Pharmacokinetics of oral Tenofovir Disoproxil Fumarate in pregnancy and lactation, a systematic review

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SUMMARY

Background: Tenofovir Disoproxil Fumarate (TDF), the oral prodrug of Tenofovir (TFV), is advocated in pregnancy to close the gap on prevention of mother to child transmission (PMCT) from failure of hepatitis B immunoglobulin and vaccination, principally in women with high hepatitis B DNA load ($>200,000$ IU/ml). The pharmacokinetics of TDF monotherapy for PMCT-HBV may be important if deployment is to emulate the success of multiple-ARVs for PMCT-HIV in resource constrained settings.

Methods: This systematic review followed a protocol and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) guidelines. We included studies that enrolled pregnant women who received oral TDF therapy as monotherapy or in combination with other antiretrovirals (ARVs): irrespective of the reason for receiving the drug (e.g. HIV, HBV or pre exposure prophylaxis); and reported pharmacokinetics.

Results: The AUC, $C_{\max}$ and C_{last} of TFV were mostly decreased in the second and third trimester compared to first trimester or post-partum values. In none of the included manuscripts in this review was the non-pregnant HBV threshold of $C_{\max}$ of 300 ng/ml for sampling in pregnancy reached, but the EC_{50} of TFV is lower for treatment of HBV compared to HIV. In ingested breastmilk, the TFV concentration in infants was 0.03% of the recommended infant dose.

Conclusion: Most knowledge of pharmacokinetic of TFV in pregnancy results from studies on HIV frequently involving multiple antiretrovirals. Increased TFV clearance occurred in the second and third trimester when optimal TFV concentrations are required to maximize suppression of HBV in the finite window before birth. Dose or duration adjustments will be better conceptualized with concurrent analysis of the PK of TFV monotherapy and Hepatitis B pharmacodynamics in pregnancy.

50 BACKGROUND

51 Over 3% of the world populations are infected with Hepatitis B virus (HBV), which is the most significant risk factor
52 for hepatocellular carcinoma. Mother to child transmission (MTCT) is the main route of acquisition [1-4] and the risk
53 of infection is linear to maternal viral load at birth [5]. Perinatal infection occurs in 70–90% of babies born to women
54 with HBe Antigen-positive (HBeAg) HBV and 0–30% in those who are HBeAg-negative [1]. Even with optimal
55 preventive strategies, hepatitis B immunoglobulins (HBIG) after birth in HBeAg-positive mothers in addition to HBV
56 vaccination, MTCT occurs in an estimated 8–32% of cases [6, 7]. Unfortunately in low-income countries (LIC) where
57 HBV is most prevalent, HBIG is usually not provided because of access (including homebirth), complexity of
58 production and delivery and short shelf life, cost, and the need for a cold chain [8].

