CLINICAL STUDY PROTOCOL

Study Title:	Study on **P**harmacokinetics of newly developed **AN**tiretroviral agents in HIV-infected preg**NA**nt women (**PANNA**).
Sponsor:	Radboud university medical center Geert Grooteplein 10 6525 GA Nijmegen, The Netherlands
Investigator/ Conductor	Prof. Dr. David M. Burger Radboud university medical center Dept. of Pharmacy Geert Grooteplein 10 6525 GA Nijmegen, The Netherlands
Protocol ID:	UMCN-AKF 08.02
EudraCT number:	2008-006158-16
Monitor:	Angela Colbers and Monique Roukens
Biostatistician:	Dr R. Donders, Radboud university medical center
Project Manager:	Angela Colbers, PhD. And Wendy van der Wekken, MD Dept. of Pharmacy 864 Radboud university medical center Geert Grooteplein 10, 6525 GA Nijmegen, The Netherlands Telephone: (+31) 6 25248088

Medical Investigators:	Deleted because of privacy
Protocol Version/Date:	Final version 8: 25 April 2023

CONFIDENTIALITY STATEMENT

The information contained in this document is provided to you in confidence as an investigator, potential investigator, or consultant, for review by you, your staff, and an applicable Institutional Review Board or Independent Ethics Committee. The information is only to be used by you in connection with authorized clinical studies of the investigational drugs described in the protocol. You will not disclose any of the information to others without written authorization from the Investigator, except to the extent necessary to obtain informed consent from those persons to whom the drug may be administered.

STUDY ACKNOWLEDGEMENT

Study of **P**harmacokinetics of newly developed
ANtiretroviral agents in HIV-infected preg**NA**nt women
(**PANNA**).

Protocol UMCN-AKF 08.02 – Final version 8 25 April 2023

INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.
I will provide all study personnel under my supervision copies of the protocol. I will discuss this material with them to ensure that they are fully informed about the drugs and the study.

_____

Prof. Dr. David Burger

Signature

Date

_____

Name investigator (if required)

Signature……

Date……

Protocol Synopsis

Title of Study:	Study of **P**harmacokinetics of newly developed **AN**tiretroviral agents in HIV-infected preg**NA**nt women (**PANNA**).
Study Centers Planned:	Erasmus Medical Center Rotterdam, The Netherlands Radboud university medical center, Nijmegen, The Netherlands University of Cologne, Germany, **– no dolutegravir and no inclusion of infants** University of Bonn, Germany**– no dolutegravir and no inclusion of infants** Hospital Universitari Germans Trias I Pujol, Badalona, Spain C&W Hospital, London, UK IMPERIAL COLLEGE HEALTHCARE NHS TRUST, London, UK IRCSS, Roma, Italy University of Padua, Italy Academisch Medisch Centrum, Amsterdam, The Netherlands St James's Hospital Dublin, Ireland Klinik für Geburtsmedizin; Charité Universitätsmedizin, Berlin, Germany**– no dolutegravir and no inclusion of infants** Klinikum der Universität München, Frauenklinik Innenstadt, München, Germany**– no dolutegravir and no inclusion of infants** Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany**– no dolutegravir and no inclusion of infants** Hospital Virgen de las Nieves, Granada, Spain Saint-Pierre University Hospital, Brussels, Belgium Mater Misericordiae University Hospital Dublin, Ireland BSUH NHS TRUST, Lawson Unit, Royal Sussex County Hopsital, Brighton, UK (satellite site) St Elisabeth Ziekenhuis, Tilburg, The Netherlands CHU Hôtel Dieu, Nantes, France University Medical Centre Utrecht, Utrecht, the Netherlands University MEdical Centre Groningen, the Netherlands Curaçao Medical Center, Willemstad, Curaçao

Objectives:	The primary objective of this study is to describe the pharmacokinetics of antiretroviral agents for which no or only limited pharmacokinetic data during pregnancy are available, in the 3rd trimester of pregnant HIV-infected women and at post-partum. And in the 2nd trimester for raltegravir once-daily regimen and dolutegravir regimen. In addition, the pharmacokinetics will be determined in the infant as well in case of post-exposure prophylaxis with one of the agents tested (not performed in Germany). Secondary objective of this study is to describe the safety of the antiretroviral agents during pregnancy and the efficacy in terms of viral load response of the mother and prevention of mother to child transmission. To describe breastmilk transfer in case of breastfeeding and exposure in child if breastfeeding. *For the raltegravir once-daily regimen the following additional objectives apply:* To describe pharmacokinetics in the infant after birth a time point of blood sampling for regular care (not performed in Germany). To describe the safety of the antiretroviral agents, in this case specifically the reformulated once-daily raltegravir tablets, during pregnancy and the efficacy in terms of viral load response of the mother and prevention of mother to child transmission. To investigate the relation between progesterone levels and raltegravir pharmacokinetic changes in pregnancy. *For the cabotegravir/rilpivirine LA regimen, the following additional objectives apply:* To describe pharmacokinetics over the duration of pregnancy by trough samples prior to each injection. To describe pharmacokinetics in the infant after birth a time point of blood sampling for regular care. To describe breastmilk transfer in case of breastfeeding for cabotegravir/rilpivirine and exposure in child if breastfeeding.
Study Endpoints	Primary endpoints: $AUC_{0\text{-}tau}$; C_{max}; C_{trough}, t_{max}, t_{half} for pregnant women. Comparison between these parameters during pregnancy and the parameters after pregnancy using mixed model analysis . C_{trough} in pregnancy should be above the minimal effective plasma concentration. Cord blood/maternal blood concentration ratio at delivery. Determination of half life in infants after in utero exposure if applicable (washout half life). Breastmilk/maternal plasma ratio in case of breastfeeding and exposure in child if breastfeeding. Secondary endpoints: Description of maternal viral suppression during pregnancy and postpartum. Description of infection status of infants at birth. Description of SAEs and AEs reported during pregnancy and postpartum.

Study Design:	This is a non-randomized, open-label, parallel-group, multi-center phase-IV study in HIV-infected pregnant women recruited from HIV treatment centers in Europe.
Number of Subjects Planned:	16 per agent
Target Population:	HIV-infected pregnant women who have an indication for treatment with a cART regimen containing a newly developed antiretroviral agent for which there is insufficient data available on the pharmacokinetics during pregnancy. The selection of the antiretroviral agents is at the discretion of the treating physician.
Duration of the study:	Approximately 14 weeks: At least two weeks prior to Week 33 (screening) of pregnancy up to approximately 6 weeks post-partum. For the raltegravir once daily sub-study and dolutegravir regimen, the duration will be appr. 26weeks (week 20 of pregnancy to post-partum). For the cabotegravir/rilpivirine LA regimen the duration will be 9 months of pregnancy until maximally 6 months after delivery (postpartum curve over a period pf 2 months after the first or second injection after delivery).
Diagnosis and Main Eligibility Criteria:	A maximum of 16 (evaluable) female subjects per agent Inclusion criteria: 1. HIV-infected as documented by positive HIV antibody test and confirmed by an antigen test. 2. Subject is at least 18 years of age at screening. 3. Subject is able and willing to sign the Informed Consent Form prior to screening evaluations. 4. Treated with a cART regimen containing at least one agent which is mentioned in Appendix 1; this agent has been taken for at least 2 weeks before the day of first PK curve evaluation. 5. Subject is pregnant 6. Subject is able to adhere to food intake recommendations, if applicable. Exclusion criteria: 1. Relevant history or current condition that might interfere with drug absorption, distribution, metabolism or excretion. 2. Inability to understand the nature and extent of the study and the procedures required. 3. Presence of grade III/IV anemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL). 4. Using oral cabotegravir/rilpivirine.

Study Procedures/ Frequency:	*Pharmacokinetics*	In the second (raltegravir QD sub-study and dolutegravir regimen only, preferably at week 20), third trimester (preferably gestational age Week 33) and at at least 2 weeks post partum (preferably before 6 weeks) blood samples (6 mL) will be taken for measurement of plasma drug concentration on T=0 (prior to dosing), and T= 0.5, 1, 2, 3, 4, 6, 8, 12 and 24h (24h sample only in case of QD regimen) post-dosing (9 or 10 samples). In case of the treatment with atazanavir/r 300/100mg QD an additional trough blood sample will be taken 12-16 weeks post partum. At delivery (if possible) a cord blood sample will be taken and at the same time a blood sample from the mother will be taken. In case the infant needs post-exposure prophylaxis with at least one of the agents or for the raltegravir QD sub-study, in Appendix 1 sparse PK sampling is optional. If the mother decides to breastfeed, also breast milk samples at 2, 4, 6, 12 and 24 hours after dosing will be collected on the postpartum PK day. For cabotegravir/rilpivirine regimen: Maternal PK curve after the injection from 24 weeks gestational age: 5 samples will be drawn: one prior to injection, and at day 3, 7, 28 and 56 (in case of 2 monthly injections). Maternal PK curve after the first or second injection postpartum (at least 4 weeks after delivery). 5 samples will be drawn: one prior to injection, and at day 3, 7, 28 and 56 (in case of 2 monthly injections). If the mother decides to breastfeed, also breast milk samples at the same postpartum timepoints and one infant blood sample (extra consent) will be collected on the postpartum PK day. Additionally a maternal blood sample will be taken at a maximum of 7 days prior to each injection during pregnancy (after informed consent and inclusion screening).

	Efficacy	
	Viral load and CD4 count	At screening and on the day of PK evaluation. Collection HIV status of infant(s).
	Safety	
	Medical history, physical examination	At screening.
	Laboratory safety	Biochemistry and hematology evaluation, urinalysis, at screening, and during the day of PK evaluation
	Pharmacogenetics	Raltegravir QD sub-study only: UGT metabolism status
	Body weight	At the day of PK evaluation
	Adverse events	From screening until last assessment
	Admission	From T=0 to T=12h during the day of the PK evaluation.
		For cabotegravir/rilpivirine regimen only short visits apply.

Test Products, Dose, and Mode of Administration:	See Appendix 1. All medication is taken according to food instructions and in combination with other antiretroviral agents at the discretion of the treating physician.	
Data analysis:	*Bioanalysis*	Plasma concentrations of antiretroviral agents will be measured in all available samples by means of a validated HPLC-UV or LC-MS/MS methods.
	Pharmacokinetics	AUC0-tau (dosing interval); C_{max}; C_{min}, t_{max}, t_{half} for pregnant women. Comparison between these parameters during pregnancy and the parameters after pregnancy. Cord blood/maternal blood concentration ratio will be determined. In case of breastfeeding: milk/maternal plasma ratio will be calculated and infant exposure will be assessed. Determination of pharmacokinetic parameters in infants if applicable.
	Safety and demographics	Tabulation and descriptive statistics for subject characteristics. Tabulation of adverse events and serious adverse events, physical examination, medical history, body weight, vital signs and biochemistry, hematology and urinalysis data.
	Antiviral efficacy	Frequency table of HIV-1 RNA viral load per time point and HIV-infection status of the infants. Tabulation and descriptive statistics for CD4+ lymphocyte counts.

This study is being performed in compliance with the guidelines of Good Clinical Practice (GCP) and all essential documents are being archived.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Background

It is generally accepted that HIV-infected pregnant women should receive treatment to prevent the transmission of HIV from mother-to-child. There are, however, no formal guidelines what combinations are to be preferred. The panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission state in their most recent version (November 2017, with update 24 December 2019) of their Recommendations for Use of Antiretroviral Drugs in Pregnant HIV-1-Infected Women for Maternal Health *and* Interventions to Reduce Perinatal HIV Transmission in the United States, [1] that:
All pregnant women living with HIV should receive ART, initiated as early in pregnancy as possible, to prevent perinatal transmission regardless of plasma HIV RNA copy number or CD4 T lymphocyte count. Maintenance of a viral load below the limit of detection throughout pregnancy and lifetime of the individual living with HIV is recommended.

