Quantifying the impact of nevirapine-based prophylaxis strategies to prevent mother-to-child transmission of HIV-1:

A combined pharmacokinetic, pharmaco- and viral dynamic analysis to predict clinical outcomes

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Abstract

Nevirapine single dose (sd-NVP) and extended NVP prophylaxis are widely used in resource-constrained settings to prevent vertical HIV-1 transmission. We assessed the pharmacokinetics of sd-NVP in 62 HIV-1 positive Ugandan pregnant woman and their newborns, taking sd-NVP prophylaxis to prevent mother-to-child HIV-1 transmission. Based on this data we developed a mathematical model system to quantify the impact of different sd-NVP regimens at delivery and extended infant NVP prophylaxis (6, 14, 21, 26, 52, 78, 102 weeks) on the 2 year risk of HIV-1 transmission and development of drug resistance in mothers and their breast-fed infants.

Pharmacokinetic parameter estimates and model-predicted HIV-1 transmission rates were very consistent with other studies. Predicted 2 year HIV-1 transmission risks were 35.8% without prophylaxis, 31.6% for newborn sd-NVP, 19.1% for maternal sd-NVP and 19.7% for maternal/newborn sd-NVP. Maternal sd-NVP reduced newborn infection predominately by trans-placental exchange, providing protective NVP concentrations to the newborn at delivery, rather than by maternal viral load reduction. Drug resistance was frequently selected in HIV-1 positive mothers after maternal sd-NVP.

Extended newborn NVP prophylaxis further decreased HIV-1 transmission risks but indicated an overall decline in cost-effectiveness for increasing durations of newborn prophylaxis. The total number of newborn infections with resistant virus was not increased by extended newborn NVP prophylaxis.

The developed mathematical modeling framework successfully predicted the risk of HIV-1 transmission and resistance development and can be adapted to other drugs/drug combinations to a priori assess their potential in reducing vertical HIV-1 transmission and resistance spread.
Introduction

HIV-1 infection remains a serious health care problem worldwide. In 2009, approximately 370,000 children became infected with HIV-1 (53). Mother-to-child transmission rates of HIV-1 in untreated breastfeeding populations in resource-limited settings ranged from 25% to 48%, accounting for the vast majority of pediatric AIDS (12). Vertical transmission of HIV-1 may occur during pregnancy (5%-10%), during birth (10%-20%), and via breastfeeding (10%-20%) (12).

Intrapartum and newborn single dose nevirapine (NVP) significantly reduce transmission of HIV-1 from the mother to the child (23) and are essential components of HIV-1 prevention strategies in many resource-constrained settings (57-58). However, the exact mechanism of HIV-1 prevention by NVP during intrapartum transmission remains unknown. Furthermore, owing to its long half-life, NVP frequently selects drug resistant viral strains in HIV-infected mothers (17, 22), which can compromise the efficacy of follow-up maternal and newborn antiretroviral treatment (ART) (8, 25, 29, 39).

In many resource-constrained settings, breastfeeding is critical for infant survival (59). Reduction of HIV-1 transmission by short-course antiviral prophylaxis is frequently impaired by subsequent infection during the breastfeeding period (24, 42). Extended newborn NVP prophylaxis has shown to reduce HIV-1 transmission via breastfeeding (3, 6, 27, 38) and current WHO guidelines for the prevention of mother-to-child transmission recommend the use of NVP throughout the entire breastfeeding period (57), which can be as long as 2 years. Clinical trial data on extended prophylactic newborn NVP are currently only available for durations of 6 weeks and 6 months (3, 10, 38). However, to evaluate the effectiveness of extended newborn NVP, a quantification of the HIV-1
transmission risks after different durations of extended NVP prophylaxis in newborns is required.

In the present study, NVP plasma data of 62 Ugandan mothers and newborns who took NVP single dose prophylaxis were simultaneously analyzed in a single integrated population pharmacokinetic model for both populations; the present work extends a previously published pharmacokinetic study, which analyzed woman and newborn NVP concentrations separately (28). The aim of this work was to combine pharmacokinetic and pharmacodynamic analysis by developing a single mathematical modeling framework. The framework should be used to predict the impact of various single and extended NVP-based prophylaxis regimens on the cumulative risk of vertical HIV-1 transmission and on selection of NVP-resistant virus.