59 Maternal Tenofovir Disoproxil Fumarate (TDF) therapy is one strategy under consideration to reduce MTCT. Pregnant
60 women could be treated with oral TDF during the course of their pregnancy and, if no further availability, it can be
61 stopped 1 month post-partum. TDF is an oral prodrug of tenofovir (TFV), a nucleotide analogue that was developed
62 and has been widely used as an antiretroviral drug against Human Immunodeficiency Virus (HIV) and HBV. TDF has
63 an oral bioavailability (F) estimated at 20-30%, is formulation dependent [9]. Following absorption and distribution,
64 TFV is converted intracellularly to its active anabolite tenofovir diphosphate (TDP). There is no efficacy threshold
65 level for TFV in HIV, but thresholds for HBV that have been used included a C_{max} of 300 ng/ml and a $t_{1/2}$ of 17 hours
66 (tested in non-pregnant males and females) [10]. The elimination of plasma or serum TFV is more rapid ($t_{1/2}$ 12-16
67 hours) than the bioactive intracellular TDP ($t_{1/2}$ 87 hours in peripheral blood mononuclear cells (PBMCs), and 96 hours
68 in hepatocytes) [11]. This $t_{1/2}$ of TFV in PBMCs is helpful since TFV is pharmacologically “forgiving” in the context
69 of poor adherence. TFV exhibits long-lasting anti-HBV activity in cell culture, but has an in vitro 50% effective
70 concentration (EC_{50}) that is lower for treatment of HBV compared to HIV (0.03 ± 0.02 μ g/ml with continuous
71 exposure) [12]. TDF is widely regarded as safe in pregnancy after extensive use in HIV-positive pregnant women [13].
72 Both the pharmacokinetics (PK) [14] and pharmacodynamic (PD) properties [15] of drugs may be affected by
73 physiological changes of pregnancy and reduced exposure has been reported for antiretroviral drugs in pregnancy [14,
74 16] and in lactation [17]. TDF suppression of HBV for prevention of MTCT starts in pregnancy which sets a constraint
75 on the time frame available to reduce the viral load before birth. While a systematic review and meta-analysis supports
76 high efficacy of TDF from 28 weeks given with HBIG and the birth dose to reduce MTCT; HBV DNA remains
77 detectable in 2-38% of participants which is important because the threshold of HBV DNA load to prevent MTCT of
78 HBV without HBIG (reality for resource limited settings) is unknown [19]. Rebound of HBV DNA to pre-treatment
79 levels within 4-8 weeks occurs following short-course TDF before protective infant HBV antibody levels can be

acquired [20]. This may be important in resource-constrained settings where breastfeeding remains the only source of infant nutrition and HBIG and HB birth dose administration are frequently not provided. The objective of this systematic review was to summarise the PK of oral TDF for PMTCT of HBV and HIV in pregnant and lactating women. The PK of TDF monotherapy for PMCT-HBV may be important before widespread deployment in low resource settings where optimal provision of HBIG and birth dose are problematic.

METHODS

This systematic review followed a protocol and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA) guidelines.

Protocol and registration

This review was registered in advance in PROSPERO (International prospective register of systematic reviews). Registration number: CRD42018082352.

Eligibility criteria

We included studies that enrolled pregnant women who received oral TDF therapy as monotherapy or in combination with other antiretrovirals (ARVs), irrespective of the reason for receiving the drug (e.g. HIV, HBV or pre exposure prophylaxis), and details of the reported pharmacokinetics. Both English and non-English-language studies were included.

Information sources

We searched for publications in Medline, Embase and Ovid Cochrane Central Register of Controlled Trials from 1980 to 15 November 2018. Controlled vocabulary supplemented with keywords was used to search for pharmacokinetic studies of TDF in pregnancy. A manual search of bibliographies of the included studies and relevant systematic reviews was conducted. We also contacted people in the field to make sure we did not miss unpublished papers. The last search was performed on 15 November 2018.

The complete search is listed in the supplementary material.

Study selection

Two independent reviewers screened in duplicate titles and abstracts for potential eligibility. Disagreements were reconciled by consensus or by a third reviewer. Articles that were selected were first screened for eligibility using the title and abstracts. Manuscripts were excluded during the process that did not meet our patient, intervention, control, outcome (PICO) objectives.

112

113 **Data extraction**

114 For each study, data extraction was done in duplicate using a standardized, pretested form. A third reviewer compared
115 data and resolved inconsistencies by referring to the full text of the articles.

116

117 **Data items**

118 The exposure parameters used were those that were previously reported as being essential for dosing decision support in
119 pregnancy, i.e. area under the concentration-time curve (AUC, AUC_{∞} as total exposure, or within an interval e.g. in the
120 case of daily dosing as AUC_{0-24}), minimal (C_{trough}) and maximal (C_{max}) plasma concentrations [14]. Other distribution
121 parameters collected were standard pharmacokinetic measures including: volume of distribution (V_d), fraction of drug
122 bound and unbound to plasma proteins (f_b and f_{ub}); and elimination parameters (half life ($t_{1/2}$) and clearance (CL).

