of EFV and nevirapine) (69, 70), CYP3A4 and UGT (important enzymes in metabolism of DTG). Interaction with tropical diseases treatment should be anticipated in HIV treatment because of shared regional prevalence and concomitant treatment of diseases together in sub Saharan Africa. Investigating drug interaction for TB, malaria, parasitic infections, helminths, neglected tropical diseases and all other tropical diseases prior to introduction of new ART will ensure safety in the sub-Saharan Africa population.

1.10 Pharmacology of HIV Drugs

Description of zidovudine antiviral activity in 1985 and subsequent approval for HIV treatment in 1987 by US Food and Drug Administration (FDA) paved way for several advances in the treatment of HIV (71). Another major milestone was also achieved in 1996 where a combination of three highly active antiretroviral therapy (HAART) introduced for use in treating HIV treatment. For improved efficacy and prevention of resistance to monotherapy with (HAART), combination therapy with three drugs (triple therapy) was subsequently introduced. Triple therapy (three drugs from two different classes) revolutionised outcomes of HIV treatment and several modalities are currently used to optimise health outcomes of PLWH. Understanding the life cycle

of HIV virus has been the basis of antiretroviral drug development, presenting ten (10) potential targets of interfering with the life cycle as targets for ARV development. Ability to interfere with any of the ten (10) steps of viral replicative cycle reduces the viral load (72). Based on these targets, ARVS can be classified into the following: Entry inhibitors, Nucleoside/nucleotide reverse transcriptase inhibitors, Non-nucleotide reverse transcriptase inhibitors, protease inhibitors and integrase inhibitors (*figure 1*).

Figure 1: 1.10 HIV Life cycle



HIV life cycle and Ten Targets for antiretroviral drug development. These Targets are: Virus adsorption, Virus-cell fusion, uncoating, reverse transcription, integration, transcription, translation, proteolytic processing, viral protein and RNA assembly and budding. . These targets have been used over the years for development of ARVS. HIV is a retrovirus which depends on reverse transcription of viral RNA and integration of proviral DNA to host (human genome) to progress in the cycle. Targeting these different stages halt the process and cause viral load suppression, with a corresponding immunological recovery, since the virus attacks the CD4 lymphocyte.

1.10.1 HIV Entry Inhibitors

These are group of ARV that target multistep process of HIV entry to CD4+ T lymphocyte by inhibiting viral envelop glycoproteins (gp120 and gp41) and targeting CD4+ T lymphocyte chemokine co-receptors (CCR5 and CXCR4) (73). The process of viral interaction with CD4+ T lymphocyte results in delivery of viral core and RNA into the cytoplasm of host cell (73). The HIV core entry begins with binding of gp120 to cellular receptor of CD4+ T lymphocyte. The interaction result in a conformational change of gp120 and binding to co-receptors (CCR5 and CXCR4) that subsequently trigger gp41 to interact with fusion peptide, bringing the virus and CD4 cell membrane to fuse. Entry inhibitors are classified into fusion inhibitor (enfuvirtide) and chemokine co-receptors (CCR5 and CXCR4 receptor) antagonist (CCR5 inhibitors: maraviroc, cenicriviroc in phase II clinical trials).

The fusion inhibitor class has enfurvitide as its only member. This is a synthetic oligopeptide that targets gp120 and gp41 (74, 75). After binding of gp120 to receptors on cell surface of CD4 cell, the transmembrane protein gp41 mediates fusion with cellular membrane by causing a conformational change in viral core structure and membrane fusion. Enfurvitide inhibits helical regions 1 and 2 (HR1 and HR2) of gp41 preventing conformational change and inability of gp120 to block fusion of viral core membrane, preventing entry. It is administered by subcutaneous injections and has minimal toxicity, but side effect of injection site reaction is a problem that discourages its use.

The chemokine (CCR5) receptors antagonists, such as maraviroc, are CCR5 antagonists that block the MIP 1 alpha and RANTES mediated cell signalling pathway.

Another member of this group is cenicriviroc (In phase II clinical trials). Tropism is required before clinical use because efficacy is determined by the presence of X4 or R5 strains of virus. Side effects include: Orthostatic hypotension in some cases and more frequently headache, asthenia, dizziness, gingivitis and nausea (76).

1.10.2 NRTI: Chemistry, Mechanisms of action, Side effects, Pharmacogenetics

Nucleoside and Nucleotide reverse transcriptase inhibitors are synthetic analogs of de-oxyribo and ribo-nucleosides or nucleotide (tenofovir). They exert their activity by mimicking nucleotides, and subsequent inhibition of the reverse transcriptase enzyme through its P66 subunits. NRTI inhibit both RNA and DNA polymerase activity, but do not have any action on RNase activity. They are activated intracellularly by phosphorylation to their active form in order to exert antiviral activity. Commonly used members of this group include: abacavir, zidovudine, lamivudine, emtricitabine and tenofovir. They are recommended as the NRTI backbone of WHO first line regimen (60). Side effects include: HLA associated hypersensitivity with abacavir, nephrotoxicity and bone toxicity with tenofovir, and zidovudine associated anaemia. They are mostly eliminated by the kidneys via drug transporters that can be influenced by mutations and polymorphism of drug transporter genes.

Tenofovir (TFV): This is an acyclic nucleoside phosphonate analogue of adenosine 5'-monophosphate administered as a prodrug tenofovir disoproxil fumarate or tenofovir alafenamide to enhance stability for formulation and oral bioavailability and absorption(77). Following administration, tenofovir is cleaved by esterase hydrolysis before absorption (78, 79). It competes with the natural substrate deoxyadenosine 5'-triphosphate for incorporation into DNA during HIV transcription.

It has activity against retroviruses and hepadnaviruses and it is also used in the treatment of hepatitis B infections. It is not a substrate of CYP enzymes and has no relevant clinical interactions with most ART, except atazanavir and didanosine (79). It is eliminated predominantly unchanged via renal transporters (80).

Emtricitabine (FTC): This is a cytidine analogue commonly prescribed in combination with TFV for treatment of HIV, or HBV (81). It has a low barrier to resistance and as such it is rarely prescribed alone, but it has synergistic potency in combination with TFV. It is eliminated unchanged via renal transporters in the kidney and well tolerated by patients. Side effects include lactic acidosis and increased risk of liver damage in chronic liver disease.

Zidovudine (ZDV): Zidovudine was the first licenced antiretroviral and was initially prescribed alone as monotherapy before the era of combination therapy (cART). As reverse transcriptase inhibitors their antiviral activity is aimed at competing with essential substrates of proviral DNA (82). Its major side effect is haematological, usually associated with anaemia and other haematological complications (83, 84). Complication such as stillbirths, preterm delivery, small for gestational age, and neonatal death have also been reported in infants exposed to ZDV during pregnancy and breastfeeding (85)

Abacavir (ABC): This NRTI is associated with HLA-B*5701 hypersensitivity and should be avoided in HLA-B*5701 positive patients (86, 87). It is highly potent against HIV-1 virus and commonly prescribed in combination with lamivudine (88, 89). Abacavir is a substrate of human ABCB1 and ABCG2. While ABCB1/ABCG2 have a modest effect on its transplacental kinetics at term, it is frequently used in pregnant women and

have minimal adverse effect on the baby and mother during pregnancy (88, 90). It is metabolised in the liver and eliminated via the kidneys.

Lamivudine (3TC): This NRTI exacts its antiviral activity via an intracellular anabolite lamivudine 5'-triphosphate (lamivudine-TP), eliminated slowly from the cell with a half-life of 15 to 16 h (91). Over 70% of lamivudine is excreted unchanged via the kidneys, and plasma concentration is known to increase in renal pathology (92). It is well tolerated but some side effects include lactic acidosis, which can be associated with severe adverse events (92). Pharmacokinetics can be influenced by drug-drug interactions (93), pregnancy (94), renal pathology and pharmacogenetic polymorphisms in some transporters (95).

1.10.3 NNRTI: Chemistry, Mechanisms of action, Side effects, Pharmacogenetics

This group of compounds, like the NRTI, also inhibit the activity of the reverse transcriptase enzyme by binding non-competitively to a different receptor binding site to cause disruption of the enzyme at it catalytic site. Unlike the NRTIs, this class of compounds do not require activation since they are not prodrugs. Highly bioavailable, but can easily develop resistance, so they are recommended for use with other potent ARVs. They are metabolised by many cytochrome P 450 enzymes. Side effects include: rash (especially after discontinuation of therapy), hepatotoxicity (most common with nevirapine), CNS symptoms and exacerbation of depression

(associated with efavirenz). Some examples include: efavirenz, nevirapine, delarvidine, etravirine and rilpivirine.

Efavirenz (EFV): This is a highly potent reverse transcriptase inhibitor with sustained antiviral activity when used in combination with other ART (96). It is commonly prescribed with other NRTI for the treatment of HIV. Due to its ability to cross the blood brain barrier in significant amount, EFV exhibits significant central nervous system (CNS) side effects (97). EFV is predominantly metabolised by CYP enzymes in the liver and plasma concentration is influenced by enzyme inducers such as rifampicin. It is associated with significant interpatient variability and could sometimes require therapeutic drug monitoring to ensure optimal dosing (98, 99). It is metabolised significantly by CYP 2B6 and polymorphisms of the enzyme impacts on plasma drug levels(100). Glucuronidation by UGT2B7 and polymormophisms of ABCB1 genes are all associated with changes in EFV concentrations (101, 102). CYP 3A4 induction and polymorphisms of CYP2A6 also influences EFV concentration (103).

Nevirapine (NVP): This is a dipyridodiazepinone compound potent in the treatment of HIV (104). It is a selective noncompetitive inhibitor of the reverse transcriptase enzyme and an inducer of CYP P450 enzyme (104, 105). It could be associated with significant hypersensitivity reaction (Stevens Johnson syndrome) (106, 107) and interact with some anti TB medications such as bedaquiline (108).

Rilpivirine (RPV): This is a potent NNRTI with diarylpyrimidine structure that enables tight allosteric binding to reverse transcriptase enzyme (109, 110). After oral administration, C_{max} is achieved 4-5 h (111). Terminal half-life is 34-55 h enabling once daily dosing (111, 112). It is over 99% protein bound and plasma exposure is affected

by protein rich drinks (112). Rilpivirine is a CYP 3A4 inducer, primarily metabolised by CYP 3A, and clearance is affected by drugs metabolised by the same pathway. Its absorption is pH dependent, and should not be administered with proton pump inhibitors and H2-receptor antagonist (112). Sustained released injectable formulation makes it potentially good in improving adherence in HIV patients (109, 113).

1.10.4 PI: Chemistry, Mechanisms of action, Side effects, Pharmacogenetics

Protease inhibitors mimic protease enzyme and inhibit maturation of viral proteins by replacing peptide bonds with hydoxyethylene bond that cannot be easily split by hydrolase enzyme. Thereby inhibiting cleavage of peptides, that is required for maturation of the viral precursor proteins. They mostly have a central core with a hydroxyethylene bond. They interact with the enzyme by fusion with the central cavity to inhibit peptide formation and halt viral replication. Commonly used members include: darunavir (DRV), atazanavir (ATV), lopinavir (LPV) and ritonavir (RTV) (which is mostly used for pharmacological boosting). Other members of the group include: saquinavir, indinavir, amprenavir, nelfinavir and fosamprenavir. They are mostly metabolised by the cytochrome P450 enzymes. Common site effects include: dyslipidaemia, insulin-resistance, cerebrovascular diseases and lipodystrophy/lipoatrophy. They are commonly administered with a pharmacological boaster, an inhibitor of CYP 3A4 to prolong drug half-time.

Darunavir (DRV): It is a non-peptide protease inhibitor with high potency and resistance barrier that inhibit HIV gag and gag pol protein to prevent viral maturation (114, 115). Darunavir is rapidly absorbed and bioavailability is increased with food. It

reaches maximum plasma concentration (C_{max}) rapidly in 2.5-3 h after oral administration of low dose ritonavir boosted darunavir (114, 115). It is better tolerated than LPV (115), and recommended as an alternative second line regimen by WHO for HIV treatment. Its use for treatment of HIV is limited compare to ATZ and lopinavir (LPV) due to evidence gap especially in pregnancy and breastfeeding (60). It interacts with both inducers, inhibitors and substrates of CYP3A4.

Lopinavir (LPV): Is a peptidomimetic inhibitor of protease enzyme. It is administered with small amount of ritonavir for CYP 3A4 inhibition and increased plasma concentration and activity of the drug (116). It can potentially interact with other drugs via CYP enzymes, as seen in other protease inhibitors (116). It is therapeutically effective and has a high resistance barrier. It is well tolerated by patients, but associated with some disturbing side effects such as lipodystrophy, changes of body fat distribution (especially redistribution from arms, legs, face, neck, breasts, and waist), insulin resistance and metabolic syndromes. These LPV associated side effects could be attributed to its role in decreasing glucose uptake by both adipose tissue and muscle, resulting in lipoatrophy and glucose intolerance (117).

Atazanavir (ATV): Exacts its antiviral activity by inhibiting viral gag and gag-pol polyprotein processing in HIV infected cells to prevent maturation of virions (118, 119). Unlike other protease inhibitors, ATV is an inhibitor of UGT1A1, and can potentially interact with integrase inhibitors with resulting pharmacological boosting of the activity of integrase inhibitors or toxicities (120). It was thought to be associated with increase cardiovascular risk (121), however recently some studies have also reported the contrary, when compared with other PI. Cobicistat, inhibits CYP 3A4 to increase bioavailability of PI. It is currently used for pharmacological

boosting of ATV (122), non-active against HIV, but better tolerated and devoid of side effects such as lipodystrophy and other metabolic syndromes.

Ritonavir (RTV): This is a protease inhibitor with potent antiviral activity. It has a long half-life compared to other PI enabling twice daily dosage. It is well tolerated, well absorbed and reaches C_{max} within 2-4 hours after oral administration (123). It is a potent CYP3A4 inhibitor and currently co-formulated in small quantity with other PI to prolong their activity (124). Its advantages in pharmacological boosting via CYP3A4 inhibition is considered for application in surgery, cancer medicine and treatment of hepatitis C (125, 126).

1.10. 5 Integrase inhibitors (INSTI)

These are a group of compounds that inhibit the strand transfer of viral DNA to host DNA by preventing nicking, ligation and covalent linkage of viral and host DNA in the nucleus (127). They inhibit the active site of the enzyme after 3' processing and chelate divalent metal ions by catalytic triad of Asp-Asp-Glu. The integrase enzyme is a protein consisting of three functional domains, encoded by gag and pol genes. Activity of the INSTI is mediated by polyvalent metals and metabolised via uridine 5'-diphospho-glucoronosyltransferase (UGT) enzymes (128, 129). Members of this class of drugs include raltegravir (RAL), elvitegravir (ELV), dolutegravir (DTG) and Bictegravir (BIC).

Raltegravir (RAL): This is the first generation of integrase inhibitors that targets proviral DNA-strand transfer. It is potent against HIV virus resistant against other

antiviral agents (130, 131). It is metabolised mainly via UGT1A1 with minor contributions from CYP3 A4. Its half-life is approximately 9 h, dose is once daily and is well tolerated. Plasma concentration is affected by fatty meals (132) and pregnancy is associated with lower drug exposure to mother but not clinically significant to warrant dose adjustment (133).

Elvitegravir (ELV): This is a potent integrase inhibitor for HIV treatment commonly boosted using a CYP 3A4 inhibitor cobicistat for once daily dosing. It is mainly metabolised via CYP 3A4 and coadministration with CYP3A4 enzyme inhibitor to prolong its half-life. Following oral administration, ELV is rapidly absorbed within 1-2.5 h and with cobicistat or ritonavir boasting, half-life could be prolonged from 3.5 h to 9.5 h (134). ELV PK is influenced by several health conditions such as liver disease, renal disease and age. It is also influenced by food and drug-drug interactions.

Dolutegravir (DTG): DTG was approved in 2013 by the FDA and is currently under evaluation for safety in pregnant women and neonates (DOLPHIN 1&2 ClinicalTrials.gov Identifier: NCT02245022 & NCT03249181). It is metabolised via UGT1A1 with minor contributions from CYP 3A4. It has a high genetic barrier to resistance and is well tolerated by patients (135). Following oral administration, it does not require pharmacological boasting like ELV. Steady state half-life is approximately 14 h enabling once daily dosing.

In the 2016 WHO Guidelines, integrase inhibitors were recommended for wider use following a systematic review which reported that NRTI+INST are more effective compared to EFV+NRTI combination in ART naïve individuals (60). Lower potential for drug resistance, high genetic resistance barrier and shorter time to viral suppression compared to EFV made it to be recommended for wider use in low and middle income

countries. These led to the recommendation of DTG+NRTI combination as a first line regimen of treating HIV (60).

However, WHO and ViiV Healthcare issued a statement of potential increase in risk of neural tube defect among women who become pregnant while on DTG recently. This is because four (4) cases of neural tube defects were reported out of 426 neonates of women who became pregnant while on DTG. This was considered to be high (0.9%) compared to 0.1% risk previously reported for other ART. WHO stated that until there are more data to guide recommendations, women should continue current treatment, but pharmacovilance programs should be strengthen including monitoring of birth outcomes (136).

Bictegravir (BIC): This is a newly licenced integrase inhibitor with activity against all strains of viruses against integrase inhibitors (137). It is currently coformulated with FTC and TAF for a once daily dose. Current available data shows consistency in PK with other INSTI (7, 138). Primarily metabolised by UGT1A1 and minor contribution by CYP 3A4 (138). It is well tolerated and efficacy is non inferior to fixed dose of DTG, ABC and 3TC (138).

1.10.6 Other new treatment and strategies of integrase inhibitors

Cabotegravir: Is a potent INSTI, a structural analogue of DTG formulated as oral tablets and injectable nanosuspension for treatment and prevention of HIV (139). Nanoformulation prolongs the half-life to approximately 40 days enabling monthly intramuscular administration. It is currently in Phase III clinical trials and is a promising option to reduce frequency of dosing. As an analogue of DTG, it is metabolised via UGT1A1, and phase 1 and 2 clinical studies have established that it

has a low propensity to drug-drug interactions (140). Evaluation of tolerability revealed that it is well tolerated and accepted by patients despite minor discomfort at the injection site (141). Currently several clinical trials are ongoing to establish pharmacokinetic safety of the drug (142, 143).

Nanoformulations: for paediatric use and for development of long acting injectable ART are currently evaluated for future use (144). Other strategies of HIV treatment include increasing the role of pharmacogenetics, personalised medicine and medical robotics in management of patients.

Drug Class	Regarding food	Dose (mg)	Steady State (days)	AUC GM (µg.h/mL)	C_{max} GM (µg/mL)	C_{24} / C_t/C_{min} GM (µg/mL)	Renal Clearance (Cl/F (L/h))	T½ (h)
NNRTI								
EFV	Without food	600 OD	6-7	57.90	4.07	1.76	<1% unchanged	40-55
Rilpivirine	With food	25 OD	10-15	2096 (198-7307)***	204±76 ***	73 (2-288) ***	<1% unchanged	45-50
NVP	With or without food	200 b.d/ 400* OD	14-28	109.0(96.0-143.5)	5.74 (5.00-7.44)	3.73 (3.20-5.03)	<3% unchanged	25-30
NRTI								
ABC	With or without food	300 b.d		6.02±1.73	3.0±0.89	0.01	<2%	1.5
ABC	With or without food	600 OD		11.95±2.51	4.26±1.19		<2%	1.5
AZT	With or without food	300 b.d		2.24	2.29	0.02	Exceeds Cr clearance	1.1
3TC	With or without food	150 b.d		4.7	1.2	0.09	>70% renal	5-7
3TC	With or without food	300 OD		8.9	2.0	0.04	>70% renal	5-7
TDF	With or without food	245 OD		3324 ***	326 ***	64.4 ***	32±10% unchanged	˜12-18
FTC	With or without food	200 OD		10.0±3.1	1.8±0.7	0.09±0.07	˜86% renal	10
PI								
ATV	With food	300/100® OD		654 ***	4466 ***	654 ***	7% unchanged in kidneys	8.6

ATV	With food	400 OD		273 ***	3152 ***	273 ***	7% unchanged in kidneys	6.5
DRV	With food	600/100® b.d		116796±33594,114302 ±32681,124698±32286 ***	6500 ***	3490±1401, 3386±1372, 3578±1154 ***	13.9% (r)	15 ®
DRV	With food	800/100 ® or 800/150 © OD		93026±27050, 93334±28626 (r) or 100152±32042, 87909 (20232), 85972 (22413) ©***	8826 *** (C)	2282±1168, 2160±1201(r) Or 2043±1257, 1899 (759), 1813 (859) © ***		9.4
Ritonavir	With food	100 b.d		6.2	0.89	0.22	3.5% unchanged	˜5
Lopinavir	With food	400/100®b. d		82.8±44.5	9.6±4.4	5.5±4.0	<3%	5-6
INSTI								
RAL	With or without food	1200 OD						
RAL	With or without food	400 b.d	2	6.91	2.17	68.6 ***	32% of dose	˜9
Elvitegravir	With fat meals	150 OD	Expected 3-4 days	23.0±7.5 © 18.0±6.5 (r)	1.7±0.4 © 1.5±0.37 ®	0.45±0.26 © 0.35±0.20 ®	Minor route	˜12.9 © ˜8.7-13.7 ®
Dolutegravir	With or without food	50 OD	˜5	53.6	3.67	1.11	31% of oral dose	˜14
Bictegravir	With or without food	50 OD	10	102 (26.9%) **	6.15 (22.9%) **	2.61 (35.2%)**	35% renal clearance	17.3
CCR5 INHIBITORS								
maraviroc	With or without food	300 b.d		2908 (H), 2550 (a), 1513 (e) ***	888 (H), 618 (a), 266 (e) ***	43.1 (H), 33.6 (a), 37.2 (e) ***	˜8% unchanged of oral dose	13.2

(*) modified released, (©) boosted with cobicistat, (®) boosted with ritonavir, (˜) approximately, (%) Percentage, (µg) microgram, OD once daily, (b.d) twice daily, (e) treatment experienced HIV patients (a) Asymptomatic HIV patients, (mL) millilitre, (ng) nanogram, (**) mean (%CV), (Cr) creatinine, (***) ng/mL or ng.h/mL

Table 1: Summary of ART Pharmacokinetics

1.11 Pharmacogenetics of ART

Pharmacogenetics describe the genes that determine responses to drugs and mutations in different individuals that significantly influence how individuals respond to drugs due to several factors (145). Variability in drug responses observed and interactions are influenced by mutations in genes. Mutations in genes result in polymorphisms that can impacts on enzymes or transporters functions (146, 147). A relevant pharmacogenetics impact of genetic polymorphisms in HIV medicine is ABC hypersensitivity associated with HLA-B*5701 (148), where presence of the gene is associated with a 2.7% incidence of hypersensitivity (148). Other associated adverse events include: increased EFV associated CNS toxicity with CYP 2B6 poor metabolisers, and NVP associated hypersensitivity reactions (149). Correlation of certain HLA alleles with hypersensitivity and associated Stevens Johnson Syndrome (SJS) are all examples of the role of pharmacogenetics in ART (145). Polymorphisms in genes associated with cytochrome P450 enzymes, UGT enzymes and transporters responsible for metabolism of ART have significant influence on drug exposure, interactions and safety (149).

1.11.1 Genetic polymorphisms:

Mutations in genes that encode metabolic enzymes and transporters gives rise to genes that alter drug metabolism. Polymorphisms of CYP enzymes, transporters, and UGT enzymes impact on ART exposure. Polymorphisms in CYP enzymes (2B6, 2D6, 3A4,) significantly affects the PK of ART such as EFV, NVP and PI. Nucleotide polymorphisms of transporters such as ABC and SLC also affect TFV, FTC and 3TC PK. Other enzyme polymorphisms such as UGT1A1 polymorphism have significant impact on DTG, RAL and ATV exposure.

1.11.2 Cytochrome P450 Enzymes (CYP)

Cytochrome P450 enzymes (CYP) are microsomal mixed function oxidase system of enzymes involve in biotransformation of therapeutic drugs to render them soluble for elimination (150). Multi-allelic genetic polymorphisms influenced by several factors leads to pharmacogenetic phenotypes that influence metabolism of the drugs (151), a major source of variability in drug pharmacokinetics and pharmacodynamics. These enzymes are involved in metabolism of several classes of ART including NNRTI, PI, and INSTI. The NNRTI EFV is primarily metabolised via CYP 2B6, with secondary contributions from CYP 2A6, and minor contributions from CYP 1A2, and CYP 3A4/5. CYP 3A4 and 2B6 are the major enzymes in NVP metabolism, with minor contributions from CYP 3A5, 2D6 and 2D9 (152). PIs are metabolised mainly via CYP3A4 with minor contributions from other CYP enzymes, UGT and transporters depending on the protease inhibitor (149). Integrase inhibitors, though not primarily metabolised by CYP enzymes, have minor contributions of CYP 3A4, and ELV is predominantly metabolised through CYP 3A pathway. These enzymes are prone to mutation and polymorphisms with significant induction of variability on drug PK between individuals, resulting in either reduced function or increased elimination (153).

1.11.3 Transporters

These are membrane polypeptides that facilitate transport of drugs across membranes. They significantly regulate drug pharmacokinetics via the subclasses of transporters (154). Transporters are classified as ATP binding cassette (ABC), or Solute carriers (SLC) transporters. The ABC transporters are further sub classified as P-glycoprotein (encoding for multigene family of ABCB1 and ABCB2), Multidrug resistance associated proteins (MRP belonging to ABCC subfamily), breast cancer resistant protein (BCRP encoded by ABCG2). The SLC

transporters are further classified into Organic anion transporting polypeptides (OATPs) responsible for drug uptake into cells, Organic anion (OAT) and cation (OCT) transporters. Other SLC transporters include: Concentrative (CNT) and equilibrative nucleoside transporters (ENT), belonging to the SLC28 and SLC29 subfamilies (155). Transporters play important role in the PK properties of ART (156) and mediate transport across different barriers in the body including: blood brain barriers, blood-intestinal mucosal barriers and blood testicular barriers (157). NRTI depends on renal transporters for their elimination and can be influenced by polymorphisms.

1.11.4 UGT enzymes

UGT enzymes are a family of membrane proteins encoded by genes located in chromosome 2q37.1 for UGT1 and 4q13.2 for UGT2 (158). Important in ART is UGT1 which is involved in metabolism of integrase inhibitors and PIs. These enzymes are involved in biotransformation and solubilisation of ARVS before elimination from the body. The enzymes also mediates drug interactions of co-administered drugs metabolised by the same pathway. UGT1A1 is the major metabolising enzyme of DTG, raltegravir and ATV. Mutations and resultant genetic polymorphisms in this enzyme significantly affects drug PK (159).

ART	Substrate	Enzymes (CYP 450 and UGT)		Transporters	
		Inhibitor	Inducer	Inhibitor	Inducer
NNRTI					
EFV	CYP3A4, 2B6 (minor)	CYP 2C9, CYP 2C19	CYP 3A4 (potent), CYP 2B6, UGT1A1		
Rilpivirine	CYP3A4 (major), CYP 2C19, 1A2, 2C8/9/10 (minor)		CYP 2C19, (moderate), CYP1A2, 2B6 and 3A4 (weak)	OCT2	
NRTI					
Tenofovir –DF	P-gp, MRP4, BCRP			MRP1, MRP2, MRP3, Pgp	
Tenofovir Alafenamide	P-gp, BCRP; minimal metabolism via 3A4	3A4 (weak- in vitro)			
FTC	MRP1			MRP1, MRP2, MRP3, Pgp	
Abacavir (ABC)	Pgp, MRP4, BCRP			MRP1, MRP2, BCRP	
3TC	BCRP			MRP1, MRP2, MRP3	
PI					
ATV	Mainly CYP3A P-gp, MRP1	3A4, UGT1A1 >>2C8		P-gp, MRP1, OATP1B1, OATP1B3, BCRP	
Darunavir	Mainly CYP3A, P-gp	CYP3A4	CYP1A2		
Ritonavir	CYP3A4, P-gp, MRP1	CYP3A4 (potent)> >2D6* >2C9 >2C19 >2A6 >1A2>2E1.	UGT, CYP1A2, CYP2C9/19, 2B6	P-gp, OATP1B1, OATP1B3, BCRP, OATP2B1, OCT2	
Lopinavir	CYP3A4	CYP3A4, CYP2D6	UGT, CYP1A2		
INSTI					
Raltegravir	UGT1A1	NIL	NIL		
Elvitegravir	CYP3A4		CYP2C9 (modest)		
Dolutegravir	UGT1A1 (major), CYP3A4 (10-15%)			OCT2 (strong), MATE1	
HIV CCR5 INHIBITORS					
Cenicriviroc	CYP3A4, 2C8.	unknown	NIL		
Maraviroc	CYP3A4, P-gp	NIL	NIL		

[156], https://hivclinic.ca/.../ARV-and-DAA-pharmacokinetic-characteristics-summary-table...

Table 2: Substrates, inhibitors and inducers of ART

1.12 Thesis Hypothesis:

Dolutegravir remains safe and effective across diverse population when deployed as a first line ART in sub-Saharan Africa.

1.13 Thesis Aims:

Following the WHO recommendation of DTG as first line ART and the likelihood of this regimen being rolled out for widespread use in sub-Saharan Africa, DTG safety in various thematic areas of care will need to be evaluated to ensure limited adverse event and optimal therapeutic benefit to patients. Therefore, this thesis aimed at the following:

Developing and validating a novel method of quantifying DTG in dried breast milk spots to be used for evaluating drug safety in breastfeeding. The developed method was subsequently applied in an ongoing DolPHIN 1&2 clinical trial (NCT02245022) to quantify the amount of drug excreted in breast milk. DTG concentrations were also quantified in plasma and PK of DTG in corresponding mothers evaluated as part of the clinical trials to determine the PK safety of DTG in pregnant women and their breastfed neonates.

Evaluating the drug-drug interactions between DTG and commonly prescribed antimalarial agents, collectively known as artemisinin combination therapy (ACT) (artemether lumefantrine (AL) and artesunate amodiaquine (AQ)). Since malaria is also highly prevalent in sub-Saharan Africa accounting for approximately 445 000 malaria related deaths in 2016 (160). Co-infection with malaria is likely to be a common encounter among HIV patients in this region.

Evaluating drug transporters with high allele frequency in a Nigerian pregnant and postpartum women population to determine the pharmacogenetic impact of pregnancy on

TFV and FTC PK. This is because these two NRTI are widely used and are likely to be co-formulated with DTG for PMTCT and general population in the region.

Finally, to evaluating the prevalence of herbal medication use in relation to ARV initiation and contamination of herbal medications with ART.

Chapter 2

CHAPTER 2

Dolutegravir: A Review of Clinical Pharmacokinetics and Pharmacogenetics, Drug-Drug Interactions and Safety in Diverse Patient Populations

2.1 Introduction

Dolutegravir (DTG) is an integrase strand transfer inhibitor (INSTI) recommended by WHO in 2016 guidelines as an alternative for first line treatment of HIV (161). It demonstrated safety and efficacy against INSTI resistant viruses in early studies (135), and efforts are currently made for affordable generic formulations in low and middle income countries (LMIC). South Africa, Kenya and about 90 other LMIC launched plans and agreed with other global stakeholders to make DTG available and accelerate rollout to intensify and ensure universal access to treatment (162). Lack of pharmacokinetic (PK) safety data in pregnancy and breast feeding, safety in children < 12 years, and PK drug-drug interaction data with some antimicrobials such as rifampicin and antimalarials make approval for use in these clinical condition delayed (159, 163-165). Furthermore, dramatic physiological changes of pregnancy and pharmacogenetics /pharmacokinetics (PGx/PK) impacts on safety and disposition of drug in many ways (128, 166). Diverse genetic polymorphisms across different populations also influence drug-drug interactions, safety and efficacy of DTG (128, 167).

2.2 Pharmacokinetics

DTG is characterised by a highly stable and predictable pharmacokinetic (PK)-pharmacodynamic relationship due to limited variability in PK parameters (159, 168). It is readily absorbed after oral administration and can be influenced by food (fasted, low fat, moderate and high fat diet) (169). At plasma steady state, DTG exposure significantly correlate with HIV RNA viral suppression, with a linear relationship between PK parameters and viral suppression (168). DTG distribution in cervical and vaginal tissue is significantly high (mostly above the protein binding-adjusted 90% inhibitory concentration (IC_{90}) (170), and it is widely distributed in other body tissues and fluids including central nervous system (CNS) and cerebrospinal fluid (CSF) (171). Its half-life is approximately 14 h and steady state is achieved in approximately 5 days (172). Interactions with mineral supplements containing divalent cations (ferrous fumarate and calcium carbonate, magnesium and aluminium) results in decreased drug exposure (173, 174). Other pharmacological agents could potentially interact with DTG since it is a lifelong HIV treatment (175-177). Transplacental transfer, neonatal toxicities and concerns of secretion in breast milk are currently evaluated in different clinical studies (PANNA, IMPAACT, and DOLPHIN etc) (178-180). DTG was reported to accumulate in neonate (four times the accepted trough concentration) but adverse events reported in the neonate were thought to be low compare to the benefits of the drug in prevention of vertical transmission of HIV (166).

PK of DTG can be influenced by disease conditions including renal and liver disease. However, no significant changes in DTG PK were observed in the few published reports of both renal and mild to moderate liver impairment (181-183). Pregnant women, children and the elderly

also experience associated changes in DTG PK. The immature UGT enzymes in children lead to decreased metabolic efficiency, resulting in DTG accumulation; as such DTG is only recommended for children aged greater than12 years (184, 185). Though these impacts may not be sufficient to warrant dose adjustment (182, 186), there should be cautious use of DTG in these group of patients especially when plasma concentration cannot be monitored. There are several ongoing PK and PGx studies of DTG in healthy volunteers, people living with HIV (PLWH) and special populations (pregnant women, children and elderly), to evaluate drug safety , which will help in providing sufficient safety data for regulatory institutions and approvals for use in the mentioned populations (166, 182, 187).

2.3 Pharmacogenetics (PGx)

DTG is not a known drug-metabolising enzyme inducer or inhibitor, but it is metabolised via minimal cytochrome P 450 enzyme (CYP 3A4) contributions and predominantly Uridine 5'-diphospho-glucuronosyltransferase (UGT1A1) enzymes. It has the potential to be influenced by enzyme polymorphisms. The potential impact of such polymorphisms is to cause a change in plasma drug concentrations, drug-drug interactions and suboptimal drug exposure or toxicities. It is an inhibitor of organic cation transporter 2 (OCT2), and increase possibility of drug-drug interaction with OCT2 substrates (examples: metformin, prazosin, dopamine, cimetidine) by decreasing the excretion of drugs that utilise the OCT2 pathways, with resultant increase in plasma concentration and risk of toxicities (188). UGT1A1 is a highly polymorphic enzyme responsible for glucuronidation of DTG to its soluble form. CYP3A4 minor contribution to DTG oxidative biotransformation pathway is via oxidative defluorination and glutathione substitution to soluble product for excretion (189). The UGT1A1 enzyme pathway is primarily responsible for biotransformation of DTG to the inactive ether glucuronide metabolites (189). It is highly polymorphic and functional polymorphisms

in UGT1A1 could result in decrease enzyme activity which impact on DTG elimination and drug accumulation in the body (163, 164, 190, 191). Some polymorphisms of UGT1A1 have been reported to be associated with reduced metabolic activity of the enzyme (159). Poor metabolisers with UGT1A1 *28/*28, *28/*37 and *37/*37 genotypes were associated with decrease clearance (CL/F), increase C_{max} and increase AUC, but not significant enough to warrant any dose adjustment (159).

This review aims at collating different DTG PK studies to determine the mean DTG PK parametres. It aimed to collate DTG PK data of DTG administered alone for both HIV-positive and healthy volunteers in different clinical settings. It also aimed at evaluating DTG PK changes in pregnant women, children <12 years, the elderly, kidney disease and liver disease patients.

2.4 Data source and study selection

Relevant studies were identified from PubMed, Web of Science, Google Scholar and abstract presentations from conferences (CROI 2016) from 2012-2018 that evaluated the PK of DTG alone as an arm of a study (Table 1& figure 1). Search key words were DTG and PK (PubMed, Web of Science and Google scholar). Studies were screened and duplicates removed by checking in the search engines. Eligibility criteria included: All studies that evaluated the PK of DTG in plasma in human subjects, reported on the PK of administered alone, even if the study also reported on DTG co-administered with other drugs, studies reported in English and described the PK of a 50 mg once daily dose. Studies considering treatment switching or viral response in relation to dose without careful attention to PK analysis and twice daily 50 mg dose or <50 mg dose were excluded.

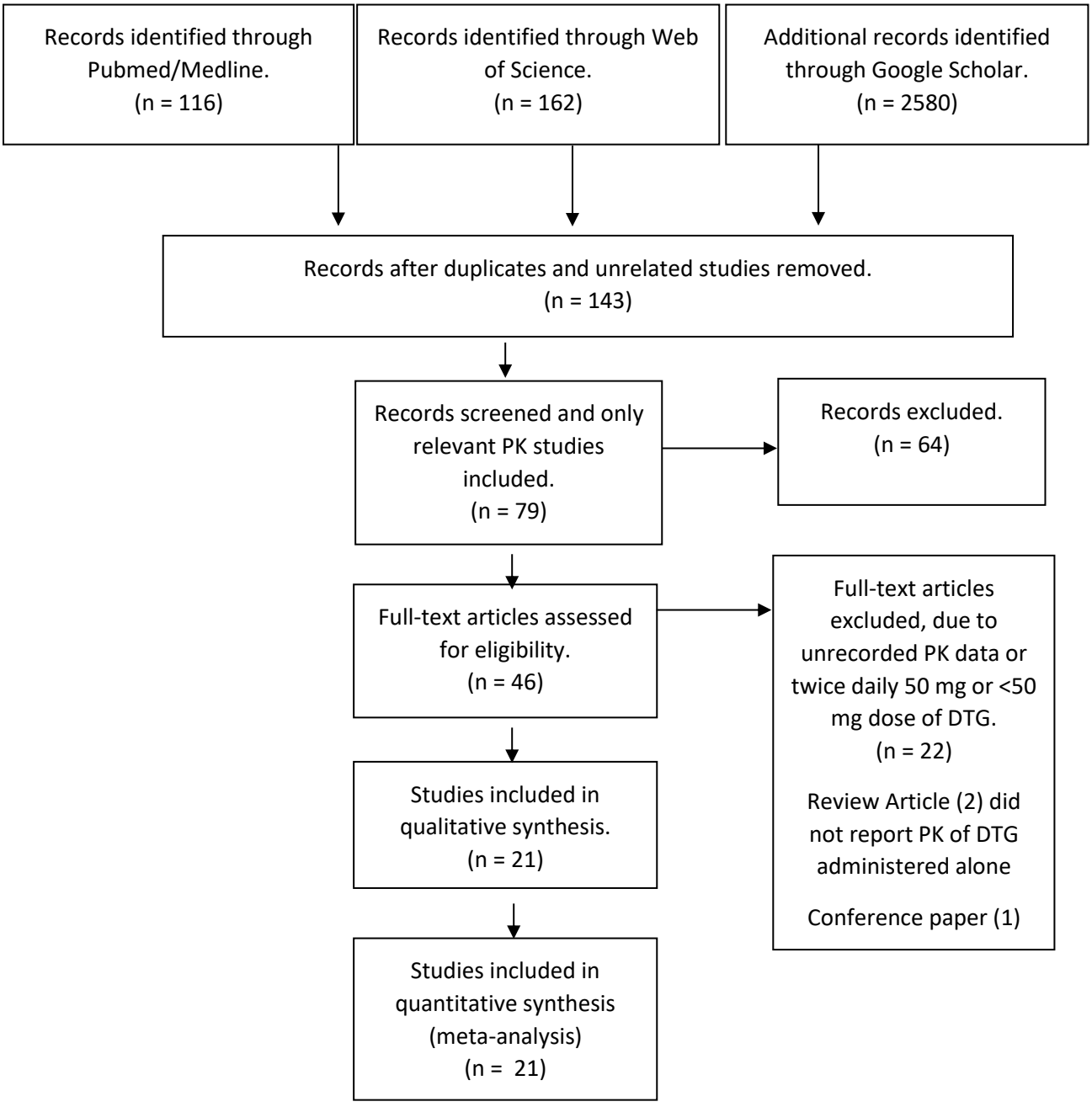


Figure 1: Flow Diagram of stages of literature search

2.5 Data extraction and statistical analysis

Statistical advice was obtained to determine the appropriate statistical methods required for this review. The advice included: The appropriate extraction method, how the data should be analysed and studies to be excluded. Since the PK data were heterogeneous, comparing DTG PK of different drugs and mineral components of diverse chemical structures and properties, meta-analysis cannot be used. Furthermore, it is important that PK data of HIV patient should be evaluated separately from that of the healthy volunteers because of impact of HIV on drug PK.