Some women may become pregnant and present for obstetrical care while receiving ART for their own health. In these cases, the choice of active drugs with known safety data in pregnancy may be more limited. In general, women who are already on a fully suppressive regimen should continue their regimens. Treatment with efavirenz should be avoided during the first 8 weeks of pregnancy (the primary period of fetal organogenesis) whenever possible. Regarding dolutegravir, in 2018 a large observational study detected a 0.9% risk of neural tube defects (NTDs) in infants delivered by women receiving dolutegravir around conception or early in the first trimester of pregnancy. This was considered a substantial risk relative to 0.1% NTDs observed with other antiretrovirals. This observation led to a recommendation at that time that dolutegravir should only be used in adolescent girls and women of child-bearing potential together with consistent and reliable contraception. Now, in 2022, more data are available: Human experience from a birth outcome surveillance study in Botswana shows a small increase of neural tube defects; 10 cases in 5,860 deliveries (0.11%; 95% CI 0.06%, 0.19%) to mothers taking dolutegravir-containing regimens at the time of conception compared to 25 cases in 23,664 deliveries (0.11%: 95% CI

0.07%, 0.16%) to women exposed to non-dolutegravir regimens at the time of conception. [2]

The incidence of neural tube defects in the general population ranges from 0.5-1 case per 1,000 live births (0.05-0.1%). Most neural tube defects occur within the first 4 weeks of embryonic development after conception (approximately 6 weeks after the last menstrual period). If a pregnancy is confirmed in the first trimester while on dolutegravir, the benefits and risks of continuing dolutegravir versus switching to another antiretroviral regimen should be discussed with the patient taking the gestational age and the critical time period of neural tube defect development into account.

Data analysed from the Antiretroviral Pregnancy Registry do not indicate an increased risk of major birth defects in over 600 women exposed to dolutegravir during pregnancy but are currently insufficient to address the risk of neural tube defects.

This led to the following guidance:

Women of childbearing potential (WOCBP) should be counselled about the potential risk of neural tube defects with dolutegravir (see below), including consideration of effective contraceptive measures. If a woman plans pregnancy, the benefits and the risks of continuing treatment with dolutegravir should be discussed with the patient.[3]

[4]:

PANNA investigators should adhere to these recommendations. In Germany, after consultation with the ethics committee and national authorities, no patients on dolutegravir will be included (since September 2018).

The use of the darunavir/cobicistat combination in pregnancy was associated with an average reduction in plasma darunavir concentrations of approximately 50% in pregnancy compared to postpartum, with concentrations in some individual pregnant women reduced by as much as 90%. Low darunavir exposure has been associated with an increased risk of treatment failure and may therefore increase the risk of HIV transmission to the infant. As a result, the FDA and EMA recommend that darunavir/cobicistat should not be initiated during pregnancy and to switch women becoming pregnant while using darunavir/cobicistat to an alternative regimen, for instance darunavir/ritonavir.[5] Because of the availability of an alternative (darunavir/ritonavir), this warning led to closure of the darunavir/cobicistat arm in PANNA.

The use of the elvitegravir/cobicistat combination in pregnancy was associated with an average reduction in plasma elvitegravir concentrations of approximately 44% in pregnancy compared to postpartum, with concentrations in some individual pregnant women reduced by as much as 89%. Low elvitegravir exposure has been associated with an increased risk of treatment failure and may therefore increase the risk of HIV transmission to the infant. As a result, the FDA and EMA recommend that elvitegravir/cobicistat should not be initiated during pregnancy and to switch women becoming pregnant while using elvitegravir/cobicistat to an alternative regimen.[5] This warning led to closure of the elvitegravir/cobicistat arm in PANNA.

Other HIV-infected women may not be receiving ART at the time they present for obstetrical care. Some women have never received ARV drugs, while others may have taken ARV drugs for treatment that was stopped or for prophylaxis to prevent perinatal transmission of HIV in prior pregnancies or for pre- or post-exposure prophylaxis. Considerations for initiating therapy in pregnant women differ, depending upon whether ARV drugs are currently indicated for maternal health or solely for fetal protection.
Initiation of treatment should be done as early as possible. Delayed initiation of prophylaxis until after the first trimester of pregnancy can be considered in women who are receiving ARV drugs solely for prevention of perinatal transmission, but earlier initiation of prophylaxis may be more effective in reducing perinatal transmission of HIV.
Continue treatment *intra partum* and *post partum* (in woman not requiring cART treatment for their health: evaluate need for continuing the combination regimen postpartum. Following delivery, considerations for continuation of the mother's ARV regimen are the same as for other nonpregnant individuals.

Efavirenz should be avoided (in the first trimester, at least the first 8 weeks of pregnancy) due to its potential teratogenicity, according to the package insert. Avoid combination of d4T/ddI, due to its suspected adverse potential for the mother (lactic acidosis).Nevirapine use has been associated with an increased risk for rash and/or hepatotoxicity: however, NVP treatment should be continued if the patient is virologically suppressed and tolerating the regimen.

The (relative) contra-indications for the NNRTIs during pregnancy make a PI based regimen or an Integrase Strand Transfer Inhibitor (INSTI) regimen the most rational choice.
There are, however, a number of clinical scenarios, where a treating physician needs to consider alternative medications. Most relevant is the presence of NRTI, NNRTI, PI or INSTI mutations due to previous failure of ARV regimens or the transmission of resistant virus. In such situations, physicians may consider the use of alternative antiretroviral agents for which there may not be extensive experience during pregnancy.

One of the unanswered questions in such a situation is the optimal dose of an antiretroviral agent during pregnancy. It is well-known that pregnancy may induce changes in the pharmacokinetic profile, for instance by an increase of the volume of distribution, increased hepatic blood flow, reduced protein binding, affecting metabolism (CYP450 expression is variable) or reduced absorption of the gastro-intestinal tract. Most of these physiological changes result in lower exposure during pregnancy, especially in the third trimester [6].

Some agents should preferably not be used during pregnancy due to teratogenicity (efavirenz, dolutegravir). However, efavirenz or dolutegravir could accidentally be used during pregnancy (even the first trimester), because the woman gets pregnant unplanned. British Guidelines [7] state: As a general principle, the mother is encouraged to continue on the cART regimen she conceived on (if she has a undetectable viral load on this regimen), even if it contains efavirenz. Therefore, we included efavirenz to the list of agents to be investigated in the UK and Ireland only. Women starting dolutegravir after the first trimester of pregnancy, or who became pregnant on dolutegravir by accident and were not switched to another regimen, may be included in the protocol. These women are advised to use adequate contraception during dolutegravir use after delivery, or switch to another regimen after pregnancy to avoid dolutegravir use around possible new conception.

For the agents under study in this protocol pharmacokinetic data during pregnancy are not available or limited [8].

To guide physicians how to dose these agents in case they need this agent in an HIV pregnant woman, we want to evaluate the pharmacokinetics of these agents in this patient population.As this is an ongoing project, which started in 2008, pharmacokinetic data from several antiretroviral agents from the initial list have been collected and published [9-14]. On the other hand, new antiretroviral agents came on the market and have been added to the list. Raltegravir is one of the preferred agents to be used during pregnancy as the 400mg BID dose. Merck has developed a once-daily regimen: 1200mg once daily. A new tablet formulation has been developed for this regimen: 600mg tablet. This tablet has shown to be non-inferior to the standard 400-mg twice-daily dose at 48 weeks in antiretroviral-naive patients in the international ONCEMRK trial [15]. For this regimen no data of pregnancy influence on pharmacokinetics are available.
In the near future women will become pregnant while using cabotegravir/rilpivirine long acting combination therapy for HIV, and potentially these women opt to breastfeed their babies. Very limited data are available regarding pharmacokinetic changes in pregnancy for these drugs and we have no information on the placental transfer of cabotegravir or breastmilk transfer of both drugs. And if cabotegravir passes the placenta, the newborn is expected to be exposed to cabotegravir until appr. 5-7 days after birth.

In general there is a call for action to generate pharmacokinetic and preliminary safety data of antiretrovirals in pregnancy during the clinical development phase, and at least as soon as possible thereafter. FDA and EMA organized workshops in 2020 and WHO in cooperation with IMPAACT recently organized a workshop to discuss enrolment of pregnant women in registrational studies with new antiretrovirals.
For oral rilpivirine we know that pregnancy affects rilpivirine levels to trough levels that are very near to the lowest effective concentration. Recently rilpivirine exposure in pregnancy after monthly IM dosing was reported for 3 cases [16], however, all patients stopped rilpivirine IM treatment after pregnancy was confirmed. Therefore, only the tail could be investigated. For these women, plasma concentrations seemed not to be different from non-pregnant women, however, the data are limited and do not permit full assessment of the pregnancy effect. The same is true for cabotegravir: 3 cases, PK tail only and monthly injections [17] .

If the rilpivirine and/or cabotegravir concentrations drop in pregnancy, especially in the once in 2 months injection regimen, recommendations for monthly dosing could be a result.
Furthermore it is important to know cabotegravir and rilpivirine exposure in the newborn and if and how long the newborn is exposed to potentially effective amounts of rilpivirine and cabotegravir. It might be long and high enough to be used as, or add to perinatal prophylaxis, this is speculative, but the study will provide information on newborn exposure.

According to (European) peri-natal guidelines a 4-week neonatal component of the zidovudine chemoprophylaxis regimen is generally recommended for HIV-exposed neonates when the mother has received standard combination antiretroviral therapy (cART) during pregnancy with consistent viral suppression to reduce perinatal transmission of HIV. Newborns at higher risk of HIV acquisition should receive a combination ARV regimen (ARV prophylaxis or empiric HIV therapy based on clinician assessment of risk).
The optimal prophylactic regimen for newborns of women with resistant virus is unknown. The use of ZDV in combination with other antiretroviral drugs effective in the mother may be used in the newborns, depending on the availability of dosage formulation suitable for dosing in infants. However, the efficacy of this approach for prevention of transmission has not been proven. Generally there is a lack of data of pharmacokinetics of antiretroviral agents in neonates. If the infants of mothers in this study have to be treated with an agent under study it will be very useful for the treatment of infants in the future to collect pharmacokinetic data from the child as well.
Recently women living with HIV are given the option to breastfeed their children, under certain circumstances. For example, UK (BHIVA) guidelines report: In the UK and other high-income settings, the safest way to feed infants born to women with HIV is with formula milk, as there is on-going risk of HIV exposure after birth. We therefore continue to recommend that women living with HIV feed their babies with formula milk (but see also section 9.4.4). Section 9.4.4 states: Women who are virologically suppressed on cART with good adherence and who choose to breastfeed should be supported to do so, but should be informed about the low risk of transmission of HIV through breastfeeding in this situation and the requirement for extra maternal and infant clinical monitoring.

When a woman decides to breastfeed, she and her infant should be reviewed monthly in clinic for HIV RNA viral load testing during and for 2 months after stopping breastfeeding. Maternal cART (rather than infant pre-exposure prophylaxis [PrEP]) is advised to minimise HIV transmission through breastfeeding and safeguard the woman's health [18]. Similar guidances are in place for the Netherlands (PHON) [19] and Suisse [20]
For most antiretrovirals passage into breastmilk is unknown. Therefore, if a mother decides to breastfeed and is using at least one of the compounds mentioned in appendix 1, we will collect breastmilk, next to maternal plasma at the postpartum visit (and a blood sample of the infant for cabotegravir/rilpivirine LA regimen, after extra informed consent).