123 **Risk of Bias Assessment**

124 Two reviewers assessed the risk of bias (i.e., systematic error) independently using the Cochrane Risk of Bias
125 assessment [21]. The quality of evidence (i.e., certainty in the estimates) was evaluated using the Grading of
126 Recommendations Assessment, Development, and Evaluation approach (GRADE) [22].
127 Criteria used to evaluate quality of evidence were risk of bias, indirectness (surrogate outcomes), imprecision (wide
128 confidence intervals), inconsistency (heterogeneity), and publication bias [23].

129

130 **RESULTS**

131 The initial search resulted in 668 citations of which 62 were duplicates. We eventually included 11 studies that were
132 published between 2008 and 2018 (Table 1). Six studies informed about TDF PK during pregnancy [24-29] and five on
133 drug concentrations of which three were after a single dose of drugs during childbirth [30-34]. The average weighted
134 kappa for study selection was 0.71 (good, supplementary table 1). The study selection process and reasons for
135 exclusions are shown in Figure 1.

136 The breastmilk search resulted in 107 citations of which 11 were duplicates. We included four studies in our final
137 analyses (Figure 2). These studies were all conducted in Africa. Three studies included HIV infected women [35-37]
138 and one included HIV negative women who received TDF as part of Pre Exposure Prophylaxis (PrEP, [17]).

139

140 **Assay Methodologies**

141 The PK parameters were tested with high performance liquid chromatography (HPLC) with fluorescence detection
142 which has been validated according to the International Conference of Harmonisation Guidelines in terms of accuracy,

143 precision, specificity, robustness, limits of detection and quantitation, and other aspects of analytical validation [38] or
144 Liquid chromatography–mass or tandem-mass spectrometry (LC-MS/MS) methods, validated for the multiplexed
145 quantification of TAF and TFV [39] (Supplementary Table 2). The analyzed PK samples were of maternal plasma, cord
146 blood, infant plasma, amniotic fluid and breastmilk; and presented as concentration ratios.

147

148 **Risk of bias within studies**

149 The quality assessment of the studies was based on the GRADE scoring system, (Supplementary Table 3). In this
150 system the studies were scored on study limitations (low, medium or high level), directness, consistency, precision and
151 reporting bias. Most of the studies did not report how patients were selected and did not report the proportion of patients
152 who agreed to participate resulting in medium levels of study limitations. There were no reporting biases detected.

153

154 **Results of individual studies**

155 Pharmacokinetics during pregnancy

156 Five publications described steady-state PK parameters [25-29]. A computer simulated modeling study was also
157 included [24] (Table 2). Of the five steady state PK studies, four included HIV-infected or non-infected pregnant
158 females treated with 300mg TDF in steady state, either as PrEP or as treatment, and only Cressey et al. included HBV
159 infected women. The non-compartmental analyses (NCA) of all publications indicated that the AUC/f and C_{max} values
160 were significantly lower in pregnancy compared to non-pregnant women.

161 The study by Colbers et al. showed that 26% of the patients receiving TDF in pregnancy did not meet the threshold of
162 2,000 ng/ml*h AUC₀₋₂₄ (defined as the threshold for TFV efficacy for HIV being the 10th percentile in non-pregnant
163 controls) in the third trimester compared with only 4% of the patients in the post-partum period. One in nine patients
164 that had an AUC below the threshold had a detectable HIV viral load at delivery compared to six out of 25 with AUC
165 above the threshold [27].

166 Colbers et al. showed that the C_{max} returned to normal after delivery [27]. Best et al. showed a similar trend, however
167 this was not statically significantly different between trimesters and the postpartum period [26]. Accordingly the C_{last}
168 reported in the various studies was decreased in pregnant women and normalized postpartum [28]. Best et al. and
169 Colbers et al. showed a decrease of 20% and 23% in AUC/f comparing the third trimester and post-partum, respectively
170 [26, 27]. These studies estimated an increased oral clearance (Cl/f) of TFV in the third trimester compared with
171 postpartum of 57-55 l/H versus 46-43 l/H, respectively. Thus the oral clearance (Cl/f) of TFV increased significantly in
172 comparison to post- partum and non-pregnant women and the AUC is inversely correlated with clearance.