The review identified 21 studies where DTG PK was evaluated after 50 mg DTG was administered alone. A total of 640 volunteers (327 HIV infected and 313 healthy volunteers) were evaluated in the 21 studies. Extracted data included: Sample size, healthy volunteers or HIV infected, nutrition state (fed or fasted), steady state or Single dose PK, (GM (IQR) or GM (%CV) of AUC, C_{max}, C_{24} or C_t, Cl/F (L/h), T_{max} (h) Median (range), and half-life (h). Pooled geometric means (GM) and interquatile range (IQR) or GM and percentage coefficient of variance (%CV) of the evaluated pharmacokinetic parametres were evaluated for highest and lowest measured PK parameter using Microsoft Excel (2013).

2.6 Results

A total of 32 'DTG alone' study arms from 21 clinical studies were evaluated including 379 subjects (122 HIV infected and 257 healthy volunteers) were evaluated. Abstracts included were 2, and the highest evaluated AUC_{24} (GM (IQR)) µg/mL and GM (%CV) were 71.1 (13.05, 84.96), and 68.186 (%CV 30), C_{max} (GM (IQR)) µg/mL or GM (%CV) 5.10 (3.75 - 7.23) and 5.29 (15.8%), C_{24} or C_t (GM (IQR)) µg/mL and GM (%CV) 1.70 (0.76 - 2.00) and1.500 (24.0), Cl/F

(L/h) (GM (IQR) and GM (%CV) 1.27 (1.02-1.94) and 1.2 (CV 39%), Tmax (h)/Med (range) 4.0 (1.0–4.0), t½ (h) 14.5 (13.1, 16.1) (*Table 1*).

Study Title and Trial registrationnumber	**Sample size**	**Instructions regarding food**	**AUC GM (IQR) ug*h/mL**	**C_{max} GM (IQR) ug/mL**	**C_{24} or C_t GM (IQR) ug/mL**	**Cl/F (L/h) GM (IQR)**	**Tmax (h) Med (range)**	**T½ (h)**
SPRING 1: ClinicalTrials.gov: NCT00950859 (192)	15[b]**	Without regard to food	48·1 (40%)	3·4 (27%)				
Lowered DTG in pregnant woman Case report (193)	1[b]**		48	4.0,	0.9			12 h
DTG Postpartum woman, Case report (193)	**		106	5.4	3.3			22 h
IMPAACT P1026s Second Trimester (180)	9[b]**	With or without food	58.4 (47.6 - 64.5)	4.5 (3.8 - 5.2)	0.8 (0.6 - 1.9)	0.8 (0.7 - 1.0)	2 (2 - 4)	10.5 (8.7 - 12.6
IMPAACT P1026s Third Trimester (180)	15[b]**	With or without food	48.7 (40.3-57.6)	3.9 (3.3-4.4)	0.9 (0.7-1.2)	1.0 (0.8-1.2)	4 (2-4)	11.2 (10.3-13.0)
IMPAACT P1026s Postpartum (180)	9[b]**	With or without food	71.1 (5.0 - 83.1)	5.1 (3.7 - 7.2)	1.7 (0.9 - 2.2)	0.7 (0.6 - 0.8)	2 (2 - 4)	12.3 (10.5 - 15.6)
IMPAACT 1093: DTG in 6-12 Year Old HIV Infected Children: 48-Week Results (194)	21[b]**		50.4 (63%)		0.9 (89%)			
IMPAACT P1093 48 WEEKS (195)	22[b]**	Standard research food	43.1 (13.1, 84.9)	3.4 (1.1, 6.0)	0.9 (0.2, 2.1)	78.0 (0.5, 3.8)	3.0 (1.0, 6.0)	11.8 (8.2, 24.8)
Single and multiple dose PK of DTG in the genital tract of women. Clinical	8[b]*	Standard research food	55.4 (43.3–62.2)	3.7 (3.4–5.2)	0.1 (0.1–0.1		2.5 (1.3–4.0)	14.8 (13.6–16.1)

trial identifier NCT01404806(170)								
Effects of boceprevir and telaprevir PK of DTG NCT01563328 Cohort 1 (196)	16[b]*	Moderate fat meal.	61.5 (53.5, 70.8)	4.6 (4.1, 5.1)	1.3 (1.1, 1.5)		2.5 (1.0, 4.0)	13.2 (12.0, 14.5)
Effects of boceprevir telaprevir PK of DTG ClinicalTrials.gov (NCT01563328 Cohort 2 (197)	16[b]*	Moderate fat meal	68.9 (60.3, 78.6)	4.9 (4.4, 5.6)	1.5 (1.3, 1.9)		3.0 (1.0, 4.0)	14.5 (13.1, 16.1)
Drug-Drug Interaction Period 1 (177)	12[b]*	Standard research food	66.6 (17.0)	5.3 (15.8)	1.5(24.0)		3.0 (1.0, 6.0)	15.5 (1.8)
Increase in DTG trough, but equivalent total DTG exposure with simeprevir in HIV/HCV NCT02404805 (198)	24[b]*	Standard research food	68.2 (30)	5.2 (23)	1.3 (38)	7.3(24)	2.5 (41)	12.8 (17)
Antiviral activity, safety, and PK/PD of DTG (168)	10[b]**		43.4 (20)	3.3 (16)	0.8(26)		2.0 (0.9–4.0)	12.0 (22)
PK of DTG co-administered with acid-reducing agents and multivitamins in healthy volunteers (199)	16[a]*	Standard research food	34.6 (31)	2.0 (25)	0.5 (38)			
PK of DTG co-administered with acid-	12[a]*	fasted	31.0 (53)	1.8 (44)	0.5 (63)			

reducing agents and multivitamins in healthy volunteers (199)								
Comparative Clinical PK/ PD [9]	10[b]**		43.4 (20)	3.3 (16)			2.0 (0.9–4.0)a	11–12
DTG PK with and without DCV (200)	12[b]*		35.7 (34.7)	2.6 (32.0)	0.7 (41.3)	1.4 (34.7)		
PK of DTG+$CaCO_3$ (173)	12[a]*	fasted	35.6 (62.3)	1.9 (45.9)	0.5 (66.0)		3.0 (0.5, 6.0)	13.9 (32.8)
PK of DTG + Ferrous Fumarate (173)	11[a]*	fasted	33.6 (39.6)	1.7 (40.6)	0.5 (40.8)		3.0 (1.0, 6.0)	
Effects of enzyme inducers EFV & tipranavir/ritonavir on the PK of DTG ClinicalTrials.gov (NCT01068925 (201)	12[b]*		42.3 (39)	3.1 (30)	0.9 (53)		2.0 (1.0–4.0)	
Effects of enzyme inducers EFV & tipranavir/ritonavir on the PK of DTG ClinicalTrials.gov NCT01098526 (201)	14[b]*		64.5 (28)	4.5 (23)	1.4 (40)		3.0 (2.0–3.0)	
Effect of fosamprenavir-ritonavir on the PK of DTG ClinicalTrials.gov (NCT01209065) (202)	12[b]*		32.9 ±13.9	2.5 ± 0.9	0.6 ± 0.3	1.7 ± 0.5	2.0 (1.0–4.0)	12.3 ± 2.4
Effects of Etravirine on the PK DTG (203)	15[b]*		60.4 (22)	4.3 (19)	1.2 (29)	0.8 (22)	3.0(1.0–4.1)	12.4 (21)

Effects of Ritonavir-Boosted PI on the PK DTG Cohort (203)	8[b]*		47.8 (19)	3.5 (12)	0.9 (33)	1.1 (19)	3.5 (2.0–4.0)	11.3 (18)
Effects of Ritonavir-Boosted PI on the PK DTG Cohort 1(203)	9[b]*		45.2 (22)	3.3 (26)	0.9 (40)	1.1 (22)	3.0 (1.0–12.0)	10.4 (17)
Effect of CBZ on DTG PK ClinicalTrials.gov (NCT01967771(204)	16[b]*		53.8 (21.4)	4.1 (14.4)	1.2 (39.1)	0.9 (21.4)		12.9 (23.8)
Effect of prednisone on PK of DTG ClinicalTrials.gov (NCT01425099) (205)	12[b]*		61.3 ±20.5	4.4 ±1.0	1.4 ± 0.7	0.8 ±0.2	4.0 (1.0–4.0)	14.1 ±3.0
PK of DTG in With Hepatic Impairment (206)	8[a]*		40.3 (15.1)	1.9 (0.8)	0.6 (0.22)	1.4 (0.7)	3.0 (1.0–4.0)	15.3 (3.9)
PK of once-daily DTG and ritonavir-boosted darunavir AUC12 (207)	10[b]**	Standard meal	26.8 (22.4–28.4)	3.43 (2.9–4.0)	0.6(0.4–0.2)			
PK Interaction of Doravirine and DTG (175)	12[a]*	Standard research food	42.9 (37.0, 49.6)	3.1 (2.5, 3.6)	1.0 (0.8, 1.2)		.5 (0.5, 3.0)	

[a] 50 mg dose steady state PK, [b]50 mg dose single dose PK, *Healthy volunteers, ** HIV 1 infected, DTG: Dolutegravir, PK: Pharmacokinetics, AUC: Area under the Concentration time curve, CBZ: Carbamazepine, EFV: Efaverenz, DCV: daclatasvir, HCV: hepatitis C virus, PD: Pharmacodynamics

Table 1: Summary of Dolutegravir Pharmacokinetics administered alone in different studies

2.7 Quality Assessment

The quality of included studies and risk of bias was assessed using the Newcastle-Ottawa quality assessment scale. This assessed adequacy of case definition, representation, definition of controls and end point in the selection process. Comparison of exposure and outcomes were all evaluated and scored as recommended by the scale (208).

2.8 Discussion

This review evaluated the PK data of various clinical studies that assessed the pharmacokinetics of DTG in healthy volunteers and HIV infected patients. PK data reported by different clinical studies were collected and evaluated for minimum and maximum reported PK parameter. Pharmacogenetics drug-drug interaction data were also pooled to determine effect of other treatment on DTG exposure and efficacy. Use of DTG in different population groups such as paediatric, pregnancy, liver and kidney disease were also evaluated for any changes and reported.

2.8.1 Safety in Special Populations (pregnant and breast feeding women, children and elderly)

Paucity of information on DTG safety in paediatric population, pregnant women, the elderly, some chronic diseases and organ failure such as liver and kidneys is a recognised research gap. This was recognised as a major mitigating factor against approvals by regulatory authorities in resource-limited countries. Although there are several ongoing clinical studies aimed at answering some of the unknown safety concerns of DTG use in these populations, lack of adequate monitoring tools is discouraging clinicians from LMIC from prescribing it until adequate information is made available.

2.8.2 Effect of pregnancy on DTG disposition

Physiologic changes impact on drug disposition (209), therefore following a 50mg once daily dose of DTG at steady state in pregnant women, exposure is reduced compared to non-pregnant women, but trough concentrations were well above EC_{90} of non-pregnant women in phase 2 and 3 clinical trials (166, 179, 185, 210). It is well tolerated and viral load suppression is achieved within few weeks of commencing therapy. If prescribed with suspected or known enzyme inducers such as rifampicin in treatment of pregnant women with TB/HIV coinfection, twice daily prescription is considered, and should be use with caution due to limited safety evidence (211)[22].

2.8.3 Dolutegravir PK in Neonates and children

DTG is highly transferred through the placenta to the neonates, and has been observed to be high in a case report of a premature neonate and other clinical trials (210). But despite high concentrations in neonates and 4 fold longer half-life, it is well tolerated (166, 179, 184). Safety concerns are currently further evaluated in DOLPHIN 1&2 clinical trials. Poorly developed UGT1A1 and low enzyme activity in neonate is suspected to significantly contribute to the accumulation. The common indicator of UGT1A1 activity is bilirubin where decrease enzyme activity is associated with hyperbilirubinaemia as seen in Gilbert syndrome and Crigler-Najjar syndrome associated polymorphic UGT genes (187), but relationship with DTG is not yet evaluated and is unknown. Africans are recognised to have less-efficient UGT1A1 promoters predicting higher average bilirubin levels, but bilirubin elimination is also thought to be influenced by other environmental factors such as ultraviolent radiation therefore the need for further evaluation (212).

2.8.4 Dolutegravir Excretion in Breast milk

Breast milk and transplacental transfer of DTG is not fully understood, and was thought to be protective against HIV infection (178), but previous reports shows that infants who became infected during breast feeding despite ART, were at risk of multiclass drug resistance (48, 49). DTG safety in pregnancy and breast feeding is also a concern and is currently being evaluated in several clinical trials (178). Clinical data on excretion of DTG in BM, and implication on infants health is limited (209), but excretion of drugs in breast milk is known to occur especially during the colostral phase (3-4 days after delivery) due to increased size of pores in the mammary epithelium (213). Transplacental transfer of DTG to neonates is high and DTG concentration persist in neonates after delivery for sometimes.

2.8.5 DTG in Liver and Renal disease

UGT1A1 and CYP3A4 are produced in the liver, and excreted unchanged in the kidneys, however there are currently no reports of significant changes in drug elimination that will require dose modification (182, 186). Post marketing surveillance and some other pharmacovigilance studies should be conducted to further evaluate the impact of liver and renal diseases in elimination of DTG since clinical studies in renal and liver diseases are difficult to conduct.

2.8.6 Pharmacokinetic of DTG in HIV infected and healthy volunteers

DTG is well tolerated by HIV-1 infected patients. PK parameters were variable in different reports but differences were insignificant between DTG PK in healthy volunteers and HIV infected patient. Although the reason for the observed differences in the reported PK

parametres is unknown, several factors such as food, population variability and research conditions could be responsible for the differences. The highest AUC_{0-24} of DTG reported in a single case report by the PANNA network of postpartum period was 106 µg/mL. Otherwise, highest AUC_{0-24} was GM (IQR) 71.1 (58.0 - 83.1) ug*h/mL, reported by the postpartum arm of IMPAACT studies (180). The lowest AUC_{0-24} was 26.81 (22.44–28.46) µg*h/mL reported by the DUALIS study. Similarly, $C_{24\ or\ t}$ of PANNA network of the same single case report of the postpartum women reported the highest trough concentration reported (3.3 µg/mL), while the lowest recorded trough concentration was 0.12 (0.10–0.15) µg/mL (*Table 1*).

2.8.7 Drug-Drug Interactions

Patient exposure to DTG can be influenced by enzyme inducers such as rifampicin. Rifampicin is an inducer of CYP3A4 and UGT1A1, the principal enzymes involve in the metabolism of DTG (214). Other inducers of CYP3A4 and UGT1A1 such as carbamazepine, phenytoin and phenobarbital also affect DTG PK. Its use with the antiretroviral etravirine is contraindicated due to significant reduction of DTG exposure via the same pathway. DTG inhibits renal transporters OCT2 (strong) and multidrug and toxin extrusion transporter 1 (MATE1), resulting in increased exposure and risk of toxicity. As an integrase inhibitor, it is chelated by polyvalent metals such as magnesium, aluminium, zinc and calcium, reducing the bioavailability of the drug (174, 214).

2.9 Conclusion

Variable DTG PK were reported by different clinical studies, influenced by factors such as food, research conditions and population variability. No significant differences were observed

between HIV infected and non HIV infected participants and DTG shows linear PK parametres. Current clinical trials to assess some drug-drug interactions and safety in pregnant and breastfeeding women will help in providing information for its wider use.

CHAPTER 3

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CHAPTER 3.

Validation and Clinical Application of a Novel Method for the Quantification of Dolutegravir in Dried Breast Milk Spots (DBMS) using LC-MS

3.1 Background

It is recommended globally that HIV-positive women receive antiretroviral therapy (ART) throughout pregnancy and breastfeeding, or for life, irrespective of clinical disease stage or CD4 count (24). In untreated HIV, breastfeeding is associated with increased risk of mother-to-child transmissions of HIV (51, 52, 215). Despite this risk, exclusive breastfeeding among HIV seropositive women in low and middle income countries is recommended by the WHO (53, 216-218), as formula feeding is associated with high infant mortality. Excretion of exogenous chemicals via the mammary epithelial cells especially during the colostral phase (3-4 days after delivery) is known to be high (213, 219), which implies that breastfeeding mothers may expose their babies to clinically significant antiretroviral (ARV) concentrations over a prolonged period (220). Furthermore, infants of mothers receiving ART who acquire HIV via breast milk (BM) have been shown to present with high rates of drug resistance, thus compromising their future treatment options (48, 49, 221-223). It is therefore essential to determine ARV pharmacokinetics (PK) and transfer into BM in order to assess the potential risk of harmful exposure to the infant through breastfeeding, and limit the development of drug resistance.

The PK of ARV in BM and breast-fed infants can be determined by measuring concentrations in paired maternal plasma (MP), infant plasma (IP) and BM, and expressed as BM:MP and IP:BM ratios. BM transfer has been assessed for a number of ARV, including the nucleoside (NRTI: abacavir, emtricitabine, lamivudine, stavudine, tenofovir, zidovudine) and non-nucleoside reverse transcriptase inhibitors (NNRTI: efavirenz, etravirine and nevirapine) and protease inhibitors (PI: atazanavir, indinavir, lopinavir, ritonavir) (224-226). However, PK data on more recently licenced ARV are lacking (178, 227, 228).

Analytical methods used for measuring ARV in BM are limited. At the current time, there are a total of 19 published methods, however, the majority use HPLC-UV (229), with only a few studies employing the more sensitive and specific technique of LC-MS (229, 230). To date, only analytical methods for the following ARV have been developed: Lamivudine, stavudine, efavirenz, indinavir, lopinavir, nevirapine, ritonavir and zidovudine. However, most of these methods are in fact modifications of previously validated assays used for plasma and serum and provide limited detail on how the BM samples were collected and stored, the type of calibration matrix and extraction method used. Furthermore, data to support the stability of these drugs in BM following sample processing and long-term storage are limited, or completely lacking. The majority of BM methods published used structurally related (analogue) internal standards(231). However, ideally stable isotopically labelled internal standards (SIL-IS) should be used to correct for matrix effects, as they share the same physicochemical properties and co-elute with the unlabelled analyte of interest. The commercial availability of these compounds is continuing to increase, such that they are now routinely used for LC-MS analysis.

Quantification of ARV from BM presents a number of analytical challenges: BM can vary in fat content throughout the feeding time, and the nutritional intake of the mother may also cause variation in BM fat content (232). Analytical methods previously described for determination of ARV in BM predominantly use liquid fractions of whole or skimmed milk which require more intensive and time consuming sample clean up procedures, such as Solid Phase Extraction (SPE) (233). Physicochemical properties influence the partitioning of drug into BM which contains variable lipid, aqueous and protein fractions. Indeed, significant differences in drug extraction recoveries between whole and skimmed have been reported which may result in misleading results, especially as whole milk is more likely to reflect the fraction ingested by the breastfeeding infant. For example, Shapiro and colleagues noted recoveries of drug between skimmed and whole BM to be 72% vs 95% for lamivudine, 103% vs 94% for zidovudine and 99% vs 89% for nevirapine, and in light of this variability elected to report whole milk concentrations(234).

Dried Breast Milk Spots (DBMS), whereby a relatively small (<50µL) volume of BM is spotted directly onto an inert filter card, offer several advantages for drug quantification as compared with liquid BM. Use of DBMS enables quantification of drugs in whole BM, which is a direct reflection of the milk ingested by the breastfeeding infant. DBMS are also more suitable for collection and storage in low resource settings as they can be stored and shipped at ambient temperatures without the need for refrigeration or dry ice during transit. We have previously developed and validated DBMS methodologies for the NRTI (emtricitabine, lamivudine, tenofovir) and NNRTI (efavirenz and nevirapine) (235-237).

The integrase inhibitor Dolutegravir (DTG) was licensed by food and drug administration (FDA) in 2013 for the treatment of HIV infection (24), and more recently, recommended as a first

line treatment of HIV in the 2016 WHO guidelines (24). Thus global uptake and the number of pregnant women and breastfeeding infants exposed to DTG in limited resource settings is predicted to increase significantly over the coming years.

Well-designed PK studies and robust analytical methods for quantifying DTG in BM are therefore needed to address these safety concerns in vulnerable patient populations. Our aim was to develop and validate an accurate, sensitive and robust LC-MS method for quantification of DTG in BM using dried breast milk spots (DBMS), with application of the method in breastfeeding mother and infant pairs.

3.2 Materials and methods

3.2.1 Reagents and Equipment:

DTG and deuterated internal standard (IS) Dolutegravir-d5 (DTG-d5) were purchased from Toronto research chemicals (Toronto, ON, Canada). LC-MS grade Methanol (99.9%), formic acid, Propan-2-ol and Tert-butyl methyl-ether (TBME) were obtained from Sigma Aldrich, UK. Deionised water (18.2 MΩ purity) was produced from Elga® Option S water purification units, Purelab® and Classic Ultraviolet water filtration (UVF) purifier (Elga LabWater, UK). Whatman 903 Protein Saver cards were obtained from GE Health Care, UK. Blank human breast milk (HIV negative breast milk from healthy donors not on any medication and not spiked with any drug or ART) was donated by volunteers through the Wirral Mothers' Milkbank and transferred to the lab for use with approval from Liverpool Women Hospital.

The HPLC system consisted of an Accela autosampler (set at a tray temperature of 10°C) and an Accela pump (Thermo Scientific, Hemel Hempstead, UK). A reverse phase C_{18} Waters XBridge (3.5µm: 2.1 x 50 mm) column (waters corporation, U.S.A) set at 40°C oven temperature and interfaced with a 2 µm C_{18} Quest column saver (Thermo Fisher Scientific, Hemel Hempstead) was used to resolve the analytes. A Thermo Quantum Access mass spectrometer with a heated electrospray ionisation source (Thermo Fisher Scientific, Hemel Hempstead) was used for quantification. TSQ Tune software was used for compound tuning and optimisation, and LCquan (version 2.7 Thermo Fisher Scientific, Hemel Hempstead) was used for data acquisition, data processing and reporting.

3.2.2 Chromatographic and mass spectrometry conditions

Mobile phase A (0.1% formic acid in deionised water) and B (0.1% formic acid in methanol) were programmed at flow rate of 500 µL/min to generate a solvent gradient for chromatographic separation. The Initial gradient was started with 80% mobile phase A and held for 0.5 minutes and then decreased to 20% mobile phase A for 2.5 min, during which the analytes were eluted. Subsequently the column was washed with 100% mobile phase B for 2 min and then re-equilibrated to to its original conditions over a total run time of 9 minutes. The injection volume was 5 µL and needle was washed with IPA: water [1:1(v/v)] after each injection.

The mass spectrometer used electrospray ionisation (ESI) set in a positive ionization mode using selective reaction monitoring (SRM). The Spray voltage was 29 V, the capillary temperature was 300^{o}C and vaporisation temperature 350^{o}C, respectively. The sheath and auxillary gas pressures were set at 50 and 10 arbitrary units. Argon was used as a collision gas and delivered at a pressure of 1.5 mTorr. The compounds were tuned using a concentration of 1 µg/mL in methanol via direct infusion into the mass spectrometer at a flow rate of 5µL/min. The m/z transitions were 420.1 → 277.1m/z for DTG and 425.1 → 276.9 for DTG-d5 with collision energies of 29 and 27 V, respectively. The scan width was set at 0.01 m/z and the dwell time at 0.1 seconds.

3.2.3 Preparation of stock solutions, standards, QCs, and internal standards of DBMS and DPS

A primary DTG stock solution (1mg/mL) was prepared in methanol and stored in the refrigerator at 4^{o}C until use. This stock was subsequently serially diluted with methanol and

water [1:1 (v/v)] to obtain intermediate solutions of 200, 50, and 2.5 µg/ml. The intermediate solutions were then used to spike into the blank human BM for preparation of the calibration standards (10 levels; 0, 10, 25, 50, 200, 500, 1000, 2000, 3200 and 4000 ng/ml) and internal QC (LOQ = 30ng/mL, MQC = 455 ng/mL and HQC = 3700 ng/mL). Exactly 30µL of each calibrator and QC was spotted onto the middle of the 12mm circle of the protein saver cards. The spots were then dried overnight at ambient temperature before sealing in a ziplock bag with desiccant sachets, and stored at -40^{o}C until use.

A 1mg/mL DTG-d5 stock solution was prepared in methanol and stored at 4^{o}C. On the day of analysis a working internal standard solution (250 µg/mL) was prepared in 50:50 (v/v) methanol:water.

3.2.4 Sample pre-treatment

This method employed a liquid-liquid extraction process to release the drug from the DBMS and remove matrix interferences (238). Prior to extraction, 20 µL of DTG-d5 (2.5 µg/mL) was spotted directly onto each 12 mm circle of the DBMS using an electronic pipette and allowed to air dry for 20 minutes. After drying the spots, the entire 12 mm circle of the DBMS were punched and folded into clean 7 mL screw capped glass tubes and pre-soaked in 500 mL of 1.8 mg/mL EDTA in water for 10 minutes.

2 mL of TBME was added to each sample and the tubes capped and tumbled for 30 minutes. The samples were centrifuged at 3398g for 10 minutes to separate the precipitated protein

and water from TBME containing drug. Using a cryobath, containing dry ice and methanol, the protein, water and paper were frozen out, and non-frozen supernatant decanted into clean 12x75mm glass tubes and evaporated to dryness using nitrogen drier for 1 hour. Finally, samples were reconstituted using 500 µL of mobile phase [80:20 methanol: water (v/v) in 0.1% formic acid] and vortexed for 5 seconds. 100 µL of reconstituted sample was transferred to 300 µL autosampler vials, sealed with crimp caps (11mm aluminium PTFE/ silicone natural) and loaded onto the autosampler tray for subsequent injection to the LC-MS system.

3.2.5 Method validation

The LC-MS method was validated according the stipulated FDA and EMA guidelines acceptance criteria for bioanalytical assay development and validation (239, 240) .

3.2.5.1 Selectivity

Selectivity was evaluated by comparing the amount of interference from six different blank DBMS in relation to the lowest calibrator (LLOQ). Area responses of interfering noise at the retention time of DTG were accepted if the interference was less than 20% of the mean response of the LLOQ (n = 6). For the internal standard, less than 5% of the mean response areas in the 6 LLOQ samples was deemed to be acceptable.

3.2.5.2 Carryover

Carryover was determined through injection of DBMS calibrators at the upper limit of quantification (ULOQ) followed by 4 blank DBMS on 7 separate occasions. Carryover was then

expressed as a percentage of the LLOQ and ULOQ, as per the EMA guidelines, and should not be >20% of the LLOQ.

3.2.5.3 Precision and accuracy

Precision and accuracy was determined by analysing five different assay batches, consisting of a calibration curve and 6 LLOQ, LQC, MQC, and HQC (separate extractions), run over 5 days. Deviation of ≤ ±15% of measured analyte from nominal concentrations was accepted, except at LLOQ where ≤ ±20% was accepted. Accuracy was defined as the percentage deviation of measured analyte from nominal concentration, and precision as a percentage coefficient of variation (%CV). Greater than or equal to 75% of calibration standards and 67% of QC in each run were required to have a deviation of ≤±15% from their respective nominal concentrations.

3.2.5.4 Recovery and matrix effect

The percentage recovery and matrix effects were determined quantitatively in accordance to the recommendations of Matuszewski et al (241). The peak area responses of six replicates of pre-extracted DBMS QC (C), post-extracted DBMS blanks spiked at an equivalent QC concentration (B) and DTG spiked directly in mobile phase (A) without matrix, were compared. Overall % recovery (process efficiency; %PE) was determined by comparing the peak area response of pre-extracted DBMS to the peak area response of DTG spiked at an equivalent concentration in mobile phase (C/A*100). The % matrix effect (%ME) was calculated by comparing DTG spiked into blank DBMS extracts with the peak areas at an equivalent concentration in mobile phase (B/A*100). The IS-normalized recovery (%Analysis RE) was calculated by comparing the peak area ratios of the pre-extracted (C2) and post-extracted (B2) DBMS QC (C2/B2*100).

Given that DTG is known to bind to free metals, the effect of EDTA (a chelating agent) on DTG recovery from BM was evaluated by comparing recovery of drug from DBMS pre-soaked in 1.8mg/mL EDTA in water against DBMS pre-soaked with water without EDTA, prior to liquid liquid extraction. Furthermore, the impact of different IS addition procedures on DTG recovery from DBMS were assessed. The IS was pipette either directly onto the DBMS, or added to the extraction solution.

3.2.5.5 Dilution Integrity

Six replicates (n=6) of DBMS were prepared at approximately 1.75 times the assay ULOQ (approximately 7000 ng/mL). Blank DBMS were also extracted and used to dilute the extracted dilution integrity DBMS by a factor of two and four in order to derive a concentration that falls within the calibration range. The resulting values were then back-calculated with the appropriate dilution factor.

3.2.5.6 Stability

Stability of DTG in DBMS (QC samples; 6 per level) for different storage and processing conditions was evaluated, including bench top stability, heat treatment, autosampler stability, re-injection reproducibility, processed and long term stability.

Bench top stability (storage of DBMS at room temperature for 5 days) was evaluated for QC by comparing with freshly prepared samples. Heat stability was assessed by heating DBMS at 50^{o}C for 1 hour before analysis, and autosampler stability was assessed by injecting samples after 24 hours of storage in the autosampler. The stability of processed (extracted) samples

was assessed by keeping the processed samples in the fridge (4^{o}C) for 24 hours before analysis. Long term stability was evaluated by storing DBMS at ambient conditions for up to one year. In all cases, concentrations in QC samples were read off freshly prepared DBMS standards and QC. Re-injection reproducibility was assessed by re-injecting samples left in the autosampler for 72 hours and results of the two runs compared for reproducibility.

3.3 Clinical Application

The validated method is currently being utilised for determination of DTG in DBMS, obtained as part of the ongoing DOLPHIN 1 clinical trial (NCT02245022) which seeks to assess the safety and pharmacokinetics of DTG in African HIV-infected pregnant mothers and their neonates. HIV-infected pregnant women are randomised at a number of sites, including the infectious disease Institute Makere University, Kampala Uganda and the Desmond Tutu HIV Foundation, Gugulethu Community Health Center, Cape Town South Africa, to receive either a DTG-based regimen or a standard of care regimen (not containing an integrase inhibitor).

Paired maternal plasma (MP) and BM samples were collected at two time points from breastfeeding mothers (1 Ugandan; 1 South African) at approximately 2 weeks post-partum. Plasma samples were also collected from the infant (IP) on two occasions to evaluate neonatal DTG exposure; one timed relative to a feed at the anticipated maternal Tmax, and one at a random time point relative to maternal dosing. In addition, paired MP, IP and BM samples were collected at 3 time points post dose following the mother's discontinuation of DTG (and transfer to standard of care).

BM samples were collected in EDTA containers and exactly 30 µL spotted on Whatman 903 protein saver cards. The cards were subsequently air dried and sealed in individual ziplocked

bags with desiccant. Plasma and DBMS were shipped on dry-ice to the University of Liverpool Bioanalytical Facility for analysis. DBMS were stored at -40◦C analysed according to the above sample pre-treatment method. DTG concentrations in plasma (MP and IP) were analysed using a previously described and fully validated LC-MS methodology (242). Ratios between DTG concentrations measured in breast milk and maternal and infant plasma samples were calculated arithmetically and expressed as BM:MP and IP:BM ratios.

3.4 Results and Discussion

3.4.1 Method Development

3.4.1.1 Chromatographic and mass spectrometry conditions

DTG and DTG-d5 were eluted from the reverse phase column at approximately 1.8 minutes, respectively. Representative chromatograms obtained from extracted DBMS (blank and LLOQ) are shown in Figure 1. The m/z transitions were 420.1 → 277.1m/z for DTG and 425.1 → 276.9 for DTG-d5 with collision energies of 29 and 27 V, respectively. The scan width was set at 0.01 m/z and the dwell time at 0.1 seconds.

3.4.1.2 Sample pre-treatment

Due to the small volume of BM sample (30µL) applied to each circle, the entire spot was punched and subsequently extracted in order to ensure a high signal intensity on the LC-MS. Punching the entire circle after spotting with known volume also ensures reliable comparison with an equivalent volume of liquid sample, making it more suitable for use in therapeutic drug monitoring (TDM) and PK studies. However, a limitation of using the entire DBMS, as opposed to a sub-punch, is that the bioanalyst is solely reliant on the expertise of the

technician at the site/clinic to accurately pipette the required volume on the filter card. This poses an additional challenge in Resource Limited Settings, where volumetric pipettes are unlikely to be maintained and calibrated to same standards as accredited research laboratories. Furthermore, regional variability, inter-operator differences in pipetting technique and on-site training of staff at investigator sites was shown to impact on the reproducibility of drug (efavirenz) quantification from Dried Blood Spots (DBS), as was observed in a global multicentre trial conducted across diverse health resource settings (ENCORE 1) (243).

Taking a DBMS sub-punch of a pre-defined diameter (e.g. 3-6 mm) is advantageous as study personnel do not need to apply an accurate volume to the card, so the technique is less reliant on operator skill. However, during early method development it was found that sub-punches and smaller BM volumes (<30µl) did not provide a high enough response at the assay LLQ (10 ng/ml or 0.3 ng/DBMS sample). Furthermore, BM is colourless on the card, therefore, punching the entire circle ensures that the entire sample is utilised to measure the drug.

However, use of protein saver cards are also known to be associated with challenges in bioanalysis such as a heterogeneous drug/matrix distribution on the cards, variable drug quantification and potential chemical interference from pre-treated cards [18-20]. Here, such issues were circumvented by punching the entire circle of the DBMS, and adjusting the reconstitution volume of the processed samples to reduce matrix interference. Other measures employed to optimise drug recovery were use of 1.8mg/mL EDTA in water to pre-soak punched cards, which was shown to increase recovery by approximately 50% (Table 2). Reasons for this observation is not certain, but binding of integrase inhibitors to polyvalent metals (Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+}) rich in breast milk is well documented [21,22]. EDTA

chelates polyvalent metals by forming complexes with the metals. Therefore addition of EDTA to DBMS samples during processing likely increases the availability of free drug and recovery of free drug from the matrix. Further studies are required to understand the clinical implication of how BM rich in free metals affects the absorption and disposition of DTG in the breastfeeding infant.

Addition of internal standard to liquid BM prior to spotting on cards is the optimal method for correcting variability and ensuring process efficiency during sample preparation and analysis (244). However, such an approach is challenging in resource limited countries, particularly in a field based setting, due to lack of standard calibrated pipettes and potential operator variability. Thus the only feasible option was to either spot the IS directly on card immediately prior to extraction or add the IS in the pre-soak solution before extraction. The addition of IS directly onto the DBMS was found to improve the overall recovery of DTG and reproducibility of the method compared with adding IS in solution (Table 2).

3.4.2 Method Validation

3.4.2.1 Selectivity

The background interference from extracted blank DBMS (n=6) at the retention time of DTG was on average <20% of the signal intensity of the assay LLOQ. Similarly, the background noise in blank DBMS was <5% of the signal intensity of the internal standard (DTG-d5) for all 6 batches tested.

3.4.2.2 Carryover

Mean % carryover into the extracted blanks (n=7) after injection of a ULOQ sample (4000 ng/mL) was 62.74% and 0.17%, 23.32% and 0.06%, 19.32% and 0.05% and 14.03% and 0.04% of the LLOQ and ULOQ after injection of first, second, third and fourth extracted blanks, respectively. Due to the observed carryover, four extracted blanks were incorporated after every injection of ULOQ or QCs and two blanks inserted between patient samples as precaution to prevent cross contamination between incurred samples.

3.4.2.3 Precision and accuracy

Five independent validation batches were run over 5 days, with each assay run consisting of a zero blank, 9 calibration standards (in duplicate) and 6 QC (LQC, MQC and HQC). The calibration was quadratic with a weighing factor of $1/X^2$, and linearity maintained from 10 ng/mL to 4000 ng/mL. The Mean regression coefficient (r^2 ±SD) was 0.9962 ± 0.0018. Inter- and intra-day accuracy and precision were within ±15% for all QC levels and ±20% for the LLOQ, as shown in Table 1.

3.4.2.4 Recovery and Matrix effect

Mean (±CV %), percentage recovery (%RE), process efficiency (%PE) and matrix effect (%ME) of DTG from DBMS are summarised in Table 2: The mean % RE, PE and ME (%CV) of DTG from DBMS pre-soaked with EDTA, and IS spotted directly onto the spot was 105.07 (11.23), 101.52 (11.09) and 96.83 (8.18), respectively. The high % recoveries of DTG and limited effect of the sample matrix are likely attributed to the inherent properties of the filter cards and the fact

that only a small volume (30µL) of sample matrix is applied. One suspected explanation is that complex biopolymers are formed between the cellulose of the protein saver cards and the proteins, peptides and polysaccharides in BM, which stick together to essentially "hold" the matrix components within the card thereby making extraction of free drug and sample processing much easier (245, 246)

Use of protein saver cards are known to be associated with challenges in bioanalysis such as a heterogeneous drug/matrix distribution on the cards, variable drug quantification and potential chemical interference from pre-treated cards (178, 228, 235). Here, such issues were circumvented by punching the entire circle of the DBMS, and adjusting reconstitution volume of the processed samples to reduce matrix interference. Other measures employed to optimise drug recovery were use of 1.8mg/mL EDTA in water to pre-soak punched cards, which was shown to increase recovery by approximately 50% (Table 2). Reasons for this observation is not certain, but binding of integrase inhibitors to polyvalent metals (Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+}) rich in breast milk is well documented (236, 237). Further studies are required to understand the clinical implication of how BM rich in free metals affects the absorption and disposition of DTG in the breastfeeding infant.

Addition of internal standard to liquid BM before spotting on cards is the optimal method for correcting variability and ensuring process efficiency during sample preparation and analysis (244). However, such an approach is challenging in resource limited countries, particularly in a field based setting, due to lack of standard calibrated pipettes and potential operator variability. Thus the only feasible option was to either spot the IS directly on card immediately prior to extraction or add the IS in the solution before extraction. The addition of IS directly

onto the DBMS was found to improve the overall recovery of DTG and reproducibility of the method compared with adding IS in solution (Table 2).

3.4.2.5 Dilution Integrity

Result of dilution integrity of DTG DBMS method after 1:2 and 1:4 dilution of samples >175% of ULOQ (approximately 7000 ng/mL) with extracted blank DBMS was within 15% of anticipated concentration with % CV <3%.