1.2 Rationale for the Current Study

Due to the potential for pregnancy-induced changes in the pharmacokinetics of medication, one cannot assume that the currently licensed doses of the medication to be tested under this protocol lead to adequate exposure in an HIV-infected pregnant woman. For the agents under study no or limited pharmacokinetic data during pregnancy are available. As the changes in pharmacokinetics during pregnancy are most prominent in the third trimester a pharmacokinetic curve will be recorded in the third trimester after attaining steady state. In case there is no significant effect on the pharmacokinetics during the 3rd trimester, it can be assumed there is also no effect in the 2nd or 1st trimester. In case a significant effect, in particular a reduction, is seen in the plasma concentrations of one or more of the agents in the 3rd trimester, a follow-up study may be indicated to investigate the pharmacokinetics of these agents also during the 1st or 2nd trimester and/or to investigate an adjusted dose of the agents affected.

To reduce the variability the pharmacokinetic curves in the third trimester will be compared to the curves recorded after delivery in the same women (intra-subject comparison). Females receiving the medication mentioned in Appendix 1 are in most cases receiving medication for the treatment of their HIV-infection, not only to prevent mother to child transmission of the HIV-virus. Therefore it is likely that the women will continue this treatment after delivery.
To determine the placenta passage of the antiretroviral agents under study a cord blood sample will be taken at delivery (if possible). Furthermore, there is a lack of data of pharmacokinetics of antiretroviral agents in neonates. For some new antiretrovirals

washout samples from the neonate will be collected (i.e. assess infant exposure through the mother). If the infants of mothers in this study have to be treated with an agent under study it will be very useful for treatment of neonates in the future to collect pharmacokinetic data from the child as well. In regular care neonates with a chance on HIV-infection will regularly be checked for HIV-RNA in the blood. PK samples will be taken at the time points of blood collection for this purpose or if blood is collected for laboratory safety (for regular care).
As breastfeeding is expected to become more common in the European setting, transfer of antiretrovirals into breastmilk will be assessed, as well as infant exposure if a woman, using at least one of the compounds mentioned in appendix 1, decides to breastfeed her infant.

The agents under study in this protocol are not first-line agents in the recommendations for the treatment of HIV-infected pregnant women. There may be, however, clinical scenarios in which a treating physician may need to prescribe one of the agents, and then the optimal dose during pregnancy should be known.

Recently women living with HIV are given the option to breastfeed their children, under certain circumstances. For most antiretrovirals passage into breastmilk is unknown. Therefore, if a mother decides to breastfeed and is using at least one of the compounds mentioned in appendix 1, we will collect breastmilk, maternal plasma and infant plasma (for cabotegravir/rilpivirine regimen only) at at least one visit they have to report to the hospital for viral load checks (generally done monthly during breastfeeding period).

The rationale for the current study is thus to evaluate the pharmacokinetics of agents with unknown (or unclear) pharmacokinetic profiles during pregnancy when their use is indicated in pregnant women by the treating physician.

2 OBJECTIVE

The primary objective of this study is to describe the pharmacokinetics of antiretroviral agents, for which no or limited available pharmacokinetic data during pregnancy is available, in the 3rd trimester of pregnant HIV-infected women and at post-partum. And in the 2nd trimester for raltegravir once-daily regimen and dolutegravir regimen.

In addition, the pharmacokinetics will be determined in the infant as well in case of post-exposure prophylaxis with one of the agents tested (not performed in Germany).

Secondary objective of this study is to describe the safety of the antiretroviral agents during pregnancy and the efficacy in terms of viral load response of the mother and prevention of mother to child transmission. To describe breastmilk transfer in case of breastfeeding and assess exposure in child if breastfeeding.

For the raltegravir once-daily regimen the following additional objectives apply:

To describe pharmacokinetics in the infant after birth a time points of blood sampling for regular care (not performed in Germany).

To describe the safety of the antiretroviral agents, in this case specifically the reformulated once-daily raltegravir tablets, during pregnancy and the efficacy in terms of viral load response of the mother and prevention of mother to child transmission.

To investigate the relation between progesterone levels and raltegravir pharmacokinetic changes in pregnancy.

For the cabotegravir/rilpivirine LA regimen, the following additional objectives apply:

To describe pharmacokinetics over the duration of pregnancy by trough samples prior to each injection.

To describe pharmacokinetics in the infant after birth a time point of blood sampling for regular care.

To describe breastmilk transfer in case of breastfeeding for cabotegravir/rilpivirine and exposure in child if breastfeeding.

The study endpoints are:

Primary endpoints:

$AUC_{0\text{-}tau}$; C_{max}; C_{trough}, t_{max}, t_{half} for pregnant women. Comparison between these parameters during pregnancy and the parameters after pregnancy using mixed model analysis .

C_{trough} in pregnancy should be above the minimal effective plasma concentration.

Cord blood/maternal blood concentration ratio at delivery.

Determination of half life in infants after in utero exposure if applicable (washout half life).

Breastmilk/maternal plasma ratio in case of breastfeeding and assessment of exposure in child if breastfeeding.

Secondary endpoints:

Description of maternal viral suppression during pregnancy and postpartum.

Description of infection status of infants at birth.

Description of SAEs and AEs reported during pregnancy and postpartum.

3 STUDY DESIGN

3.1 Overall Study Design

This is a non-randomized, open-label, parallel-group, multi-center phase-IV study in HIV-infected pregnant women recruited from HIV treatment centers in Europe.

Patients treated with one or more of the agents listed in Appendix 1 during pregnancy will be screened and the PK evaluation will take place in the second (preferably week 20) for raltegravir QD sub-study and dolutegravir regimen, third trimester (preferably Week 33) of the pregnancy, representing the 2nd, 3rd trimester and at least 2 weeks post-partum (if medication use continues after delivery). Patients will use a cART regimen containing one or more of the agents mentioned in Appendix 1 during pregnancy (at least two weeks prior to the first PK evaluation) and treatment is expected to continue post-partum. If possible a cord blood sample and matching maternal blood sample will be taken at delivery. Breastmilk and maternal blood (and infant blood in case of cabotegravir/rilpivirine LA, and after extra informed consent) will be taken at at least one postpartum visit, if the mother is breastfeeding. Safety and antiviral efficacy (including MTCT) will be evaluated too.

3.2 Rationale for Study Design

See also section 1.2 of this protocol.
For the ARVs under study no or limited data on pharmacokinetic behavior during pregnancy are available. The ARVs under investigation are not the first choice ARVs to be used during pregnancy, therefore we only include women who need to use the selected ARVs according to their treating physician. As the number of pregnant patients using these ARVs is sparse, sufficient patients can only be enrolled performing this study as a multi-center study. To minimize the variability the patients will be their own control (PK curve in the second (for raltegravir QD sub-study and dolutegravir regimen), third trimester and post partum).

The PK sample times are identical for all agents tested, except for the extra sample at 24h post dosing for the agents with a QD regimen and for the cabotegravir/rilpivirine LA

regimen as the half life is much longer than other oral ARVs. The T_{max} of the agents taken orally ranges from 1-5 hours, with most of the agents showing the peak plasma concentrations at appr. 2 hours after dosing.

For breastmilk collection: this will be done at a postpartum. The ratio between milk and maternal plasma will be determined and the exposure of the infant will be extrapolated to infant dosages. This will be done for minimally one sample, or preferably simultaneous milk sampling to plasma sampling when the woman attends for the PK curve during the postpartum period (only one infant blood sample will be collected for cabotegravir/rilpivirine LA regimen only, and after extra informed consent).

4 SUBJECT POPULATION

4.1 Number of Subjects and Subject Selection

A maximum of 16 evaluable subjects per agent will be included in this study. For an agent with more than one possible dosing regimen PK analysis will be performed after 16 patients have been included on the agent (all dosing regimens together). If deemed necessary more patients on a certain regimen can be included after this analysis. If one subject uses more than one agent from the list in Appendix 1, this subject will be included for all agents taken.

4.2 Inclusion Criteria

Subjects must meet *all* of the following inclusion criteria to be eligible for participation in this study.

1. HIV-infected as documented by positive HIV antibody test and confirmed by an antigen test.
2. Subject is at least 18 years of age at screening.
3. Subject is able and willing to sign the Informed Consent Form prior to screening evaluations.
4. Treated with a cART regimen containing at least one agent which is mentioned in Appendix 1; this agent has been taken for at least 2 weeks before the day of first PK curve evaluation.
5. Subject is pregnant.
6. Subject is able to adhere to food intake recommendations, if applicable.

4.3 Exclusion criteria:

Subjects who meet one or more of the following criteria cannot be selected:

1. Relevant history or current condition that might interfere with drug absorption, distribution, metabolism or excretion.
2. Inability to understand the nature and extent of the study and the procedures required.
3. Presence of grade III/IV anemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL).
4. Using oral cabotegravir/rilpivirine.

4.4 Prohibitions

Subject may not use grapefruit or Seville oranges containing food or juice from 7 days before the PK blood sampling days until the last sample taken.

4.5 Removal of Subjects from Therapy or Assessment

A subject may decide to withdraw from the study at any time. If so, the Investigator must be informed immediately. The Investigator may decide to terminate participation of a subject if: it is difficult to obtain blood samples; there has been a violation of the protocol; if a serious adverse event occurs or if it is in the best interest of the subject that she is withdrawn. If there is a medical reason for withdrawal the subject will remain under the care of the Investigator until the problem prompting withdrawal has been resolved or until referral to her General Practitioner. On the basis of the occurrence of adverse events, the Investigator may decide to discontinue the study.

4.6 Replacement of Subjects

The following definitions apply:
Screening failure: subject that does not comply with the in- and exclusion criteria at some point before the first pharmacokinetic curve taken.
Entered/included: first pharmacokinetic sample taken.
Dropout: subject withdrawn from the study after collection of at least one pharmacokinetic blood sample.

Replacement occurs as follows:
Screening failures: are replaced with a new subject.
Dropout: in general, included subjects (for example withdrawn from the study by the Investigator on ground of adverse events) that had at least one curve taken will not be replaced.

4.7 Procedure for Dropout patients

To minimise the number of dropouts after delivery the following procedure will be followed: a patient will be contacted three times, these attempts will be documented. If a patient withdraws her participation, that will be documented. The study staff will ask for

permission to report the relevant safety information of the infant in the study documents. This will contain: date of birth, body weight at birth, gender, results PCR test and whether congenital abnormalities occurred.

If a patient cannot be reached by phone for the postpartum visit and the study staff has access to the delivery information, the same information will be collected.

5 STUDY DRUGS

5.1 Overview

Subjects will be included if their cART contains at least one of the agents mentioned in Appendix 1 of this protocol. Subjects will not be switched to this medication to participate in this study. All medication is taken according to food instructions and in combination with other antiretroviral agents at the discretion of the clinical physician. Females receiving the medication mentioned in Appendix 1 are in most cases receiving medication for the treatment of their HIV-infection, not only to prevent mother to child transmission of the HIV-virus. Therefore it is likely that the women will continue this treatment after delivery.
Some of the compounds on the list in Appendix 1 might not be available in all countries participating in the PANNA study. The study will be opened for these compounds from public availability onwards. No medication will be provided by the sponsor.

Prior to the first pharmacokinetic curve taken the subjects should have used the medication (on a stable dose) for at least two weeks.

If the newborn needs post-exposure prophylaxis with one of the agents listed in Appendix 1 sparse pharmacokinetic blood sampling of the child will be proposed.

5.2 Identity of Study Drugs

Agents mentioned in Appendix 1, licensed in the various countries participating in this study. Some of the compounds on the list in Appendix 1 might not be available in all countries participating in the PANNA study. The study will be opened for these compounds from public availability onwards. No medication will be provided by the sponsor. All agents will be provided to the patients by their community pharmacist. Batch numbers and expiry dates of the medication used on the PK blood sampling day will be reported.

