

**Study on PhArmacokiNetics of first liNe
Antiretrovirals in healthy Breastfeeding
volunteers or women living with HIV and
already on antiretrovirals
(PANNA-B PK)
(27th of March 2025)**

| Protocol changes | |
|--------------------|---|
| <i>Date</i> | <i>Changes</i> |
| 03-01-2023 | 7.5 Limit added to number of dropouts |
| 05-01-2023 | 7.3.3. Screening for eligibility added |
| 05-01-2023 | 7.3.5. Description added on screening for AE and the handling screening and safety of repeating participant |
| 26-01-2023 | Removed possibility for participants to participate multiple times |
| 25-04-2023 | Added possibility to participate and sustain from feeding infant breastmilk during 4 days after ingestion of study medication |

| | |
|------------|--|
| 13-06-2023 | <p>Adapted language: 'sustain' from breastfeeding has been changed to 'abstain' from breastfeeding</p> <p>Added that the treatments will be applied in a sequential order (second arm will start when first arm is fully recruited), see chapter 3</p> |
| 02-01-2024 | Adjusted the timing of the safety visit. |
| 15-02-2024 | Added phone call 7 days after study visit to check for adverse events |
| 07-03-2024 | <p>Added possibility to participate if already using an antiretroviral regimen</p> <p>Added measurement of breastmilk pH</p> |
| 27-03-2025 | <p>Removal of raltegravir as study-arm</p> <p>Adjustment of sample size for doravirine study-arm</p> |

PROTOCOL TITLE: Study on pharmacokinetics of first line antiretrovirals in healthy breastfeeding volunteers or women living with HIV and already taking antiretrovirals

| | |
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TABLE OF CONTENTS

| | |
|---|----|
| 1. INTRODUCTION AND RATIONALE | 10 |
| 2. OBJECTIVES | 13 |
| 3. STUDY DESIGN | 13 |
| 4. STUDY POPULATION | 15 |
| 4.1 Population | 15 |
| 4.2 Inclusion criteria | 15 |
| 4.3 Exclusion criteria | 15 |
| 4.4 Sample size calculation | 16 |
| 5. TREATMENT OF PARTICIPANTS | 18 |
| 5.1 Investigational product/treatment | 18 |
| 5.2 Use of co-intervention | 18 |
| 5.3 Escape medication | 19 |
| 6. INVESTIGATIONAL PRODUCT | 19 |
| 6.1 Name and description of investigational products | 19 |
| 6.2 Summary of findings from non-clinical studies | 19 |
| 6.3 Summary of findings from clinical studies | 19 |
| Efficacy data is summarized in chapter 5.1 of the SmPC. | 19 |
| 6.4 Summary of known and potential risks and benefits | 19 |
| 6.5 Description and justification of route of administration and dosage | 19 |
| 6.6 Dosages, dosage modifications and method of administration | 19 |
| 6.7 Preparation and labelling of Investigational Medicinal Product | 20 |
| 6.8 Drug accountability | 20 |
| 7. METHODS | 21 |
| 7.1 Study parameters/endpoints | 21 |
| 7.1.1 Main study parameter/endpoint | 21 |
| 7.1.2 Secondary study parameters/endpoints | 21 |
| 7.1.3 Other study parameters | 21 |
| 7.2 Randomisation, blinding and treatment allocation | 22 |
| 7.3 Study procedures | 22 |
| 7.3.1 Screening for eligibility | 22 |
| 7.3.2 Preparation and maintenance for blood drawl | 22 |
| 7.3.3 PK analysis of blood | 22 |
| 7.3.4 PK analysis of breast milk | 22 |
| 7.3.5 Other procedures for safety assessment | 23 |
| 7.4 Withdrawal of individual participants | 23 |
| 7.5 Replacement of individual participants after withdrawal | 23 |
| 7.6 Follow-up of participants withdrawn from treatment | 23 |
| 8. SAFETY REPORTING | 24 |
| 8.1 Temporary halt for reasons of subject safety | 24 |
| 8.2 AEs, SAEs and SUSARs | 24 |
| 8.2.1 Adverse events (AEs) | 24 |

| | | |
|-------|---|----|
| 8.2.2 | Serious adverse events (SAEs)..... | 24 |
| 8.2.3 | Suspected unexpected serious adverse reactions (SUSARs) | 25 |
| 8.3 | Annual safety report | 25 |
| 8.4 | Follow-up of adverse events..... | 26 |
| 8.5 | Data Safety Monitoring Board (DSMB) / Safety Committee | 26 |
| 9. | DATA ANALYSIS | 26 |
| 9.1 | Bioanalysis | 26 |
| 9.2 | Assay validation | 26 |
| 9.3 | Statistical analysis | 27 |
| 9.4 | Pharmacokinetic analysis | 27 |
| 10. | ETHICAL CONSIDERATIONS..... | 27 |
| 10.1 | Regulation statement | 27 |
| 10.2 | Recruitment and consent..... | 27 |
| 10.3 | Benefits and risks assessment, group relatedness | 27 |
| 10.4 | Compensation for injury | 28 |
| 10.5 | Incentives | 28 |
| 11. | ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION | 29 |
| 11.1 | Handling and storage of data and documents | 29 |
| 11.2 | Monitoring and Quality Assurance..... | 29 |
| 11.3 | Amendments | 29 |
| 11.4 | Annual progress report..... | 30 |
| 11.5 | Temporary halt and (prematurely) end of study report..... | 30 |
| 11.6 | Public disclosure and publication policy..... | 30 |
| 11.7 | Sample destruction after study completion | 31 |
| 12. | STRUCTURED RISK ANALYSIS..... | 31 |
| 13. | REFERENCES | 32 |