3.4.2.5 Stability

Stability of DTG extracted from DBMS was determined under multiple conditions which are summarised in Table 3. DTG was stable in DBMS after 5 days on the bench at room temperature, when refrigerated for 24 hours at 4^{o}C, during processing, and when stored in the autosampler for up to 24 hours. DTG was stable after heating DBMS at 50^{o}C in oven for 1 hour and results were reproducible when samples were re-injected after 72 hours (reinjection reproducibility). Long term stability was also demonstrated; DTG DBMS were shown to be stable when stored at ambient temperature for a period of 1 year. All evaluated conditions for standards and QC were within ±15% of their respective nominal concentrations. These stability data indicate that DBMS are stable at high temperatures and over a long period of time. Therefore DBMS are ideal in middle and low income countries where stable power cannot be guaranteed, since refrigerators or freezers are not necessary for storage of DBMS**.**

2.4.3 Clinical Application

Steady state DTG concentrations in BM and MP taken at 2 weeks post-partum in a Ugandan mother, were 154.2 and 3786.2 ng/mL and 40.9 and 1210.7 ng/mL at 4 and 24 hours post

dose, resulting in BM:MP ratios of 0.05 and 0.04, respectively. Time matched IP concentrations in the breastfeeding infant were 67.8 ng/mL and 75.5 ng/mL at 4 and 24 hours post dose, resulting in IP:BM ratios of 0.44 and 1.85. Similarly, steady state BM and MP collected at post-partum in a South African mother at 3 and 24 hours post dose were 116.3 and 3029.5 ng/mL and 17.7 and 603.3 ng/mL, with BM:MP ratios of 0.04 and 0.03, respectively. The IP concentration at 24 hours post the DTG dose was 16.3 ng/mL (IP:BM =0.92). In both women, DTG BM concentrations fell below the assay limit of quantification (<10 ng/ml) when sampled 2 days (Ugandan mother) and 9 days (South African mother) after the women stopped DTG therapy, whereas corresponding MP (103.8 ng/ml) and IP (58.6 ng/ml) concentrations remained detectable in the Ugandan mother and her infant for up to 2 days post DTG treatment cessation. These results should be interpreted with caution due to small number of samples evaluated. Transfer of DTG from the mother's circulation to BM is potentially governed by its high (>98%) protein binding and thus sole passage of the unbound (free) form (247, 248). The influence of transporters and enzymes on DTG transfer across the mammary epithelium to the breastfeeding infant is unknown(178), however immature metabolic pathways of UGT1A1 and CYP enzymes may be responsible for the relatively high levels of DTG seen in the infants (180). The metabolising enzymes in infants reach adult function between 6 months to first year of life (249, 250).

3.5 Conclusion

A sensitive, specific and reproducible electrospray ionization LC–MS methodology has been developed and validated for the accurate measurement of DTG in dried breast milk spots (DBMS). Assay validation experiments such as extraction recovery, matrix effects and short- and long-term stability have been discussed. The method has been used for the quantification of DTG in DBMS as part of an ongoing clinical trial investigating the safety and pharmacokinetics of DTG in pregnant and breastfeeding mothers and their infants. DBMS samples from two breastfeeding mothers (Uganda and South Africa) paired with mother and infant's plasma concentration, were analysed. The concentration of DTG secreted in BM was approximately 2-4% of the maternal plasma concentration at all analysed time points.

3.6 Future Perspective

Dolutegravir is emerging globally as an acceptable first line treatment of HIV. It was recommended by WHO guideline for treatment of HIV in 2016 (224), and has been rolled out in many western countries, while efforts are being made to make it available in low and middle income countries. High prevalence of HIV among women of reproductive age in resource limited countries (225), requires sufficient safety and pharmacokinetic data to ensure protection of neonatal and paediatric patients from adverse events, such as toxicity or acquired drug resistance, that may occur during breastfeeding. Robust analytical methods for measurement of drugs in localised body fluids like BM are a significant component of pharmacokinetic studies that aim to characterise antiretroviral distribution into anatomical sites relevant to HIV transmission. This assay will provide a tool for evaluating DTG secretion in BM and implication in neonatal safety and prevention of vertical transmission during breastfeeding.

The preparation and extraction of liquid breast milk is generally cumbersome and often requires solid phase extraction due to the high amount of fats, polysaccharides and proteins in BM (227). Although cellulose of protein saver cards is known to be inert, we suspect that cellulose potentially interact with fats, polysaccharides and proteins in BM to form biopolymers that stick together and can be easily frozen down to ensure good sample cleaning (245, 246). In contrast, this method requires only 30µL of BM spot and samples can be easily shipped and stored at ambient temperatures. Although automated volumetric absorptive microsampling technologies are fast replacing conventional methods of sampling and analysis, it will take a long time for these to be available in resource limited countries (226).

Acknowledgements

We wish to thank the study participants and clinical staff at the study sites.

Ethical conduct of research

Ethical approval to source blank human breast milk from the Wirral Mothers' Milk Bank, Clatterbridge Hospital, Wirral, UK; was granted by the University of Liverpool Research Ethics Committee.

DOLPHIN -1: Ethical approvals were obtained from all institutions involved (University of Liverpool Research Ethics committee, Joint Clinical Research Centre Uganda, Ugandan National council of Science and Desmond Tutu HIV Foundation, Gugulethu Community Health Centre, Cape Town Ethics and Research Committee). The study protocol and the material transfer agreement were approved by the University of Liverpool Research Ethics Committee, UK and the Joint Clinical Research Centre IRB, Uganda and the Uganda National Council for Science and Technology (HS1675).

Table 1: Precision and Accuracy for DTG DBMS

Nominal Concentrations (ng/mL)	**Mean (SD)**	**Precision (CV%)**	**Accuracy (%)**
Inter-assay			
LLOQ	10.39 (1.09)	10.53	3.88
LQC (30)	26.00 (2.77)	10.64	-13.34
MQC (455)	440.14 (27.41)	6.23	-3.27
HQC (3700)	3762.67 (213.45)	5.67	1.69
Intra-assay			
LLOQ	10.05 (0.69)	6.83	0.53
LQC (30)	25.8 (1.93)	7.50	-13.95
MQC (455)	432.45 (13.08)	3.03	-4.96
HQC (3700)	3703.91 (169.50)	4.58	0.11

Table 2. Recovery and Matrix Effect for DTG DBMS following liquid-liquid extraction with and without addition of EDTA, and the Internal Standard spiked in the extraction solution or directly onto the DBMS filter paper.

Method	Nominal QC Concentration (ng/mL)	%ME (SD)	%RE (SD)	%PE (SD)	%Analysis RE (SD)
		(B/A*100)	(C/B*100)	(C/A*100)	(C2/B2*100)
DBMS extracted with IS added to the extraction solvent	**30 (LQC)**	109.48 (7.80)	35.816 (4.15)	39.21 (5.99)	37.38 (7.94)
	455 (MQC)	129.63 (7.55)	50.53 (1.53)	65.51 (3.30)	55.66 (7.085)
	3700 (HQC)	142.95 (10.32)	39.41 (2.69)	56.33 (4.01)	41.33 (3.41)
	Overall Mean (%CV)	127.35 (13.24)	41.92 (7.96)	53.68 (13.35)	42.07 (25.67)
DBMS pre-soaked with EDTA, and IS added to the extraction solvent	**30 (LQC)**	90.37 (3.07)	102.23 (9.56)	92.38 (11.50)	98.64 (8.59)
	455 (MQC)	74.27 (7.49)	104.81 (8.46)	77.842 (6.48)	64.74 (5.63)
	3700 (HQC)	85.29 (6.93)	99.26 (11.97)	84.67 (11.49)	71.15 (9.73)
	Overall Mean (%CV)	83.31 (8.12)	102.10 (11.92)	84.97 (13.58)	78.18 (23.03)
DBMS pre-soaked with EDTA, and IS spotted directly onto the spot	**30 (LQC)**	97.10 (4.02)	92.94 (10.27)	90.24 (12.11)	102.10 (12.87)
	455 (MQC)	88.46 (8.62)	114.82 (14.33)	101.56 (11.08)	86.84 (10.58)
	3700 (HQC)	104.93 (12.03)	107.47 (15.18)	112.77 (7.45)	78.15 (10.12)
	Overall Mean (%CV)	96.83 (8.18)	105.07 (11.23)	101.52 (11.09)	89.03 (13.62)

A=Mean peak area response of analyte in mobile phase; B= Mean peak area response of analyte spiked after extraction of matrix (Spiked post extraction of blank plasma); C= Mean peak area response of analyte spiked prior to extraction (spiked pre-extraction); %ME= Matrix effect, defined as ratio of mean peak area of analyte spiked post extraction (B) to mean peak area of analyte in mobile phase (A) X 100; %RE= Extraction yield, derived by dividing the mean peak area response of analyte spiked pre-extraction (C) with mean peak area response of analyte spiked post extraction (B) and multiplying by 100; %PE= Process efficiency calculated by dividing mean peak area response of analyte spiked pre-extraction (C) by mean peak area of analyte in mobile phase (A) and multiplying by 100; B2 is defined as response ratio of analyte spiked post extraction and internal standard; C2 is the response ratio of analyte spiked pre-extraction and internal standard; %Analysis RE calculated by dividing the mean response ratio of analyte spiked pre-extraction (C2) to mean response ratio of analyte spiked post extraction (B2), multiply by 100;

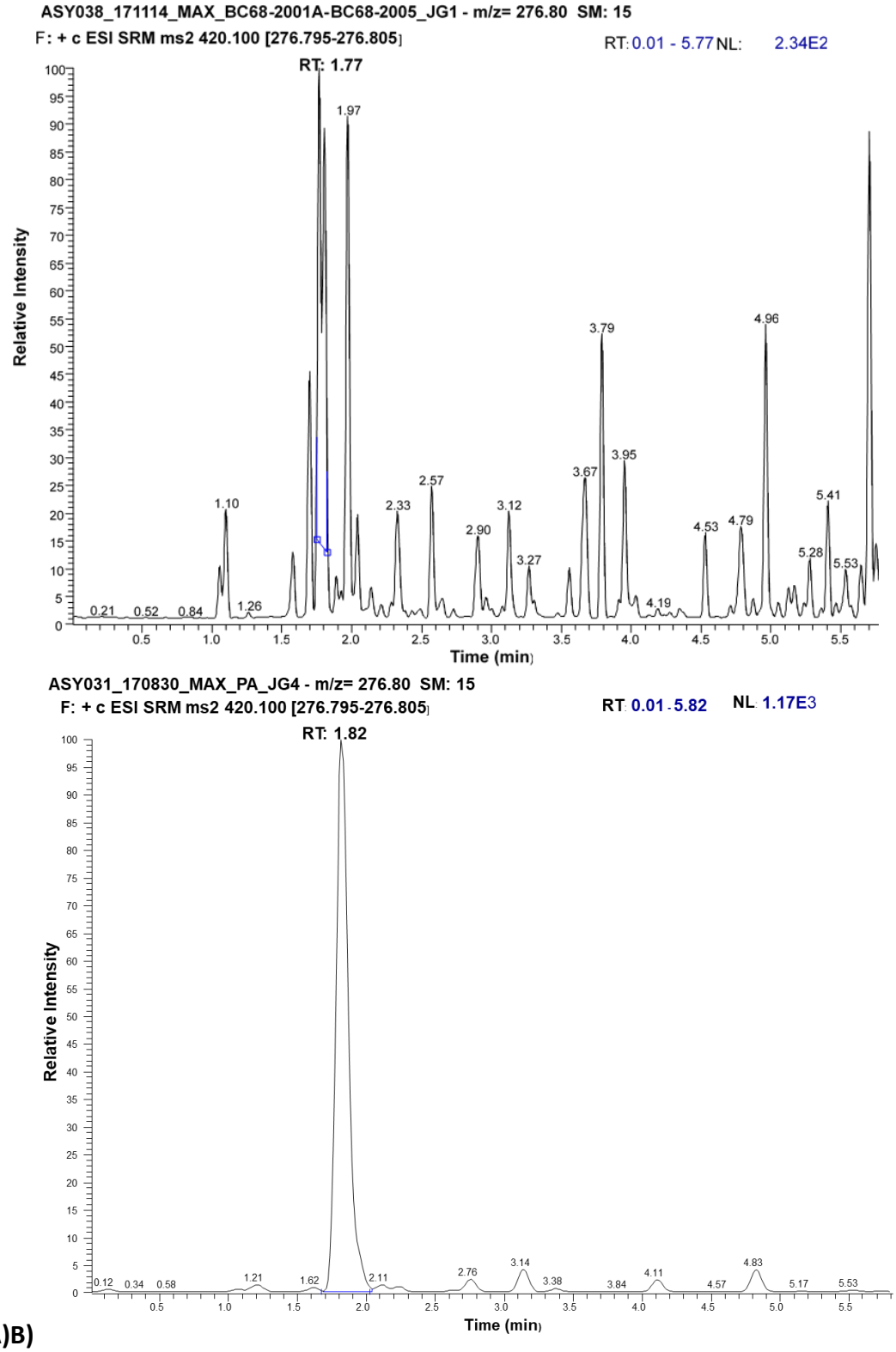
Table 3. Short and long term stability of DTG DBMS

Stability	QC	Precision (%CV)	Accuracy (%bias)
Bench top (RT; 5 days)	LQC	4.70	0.37
	HQC	3.90	-7.59
Processed stability (4°C; 24 hr)	LQC	10.47	1.26
	HQC	7.05	1.26
Heat treatment (50°C; 1 hr)	LQC	4.78	8.96
	MQC	10.41	-4.78
	HQC	5.65	6.78
Autosampler stability (10°C; 24 hr)	LQC	5.79	-10.29
	HQC	4.04	-9.61
Long term stability (RT; 12mths)	LQC	9.32	2.47
	HQC	3.11	16.53
Re-injection Reproducibility (72 hr)	LQC	12.20	-2.84
	MQC	6.35	-5.73
	HQC	3.67	-0.89

RT = room temperature

Figure 1: Chromatograms of (A) a blank DBMS and (B) the assay lower limit of quantification (LLOQ)

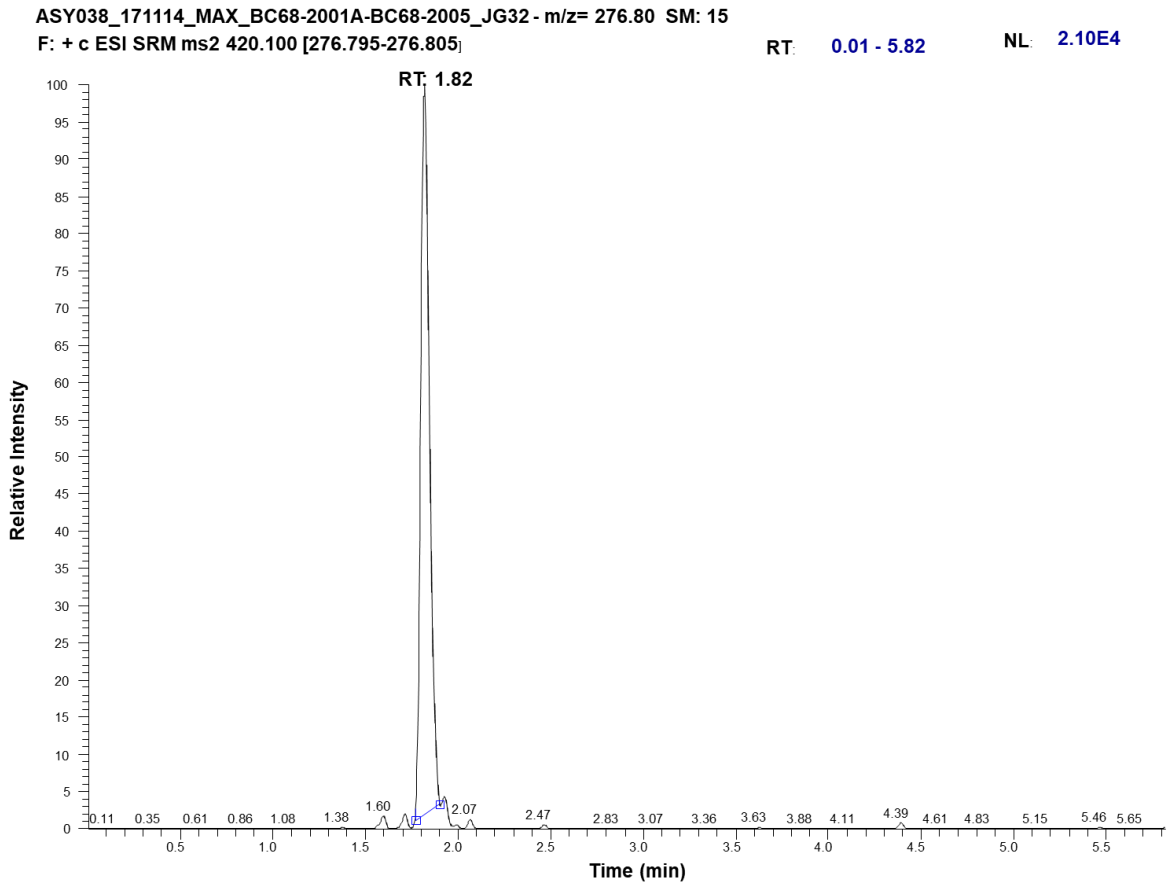
Figure 1:



A)B)

Figure 2: Chromatogram of patient DBMS sample taken at 4 hours post the dolutegravir dose

Figure 2.



CHAPTER 4

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CHAPTER 4

Safety and Pharmacokinetics of Dolutegravir in Pregnant HIV Mothers and their Neonates

4.1 Introduction

Dolutegravir (DTG) is a potent integrase inhibitor that can reduce risk of mother to child transmission (MTCT) of HIV during pregnancy by rapidly suppressing viral load. Impact of pregnancy on DTG pharmacokinetics (PK) and safety is unknown and requires evaluation before it is widely rolled out for use in pregnancy. HIV burden among women of reproductive age on the other hand is significant with an estimated 17.8 million women age 15-24 years currently living with HIV and over 1.5 million HIV positive women in sub-Saharan Africa get pregnant annually (251, 252).. Women constitute over 56% of new adult infections, a large proportion between age 15-25 years SSA (253). Despite efforts to intensify ART access, high proportion of HIV positive women still get pregnant prior to commencing ART (254), predisposing there neonates to risk of HIV in-utero or during delivery.

Great strides have been made in integrating HIV and antenatal care (255), but many women still present at third trimester of pregnancy or in labour with high viral loads, so there is limited time to achieve viral suppression with ART before labour, further increasing the risk of vertical transmission during delivery. Integrated antenatal care

(ANC) includes screening of blood born viruses (HIV, HCV, HBV and syphilis) as part of routine antenatal care in most sub-Saharan African countries (256). This practice has enabled diagnosis of many women during antenatal visits, and ART treatment initiated accordingly to prevent transmission to their neonates. Late presentation therefore allows little or no time for adequate virological suppression before delivery if HIV diagnosed during antenatal care. The widely available NNRTI based regimen [standard of care (SoC)] approved in most sub-Saharan African countries for treatment of women during pregnancy is NNRTI (EFV) plus 2 NRTI which can take approximately 84 days (or 2-3 months) to successfully suppress the viral load below 50 copies/mL (257). Thus, women presenting late for ANC who are prescribed this regimen are at risk of reaching term and labour without successful viral suppression below detectable levels. Previous practice of option B in PMTCT which recommended that ART (NNRTI (EFV or NEV) plus 2NRTI) is stopped 6 weeks after delivery or cessation of breast feeding (258), also suggest that there may be associated drug resistance from these medications in a significant number of women from previous exposure (259), further making viral suppression before labour unlikely. Compounding to the problem in this region is the lack of facilities for resistance testing which is not part of the routine ART investigations. Therefore diagnosis of resistance prior to commencing ART is difficult, and relies on lack of clinical response.

DTG an integrase strand inhibitor (INST) with a plasma half-life ($t_{1/2}$) of approximately 14 h, maximum plasma concentration (C_{max}) 3.67 µg/mL, minimum plasma concentration (C_{min})1.11 µg/mL and 24 h area under the concentration curve (AUC_{0-24}) 53.6 µg.h/mL after a once daily dose of 50 mg of DTG respectively from a

population pharmacokinetic data (172). It is highly potent and rapidly suppresses viral load (approximately 28 days) after drug initiation, with a corresponding immunological recovery within a short duration of time (260). It also has a high resistance barrier, as individuals previously resistant to NNRTI and other INST such as raltegravir (RAL) were found to respond to DTG with efficient viral suppression within 2 weeks after drug initiation (135, 261). It is well tolerated by many patients and known to have minimal side effects (192). Pregnancy and breastfeeding data are limited and this was recognised as a research gap by WHO in the 2016 guidelines (262). Therefore they recommend cautious use in pregnancy, breastfeeding and in children <12 years (262). Fear of teratogenicity, transplacental drug transfer and feotal toxicities are limitations to its use in pregnancy for prevention of MTCT of HIV. Limited Pregnancy and breastfeeding safety data is a major factor mitigating national approval of DTG due to the difficulty of asserting safety during this period.

DOLPHIN-1 (NCT02245022) was a multicentre, multinational randomised controlled trials of newly diagnosed HIV positive pregnant women initiating treatment between 28-36 weeks gestation in Uganda and South Africa that randomised women into 2 groups (DTG group and EFV (standard of care) group). This study aimed at evaluating the steady-state pharmacokinetics of DTG in third trimester of pregnancy and two weeks post-partum. It also evaluated the transfer of DTG in breast milk during treatment and infant exposure during breast feeding, both within the dosing interval and following drug cessation. Other aims of the study were to evaluate the viral load (VL) response, safety and tolerability of DTG by pregnant and breastfeeding HIV infected women.

4.2 Methods

4.2.1 Study Design

DOLPHIN-1 was an open labelled randomised trial of DTG vs EFV-containing ART in pregnant women initiating treatment in late (28-36w) pregnancy and postpartum. It evaluated VL, safety and tolerability of DTG, transplacental neonatal exposure, breast milk exposure and DTG elimination in neonates. Women were randomised 1:1 to EFV (SoC) or a DTG arm. Those randomised to the EFV arm were commenced on EFV containing ART immediately as recommended by the guidelines, while those on the DTG arm received EFV immediately after referral and were subsequently switched to a DTG containing regimen within 7 days of initiating EFV based regimen. Subsequently, the women were switched to SoC regimen after the postpartum visit. Breast milk samples, cord blood at delivery and maternal plasma after cessation of DTG were also collected in the study for evaluation of cord: maternal ratio (Cord: MP), infant plasma: maternal plasma ratio (IP:MP), breast milk : maternal plasma ratio (BM:MP). VL and other recommended antenatal care were monitored throughout pregnancy and standard care was ensured throughout the study period. Baseline demographics, clinical and laboratory data, birth outcome data were recorded, and all infants were exclusively breastfed according to study protocol. The inclusion criteria of the study were: Ability to provide signed informed consent after adequate information provided and required precautions explained to the patient, willingness to participate, age 18 years and above, pregnant and untreated HIV infection in late pregnancy at ≥28 – 36 weeks gestation. Exclusion criteria were: Received antiretroviral drugs in previous 6 months, if ever received INST, serum haemoglobin < 8.0 g/dl, elevations in serum levels of alanine aminotransferase (ALT)

>5 times the upper limit of normal (ULN) or ALT >3xULN and bilirubin >2xULN (with >35% direct bilirubin), eGFR < 50mL/min, active Hepatitis B infection, history or clinical suspicion of unstable liver disease, or subjects with severe liver disease, severe pre-eclampsia (e.g. HELLP), or other pregnancy related events such as renal or liver, abnormalities (e.g. grade 2 or above proteinuria, elevation in serum creatinine (above 2.5 x ULN), total bilirubin ALT or AST), paternal non-consent (where disclosure to male partner has been made) and clinical depression or clinical judgement suggests increased risk of suicide.

The primary endpoint was pharmacokinetics of DTG and secondary endpoint were viral load changes, tolerability and safety. The sample size was derived from studies of non-pregnant HIV infected adults because of lack of pregnancy data (168), with the assumption that CV 20% will give a > 80% power (168). Clinical adverse events were recorded as recommended by DAIDs (263).

4.2.2 Sample collection

For the DTG arm, patients were initiated on once daily 50mg DTG (DTG 50 mg +2 NRTI) with sampling on days 1, 7 and 14 for baseline, one week and 2 weeks VL monitoring. Rich PK sampling (0-24h) was done on day 14 and 2 weeks post-partum. Similarly, samples were also collected at the same time points for viral load monitoring in the EFV arm (*Figure 1*). Breast milk samples were also collected postpartum at 3-5 h post dose, 24 h post dose and 1-3 days post DTG cessation. Maternal and infant plasma samples were also collected at 3-5 h post dose, 24 h post dose and 1-3 days post DTG cessation. Plasma samples and breast milk samples were collected and stored at -80^{o}C until shipment to appropriate laboratory for analysis.

All sample collection and processing at the sites of the trials were conducted by designated technical staff. PK and measurement of drug levels was done in Liverpool bioanalytical facility, while plasma and BM VL were analysed at National health laboratory service, Division of Virology Cape Town South Africa. Other baseline investigations and evaluation of participants were done at the study site.

4.2.3 Bioanalysis of DTG

DTG bioanalysis was performed within 3 h of removal of the samples from the -40 freezer. Samples were thawed, and analyse using equal volume (100µL) of sample as used in the calibration standards and dolutegravir-d5 internal standard. Tert-butyl-methyl-ether (TBME) was used for extraction and the samples were subsequently dried in a nitrogen drier. Samples were reconstituted in required proportion of methanol: water and 0.1% formic acid (80:20 v/v) for subsequent injection onto the LC-MS. The analytes were resolved using reverse phase C_{18} XBridge waters (3.5µm: 2.1x50mm) column (Waters Cooperation USA) and quantified by a Thermo TSQ Access triple quadrupole mass spectrometer. Data acquisition was performed using LQUAN software (Thermo Scientific) (242). The dried breast milk spots (DBMS) were analysed by spotting 30 µL of breast milk sample on a 12 mm circle of a whartman protein saver card and allowed to dry. Dolutegravir-d5 was spotted on the card and allowed to dry prior to processing as described in chapter 3. My role in this trial was to validate and develop a novel method for quantifying DTG in BM spots and analysis of samples, plasma samples analysis and non compartmental modelling of the measured DTG concentrations to derive PK parametres. All analysis were done under the supervision of senior laboratory personals.

4.2.4 Statistical analysis

DTG PK parameters (C_{max}, T_{max}, $t_{1/2}$, $C_{min,}$ C_{0-24} and AUC_{0-24}), were calculated using WinNonlin, Phoenix, (version 6.1, Pharsight, Mountain View, CA) for non-compartmental PK analysis of data. Other statistical analysis of associations and relationships were done using Geometric means, Geometric mean ratios and parametric test (t-test).

4.2.5 Ethical Approvals

The study adhered to the Helsinki declaration and current applicable regulatory requirements. Research ethics as stipulated in the protocol and compliance with ICH-GCP were strictly followed to the latter. Protocol and ethical approval were obtained from Joint Clinical Research Centre Institutional review board, Ugandan National Council for Science and Technology and Research integrity, The Governance and University of Liverpool research ethics committee, Liverpool UK and Desmond Tutu hospital research committee. A signed inform consent including paternal consent was obtained before enrolling participants after carefully providing information on the study, the risk and precautions that participants are required to adhere to. Participants were also free to withdraw from the study at any time they deem risky to continue with the study or to be withdrawn by the clinician if he/she judge participant to be at risk of complications.

4.3 Results

At the time of writing this thesis, only 16 [8 on DTG and 8 on EFV arm (SoC)] VL and safety data were available and a total of 28 PK data of both third trimester and postpartum mothers. Median gestational age was 29.5w (range 28-35w) at enrolment. Baseline VL was $\log_{10}$ 4.15 (2.43-4.88) copies for DTG, and $\log_{10}$ 4.13 (2.88-6.07) copies for EFV. The proportion of VL reported as <50 copies/mL or undetectable at 2 weeks and at 4 weeks of therapy was 5/8 and 4/8 in mothers on DTG, and 1/5 and 2/7 in mothers on EFV, respectively. At 2 weeks post-partum 5/6 and 4/7 mothers on DTG and EFV had VL <50 copies. Two mothers in the DTG arm were withdrawn for virological failure; the first had no detectable drug in plasma and was non-adherent, the second had evidence of 3 class drug resistance (reverse transcriptase (RT) and protease mutations) (*Table 1*).

4.3.1 Maternal DTG Pharmacokinetic in Third Trimester of Pregnancy and 2 weeks postpartum

DTG PK parametres $t_{1/2}$, T_{max}, C_{max}, $C_{0\text{-}24}$ and $AUC_{0\text{-}24}$ were not different from previously reported studies (*Table 3, 4 and 5)*. Geometric mean (%CV) of the following PK parameters: $t_{1/2}$, T_{max}, C_{max}, $C_{0\text{-}24}$ and $AUC_{0\text{-}24}$ were 10.05 (24.46) h, 2.94 (52.29) h, 2534.33 (27.42) ng/mL, 641.83 (74.39) ng/mL and 35322.10 (31.98) ng.h/mL respectively in third trimester of pregnancy (n=28), and $t_{1/2}$ 11.12 (26.23) h, T_{max} 2.43 (53.27) h, C_{max} 2843.27 (23.92) ng/mL, $C_{0\text{-}24}$ 695.98 (39.61) ng/mL, and $AUC_{0\text{-}24}$ 37574.55 (26.60) ng.h/mL respectively in the postpartum period of the same women (n=27). Although overall difference between pregnancy and postpartum was not

significant, there was a slight decrease (approximately 5%) in DTG exposure during pregnancy (*Table 3, Figures 2 and 3*).

4.3.2 Infant and BM DTG exposure intrapartum, postpartum and after cessation of DTG

BM DTG exposure was evaluated 1-5 h and 24 h post maternal DTG dose and 1-3 days after cessation of DTG therapy (*Tables 6 and 7*). BM DTG transfer was approximately 3% of the maternal plasma concentration, however concentrations in BM fell below the assay limit of quantification (LLQ) (10 ng/mL) after 1-3 days of treatment cessation. Sampling closer to time of delivery has an impact on infant plasma DTG concentration due to contributions from placental transfer. Cord to maternal plasma DTG ratio (C:M) was Geometric mean (90% CI) 1.19 (1.01, 1.38), consistent with values previously reported by the PANNA studies (264). Furthermore, IP:MP ratios were higher after drug cessation (as compared with the dosing interval) due to a persistent IP concentration, whereas DTG was rapidly eliminated in the mother. Given that BM DTG concentrations after 24 h were <LLQ suggests that the persistently high DTG concentrations in infants is driven by inefficient elimination of DTG in neonates. One sample was done 9 days after cessation of DTG, and all samples (MP, IP, BM) were found to be undetectable *(Tables 6 and 7*).

4.3.3 Safety of DTG in Third Trimestre of pregnancy

A total of 4 severe adverse events (SAEs) were reported in the study as follows: One (1) stillbirth in the DTG arm, 1 grade 3 hypertension in the EFV arm, 1 baby with polydactyly in the DTG arm, and other adverse events summarised in table 2. Out of the four SAEs reported, two were in one participant in the DTG arm and two in the EFV arm: JU9 (DTG arm) had a fresh stillbirth attributed to asphyxia due to cord around the neck and liver event (ALT 5 x ULN, total bilirubin 2.3 x ULN), which resolved after discontinuation of DTG. Patient also had ingested physalis minima Linn (herbal medication) prior to event, thought to be possibly related to the event, considering our poor understanding of the role of herbal medication in drug metabolism. VR0 (EFV arm): Developed pregnancy induced hypertension necessitating induction of labour. While EK6 (EFV arm): Infant polydactyly left foot (9 digits) and syndactyly of 2nd, 3rd and 4th digits were all considered unrelated since embryological development of these structures occurred prior to 3rd trimester of pregnancy, when patients were exposed to DTG, and it occurred in patient not exposed to DTG at all.

4.4 Discussion

DTG steady state exposure after 50 mg once daily dose in third trimester of pregnancy decreased by approximately 5% compared to postpartum period, and was not statistically significant. This was because postpartum sampling was done median 8

days (2-28 days) and does not reflect return to normal physiology. Unlike other reports of PANNA and IMPAACT 1026, there was a significant difference between third trimester pregnancy and postpartum because sampling was done ≥2 weeks postpartum (*Tables 3, 4, 5*).. C_{trough} concenctrations were all above the protein adjusted 90% inhibitory concentration (IC_{90}) of DTG which is 64ng/mL. Minimum effective concentration (MEC) of DTG is unclear, but only 9 out of 28 samples had C_{trough} below reported MEC of 324ng/mL. A value derived from a 10 days ranging studies of DTG monotherapy (168, 176). Despite decrease in DTG exposure during pregnancy [AUC_{0-24} = 35322.10 (19195.73, 67922.24) ng.h/mL], undetectable viral loads (<50 copies/mL) were still achieved within 2 weeks after commencing DTG. Intrapartum and postpartum Infants exposure to DTG is governed by transfer of DTG through membranes. DTG is able to pass through the placental membrane in utero, resulting in high infant exposure that persist in high concentration even after delivery. Postpartum DTG exposure is mainly through breast milk, which is determined by the amount of DTG excreted in BM. Transplacental transfer of DTG in utero was high during pregnancy as previously reported [132]. Median ratio of cord to plasma concentration at delivery was 1.23 suggesting high transplacental transfer. This is consistent with previous reports of high DTG transfer across the placenta to foetus resulting in high in utero exposure of neonates to DTG. Elimination of DTG in neonates was prolonged compared to the postpartum women (undetectable levels of DTG in MP and BM 1-3 days after DTG cessation) and was consistent with other reports of DTG elimination in infants (265). The persistent high levels of DTG in infants was attributed to immature metabolic pathways of UGT1A1 and CYP enzymes in the liver of infants (266-268). Transplacental transfer of DTG coupled with an

underdeveloped liver UGT1A1 and BM DTG exposure was thought to be responsible for the high neonatal DTG concentration (269). Previous reports of children exposed to low dose ART during PMTCT programmes and observation in some adults in current study who were previously exposed to ART in PMTCT, suggest that previous exposure to ART could promote resistant clades of virus against antiretroviral agents (23, 270).

4.4.1 DTG transfer to breast milk and Infant exposure

Steady state BM DTG concentrations were quantifiable at 2-4 hours and 24 hours post maternal dose in postpartum Ugandan and South African mothers. This resulted in median BM:MP ratios of 0.03 at 2-4 hours and 24 hours post maternal dose. Transfer of DTG from the mother's circulation to BM is potentially governed by its high (>98%) protein binding and thus sole passage of the unbound (free) form(247, 248).The influence of transporters and enzymes on DTG transfer across the mammary epithelium to the breastfeeding infant is unknown (178, 248). Time matched IP concentrations in breastfeeding infant 2-4 and 24 hours post dose and MP resulted in median IP:MP ratio 0.05 and 0.12 respectively. In all the women, DTG BM concentrations was less than LLQ (<10 ng/mL) when sampled 1-3 days after cessation of DTG therapy, whereas corresponding MP and IP concentrations remained detectable (>10ng/mL) in infants for up to 3 days post DTG treatment cessation. DTG MP elimination was consistent and concentrations measured were low 3 days after cessation of DTG, while IP DTG elimination was slower, giving rise to high IP:MP of 0.46 (*Table 7*). Infant Plasma concentration was not measured beyond 3 days after cessation.

4.4.2 Safety and efficiency of viral suppression of DTG in Third trimester pregnancy

Administration of medications during pregnancy is complex and risky due to potential of inducing teratogenicity to embryos in utero. But in pregnant HIV positive women, it is essential in preventing MTCT. This study was conducted in third trimester of pregnancy, and less teratogenicity is expected since organogenesis occurs in first trimester (271). A recent interim report from an ongoing NIH funded study (Tsepamo) in Botswana suggested that there could be an increased risk of neural tube defect in neonates of women who become pregnant while taking DTG. Cautious use of DTG in women of reproductive age who want to become pregnant (272) was recommended by WHO while awaiting more data. In this study, abnormalities were noted at birth, but were thought not be related to DTG. Pregnancy induced hypertension observed in one participant was a known obstetric complication of unknown cause and polydactyly and syndactyly observed in 1 neonate were not considered to be related to DTG since limb and bone development takes place in first trimester of pregnancy (271) and DTG was only administered in third trimester, therefore highly unlikely to be related to DTG. A still birth recorded, was thought to be an obstetric complication of asphyxia secondary to cord round the neck and the cause of liver event (5X elevated ALT and 2.3 times ULN bilirubin) was unclear due to the presence of other confounders in the patients such as ingestion physalis minima Linn (herbal medication), and unknown UGT1A1 genotype of the woman, since polymorphisms in UGT1A1 could affect bilirubin metabolism (212).

The majority (80%) of patients on DTG achieved an undetectable viral load (<50 copies/mL) 2 weeks after commencing DTG compared to the EFV regimen (SoC)

which was approximately 10% (*Table 1*). One patient previously exposed to ART had high level multiclass resistance to NNRTI, NRTI and PI, resulting in treatment failure and VL of 145 copies/mL after 28 days DTG, and 2217 copies/mL 2 weeks postpartum. Out of the 6 who had no problem, 4 had VL of <50 copies/mL 14 days after starting DTG and 5 out of the 5 VL measured were <50 copies/mL 2 weeks postpartum. In contrast, only 1 out of the 8 patients on SoC regimen had VL<50 copies/ mL 14 days after starting ART, and 4 out of 8 2 weeks postpartum.

4.4.3 Limitations

Lack of sufficient safety data in early pregnancy is a concern to women who become pregnant on DTG regimen especially after the recent report of Tsepamo observational study in Botswana, which reported increased incidence of neural tube defect in infants of mothers who became pregnant while on DTG. DTG safety data in first trimester is still insufficient, when most organogenesis occurs and recommendations are still inconclusive. Several questions would need to be answered which include: Would women be advised to use contraception while on DTG? Will their DTG regimen be switched to other regimen prior to becoming pregnant and continued after first trimester? Is stopping DTG a risk of promoting class resistance? What happen to women who are resistant to switch options? Are there plans to evaluate safety of DTG in children <12 years? What is the long term effect of high neonatal DTG exposure to infant born to mothers on DTG regimen? These are questions that will need to be answered in subsequent studies. This study was conducted in small sample size, postpartum sampling collected median 8 days after delivery and does not reflect return to normal physiology. Therefore no difference was observed between third trimestre and postpartum. UGT1A1 is highly

polymorphic and activity is likely to vary from one individual to another. These limitations were noted and DOLPHIN 2, a definitive trials aim at providing clarity to the limitations.

4.5 Conclusion

DTG exposure during pregnancy is sufficient to induce viral suppression to undetectable levels within 2 weeks of commencement, irrespective of reduced concentrations. It efficiently reduce the risk of vertical transmission during delivery. However transplacental in utero exposure to neonates with poorly developed UGT1A1 and persistently high foetal exposure over a long period of time is a concern since implication on foetal health and development is unknown. Still birth and liver event encountered in this study were thought not to be related to DTG, but additional safety data throughout pregnancy and postpartum period (including breastfeeding) is required. Further evaluation of pharmacogenetics of UGT1A1 and CYP enzymes, viral loads in breast milk, DTG excretion profiles in BM and safety in the paediatric age group < 12 years is required and likely to be evaluated further in DOLPHIN 2 clinical trial.

Table 1: Viral Load

Subj No.	Gestation at Enrolment	Arm	Baseline CD4	Baseline VL	VL at Rand.	Day 7 VL	Day 14 VL	Day 28 VL	2 Weeks Post-partum
1*	32	DTG	277	76721	34506	610	<100	145	2217
2	30	DTG	343	54246	7432	245	207	<100	ND
3	30	DTG	42	69117	2042	ND	<50	<50	<50
5	28	DTG	578	267	140	ND	<50	54	<50
9	31	DTG	469	6374	4147	ND	<50	<50	<50
11	29	DTG	318	13256	293	61	<50	<50	<50
12	28	DTG	296	15342	41209	ND	63169	301	<50
14^	30	DTG	514	1115	1906	3586	5055	17815	ND
4	27	EFV	117	1181787	~	ND	7455	448	292
6	29	EFV	567	143330	~	ND	1313	201	<50
7	28	EFV	605	3572	~	ND	PD	<50	<50
8	28	EFV	32	33147	~	ND	PD	835	180
10	33	EFV	502	5722	~	ND	166	181	ND
13	30	EFV	381	947	~	ND	103	74	<50
15	35	EFV	69	108975	~	ND	ND	ND	941
16	28	EFV	354	765	<50	<50	<50	<50	<50

Participant 1 had high level resistance (NNRTI, NRTI and PI) from baseline sample, suggesting previous, ART exposure

^Participant 14 had not been taking study drug, as evidenced by undetectable concentrations on the day,

of intensive PK ~Protocol did not initially require a repeat VL at randomization in the EFV arm

ND: not done; PD: protocol deviation

Table 2. Adverse events. All were of mild or moderate severity and either not related, possibly related or probably related to ART. In bold are events that occurred more than once. All events that were either possibly or probably related to study drug recovered/resolved before the end of the study except for one case of diarrhoea and the case of hyponatraemia.