173

174 The data from these studies [17,18] was used for the computer modeling [24]. The modeling study used population
175 characteristics of a certain patient population and physicochemical parameters of the drug to simulate drug plasma
176 concentrations. Oral clearance (Cl/f) was estimated to increase until an estimated gestational age (EGA) of 28 weeks
177 and then to slowly decrease again. Thus tenofovir C_{max} , C_{last} and AUC all decreased in HIV pregnant women in the
178 second and third trimester. The absorption time (T_{max}) did not change during pregnancy. Best et al. [26] estimated a
179 terminal $t_{1/2}$ of 16.1 h during pregnancy compared with 12.4 h postpartum [26] which suggests that the exposure
180 parameters are affected mainly by an increased volume of distribution, V_d . There were no data on the intracellular
181 phosphorylation kinetics of TFV in pregnancy.

182

183 TFV concentrations

184 The study from Pyra et al. [29] compared the mean concentrations of TFV during the different trimesters in pregnancy
185 and in pre-pregnant and in non-pregnant women when the drug was provided for pre-exposure prophylaxis HIV. Their
186 data suggests that after the first trimester the mean concentration of TFV decreases to below the non-pregnant
187 concentration (Figure 3). During pregnancy the TFV concentrations were 45–58% lower compared with non-pregnant
188 after adjusting for adherence. Cressey et al. [28] assessed TFV exposures in HIV uninfected HBV infected pregnant
189 women (estimated gestational age 28 weeks), and showed that TFV exposure was 20% lower in pregnancy compared
190 with postpartum. This was similar to the results reported in studies of HIV infected pregnant women.

191

192 Pharmacokinetics during childbirth

193 Three publications provide PK information after a single oral dose (600mg or 900mg) of TDF during childbirth [30-32].
194 The reported C_{max} after 600mg TDF differed widely among all studies with 234 (range 83-595) ng/ml, 310 (range 70–
195 520) and 448 (range 110-928) ng/ml. The reported AUCs of the women who received 600mg TDF were 2.73 mg/l*hr
196 (range 1.43-3.55) and 4.22 (range 2.77-24.46) mg/l*hr. The $t_{1/2}$ following the 600 mg dosage was 15.9-19.5 (range 3.3-
197 187.6) hours.

198

199 Cord blood concentrations

200 Rimawi et al. [33] compared paired maternal (delivery) and cord blood samples of 10 patients who received a TDF
201 containing ARV regimen. They found a maternal plasma TDF concentration of 94.6 ng/ml with a cord plasma of 38.5
202 ng/ml, or a median cord plasma/maternal plasma ratio of 1:2.5 (95%CI). Yeh et al. [34] measured drug concentrations
203 at delivery in maternal blood plasma, cord blood plasma, and amniotic fluid. The TFV concentration in the maternal
204 plasma was 5.0 ng/mL and in cord plasma was 30 ng/ml. These two studies report very different cord plasma / maternal
205 plasma ratios which could be due to the different assays used in the studies, LC-MS/MS and HPLC.

206

207 Breast milk.

208 In four studies, maternal plasma and breastmilk samples, taken within 30 minutes of each other, were compared (Table
209 3), three studies were done in HIV infected women. The timing of the samples in relation to the TDF dose varied from
210 1-2 hours after the maternal TDF dose to over 12 hours [35-37, 40]. The median TDF concentration in breastmilk
211 varied from 3.2 to 14.1 ng/ml with a maternal plasma concentration of 86.7 to 293.0 ng/ml. This resulted in a
212 breastmilk/maternal plasma ratio of 0.03 to 0.07. TFV was excreted in breastmilk in a very low concentration, lower
213 than the concentrations found in cord blood samples, resulting in a median amount of ingested TFV in infants of 0.03%
214 of the recommended infant dose.