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

| | |
|--------------------|---|
| ABR | General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier) |
| AE | Adverse Event |
| AR | Adverse Reaction |
| ARV | Antiretrovirals |
| BIC/FTC/TAF | Bictegravir/emtricitabine/tenofovir alafinamide |
| CA | Competent Authority |
| cART | Combination anti-retroviral therapy |
| CCMO | Central Committee on Research Involving Human Participants; in Dutch: Centrale Commissie Mensgebonden Onderzoek |
| CV | Curriculum Vitae |
| CV% | Coefficient of variation |
| CRF | Case Report form |
| DOR | Doravirine |
| DSMB | Data Safety Monitoring Board |
| EU | European Union |
| GCP | Good Clinical Practice |
| GDPR | General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG) |
| IB | Investigator's Brochure |
| IC | Informed Consent |
| IMP | Investigational Medicinal Product |
| IMPD | Investigational Medicinal Product Dossier |
| METC | Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC) |
| PK | Pharmacokinetic(s) |
| PLWH | People living with HIV |
| (S)AE | (Serious) Adverse Event |
| SPC | Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst |
| Sponsor | The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical |

| | |
|--------------|--|
| | company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party. |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| UAVG | Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG |
| WMO | Medical Research Involving Human Participants Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen |

SUMMARY

Rationale: Although current guidelines advise against breastfeeding while using antiretrovirals in people living with HIV, some women choose to breastfeed because advantages of breastfeeding may exceed the possible risk of HIV transmission to the newborn. However, no sound recommendation can be made on which antiretrovirals are most suitable during breastfeeding, because not too little data on penetration of these drugs in breastmilk exist. Too high concentrations may lead to infant toxicity and too low concentrations may lead to development of resistance in case the infant inadvertently becomes infected with the virus.

Objective: to determine the concentration of currently often used ARV (doravirine, and bictegravir, tenofovir alafenamide, emtricitabine) in breast milk after administration of a single dose

Study design: This is a single centre, single dose, open label, pharmacokinetic study in healthy volunteers and women living with HIV and using antiretroviral drugs.

Study population: Adult, healthy breastfeeding volunteers willing to end or abstain from breastfeeding during study. Also, breastfeeding women living with HIV and already using antiretroviral drugs will be recruited to examine their current regimen, or to add on a single dose of study drug if no interactions are to be expected.

Intervention: Administration of one dose of either doravirine (DOR) 100mg, or a combination of tenofovir alafenamide 25mg, emtricitabine 200mg and bictegravir 50mg (BIC/FTC/TAF).

Main study parameters/endpoints: Area under the plasma and milk concentration curve are used to calculate milk to plasma ratio.

Nature and extent of the burden and risks associated with participation, benefit and group

relatedness: Participants will not directly benefit from this study, but will contribute to knowledge on breastmilk transfer of ARV and possibly enable people living with HIV to make an informed decision on breastfeeding while using these medications.

No harm is expected from participation in this study, but possible side effects should be anticipated. Known side effects of DOR are nausea (4%) and headache (3%), abnormal dreams and insomnia (1-10%), dizziness and somnolence and fatigue (1-10%). BIC/FTC/TAFs known side effects are: headache (5%), diarrhea (5%) and nausea (4%), depression and abnormal dreams and fatigue (1-10%), suicidal ideation (0,1-1%), angio-edema (0,1-1%) and Steven Johnson syndrome (0,01-0,1%) and osteonecrosis (0,01-0,1%). Due to the fact that only one dose of the drugs will be ingested, the risk of development of one or more of these side effects is considered to be low.

Participation of women living with HIV and already taking antiretroviral drugs and taking an extra dose of DOR for study purposes, are not expected to experience any, or at least minimal adverse events, as only a single dose is to be administered. As their infants are already exposed to antiretroviral drugs through breastmilk, the extra exposure of a single dose of study drug is deemed to be futile.

Participation in this study requires participants to be admitted for 12 hours, a visit the next morning and a return visit 7 days later. During the sampling day an intravenous indwelling catheter is installed for collection of blood samples. A total volume of 25-50ml of blood, 2 urine samples and 6 breastmilk samples (expressed using a personal electronic pump) are collected. No harm is to be expected from these sample collection procedures.

1. INTRODUCTION AND RATIONALE

Worldwide, 19,7 million women are living with HIV (1). An estimated 1,3 million of them become pregnant each year (2). The risk of transmitting the virus to their infants is drastically reduced by the implementation of guidelines on use of antiretroviral therapy (ART) in pregnancy(3, 4), but a slight risk remains, especially during delivery and in smaller extent through breastfeeding.

Data on HIV transmission through breastfeeding mainly originates from African countries and range from 0-12% (5-7). No cases of mother-to-child transmission (MTCT) have been reported in Europe, but these cohorts might have been too small to detect these events(8-10).