Stem Organ Class	Preferred Term (n)	Total	ITT		As Treated	
			DTG	EFV	DTG	EFV
Blood and lymphatic system disorders	Anaemia (1)	1	1	0	1	0
Cardiac disorders	Tachycardia (1)	1	0	1	0	1
Gastrointestinal disorders	**Nausea (6)**, **Vomiting (6)**, **Diarrhoea (3)**, Abdominal pain lower (1), Dyspepsia (1),	17	13	4	7	10
Infections and infestations	**Upper respiratory tract infection (3)**, **Urinary tract infection (6)**, Gastroenteritis (1), Lower respiratory tract infection (1), Nasopharyngitis (1), Oral candidiasis (1), Streptococcal urinary tract infection (1), Vulvovaginal candidiasis (1), Wound sepsis (1)	16	7	9	5	11
Injury, poisoning and procedural complications	Laceration (1)	1	0	1	0	1
Investigations	Blood pressure increase (1)	1	1	0	1	0
Metabolism and nutrition disorders	Decreased appetite (1), hypoglycaemia (1), hyponatraemia (1)	3	1	2	1	2
Musculoskeletal and connective tissue disorders	Arthralgia (1)	1	1	0	1	0
Nervous system disorders	**Dizziness (6)**, **Headache (4)**, Syncope (1)	11	9	2	4	7
Pregnancy, puerperium and perinatal conditions	**Premature labour**[†] **(2)**, Gestational hypertension(1)	3	1	2	1	2
Psychiatric disorders	Abnormal dreams, nightmare	2	2	0	1	1
Renal and urinary disorders	Proteinuria (1)	1	1	0	0	1
Respiratory, thoracic and mediastinal disorders	**Cough (2)**, Hiccups (1)	3	2	1	2	1
Skin and subcutaneous tissue disorders	Rash (1), Rash papular (1)	2	1	1	0	2
Total		63	40	23	24	39

DTG = dolutegravir + 2 NRTIs; EFV: efavirenz-based standard of care; ITT = intention to treat; n = number of events. [†] 35 and 36 weeks of gestation.

Table 3: Maternal DTG Pharmacokinetic Parametres Geometric means (Geomean) and Geometric mean ratios (GMR) of women in third trimester pregnancy and Postpartum

DTG PK Parametres	Pregnant at 3rd Trimester (n=28)	Postpartum (n=27)	GMR (90% CI)
C_{max} (ng/mL)	2534.33 (1461.99, 3986.37)	2843.27 (1397.94, 4224.19)	0.91 (0.82, 1.01)
T_{max} (h)	2.94 (2.40, 3.47)	2.63 (2.04, 3.21)	1.23 (0.82, 1.82)
AUC_{0-24} (ng.h/mL)	35322.10 (19195.73, 67922.24)	37574.55 (14933.21, 59633.24)	0.95 (0.74, 1.23)
C_{0-24} (ng/mL)	641.83 (188.16, 3087.91)	695.98 (204.05, 1443.35)	0.93 (0.76, 1.14)
$t_{1/2}$ (h)	10.05 (6.50, 16.74)	11.12 (6.13, 17.34)	

Table 4: IMPAACT P1026s (266)

Parameter	Third trimester (30-38 wks)	Postpartum (6-32 wks)	GMR (90% CI) T3/PP		
n subjects	28	22	22		
$C_{24\ ng/ml}$	930 (680-1340)	1280 (800-1950)	0.66	0.52	0.84
$C_{max\ ng/ml}$	3540 (2660-4240)	4850 (3830-5970)	0.75	0.64	0.88
$AUC_{24\ ng.h/ml}$	49200 (36400-62000)	65000 (47800-88400)	0.71	0.63	0.81

Values are expressed as median (IQR)

Table 5: PANNA (264)

Parameter	Third trimester (31-38 wks)	Postpartum (3-7 wks)	GMR (90% CI) T3/PP		
n subjects	8	5	5		
$C_{24\ ng/ml}$	700 (109)	1100 (71)	0.66	0.32	1.36
$C_{max\ ng/ml}$	3400 (33)	3000 (41)	1.07	0.78	1.47
$AUC_{24\ ng.h/ml}$	42900 (39)	44800 (56)	0.95	0.60	1.48

Values are expressed as geometric mean (CV%)

Table 6: Infant and BM DTG exposures 2-4 and 24 hours dose postpartum

IDI Uganda			
Postpartum			
Post-delivery (days)	**GM (90%CI)**	**Post-delivery (days)**	**Median (min-max)**
	5.6 (3.6, 7.5)		6.0 (2.0-14.0)
[DTG]	**GM (90% CI)**	**Ratio**	**GM (90%CI)**
$Infant_{max\ ng/ml}$	199.64 (123.79,275.50)	$IP:MP_{max}$	0.08 (0.05,0.11)
$Infant_{trough\ ng/ml}$	151.06 (103.24,198.88)	$IP:MP_{trough}$	0.23 (-0.02,0.49)
$BM_{max\ ng/ml}$	64.67 (39.42,89.92)	$BM:MP_{max}$	0.03 (0.02,0.04)
$BM_{trough\ ng/ml}$	23.55 (17.31,29.79)	$BM:MP_{trough}$	0.03 (0.02,0.04)
South Africa			
Postpartum			
Post-delivery (days)	**GM (90%CI)**	**Post-delivery (days)**	**Median (min-max)**
	10.1 (8.6,11.7)		11.0 (7.0,18.0)
[DTG]	**GM (90%CI)**	**Ratio**	**GM (90%CI)**
$Infant_{max\ ng/ml}$	58.47 (-29.83,146.77)	$IP:MP_{max}$	0.02 (-0.01,0.06)
$Infant_{trough\ ng/ml}$	50.48 (-8.62,109.57)	$IP:MP_{trough}$	0.06 (-0.05,0.18)
BM_{max} ng/ml	75.85 (64.78, 86.92)	$BM:MP_{max}$	0.03 (0.03, 0.04)
Bm_{trough} ng/ml	23.78 (19.00, 28.56)	$BM:MP_{trough}$	0.03 (0.02, 0.04)
Combined South Africa and Uganda			
Postpartum			
Post-delivery (days)	**GM (90%CI)**	**Post-delivery (days)**	**Median (min- max)**
	7.9 (6.4, 9.3)		8.5 (2.0, 18.0)
[DTG]	**GM (90%CI)**	**Ratio**	**GM (90%CI)**
$Infant_{max\ ng/ml}$	110.73 (49.79, 171.66)	$IP:MP_{max}$	0.05 (0.02, 0.07)
$Infant_{trough\ ng/ml}$	87.32 (47.41, 127.24)	$IP:MP_{trough}$	0.12 (-0.02, 0.26)
BM_{max} ng/ml	70.47 (57.65, 83.29)	$BM:MP_{max}$	0.03 (0.03, 0.04)
BM_{trough} ng/ml	23.78 (19.00, 28.56)	$BM:MP_{trough}$	0.03 (0.02, 0.04)

IP = infant plasma, MP = maternal plasma, CD = cord blood, BM= breast milk

Table 7: Infant and BM DTG exposures 1-3 days after DTG cessation

IDI Uganda			
Postpartum (1-3 days post cessation of DTG)			
Post-delivery (days)	**GM (90%CI)**	**Post-delivery (days)**	**Median (min-max)**
	9.0 (7.0, 11.0)		8.5 **(**5.0-18.0)
Post final dose (days)	**GM (90%CI)**	**Post final dose (days)**	**Median (min-max)**
	1.9 (1.5, 2.3)		2.0 (1.0, 3.0)
[DTG]	**GM (90%CI)**	**Ratio**	**GM (90%CI)**
$Infant_{1\text{-}3days\ ng/ml}$	60.24 (14.90, 105.59)	$IP:MP_{max}$	0.73 (0.27, 1.19)
$BM_{1\text{-}3days\ ng/ml}$	**<LLQ**	$BM:MP_{max}$	**<LLQ**
South Africa			
Postpartum (1-3 days post cessation of DTG)			
Post-delivery (days)	**GM (90%CI)**	**Post-delivery (days)**	**Median (min-max)**
	13.9 (12.0, 15.8)		13.0 (10.0, 25.0)
Post final dose (days)	**GM (90%CI)**	**Post final dose (days)**	**Median (min-max)**
	2.1 (1.2, 3.0)		2.0 (1.0, 9.0)
[DTG]	GM (90%CI)	Ratio	GM (90%CI)
$Infant_{1\text{-}3days\ ng/ml}$	34.18 (9.16, 59.20)	$IP:MP_{max}$	0.33 (-0.46, 1.11)
$BM_{1\text{-}3days\ ng/ml}$	**<LLQ**	$BM:MP_{max}$	**<LLQ**
Combined Uganda and South African study sites			
Postpartum (1-3 days post cessation of DTG)			
Post-delivery (days)	**GM (90%CI)**	**Post-delivery (days)**	**Median (min-max)**
	11.5 (9.9, 13.1)		12.0 (5.0, 25.0)
Post final dose (days)	**GM (90%CI)**	**Post final dose (days)**	**Median (min-max)**
	2.0 (1.5, 2.5)		2.3 **(**1.0-9.0)
[DTG]	**GM (90%CI)**	**Ratio**	**GM (90%CI)**
$Infant_{1\text{-}3days\ ng/ml}$	44.22 (19.34, 69.11)	$IP:MP_{max}$	0.46 (-0.02, 0.95)
$BM_{1\text{-}3days\ ng/ml}$	**<LLQ**	$BM:MP_{max}$	**<LLQ**

IP = infant plasma, MP = maternal plasma, CD = cord blood, BM= breast milk, LLQ =

lower limit of quantification

***Figure 1*: Study Design. Intensive PK, infant and maternal viral load sampling time points**

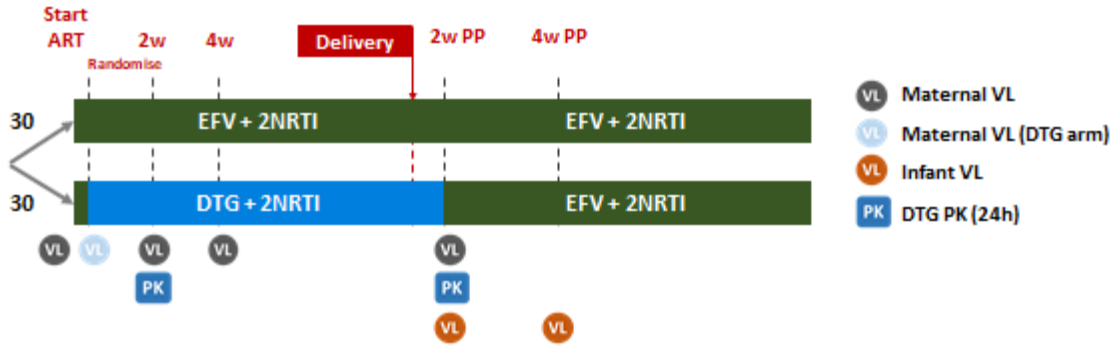


Figure 2: Paired PK profiles of DTG concentrations during third trimester pregnancy and postpartum period

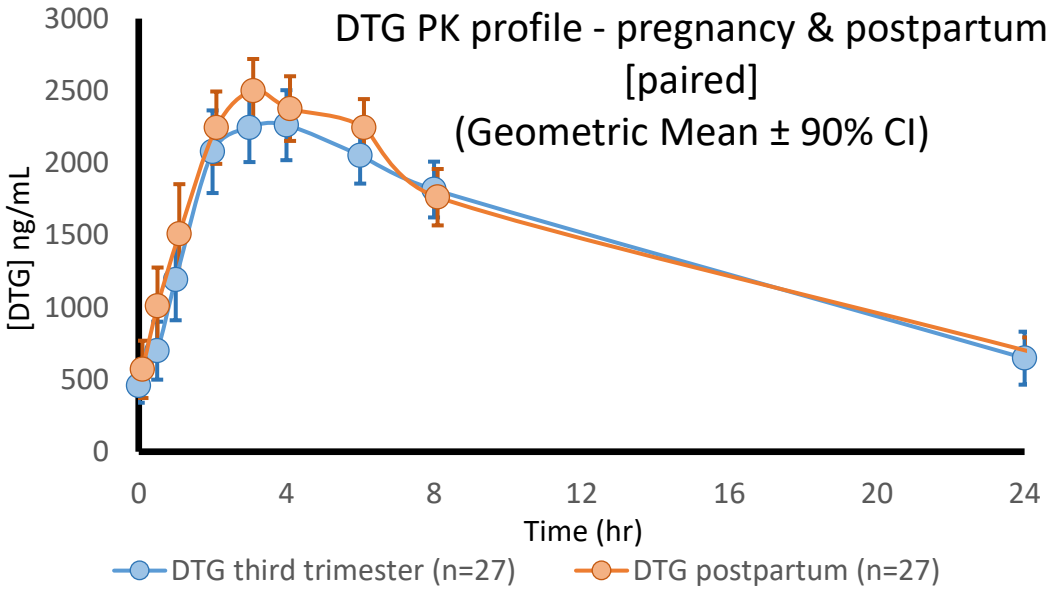
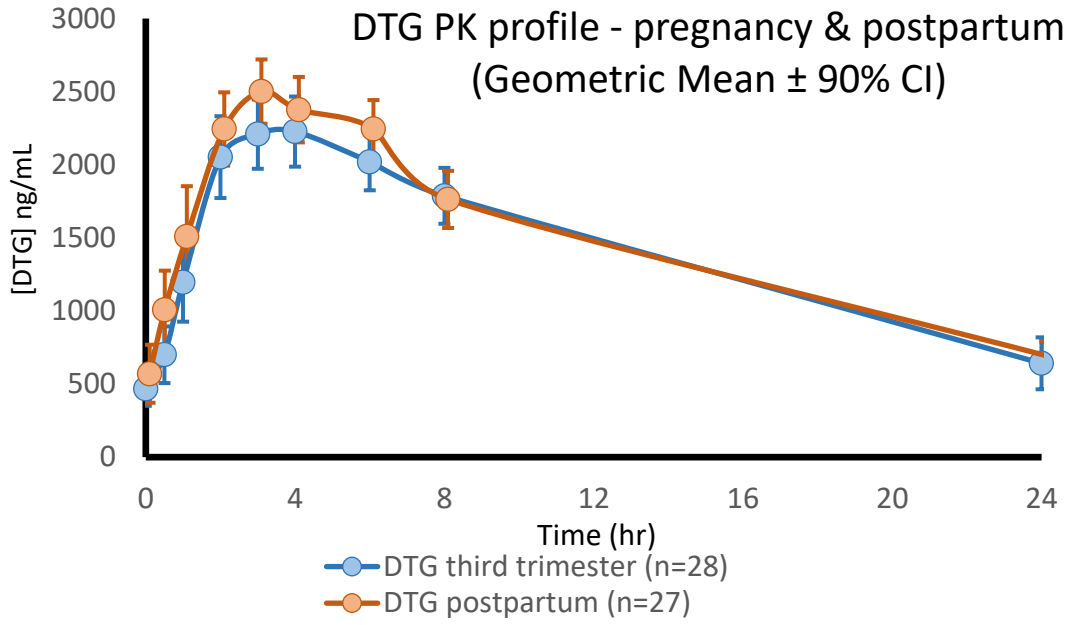


Figure 3: Unpaired PK profiles of DTG concentrations during third trimester pregnancy and postpartum period



CHAPTER 5

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CHAPTER 5

DOLUTEGRAVIR INTERACTIONS WITH ARTEMETHER-LUMEFANTRINE AND ARTESUNATE-AMODIAQUINE

5.1 Introduction

Malaria and HIV are highly prevalent in sub-Saharan Africa, causing high number of deaths especially among children and immunocompromised patients (160). In 2016, 212 million new cases of malaria were reported globally of which 90% were from Africa (160, 273). Also in 2015, 429,000 malaria related deaths were recorded, and 92% were from Africa (160, 273). Over 25 million people are currently living with HIV in sub-Saharan Africa, and 730,000 AIDS related deaths were reported in 2016 (3, 274). Therefore evaluating the safety of simultaneous treatment of the two diseases in the population is urgently needed, particularly before dolutegravir (DTG) is rolled out and widely prescribed in the region. Important in the natural history of these tropical diseases is while HIV is chronic in nature requiring lifelong treatment, malaria treatment is short duration, approximately 3 days. This makes co-administration of the two treatments and potential drug interaction an important challenge since DTG will not be stopped while malaria is been treated. Drug interactions with antiretroviral (ARV) and other antimicrobial medications is a well recognised challenge in HIV patients on ARV treatment especially as both drug classes are metabolized via similar pathways. Malaria treatment with artemisinin containing

therapies (ACTs, the most commonly prescribed group of antimalarials globally) in HIV patients on ARVs is common in the region, but there have been limited number of interaction studies (275, 276). ACT are known to interact with several classes of drugs which have been reported in several studies (277). Depending on the interaction, activity of the antimalarial is either associated with sub-optimal pharmacotherapy, with resultant decrease in clearance of plasmodia species and promoting recrudescence of malaria or enhanced activity with associated toxicities (278, 279).

It is worth nothing that HIV associated immunosuppression promotes infection with microbial agents, severe disease and poor outcomes (280). In malaria endemic regions, malaria/HIV co-infection is common, and presents with severe forms of malaria (280). New treatment of either disease need to be evaluated for safety of co-administration because of potential drug interactions (63, 278, 281). Sub-Saharan Africa is malaria endemic, so malaria is one of the most commonly encountered clinical conditions. Simultaneous management with HIV is complex due to potential drug-drug interactions (279, 282) and polypharmacy from multiple pills. Pharmacokinetic evaluation of the potential interaction of DTG with commonly prescribed antimalarial drugs [Artemether lumefantrine (AL) and artesunate-amodiaquine (AS-AQ)], as sample of ACT is a relevant and important study that will ensure evidenced based best practices in the region when using DTG and ACT simultaneously for treating HIV patients. Treatment should aim at reducing therapeutic failure, recrudescence rate of malaria treatment and drug safety. ACT are effective in treatment of malaria and minimal resistance have been reported against

the drugs (283-285). Likewise DTG is potent antiretroviral agent and favoured for a potentially wider choice as first line treatment of HIV (60). It sufficiently suppresses viral loads to undetectable levels (<50 copies per mL) within few weeks (VIKING and SAILING trials) (286-289).

Genetic variations of highly polymorphic UGT1A1 (major enzyme involve in the metabolism of DTG) in African populations (290), supports evaluation of these drugs in diverse ethnic groups to establish safety profiles in all populations in case of genetic variability in drug metabolism (187, 291, 292). The major metabolizing enzyme of DTG is UGT1A1 with a 10-15% contribution of CYP enzymes (CYP 3A4) (128). Artemisinin derivatives (artemether and artesunate) are converted to dihydroartemisinine (DHA) by CYP 3A4/5, 2B6 and 2A6 for artesunate (293). They in turn undergo glucoronidation by UGT1A1, to improve solubility for subsequent elimination (293). Both artemisinins and their metabolite DHA have antimalarial properties and induce rapid parasite clearance. Lumefantrine, from the halofantrine class of drugs is metabolized via CYP 3A4 and contributions from CYP 3A5 to its metabolite desethyl-lumefantrine (DBLF) (294, 295). Lumefantrine is long acting (half-life ($t_{1/2}$) 3-6 days) and highly lipophilic, its bioavailability is increased by fatty meals and is highly variable. Both AL and DBLF are active against plasmodium (293). Amodiaquine is a 4-aminoquinoline with short elimination $t_{1/2}$ of approximately 4-5 h (296, 297). It is converted to desethyl-amodiaquine (DEAQ), a potent metabolite with a terminal elimination $t_{1/2}$ of 6-18 days (297). Amodiaquine undergoes oxidative bio-activation to its metabolite N-desethyl-amodiaquine (DEAQ) via CYP enzymes (including CYP3A4, CYP2D6, CYP2C8 and CYP2C9) in the liver. DEAQ bio-activation is

also thought to be mediated via CYP2C9, CYP2D6 and CYP3A4 (298). Because of long elimination $t_{1/2}$ of lumefantrine, amodiaquine and metabolites DEAQ and DBLF, recrudescence is prevented in malaria treatment due to their prolonged activity (299, 300). Therefore combined effect of rapid parasite clearance from artemisin derivatives and prolonged clearance of parasites by the long-acting agents makes ACT an effective treatment option for malaria.

Lack of adequate DTG safety data is still a concern and limits use in settings where patients cannot be properly monitored. Evaluating the pharmacokinetic safety of co-administered DTG and ACT will contribute significantly to approval process by regulatory authorities. In view of the significance of simultaneous malaria and HIV treatment in the region, we sought to evaluate the pharmacokinetic drug-drug interaction of DTG and ACT (AL and AS-AQ) in healthy volunteers to determine if DTG can be safely use for treatment of HIV simultaneously with malaria in an African population.

5.2 Methods

5.2.1 Study Design

DolACT (Clinical trials Identifier: NCT02242799) was a prospective, open label, fixed sequence healthy volunteer study of Ugandan HIV negative male or female (consent to use contraception throughout the trial period) subjects at Infectious Diseases Institute, Makerere University College of Health Sciences, that evaluated the

pharmacokinetic interactions between DTG and artemisinin based combination therapies (AL and AS-AQ). The study was divided into study A and B due to the long $t_{1/2}$ of antimalarial metabolites. Study A, a cross over design, randomized (1:1) 14 patients into two sequences of 7 patients each (sequence 1 and 2), calculated to have a power of >80% to detect changes in AUC outside the FDA limit of bioequivalence for DTG, with the assumption that LF CV ≤30% and ≥32% changes in DHA. Study B, a parallel group study design randomized 30 subjects into 2 equal arms of 15 each (arms 1 and 2) (*Figure 1*) was similarly calculated to yield 80% power to detect AUC difference of >25-30% (DTG and DEAQ), and a ≥42% change in DHA. Study A (AL study group): Sequence 1 (S1)subjects received 6 doses of AL (80 mg artemether and 480 mg lumefantrine) over 3 days, with rich PK sampling on day 3 (AL alone). Thirteen (13) time points (0-264 h post dose) were collected followed by a 21 day washout, after which a once daily 50mg of DTG was administered for 6 days with rich PK sampling (DTG alone) on day 34, and 8 time points (0-24h post dose) collected. AL and DTG were then administered for 3 days with PK (DTG and AL combined) on day 38, and vice versa for S2. Study B subjects were randomised to Arm 1 or Arm 2 (A1/A2) due to the long $t_{1/2}$ of amodiaquine and its metabolites, making retention of volunteers unfeasible in a cross over design . A1 subjects received AS-AQ (4 mg/kg body weight of artesunate and 10 mg/kg body weight of amodiaquine) once daily for 3 days with rich PK sampling (AS-AQ alone) on day 3 and 13 time points (0-264 h). A2 subjects received 7 days of once daily 50 mg of DTG with PK sampling (DTG alone) on day 7 of 8 time points (0-24 h) , followed by 3 days of DTG and AS-AQ once daily and PK (AS-AQ and DTG in combination) on day 10.

Inclusion criteria were: Well informed and signed consent, willingness of participants to comply with all study requirements and schedules, age ≥ 18 years, weight ≥40kg, negative malaria blood film screening, HIV antibodies negative, willingness to use mosquito bednet throughout the study period and willingness to use barrier contraception throughout study duration. Exclusion criteria were abnormal creatinine levels, serum alanine transaminases, pregnant women or unwilling to use required contraception, abnormal ECG (prolong QT), known injection drug user, hepatitis B surface antigen positive, participant judged by clinician to likely be poorly adherent to treatment, other neurological, gastrointestinal and respiratory symptoms judged by clinician to be worsened by participating in the trial. The primary endpoint of this study were changes of pharmacokinetic parameters of antimalarials and DTG, and secondary endpoint were safety and tolerability of the drugs when co-administered. All clinical and laboratory adverse events were reported using DAIDs criteria (263).

5.2.2 Sample Collection

All drug administration were supervised and administered with moderate fatty meals to enhance absorption (301, 302). DTG steady state PK measurements were taken at 0, 1, 2, 3, 4, 8, 12 and 24 hours post dose while AL and AS-AQ PK measurement were taken at 0, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96 ,168 and 264 h post dose, and 0, 1, 2, 3, 4, 8, 12, 24, 48, 72, 96, 120, 228, and 624 h post dose respectively. Blood samples were collected in lithium heparin collection tubes for AL and AS-AQ PK measurements to minimize ex-vivo degradation of artemisinins to DHA by plasma esterases (303), and ethylenediaminetetraacetic acid (EDTA) tubes to chelate polyvalent metals (242) for

DTG PK measurements. Blood samples were delivered to the laboratory within 15 minutes of collection for separation of plasma within 3 hours of collection of blood samples. Approximately 2 mL of plasma was collected and stored at -80 ^{o}C until shipment (on dry ice) to the Bioanalytical Facility at The University of Liverpool for DTG analysis and Wellcome trust Mahidol Oxford Tropical Medicine Research Unit (MORU) for ACT analysis, using liquid chromatography-mass spectrometry (LC-MS), .

5.2.3 Safety assessments

Safety assessment included: Medical history, physical examination, electrocardiogram (ECG), urine pregnancy test, rapid malaria and HIV tests and safety bloods (haemoglobin, white cell count, platelets, urea, creatinine, electrolytes, ALT). Standard 12-lead ECG was performed at screening, intensive PK and at end of study. Safety blood investigations were repeated at every intensive PK visit and prior to discharge from the study. All laboratory and clinical abnormalities were noted and graded for severity according to the U.S National Institutes for Health Division of AIDS (DAIDS) Table for Grading Severity of Adult and Pediatric Adverse Events.

5.2.4 Ethical Approvals

This study was conducted in accordance with the principles of Helsinki declaration and current applicable versions of regulatory requirement. The study strictly adhered to the standards of research ethics as stipulated in the protocol and compliance with the ICH-GCP requirements. Protocol and ethical approval were obtained from Joint Clinical Research Centre Institutional review board, Ugandan National Council for Science and Technology and Research integrity and Governance and University of Liverpool research ethics committee, Liverpool UK. A signed inform consent was

obtained from each volunteer after clearly explaining the risk associated with the study and expected precautions subject were required to adhere to. Participants were free to withdraw from the study at any time they deemed risky to continue with the study.

5.2.5 Bioanalysis

DTG samples were analysed using a validated LC-MS method for measuring DTG in plasma [calibration range 10-4000 ng/ml and precision of 5% at low quality control (LQC)] at the Liverpool bioanalytical facility (BAF), A GCP accredited laboratory that participate in external Quality Assurance programmes. In brief, DTG was extracted using a liquid-liquid extraction method with tert-butyl-methyl ether (TBME) containing a stable isotope labelled internal standard dolutegravir-d5. Plasma samples were extracted, dried in a nitrogen drier and reconstituted in required proportion 80:20 (v/v) methanol/water, and 0.1% formic acid. The sequence was acquired using LQUAN software (Thermo Scientific, Hemel Hempstead, UK) and analytes resolved using reverse phase C_{18} XBridge (3.5µm: 2.1x50mm) column (Waters, Corporation, U.S.A) for quantification by a Thermo TSQ Access triple quadrupole mass spectrometer.

DHA, artemether, artesunate, lumefantrine, DBLF, amodiaquine and DEAQ for AL and AQ-AS were analysed at MORU tropical health Network Laboratory Bankok, also a GCP accredited laboratory that participate in external Quality Assurance programmes using validated LC-MS methods (calibration ranges 1.14-575 ng/mL for DHA and artemether (assay coefficient of variation < 6%) , 7.77-23000 ng/mL for lumefantrine

(coefficient of variation < 6%) and 0.808-884 ng/mL for DBLF(coefficient of variation < 6%). Calibration ranges of DHA in study B was 1.57-2875 ng/mL (coefficient of variation < 7%), artesunate was 0.952-837 ng/mL (coefficient of variation < 7%), amodiaquine was 0.864-302 ng/mL and DEAQ was 1.13-702 ng/mL (coefficient of variation < 8%) respectively (303-305).

5.2.6 Statistical analysis

5.2.6.1 Study A

Pharmacokinetic parameters for DTG [Maximum plasma concentration (C_{max}), time to maximum concentration (T_{max}), $t_{1/2}$, minimum plasma concentration in 24 hours (C_{0-24}) and area under the concentration curve (AUC_{0-24})], artemether [C_{max}, T_{max}, $t_{1/2}$, plasma concentration from time zero to trough concentration (C_{0-t}) and area under the concentration curve from time zero to trough concentration (AUC_{0-t})] and DHA (C_{max}, T_{max}, $t_{1/2}$, C_{0-t} and AUC_{0-t}), and lumefantrine and DBLF (C_{max}, T_{max}, $t_{1/2}$, $C_{0-t,}$ AUC_{0-t}) were estimated using non-compartmental analysis (WinNonlin, Phoenix, version 6.1, Pharsight, Mountain View, CA). Subject changes were assessed using geometric mean ratios (GMR) with 90% confidence intervals (GMR 90% CI), by log-transformation of PK data and geometric mean ratios (GMR), with 90% CI evaluated using paired (Study A) or unpaired (Study B) t-tests which were then back-transformed to absolute ng/mL concentrations.

5.2.6.2 Study B

Pharmacokinetic parameters for DTG (C_{max}, T_{max}, $t_{1/2}$, C_{0-24} and AUC_{0-24}), artesunate (C_{max}, T_{max}, $t_{1/2}$, C_{0-t} and AUC_{0-t} and DHA (C_{max}, T_{max}, $T_{1/2}$, C_{0-t} and AUC_{0-t}), amodiaquine

and DEAQ (C_{max}, T_{max}, $t_{1/2}$, C_{0-t} and AUC_{0-t}) were calculated using (WinNonlin, Phoenix, version 6.1, Pharsight, Mountain View, CA) and geometric means and GMR to determine associations. AS-AQ PK data were log-transformed and the 90% CI derived using an unpaired t-test, after which the values were back-transformed to absolute ng/mL concentrations. An analysis of variance (ANOVA) was performed by SPSS (Windows Standard version 22, SPSS, Chicago) on PK parameters (AUC_{0-t}, C_{max}, C_{24}) to assess potential sequence and period related effects.

5.3 Results

A total of 48 adult male or female subjects age 21-34 years were recruited in the study, and 39 completed the study. Study A sequence 1 and 2 had 14 volunteers randomized to sequence 1&2 of 7 each in a crossover study design which evaluated the pharmacokinetic interaction of AL and DTG. In Study B (Arm 1 and 2) 13 subjects were randomized to arm1 and 12 subjects in arm 2 in a parallel study design that evaluated the pharmacokinetic interaction of AS-AQ and DTG. All subjects were healthy by history, vital signs, physical examinations, electrocardiograms and laboratory results. There was good adherence to drugs and drugs were well tolerated by subjects throughout the study period (*Table 5*).

5.3.1 Study A

5.3.1.1 Impact of Artemether lumefantrine on Dolutegravir Pharmacokinetics

Artemether lumefantrine had no significant impact on DTG AUC_{0-24} C_{max} or T_{max} in both study arms (*Table 1 and figure 2*). There was a 37% decrease in the DTG C_{0-24}

[0.63 (0.48, 0.82)], however this was thought to be driven by an unexplained rise in $C_{0\text{-}24}$ values of some subjects in DTG alone arm as shown by the individual PK plots; (*figures 4 and 6*) who appeared to have a spike in the DTG concentrations after the C_{12} time point. Despite a statistically significant decrease in the DTG $C_{0\text{-}24,}$ the minimum measured $C_{0\text{-}24}$ concentration of 1543.11 ng/mL was higher than the mean trough concentration (1100 ng/ml) reported in prior phase II trials (172, 306, 307). Geometric means (%CV) of the following pharmacokinetic parameters: $t_{1/2}$, T_{max}, C_{max}, $C_{0\text{-}24}$ and $AUC_{0\text{-}24}$ in DTG alone arms were 24.29 (61.26) h, 3.94 (103.65) h, 5018.44 (22.24) ng/mL, 2456.50 (34.10) ng/mL and 78753.39 (22.73) ng.h/mL respectively. When DTG was administered with AL, the PK parameters [Geomean (%CV)] were 13.01 (42.67) h, 3.00 (68.61) h, 5216.45 (25.02) ng/mL, 1543.11 (54.27) ng/mL and 73738.47 (30.48) ng.h/mL respectively (*Table 1 and Figures 2-7*), and there were no significant difference from the DTG only phase. The ANOVA showed no significant sequence effect upon DTG pharmacokinetics, but there was a significant period effect (DTG alone versus. DTG plus AL) for DTG C_{24} in both arms (p=0.025).

5.3.1.2 Impact of DTG on PK of AL and its metabolites (Artemether, Dihydroartemisinin (DHA), lumefantrine and Desbutyl-lumefantrine (DBLF))

DTG administration had no significant impact on artemether, DHA, lumefantrine (LF) and desbutyl-lumefantrine (DBLF) PK parameters (*Table 3 & figure 8*). The $t_{1/2}$, T_{max}, C_{max}, $C_{0\text{-}t}$ and $AUC_{0\text{-}t}$ of parent drug and metabolite were not significantly altered with or without DTG. Geometric means (%CV) of artemether for the following: $t_{1/2}$, T_{max}, C_{max}, $C_{0\text{-}t}$ and $AUC_{0\text{-}t}$were: 4.92 (62.69) h, 2.03 (40.31) h, 31.93 (64.43) ng/mL, 2.88 (69.09) ng/mL and 129.58 (70.15) ng.h/mL for AL alone, and 7.28 (55.92) h, 2.16

(39.40) h, 27.88 (93.12) ng/mL, 2.05 (105.97) ng/mL and 136.44 (85.19) ng.h/mL for AL plus DTG, respectively. The geometric means (%CV) of artemether metabolite (DHA) PK parameters ($t_{1/2}$, T_{max}, C_{max}, C_{0-t} and AUC_{0-t}) were 2.54 (39.12) h, 2.31 (31.13) h, 110.43 (34.36) ng/mL, 3.01 (68.24) ng/mL and 389.26 (25.37) ng.h/mL for AL alone and 3.01 (95.80) h, 2.70 (53.91) h, 89.91 (42.50) ng/mL, 3.97 (79.21) ng/mL and 357.26 (47.85) ng.h/mL for AL plus DTG respectively (*Table 3 and Figures 8-28*).

Lumefantrine (LF) and its metabolite DBLF were also not impacted by DTG. Geometric means (%CV) of the following LF PK parameters ($t_{1/2}$, T_{max}, C_{max}, C_{0-t} and AUC_{0-t}) were 83.44 (18.87) h, 3.92 (64.52) h, 9975.96 (35.89) ng/mL, 6638.10 (38.99) ng/mL, 280.05 (30.43) ng/mL and 389350.00 (31.08) ng.h/mL for AL minus DTG, and 86.13 (24.66) h, 6.48 (121.62) h, 11203.12 (32.21) ng/mL, 7797.55 (31.64) ng/mL, 302.93(29.87) ng/mL and 429736.05 (25.38) ng.h/mL for AL plus DTG respectively. LF metabolite DBLF geometric mean (%CV) $t_{1/2}$, T_{max}, C_{max}, C_{0-t} and AUC_{0-t} were 141.57 (17.85) h, 4.78 (54.86) h, 51.75 (54.36) ng/mL, 46.68 (54.71) ng/mL, 11.27 (49.72) ng/mL and 6299.72 (48.12) ng.h/mL for AL minus DTG, and 162.12 (36.28) h, 9.52 (91.94) h, 49.95 (35.87) ng/mL, 46.16 (36.38) ng/mL, 11.34 (31.29) ng/mL and AUC_{0-t} 6048.71 (29.36) ng.h/mL for AL plus DTG respectively (*Table 3 and Figures 8-28*). The ANOVA showed no evidence of a significant sequence or period effect upon AL pharmacokinetics.

5.3.2 Study B

5.3.2.1 Impact of AS-AQ on DTG Pharmacokinetics

Co-administration of AS-AQ with DTG resulted in an approximate 42% and 24% decrease in DTG C_{24} and AUC_{0-24} [0.58 (0.50, 0.69) and 0.76 (0.69, 0.84)] respectively (*Table 2 and Figures 2*). However, despite the decreased DTG concentration, the

minimum measured concentration (1543.11 ng/mL) was higher than the reported trough concentration derived from population modelling (172). Therefore although statistically significant, it is not clinically significant to warrant any recommendation for dose adjustment when administered together. DTG was well tolerated with AS-AQ and geometric mean (%CV) of PK parameters $t_{1/2}$, T_{max}, $C_{0\text{-}24}$, C_{max} and $AUC_{0\text{-}24}$ were 16.21 (34.77) h, 3.69(49.49) h, 2174.32 (51.70) ng/mL, 5114.41 (22.18) ng/mL and 77936.06 (26.34) ng.h/mL for DTG alone, and 13.31 (29.22) h, 2.66 (58.60) h, 1271.93 (37.90) ng/mL, 4666.70 (31.27) ng/mL, 59490.93 (24.06) ng.h/mL respectively for AS-AQ plus DTG (*Table 2, figures 2 and 30-32*) .

5.3.2.2 Impact of DTG on AS-AQ PK and metabolites (Dihydroatemisinin (DHA), and Desethylamodiaquine (DEAQ))

AS-AQ is a combination of two potent antimalarials artesunate and amodiaquine. Although statistically significant changes were observed in the DTG PK parameters C_{24} and $AUC_{0\text{-}24}$ when co-administered, no significant impact was observed for the antimalarial and their metabolites (*Table 4 and Figure 29-36*). When AS-AQ was administered alone, the geometric mean (%CV) of artesunate PK parameters $t_{1/2}$, T_{max}, C_{max}, $C_{0\text{-}t}$, and $AUC_{0\text{-}t}$ were 1.85 (104.15) h, 1.17 (65.37) h, 61.29 (60.56) ng/mL, 0.72 (162.19) ng/mL, and 128.38 (55.19) ng.h/mL respectively. DHA PK parameters were $t_{1/2}$ 2.22 (170.10) h, T_{max} 1.58 (52.38) h, C_{max} 217.66 (52.8) ng/mL $C_{0\text{-}t}$ 3.56 (157.48) ng/mL, and $AUC_{0\text{-}t}$ 788.25 (42.45) ng.h/mL. Amodiaquine geometric mean (%CV) PK parameters $t_{1/2}$, T_{max}, C_{max}, $C_{0\text{-}t}$, and $AUC_{0\text{-}t}$ were 15.83 (20.80) h, 2.36 (94.28) h, 17.97 (33.44) ng/mL, 0.43 (0) ng/mL, and 256.14 (27.25) ng.h/mL and DEAQ were $t_{1/2}$ 243.68 (11.77) h, T_{max} 2.68 (57.01) h, C_{max} 393.96 (35.47) ng/mL, $C_{0\text{-}t}$ 15.13 (21.29)

ng/mL and AUC_{0-t} 31492.83 (18.92) ng.h/mL. When co-administered with DTG, geometric means (%CV) artesunate PK parameters ($t_{1/2}$, T_{max}, C_{max}, C_t and AUC_{0-t}) were 1.17 (50.54) h, 1.66 (56.54) h, 52.01 (67.78) ng/mL, 0.53 (61.36) ng/mL and 115.71 (50.32) ng.h/mL respectively. DHA PK parameters were $t_{1/2}$ 1.60 (23.97) h, Tmax 2.02 (46.90) h, C_{max} 290.43 (56.64) ng/mL, C_{0-t} 3.33 (171.73) ng/mL and AUC_{0-t} 946.78 (38.23) ng.h/mL. PK parameters for amodiaquine were $t_{1/2}$ 14.79 (29.58) h, T_{max} 1.97 (50.59) h, C_{max} 19.17 (33.46) ng/mL, C_{0-t} 0.43 (0) ng/mL and AUC_{0-t} 225.02 (23.68) ng.h/mL and DEAQ were $t_{1/2}$ 182.45 (40.74) h, T_{max} 3.38 (54.68) h, C_{max} 385.57 (20.66) ng/mL, C_{0-t} 232.56 (24.61) ng/mL and AUC_{0-t} 26943.12 (29.72) ng.h/ml respectively (*Table 4 and Figures 29 and 33-36*).