5.3 Method of Assigning Subjects to Treatment Groups

Randomization is not applicable in this study. The Department of Pharmacy of the Radboud university medical center will maintain an inclusion list and provide the CRF numbers to the sites.

5.4 Selection and Timing of Dosing and Dietary

The treatment is regular care treatment of patients needing cART for the treatment of HIV infection or PMTCT. The dosages of the drugs are the standard dosages in clinical use. Dosages prescribed will be reported.

On the PK blood sampling days the morning dose will be taken at the study center. The time point of the last dose taken prior to the dose on the sampling day will be recorded. Food intake recommendations for the specific drugs will be followed; in the CRF food recommendations per agent will be provided. The medication will be taken 5 minutes after the start of the breakfast (if applicable) for oral medication.
For cabotegravir/rilpivirine LA regimen the time (if related to PK curve) and dose of injection and the needle length used will be reported for every injection during pregnancy and postpartum when in the study. Also the date of the injection prior to inclusion in PANNA will be reported. No food restrictions apply.

5.5 Blinding

As this is an open study, blinding procedures are not applicable.

5.6 Prior and Concomitant Therapy

Medication will be taken in combination with other antiretroviral agents at the discretion of the clinical (treating) physician. Use of non-ARV concomitant medication will be done according to SPC at the discretion of the clinical (treating) physician. For two weeks preceding the pharmacokinetic curve and during the entire study, all concomitant medication will be recorded in the subject's Case Record Form (CRF).

5.7 Treatment Compliance

The subjects will be asked for their adherence during the last two weeks prior to the pharmacokinetic curves. No pill count will be performed, as the treatment is part of normal clinical care. The time point of oral medication intake the day before the PK blood sampling day will be recorded and the medication intake on the PK blood sampling day will take place at the study centre.

6 STUDY PROCEDURES

6.1 Inclusion Screening

Inclusion screening will be done if possible within 4 weeks prior to the first pharmacokinetic curve (or sample in case of cabotegravir/rilpivirine LA regimen) taken. To minimize the number of extra visits for the patient, screening can be done on the first PK collection day, provided that the in- and exclusion criteria are met, especially recent haemoglobin levels should be available:

At inclusion screening the following will be performed and documented, to establish eligibility for inclusion:

1. Medical history, including hepatitis infections and registered resistance, smoking and drug/alcohol habits.
2. Physical examination (including body height and weight).
3. HIV-1 RNA and CD4+ lymphocyte determination according to local procedures. Results of <2 months prior to screening can be used.
4. Serum biochemistry including lipid panel, glucose, hematology evaluation, urinalysis (for details see section 6.4.1).

Subjects meeting all of the inclusion criteria and none of the exclusion criteria will follow the study procedures as detailed in Appendix 3.

6.2 Study Flow Chart

The study flow chart is given in Appendix 3.

Subjects will visit the center for a screening visit and for two PK blood sampling days: third trimester (preferably Week 33) of pregnancy and at least 2 weeks post partum (preferably before 6 weeks). For cabotegravir/rilpivirine subjects will have 5 samples collected over a period of 8 weeks after the injection at at least 24 weeks gestation and postpartum, every visit for injection during pregnancy a trough sample will be collected. If the research site has the facilities in place, these samples can be collected during home visits.

In case of the treatment with atazanavir/r 300/100mg QD an additional trough blood sample will be taken 12-16 weeks post partum, preferably during a routine clinical visit. For the raltegravir once-daily sub-study and dolutegravir regimen an additional pharmacokinetic curve will be taken in the second trimester of pregnancy, if start of, or switch to once-daily treatment started in the second trimester.
In case the infant is included for PK blood sampling, extra visits will take place in Week 1 and 3 post partum. At delivery (if possible) a cord blood sample will be taken and at the same time a blood sample from the mother will be taken.
For the raltegravir once-daily sub-study, after birth 1mL samples will be collected from the newborn for pharmacokinetic purposes at time points blood is collected for regular care, with a maximum of 3 samples and up to maximally 21 days after birth. Bilirubin levels will be reported, as monitored in regular care for all children.

During the entire study, a total volume of appr. 270 mL of blood (depending on dosing frequency: BID or QD and the blood volume to be collected for laboratory safety and efficacy per center) will be sampled from the mother. This volume includes all samples for virological efficacy, safety and pharmacokinetics. In case the infant is included for PK blood sampling a maximum of 3 mL of blood will be sampled from the infant.

6.3 Blood Sampling for Pharmacokinetics, HIV-1 RNA and CD4

For the determination of the plasma concentrations of the agents mentioned in the list in Appendix 1, except for cabotegravir/rilpivirine LA regimen (and their metabolites if applicable), blood samples of 6 mL to obtain appr. 3 mL of plasma will be collected in EDTA hard plastic tubes (**without gel**) just before drug intake (pre-dose) and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24h (24h sample only in case of QD regimen) post-dosing (9 or 10 samples) in the second trimester (raltegravir once-daily sub-study and dolutegravir regimen), third trimester (preferably in Week 33 GA) and at at least 2 weeks post partum (preferably before 6 weeks). In case of the treatment with atazanavir/r 300/100mg QD an additional trough blood sample will be taken 12-16 weeks post partum.
At delivery (if possible) a cord blood sample will be taken and at the same time a blood sample from the mother will be taken. The umbilical cord will be clamped (if possible within 1 min) after delivery of the infant. The cord will be wiped free of any maternal blood and cleaned with alcohol followed by betadine. After clamping, a sample of cord blood (6

ml) will be collected from the umbilical vein in a syringe as soon as possible after delivery (before delivery of the placenta) and will be transferred into an EDTA hard plastic tube (without gel).
If extra informed consent was given for cabotegravir and rilpivirine also a (heelstick) blood sample will be taken from the newborn after birth.
The exact times of sampling will be recorded in the case report forms.

In case the newborn needs post-exposure prophylaxis with (at least) one of the agents mentioned in Appendix 1, sparse PK blood sampling will be performed at time points when blood sampling for regular care (determination of HIV infection (viral load) or laboratory safety) is being performed (if both legal representatives agree). The proposed blood sampling time points are: in Week 1, 3 and between 4 and 6. One blood sample (1 mL) will be taken by venepuncture per collection day. The body weight of the child and the time point of dosing and blood sampling will be recorded.

For the raltegravir once-daily sub-study, after birth 1mL samples will be collected from the newborn for pharmacokinetic purposes at time points blood is collected for regular care, with a maximum of 3 samples and up to maximally 21 days after birth.

If the mother decides to breastfeed, also breast milk samples after 0 (expression before dosing), 2, 4, 6, 12 and 24 hours after dosing will be collected on the postpartum PK day. For pharmacokinetic analysis of milk, samples of whole breast milk will be collected from both breasts with the use of a personal electric pump. After the milk is collected, it is gently mixed and the necessary aliquots (a minimum of 10 mL) for assay will be saved using proper storage methods. The timing of sampling relative to dose and the total volume of the sample will be reported in the case report form (CRF).Samples will be stored at ≤ -80°C until shipment to the laboratory in Nijmegen. The rest of the milk can be used to feed the baby.

On the pharmacokinetic sampling day an indwelling Venflon® I.V. cannula will be inserted in a peripheral vein of each subject by a physician or an authorised nurse to facilitate repeated blood sampling. The cannula will be removed at the latest prior to the end of

the 12 hours period of PK sampling. The subsequent blood sample(s) will be drawn by venepuncture.
After each blood sample collection, the cannula will be flushed with a 0.9% NaCl solution in order to maintain patency. Before each blood sample collection, approximately 1 mL blood will be discarded. The blood samples will be mixed and stored in the refrigerator immediately. Blood samples will be centrifuged at about 2500 g (3800 rpm for a normal laboratory centrifuge) for 10 minutes preferably at 4°C. Plasma will be divided and transferred to labeled polypropylene tubes (supplied by UMC Nijmegen) and stored at ≤ -18°C at the local analytical laboratory. Samples will be shipped in frozen condition (dry-ice) to the analytical laboratory of the UMC Nijmegen unless the local analytical laboratory can demonstrate acceptable accuracy according to external QC program (KKGT).

For cabotegravir/rilpivirine LA regimen:

Blood samples of 6 mL to obtain appr. 3 mL of plasma will be collected in EDTA hard plastic tubes (**without gel**).

- Maternal PK curve after the injection from 24 weeks gestational age: 5 samples will be drawn: one prior to injection, and at day 3, 7, 28 and 56 (in case of 2 monthly injections). 5 samples will be drawn: one prior to injection, and at day 3 (+/- 2 days), 7 (+/- 2 days), 28 (+/- 7 days) and 56 (+/- 7 days, but before the next injection). The 28 days sample will be taken a maximum of 7 days before the next injection in case of monthly dosing, the 56 days sample is not applicable in that cases. The 56 days sample will be taken a maximum of 7 days before the two months injection, in the 2 monthly injection regimen.

- Maternal PK curve after the first or second injection postpartum (at least 4 weeks after deliv-ery). 5 samples will be drawn: one prior to injection, and at day 3 (+/- 2 days), 7 (+/- 2 days), 28 (+/- 7 days) and 56 (+/- 7 days, but before the next injection). The 28 days sample will be taken a maximum of 7 days before the next injection in case of monthly dosing, the 56 days sample is not applicable in that cases. The 56 days sample will be taken a maximum of 7 days before the two months injection, in the 2 monthly injection regimen.

- If the mother decides to breastfeed, also breast milk samples at the same postpartum timepoints and one infant blood sample (extra consent) will be collected on the postpartum PK days, procedure see above.

- Additionally a maternal blood sample will be taken prior to each injection during pregnancy (after informed consent and inclusion screening).

- Maternal and cord blood samples will be collected at delivery (if possible).

- A minimum of two and maximum of four blood samples (1 mL) of the infant will be taken up to a maximum period of 7 days after birth at a moment blood collection will take place in standard of care, for infants that are not breastfed.

The blood samples will be mixed and stored in the refrigerator immediately. Blood samples will be centrifuged at about 2500 g (3800 rpm for a normal laboratory centrifuge) for 10 minutes preferably at 4°C. Plasma will be divided and transferred to labeled **amber** polypropylene tubes (supplied by UMC Nijmegen) and stored at ≤ -40°C at the local analytical laboratory. Samples will be shipped in frozen condition (dry-ice) to the analytical laboratory of the UMC Nijmegen unless the local analytical laboratory can demonstrate acceptable accuracy according to external QC program (KKGT).

6.3.1 Pharmacogenetics – raltegravir once-daily sub-study only

If consented by the subject, a 6 mL EDTA blood sample will be collected for UGT genotype determination.

6.3.2 HIV-RNA and CD4+ lymphocyte measurements

HIV-1 RNA and CD4+ lymphocyte measurements are performed at screening (results of <2 months before screening are acceptable) and at each pharmacokinetic blood sampling day (for cabotegravir/rilpivirine LA regimen on the first day of the PK curves, and all maternal (and infant if applicable) viral loads collected in regular care during the trial period are reported). Blood samples for determination of CD4+ lymphocytes and HIV-

1 RNA will be taken according to local standard procedures. Samples will be transferred to the local laboratory.

6.4 Safety Assessments

6.4.1 Biochemistry, Hematology, and Urinalysis

Blood samples for serum biochemistry including glucose, hematology and a urine sample for urinalysis will be taken at screening and at each PK sampling day (pre-dose). The blood samples will be taken according to local standard procedures. If at delivery serum biochemistry, hematology urinalysis, CD4+ lymphocyte count and/or viral load is determined for regular care, the results will be reported in the CRF. No blood sample will be taken for the study.