So uncertainty on the actual risk on MTCT through breastfeeding in Western countries remains. Therefore, European and American guidelines recommend against breastfeeding for HIV-1 infected women(11, 12). However, many benefits from breastfeeding might outweigh this potential risk. Breastfeeding is associated with a reduced risk of childhood infections and development of obesity and diabetes mellitus and it positively influences cognitive development. Also mothers benefit from breastfeeding their infants, because it reduces the risk of breast- and ovarian cancer and type 2 diabetes(13). Many sociocultural and psychological factors should be considered as well. Mother-child bonding, forming of a maternal identity, self-image and acceptance by social environment influence a mother's choice on ways to feed her child(14-16). The exact amount of HIV-1 infected mothers who rather breastfeed, is not established. A survey among 93 health care providers in the US showed that 75% of these caregivers cared for women who asked whether they could breastfeed and 29% eventually cared for women who – despite recommendations – actually chose to breastfeed(17).

To make a well thought-out decision on child-feeding, the HIV-infected mother and her treating physician should have access to data on which ART suits the needs of mother- and child best. Detectable concentrations of ART might lead to infant toxicities or – in the case MTCT has occurred – development of a resistant virus(18, 19). Despite rules on labeling drugs with information on pregnancy and lactation(20), clinically relevant information on drug labels of newly approved drugs is still lacking(21). It can take up to six years for such information to become available from post marketing studies (22). Only for a few antiretrovirals (ARV) such information exists (see table 1).

Therefore, the purpose of this study is to establish the concentrations of currently often used ARV (dorzavirine, and bictegravir with emtricitabine and tenofovir alafenamide) in breast milk. Due to ethical reasons, healthy volunteers who are willing to stop breastfeeding (temporarily) during the study will be recruited. The maximum elimination half life of the study drugs is 18 hours, so interruption of breastfeeding for four days (5 times the elimination half life) will ensure the blood – and therefore – breastmilk concentrations will be expected to be zero. In this way the risk of exposure of infants to antiretroviral drugs is minimized.

| Table 1: Summary of available lactation studies | | | | | | | | |
|---|--------------------------------|-------------------------|----------------|--------------------------|-----------------|----------------|--|--|
| Treatment | Milk/maternal plasma ratio | | | Infant plasma/milk ratio | | | RID (dosing) | Data amount + REF |
| | Mean | CI | I ² | Mean | CI | I ² | | |
| ISTIs | | | | | | | | |
| DTG | 0.04 ; 0.03 ; 0.02 | 0,03-0,05 | N A | Median IDD = 0,02 | 0,01- 0,02 | - | 0,78-1,33 | BM/MP = Two studies + case report (23-25) |
| RAL ^A | 0.46 ; 0.55 ; 0.39 | 0.39-0.42 | N A | Median IDD = 0.25 | 0.15- 0.32 | - | 0.12 | BM/MP = Case report (26) and single study (n=14) (25) |
| BIC | 0.01 | 0,01-0,01 | N A | Median IDD = 0.01 | 0.01- 0.01 | | NA | BM/MP + IDD + RID = Single study (n=3) (25) |
| PIs | | | | | | | | |
| ATZ ^B | 0.08 ; 0.06 | NG | N A | - | - | - | - | BM/MP = Two studies (27) |
| DRV ^B | 0.12 | 0.10-0.14 | N A | Median IDD = 0.05 | 0.04- 0.05 | - | 0.11-0.12 | BM/MP + IDD + RID = Single study (n=4) (25) |
| RTV | 0.17 | 0.15-0.19 | 94 % | 0.19 | NG | NA | 0.42% (100mg BID) | BM/MP = Pooled (27) IP/BM = Single study(27) RID (27) |
| NRTIs | | | | | | | | |
| ABC | 0.85 ; 1.03 | 0.78-1.66 | N A | Median IDD = 0.01 | 0.01- 0.02 | NA | 0.1 - 0.88% (300mg BID); 0.17- 0.46% | BM/MP = Two studies (25, 27) IP/BM = Single study (27) IDD (25) RID (25, 28) |
| FTC | 3.01 ; 3,92 | 2.06-3.38; 2,34-5,55 | N A | Median IDD = 0.12 | 0.07- 0.17 | - | 0.93 – 3.57% (400mg once, to 200mg/d); 2.38-5.59% | RID = single study (25) BM/MP = two studies (25, 29) |
| TAF | 4.09 | 3.38-4.92 | N A | Median IDD = 0.007 | 0.006- 0.008 | - | NA | Single study (n=5) (25) |

| | | | | | | | | |
|--|-------------------|-------------------------|--------------------|--|---------------------------------|---------|--|--|
| TDF | 0.08 | 0.06-0.10 | N A | IP=ND in most cases, Median IDD = 0.001 | 0.0001- 0.002 | NA | 0.02 – 0.03% (600mg once, to 300mg/d); 0.01-0.02% | IP/BM = Multiple studies (30) BM/MP = single study (n=24) (25) RID (28) |
| 3TC | 0.93 ; 3.74 | 0.88-0.98; 2.77-4.73 | 98 %; N A | 0.02; median IDD = 0.20 | 0.01- 0.02; 0.12- 0.24 | 88 % | 0.49-6.4% (150mg BID); 2.95- 6.10% | BM/MP = Pooled (27) and later published (n=11) (25) IP/BM = Pooled (27) IDD (25) RID (25, 28) |
| NNRTIs | | | | | | | | |
| EFV | 0.86 | 0.70-1.11 | - | Median IDD = 0.41 | 0.32- 0.48 | - | 1.07-1.61% | BM/MP + IDD + RID = Single study (n=3) (25) |
| NVP | 0.70 | 0.68-0.75 | - | Median IDD = 0.41 | 0.29- 0.42 | - | 2.38-3.51% | BM/MP + IDD + RID = Single study (n=5) (25) |
| RPV | 1.08 | 1.1-1.1 | - | Median IDD = 0.02 | 0.02- 0.02 | | NA | BM/MP + IDD + RID = Single study (n=2) (25) |
| <p>A. First value calculated at 4 months postpartum, second value at 8 months postpartum</p> <p>B. These regimens are given with RTV, a pharmacologic enhancer. RTV is not prescribed alone.</p> <p>ABC = Abacavir; ATZ = Atazanavir; BM = Breastmilk concentration; DNV = Darunavir; DTG = Dolutegravir; EFV = Efavirenz, FTC = Emtricitabine; IDD = infant daily dose (mg/kg), IP = Infant plasma concentration; ISTI = Integrase Strand Transfer Inhibitors; NA = not applicable; ND = Not detectable; NG = not given; NRTI = nucleoside/nucleotide transcriptase inhibitor; NNRTI= non- nucleoside/nucleotide transcriptase inhibitor, NVP = neviraprine, PI = Protease Inhibitor; RAL= Raltegravir; REF = reference; RID = Relative infant dose; RTV = Ritonavir; TAF = Tenofovir alafenamide; TDF = Tenofovir disoproxil fumarate; 3TC = Lamivudine.</p> | | | | | | | | |