5.4 Discussions

We report for the first time that AL and AS-AQ combinations are both well tolerated and can be safely co-administered to patients receiving DTG. The study evaluated the PK interaction of AL and DTG and found changes in PK parameters of DTG, artemether, DHA, lumefantrine or DBLF when co-administered in healthy volunteers. The statistically significant decrease in DTG C_{0-24} by 37% was difficult to explain. Artemether and its metabolite DHA are known to induce CYP 3A4 and artemisin autoinduction is detectable for up to a week after administration which can affect medications pharmacology even after several days (308). Additional administration of DTG prior to next dosing was unlikely, because exact pills were given to participants and drug administration was closely monitored.

AS-AQ and DTG interaction resulted in statistically significant decrease in DTG C_{0-24} and AUC_{0-24} by 42% and 24% respectively, but in both instances, C_{0-24} (1.27 µg/mL) in

the AS-AQ +DTG study group was still above minimum concentrations (C_{min}) (1.11 µg/mL after a once daily 50mg dose) reported in previous trials steady state DTG C_{min} and population PK (172, 309).

Decrease in DTG $C_{0\text{-}24}$ (AL arm) and DTG $C_{0\text{-}24}$ and $AUC_{0\text{-}24}$ (AS-AQ arm) is unlikely to be clinically significant to warrant dose adjustment since. Subjects who received DTG with AL or AS-AQ, with DTG had comparable C_{trough} to or above 1100ng/mL (192). (The mean C_{trough} observed in previous DTG phase III adults trials and FDA approval trial study). The target minimum effective concentration for DTG is unknown, although a DTG minimum effective concentration (MEC) of 324ng/mL has been proposed (168, 176). A value derived from a phase II ten days dose ranging study of DTG monotherapy, found to be associated with virological efficacy. $C_{0\text{-}24}$ (C_{trough}) in all subjects were higher than previously reportedclinical trials (195), and were considerably higher than the estimated protein adjusted IC_{90} (64 ng/ml) of DTG (176). Assuming DTG concentration of less than 324 ng/mL is considered to be the sub-therapeutic level of DTG and given the $t_{1/2}$ of approximately 14 h, it will require about 2 missed doses (48-72 h post dose) for DTG C_{trough} concentration to be less than 324 ng/mL (170, 310), and since antimalarial treatment is just for three days, DTG decrease is not expected to be below 324ng/mL before completion of malaria treatment.

Previous reports of DTG PK has been predominantly in Caucasian populations or mixture of different ethnicities, but this study was exclusively conducted in African population and high plasma concentration could be associated with some genetic polymorphisms. Therefore evaluation of other factors such as polymorphisms of CYPS

and UGT1A1 enzymes to determine potential genetic reason for the higher concentrations measured will help answer some of the unexplained findings.

Previous reports of interactions of AL and AS-AQ with other antiretrovirals have suggested potential interaction of artemether and artemisinin with inhibitors of CYP3A4 such as Cobicistat, ritonavir, and elvitegravir (308, 311). DHA was also reported to be largely glucuronidated via UGT1A9 and DTG is a substrate of UGT1A9, suggesting potential interaction of DTG with drugs metabolized via these pathways, but that was not observed in this study (279). DTG was therefore observed to have a low propensity to drug-drug interaction and there was no drug-drug interaction between DTG and ACT. Therefore no dose adjustments is required in co-administration of the two medications.

This study was conducted in healthy volunteers who have negative malaria smear and are HIV negative. However, drug absorption in HIV positive patients differs from HIV negative subjects (293, 312, 313). It is influenced by changes in gut mucosa, microbiome and other pathological changes induced by HIV. Antimalarial PK and PD is also highly variable in malaria patients (314). The PK-PD changes in the course of treatment is influenced by parasite density (315). This makes antimalarial PK evaluation in malaria patients difficult. Conducting studies in healthy subjects provides baseline information for PK-PD modelling of antimalarial AL and AS-AQ that can be used in a population PK model to predict PK-PD parameters in an infected patients.

All adverse events (AEs) were Grade 1 or 2 in severity with more predominantly gastro-intestinal AEs, among participants who received AS-AQ, and no serious

adverse events were reported. Haematological (haemoglobin, white blood cells and platelets), biochemical (electrolytes, creatinine, urea and ALT) and electrocardiographic (QT interval) parameters evaluated throughout the trial period for both AL and AS-AQ were within normal ranges and no safety concerns were encountered throughout the trial. Although the chemical similarity of halofantrine and lumefantrine, was a potential risk of QT prolongation and arrhythmias among AL arm subjects, none was encountered throughout the study. Other established side effects of artemether lumefantrine such as headache, loss of appetite and generalized body weakness were also well tolerated. Known side effects of amodiaquine such as nausea, vomiting, vertigo and generalized body weakness were minimal and well tolerated by volunteers, so was also the side effects of DTG.

5.5 Conclusion

This Study is relevant and timely considering the planned roll out of DTG in low and middle income countries. The data will guide co-administration of DTG and ACT in HIV patients, without risk of inadequate parasite clearance and recrudescence from sub-optimal drug exposure. The short duration of antimalarial therapy, and clinically insignificant impact of DTG on ACT concentration makes it safe for DTG to be co-administered with ACT. This data will provide useful information that will guide national policy makers and DTG approval process in malaria endemic regions of the world.

Figure 1: Schedules and procedures of study

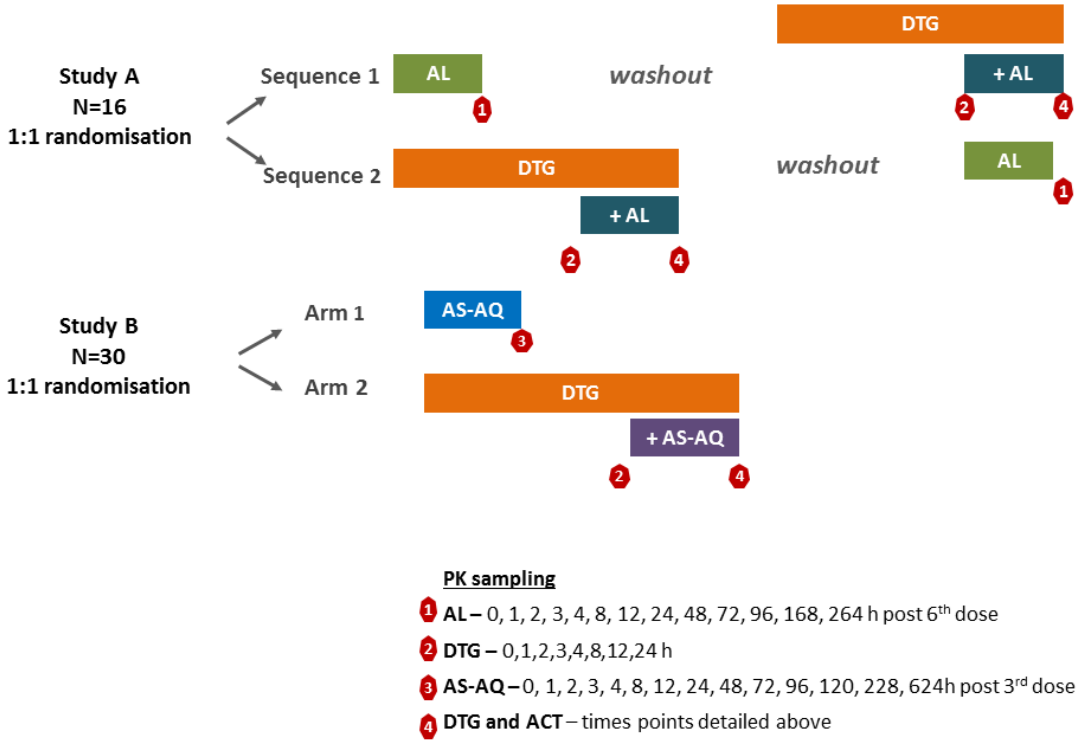


Table 1: Pharmacokinetic parameters [geometric mean (90% CI)] and Geometric Mean Ratio (GMR) of DTG in Study A (DTG+AL)

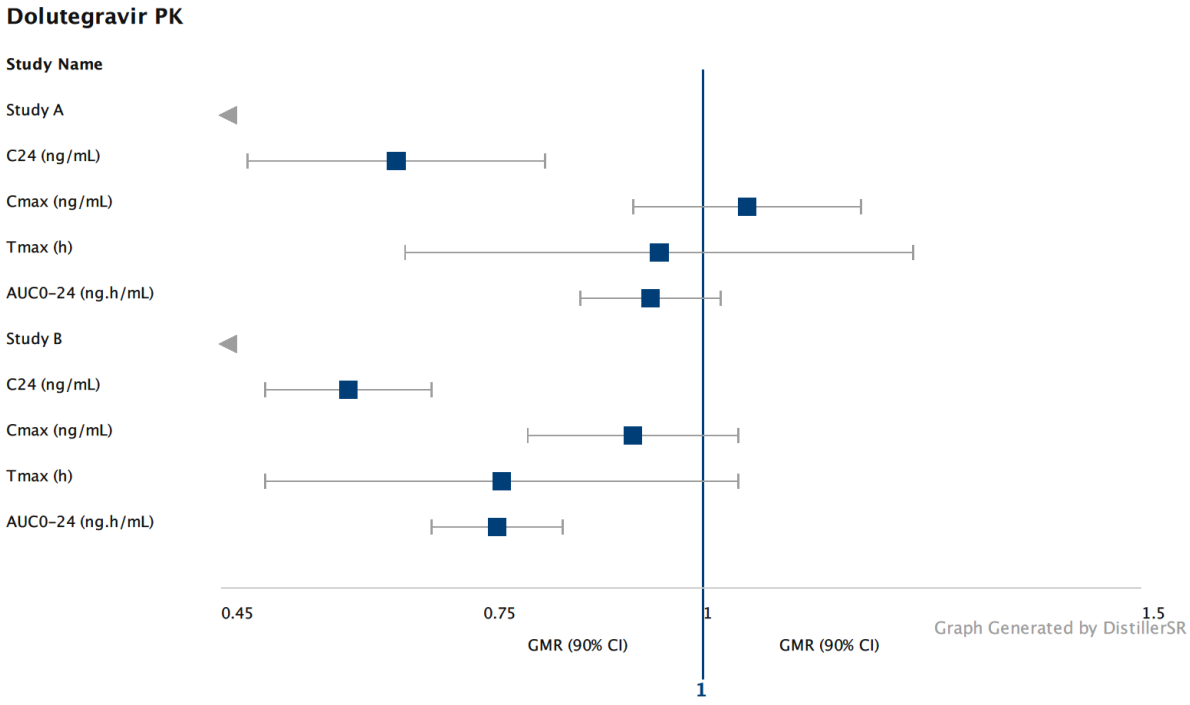
Study A (n= 14)	DTG alone [GM (90% CI)]	DTG+ AL [GM (90% CI)]	GMR (90%CI)
C_{24} (ng/mL)	2456.50 (2062.40, 2850.61)	1543.11 (1121.59, 1964.64)	**0.63 (0.48, 0.82)**
C_{max} (ng/mL)	5018.44 (4511.68, 5525.19)	5216.45 (4623.51, 5809.38)	1.04 (0.92, 1.18)
T_{max} (h)	3.94 (1.41, 6.46)	3.00 (1.89, 4.10)	0.90 (0.66, 1.24)
AUC_{0-24} (h.ng/mL)	78753 (70615, 86891)	73738 (63420, 84056)	0.94 (0.86, 1.02)

Table 2: Pharmacokinetic parameters [geometric mean (90% CI)] and Geometric Mean Ratio (GMR) of

DTG in Study B (DTG+AS-AQ)

Study B (n=12)	DTG alone [GM (90% CI)]	DTG + AS-AQ [GM (90% CI)]	GMR (90%CI)
C_{24} (ng/mL)	2174.32 (1567.34, 2781.30)	1271.93 (1025.47, 1518.40)	**0.58 (0.50, 0.69)**
C_{max} (ng/mL)	5114.41 (4562.16, 5666.66)	4666.70 (3940.04, 5393.36)	0.91 (0.80, 1.04)
T_{max} (h)	3.69 (2.72, 4.65)	2.66 (1.82, 3.50)	0.72 (0.50, 1.04)
AUC_{0-24} (h.ng/mL)	77936 (67804, 88068)	59490 (52479, 66502)	**0.76 (0.69, 0.84)**

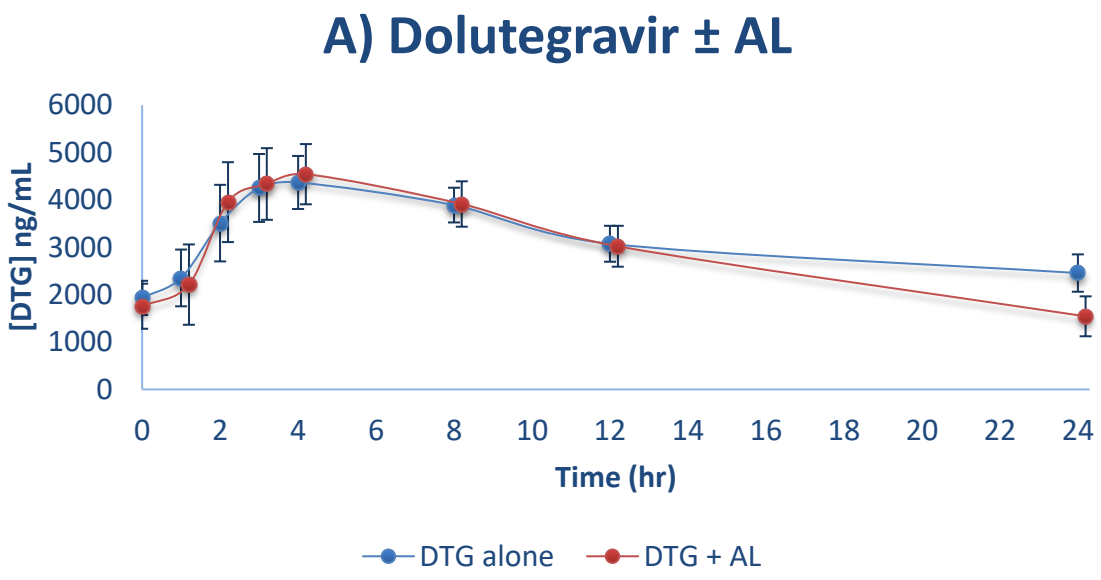
Figure 2: Geometric mean ratios (90% Confidence intervals) of Dolutegravir in study A and B (GMR; Geometric mean ratio, LCL; lower confidence level, UCL; upper confidence level)



Pharmacokinetic Summary of DTG: <u>Study A</u>

Geometric mean (90% confidence intervals) pharmacokinetic profile

Figure 3. Summary of DTG PK Data (DTG ± AL)



STUDY A: SEQUENCE 1 (n=7)

Individual PK profiles [Geometric mean shown by solid black line]

Figure 4. DTG Alone

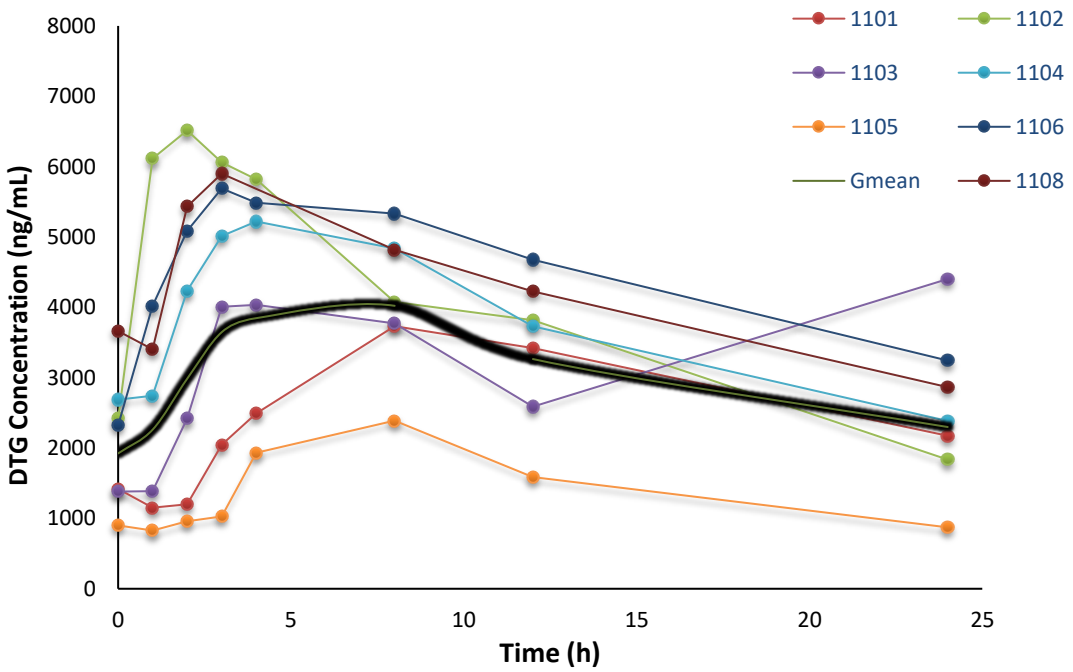
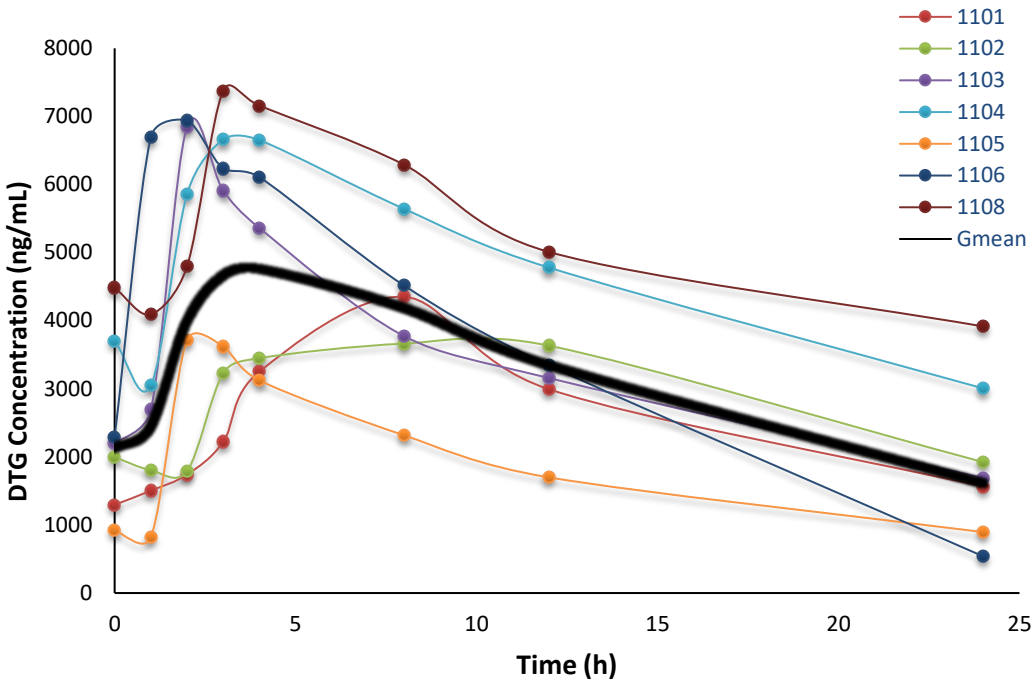


Figure 5. DTG +Artemether Lumefantrine



STUDY A: SEQUENCE 2 (n=7)

Individual PK profiles [Geometric mean shown by solid black line]

Figure 6. DTG Alone (Day 6; n=7)

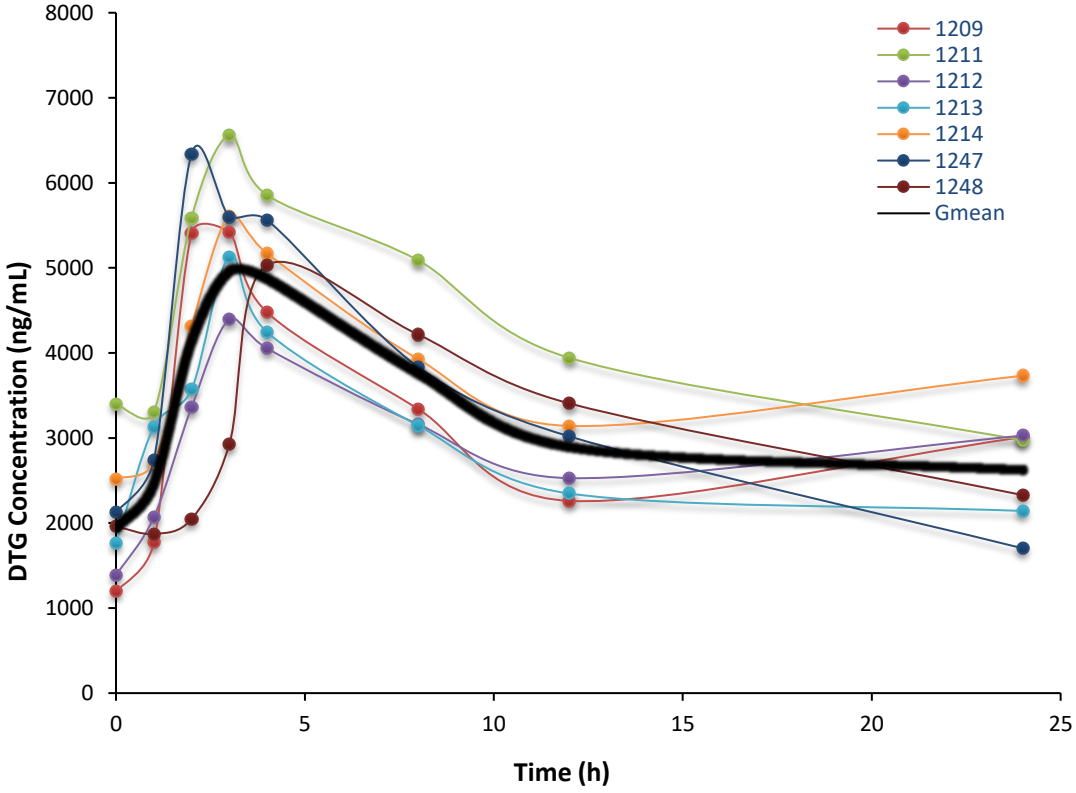


Figure 7. DTG +Artemether Lumefantrine (Day 10; n=7)

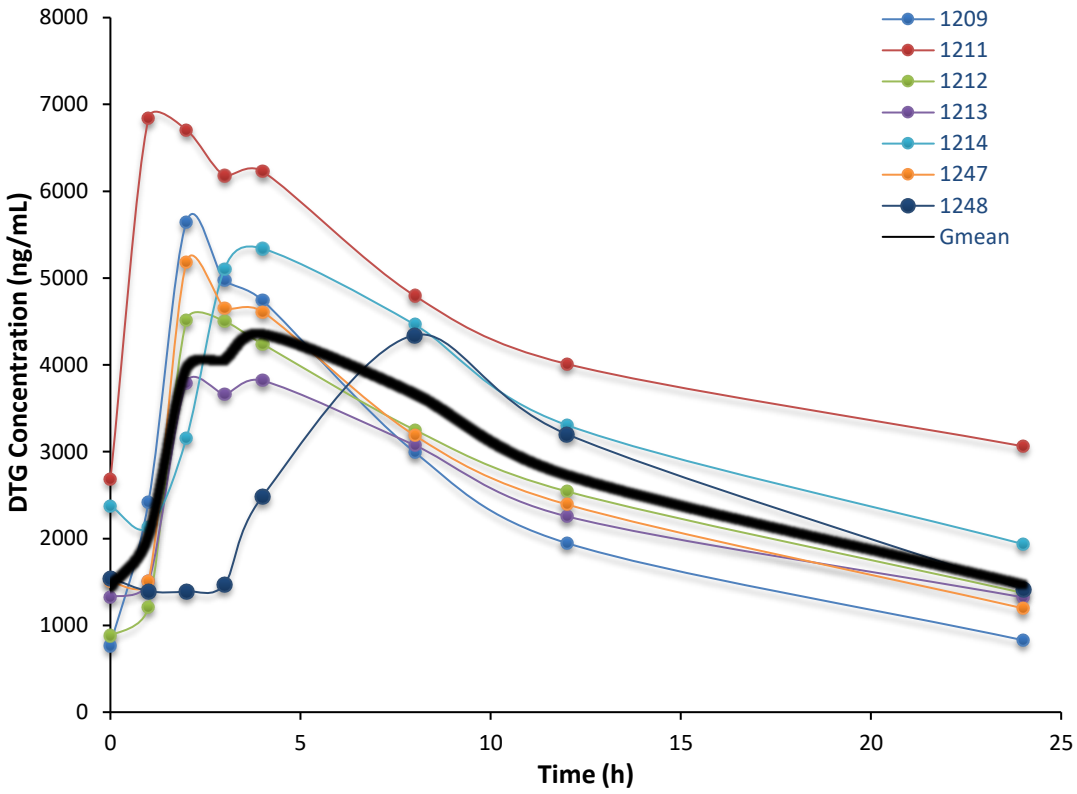
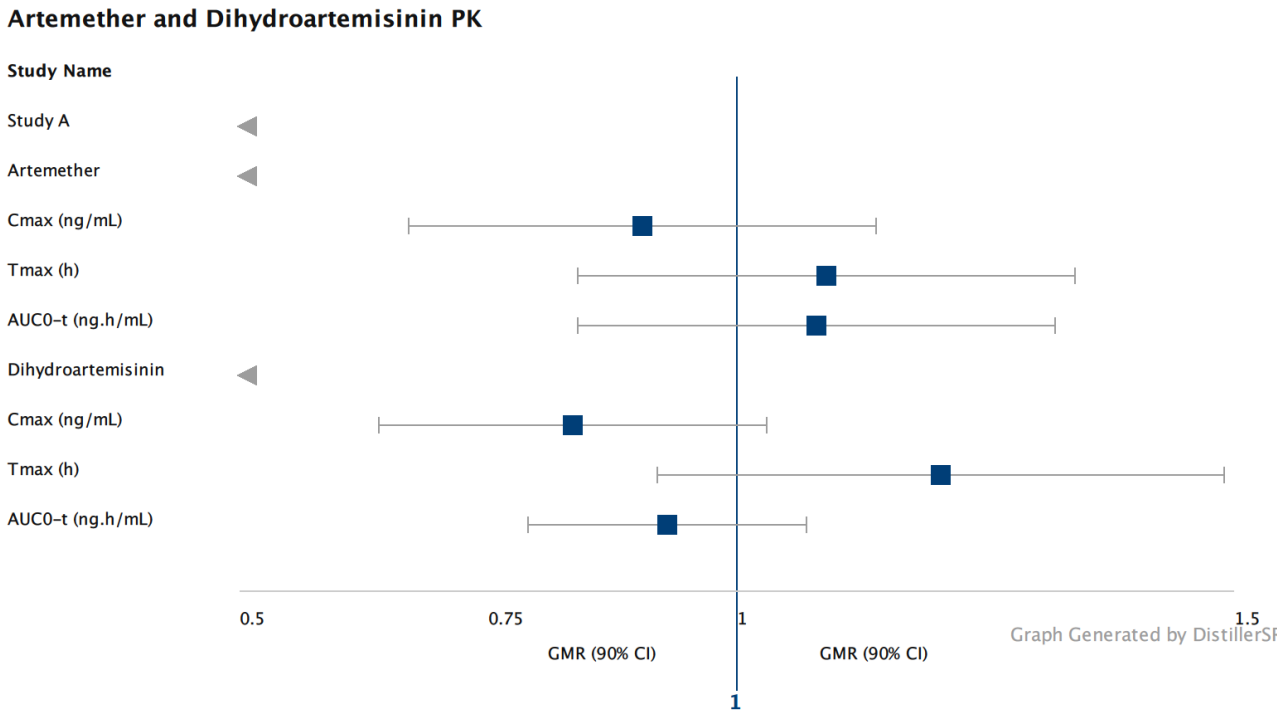
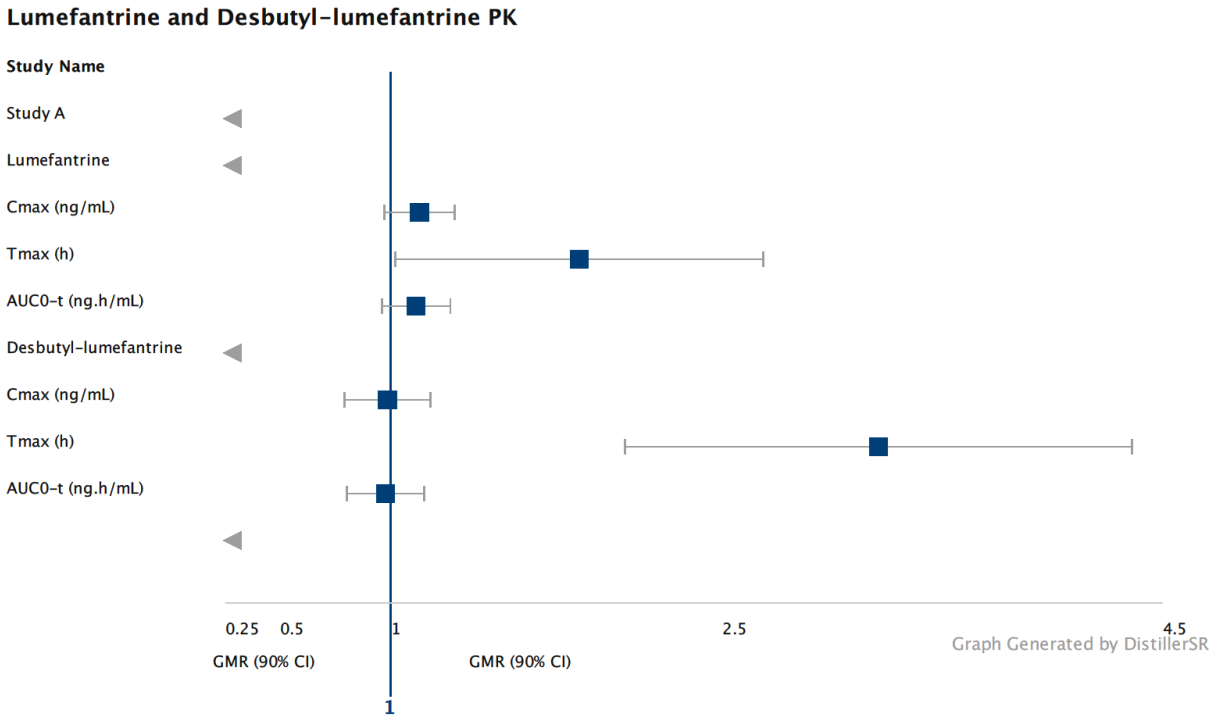


Table 3: Pharmacokinetic parameters [geometric mean (90% CI)] and Geometric Mean Ratio (GMR)
Artemether and Lumefantrine

Study A (n=14)	**AL alone**	**AL+ DTG**	**GMR (90%CI)**
Artemether			
C_{max} (ng/mL)	31.93 (20.60, 43.26)	27.88 (10.30, 45.47)	0.87 (0.67, 1.14)
T_{max} (h)	2.03 (1.64, 2.43)	2.16 (1.75, 2.56)	1.06 (0.84, 1.34)
AUC_{0-t} (ng.h/mL)	129.58 (79.35, 179.82)	136.44 (60.29, 212.59)	1.05 (0.84, 1.32)
Dihydroartemisinin			
C_{max} (ng/mL)	110.43 (92.86, 127.99)	89.91 (71.07, 108.74)	0.81 (0.64, 1.03)
T_{max} (h)	2.31 (1.98, 2.64)	2.70 (1.99, 3.42)	1.17 (0.92, 1.49)
AUC_{0-t} (ng.h/mL)	389.26 (344.52, 434.01)	357.26 (274.89, 439.63)	0.92 (0.79, 1.07)
Lumefantrine			
C_{max} (ng/mL)	9975.96 (8318.44, 11633.47)	11203.12 (9533.23, 12873.02)	1.12 (0.97, 1.29)
T_{max} (h)	3.92 (2.49, 5.35)*	6.48 (1.43, 11.54)[#]	**1.65 (1.02, 2.69)**
AUC_{0-t} (ng.h/mL)	389350.00 (333607.59, 445092.41)	429736.05 (379911.49, 479560.61)	1.10 (0.96, 1.27)
Desbutyl-lumefantrine			
C_{max} (ng/mL)	51.75 (37.50, 66.00)	49.95 (41.54, 58.35)	0.97 (0.79, 1.18)
T_{max} (h)	4.78 (3.43, 6.12)	9.52 (4.48, 14.56)[#]	**3.00 (2.06, 4.36)**
AUC_{0-t} (ng.h/mL)	6299.72 (4803.87, 7795.57)	6048.71 (5235.34, 6862.09)	0.96 (0.80, 1.15)

Figure 8: Geometric means (90% Confidence Intervals) of Artemether DHA, Lumefantrine and DBLF (GMR; Geometric mean ratio, LCL; lower confidence level, UCL; upper confidence level)



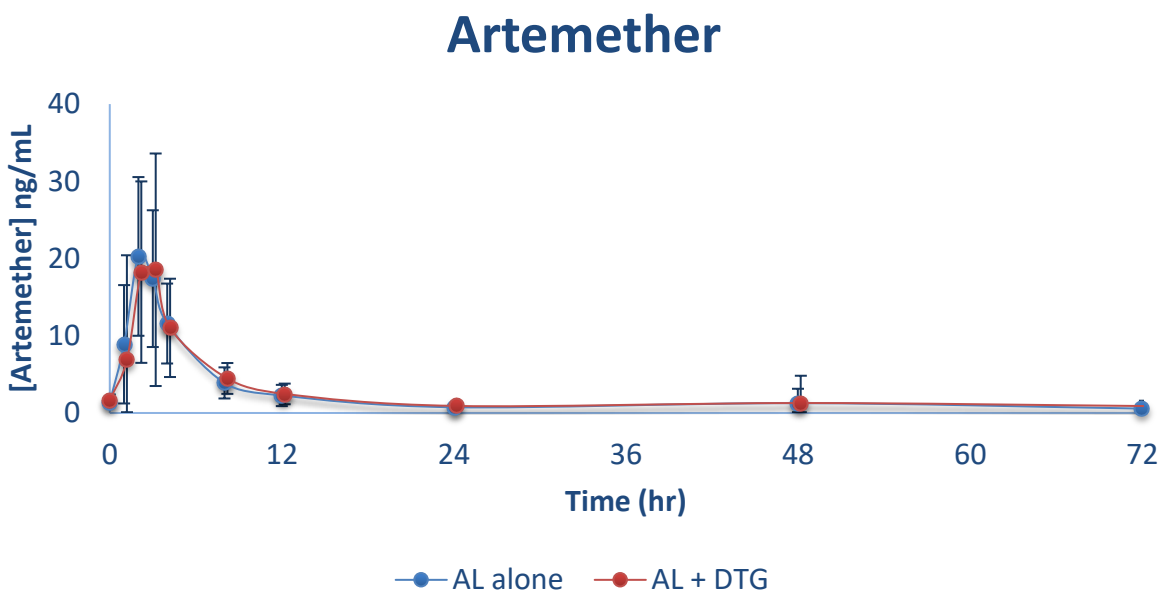


Pharmacokinetic Summary (Artemether-lumefantrine; AL)

Artemether

Geometric mean (90% confidence intervals) Artemether pharmacokinetic profile

Figure 9: AL ± DTG



SEQUENCE 1 (n=7)

Individual Artemether PK profiles [Geometric mean shown by solid black line]

Figure 10: AL Alone

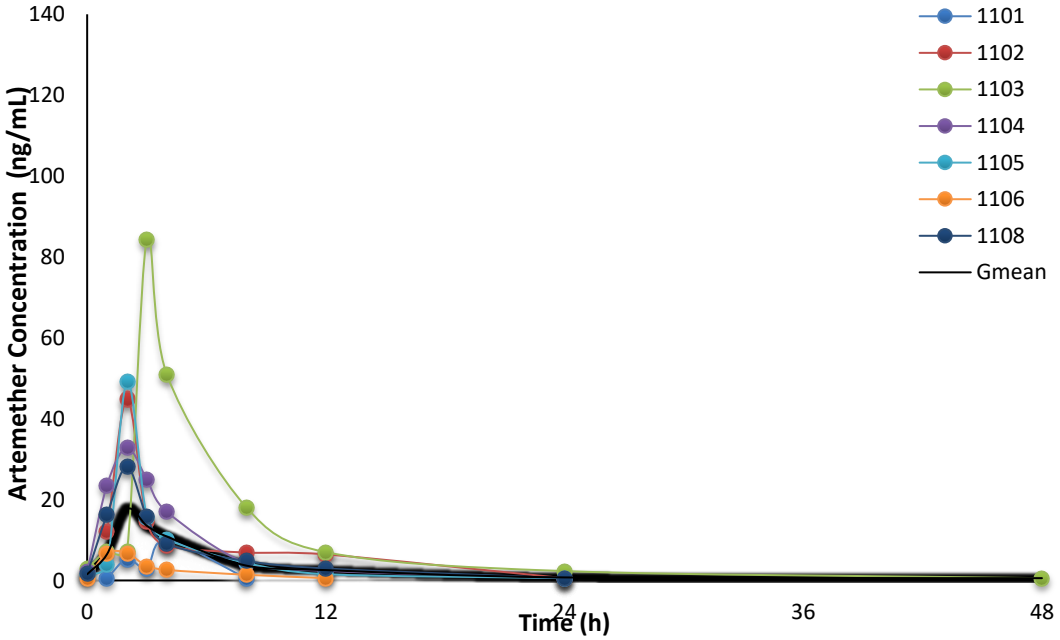
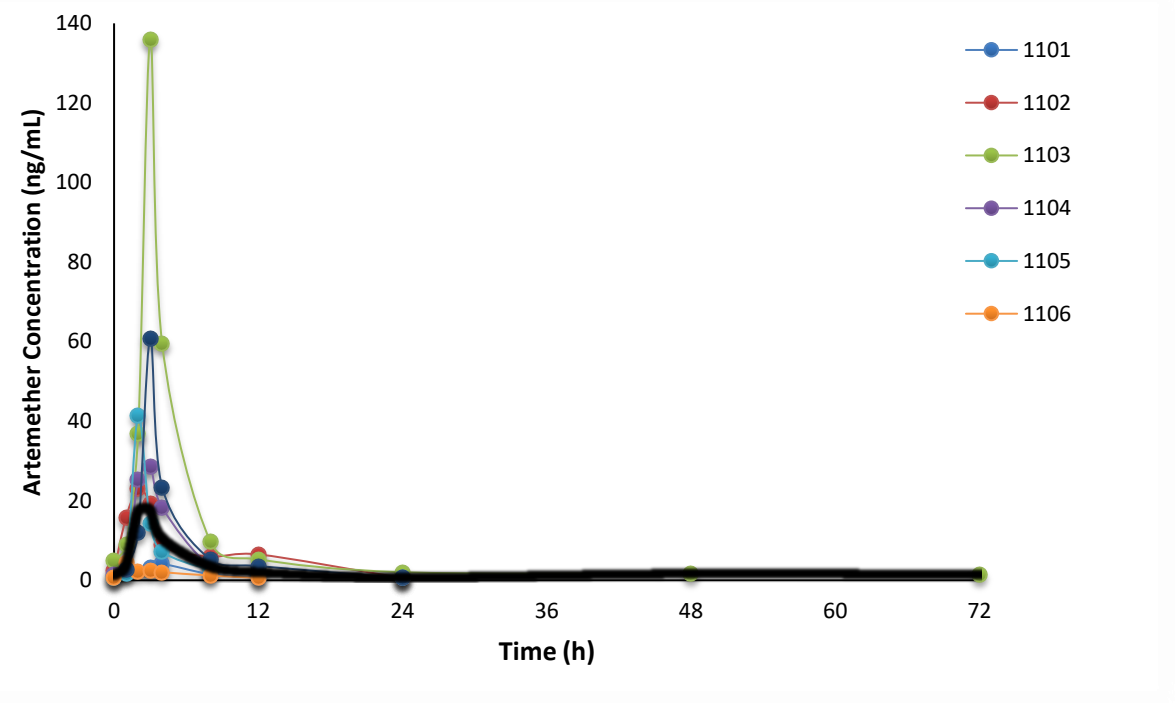