215

216 **DISCUSSION**

217 In order to prevent MTCT of HBV and HIV in pregnancy, the viral load needs to be low or preferably undetectable
218 particularly at the time of birth. HBV DNA suppression may be compromised by lower exposure to TDF in later
219 pregnancy. In this review the previously stated threshold for the C_{max} of 300 ng/ml for HBV suppression was not
220 achieved. This review shows that the C_{mean} and C_{max} of TFV decrease during pregnancy. With T_{max} remaining the same,
221 the absorption of the drug appears to be unaffected. The decrease in C_{max} could be a result of the increasing V_d , due to
222 an physiological increase of total body water and extracellular fluid during pregnancy [14]. In one published manuscript
223 this led to a sufficient C_{max} in the first trimester, but C_{max} then decreased as the pregnancy evolved [14-16]. Drug
224 elimination is increased during pregnancy with increased clearance until a gestation of approximately 28 weeks after
225 which it slowly decreases [26, 27, 41]. Since TDF is excreted mainly by glomerular filtration, its renal clearance is
226 expected to parallel changes in creatinine clearance during pregnancy [15]. As the AUC is dependent on the clearance,
227 an increase in the creatinine clearance results in a decrease in TFV AUC per trimester of the pregnancy. This review
228 supports conclusions of Benaboud et al., that dosing escalation be considered from 2nd trimester to achieve similar
229 exposure to non-pregnant adults with the caveat that clinical experience of more than 300 mg daily is limited [41].
230 Dense and sparse PK sampling under TDF treatment correlated with HBV DNA load across gestation and post-partum
231 (or pre-pregnancy) are required to determine if dose adjustment is needed. If this is needed, it needs to be considered
232 that TDF follows a dose-linear PK [9].

233

234 **Effect of pharmacokinetics on HBV DNA suppression**

235 While this review shows reduce exposure of TDF in pregnancy the implications of this altered PK on HBV DNA
236 suppression are unclear because of unknowns in the PK PD relationships in the initial clearance phase and the treatment
237 duration factor. Previously it has been suggested that the decrease of AUC, C_{max} and C_{min} that occurs in pregnancy does

not affect the viral load in HIV patients [27]. However, the women enrolled were taking multiple antivirals, making it unclear if it was the TDF suppressing the virus or one of the other antivirals. Moreover, co-formulated TDF increases the AUC, C_{max} and C_{min} of TFV [42].

Treatment with TDF can reduce the HIV or HBV viral load with an adequate length of time before delivery. In non-pregnant chronic HBV infected patients treated with TDF, maximal suppression of HBV DNA plateaus at approximately 32 weeks with good compliance and with a 4 week viral load reduction of approximately 3.9-4.0 $\log_{10}$ IU/ml [43-45]. This reduction is higher than reported in pregnancy, where the HBV DNA decreases between 2.75 and 3.52 $\log_{10}$ IU/ml after 4 weeks of TDF therapy with up to 32% of mothers with an HBV DNA >200,000 IU/ml at delivery [6, 46-48]. If started earlier in pregnancy (gestation of 24 weeks), the viral decline can reach 4.08- 5.23 $\log_{10}$ IU/ml until childbirth, however, in LIC women tend to present later in pregnancy [49]. The HBV DNA viral load needed to omit HBIG is unknown but potentially negated by longer dosing prior to childbirth.

In studies on the PK and PD of TFV in HIV infected individuals, dose escalating data from 75, 150, 300 and 600mg showed a dose-proportional increase in viral suppression until 300mg, the 600 mg dose could not produce a steeper viral decay compared to the 300mg dosage [43, 50]. The C_{max} were proportional to the dosage with 375 ng/ml vs 573 ng/ml after 8 hours for 300mg and 600mg respectively. The studied plateau TFV concentrations in the healthy individuals was higher compared to the described PK data in pregnancy. This suggests that in healthy individuals the maximum achievable HIV viral decay is with 300mg but this might be different in pregnancy when the drug concentrations are lower; or different for HBV. Increasing the dosage of TDF to get a C_{max} as observed in healthy individuals might produce a steeper viral decay and, as toxicity is related to drug concentrations, would not necessarily increase risks.

More data is needed in the PK/PD of TDF in pregnancy, in particular on the impact of variable circulating blood concentrations on the clearance rate of HBV. This information could guide recommendations on dosing and the duration of treatment in the prevention of MTCT particularly when optimal delivery of HBIG and birth dose vaccination are compromised.