2. OBJECTIVES

Primary Objective: to determine the concentrations of currently often used ARV (doravirine, and bictegravir, tenofovir alafenamide, emtricitabine) in breast milk after administration of a single dose

Secondary Objective:

- to determine pharmacokinetics (e.g. half life, clearance, volume of distribution; see chapter 7.1.2.) of abovementioned ARV in breast milk
- to estimate absolute and relative infant dosage through breastmilk

3. STUDY DESIGN

This is a single centre, single dose, open label, pharmacokinetic study in healthy volunteers and breastfeeding women living with HIV and using antiretroviral drugs.

Participants will be screened for inclusion using the in- and exclusion criteria. Once included, they will be admitted at the study centre for a duration of 12hours and return for sample collection 24hours after ingestion of study medication. During this sampling day, plasma will be collected at t=0 (pre-dose), 1, 2, 3, 4, 5, 6, 8, 10, 12, 24 hours post ingestion of a single dose of doravirine 100mg or a single dose of the combination tablet containing 50mg bictegravir, 200mg emtricitabine and 25mg tenofovir alafenamide. Treatments will be tested in a sequential order. Breastmilk expression is planned at 0, 2, 4, 6, 12, 24h after dosing. At the 24h sampling time point, a safety assessment will be performed. 7 days after the study visit the participants will be contacted by telephone to inquire for any adverse events.

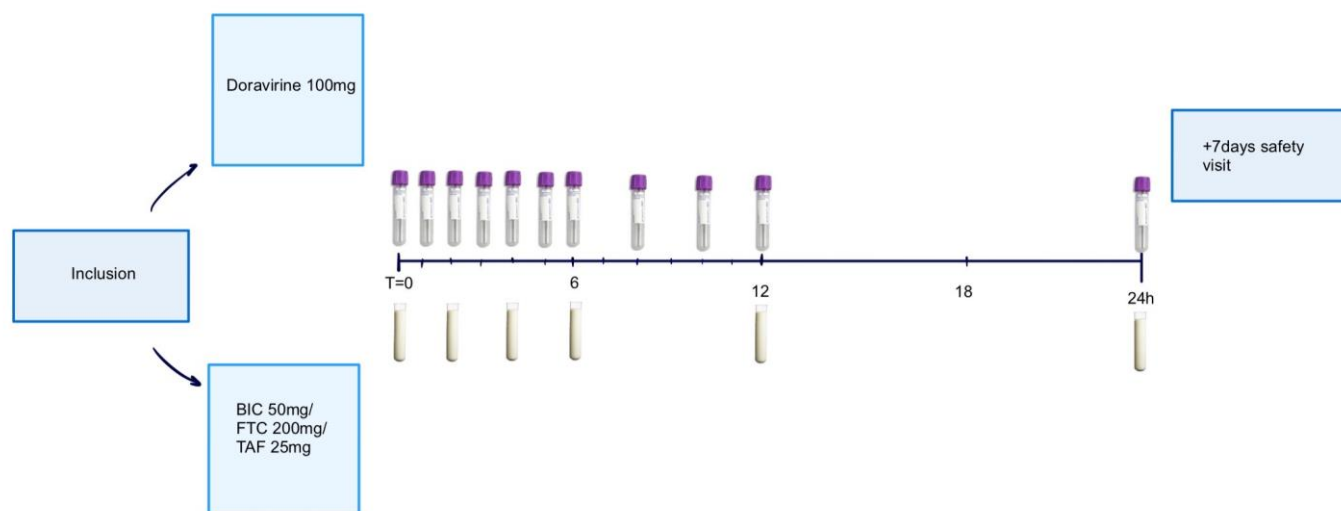


Figure 1: overview of study design

4. STUDY POPULATION

4.1 Population

Three types of participants are eligible for inclusion:

- Healthy volunteers → in- and exclusion criteria 4.2.1 and 4.3.1.
- Women living with HIV and using antiretroviral therapy currently under investigation (DOR, or BIC/FTC/TAF) → in- and exclusion criteria 4.2.2 and 4.3.2.
- Women living with HIV and using antiretroviral therapy and willing to ingest a single dose of DOR on top of their own regimen → in- and exclusion criteria 4.2.3 and 4.3.3.

Women living with HIV and using antiretroviral drugs using either DOR or BIC/FTC/TAF may choose to participate in study using their own regimen, or to ingest one of the drugs under investigation on top of their regular regimen.