All laboratory test results will be reviewed and signed by the (Sub-)Investigator. All laboratory results will be filed as source documents at the clinical site.

6.4.1.1 Serum biochemistry

Venous blood samples will be taken for the determination of ASAT, ALAT, gamma-GT, alkaline phosphatase, creatinine kinase, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, creatinine, glucose, albumin and α-1-acid glycoprotein (if available). For the raltegravir once-daily sub-study, during each visit a sample will be taken for progesterone analysis (2 mL sample dry tube with gel). Samples will be stored at room temperature until transfer to the local clinical chemistry lab.
Neonates bilirubin levels will be reported, as monitored in regular care, in case the mother was treated with raltegravir or other UGT1A1 substrate.

6.4.1.2 Hematology

A sample venous blood sample will be collected for hemoglobin, hematocrit, red cell count (RBC), platelet count, total white cell count (WBC), and differential white cell count. Samples will be stored at room temperature until transfer to the local clinical chemistry lab.

6.4.1.3 Urinalysis

A midstream urine sample will be collected for the analysis of glucose, protein, blood, leukocytes, and nitrite. If positive, microscopic examination will be done by the local lab.

6.4.2 Determination of HIV-infection status of infant

The HIV status of the newborn infant will be collected from the treating physician. Infants born from HIV-infected women are tested for HIV-infection in regular care (DNA PCR). The treating physician will be asked to provide the HIV-infection status to the investigator. Also the gender, birth weight and if applicable congenital abnormalities will be collected and relevant laboratory (biochemistry, hematology) parameters related to safety and/or efficacy of the antiretroviral drugs.

6.5 Safety Reporting

Subjects will be monitored for adverse events (AEs) from signing informed consent until the last visit by the medical and nursing staff of the trial centre. Subjects should voluntarily report any AEs or in response to general questioning (e.g. ‘How has your health been since the last visit?’).
All AEs occurring between signing informed consent and the end of the trial will be reported.
ICH GCP requires that both investigators and sponsors follow specific procedures when reporting adverse events/reactions in clinical studies. These procedures are described in this section of the protocol. Section 6.5.1 lists definitions, section 6.5.2 gives details of the institution/investigator responsibilities and section 6.5.3 provides information on Radboud university medical center responsibilities as the sponsor.

6.5.1 Definitions

The definitions of the EU Directive 2001/20/EC Article 2 based on ICH GCP apply in this study protocol. These definitions are given in Table 1:

Table 1 Terms and definitions for adverse events

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a patient or clinical study subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered.
Unexpected Adverse Reaction (UAR)	An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in: The Summary of Product Characteristics (SPC) for that product (for products with a marketing authorisation) The Investigator's Brochure (IB) relating to the study in question (for any other investigational product)
Serious Adverse Event (SAE) **Serious Adverse Reaction (SAR)** **Suspected Unexpected Serious Adverse Reaction** **(SUSAR)**	Respectively, any adverse event, adverse reaction or unexpected adverse reaction that: • results in death • is life-threatening* • requires hospitalisation or prolongation of existing hospitalisation** • results in persistent or significant disability or incapacity • consists of a congenital anomaly or birth defect • other medically important/clinically significant events***

*The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
** Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, including elective procedures that have not worsened (for example planned caesarian section), do not constitute an SAE.
***Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

6.5.2 Institution/Investigator Responsibilities

All non-serious AEs/ARs, whether expected or not, should be reported in the toxicity (symptoms) section of the appropriate CRF. The severity (i.e. intensity) of all AEs/ARs (serious and non-serious) in this study should be graded using the toxicity gradings in Appendix 4.

6.5.2.1 Investigator Assessment

(a) Seriousness

When an AE/AR occurs the investigator responsible for the care of the patient must first assess whether the event is **serious** using the definitions given in Table 2. If the event is serious and not exempt from expedited reporting, then an SAE form must be completed and sponsor notified.

(b) Causality

The investigator must assess the causality of all serious events/reactions in relation to the study therapy using the definitions in Table 2. There are 5 categories: unrelated, unlikely, possible, probable and definitely related. If the causality assessment is unrelated or unlikely to be related the event is classified as a SAE. If the causality is assessed as either possible, probable or definitely related then the event is classified as a SAR.

Table 2 Definitions of causality

Relationship	Description	Event Type
Unrelated	There is no evidence of any causal relationship	SAE
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the study medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatment).	SAE
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the study medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).	SAR
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.	SAR
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.	SAR

An SAE considered related to study participation (e.g., procedures, invasive tests,) even if it occurs during the pre- or post-treatment period, will be reported to the sponsor on an SAE form. The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements relating to the reporting of incidents and near-incidents to regulatory bodies, and the ethics committees.

(c) Expectedness

If the event is a SAR the sponsor must assess the expectedness of the event. If a SAR is assessed as being unexpected it becomes a SUSAR.

(d) Notification

Investigators must notify the sponsor of all SAEs occurring from the time of signature of informed consent until the last protocol assessment. SARs and SUSARs must be notified to the sponsor indefinitely (i.e. no matter when they occur after randomisation).

6.5.2.2 Notification Procedure

The SAE form should be completed by the responsible investigator. The investigator should assess the SAE for the likelihood that that it is a response to study drug or other medication. In the absence of the responsible investigator the form should be completed and signed by a member of the site study team and faxed to the sponsor immediately. The responsible investigator should check the SAE form, make changes as appropriate, sign and then re-fax to the sponsor as soon as possible. The initial report shall be followed by detailed, written reports.

Send the SAE form by email (within 24 hours or next working day) to:

David Burger David.Burger@radboudumc.nl

And

Angela Colbers Angela.Colbers@radboudumc.nl

The sponsor will notify the local ethics committee according to the local requirements.

Follow-up of SAEs: In the case of an SAE the subject must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until the event

has stabilized. Follow-up may continue after completion of protocol treatment if necessary. Follow-up information is noted on another SAE form by ticking the box marked 'follow-up' and faxing to the sponsor as information becomes available. Extra, annotated information and/or copies of test results may be provided separately. The patient **must** be identified by study number, date of birth and initials only. The patient's name **should not** be used on any correspondence.

6.5.3 Sponsor Responsibilities

The sponsor will report the SAEs through the web portal ToetsingOnline to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

The local ethics committees will be notified by the sponsor following the same reporting guidelines:

-SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.

- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.

- A list of all SARs (expected and unexpected) must be reported annually.

If applicable the sponsor will notify the firm producing the ARV on the SUSARs and SARs.

6.5.4 Satellite sites

Satellite sites are sites which will enrol patients, perform screening assessments (including informed consent procedure) and collect 24h PK blood sample of the PK curve, or entire curves in case of cabotegravir/rilpivirine LA regimen. The collection of the blood samples of the pharmacokinetic extensive sampling days will be done by one of the other centres of the study.

6.5.5 Antiretroviral pregnancy registry

Investigators are encouraged to register included subjects with the Antiretroviral Pregnancy Registry (APR) prospectively as soon as possible after the subject starts treatment as part of the study on Day 1, and before the pregnancy outcome (otherwise there is less likelihood of the subject being included in the APR). More information including copies of applicable CRFs and fax numbers are available at www.apregistry.com.

7 DATA ANALYSIS

7.1 Sample Size Calculation

Sample size calculation is based on an expected intrasubject variability of area under the curve (AUC0-tau) of ≤35%. A sample size of 8 (number of pairs) is required to achieve a power of 80% and a level of significance of 10% (2-sided) for detecting a mean of the differences of 30% (for a geometric mean ratio confidence interval [CI] of 0.70–1.43) between pairs and assuming an intrasubject variation of 35%.
To account for possible dropouts, our aim is to include 16 patients.
There is an expected number of pregnancies in HIV-infected women per centre between 5 and 40 per year (communicated data). As the agents under protocol are not frequently used in HIV infected females, this study will be performed in several centers in Europe. The European centres will participate in this study, together treating >2500 HIV infected females with an expected number of 300 pregnancies / year.

7.2 Data Collection and Data Management

All data obtained in the clinical study described in this protocol will be recorded on CRFs or study specific entry forms. Data will be partly collected as source data only, and it will be clearly documented in the Study Documentation file which data will be collected as source data only.
Some data collected in standard care prior to participation can be used to complete screening data. Therefore dates of blood collection (viral load / CD4 lymphocyte count) can be before signature of informed consent.

The database will be constructed by the RadboudUMC Nijmegen. Data from working copies of CRFs, obtained from the Investigator, will be directly entered into the database management system.

The original CRF will be submitted to Data Management after a subject completed the study, including the data corrections (a copy of CRF pages used to collect source data will be provided). To eliminate entry errors and other inconsistencies between the data on the CRF and those in the database, Data Management will validate the data as follows:

by entering numerical data twice into the database (double data entry), and by rereading comment fields, or by single data entry and 10% QC. Remaining queries will be solved on data clarification forms and updating of the database. These forms, signed by the Investigator, together with the original CRFs will complete the raw data set.

7.3 Bioanalysis

RadboudUMC has established or will establish HPLC/UPLC/LC-MS/MS assays for the agents mentioned in Appendix 1 [21]. Raltegravir total and unbound plasma concentrations will be determined in all samples, and the concentration of the main glucuronide metabolite of raltegravir (M2) will also be determined. For cabotegravir and rilpivirine in the LA regimen, also unbound concentrations will be determined in maternal plasma samples. Assays are developed for analysis in breastmilk if applicable. For other highly protein bound ARVs unbound plasma concentrations may be analysed and metabolites will be analysed if relevant and possible (and no additional blood collections are required). The assays will be externally validated by the International Interlaboratory Program for the Quality Control of Therapeutic Drug Monitoring in HIV Treatment [22, 23]. Local analytical laboratories are allowed to measure the plasma drug concentrations as soon as they can demonstrate acceptable accuracy in the KKGT program during the last 2 years prior to participation in the study. Acceptable accuracy is defined as a performance within 80-120% of the nominal concentrations for at least 4 out of the 6 QC samples per year, while the two abnormal results are not allowed to be from the same concentration level (low, medium, high). All antiretroviral drugs used by the included subjects can be determined.

7.4 Pharmacokinetic Analysis

Pharmacokinetic parameters will be calculated using WinNonlin (Pharsight Corporation, CA, USA).

Individual and mean plasma concentrations will be presented. Overlay presentations will be given to illustrate inter-subject variability. Descriptive statistics will be calculated for the plasma concentrations at each sampling time.

For an agent with more than one possible dosing regimen PK analysis will be performed after 16 patients have been included on the agent (all dosing regimens together). If deemed necessary more patients on a certain regimen can be included after this analysis.

The primary comparison will be made between $AUC_{0\text{-}tau}$ (AUC over a dosing interval), C_{max} and C_{trough} values of the agents in the 2nd (RAL QD substudy and dolutegravir regimen) or 3rd trimester (test) versus these values in the same subjects at post partum (reference), respectively. Geometric Mean Ratios of AUC, C_{max} and C_{min} of test vs. reference will be calculated. If the 90% confidence interval fall completely within the 0.80 – 1.25 range that is used for bioequivalence, it is concluded that there is no influence of pregnancy on the pharmacokinetic parameters. For clinical relevance wider acceptance ranges may be applied, for example a 30% change is usually acceptable.
Ctrough in pregnancy should be above the minimal effective plasma concentration for at least 80% of trough samples.
PK parameters in pregnancy and postpartum will be compared to historical controls.
Cord blood/maternal blood concentration ratio will be determined.
Breastmilk/maternal blood concentration ratio will be determined.
Infant daily dose will be assessed for breastfed infants using the following calculation: (breastmilk/maternal plasma concentration ratio) * maternal plasma concentration * average milk intake volume (150 mL/kg/day).