Figure 11: DTG +AL



SEQUENCE 2 (n=7)

Figure 12: AL Alone

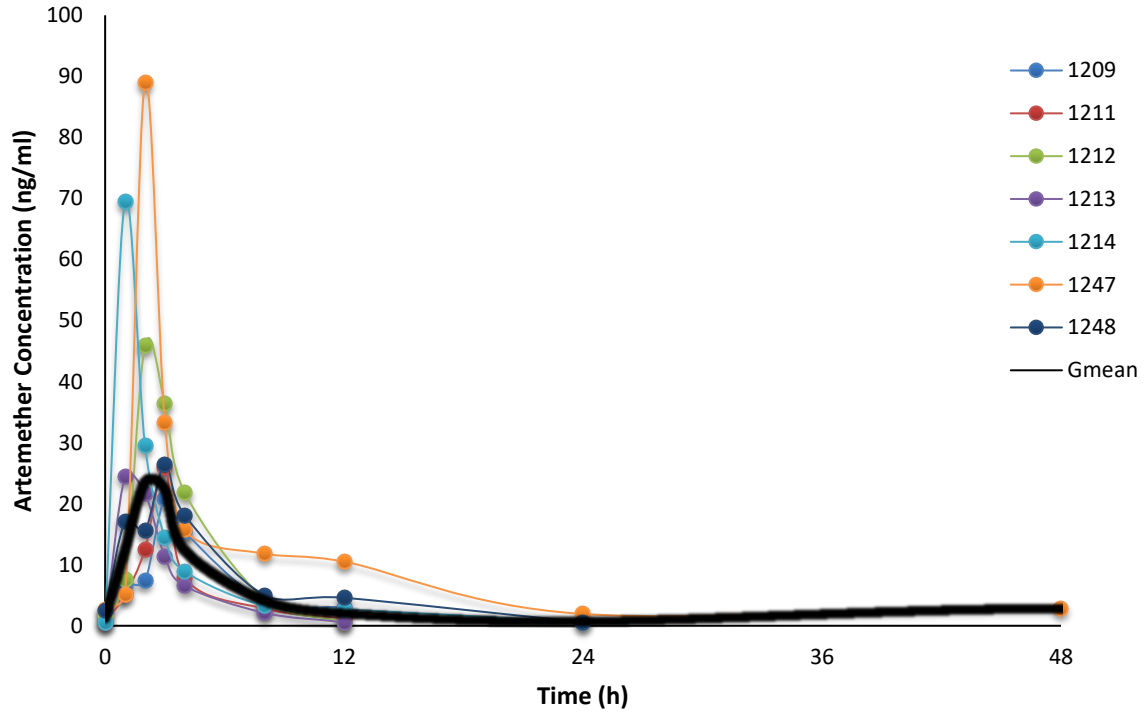
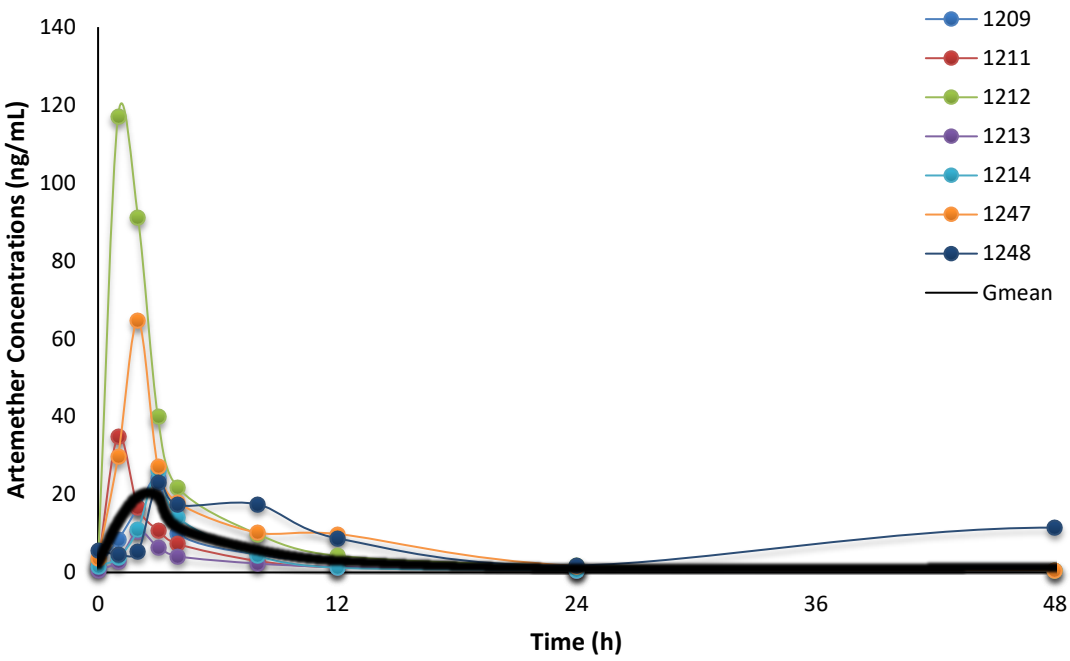


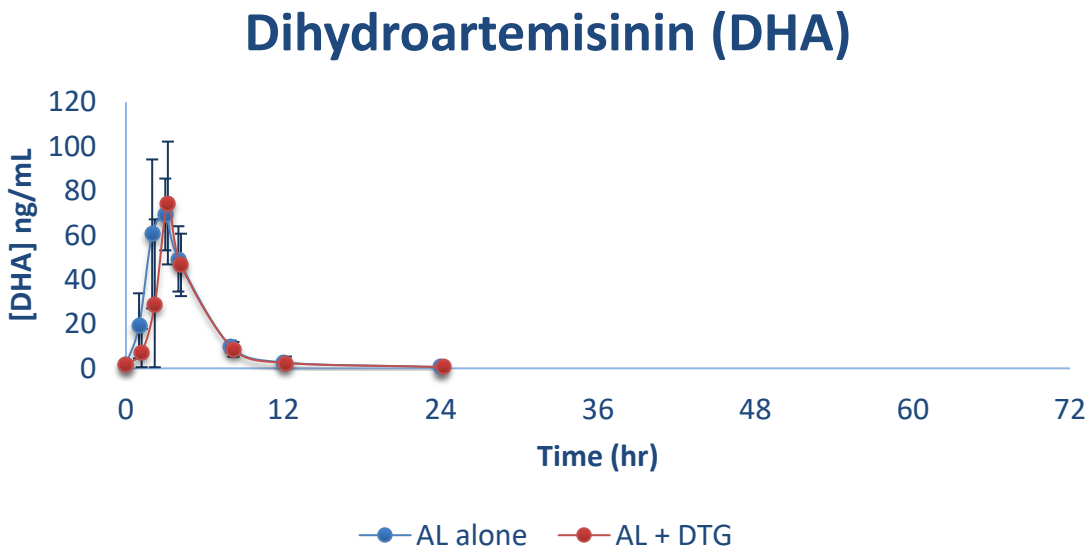
Figure 13: DTG +AL



Dihydroartemisinin (DHA)

Geometric mean (90% confidence intervals) DHA pharmacokinetic profile

Figure 14: AL ± DTG



Individual DHA PK profiles [Geometric mean shown by solid black line]

SEQUENCE 1 (n=7)

Figure 15: AL Alone

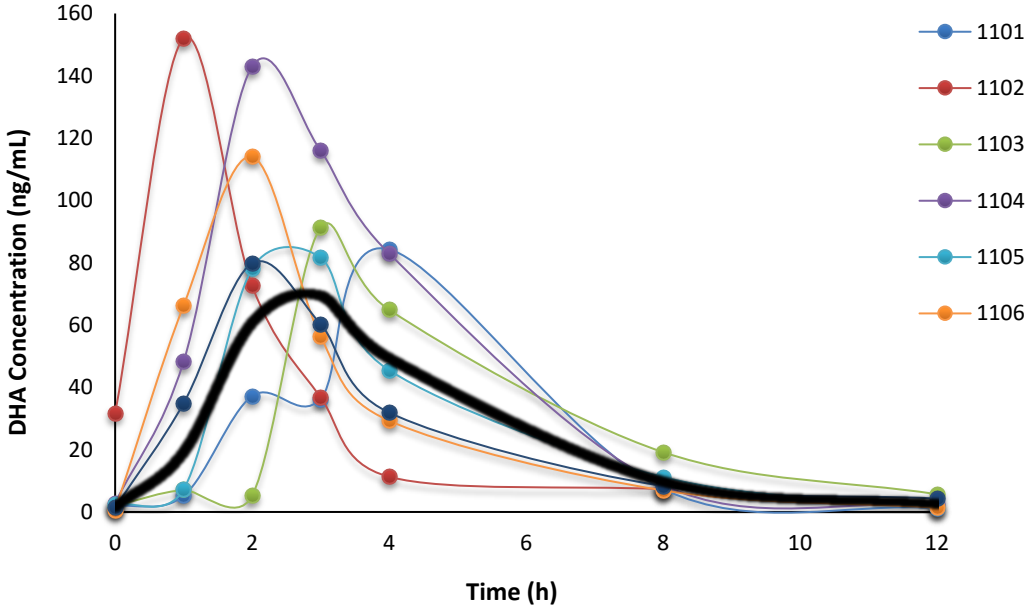
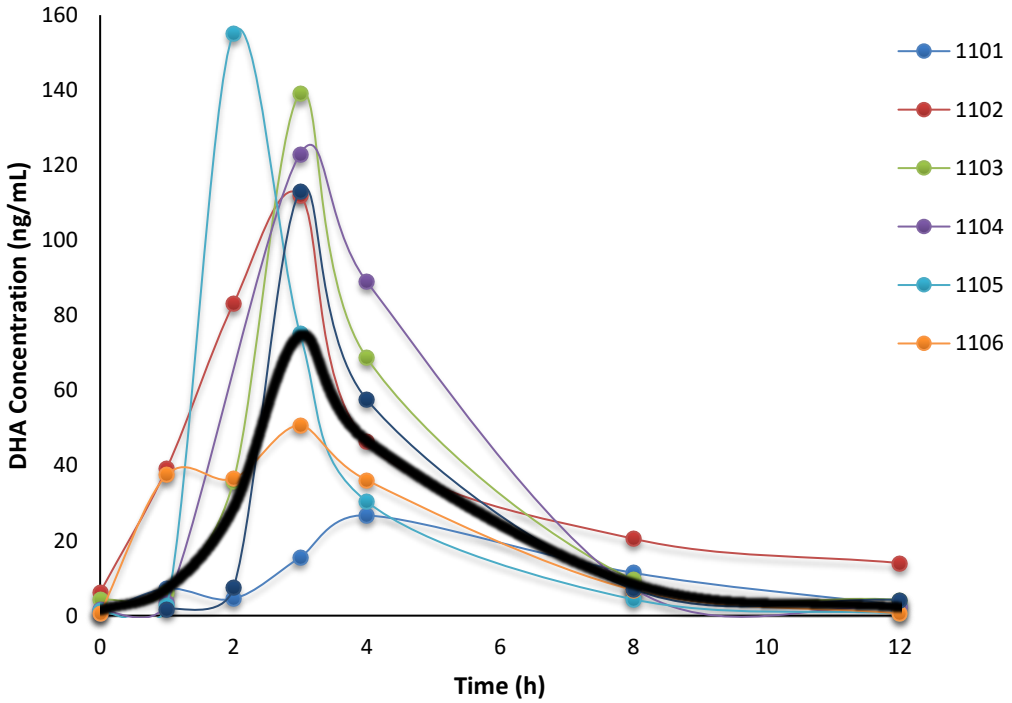


Figure 16: DTG +AL



STUDY A: SEQUENCE 2 (n=7)

Figure 17: AL Alone

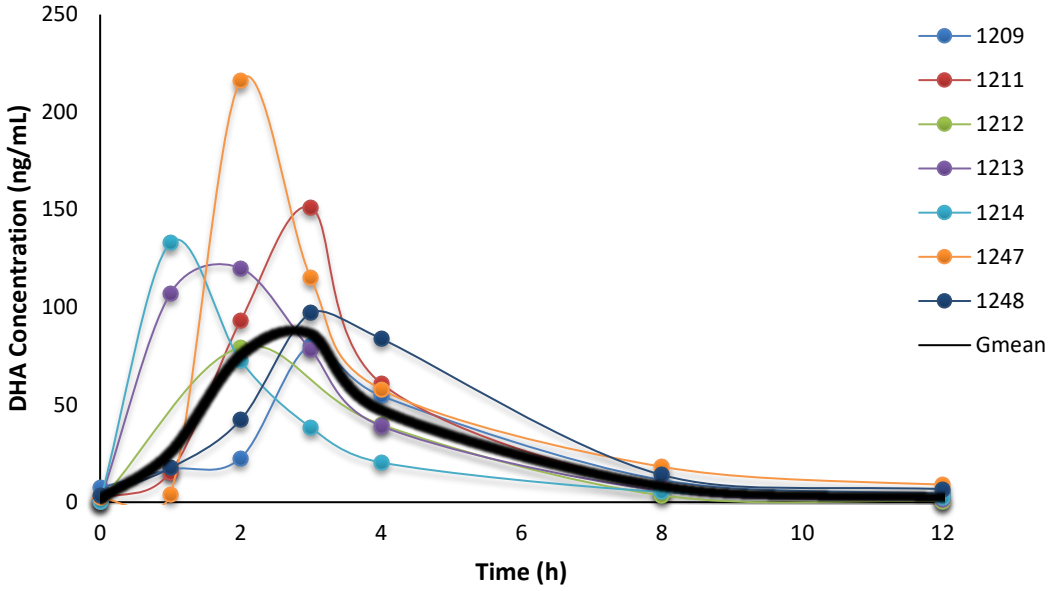
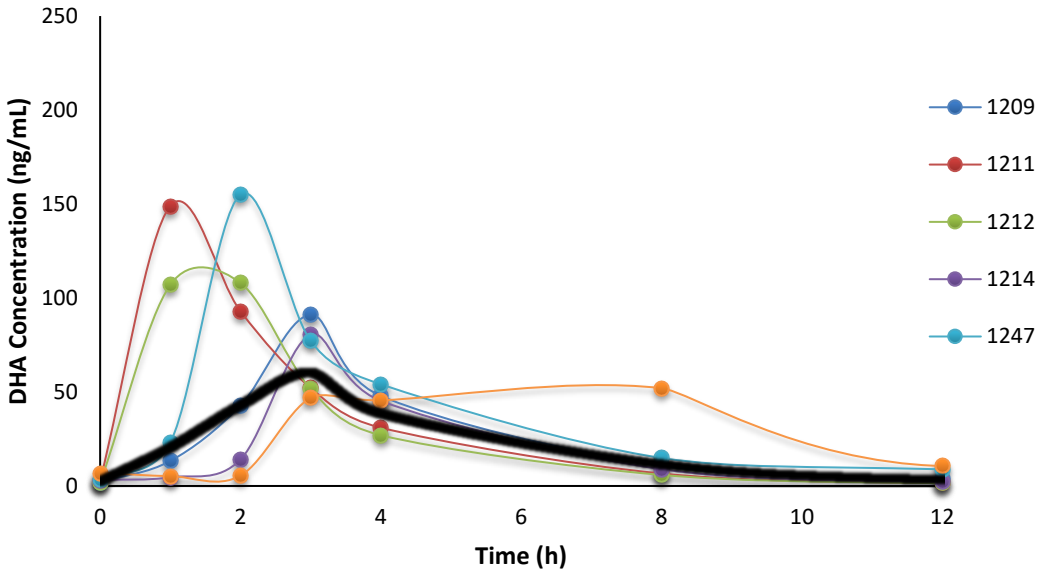
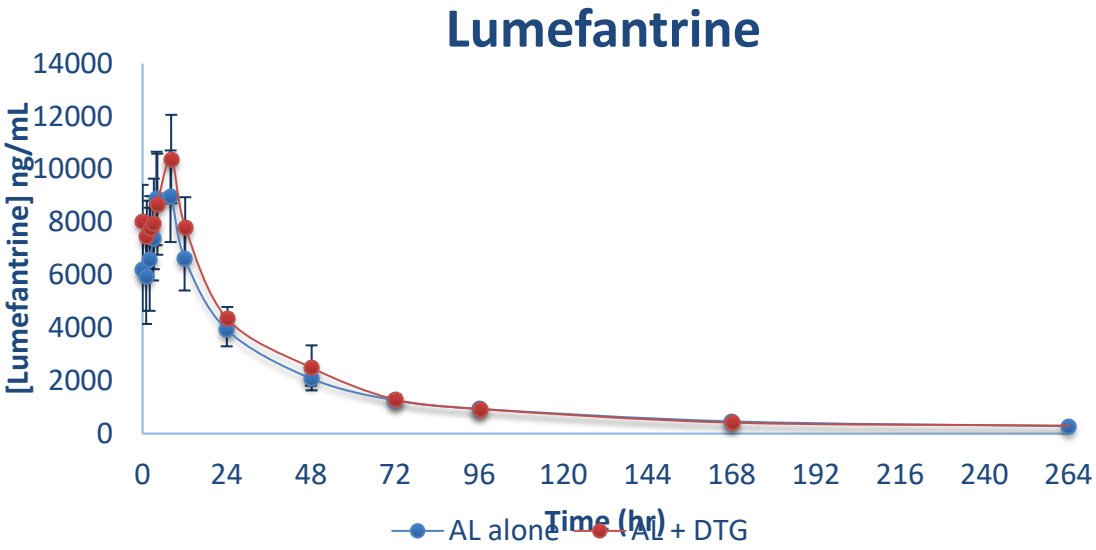


Figure 18: DTG +AL



Lumefantrine ***Geometric mean (90% confidence intervals) pharmacokinetic profile***
Figure 19: AL ± DTG



Individual Lumefantrine PK profiles [Geometric mean shown by solid black line]

<u>SEQUENCE 1 (n=7)</u> Figure 20: AL Alone

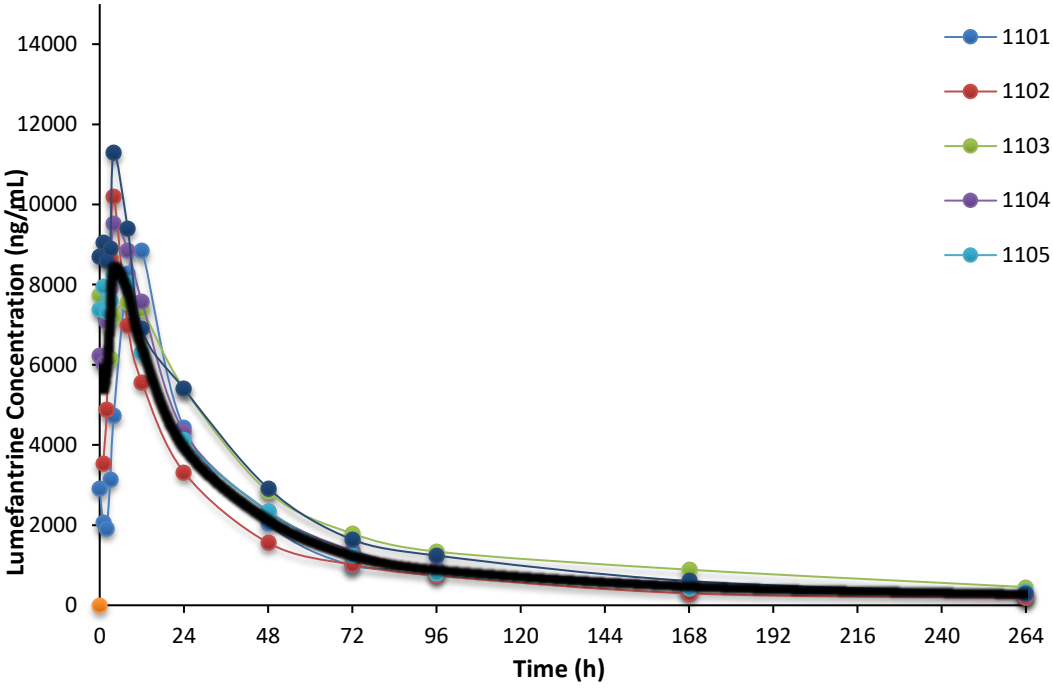
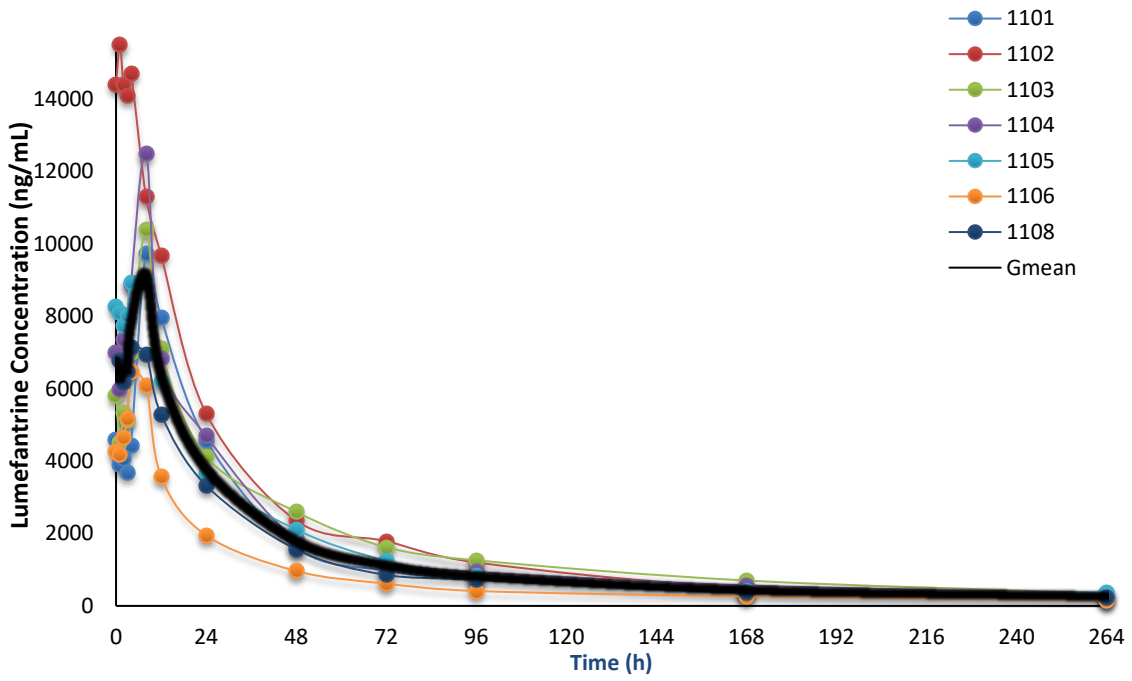


Figure 21: DTG +AL



SEQUENCE 2 (n=7)

Figure 22: AL Alone

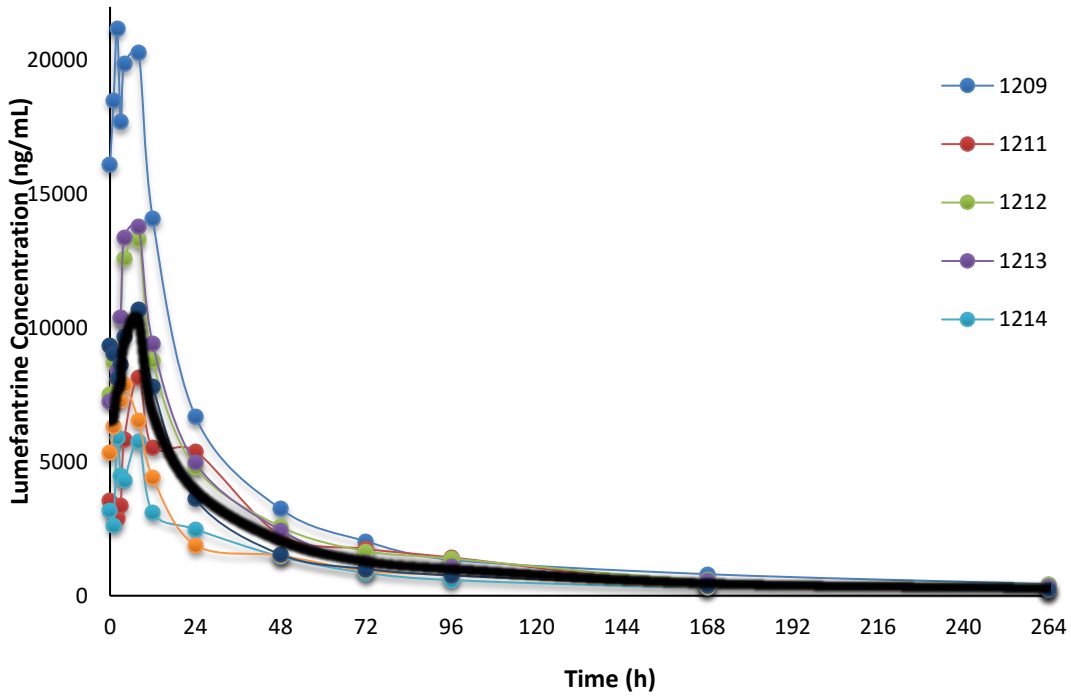
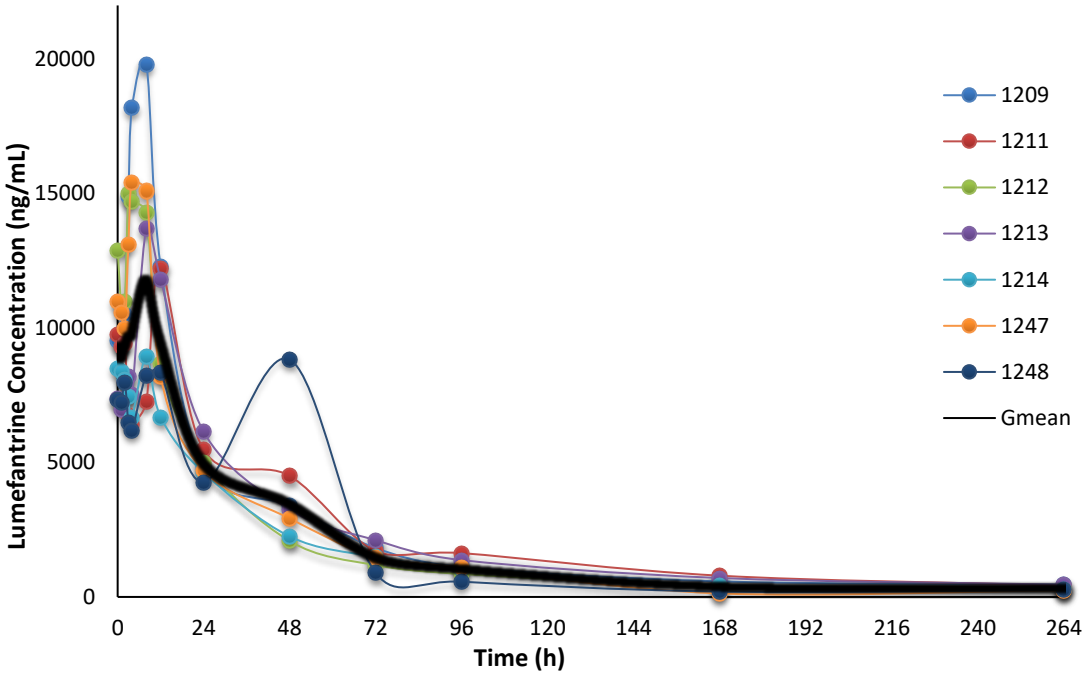


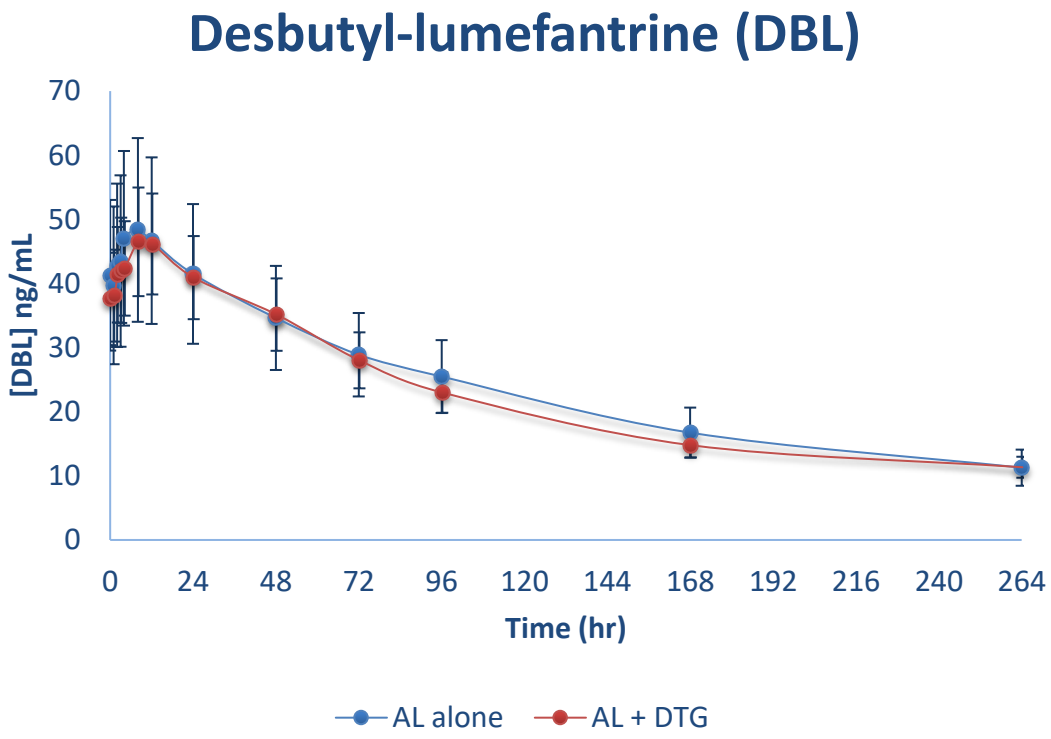
Figure 23: DTG +AL



Desbutyl-lumefantrine (DBL)

Geometric mean (95% confidence intervals) pharmacokinetic profile

Figure 24: AL ± DTG



Individual DBL PK profiles [Geometric mean shown by solid black line]

SEQUENCE 1

Figure 25: AL Alone

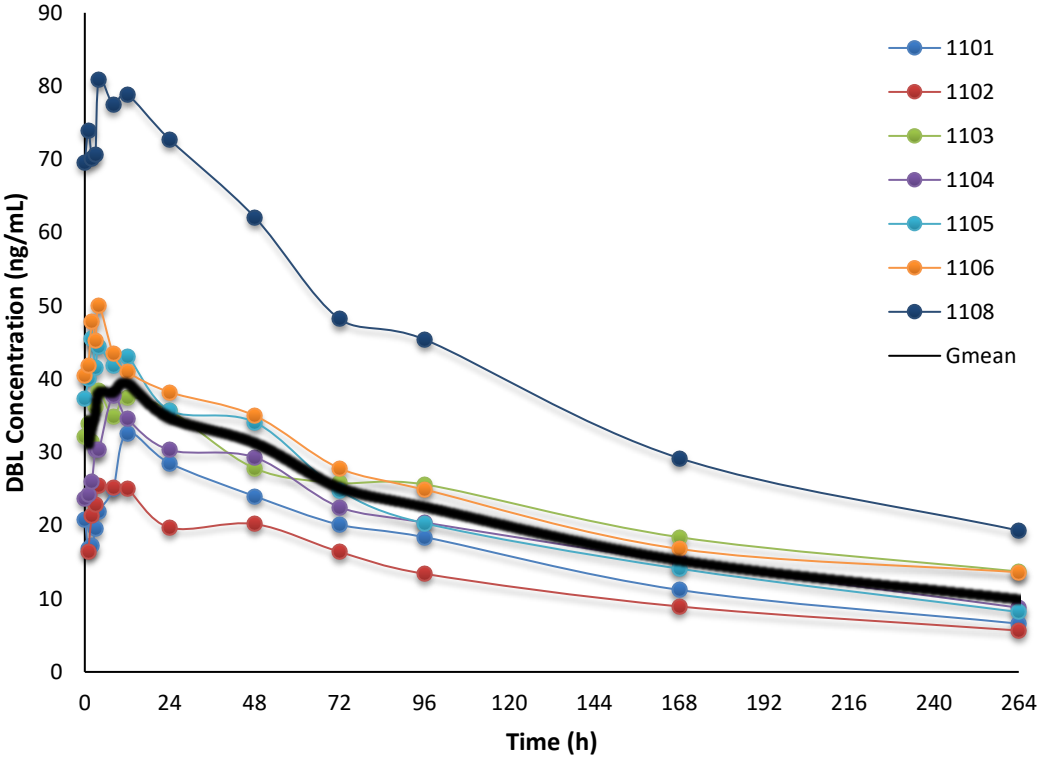
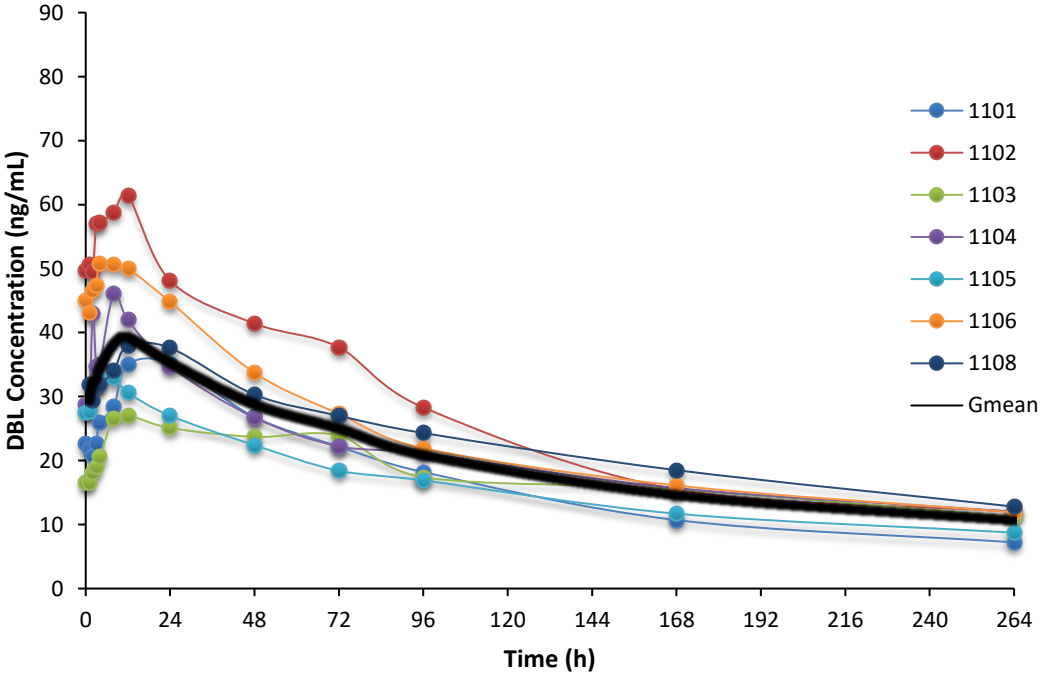