4.2 Inclusion criteria

4.2.1. Healthy volunteers

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- At least 18 years of age at the moment of screening
- At least 10 days post partum
- At the end of breastfeeding period; subject is able to produce breastmilk at least two times a day and is no longer feeding infant at start of study or willing and able to abstain from breastfeeding during four days after ingestion of study medication
- Able and willing to sign an informed consent

4.2.2. Women living with HIV using either DOR or BIC/FTC/TAF

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- At least 18 years of age at the moment of screening
- At least 10 days post partum
- Able and willing to sign an informed consent

4.2.3. Women living with HIV using antiretroviral drugs and able to ingest a single dose of DOR

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- At least 18 years of age at the moment of screening
- At least 10 days post partum
- Able and willing to sign an informed consent

4.3 Exclusion criteria

4.3.1. Healthy volunteers

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Relevant co-medication or comorbidity that might interfere with drug absorption, distribution, metabolism or excretion
- Inability to take drugs according to the instructions (i.e. with food)
- Presence of positive HIV screening or HIV RNA
- Presence of HBsAg or HBcAg without anti-HBs
- Presence of grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL).
- Presence of hereditary forms of severe galactose intolerance, total lactase deficiency or glucose-galactose malabsorption

4.3.2. Women living with HIV and taking either DOR or BIC/FTC/TAF

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Relevant co-medication or comorbidity that might interfere with drug absorption, distribution, metabolism or excretion, especially the use of ritonavir/cobicistat boosted protease inhibitors are expected to interfere with study drugs and are therefore excluded.
- Inability to take drugs according to the instructions (i.e. with food)
- Presence of grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL).
- Presence of hereditary forms of severe galactose intolerance, total lactase deficiency or glucose-galactose malabsorption

4.3.3. Women living with HIV using antiretroviral drugs and able to ingest a single dose of DOR

- Relevant co-medication or comorbidity that might interfere with drug absorption, distribution, metabolism or excretion; especially participants whose regular ARV regimen contains ritonavir or cobicistat
- Inability to take drugs according to the instructions (i.e. with food)
- Presence of grade III/IV anaemia (i.e. Hb <4.6 mmol/L or <7.4 g/dL).
- Presence of hereditary forms of severe galactose intolerance, total lactase deficiency or glucose-galactose malabsorption

Participation to other studies is not an exclusion criterion per se, when other in- and exclusion criteria are met and the other study does not interfere with the outcomes of the current study.

4.4 Sample size calculation

No formal statistical comparison will be performed, as the main objective of this study is to determine concentrations in breastmilk transfer of the aforementioned drugs,

Considering a coefficient of variation (CV%) of 30%, 12 participants are estimated to be required to reliably establish the pharmacokinetic (PK) parameters for BIC/FTC/TAF in breastmilk. For doravirine a sample size of 8 is deemed sufficient, because interim analysis of data collected so far has shown a CV% of less than 30%. In addition, awaiting results of this study, a physiology based

pharmacokinetic model was developed in which doravirine transfer into breastmilk was simulated. Results from this study are similar to those seen in the samples collected thus far in current study, The combination of these two data sources creates a reliable determination of doravirine pharmacokinetics in breastmilk.

Women are recruited from the child consultation clinics, infant day care facilities, through lactation specialists and midwives in the Nijmegen area. Also, approved promotion material is shared through the research platform of Radboudumc and nearby hospitals as well as social media (Instagram en LinkedIn).

5. TREATMENT OF PARTICIPANTS

5.1 Investigational product/treatment

Participants will either receive one administration of DOR 100mg, or a single tablet containing a combination of bictegravir 50mg, tenofovir alafenamide 25mg and emtricitabine 200mg (BIC/FTC/TAF).

If a participant is already using an antiretroviral regimen containing one of the study drugs, the participant may choose to ingest one extra study drug on top of her existing regimen. However, participants who use DOR on a daily basis, will not receive additional BIC/FTC/TAF, to prevent additional exposure to the NRTIs FTC/TAF, because their regimen most likely already contains two NRTIs.

For participants using DOR or BIC/FTC/TAF or other ART on a daily basis, with or without add on study drugs, observed intake of the daily regimen will take place at the study centre.

DOR is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and – in combination with other ARV – indicated for treatment of HIV-1 infection without evidence for NNRTI class resistance.

Bictegravir is an integrase inhibitor and tenofovir alafenamide and emtricitabine belong to the class of nucleoside reverse transcriptase inhibitors. In combination, these drugs are indicated for the treatment of a HIV-1 infection.

5.2 Use of co-intervention

Co-ingestion of (medicinal) products that have the capacity to induce or inhibit CYP3A is prohibited up till 7 days before participation in the study. Simultaneous ingestion of study medication and certain supplements may influence absorption and is therefore avoided. Examples of such products are summarized in table 1. Co-medication will be judged during screening.

Table 1: Examples of prohibited co-medication and products

| Examples of prohibited co-medication and products |
|--|
| Ritonavir or cobicistat |
| Carbamazepin, oxcarbazepine, phenobarbital, phenytoin |
| Rifampicin, rifapentine, rifabutin |
| Mitotane |
| Grape fruit or Seville oranges |
| Saint John's wort |
| Ketoconazole, fluconazole, itraconazole, posaconazole and voriconazole |
| Diltiazam, verapamil |
| Anti-acids containing aluminium or magnesium |
| Oral iron and calcium supplements |

Standardized meals, drinks and snacks will be provided to the subject during sampling day.