Newborn PK
Plasma concentrations of raltegravir, unbound raltegravir and M2 will be reported per time point after delivery. If more than two samples are available for a subject the half-life will be assessed. For non-breastfed infants this also applies for cabotegravir/rilpivirine LA regimen.

For several compounds population PK and/or Physiologically Based Pharmacokinetic Models will be developed, using NONMEM and Simcyp.

UGT genotype
UGT1A1 genotype will be assessed, where UGT1A1*28/*28 genotype is associated with decreased activity of UGT1A1 and UGT1A1*1/*1 with normal UGT1A1 activity.

Genotypes will be described and linked to raltegravir pharmacokinetics and used as a covariate in population PK analysis.

Progesterone levels
Maternal progesterone concentrations will be determined at all visits during pregnancy and at the postpartum visit. Descriptive statistics will be presented and the results can be used as covariate in population PK analysis.

7.5 Statistical Analysis

In general, all subjects who completed the study will be included in the statistical evaluation for demographics, pharmacokinetics, safety and virological efficacy.
Statistical analysis will be carried out using SPSS® software. Descriptive statistics will be calculated using Excel software [Microsoft Corporation].

8 RESPONSIBILITIES

8.1 Investigator Responsibilities

8.1.1 Good Clinical Practice

The investigator will ensure that this study is conducted in full compliance with the principles of the “Declaration of Helsinki” (as amended in Tokyo, Venice, Hong Kong, South Africa and Scotland), ICH guidelines, or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the study subject.

8.1.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC) Approval

This protocol and any accompanying material to be provided to the subject (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) will be submitted, by the investigator, to an IEC. Approval from the committee must be obtained **before** starting the study and should be documented in a letter to the investigator specifying the protocol number, protocol version, documents reviewed, and date on which the committee met and granted the approval.
Any modifications made to the protocol after receipt of IEC approval must also be submitted to the committee for approval prior to implementation.

8.1.3 Informed Consent

It is the responsibility of the investigator to obtain written informed consent from each individual participating in this study after adequate explanation of the aims, methods, objectives, and potential hazards of the study and prior to undertaking any study-related procedures. Informed consent forms will be written in the local language for each country. For the blood sampling of the infants a separate informed consent form has been written and this will be used only in case an infant needs treatment with one of the agents in Appendix 1 or for washout sample collection (the raltegravir QD sub-study or cabotegravir/rilpivirine LA regimen). This also applies when infant blood samples are collected if the mother breastfeeds and participates in the breastfeeding substudy (for

cabotegravir/rilpivirine LA only). Two legal representatives of the infant will have to consent for the participation.

The investigator must utilize an IEC-approved consent form for documenting written informed consent. Each informed consent will be appropriately signed and dated by the subject and the persons obtaining consent.

8.1.4 Confidentiality

The investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only an identification code (i.e., not names) should be recorded on any form submitted to the sponsor and IEC. The investigator must keep a screening log showing codes, for all subjects screened and for all subjects enrolled in the study.
This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from the Investigator. The investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain. Data can be shared with parties outside of the EU, but the handling of personal data should comply with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation.

8.1.5 Study Files and Retention of Records

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into 2 separate categories: (1) investigator's study file, and (2) subject clinical source documents.
The investigator's study file will contain the protocol/amendments, CRF and query forms, IEC and governmental approval with correspondence, informed consent, drug records, staff curriculum vitae and authorization forms, and other appropriate documents and correspondence.

Subject clinical source documents (usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs) would for example include subject hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, electrocardiogram (ECG), electroencephalogram (EEG), X-ray, pathology and special assessment reports, consultant letters, screening and enrollment log, etc.

All data (including CRFs) generated in connection with this study, together with a hard copy of the final report, will be kept in the archives of the Radboud university medical center for at least 15 years after the study has been completed. Source documents will be archived for at least 15 years by the study centre. All data will be available for inspection by authorized persons. The investigator must be notified prior to destroying any clinical study records.

8.1.6 Case Report Forms

For each subject enrolled, a CRF must be completed and signed by the principal investigator or co-/sub-investigator within a reasonable time period after data collection. This also applies to records for those subjects who fail to complete the study (even during a pre-randomization screening period if a CRF was initiated). If a subject withdraws from the study, the reason must be noted on the CRF. If a subject is withdrawn from the study because of a treatment-limiting adverse event, thorough efforts should be made to clearly document the outcome.

8.1.7 Drug Accountability

The subjects will be asked for their compliance during the last two weeks prior to the pharmacokinetic curves. No pill count will be performed, as the treatment is part of normal clinical care.

The investigator will report the batch number and expiry date of the medication used on the PK sampling day. On this day medication intake will be supervised.

As drugs are not provided for this study, but as part of standard care, no disposal/destruction requirements are necessary.

8.1.8 Inspections

The investigator should understand that source documents for this study should be made available to regulatory authority or health authority inspectors.

8.1.9 Protocol Compliance

The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

8.1.10 Insurance Cover

According to the Dutch law (WMO) the sponsor is obliged to have an insurance for compensation of subjects entered in clinical studies in the Netherlands who experienced study related injury or death. Each participating center is obliged to arrange such an insurance for participants from their center. Information on the insurance is part of the patient information.

8.2 Sponsor Responsibilities

8.2.1 Protocol Modifications

Protocol modifications, except those intended to reduce immediate risk to study subjects, may be made only by the sponsor. All protocol modifications must be submitted to the IEC in accordance with local requirements. Approval must be obtained before changes can be implemented.

8.2.2 Study Report and Publications

The investigator will submit any proposed publication or presentation along with the respective scientific journal or presentation forum at least 7 days prior to submission of the publication or presentation to all co-investigators listed as co-author.

8.3 Joint Investigator/Sponsor Responsibilities

8.3.1 Access to Information for Monitoring

In accordance with International Conference on Harmonisation Good Clinical Practice (ICH-GCP) guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the CRFs for consistency.

The monitor is responsible for routine review of the CRFs at regular intervals throughout the study, to verify adherence to the protocol, and the completeness, consistency and accuracy of the data being entered on them. The monitor should have access to any subject records needed to verify the entries on the CRFs. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved.

8.3.2 Study Discontinuation

The investigator reserves the right to terminate the study at any time. Should this be necessary, the investigator will arrange discontinuation procedures. In terminating the study, the investigator will assure that adequate consideration is given to the protection of the subjects' interests.

9 APPENDIX 1. TEST PRODUCTS, DOSE, AND MODE OF ADMINISTRATION

** arms will open once registered by EMA and available in a country

Drug name	Class	regimen	Dose	Food	PK data in pregnant women available?	DHHS recomm.	to be determined in plasma
Etravirine, Intelence, TMC125	NNRTI	BID	200mg	With	Limited	Insufficient data to recommend routine use ART-navie pregnant women. Insufficient data to assess for teratogenicity in humans. PK data in pregnancy (n = 26) suggest 1.2–1.6 fold increased etravirine exposure during pregnancy. No change in dose indicated.	etravirine
Rilpivirine, Edurant, TMC278	NNRTI	QD	25mg	With a meal	Limited	Alternative NNRTI. RPV not recommended with pretreatment HIV RNA >100,000 copies/mL or CD4 cell count <200 cells/mm3. Do not use with PPIs. PK data available in pregnancy but relatively little experience with use in pregnancy. Routine dosing adjustment in all women is not recommended for RPV during pregnancy. Individual patients should be closely monitored.	rilpivirine
Emtricitabine, Emtriva or FTC	NRTI	QD	200mg	NR	Yes	Preferred NRTI (FDC with TDF). PK of FTC not significantly altered in pregnancy. No change in FTC dose indicated.	emtricitabine
Tenofovir, Viread, TDF	NtRTI	QD	245mg	With	Yes	Preferred NRTI (FDC with FTC). AUC lower in third trimester than postpartum but trough levels adequate No change in dose indicated.	tenofovir
Abacavir, Ziagen, ABC Abacavir, Ziagen, ABC	NRTI	QD BID	600 mg 300 mg	No	Limited	Preferred NRTI (in FDC with 3TC). ABC/3TC with ATV/r or with EFV is not recommended if pretreatment HIV RNA >100,000 copies/mL.	abacavir

Drug name	Class	regimen	Dose	Food	PK data in pregnant women available?	DHHS recomm.	to be determined in plasma
						PK not significantly altered in pregnancy. No change in dose indicated.	
Atazanavir, Reyataz, Evotaz	PI	300mg/100mg RTV QD 400mg QD 400/100mg RTV QD	300/150mg COBI	With	Limited	Preferred PI. ATV concentrations reduced during pregnancy; further reduced when given concomitantly with TDF or H2-receptor antagonist. Must be given as low-dose RTV-boosted regimen in pregnancy.Use of ATV not recommended for treatment experienced pregnant women taking TDF and an H2-receptor antagonist. Evotaz: No PK studies in human pregnancy.	atazanavir
Fosamprenavir, Telzir, FPV	PI	BID QD	700mg/100mg RTV 1400mg/200mg RTV	NR	Limited	Safety and PK data in pregnancy are insufficient to recommend use during pregnancy in ARVnaive patients. Recommended to be given as low-dose RTV-boosted regimen.	amprenavir
Tipranavir, Aptivus, TPV	PI	BID	500mg/200mg RTV	With	No	Insufficient data to recommend routine use ART-navie pregnant women. Safety and PK data in pregnancy are insufficient to recommend use during pregnancy in ARVnaive patients. Must give as low-dose RTV-boosted regimen.	tipranavir
Raltegravir, Isentress	integrase inhib	BID QD	400mg 1200mg	NR	Limited	Preferred INSTI. PK data available and increasing experience in pregnancy. Rapid viral load reduction.	Raltegravir, + metabolite

Drug name	Class	regimen	Dose	Food	PK data in pregnant women available?	DHHS recomm.	to be determined in plasma
						Useful when drug interactions with PI regimens are a concern. Twice-daily dosing required. The QD dosing regimen has not been investigated in pregnancy.	
Enfuvirtide, Fuzeon	entry inhibitor	BID	90mg	NR	No	Insufficient data to recommend routine use ART-navie pregnant women. Safety and PK data in pregnancy are insufficient to make dosing recommendations.	enfuvirtide
Maraviroc, Celsentri	entry inhibitor	BID	300mg*150mg / 600mg in case of interactions	NR	Limited	Insufficient data to recommend routine use ART-navie pregnant women. Safety and PK data in pregnancy are insufficient to make dosing recommendations.	maraviroc
Efavirenz, Stocrin, EFV **(UK and Ireland only)**	NNRTI	QD	600mg	NR	Limited	Preferred NNRTI, Preferred regimen in women who require co-administration of drugs with significant interactions with PIs or the convenience of co-formulated, single-tablet, once-daily regimen. Use of EFV should be avoided in the first 8 weeks of pregnancy. AUC decreased during third trimester, compared with postpartum, but nearly all third-trimester participants exceeded target exposure. No change in dose indicated.	Efavirenz
Dolutegravir, Tivicay*	Integrase inhib	QD	50mg	NR	Limited	Preferred agent in prepgnancy, alternative when planning pregnancy.	Dolutegravir, total and free + metabolites
Tenofovir alafenamide, TAF, Descovy, Genvoya, Odefsey	NRTI	QD	200/10mg or 200/25mg (FTC/TAF) 150/150/200/10mg (EVG/COBI/FTC/TAF) 25/200/25mg	With	No	Preferred dual NRTI-backbone.	TAF and TFV