TDF in breastfeeding

TFV concentrations in breastfeeding infants are mostly undetectable or lower than considered of clinical significance. These findings corroborate with findings from animal studies [51] and suggests that usage of TDF in breastfeeding women is safe for the infant but of no therapeutic value. Most liver disease societies including American Association for the Study of Liver Diseases and European Association for the Study of the Liver support allowance of breast feeding when mothers were taking TDF. In a resource-constrained setting the risk of infant infection from lactation

269 (cracked nipples) due to HBV rebound (typically 4-8 weeks) after TDF cessation at one month post-partum and before
270 protection from infant vaccination is established (when HBIG and birth dose are not accessible), is unknown.

271

272 **Limitations**

273 This systematic review has several limitations including few available studies most of which are on HIV. The number
274 of studies is disproportionally low compared to the number and ethnicities of pregnant women with HIV and HBV
275 infections worldwide. The two studies reporting on the drug concentrations used different assays to measure the PK
276 which makes comparison difficult. The impact of pharmacogenetics is not reviewed, although it is known that genetic
277 variation of HBV might impact the PK. Lastly, the studies reporting on pharmacokinetics in pregnancy are all based in
278 Europe or USA. This makes the results difficult to translate to patients elsewhere, although no difference in PK has
279 been reported for TFV between these populations in non-pregnant adults.

280

281 **CONCLUSION**

282 This main two highlights of this review are firstly the limited data on the pharmacokinetics of TDF monotherapy
283 treatment for HBV in pregnant women which suggest lower concentrations of TFV in second and third trimester of
284 pregnancy compared to first trimester and non-pregnant status. Secondly, studies to date have occurred in settings
285 where other HBV risk reduction measures (birth dose, HBIG, TDF during lactation) were optimized and as these are not
286 routinely provided in resource-constrained settings consideration for ensuring the most favourable exposure before
287 childbirth is useful.

288

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294 **CONFLICT OF INTEREST**

295 EJS declares to have received travels grants from Abbvie and Gilead.

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298

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421 Figure 1. PRISMA Flow Diagram for pharmacokinetic studies on TDF in pregnancy

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424 Figure 2. PRISMA Flow Diagram for pharmacokinetic studies on TDF in breastmilk

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429 Figure 3. TFV_{mean} concentration in pregnant (n=37) and non-pregnant (n=97) women in tenofovir pre-exposure
430 prophylaxis [29]

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433 Light grey: TFV_{mean} in women that had sampling done before and during pregnancy, as per manuscript - these are the
434 same women;

435 Black stripes: TFV_{mean} in women that did not had sampling done before pregnancy, as per manuscript - these are the
436 same women;

437 100% medication event monitoring system and unadjusted for dose; TFV-tenofovir

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Table 1. Summary of evidence

Author	Country (period)	Type of study	Study Population PW- pregnant women	Intervention (drug (manufacturer), timing and dose)	PK model	PK variables	Steady state/single dose	GRADE score
Benaboud [25]	France	Observational drug monitoring	186 PW and 46 non-PW with HIV, EGA 28-38 weeks	TDF oral 300mg OD with other ARV	One and two compartment model	V _c , Q, V _p , k _a , C _{min} , AUC, CL/F	Steady state	Moderate
Best [26]	USA clinics from the P1026s cohort (Nov 04-Sep 08)	Multicenter, prospective non blinded	37 PW with HIV infection, EGA 20-38 weeks	TDF oral 300mg OD with other ARV	Two compartment model	V _d /F, C _{max} , t _{max} , C ₀ , C ₂₄ , AUC, t _{1/2} , CL/F,	Steady state	High
Colbers [27]	PANNA network (Nov 08-Jan 12)	Non randomized, open label, prospective, multicenter	34 PW with HIV, EGA 28-38 weeks, median 33 weeks	TDF oral 300mg OD with FTC	Non compartment model	C _{max} , C ₂₄ , t _{max} , AUC, t _{1/2} , CL/F	Steady state	High
Cressey [28]	Thailand (Jan 2013-Dec 2016) and PANNA network	Data were combined from two trials	154 PW with HBV, EGA 28 weeks	TDF oral 300mg OD with and without FTC	Two compartment model	AUC, C ₂₄ ,	Steady state	Moderate
De Sousa Mendes [24]	France (2014)	PK modeling based on physiology	Male volunteers	TFV, FTC, 3TC	Physiologically-based pharmacokinetic	V _{ss} , C _{max} , t _{max} , AUC, Cl/F	Simulation	Moderate