Figure 26: DTG +AL



SEQUENCE 2

Figure 27: AL Alone

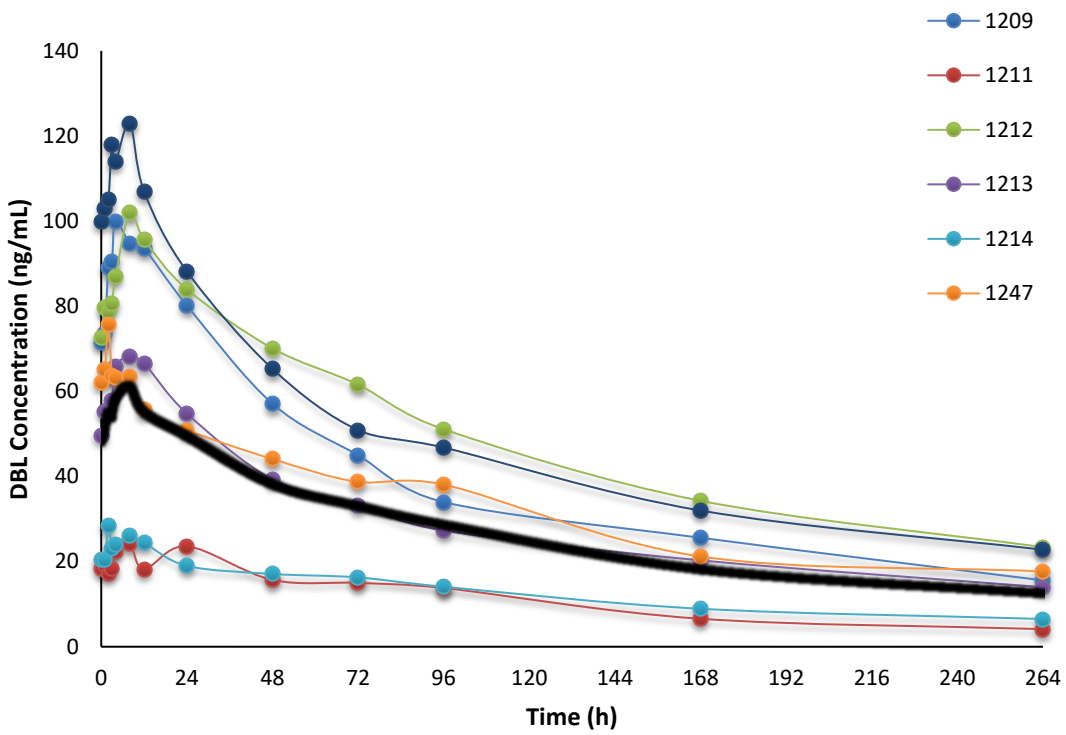


Figure 28: DTG +AL

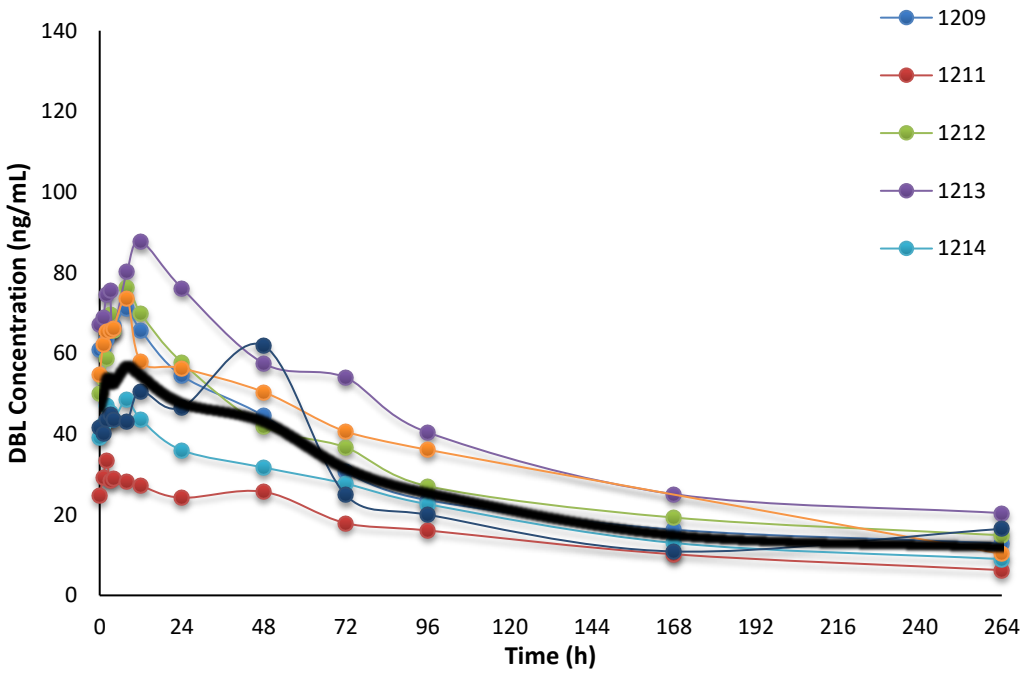
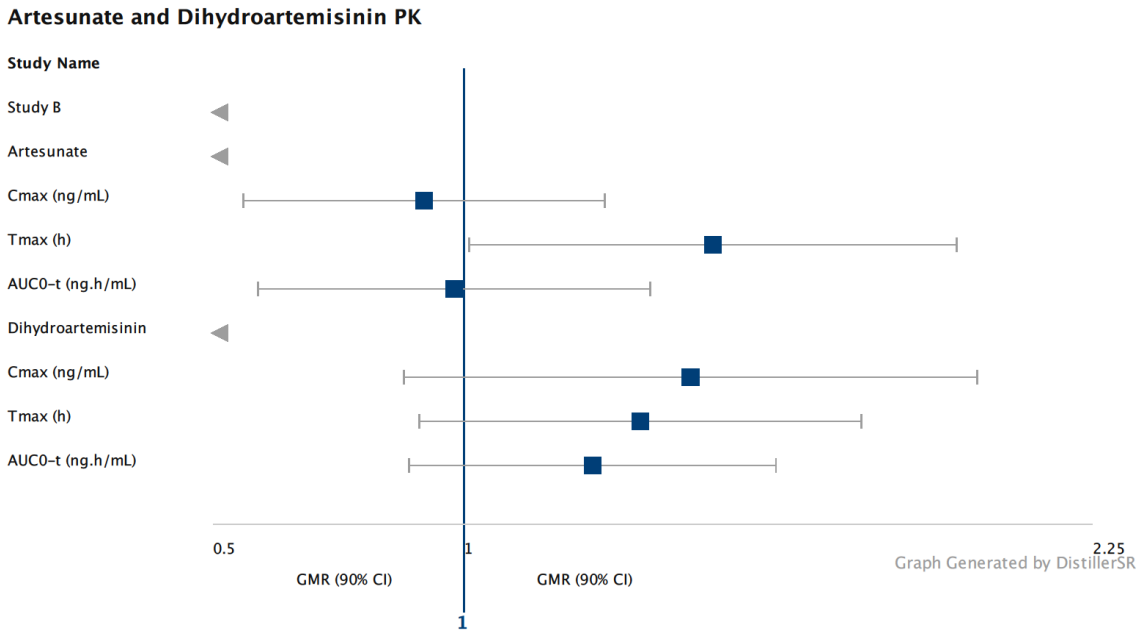
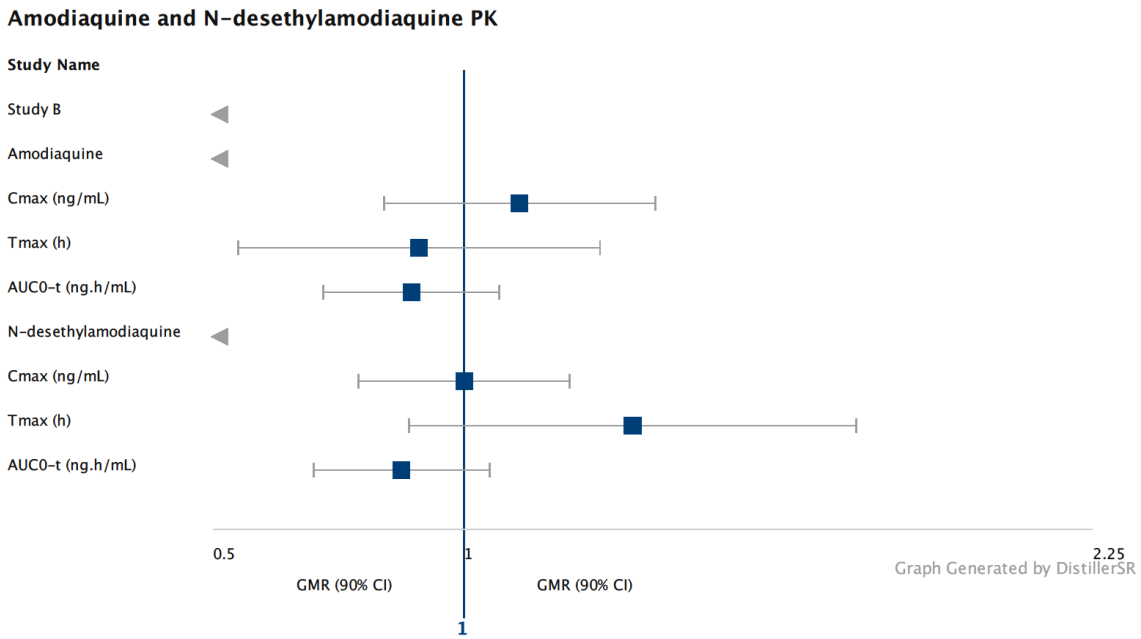


Table 4: Pharmacokinetic parameters [geometric mean (90% CI)] and Geometric Mean Ratio (GMR) of AS-AQ

Study B	**AS-AQ alone (Arm 1, n=13)**	**AS-AQ + DTG (Arm 2, n=12)**	**GMR (90% CI)**
Artesunate			
C_{max} (ng/mL)	61.29 (41.54, 81.04)	52.01 (31.70, 72.33)	0.85 (0.56, 1.28)
T_{max} (h)	1.17 (0.78, 1.56)	1.66 (1.14, 2.17)	**1.41 (1.01, 1.98)**
AUC_{0-t} (ng.h/mL)	128.38 (90.81, 165.94)	115.71 (83.22, 148.21)	0.90 (0.59, 1.37)
Dihydroartemisinin			
C_{max} (ng/mL)	217.66 (157.37, 277.95)	290.43 (197.26, 383.59)	1.33 (0.88, 2.02)
T_{max} (h)	1.58 (1.16, 2.00)	2.02 (1.52, 2.52)	1.28 (0.91, 1.79)
AUC_{0-t} (ng.h/mL)	788.25 (622.06, 954.43)	946.78 (760.15, 1133.40)	1.20 (0.89, 1.62)
Amodiaquine			
C_{max} (ng/mL)	17.79 (14.91, 20.68)	19.17 (15.95, 22.39)	1.08 (0.84, 1.38)
T_{max} (h)	2.36 (1.06, 3.65)	1.97 (1.43, 2.51)	0.84 (0.55, 1.27)
AUC_{0-t} (ng.h/mL)	256.14 (222.52, 289.76)	225.02 (198.93, 251.10)	0.88 (0.72, 1.07)
N-desethylamodiaquine			
C_{max} (ng/mL)	393.96 (325.91, 462.01)	385.57 (346.81, 424.33)	0.98 (0.79, 1.21)
T_{max} (h)	2.68 (1.88, 3.49)	3.38 (2.41, 4.36)	1.26 (0.89, 1.78)
AUC_{0-t} (ng.h/mL)	31492.83 (28720.90, 34264.76)	26943.12 (22913.01, 30973.23)	0.86 (0.70, 1.05)

Figure 29: Geometric means and 90% Confidence Intervals of Artesunate, DHA, Amodiaquine and DEAQ (GMR; Geometric mean ratio, LCL; lower confidence level, UCL; upper confidence level)

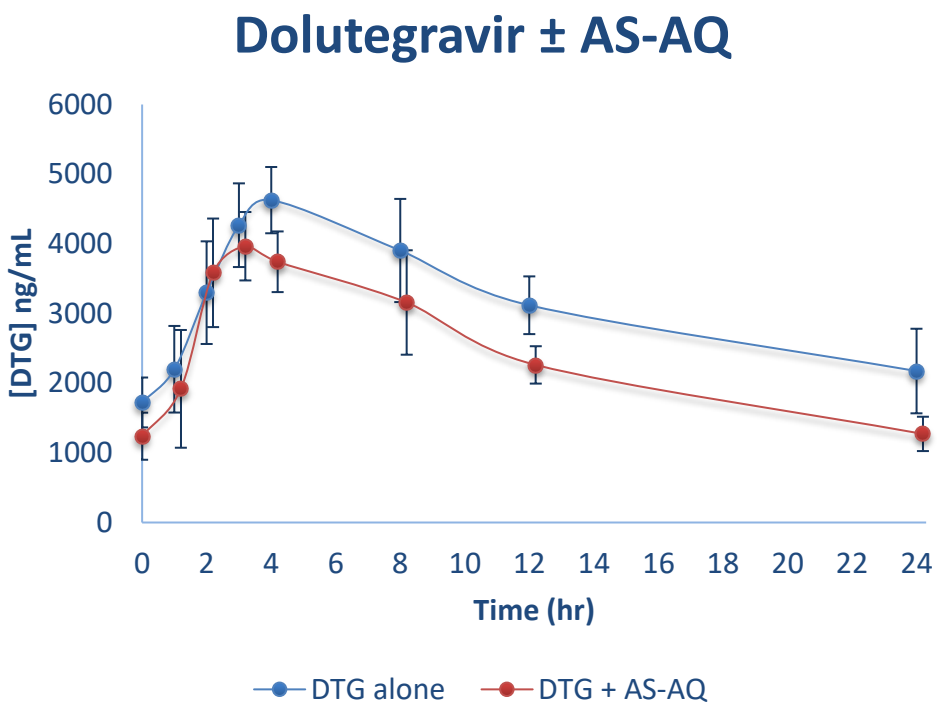




STUDY B: (n=12)

Geometric mean (90% confidence intervals) pharmacokinetic profile

Figure: 30



STUDY B: ARM 2 (n=12)

Individual PK profiles [Geometric mean shown by solid black line]

Figure 31: DTG Alone

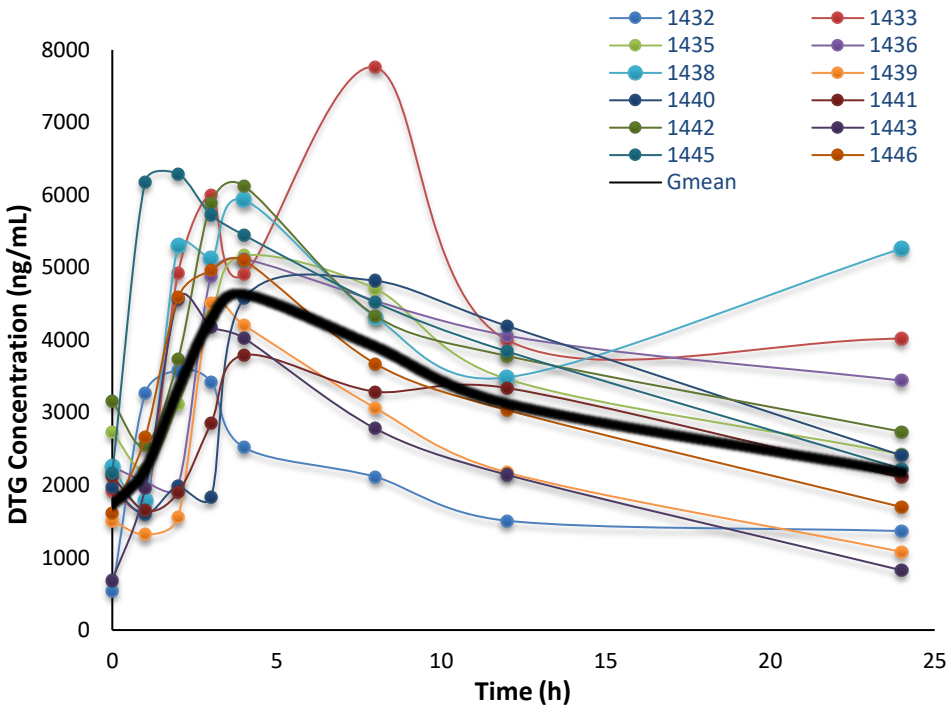
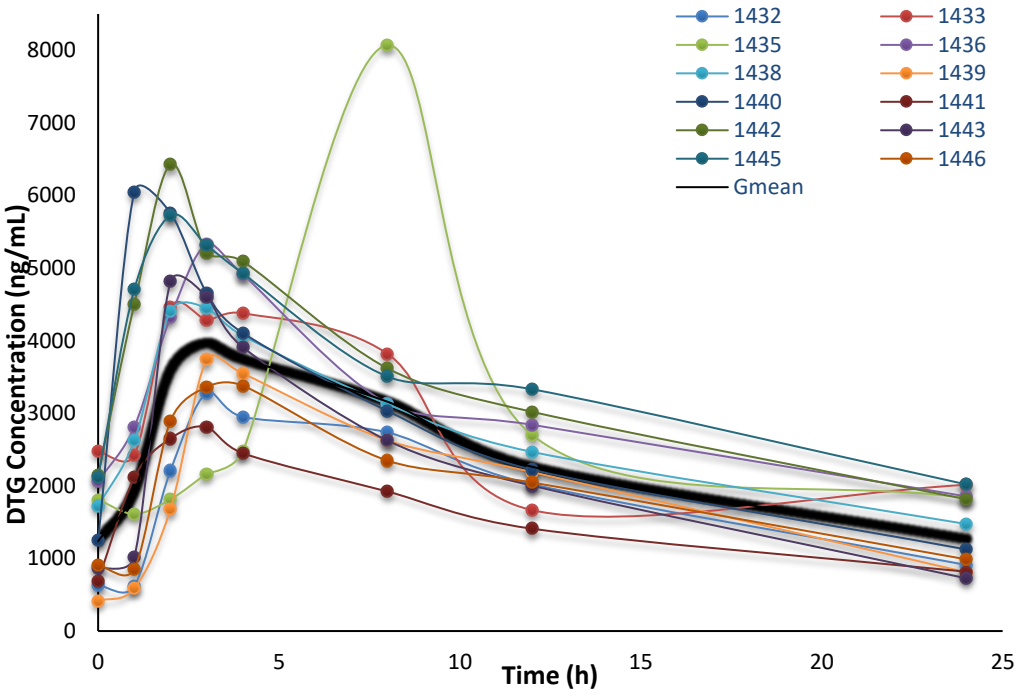


Figure 32: DTG +Artesunate Amodiaquine



Pharmacokinetic Summary (Artesunate Amodiaquine; AS-AQ)

Figure 33: Artesunate

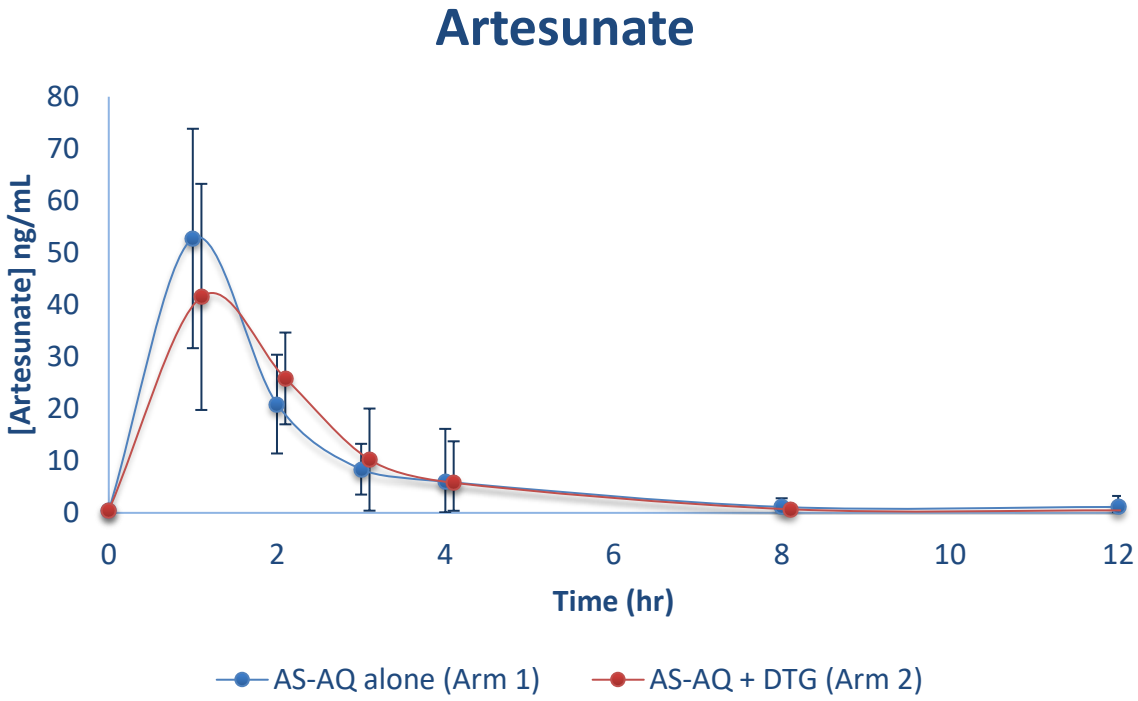


Figure 34: Dihydroartemisinin (DHA)

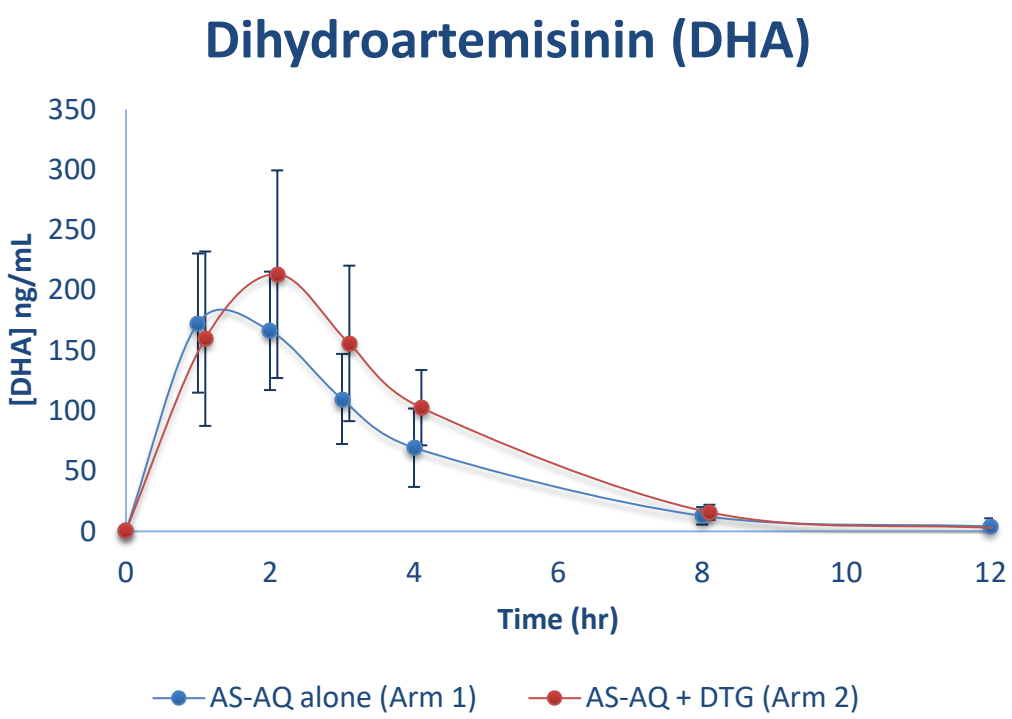


Figure 35: Amodiaquine

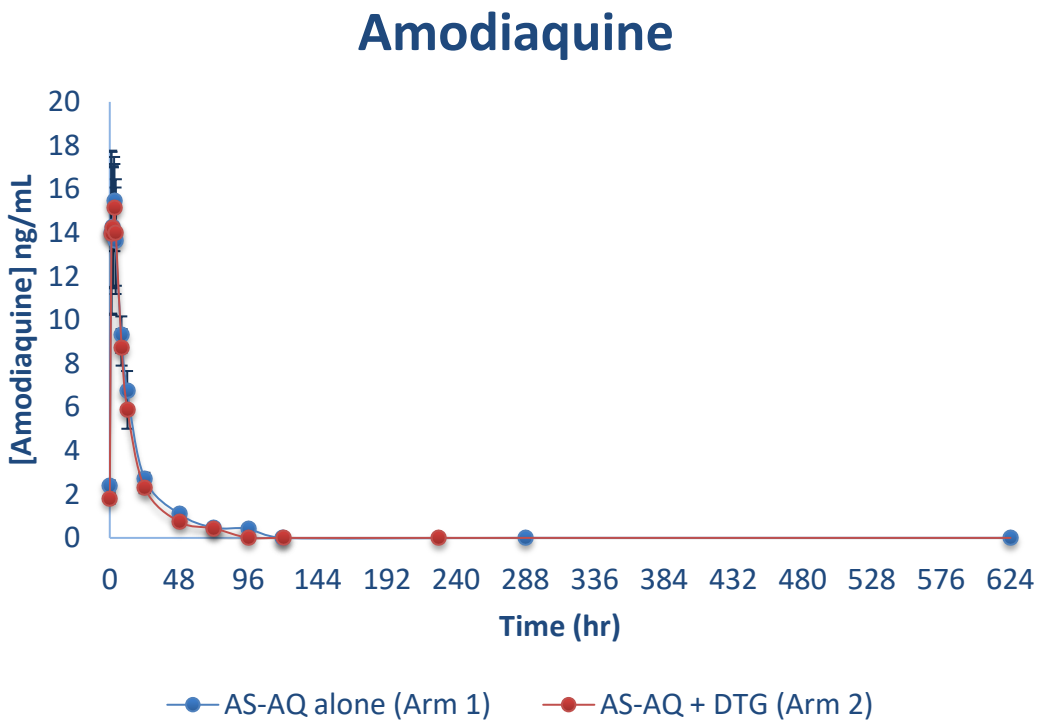


Figure 36: N-desethyl-amodiaquine

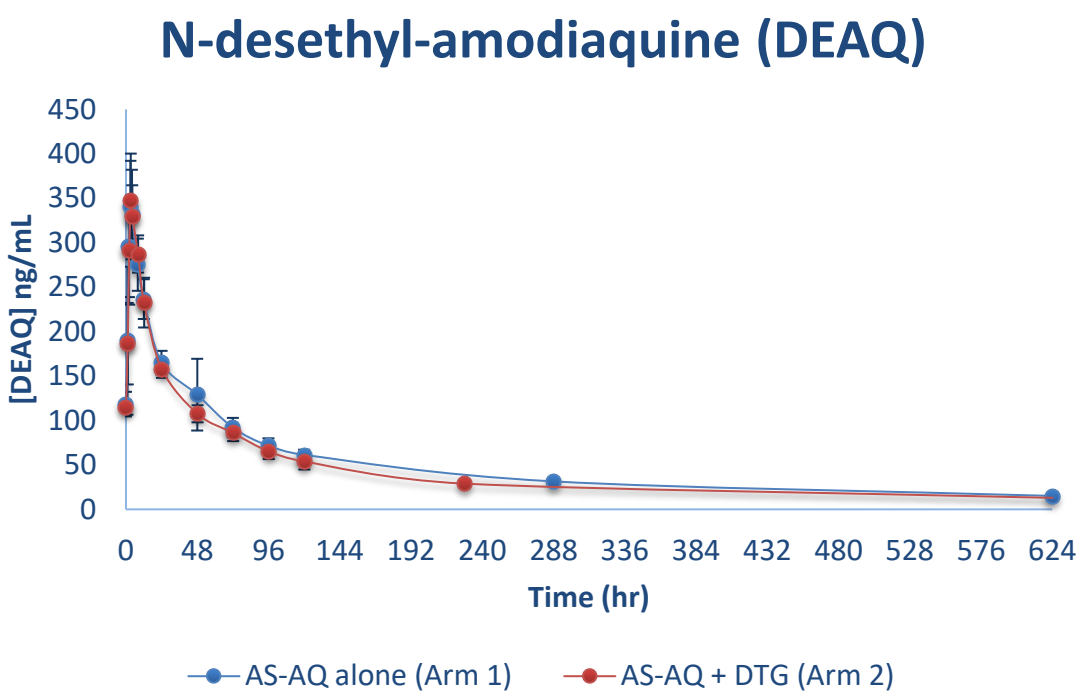


Table 5: Baseline Demographics and Laboratory markers of adverse events

Parameters (n=39) [Mean (Range)]	Study A (n=14)		Study B (n=25)	
	Sequence 1	Sequence 2	Arm 1	Arm 2 (n=12)
Age (years)	29 (21-32)	25 (23-29)	24 (23-28)	30.5 (23.5-34)
Weight (Kg)	55.5 (54-64)	59 (54-62)	59.5 (57-	60.25 (58-
BMI (Kg/m^2)	21.1 (17-	21.2 (20.1-	21.4 (19.8-	20.5 (18.95-
Haemoglobin (g/dL)	15.1 (13.3-	14.7 (13.7-	15.2 (13.5-	15.3 (14.5-
ALT (IU/L)	12 (9-20)	15 (12-17)	15 (13-19)	18 (13-20)
Total bilirubin (mg/dL)	0.7 (0.4-0.9)	0.6 (0.4-1.1)	0.5 (0.3-0.7)	0.6 (0.35-1.65)
Potassium (mmol/ L)	4.3 (3.9-4.4)	4 (3.7-4.3)	4 (3.9-4.2)	4.5 (4.3-4.75))
Urea (mg/dL)	7 (6-9)	7 (6-9)	7 (5-8)	8 (6.5-11)
Creatinine (mg/dL)	0.76 (0.59-	0.75 (0.62-	0.82 (0.67-	0.85 (0.69-
Creatinine Kinase	102 (89-	183 (129-	136 (114-	142 (132-183)
ECG corrected QT	387 (378-	415 (397-	396 (369-	400.5 (373.5-

CHAPTER 6

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CHAPTER 6

Impact of Pharmacogenetics and Pregnancy on Tenofovir and Emtricitabine Pharmacokinetics.

6.1 INTRODUCTION

Tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) in combination are the most common components of WHO-recommended first-line combination antiretroviral therapy (cART) regimens recommended for prevention of mother-to-child transmission (PMTCT) of HIV (8, 161, 316, 317) and first-line regimens for treatment of adults, adolescents and pre-exposure prophylaxis (PrEP) (161). Knowledge of anticipated pharmacokinetic (PK) parameters has been derived in non-pregnant adult (94, 172, 318), but physiologically, pregnancy alters absorption, distribution, metabolism and elimination of drugs (319). Pregnancy also induces changes in gastric pH, intestinal transit time, progesterone production and glomerular filtration rates, significantly changing pharmacokinetics (318, 319), inducing modifications in bioavailability, volume of distribution (Vd) and clearance of drug (CL) (319, 320).

Genetic polymorphisms of drug transporters are known to influence the pharmacokinetics of FTC and TDF active moiety tenofovir (TFV) during pregnancy (8, 316). Marked increases in 17 β estradiol significantly influence FTC and TFV excretion due to genetic modulation of *ABCC4* transporters (318, 321-323). ABCC4 transporters as potent inhibitors of 17 β estradiol also impact on TFV plasma exposure and TFV

elimination via the kidneys (324-326). After an initial metabolic conversion of TDF to TFV by esterase hydroxylation and subsequent intracellular phosphorylation by nucleotide kinase to tenofovir phosphate, TFV is finally excreted unchanged by glomerular filtration and active tubular secretion via renal transporters (327, 328). Likewise, FTC is also excreted predominantly unchanged via the kidneys, but excretion is predominantly influenced by *ABCC2* in both pregnant and postpartum mothers (327, 329). Excretion of TFV and FTC in kidneys and trans-placental transport is regulated by substrate specific efflux and influx transporters (328). Single nucleotide polymorphisms (SNPs) of transporters regulatory genes have also been associated with maternal exposure and viral suppression during pregnancy (172, 319, 320). These polymorphisms in *ABCC* class of transporters influences pharmacokinetics of FTC with consequential effect on drug distribution during pregnancy (325, 326, 330). For instance, FTC is an *ABCC1* substrate eliminated primarily unchanged via the kidneys (330). It is also excreted in breast milk and crosses placental membrane to the fetal compartment in significant amount, and can all be modulated by pregnancy (328).

While these changes in pharmacokinetics of widely prescribed antiretroviral (ART), including TFV and FTC, have been reported in pregnancy, only limited data is available for combined influence of pregnancy and pharmacogenetics on TFV and FTC PK (319, 321, 322, 331). This study hypothesised that combined effect of pregnancy and pharmacogenetics result in significant changes in FTC and TFV pharmacokinetics, and aimed at investigating the impact of pharmacogenetics on changes in FTC and TFV pharmacokinetics during pregnancy.

6.2 METHODS

6.2.1 Study population and selection criteria

HIV-positive pregnant and postpartum women were recruited from three hospitals in Benue State Nigeria: Bishop Murray Medical Centre, Makurdi; St. Monica's Hospital, Adikpo; and St. Mary's Hospital, Okpoga. The original study was conducted between December 2012 and October 2013 to evaluate the pharmacogenetics of pregnancy-induced changes and breastfed infants' exposure to efavirenz and nevirapine during pregnancy and postpartum, respectively (323, 332). For the present analysis, samples were selected to evaluate the influence of genetics and pregnancy on TFV and FTC. Pregnant and postpartum women taking ART regimens containing TDF and FTC were included. Participants were excluded if samples were collected within four hours of dosing. A total of 61 women (31 pregnant and 30 postpartum) were eligible and evaluated for both drug concentrations and selected SNPs.

Permission was obtained from the hospitals management and consent forms signed by participant before recruitment. Protocol and materials transfer agreements were approved by the National Health Research Ethics Committee (NHREC) Abuja Nigeria.

6.2.2 Study Design

This was an observational study of HIV-positive women conducted to evaluate the relationship between selected single nucleotide polymorphisms (SNP) and drug

concentrations in pregnant and postpartum women. Drug concentrations were quantified at single time points (4-18 h post observed dose) for each patient, and allele and genotype frequencies evaluated to determine association between TFV and FTC concentrations in pregnant and postpartum women. Five (5) *ABCC2* and *ABCC4* transporter SNPs were evaluated for impact of polymorphism on drug exposure in pregnancy and postpartum. SNPs with minor allele frequency (MAF) of ≥25% were investigated for polymorphisms in renal tubular transporters that significantly affect drug pharmacokinetics in pregnancy. The choice of SNPs of MAF ≥ 25% was because of the small sample size. The study was an additional evaluation of genetics and PK of TFV and FTC from samples of previous study where EFV and NVP pharmacogenetics and PK was evaluated. Therefore, samples that meet the study criteria were few. Drug concentrations in pregnant and postpartum women were also measured and relationship between genetic polymorphisms, TFV and FTC blood concentrations were assessed. The reference minor allele frequency of the Yoruba ethnic group, a subset of Nigerian population, was used to determine the polymorphic genes to be evaluated. Genes with minor allele frequency ≥25% include: *ABCC2* 154962860T>C, 35%, *ABCC2* 32293730T>C, 38% *ABCC4* 95020696A>G, 29%, *ABCC4* 95021537A>C, 25%, *ABCC4* 13: 95062722C>T 27% [8].

6.2.3 Tenofovir and Emtricitabine Quantification

TFV and FTC concentrations in blood was measured from dried blood spots (DBS) using validated LC-MS method (235). Assay calibration range 16- 4000 ng/mL and internal standards 2CA and TFV-d6 were used for assay proficiency. Measured drug

concentrations were used to evaluate the relationship between drug concentrations and genetic polymorphism of SNPs using a regression model.

6.2.4 Genotyping

Genotyping for *ABCC2* 12:g.154962860T>C, *ABCC2* 12:g.32293730T>C, *ABCC4* 11:g.95020696A>G , *ABCC4* 11:g.95021537A>C and *ABCC4* 13: 95062722C>T was performed by real time polymerase chain reaction (PCR) allelic discrimination using standard Taqman assays. Genotypes assignment and allelic discrimination plots were performed on a chromo4 system (Bio-Rad Laboratories, Hercules, CA) and Opticon Monitor version 3.1 software (Bio-Rad Laboratories). The PCR protocol involved denaturation of DNA at 95^{o}C for 10 minutes, 40 cycles of amplification at 95^{o}C for 15 seconds and annealing at 60 ^{o}C for 1 min.

6.2.5 Statistical analyses

Allelic and genotype frequencies were evaluated to ensure Hardy-Weinberg equilibrium was maintained. The following SNPs: *ABCC2* 12:g.154962860T>C, *ABCC4* 11:g.95020696A>G and *ABCC4* 11:g.95021537A>C were in Hardy-Weinberg equilibrium (P value >0.05) except *ABCC2* 12:g.32293730T>C (X^2 = 4.8, P<0.01) and *ABCC4* 13: 95062722C>T (X^2 = 5.05, P<0.01) respectively which compromised their interpretation. Normality was checked for continuous variables using Shapiro Wilk test, which was statistically significant (p<0.001), and variables were log transformed. Univariate linear regression models were used to determine the relationship

between drug concentrations and other variables (age, regimen, time post dose) in SPSS version 23.0. Covariates with P-value ≤ 0.2 were further entered into a multivariate model in a stepwise fashion and analysed for relationship between the groups. Missing covariate >10% were managed by excluding the sample from the regression analysis. Within groups relationships were analysed by first creating dummy variables and subsequently entered into a model to determine specific relationships with genotypes. Output of this analysis enabled determination of the relationship between drug concentrations at different time points with pregnancy, postpartum and the SNPs. All charts were plotted using Graph pad prism 5.0 (GraphPad Software Inc).

6.3 RESULTS

Pregnant and postpartum women on once daily FTC and TDF were evaluated at single time points median (range) 14 (4-18) h post dose for drug levels and associations with polymorphisms in genes using regression models. Of the 61 (31 pregnant and 30 postpartum women) evaluated, the median (range) age and weight of pregnant women was 29 (17-42) years and 57 (48-79) kg respectively, and postpartum women were 30 (18-40) years and 59 (45-73) kg respectively (*Table 1*). Women on nevirapine (NVP) regimen were 9 (14.8%) and 52 (85.2%) were on efavirenz (EFV) regimens. Linear and multiple regression (for con-founders) models, were used to adjusted for associations with time post dose, pregnancy, postpartum and drug concentrations.

Genotype frequencies of both pregnant and postpartum women were similar to previously reported genotype and allele frequencies in the region (Table 2).

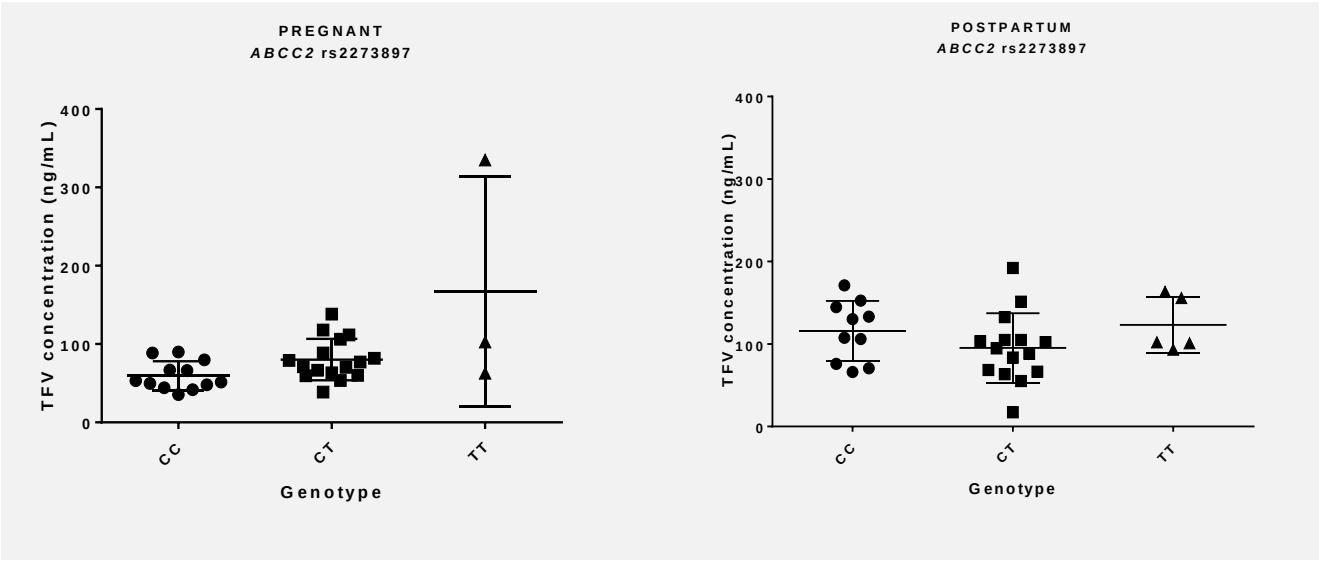
For the pregnant women, the highest TFV concentration was 334.935ng/mL, and lowest was 35.520ng/mL at 18 and 14.5 h post dose respectively. Pregnancy was found to decrease plasma concentration of TFV by a factor of $\log_{10}$ 0.13 ($\log_{10}$ β= -0.131 (-0.228, -0.034), (P= 0.009) between 7-18.5 h post dose. Over 50% of pregnant women had TFV concentration greater than IC_{50} (2.3 µM or 10ng/ml) at 18 h post dose. The highest measured concentration was 553.259ng/mL at 12 h post dose and there was no significant association of drug concentration with any SNP. *ABCC2* 12:g.154962860T>C T allele was associated with a significant increase concentration of FTC by a factor of $\log_{10}$ 0.766 in both pregnant and postpartum women irrespective of time post dose (β = $\log_{10}$ 0.766 (0.084, 1.448) (P= 0.028). There was no association of FTC concentrations with other SNPs.