Drug name	Class	regimen	Dose	Food	PK data in pregnant women available?	DHHS recomm.	to be determined in plasma
			(RPV/FTC/TAF)				
Bictegravir, BIC, Biktarvy**	INSTI	QD	50/200/25mg BIC/FTC/TAF	NR	No	Insufficient data to recommend routine use ART-navie pregnant women	Bictegravir
Doravirine, DORA, Pifeltro, Delstrigo**	NNRTI	QD	100mg 100/300/300mg DORA/3TC/TDF	NR	No	Insufficient data to recommend routine use ART-navie pregnant women	Doravirine
Cabotegravir LA, Vocabria	INSTI	Per 1 or 2 months IM	400mg in 2 mL 600mg in 3 mL	NR	Tail only	Not recommended, due to insufficient data	Cabotegravir also unbound
Rilpivirine LA, Rekambis	NNRTI	Per 1 or 2 months IM	600mg in 2mL 900mg in 3mL	NR	Tail only	Not recommended, due to insufficient data	Rilpvirine also unbound

*Not in Germany

10 APPENDIX 3: STUDY FLOW CHART

Item	Screening	2nd trimesters (preferably week 20) RAL QD/DTG	3rd trimester (preferably Week 33)	Delivery	post partum (pref. before 6 weeks)	ATV/r 300/100mg trt only: 12-16 weeks PP
Demographics	X					
Physical examination	X					
Urinalysis	X					
HIV-1 RNA	X	X	X		X	
CD4	X	X	X		X	
Blood chemistry	X	X	X		X	
Hematology	X	X	X		X	
Body weight	X	X	X		X	
Drug levels (curve)		X	X		X	
Cord blood				X		
Maternal PK sample				X		X
Breastmilk sample(s)					X	
Pharmacogenetics Ral QD sub-study only		X				
Adverse events	X		X		X	X
Concomitant medication	X		X		X	X

Infant PK blood sampling (during regular care blood sampling at the time points indicated below), not applicable for Germany

Item	Week1	Week 3	Week 4 or 6
Demographics	X		
Body weight	X	X	X
Drug levels	X	X	X
Adverse events	X	X	X
Concomitant medication	X	X	X

Cabotegravir/rilpivirine LA regimen mother

Item	Screening	Every visit for injection during pregnancy	Pregnancy PK curve (from week 24 gestational age)	Delivery	post partum (first injection, if not curve)	post partum (injection at least 4 weeks after delivery)
Demographics	X					
Physical examination	X					
Urinalysis	X					
HIV-1 RNA	X	X	X			X
CD4	X		X			X
Blood chemistry	X		X			X
Hematology	X		X			X
Body weight	X	X	X			X
Maternal PK sample		X (trough)	X (trough, day 3, 7, 28, 56)	X	X (trough)	X (trough, day 3, 7, 28, 56)
Cord blood				X		
Breastmilk (if applicable)						X (trough, day 3, 7, 28, 56)

Infant blood sampling, not in Germany					(within 7 days if not breastfeeding)	X one sample if breastfeeding
Adverse events	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X

11 APPENDIX 4: CLINICAL TOXICITY GRADES (ACTG)

	GRADE 1 TOXICITY	GRADE 2 TOXICITY	GRADE 3 TOXICITY	GRADE 4 TOXICITY
HEMATOLOGY				
HEMOGLOBIN	6.0-6.5 mmol/L	5.0-5.9 mmol/L	4.0-4.9 mmol/L	< 4.0 mmol/L
ABSOLUTE NEUTROPHIL COUNT	1.00-1.50x10^9/L	0.75-0.99 x10^9/L	0.50-0.74 x10^9/L	<0.50 x10^9/L
PLATELETS	75-99x10^9/L	50-74,99 x10^9/L	20-49,99 x10^9/L	<20x10^9/L or diffuse petechiae
PT	1.01-1.25 x upper limit of normal	1.26-1.5 x upper limit of normal	1.51-3.0 x upper limit of normal	>3 x upper limit of normal
PTT	1.20-1.66 x upper limit of normal	1.67-2.35 x upper limit of normal	2.36-3 x upper limit of normal	>3 x upper limit of normal
FIBRINOGEN	0.99-0.75 x lower limit of normal	0.74-0.50 x lower limit of normal	0.49-0.25 x lower limit of normal	<0.25 x lower limit of normal
FIBRIN SPLIT PRODUCT	20-40 mg/L	41-50 mg/L	51-60 mg/L	>60 mg/L
INTERNATIONAL NORMALIZED RATIO OF PROTHROMBIN TIME (INR)	1.1-1.5 x upper limit of normal	1.6-2.0 x upper limit of normal	2.1-3.0 x upper limit of normal	>3 x upper limit of normal
METHEMOGLOBIN	5-9.9%	10.0-14.9%	15.0-20.0%	>20%
CHEMISTRIES				
ALBUMIN, SERUM, LOW	30 g/L-<lower limit of normal	20-29 g/L	<20 g/L	not applicable
HYPONATREMIA	130-134 mmol/L	123-129 mmol/L	116-122 mmol/L	115 mmol/L and less or mental status changes or seizures
HYPERNATREMIA	146-150 mmol/L	151-157 mmol/L	158-165 mmol/L	>165 mmol/L or mental status changes/seizures
HYPOCALCEMIA (ionized calcium)	0.74-0.85 mmol/L	0.62-0.73 mmol/L replacement Rx req.	0.50-0.61 mmol/L or intensive replacement Rx Req. or hospitalization	<0.50 mmol/L or paresis or ileus or life-threatening arrhythmia
HYPERCALCEMIA (ionized calcium)	1.40-1.52 mmol/L	1.53-1.63 mmol/L	1.64-1.75 mmol/L	>1.75 mmol/L or paresis or ileus or life-threatening arrhythmia
TRIGLYCERIDES (fasting)	----------	4.52-8.47mmol/L	8.48-13.55 mmol/L	>13.55 mmol/L
CHOLESTEROL	5.17-6.19 mmol/L	6.20-7.77 mmol/L	7.78-10.34 mmol/L	>10.34 mmol/L
HYPOGLYCEMIA	3.0-3.7 mmol/L	2.2-3.0 mmol/L	1.7-2.1 mmol/L	<1.7 mmol/L or mental status changes or coma

	GRADE 1 TOXICITY	GRADE 2 TOXICITY	GRADE 3 TOXICITY	GRADE 4 TOXICITY
HYPERGLYCEMIA	6.4-8.8 mmol/L	8.9-13.8 mmol/L	13.9-27.7 mmol/L	>27.7 mmol/L or ketoacidosis or seizures
HYPERURICEMIA	446-592 µmol/L	593-716 µmol/L	717-892 µmol/L	>892 µmol/L
HYPOCALCEMIA corrected for albumin	2.10-1.94 mmol/L	1.93-1.74 mmol/L	1.73-1.53 mmol/L	<1.53 mmol/L or life-threatening arrhythmia or tetany
HYPERCALCEMIA corrected for albumin	2.65-2.88 mmol/L	2.89-3.13 mmol/L	3.14-3.38 mmol/L	>3.38 mmol/L or life-threatening arrhythmia or tetany
HYPOMAGNESEMIA	0.7-0.58 mmol/L	0.59-0.48 mmol/L or replacement of Rx req.	0.47-0.3 mmol/L or intensive Rx req. hospitalization	<0.3 mmol/L or life-threatening arrhythmia
HYPOPHOSPHATEMIA	0.64-0.78 mmol/L	0.47-0.63 mmol/L or replacement Rx req.	0.32-0.46 mmol/L intensive Rx req. hospitalization	<0.32 mmol/L life-threatening arrhythmia or CHF
HYPER-BILIRUBINEMIA	1.1-1.5 x upper limit of normal	1.6-2.9 x upper limit or normal	3-5 x upper limit of normal	>5 x upper limit of normal
HYPERKALEMIA	5.6-6.0 mmol/L	6.1-6.5 mmol/L	6.6-7.0 mmol/L	>7.0 mmol/L
HYPOKALEMIA	3.0-3.4 mmol/L	2.5-2.9 mmol/L	2.0-2.4 mmol/L	<2.0 mmol/L
BUN	1.25-2.5 x upper limit of normal	2.6-5.0 x upper limit of normal	5.1-10.0 x upper limit of normal	>10 x normal
CREATININE	1.1-1.5 x upper limit of normal	1.6-3.0 x upper limit of normal	3.1-6.0 x upper limit of normal	>6.0 x upper limit of normal or requires dialysis
CPK (CK) (not related to exercise)	1.1-2.0 x upper limit of normal	2.1-4.0 x upper limit of normal	4.1-6.0 x upper limit of normal	>6.0 x upper limit of normal
ENZYMES				
AST/SGOT	1.25-2.5 x upper limit of normal	2.6-5 x upper limit of normal	5.1-10 x upper limit of normal	>10 x upper limit of normal
ALT/SGPT	1.25-2.5 x upper limit of normal	2.6-5 x upper limit of normal	5.1-10 x upper limit of normal	>10 x upper limit of normal
GGT	1.25-2.5 x upper limit of normal	2.6-5 x upper limit of normal	5.1-10 x upper limit of normal	>10 x upper limit of normal
ALKALINE PHOSPHATASE	1.25-2.5 x upper limit of normal	2.6-5 x upper limit of normal	5.1-10 x upper limit of normal	>10 x upper limit of normal
AMYLASE	>1.0-1.5 x upper limit of normal	>1.5-2.0 x upper limit of normal	>2.0-5.0 x upper limit of normal	>5 x upper limit of normal
LIPASE	>1.0-1.5 x upper limit of normal	>1.5-2.0 x upper limit of normal	>2.0-5.0 x upper limit of normal	>5 x upper limit of normal
URINALYSIS				
PROTEINURIA	1+, 0.3-1.0 g/L	2+ , >1 g/L – 3 g/L	3+, >3 g/L	nephrotic syndrome
HEMATURIA	microscopic only, <10 RBCs	gross, no clots, 10-100 RBCs	gross, clots, > 101 RBCs	obstructive or Rx req.
CARDIAC				
CARDIAC RHYTHM	- -- - - - - -- - - -	asymptomatic, transient signs, no Rx required	recurrent/persistent, no Rx required	unstable dysrhythmia requires treatment or hospitalization
HYPERTENSION	transient inc. >20 mm, no Rx	recurrent, chronic >20 mm, Rx req.	requires outpt. acute Rx	hospitalization
HYPOTENSION	transient orthostatic hypotension, no Rx	symptoms correctable with oral fluid Rx	required IV fluids, no hospitalization required	requires hospitalization