					two compartment model			
Flynn [30]	USA and Puerto Rico	RCT	31 HIV infected PW, EGA $\geq$ 34	600mg TDF, 900mg TDF or 900/600 TDF/FTC before delivery with 2 4mg/kg TDF for infant or 4/3 mg/kg TDF/FTC for child	Non compartment model	C _{max} , t _{max} , C ₂₄ , AUC, t _{1/2} , concentration at delivery	Single dose	Moderate
Hirt [31]	ANRS 12109 study TEmAA in Africa/Asia	Modelling study	38 HIV+ PW, EGA 28-38	NVP/TDF 600mg at delivery and TDF 300mg 7 days pp	Two and three compartment models	V/F, ka, C _{min} , C _{max} , AUC, CL/F	Single dose	Moderate
Mirochnick [32]	HPTN 057, Malawi and Brazil	Open label, non-controlled intervention trial	122 HIV+ PW, 3 rd trimester, and neonates	TDF 600mg at delivery/no infant dosing. Or TDF 900mg a delivery/infant 4mg/kg or TDF 900mg at delivery/infant 6mg/kg day or TDF 600mgat delivery/infant 6mg/kg daily for 7 days	Non compartment model	C _{max} , t _{max} , C _{through} , AUC, t _{1/2} , concentration at delivery	Single dose	Moderate
Rimawi [33]	USA (Apr 15-Jun 16)	Prospective cohort pilot study	13 HIV+ PW, 2 nd trimester	EVG (or DTG) /COBI/TDF/FTC	Unknown	Drug concentrations and drug concentration ratios	Steady state	High

Pyra [29]	Uganda and Kenya (Nov 2012 - Jan 2015)	Stored plasma analyses of an open label study	37 HIV negative PW, 1 st trimester, and control group with 97 non-PW	Coformulated FTC/TDF 200 mg/300 mg (PrEP)	Unknown	Mean concentrations per trimester	Steady state	High
Yeh [34]	North Carolina (Feb 04-Dec 05)	Prospective, open label, longitudinal study	14 HIV+ PW, EGA >10 weeks	3 women TDF 300mg once daily with other ARV	Unknown	Drug concentrations and drug concentration ratios	Steady state	Moderate

Breastmilk studies

Author	Country (period)	Type of study	Study Population	Intervention (drug, timing and dose)	PK variables	Steady state/single dose	GRADE score
Benaboud[35]	Cote d'Ivoire	Prospective as part of the TEmAA study	5 exclusively breastfeeding mothers, HIV+	TDF oral 600mg at delivery (in combination with FTC and NVP) and FTC-TDF OD for 7 days	Drug concentrations in breastmilk and maternal plasma. Simulated minimal and maximal milk doses	Steady state	Moderate

Mugwanya[17]	Kenya and Uganda (Jan-Jun 2015)	Prospective, open label, single arm	50 HIV negative breastfeeding mothers	TDF oral 300mg OD (PrEP)	Drug concentration in infant plasma, maternal plasma and whole breastmilk, M/P, infant plasma drug-to-milk	Steady state	High
Palombi [36]	Malawi	Prospective as part of the Option B-Plus approach	47 HIV infected breastfeeding mothers	TDF oral 300mg OD n combination with other ARV	Drug concentration in infant plasma, maternal plasma and whole breastmilk, M/P	Steady state	High
Waitt [37]	Uganda and Nigeria	Prospective	27 HIV infected and breastfeeding mothers	TDF 300mg OD in combination with NVP, 3TC, EFV and/or FTC	Drug concentration in infant plasma, maternal plasma and whole breastmilk, M/P	Steady state	Moderate