***Table 1*: Demographic and genetic characteristics of study population**

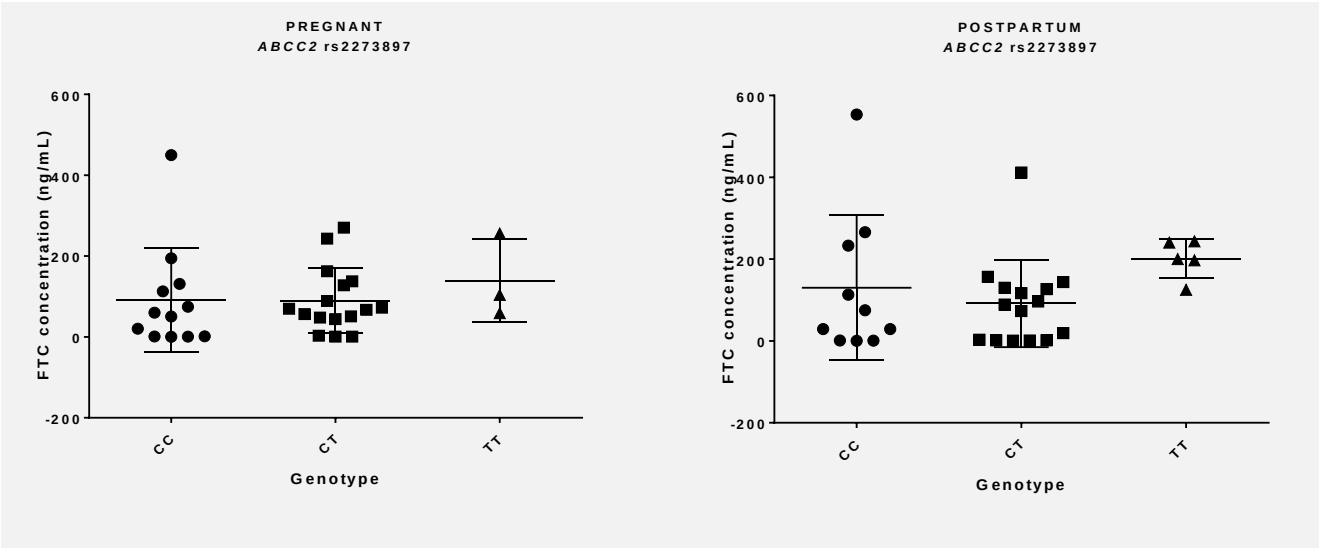
Characteristic	Pregnancy	Postpartum
Number (%) of women	31 (50.8%)	30 (49.2%)
Median (Range) • Age (years) • Weight (Kg)	 29 (17-42) 57 (48-79)	 30 (18-40) 59 (45-73)
Median (Range) CD4 Count (cells/mm^3) • Baseline CD4 count • Last CD4 Count	 234 (9-533) 443 (108-1206)	 336 (73-898) 574 (96-1290)
Median (Range) Time Post Dose (Hrs)	12 (7-18.5)	14 (4-16)
Mean (Range) drug Concentrations (ng/mL) • TFV • FTC	 80.60 (35.52-334.93) 122.95 (20.39-449.89)	 106.74 (17.23-192.16) 157.23 (19.27-553.25)
Drug Regimen • TDF/FTC/EFV • TDF/FTC/NVP	 24 (77.4%) 7 (22.6%)	 28 (93.3%) 2 (6.7%)
Median (range) Duration on regimen (months)	27 (1-48)	13 (2-36)

Genotype Frequencies		
ABCC2 12:g.154962860T>C (rs2273897)		
TT	0.13	0.23
CT	0.54	0.45
CC	0.33	0.32
MAF	0.35	0.35
ABCC2 12:g.32293730T>C (rs3749966)**		
TT	0.33	0.27
CT	0.58	0.59
CC	0.08	0.14
MAF	0.38	0.38
ABCC4 11:g.95020696A>G (rs1059751)		
AA	0.54	0.59
AG	0.46	0.32
GG	0.00	0.09
MAF	0.29	0.29
ABCC4 11:g.95021537A>C (rs3742106)		
AA	0.50	0.55
AC	0.38	0.36
CC	0.13	0.09
MAF	0.25	0.25
ABCC4 13: 95062722C>T (rs1751034)**		
CC	0.04	0.00
CT	0.50	0.59
TT	0.46	0.41
MAF	0.27	0.27

Figure 2: Tenofovir and Emtricitabine Mean (Standard Deviation) concentrations compared between genotypes in pregnant and postpartum women


PREGNANT
ABCC2 rs2273897
TFV concentration (ng/mL)
400
300
200
100
0
CC
CT
TT
Genotype

POSTPARTUM
ABCC2 rs2273897
TFV concentration (ng/mL)
400
300
200
100
0
CC
CT
TT
Genotype


PREGNANT
ABCC2 rs2273897
FTC concentration (ng/mL)
600
400
200
0
-200
CC
CT
TT
Genotype

POSTPARTUM
ABCC2 rs2273897
FTC concentration (ng/mL)
600
400
200
0
-200
CC
CT
TT
Genotype

TDF	Univariate analysis		Multivariate analysis		
Log_{10}	**P-value**	**β(95%CI)**	**P-value**	**β(95%CI)**	**% Effect**
Age (years)	0.420	0.004 (-0.007, 0.015)			
Time Post dose	0.047	0.028 (0.001, 0.055)	0.027	0.017 (0.002, 0.032)	4.0
Pregnant	0.005	-0.154(-0.259, -0.049)	0.009	-0.131 (-0.228, -0.034)	26.0
Genotype					
***ABCC2* 12:g.154962860T>C (rs2273897)**	0.106	0.141(-0.031, 0.312)	0.531	-0.081 (-0.105, 0.055)	17.02
***ABCC2* 12:g.32293730T>C (rs3749966)**	0.431	0.046 (-0.071, 0.163)			
***ABCC4* 11:g.95020696A>G (rs1059751)**	0.119	0.083(-0.022, 0.189)	0.119	0.194(-0.021, 0.179)	56.31
***ABCC4* 11:g.95021537A>C (rs3742106)**	0.166	-0.082(-0.035,0.199)	0.166	0.188 (-0.035, 0.199)	54.17
***ABCC4* 13: 95062722C>T (rs1751034)**	0.678	-0.021(-0.123,0.081)			
FTC	**Univariate analysis**		**Multivariate analysis**		
Log_{10}	**P-value**	**β(95%CI)**	**P-value**	**β(95%CI)**	
Age (years)	0.215	0.035 (-0.021,0.091)			
Time Post dose	0.941	-0.066(-1.845,1.712)			
Pregnant	0.820	0.061(-0.479, 0.602)			
Genotype					
ABCC2 12:g.154962860T>C (rs2273897)	0.112	0.724 (-0.175,1.622)	0.028	0.766(0.084,1.448)	171.39
ABCC2 12:g.32293730T>C (rs3749966)	0.665	-0.130(-0.728, 0.469)			
ABCC4 11:g.95020696A>G (rs1059751)	0.791	-0.072 (-0.615,0.471)			
ABCC4 11:g.95021537A>C (rs3742106)	0.520	0.194 (-0.407,0.746)			
ABCC4 13: 95062722C>T (rs1751034)	0.561	0.153 (-0.372,0.677)			
ABCC2 12:g.154962860T>C (rs2273897)					
	TT		CT		CC
TFV (ng/mL) Mean (Range)	139.48 (62.88-334.935)		87.48 (17.48-192.16)		92.99 (35.52-170.99)
FTC (ng/mL) Mean (Range)	178.14 (58.74-256.12)		121.85 (19.27-411.74)		159.43 (20.39-553.25)

***Table 2*: Univariate and multivariate analysis of pregnant and postpartum women combined**

6.4 Discussion

This study report for the first time the link between increased FTC blood concentration and *ABCC2* 12:g.154962860T>C T allele. An estimated 1-2increase in FTC blood concentration was observed in pregnant and postpartum women with *ABCC2* 12:g.154962860T>C T allele compared to women with CT and CC alleles. Other transporters evaluated in this study for their impact on TFV and FTC concentrations during pregnancy or postpartum were *ABCC2* 12:g.32293730T>C, *ABCC4* 11:g.95020696A>G, *ABCC4* 11:g.95021537A>C and *ABCC4* 13: 95062722C>T which showed no significant relationship with TFV or FTC concentration. All allele frequencies, genotype frequencies and common allele were consistent with previously reported genes in the region (318). TFV concentration was 26% lower in pregnant women, consistent with previous reports of PANNA and IMPAACT P1026 studies (333, 334). This is an important finding in understanding ART exposure in pregnancy. Clinicians will need to be aware that that an overall decrease in blood exposure to TFV and FTC during pregnancy may not be limited to only physiological changes of pregnancy, but other factors such as genetic polymorphisms. Therefore may require therapeutic drug monitoring and dose adjustment if available. For instance, CYP2B6 516G homozygous reported in previous EFV studies of this patient suggest a significant decrease in EFV exposure during pregnancy (330).. The significance of this findings are not clear, since we are unsure of what happen to the intracellular active metabolites of TFV but optimal drug exposure without adverse side effect is required to ensure viral suppression and adherence to drugs. Good adherence

is an important factor of ensuring treatment efficiency and prevention of increasing clades of resistant mutant virus against a wide class of antiretroviral agents.. Good adherence is an important factor of ensuring treatment efficiency and prevention of increasing clades of resistant mutants virus against a wide class of antiretroviral agents (335, 336).

This study was performed in a small sample size and PK sampling was inadequate since only single time point TFV and FTC blood concentrations were measured per patient. Furthermore, some important transporters such as *ABCC1* that may be important in the elimination of TFV and FTC were not included in the analysis. We recognised these limitations and advice further evaluation of the impact of pregnancy and pharmacogenetics on TFV and FTC across a wider population and larger sample size.

.

6.5 Conclusion

In conclusion, pregnancy and the puerperium are periods of considerable physiological changes in women, and the interplay between pregnancy and pharmacogenetics have significant impact on FTC and TFV exposure. However, this study did not find any relationship between pregnancy and pharmacogenetics on FTC and TFV PK. TFV blood concentration was decreased during pregnancy, and the pharmacogenetic relationship of FTC exposure and *ABCC2* 12:g.154962860T>C T allele was not related to pregnancy but associated with higher concentrations of FTC exposure irrespective of pregnancy

6.6 Future Perspective

In view of these findings and limitations of this study, further pharmacogenetic investigation of *ABCC1*, *ABCC2*, *ABCC4* and other transporters is required in larger population and multiple regions. Proposed intensified ART in sub-Saharan Africa and other low and middle income countries with TDF/FTC NRTI backbone will increase frequency of prescription of formulation containing TDF and FTC in pregnancy. Therefore, long term efficacy of these two important NRTI will need to be protected.

CHAPTER 7

Widespread use of Herbal Medicines by people living with HIV (PLWH) and Herbal Medicines contamination with Antiretroviral (ART) in Nigeria

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CHAPTER 7

Widespread use of Herbal Medicines by people living with HIV (PLWH) and Herbal Medicines contamination with Antiretroviral (ART) in Nigeria

Introduction

Prevalence of herbal medication use among PLWH and contamination with medicinal products and certain pharmaceutical agents has previously been reported (337), though understudied. Herbal contamination has not been described for antiretrovirals (ART), despite suspicion and poor regulations of herbal practices in sub-Saharan Africa (SSA) (338, 339). Widespread use of herbals among HIV-infected patients could be detrimental to the intensified efforts to end the HIV epidemic by 2030 (340). Use of herbal medicines could be associated with toxicities, reduced ART adherence and poor health outcomes (341-343). Previous studies of herbal medicine use among PLWH reported 63.5% use after diagnosis and 32.8% concomitant use with other pharmaceutical products including ART in Uganda and 27.5% use among PLWH prior to starting ART treatment and 4.25% of concomitant users with ART in Nigeria respectively (344, 345) .

Nigeria and South Africa account for over 40% of the HIV burden in SSA and over 3 million PLWH in 2016 were living in Nigeria (18, 346). Furthermore, poor access to health care, lack of qualified healthcare personnel, health care cost, economic status,

education and cultural beliefs are all associated with concomitant use of ART with herbal medications (347-349). Other challenges of healthcare in Nigeria include; infrastructural, administrative and logistics challenges. These factors were recognised to be responsible for high cases of paediatric HIV in 2016 due to vertical transmission as a result of lack of power to analyse viral load samples for early infant diagnosis of HIV (3). Marked variability in access to ART across the country and complex peculiarities in the HIV programmes poses a significant challenge and promotes herbal medicines utilisation by patients (346).

Globally, several studies have reported use of herbal medicines among PLWH either as part of complimentary medicines or as a component for treating other ailments (350) but few reports described the impact of herbal medication use on patient safety, adherence and health outcomes (341, 343). Although some studies reported that concomitant use of herbal medicines is significantly associated with poor adherence to ART (351), and others found no relationship between adherence to ART and herbal medication use (345, 348), herbal use in HIV treatment should be discouraged because of potential interactions with ART (352).

Herbal medicines are commonly taken by PLWH, but use in relation to the timing of ART initiation or safety is not well characterised (344, 353). Users and practitioners erroneously consider herbal medicines to be safe without any safety evaluation (354, 355). Furthermore, practitioners have little or no understanding of modern approaches to evaluation of safety of medicinal products (354, 356).

We sought to evaluate the prevalence of herbal medicine use by PLWH attending one rural and three urban HIV clinics, and related this to the time of ART initiation. In

addition, samples of traditional herbal medicines were collected from street vendors across Nigeria and were screened for the presence of ART using liquid chromatography-mass spectrometry.

Methods

a) Contamination of Herbal medications with ARVs

Herbal medicines were collected across 8 states in Nigeria between December 2014 and June 2015. The protocol for sample collection was as follows: i) street vendors from both urban and rural settings were approached by study investigators or assigned personnel, ii) herbal medicines were requested for the following indications: HIV, AIDS, fevers or non-specific symptoms (e.g. weakness) known to be associated with HIV, iii) herbal vendors were not informed that samples were to be used for research purposes to avoid any attempt to modify preparation by herbal vendors to conceal any secret, iv) only herbals sold as powders or liquids were purchased, v) instructions for use were recorded, as was the date and site.

A semi-quantitative screen for ARV contamination of herbal medicines was performed at the University of Liverpool by liquid chromatography-mass spectrometry (LC-MS) for the following ARVs: efavirenz, nevirapine, lopinavir, darunavir, ritonavir, atazanavir, emtricitabine, tenofovir and lamivudine, using a method adapted from Else *et al* (357). This method enabled the simultaneous measurement of 9 ARVs and was modified based on the suspected polarity of the different ART.

Sample pre-treatment

Herbal powders were weighed and dissolved in both water and dimethyl sulfoxide (DMSO) in order to optimise recovery due to different solubility of possible contaminants, at a stock concentration of 10 mg/mL. Working solutions were prepared by further diluting the stock (1:1) with mobile phase. Control (drug-free) mobile phase and blank herbal extracts (herbal controls) were prepared to check the background response of the LC-MS assay. Reference standards spiked with 9 ARV (efavirenz, nevirapine, lopinavir, darunavir, ritonavir, atazanavir, emtricitabine, tenofovir and lamivudine) at two concentrations (50 ng/mL and 100 ng/mL) were prepared.

Semi-quantitative LC-MS/MS:

Blanks, reference standards and unknown samples (sourced herbal extracts) were injected (10 µL) onto the LC column coupled to a triple quadrupole mass spectrometer (TSQ Ultra; Thermo Scientific, Hemel Hempstead, UK). Data acquisition and processing was performed using LC Quan^{TM} software (Thermo Scientific, Hemel Hempstead, UK). Herbal contamination was determined by semi-quantitative LC-MS assay (no internal standard or calibrators were used). An estimate of the amount of drug within contaminated samples was derived by comparing chromatographic peak areas of the samples against peak areas of known concentrations of tenofovir, emticitabine and lamivudine spiked in water after correcting for background signal from a known negative herbal sample. Two rounds of analysis (initial screening using 10 mg/mL and confirmatory tests using 50 mg/ mL) were performed before accepting the presence of ART contamination in the sample. Detectable drug was confirmed if the response (chromatographic peak area; arbitrary units) of the unknown sample

was at least five times greater than the response of the blank herbal extract (assay background). A semi-quantitative value per milligram of herbal powder was calculated based on the response of the reference standards minus the assay background interference. For samples that showed presence of drug during screening, additional confirmatory experiments were performed using higher (50 mg/mL) concentrations of herbal extract.

b) Survey of herbal medication use among PLWH

A clinical survey of PLWH attending ARV facilities in one rural (Rural Hospital Idong) and three urban facilities (Specialist Hospital Gombe, Faith Alive Foundation Clinic, Jos and Dalhatu Araf Specialist Hospital, Lafia) was conducted. Using non-probability sampling technique, 500 PLWH were surveyed from Faith Alive Foundation Clinic, 199 from Dalhatu Araf Specialist Hospital, 33 from Specialist Hospital Gombe and 10 from Rural Hospital Idong, respectively. The national prevalence rate of HIV in Nigeria is 3.2%; Kaduna, Gombe, Plateau and Nassarawa states have HIV prevalence rate of 9.2%, 8.1%, 3.4% and 2.3%, respectively. These surveyed clinics attend to over 6000 PLWH and the Faith Alive Foundation Clinic in Jos attends to over 5000 PLWH per month, while Rural Hospital Idong, a primary health care setting, attends to less than 50 PLWH. (171, 242).

Inclusion criteria were: known HIV-antibody positive patients attending clinic, any age, willing to participate in the survey. For children less than18 years old, parents were asked for consent and if willing, provided responses to the survey. Hospitalised patients, patients who were acutely unwell and any unconfirmed patient in the clinic

were excluded. We utilised a structured questionnaire adapted from Langlois-Klassen *et al* (2007) who previously evaluated herbal medication use among PLWH (344). The questionnaire was modified for our purpose and was applied to evaluate use of herbal medications across age, gender, education, employment status, date of diagnosis, date of starting herbal medications, type of herbal medication, source of recommendation for herbal use, source of procurement of herbal, reasons for use and perceived effectiveness of the herbals. The primary outcome was prevalence of herbal medication use among PLWH. Secondary outcomes were use of herbals in relation to initiation of antiretroviral drugs, differences by gender, by age, by educational attainment, employment status, and perception of benefit from herbal use. Herbal sample collection was random, and collection at different regions of the country was ensured for fair representation of different regions of the country.

Statistical Analysis

Data were analysed using IBM SPSS statistics version 22.0 (*IBM Corp. 2013*). Participants who answered positively when asked, "Do you use herbal medicine?" were considered as herbal medicine users. Data on herbal use and baseline social and demographic factors were pooled across all four centres to produce aggregate, descriptive overall frequencies at 5% level of significance (*Table 1*). We then compared social and demographic factors in PLWH who used or did not use herbal medicines. First univariate analysis was performed using binary logistic regression with herbal use as the dependent variable. Next all univariate associations with P<=0.1 were included in the final multivariable model. Groups were compared for

relationships and confounders on the dependent variable (use of herbal medication) were resolved using multiple regression models (*Table 2*).

Ethical approval

Ethics approvals for the Survey were obtained from Dalhatu Araf Specialist Hospital Lafia, Faith Alive Foundation Hospital and PMTCT Centre Jos (protocol assigned number: FAFHREC/08/34/5) and parental consent for including children was sought from parents before interviewing the parents for their children's information. Only verbal consent and permission from hospital management was obtained from patient in primary health care Idong and Gombe Specialist Hospital.

Results

A total of 742 (approximately 12% of patients receiving treatments in these facilities) consecutively attending PLWH aged 2-91 years were surveyed across the 4 centres. A total of 715 (96.4%) were adults aged 18 and above, 457 (61.6%) were female, 281 (37.9%) were males and details of gender were missing in 4 (0.9%). Further details of demographic characteristics are provided in Table 1. Of the 742 PLWH surveyed, 594 (80.05%) were receiving ART.

Prevalence of herbal medication use was 41.8% (310). Of the 310 individuals who used herbal remedies, 38.4% took herbals prior to diagnosis of HIV while 61.6% started taking herbals after HIV diagnosis (*Table 2*). Educational attainment and employment status were not determinants of herbal medication use (70% of herbal users vs 66% of non-users had secondary and tertiary education) and (70.7% of herbal users vs 65.1% non-herbal users were employed). Time from diagnosis of HIV (P=0.73) and whether individuals were receiving ART (P= 0.53) were not significantly associated with herbal medication use. PLWH who use herbals did so for a variety of reasons: to cure HIV (46.8%), or following the advice of family (67.4%) or friends (31.6%). Altogether, 40.3% said they felt herbals were ineffective for HIV while 4.8% believed themselves cured (*Table 2*). Ingestion of herbal medications while on ARVs was more likely in the elderly (OR 2.31 (95% CI 1.34, 3.99) and the employed (OR 1.23 (CI 0.89, 1.70) compared to the unemployed (*Table 3*).

As an extension to this study, 138 herbal samples were sourced from herbal vendors in diverse locations across Nigeria to assay for presence of ART. Of 138 samples collected and analysed, 3 (2%) contained detectable ART. One sample from Jos contained tenofovir and emtricitabine (estimated as 0.02 ng/mg powder and ≤0.01 ng/mg powder respectively), while two samples from Ibadan contained tenofovir (estimated as <0.01 and 0.13 ng/mg powder) and emtricitabine (estimated as <0.01 ng/mg powder respectively), with one of these also containing lamivudine (estimated as 0.02 ng/mg powder). Other ART classes were not detected.

Dosing recommendations provided by the herbal vendors are often not precise. We found a typical dose recommendation was '3 fingers' or scoops of medicinal powder in the palm of the hand, conservatively estimated as 1-2 teaspoons (1 teaspoon estimated as 5 g). Based on these recommendations a single dose of 2 teaspoons (approximately 10 g) of contaminated herbal powder could potentially deliver up to 1.3 µg of tenofovir, 0.1 µg of emtricitabine and 0.2 µg of lamivudine. Although amount of drug detected is small, repeated exposure, or exposure to higher doses with different batches or preparations, could promote drug resistance.

In Nigeria, tenofovir and emtricitabine are available in fixed-dose combination as TRUVADA®. We wanted to examine if the discrepancy in the amount of tenofovir and emtricitabine observed could have been explained by differential degradation when crushed or dissolved, or recovery during the extraction process. Recovery from dissolved TRUVADA® tablets in water at room temperature carried out after 12 weeks and 9 months respectively was 63.2% and 1.47% for emtricitabine, compared with

0.8% and 1.47% for tenofovir. There were also considerable differences in drug solubility, recovery (extraction efficiency) and matrix effects (ion suppression) between different herbal preparations, which more likely explained the differences in measured quantity between both drugs.

Discussion

Like other studies (344, 358), we observed that use of herbal medicines was widespread (*Table 1*) regardless of age, gender, educational or employment status. Approximately 594 (80%) of PLWH were receiving ART, 40% use herbal or traditional medicines and of these 40% were using these remedies prior to initiation of ART.

Of concern, a significant proportion of individuals (14.5%) use herbals prior to initiation of ART. Previous reports of low level ART exposure to neonate during PMTCT suggest that continuous low-level exposure to ART could drive resistance (48, 359). Our finding of contamination of herbals with ART is a concern, though concentration was very small and may not be sufficient to drive resistance, finding contamination in herbals is detrimental to treatment and HIV programme success. Herbal medicine use was highly prevalent in patients receiving HIV treatment (43.6% of people on ART were also taking herbals). Around half of the subjects perceived use of herbal medicines to either moderately or completely relieve symptoms such as headaches, fever, and generalised body weakness.

We found 2% of herbal medicines contained small, but detectable quantities of ART, yielding an estimated dose of 0.1-1.3 µg ingested per dosing occasion. The clinical relevance of this is unclear. However, continuous low-level exposure to drug below

PA IC_{50} can lead to resistance, provided sufficient drug is present to drive selection pressure for resistant mutants (360, 361). Considering the oral bioavailability of drugs, only small levels are likely to be achieved in this scenario since measured concentration is low. Therefore, its potential role in driving resistance is not clear. Furthermore, potential drug-drug interaction of herbals and ART as well as safety (342, 352) was difficult to ascertain since constituents of the herbal medicines were unknown (362). Although the concentrations detected were very low, actual concentrations may have been higher given the different efficiencies of extraction and degradation over time that were observed.

The finding that traditional or herbal remedies may contain pharmaceutic agents is not new. Previously, herbal medicines have been found to contain antipyretics, anti-inflammatory agents or steroids, diuretics, antidiabetics, sedatives, antihistamines and vasodilators through accidental or deliberate contamination (363). Accidental contamination may result from carryover of drug during local manufacture of medicines; however, this is less likely here, as HIV drugs are not locally co-manufactured with herbals to our knowledge. It is difficult to ascertain whether contamination was deliberate or accidental in this case. HIV drugs are dispensed free of charge through the National Treatment Programme; their use and belief that they are beneficial for the treatment of HIV disease may provide both the opportunity and the motive for any deliberate contamination. Herbalists and their medicines can play an important role in supplementing and supporting HIV treatment in treatment settings (353, 364) where weak health systems and complex cultural beliefs around symptom management (365) promote widespread herbal use. Enforcing the

regulation of herbal medicine practices (339) and re-evaluating policies guiding herbal practitioners in Nigeria is urgently required to avoid harmful practices that put the general public at risk.

Limitations

We were not able to ascertain how herbal use influenced engagement with HIV services, or adherence to ART although these are important questions to address in future research. Moreover, we were only able to assess PLWH already engaged with treatment services. There is currently no clear understanding of positive or negative effects of traditional medicines as adjunctive treatment in PLWH. However, the lack of regulation and standardisation in these preparations argues strongly for further work to confirm our findings in other countries where herbal medicines use is prevalent, and to understand whether ART contamination of herbal medicines has a negative impact on ART programmes through the generation of drug resistance. Surveys were only conducted in small population from northern Nigeria, which may vary from other regions of the country. Therefore, a larger study that aims at ensuring fair representation of all the regions of Nigeria is required.

Conclusion

Herbal medicines were erroneously perceived by users to relieve symptoms or cure disease and use was widespread amongst PLWH, often after commencing ART. Co-administering herbal medicines with ART is concerning, especially since constituents and safety profiles of the herbal medicines are unknown. Previous reports of drug-drug interaction, liver and kidney injuries due to toxicities from herbals medicines

favours urgent evaluation of policies by authorities and further evaluation of our findings by researchers to establish the best approach to monitor and enforce safe practices of herbalist for public safety of herbal products.

Table 1: Prevalence of social and demographic factors in PLWH who use, or do not use herbal medicines

Variable	Use Herbal Medications (n= 310) n (%)	Do not Use Herbal Medications (n= 432) n (%)
Age		
• <18 years	3 (1.0)	24 (5.6)
• >18 years	307 (99.0)	408 (94.4)
Gender		
• Male	119 (38.4)	162 (37.9)
• Female	191 (61.6)	266 (62.1)
• Missing data		4 (0.9)
Level of Education		
• Little or no education	81 (26.1)	139 (32.1)
• Secondary & Tertiary education	217 (70.0)	285 (66.0)
• Missing data	12 (3.9)	8 (1.9)
Employment Status		
• Employed	219 (70.7)	281 (65.1)
• Unemployed	88 (28.4)	140 (32.4)
• Missing data	3 (0.9)	11 (2.5)
Months since HIV diagnosis		
• < 6 months	146 (47.1)	136 (31.5)
• >6 months	152 (49.0)	284 (65.7)
• Missing data	12 (3.9)	12 (2.8)
Commenced ART		
• Yes	259 (83.6)	335 (77.5)
• No	45 (14.5)	78 (18.1)
• Missing data	6 (1.9)	19 (4.4)

Variable	Use Herbal Medications (n= 310) n (%)
When Did you Start Using Herbal Medications?	
• Pre-HIV Diagnosis	119 (38.4)
• Post-HIV Diagnosis	191 (61.6)
Who Recommended the herbal medications	
• Family	209 (67.4)
• Friends	98 (31.6)
• Missing data	3 (1.0)
Source of Herbal Medicines	
• City vendors	108 (34.8)
• Village Herbalist	145 (46.8)
• Others	15 (4.8)
• Missing data	42 (13.6)
Reasons For Herbal Medication Use	
• To Relieve Symptoms	108 (34.8)
• To achieve cure of Disease	145 (46.8)
• Others	15 (4.8)
• Missing data	42 (13.6)
Perceived effectiveness of herbal medication	
• No help	125 (40.3)
• Moderate relieve of Symptoms	89 (28.7)
• Completely relieved symptoms	43 (13.9)
• Cures disease (HIV) completely	14 (4.5)
• Missing data	39 (12.6)

Table 2: Source of recommendations, reasons for use, when herbal was started and perceived effectiveness among PLWH who use herbal medication (n=310)

	Univariate Analysis			Multivariate analysis		
Variable	β	Odds Ratio (OR) (95% Confidence Interval)	P-Value	β	Odds Ratio (OR) (95% Confidence Interval)	P-Value
Age	0.688	1.990(1.183, 3.347)	0.009	0.839	2.314(1.343,3.987)	0.003
Gender	-0.039	0.797(0.712, 1.298)	0.797			
Level of Education	0.248	1.282(0.930, 1.766)	0.130	0.190	1.209 (0.868, 1.686)	0.262
Employment Status	0.192	1.228 (0.887, 1.696)	0.228			
Months since HIV diagnosis	-0.688	0.502 (0.370, 0.681)	<0.001	-0.724	0.485(0.355, 0.663)	<0.001
Commenced ARV's	0.154	1.167(0.080, 1.696)	0.418			

Table 3: Association of surveyed variables and herbal medication use among PLWH

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CHAPTER 8

8.0 GENERAL DISCUSSION

Effectiveness of antiretroviral therapy (ART) in sub-Saharan Africa (SSA) within the framework of therapeutic drug safety in pregnant women and their neonates and drug-drug interactions with commonly prescribed medications is an important consideration in care of PLWH. Co-administration of ART with tropical disease treatments is common, often dependent on the assumption that there is a clinically insignificant influence of the aforementioned factors in drug exposure to patients. Most preliminary clinical studies of ART are often dependent on healthy male volunteers and population pharmacokinetic modelling to draw conclusions on safety and potential interactions. Recent advances in pharmacokinetics (PK) and pharmacogenetics have unravelled significant amount of subtle differences in the way medications are handled by different individuals of different populations, thereby requiring much more evaluation before conclusions on drugs safety profiles can be drawn. DTG, an ART agent used in HIC since 2013 for treatment of HIV, requires further evaluation before roll out to LMIC including sub-Saharan Africa. Since recommendation by WHO in 2016 guidelines as alternative first line treatment of HIV, efforts have been made for roll out in LMIC and it is currently being evaluated for safety in the sub-Saharan African populations of Botswana, Uganda and South Africa by different research groups before it is approved by for wider use in countries of the region. Pregnancy and paediatric safety data as well as drug-drug interaction data

with commonly prescribed medications for treatment of tropical diseases such as malaria is lacking for DTG. In recognition of this research gap and insufficient data, several clinical trials are currently being conducted by several research groups. Notably among these are IMPAACT P1026s, National Institute of Allergy and Infectious Diseases (NIAID) funded trial known as VESTED which evaluated the efficacy and safety of DTG-containing versus EFV-containing ART regimens in HIV-1-infected pregnant women and their infants, DOLPHIN 1 and 2, and DolACT which will provide pregnancy and paediatrics safety profiles and drug-drug interaction data. Intensified HIV treatment in regions with high prevalence is a major determinant of ambitious vision 90-90-90 success. Therefore, more effort is required by stakeholders to ensure DTG safety is evaluated and rolled out for treatment of over 25 out of the over 36 million PLWH for successful achievement of the set targets. Chapters 2 of this thesis reviewed the PK of DTG in different studies to identify baseline of safety data available. Chapter 3 developed and validated robust method of DTG measurements in DBMS, to support sample analysis in DOLPHIN 1 and 2, while chapter 5 evaluated DTG safety and drug-drug interactions with ACT (frequently prescribed antimalarials that could potentially be co-administered with DTG in PLWH). Chapters 4, and evaluated the changes in ART PK, safety, viral load changes of DTG in pregnant mothers and their neonates, as well as DTG changes in postpartum period and excretion in breastmilk of mothers in relation to their plasma concentration (DOLPHIN 1). The pharmacogenetic impact of pregnancy on FTC and TFV PK was evaluated in chapter 6 and potential role of herbal medication in influencing adherence to ART in Nigerian PLWH was evaluated in chapter 7.

DTG was hypothesised to be safe and effective across diverse populations when deployed as first line ART in sub-Saharan Africa and this thesis aimed at testing the hypothesis by evaluating DTG safety in some thematic areas of HIV treatment. This involved developing and validating robust methods of DTG measurement that can be easily applied in the region for clinical studies or therapeutic drug monitoring and studying DTG safety in the sub-Saharan African populations.

DTG was well tolerated by patients in the two studies (DolACT and DOLPHIN 1), with a linear PK in both studies (chapters 4 and 5). DTG metabolism is known to be influenced by CYP enzymes and UGT enzymes and PGx of these enzymes would be evaluated in DOLPHIN 2 for their role in influencing DTG PK in both pregnant and postpartum women. Chapter 2 was a review of DTG PK and PGx safety in different population groups as reported by different clinical studies. The reviewed data revealed limited pregnancy and paediatric safety data of DTG from early studies. This resulted in the pregnancy and paediatric clinical trials. These are essential as DTG use is likely to be increased following its recommendation for first line treatment of HIV in 2016 WHO guidelines; consequently, more studies are needed to determine safety in these population of patients to ensure safety. Clinical studies involving measurement of drug concentrations in body fluids in sub-Saharan Africa is challenging due to lack of basic infrastructures, and often require complex transport logistics to regions of the world with appropriate infrastructures and expertise for sample analyses. Chapter 3 described a method that was easy to apply in the region for sample collection and transport under ambient conditions. Recently, the use of protein saver cards by researchers for drug measurements in body fluids has increase and it is fast gaining popularity for potential use in therapeutic drug monitoring of

ART and other pharmacological agents. It is hoped that this method will contribute significantly to DTG measurement in BM. Though microsampling is also increasingly becoming popular and likely to be a LMIC friendly sample collection technique, it may take time to become readily available in the region. Therefore, this method could become a reliable and robust alternative before microsampling techniques are made available (366, 367).

Despite integrated antenatal care in sub-Saharan African countries, late booking and ART initiation in HIV infected pregnant women are common, leaving very little or no time for viral suppression before labour. Chapter 4 evaluated time to undetectable viral suppression, safety and pharmacokinetic changes of DTG during pregnancy compared to EFV. Over 50% of women achieved <50 copies/mL viral load after 2 weeks of commencing DTG compared to just about 10% of women of EFV in the interim report. At 2 weeks postpartum, 100% of women who had no resistance to DTG and adherent to medication achieved viral suppression with VL < 50 copies/mL. DTG also appeared safe as adverse events recorded (stillbirth, hypertension, polydactyly and elevated liver enzymes) were likely to be unrelated to DTG. Recently, preliminary report of DTG in pregnancy known as the Tsepamo observational study in Botswana reported an increased incidence of neural tube defect in infants of women who conceived while on DTG compared to incidence with other ART (368). Therefore, cautious use is now recommended until study is completed and more data is made available. AUC_{0-24}, C_{0-24} and C_{max} were slightly lower in pregnancy compared to postpartum but not statistically significant. Pregnancy is known to be associated with physiological changes and decrease in plasma levels of drugs seen in both chapter 4 and 6. In chapter 6 there was also an associated 1-2 fold increase in FTC

concentration 12-18.5 h post dose with *ABCC2* 12:g.154962860T>C T allele. This finding will need to be further verified since FTC and TFV are important components of the proposed co-formulation of DTG likely to be rolled out in LMIC. Evaluation of the pharmacogenetics of FTC and TFV in pregnancy will elicit better understanding of the factors influencing ART exposure during pregnancy and the general population.

Outcomes of malaria, like many diseases, could be worsen by background HIV infection or immunosuppression and vice versa. Geographical overlap of these diseases makes frequent treatment of the two diseases simultaneously likely. The potential of drug-drug interaction between the commonly prescribed antimalarials ACT and DTG was suspected since they are metabolised by similar CYP and UGT enzymes. Chapter 5 described interactions of DTG with ACT and found no need of dose adjustment or contraindication of co-administering DTG and ACT together despite statistically significant decrease in DTG C_{trough} by 37% with AL, 42% with AS-AQ and 24% decrease in AUC_{0-24} with AS-AQ. All values were still above the protein adjusted IC_{90} for the C_{trough} despite the decrease and does not require dose adjustment. No significant changes in ACT concentrations were observed making recrudescence highly unlikely. Considering the usual short duration of malaria treatment, any impact on DTG concentration is likely to be brief and within the accepted minimum concentration.

Adherence to ART is a complex challenge in treatment of HIV, due to poor ART forgiveness and easy mutation of viral targets, resulting in resistance against ART and therapeutic failure. Optimal ART adherence (>90%) is required for viral control (369, 370). Herbal medication use is a recognised driver of poor ART adherence among

PLWH (343). Herbal medication culture in a complex system of poor health infrastructures is a challenge to treatment success since details of impact of herbal medications on ART is limited. Challenges of herbal medication use are not just limited to the unknown composition of herbals cocktails, the dose and chemical composition of the compounds are also unknown. Chapter 7 described contamination of herbal medications with ART and widespread use among PLWH in a Nigerian population. The finding of over 40% use in PLWH with 38% preceding ARV initiation, 61.1% concomitantly used with ART and contamination of herbals with ART is concerning. It may serve to sabotage intensified treatment efforts and long term consequence is unknown. Further evaluation in other countries of the region to understand the trend of contamination and introduce programmes that aim at curbing the practice is required. Evaluation of the effect on ART outcomes and appropriate recommendations to regulatory organisations to prevent and/or eliminate the practice will be an important step in ensuring good outcomes. A negative effect of herbal medication use was experienced in DOLPHIN 1, where an adverse event was experienced by a participant but it was unclear whether the elevated liver enzyme were caused by the herbal medication or DTG (chapter 4).

Future perspectives (Recommendations)

- More microsampling techniques and use of portable electrospray or paper spray mass spectrometry methods that are robust and adaptable to remote areas of the world should be evaluated. This will enable samples to be evaluated by the bedside or in local laboratories and results produced faster, easier and at a reduced cost. Technologies such as handheld desorption

atmospheric pressure chemical ionization (DAPCI)(371) should be further developed and evaluated for potential use as bedside means of measuring ART in body fluids for PK studies and therapeutic drug monitoring. While these technologies are being evaluated for future use in LMIC, use of protein saver cards and automated extraction processes should be intensified for TDM since these are now readily available and the new technologies may take a while to be finally made available.

- Potential of adopting and co-opting DBMS method principles with microsampling techniques to develop easier and more robust methods of sample analyses should also be evaluated for possible use in future. Development of protein saver cards that absorb specific volumes and made in form of swab sticks will be much easier, requiring less expertise for sample collection and small sample volume.
- Use of calibrated capillary tubes or capillary tubes with dispensers will require less expertise for sample collection without compromise to the accuracy of volumetric pipetting.
- Although cellulose in protein saver cards is inert and not expected to interact with other molecules, formation of biopolymers with polysaccharides and peptides of BM is possible. Therefore collaboration with organic or polymer chemists to evaluate the formation of these biopolymers during sample processing may help to clarify the reasons why sample processing with DBMS is much easier compare to liquid BM.

- Pharmacogenetics (CYP 3A4 and UGT1A1) should be further evaluated in large clinical trials to determine the impact of UGT and CYP 3A4 on DTG exposure across different populations of the SSA region.
- Evaluation of the interaction of DTG with the treatments of other tropical diseases including treatments of neglected tropical diseases that uses CYP 3A or UGT metabolic pathways such as ivermectin, praziquantel, mebendazole and albendazole for drug-drug interactions. Drug-drug interactions with commonly prescribed antibiotics and antimicrobial agents should also be evaluated in future.
- Although, no toxic effect of DTG due to transplacental transfer to neonates has been observed so far, toxic effects of high drug exposure should be investigated for any subtle problems that could be posed by high DTG exposure in utero especially since poorly developed liver UGT1A1 promotes DTG accumulation in neonates over a long period of time. A greater than 2 fold increase in plasma half-life ($t_{1/2}$) of DTG was observed in DOLPHIN 1 and IMPAACT P1026s (ClinicalTrials.gov NCT00042289) (266). Further investigation of the risk of more accumulation in children at risk of hyperbiluribinaemia should be evaluated due to shared metabolic pathway (via UGT enzyme) for markers of increased risk of neonatal DTG exposure. Developmental dynamics of liver should also be evaluated for potential interventions that could improve liver maturity and improved enzyme development (372, 373). Safety of DTG is unknown in children, and only recommended for children >12 years. Studies should be designed to evaluate

DTG safety in children <12 years especially considering its robustness in viral control and potential benefits to affected children.

- Although it is ethically difficult to conduct clinical studies in children, its potential benefit should encourage a balanced method that combines preclinical physiological based modelling and outcomes of neonatal exposure to predict potential safety and cautious trials be conducted within an ethically acceptable framework.
- Safety of DTG use in chronic diseases of the liver and kidneys should be further evaluated.
- Further evaluation of the role of *ABCC2, ABCC1, ABCC4 and* p-glycoprotein in FTC and TFV PK and pharmacogenetics in large sample size across SSA countries will increase genetic libraries for research and improvement of clinical outcomes of HIV treatment with FTC and TFV combinations.
- Evaluation of the role of herbal medications in determining adherence to ART, prevalence of herbal medication contamination with ART and prevalence of herbal medication use among PLWH in different centres across different countries of sub-Saharan Africa and making appropriate recommendations to regulatory authorities.
- Furthermore, collaborative research with Botanist, organic phytochemists, medicinal chemist and pharmacologist to investigate the commonly utilised plant products and other natural products using evidenced based scientific techniques will provide better understanding of the role of African herbs in antiretroviral outcomes. Designing studies that describes the taxonomy of the plants, investigate the chemical composition of the herbal products, describe

the chemical structure and potential modification of the structures to form new molecules. Beyond studying the interactions and impact on antiretroviral therapy, evaluating herbal medications may provide an opportunity for synthesis of novel molecules of medicinal importance.

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