	GRADE 1 TOXICITY	GRADE 2 TOXICITY	GRADE 3 TOXICITY	GRADE 4 TOXICITY
PERICARDITIS	minimal effusion	mild/mod asymptomatic effusion no Rx	symptomatic effusion pain ECG changes	tamponade percardiocentesis or surgery required
HEMORRHAGE, BLOOD LOSS	microscopic occult	mild/no transfusion	gross blood loss, 1-2 units transfused	massive blood loss, >3 units transfused
RESPIRATORY				
COUGH	transient-no Rx	treatment associated cough, local non-narcotic Rx	treatment associated cough, narcotic Rx required	uncontrolled
SHORTNESS OF BREATH	mild, does not interfere with routine activities	moderate, interferes with routine activities req. intermittent Rx	moderately debilitating requiring nasal oxygen	severe, requiring ventilatory assistance
BRONCHOSPASM ACUTE	transient, no Rx, 70%-80% of peak flow	req. Rx normalize w/bronchodilator FEV at 50% of peak flow	no normalization w/bronchodilator FEV at 50% of peak flow, retraction	cyanosis, FEV <25% of peak flow intubated
GASTROINTESTINAL				
STOMATITIS	mild discomfort, no limits on activity	some limits on eating, talking	eating, talking very limited	unable to drink fluids; req. IV fluids
NAUSEA	transient, mild discomfort, maintain reasonable intake	mod. discomfort, sign dec of intake, some limit of activity or decreased intake <3 days	severe discomfort, no significant food intake activities limited or minimal intake ≥3 days	minimal fluid intake or hospitalization required
VOMITING	transient emesis, 2-3 per day or lasting <1 week	moderate emesis 4-5 per day or lasting ≥1 week	vomiting all food/fluids in 24 hours, orthostatic hypotension or IV fluid, Rx req.	hypotensive shock hospitalization IV fluid therapy
CONSTIPATION	Mild	moderate, Rx required	severe, Rx required, vomiting	distention with vomiting
DIARRHEA	>3 loose stools/day lasting <1 week	5-7 loose stools/day and/or nocturnal loose stools lasting ≥1 week, Rx required	orthostatic hypotension or >7 loose stools/day or req. IV fluids Rx	hypotensive shock or hospitalization, IV fluid therapy
ABDOMINAL PAIN	mild occasional transient	moderate, transient	severe, requiring analgesic	severe with guarding peritoneal signs
NEURO/NEUROMUSCULAR				
NEURO CEREBELLAR	slight incoordination Dyskiadokinesis	intention tremor, dysmetria, slurred speech nystagmus	locomotor ataxia	incapacitated
MOOD	mild anxiety or depression	mod. anxiety or depression, therapy required	severe anxiety or depression or manic; (needs assistance)	acute psychosis; incapacitated required hospitalization
NEUROCONTROL	confusion/agitation	mod. confusion/ agitation; some severe, min. Rx	sev. confusion/ agitation	toxic psychosis; hospitalization
MUSCLE STRENGTH	subjective weakness, no objective symptoms/signs	mild objective no dec in function	objective weakness; function limited	paralysis
MYOSITIS	minimal findings	Subjects must have some measures of myositis (positive EMG or muscle	Subjects must have some measures of myositis (positive EMG or muscle biopsy) and one of the following:	Subjects must have some measures of myositis (positive EMG or muscle

	GRADE 1 TOXICITY	GRADE 2 TOXICITY	GRADE 3 TOXICITY	GRADE 4 TOXICITY
		biopsy) and one of the following:		biopsy) and one of the following:
		1) mild myalgias, >6 weeks requiring nonsteroidal anti-inflammatory agents	1) moderate myalgias or muscle tenderness, >6 weeks requiring non-steroidal anti-inflammatory agents	1) severe muscle pain (myalgias) not related to exercise requiring narcotics
		2) difficulty climbing stairs or rising from a sitting position but able to ambulate without assistance	2) requires some assistance with ambulation or general activities	2) muscle weakness resulting in inability to ambulate, requiring special care and assistance with mobilization
				3) acute rhabdomyolysis with muscle necrosis and edema, moderate to severe muscle weakness with inability to ambulate or mobilize self without assistance
PAINFUL NEUROPATHY	mild discomfort; no therapy required	moderate discomfort persisting for > 72 hours; analgesic required	severe discomfort, marked antalgic gait, narcotic analgesic required, with symptomatic improvement	incapacitating, intolerable discomfort. Not improved or unable to walk despite narcotic analgesics
OTHER PARAMETERS				
FEVER oral, w/o infection, >12 hrs.	37.7-38.5C or 100.0-101.5F	38.6-39.5C or 101.6-102.9F	39.6-40.5C or 103-105F	>40.5C >105F
HEADACHE	mild, no Rx therapy	transient, mod; non-narcotic Rx req.	severe, responds to initial narcotic therapy	intractable, req. repeated narcotic therapy
FATIGUE	<25% decrease in daily activities	normal activity decrease 25-50%	normal activity decrease >50%, can't work	unable to care for self
ALLERGIC REACTION	pruritus w/o rash	localized urticaria angioedema	generalized urticaria angioedema	anaphylaxis
LOCAL REACTION	tenderness or erythema	induration <10 cm or phlebitis or inflammation	induration >10 cm or ulceration	necrosis
MUCOCUTANEOUS	erythema, pruritus	diffuse maculopapular rash dry desquamation	vesiculation, moist desquamation ulceration	exfoliative dermatitis, mucous membrane involvement suspected, Stevens Johnson or erythema multiforme, necrosis requiring surgery

12 REFERENCES

1. DHHS. Recommendations for the Use of Antiretroviral Drugs in Pregnant Women with HIV Infection and Interventions to Reduce Perinatal HIV Transmission in the United States. Available at: https://aidsinfo.nih.gov/guidelines/html/3/perinatal/0 2017; Accessed: Oct 2018.
2. Zash R, Holmes LB, Diseko M, Jacobson D, Mayondi G, Mabuta J, *et al.* Update on neural tube defects with antiretroviral exposure in the Tsepamo Study, Botswana; AIDS 2022; abstract 12759. Available at: https://programme.aids2022.org/Abstract/Abstract/?abstractid=12759 2022; Accessed: 18-11-2022.
3. EMA. Tivicay; Summary of Product Characteristics. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/002753/WC500160680.pdf 2022; Accessed: July 28.
4. EMA. Safety warning: New study suggests risk of birth defects in babies born to women on HIV medicine dolutegravir. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Press_release/2018/05/WC500249225.pdf 2018; Accessed: 08-07-2018.
5. EMA. Updated SPC Rezolsta, UK. Available at: https://www.medicines.org.uk/emc/product/3466/smpc 2018; Accessed: 08-07-2018.
6. Anderson GD. Pregnancy-induced changes in pharmacokinetics: a mechanistic-based approach. *Clin.Pharmacokinet.* 2005; 44:989-1008.
7. De Ruiter A, Mercey D, Anderson J, Chakraborty R, Clayden P, Foster G, *et al.* British HIV Association and Children's HIV Association guidelines for the management of HIV infection in pregnant women 2008. *HIV Med.* 2008; 9:452-502.
8. Roustit M, Jlaiel M, Leclercq P, Stanke-Labesque F. Pharmacokinetics and therapeutic drug monitoring of antiretrovirals in pregnant women. *Br.J.Clin.Pharmacol.* 2008.
9. Blonk MI, Colbers AP, Hidalgo-Tenorio C, Kabeya K, Weizsacker K, Haberl AE, *et al.* Raltegravir in HIV-1-Infected Pregnant Women: Pharmacokinetics, Safety, and Efficacy. *Clin Infect Dis* 2015; 61:809-816.
10. Colbers A, Best B, J. W, Stek A, Hidalgo Tenorio C, Hawkins D, *et al.* A Comparison of the Pharmacokinetics of Maraviroc during Pregnancy and Postpartum. *20th Conference on Retroviruses and Opportunistic Infections, Atlanta, Georgia, March 3 - 6, 2013 (paper #391)* 2013.
11. Colbers A, Hawkins D, Hidalgo-Tenorio C, van der Ende M, Gingelmaier A, Weizsacker K, *et al.* Atazanavir exposure is effective during pregnancy regardless of tenofovir use. *Antivir Ther* 2015; 20:57-64.
12. Colbers A, Molto J, Ivanovic J, Kabeya K, Hawkins D, Gingelmaier A, *et al.* Pharmacokinetics of total and unbound darunavir in HIV-1-infected pregnant women. *J Antimicrob Chemother* 2015; 70:534-542.
13. Colbers AP, Hawkins DA, Gingelmaier A, Kabeya K, Rockstroh JK, Wyen C, *et al.* The pharmacokinetics, safety and efficacy of tenofovir and emtricitabine in HIV-1-infected pregnant women. *AIDS* 2013; 27:739-748.

14. Schalkwijk S, Colbers A, Konopnicki D, Weizsacker K, Molto J, Tenorio CH*, et al.* The pharmacokinetics of abacavir 600 mg once daily in HIV-1-positive pregnant women. *AIDS* 2016; 30:1239-1244.
15. Cahn P, Rolón M, Figueroa M, Gun A, Patterson P, Sued O. Raltegravir (RAL) 1200 mg once daily (QD) is non-inferior to RAL 400 mg twice daily (BID), in combination with tenofovir/emtricitabine, in treatment-naive HIV-1-infected subjects: week 48 results. 21st International AIDS Conference (AIDS 2016). July 18-22, 2016. Durban, South Africa. Abstract FRAB0103LB. Available at: *http://www.aids2016.org/Portals/0/File/AIDS2016_Abstracts_LOW.pdf?ver=2016-08-10-154247-087* 2016; Accessed: 8 June.
16. Crauwels H, Rice D, Neyens M, Birmingham L, Vandermeuelen K, Van Solingen-Ristea R*, et al.* Rilpivirine Long-Acting Pharmacokinetic Tail and Pregnancy, HIV&Women workshop 2021, Abstract 14. Available at: *https://academicmedicaleducation.com/meeting/international-workshop-hiv-women-2021/abstract/rilpivirine-long-acting-pharmacokinetic-tail* 2021; Accessed: 01-06-2022.
17. Spreen W, Patel P, Thiagarajah S, Ford SL, Margolis D, Romach B*, et al.* Cabotegravir pharmacokinetic tail in pregnancy and neonatal outcomes, HIV&Women workshop 2020, Abstract 20. Available at: *https://academicmedicaleducation.com/meeting/international-workshop-hiv-women-2020/abstract/cabotegravir-pharmacokinetic-tail-pregnancy* 2020; Accessed: 01-06-2022.
18. Gilleece DY, Tariq DS, Bamford DA, Bhagani DS, Byrne DL, Clarke DE*, et al.* British HIV Association guidelines for the management of HIV in pregnancy and postpartum 2018. *HIV Med* 2019; 20 Suppl 3:s2-s85.
19. PHON. Update landelijk HIV expositie protocol neonaten, inclusief follow-up pasgeborene en kind [Dutch]. Available at: *https://nvk.nl/themas/kwaliteit/richtlijnen/richtlijn?componentid=6881287&tagtitles=Infectieziekten%2ben%2bImmunologie,Neonatologie* 2019; Accessed.
20. Health FOoP. Maladies transmissibles: recommandations de la commission fédérale pour la santé sexuelle (CFSS) en matière de prévention contre la transmission du VIH de la mère à l'enfant, OFSP-Bulletin 50 du 10 décembre 2018. Available at: *https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwj64sjW-5r4AhUqIMUKHQU-BLwQFnoECAcQAQ&url=https%3A%2F%2Fwww.bag.admin.ch%2Fdam%2Fbag%2Ffr%2Fdokumente%2Fmt%2Fp-und-p%2Frichtlinien-empfehlungen%2Feksg-mtct-hiv.pdf.download.pdf%2Fcfss-mtct-vih.pdf&usg=AOvVaw0zDCmimugPphFKjVzA8Ajv* 2018; Accessed.
21. Droste JAH, Verweij-van Wissen CPWGM, Burger DM. Simultaneous Determination of the HIV Drugs Indinavir, Amprenavir, Saquinavir, Ritonavir, Lopinavir, Nelfinavir, the Nelfinavir hydroxymetabolite M8 and Nevirapine in Human Plasma by Reversed Phase High Performance Liquid Chromatography. *Ther Drug Monit* 2003; 25:393-399.
22. Aarnoutse RE, Verweij-van Wissen CPWGM, van Ewijk-Beneken Kolmer EWJ, Wuis EW, Koopmans PP, Hekster YA*, et al.* International interlaboratory quality control program for measurement of antiretroviral drugs in plasma. *Antimicrob.Agents Chemother.* 2002; 46:884-886.

23. Droste JA, Aarnoutse RE, Koopmans PP, Hekster YA, Burger DM. Evaluation of antiretroviral drug measurements by an interlaboratory quality control program. *J Acquir Immune Defic Syndr* 2003; 32:287-291.