3TC, Lamivudine; AUC, Area under the plasma concentration-time curve; ARV, Antiretrovirals; C_{max} , Maximum (peak) plasma drug concentration; C_{min} , Minimum plasma drug concentration; C_0 , Initial (fictive) or back-extrapolated plasma drug concentration at time zero following bolus intravenous injection; C_{24} , plasma drug concentration at time 24 following bolus intravenous injection; $C_{through}$, Trough plasma concentration (measured concentration at the end of a dosing interval at steady state [taken directly before next administration]); CL/F, Apparent total clearance of the drug from plasma after oral administration; COBI, Cobisistat; DTG, Dolutegravir; EGA, Estimated Gestational Age; EVG, Elvitegravir; RFV, Efavirenz; FTC, Emtricitabine; HIV, Human Immunodeficiency Virus; k_a , Absorption rate constant (first-order); M/P, milk to plasma ratio; NVP, Nevirapine; OD, Once Daily; PK, Pharmacokinetic; PrEP, Pre Exposure Prophylaxis; PW, pregnant women; Q, intercompartmental clearance; $t_{1/2}$, Elimination half-life; TDF, Tenofovir Disoproxil Fumarate; t_{max} , Time to reach maximum (peak) plasma concentration following drug administration; V_c , central volume of distribution; V_d/F , apparent peripheral volume of distribution; V_{ss} , Apparent volume of distribution at steady state; V_p , peripheral volume of distribution;

Table 2. Pharmacokinetic outcome measurements of TDF in pregnancy

Author	C _{max} (ng/ml)			C ₂₄ (ng/ml)			AUC (ng/ml.h)		
	Second trimester	Third trimester	Post-partum	Second trimester	Third trimester	Post-partum	Second trimester	Third trimester	Post-partum
Benaboud [25]					39 (22-92)			1,600 (900-3,300)	
Best [26]	250 (202-355)	245 (207-334)	298 (200-341)	39 (34-49)	54 (40-70)	61 (45-79)	1900 (1700-2500)	2,400 (1,900-3,100)	3,000 (2,200-3,700)
Colbers [27]		280 (240-310)	330 (290-390)		52 (47-59)	66 (58-76)		2,460 (2,230-2,660)	3,170 (2,860-3,520)
De Sousa Mendes [24]		270 (120-910)							
Cressey [28]					40 (34-48)	56 (48-68)		1,830 (1,650-2,090)	2,280 (2,050-2,770)

AUC, Area under the plasma concentration-time curve; C₂₄, plasma drug concentration at time 24 following bolus intravenous injection; C_{max}, Maximum (peak) plasma drug concentration

Table 3. Drug concentrations of TDF in breastmilk

Author	C_{min} in breastmilk (ng/ml)	C_{max} in breastmilk (ng/ml)	Median ingested TFV dose mcg/kg/day	Infant concentration (ng/ml)	Concentration in maternal plasma (ng/ml)	Breastmilk/plasma ratio (M/P)
Benaboud [35]	6.8 (5.83-8.75)	14.1 (11.60-16.25)	1.4 (1.1-2.4)	undetectable		
Mugwanya [17]		3.2 (2.3-4.7)	0.47 (0.35-0.71)	94% undetectable	152.0 (56.9-321.0)	0.03 (0.01-0.05)
Palombi [36]		5.0 (0-6.1)		24 (0-51.6)	86.7 (73.7-102.6)	0.07 (0.06-0.08)
Waitt [37]		5.98 (0-8.05)			293 (176-391)	

C_{max}, Maximum (peak) plasma drug concentration; C_{min}, Minimum plasma drug concentration; TFV, Tenofovir

Supplementary material

Search strategy

Supplementary Table 1. Kappa calculation

Supplementary Table 2. Reporting methodology and limit of quantification of tenofovir assay

Supplementary Table 3. Quality assessment of the studies was based on the GRADE scoring system