In the case participants already use ART, but are accustomed to ingest their drugs at night, a scheme will be provided to switch to daily intake during 3-5 days prior to the sampling day. Also a scheme to return back to their normal routine will be provided.

5.3 Escape medication

Not applicable

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational products

- Doravirine (DOR)
- Bictegravir/emtricitabine/tenofovir alafenamide (BIC/FTC/TAF)

6.2 Summary of findings from non-clinical studies

In chapter 5.1 of the Summary of Product Characteristics (SmPC) results of in vitro studies on antiviral activity and resistance are listed under subsection “antiviral activity” and “resistance” respectively. Chapter 5.3 of the SPC summarizes preclinical safety data.

6.3 Summary of findings from clinical studies

Efficacy data is summarized in chapter 5.1 of the SmPC.

6.4 Summary of known and potential risks and benefits

Chapter 5.3 of the SmPC summarizes preclinical safety data. Chapter 4.8 of the SmPC outlines all known undesirable effects. Findings regarding fertility, pregnancy and lactation are listed in chapter 4.6 of the SmPC.

6.5 Description and justification of route of administration and dosage

Route of administration and dosages in this study are in line with manufacturers advice (SmPC).

6.6 Dosages, dosage modifications and method of administration

- Doravirine 100mg, 1 tablet, single dose, administered orally with water
- Bictegravir 50mg/emtricitabine 200mg/tenofovir alafenamide 25mg, 1 tablet, single dose, administered orally with water

6.7 Preparation and labelling of Investigational Medicinal Product

Study medication will be procured by the clinical trial unit (CTU) and stored at the Pharmacy of the Radboudumc. Packing of the medication on an individual level will be done by the Hospital Pharmacy's Clinical Trial Unit (CTU).

For the (licensed) medication preparation, we use the European Clinical Trial Regulation No 536/2014, item (57): 'Where the investigational or auxiliary medicinal product have already been placed on the market as an authorised medicinal product in accordance with Directive 2001/83/EC and Regulation (EC) No 726/2004 of the European Parliament and of the Council, as a general rule no additional labelling should be required for clinical trials that do not involve the blinding of the label.'

This means that labelling in accordance with GMP annex 13 is not necessary for the study medication in this study, and that the KNMP's guideline 'ter handstellen' in this case applies. All drugs will only be administered at the study site on the sampling day(s). Doravirine will be taken directly from the commercial package and is then stored at the CTU. The date at which the package is first opened and the subsequent expiration date (30 days after opening according to information provided by the manufacturer) is noted on the package itself. Biktarvy comes in a blisterpackage. The drug is taken directly from the commercial package and then stored at the CTU. Batch numbers, expiry dates and logs of deliveries of all drugs are recorded. As soon as the expiration date is reached, the drugs will be disposed

The CTU of the Pharmacy will be in charge of drug accountability of the study medication on a Pharmacy level (i.e. overview of the purchased and dispensed packages). Drug accountability on a participant's level will be performed at the study site. In case of a participant already using DOR or BIC/FTC/TAF and who will not be using add on drugs for study purposes, the drugs already prescribed and in possession of participant will be ingested..

6.8 Drug accountability

Shipment, disposition, return and eventual destruction of investigational medicinal products will be carried out according ICH guidelines for Good Clinical Practice (GCP).

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter/endpoint

Area under the plasma and milk concentration curve are used to calculate milk to plasma ratio.

7.1.2 Secondary study parameters/endpoints

The following pharmacokinetic parameters of ARV in plasma and/or breastmilk will be described:

- AUC_{0-tau} (AUC over a dosing interval) estimated using the trapezoidal method.
- Peak plasma concentration (C_{max})
- Time to C_{max}
- Concentration at end of dosing interval (C_{trough})
- Apparent clearance
- Apparent volume of distribution
- Half life
- Parameter estimation through compartmental modelling

7.1.3 Other study parameters

- Body weight and height at moment of participation in study and reported weight pre-pregnancy
- Use of co-medication, herbal or dietary supplements, tobacco, alcohol or illicit drugs two weeks prior to study participation
- Time post partum, gestational age at delivery
- Serum glucose
- Glomerular filtration rate calculated using CKD-EPI (31)
- Complete blood count (hemoglobin, leukocytes, thrombocytes)
- Liver panel (alanine transferase, aspartate aminotransferase, bilirubine, gamma glutamyl transferase, alkaline phosphatase)
- Albumin and α -1-acid glycoprotein
- Urine analysis (glucose, protein, blood, leukocytes, and nitrite. If positive, microscopic examination will be performed)
- Adverse effects reported by participants
- Concentrations found in breastmilk will be extrapolated to infant dosages
- pH of breastmilk
- If participants are living with HIV extra parameters will be noted:
 - Year of HIV diagnosis
 - CD4 cells
 - HIV-1 or HIV-2
 - Recent (<3 months) HIV viral load

7.2 Randomisation, blinding and treatment allocation

This is a non-randomised, open label trial. Participants will be allocated to DOR, or BIC/FTC/TAF in a consecutive manner. First 8 participants will receive DOR, followed by administration of BIC/FTC/TAF is administered in 12 other participants.

7.3 Study procedures

7.3.1 Screening for eligibility

The participants will be screened for eligibility for inclusion within 4 weeks and 3 days prior to study participation. The following procedures will be performed and documented:

- Medical history and demographics (with a focus on co-medication, drug, alcohol- and tobacco use)
- Haematological and biochemical analysis of blood (complete blood count, kidney function, liver tests, albumin)
- Urinalysis
- Serologic tests (HIV and hepatitis B screening) in case of healthy volunteers

When a subject is found eligible the study day and safety visit will be planned as soon as possible.

7.3.2 Preparation and maintenance for blood draw

At the start of the study day an indwelling intravenous canula will be installed by a certified nurse for repeated collection of blood samples. After every sample collection, the cannula will be flushed with a 0.9% NaCl solution in order to maintain patency. Prior to each blood sample collection, approximately 1 mL blood will be discarded.

7.3.3 PK analysis of blood

A total of 11 samples will be collected for PK analysis. After the first sample (pre-dose, $t=0$) is drawn, DOR or BIC/FTC/TAF is administered. Hereafter, a new sample is collected using the intravenous canula after 1, 2, 3, 4, 5, 6, 8, 10, 12, 24 hours post ingestion. Each sample contains a minimum of 2 mL of blood collected in a EDTA tube. After collection, the samples are centrifugated during 5 minutes at room temperature at 1900g and transferred to a polypropylene tube and stored at freezers with a temperature of -40°C .

7.3.4 PK analysis of breast milk

For pharmacokinetic analysis of milk, samples of whole, mature (at least 10 days post-partum) 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 1ml) for assay will be saved using proper storage methods. Expression of breastmilk is planned at 0, 2, 4, 6, 12, 24h after dosing. The timing of sampling relative to dose and the total volume of the sample will be reported in the case report form (CRF).

7.3.5 Other procedures for safety assessment

During the 24h post-dosing sampling time point, 2 samples (one in EDTA, one in lithium heparin; each filled with a minimum of 2ml) will be collected for haematological and biochemical analysis. An urine sample will be collected as well, using a plastic, sterile cup provided by the study team.

The participants will be asked if they experienced any adverse events at the 24h sampling time point and 7 days after intake of study medication. Any spontaneously reported by subject or observed by investigator will be documented.

7.4 Withdrawal of individual participants

Participants can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Other reasons for termination of participation are: protocol violation, difficulty in collecting blood-, or breastmilk samples or in the case of a serious adverse event.

7.5 Replacement of individual participants after withdrawal

The following definitions apply:

Screening failure: subject that does not comply with the in- and exclusion criteria at some point before the first samples for pharmacokinetic analysis are collected.

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 participants (for example withdrawn from the study by the Investigator on ground of adverse events) will be replaced.

7.6 Follow-up of participants withdrawn from treatment

Participants will only receive one dose of study drugs. If participants are withdrawn – defined as a dropout in section 7.5 – the subject will still be invited for the 7day post-dose safety follow up visit. In the case of an adverse event, procedures mentioned in chapter 8 section 4 of this protocol will be followed.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all participants are kept informed.

8.2 AEs, SAEs and SUSARs

8.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to DOR or BIC/FTC/TAF. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events.

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.

8.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in the SPC.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC :

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the participants involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or *ToetsingOnline* is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

8.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the participants, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

8.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached.

Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

8.5 Data Safety Monitoring Board (DSMB) / Safety Committee

This study takes place in healthy, adult volunteers and consists of the intake of a single dose of a drug that is well studied and not associated with severe drug reactions. Therefore, the installation of a data safety monitoring board or safety committee is not needed.

9. DATA ANALYSIS

9.1 Bioanalysis

RadboudUMC has established or will establish high pressure liquid chromatography (HPLC)/ ultra pressure liquid chromatography (UPLC)/ liquid chromatography with tandem mass spectrometry (LC-MS/MS) assays for ARV (32).

9.2 Assay validation

The plasma assays will be externally validated by the International Interlaboratory Program for the Quality Control of Therapeutic Drug Monitoring in HIV Treatment (33, 34). The breastmilk assay are internally validated according EMA guidelines(35). Acceptable accuracy is defined as a performance within 80-120% of the nominal concentrations for the lower limit of quantification (LLOQ) and 85-115% for low-, middle-, high- and highest level of quantification in a run of 5 samples. Acceptable precision is defined as a coefficient variation (CV%) below 20% for the LLOQ and below 15% for the other levels of quantification. The carry over effect and matrix effect are also established and should be below 20% of LLOQ and CV% <15% respectively. Stability evaluation is carried out as well for long term stability and freeze and thaw stability.

9.3 Statistical analysis

In general, all participants who completed the study will be included in the statistical evaluation for description of demographics and pharmacokinetics. No formal statistical comparisons will be performed.

Descriptive statistics will be carried out using R® software.

9.4 Pharmacokinetic analysis

For the calculation of pharmacokinetic parameters WinNonlin (Pharsight Corporation, CA, USA) will be used. Individual and mean plasma concentrations and area under the curve (AUC_{tau}) will be presented. AUC_{tau} will be calculated using the trapezoidal method. Inter-subject variability will be illustrated using overlay presentations.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (8th version, 2013), Declaration of Tapei (2016) and in accordance with the Medical Research Involving Human Participants Act (WMO).

10.2 Recruitment and consent

Participants will be recruited through the Dutch Breastmilk Bank and at child consultation clinics in Nijmegen area. The Dutch Breastmilk Bank has a continuous number of approximately 30 individuals who donate milk in their database that can be approached. These individuals are interested in enabling others to breastfeed and may be willing to participate in this study. Normally, individuals donate for approximately 3 months and are then replaced by others; on a yearly basis the Breast Milk Bank has 120 individuals on file.

The investigator will inform the subject and provide a patient information leaflet and be available for additional questions. Participants are given as much time as needed to consider participation in this study.

10.3 Benefits and risks assessment, group relatedness

Participants will not directly benefit from this study, but will contribute to knowledge on breastmilk transfer of ARV and possibly enable people living with HIV to make a better informed decision on breastfeeding while using these medications.

No harm is expected from participation in this study, but possible side effects should be anticipated. Known side effects of DOR are nausea (4%) and headache (3%), abnormal dreams and insomnia (1-10%), dizziness and somnolence and fatigue (1-10%). BIC/FTC/TAFs known side effects are: headache (5%), diarrhoea (5%) and nausea (4%), depression and abnormal dreams and fatigue (1-10%), suicidal ideation (0,1-1%), angio-edema (0,1-1%), Steven Johnson syndrome (0,01-0,1%). Due to the fact that only one dose of the drugs will be ingested, the risk of development of one or more of these side effects is considered to be low.

Participation in this study requires participants to be admitted during 24hours and will be contacted by telephone 7 days later. During the sampling day an intravenous indwelling catheter is installed for collection of blood samples. A total volume of 25-50ml of blood, 2 urine samples and 6 breastmilk samples (expressed using a personal electronic pump) are collected. No harm is to be expected from these sample collection procedures.

In participants who chose to abstain from breastfeeding for a minimum of four days, the risk of toxicity for their infants will be negligibly small. All study drugs are metabolized by first line kinetics, which means that after 4-5 times the elimination half-life of the drug, the plasma concentrations will be near to zero. Because breastmilk concentrations are mainly determined by passive diffusion from plasma, the breastmilk concentrations will most likely be lower than the plasma concentrations. So, when a near to zero concentration in plasma has been reached after 4-5 elimination half-life periods, the risk of getting exposed to toxic concentrations via breastmilk will be nihil. Therefore, participants who agree to feed their infants in an alternative way (formula, pre-pumped milk form before ingestion of study drug, donor milk etc) will have to plan this alternative feeding in advance and might need to pump during this period to retain milk-production. This might be conceived as an inconvenience.

In participants living with HIV no harm is to be expected if they ingest a single dose of DOR or BIC/FTC/TAF. Also, the exposure of their infants through breastmilk resulting from an extra single dose is regarded futile.

10.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research participants through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

10.5 Incentives

Participants will receive compensation for travel expenses and a compensation of €300,- .

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

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 Trial Master File which data will be collected as source data only. The database will be constructed by the Radboudumc in Castor (a validated data management system): it is ensured and documented that the electronic data processing system(s) conforms to the sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance. The data management system will be compliant with GCP, as set out in Title 21 CFR Part 11. A data management plan will be written.

Data from working copies of CRFs, obtained from the Investigator, will be directly entered into the database management system.

The investigator must assure that subject's anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Blood and milk samples for PK analysis will be identified by study name, patient number, date and time of sampling and sample code. CRFs do not contain identifiable information and will be coded with randomization numbers only. Information and study files that are necessary for the evaluation of the research are stored anonymously and the identification key will not be accessible by unauthorized parties.

11.2 Monitoring and Quality Assurance

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 at least 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.

11.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the participants of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

11.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of participants included and numbers of participants that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

11.6 Public disclosure and publication policy

The study will be registered to a publicly accessible registry and results database (ClinicalTrials.gov). 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.

11.7 Sample destruction after study completion

Any samples left over after analysis will be destroyed when the study is completed. Samples will be discarded in compliance with local biologic waste disposal requirements.

12. STRUCTURED RISK ANALYSIS

Participants will not directly benefit from this study, but will contribute to knowledge on breastmilk transfer of ARV and possibly enable people living with HIV to make an informed decision on breastfeeding while using these medications.

Participants are entitled to reimbursement of travel expenses and will receive a compensation of €300,- for each completed sampling day.

No harm is expected from participation in this study, but possible side effects should be anticipated. Known side effects of DOR are nausea (4%) and headache (3%), abnormal dreams and insomnia (1-10%), dizziness and somnolence and fatigue (1-10%). BIC/FTC/TAFs known side effects are: headache (5%), diarrhoea (5%) and nausea (4%), depression and abnormal dreams and fatigue (1-10%), suicidal ideation (0.1-1%), angio-edema (0,1-1%) and Steven Johnson syndrome (0.01-0.1%) and osteonecrosis (0.01-0.1%). Due to the fact that only one dose of the drugs will be ingested, the risk of development of one or more of these side effects is considered to be low.

Participation in this study requires participants to be admitted during 12hours and a return visit 24hours after ingestion of the study drug and again 7 days later. During the sampling day an intravenous indwelling catheter is installed for collection of blood samples. A total volume of 25-50ml of blood, 2 urine samples and 6 breastmilk samples (expressed using a personal electronic pump) are collected. No harm is to be expected from these sample collection procedures.

Lastly, no harm to infants of participants who temporarily stop breastfeeding is to be expected, because a stopping-period of four days is ensured, which is more than enough time for metabolization of the drugs to a nearly zero concentration. The need to plan alternative feeding options for subject's infants and the possible need to pump to maintain milk production, are inconvenient implications of temporary breastfeeding stops.

In participants living with HIV no harm is to be expected if they ingest a single dose of DOR or BIC/FTC/TAF. Also, the exposure of their infants through breastmilk resulting from an extra single dose is regarded futile.

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