Methods

Patient Characteristics and Study Design

During a program for the prevention of mother-to-child transmission of HIV-1 in western Uganda, 62 HIV-1 positive pregnant women and their newborns were enrolled for pharmacokinetic (PK) analysis after they had given informed consent and delivered at Fort Portal District Hospital (Fort Portal, Kabarole District, western Uganda). Pregnant women received a single 200 mg NVP tablet at onset of labor and newborns received 2 mg/kg NVP syrup orally within 72 h after birth (28). Ethical approval was obtained from the Uganda National Council for Science and Technology.

Median age and body weight of the pregnant women were 26 years and 56 kg [range: 16-39 yrs; 42-84 kg], respectively. Newborns had a median body weight of 3.1 kg [range: 2.0-3.9 kg]. The median time period passed between NVP intake of pregnant women...
and birth was 5.1 h [range: 0.3-24.8 h]. The median time interval between birth and NVP administration to the newborn was 0.9 h [range: 0.1-40.6 h] and 8.5 h [range: 1.3-46 h] from NVP intake of the pregnant women until the NVP administration to the newborns (28).

For PK analysis, a total of 113 plasma samples from mothers and newborns were collected over three time periods, i.e. delivery, week 1 and week 2. The geometric mean NVP concentration-time profile was previously presented (28). Here we illustrate the dispersion of the individual plasma concentrations over time for the same population (Figure 1 A). NVP concentrations were determined by a validated LC/tandem-mass-spectrometry method according to the criteria set by the FDA (FDA-Guideline, (28, 48)).

*Pharmacokinetic Analysis*

Based on the previously established pharmacokinetic models and data (28), an integrated population pharmacokinetic model was developed to simultaneously analyze NVP plasma data of mothers and newborns.

For population PK data analysis the nonlinear mixed effects modeling approach implemented in the software program NONMEM™ (Icon development solutions, version VI, 1st update, 2006) was chosen due to sparse data situation. The pharmacokinetic model was parameterized in terms of clearance(s) (CL) and volumes(s) of distribution (V) using the PREDPP subroutines (FOCE with interaction, ADVAN6 TOL5) supplied in NONMEM™. The model building process was guided by changes in the objective function value of nested models provided by NONMEM™, by precision of the PK parameter estimates (relative standard errors, RSE) and by basic goodness of fit (GOF) plots. In addition, 2000 bootstrap data sets were assessed using NONMEM™. For
model evaluation, the final PK parameter estimates were compared to the corresponding median and 95% confidence interval of the bootstrap runs. Model-based simulations for visual predictive checks (VPC) were performed by NONMEM\textsuperscript{TM} (n=1000 simulations) and the statistics of 5\textsuperscript{th}, median and 95\textsuperscript{th} percentile were calculated using R, version 2.9. The schematic structure of the final PK model for maternal and newborn data is presented in Figure 1 B. Due to the difference in drug transport processes, solid lines represent those occurring continuously over the whole time and dashed lines only those before delivery except K34 which occurs only after delivery. The NVP absorption rate constants for pregnant women (KA) and newborns (K34) were fixed to 1.34 h\textsuperscript{-1}, respectively, due to no available data during the absorption process. Prior published values of absorption rates varied between 0.013 h\textsuperscript{-1} and 3.81 h\textsuperscript{-1} (median: 1.3 h\textsuperscript{-1}) (4, 11, 16, 26).

Maternal plasma concentrations were associated to the central compartment with the volume of distribution V2 and fetal/newborn concentrations to the peripheral compartment with the volume of distribution V4. After delivery, but before the NVP administration to newborns, significant NVP concentrations were detected in the plasma of newborns. Considering the placenta-permeability of NVP (35, 37), we implemented a trans-placental exchange of NVP between pregnant woman and fetus (PCL) before the time of delivery (dashed lines in Figure 1 B). The ratio of NVP plasma concentrations between fetus and pregnant women was described by a partition coefficient, PCM. NVP elimination from the central compartment (related to the plasma of mothers) and the peripheral compartment (related to the plasma concentration in the fetus/newborn) were described by the PK parameters CL1 and CL2, respectively.
For the predictive performance of the final PK model a visual predictive check (VPC) is depicted in Figure 2 A and B. The dashed lines represent the 5\textsuperscript{th} and 95\textsuperscript{th} model-simulated percentiles and solid lines represent the model-simulated median of NVP concentrations. The VPC of mother plasma (Figure 2 A) and newborn plasma data (Figure 2 B) revealed sufficient model predictive performance for the general trend. Overall, the model-predicted variability was sufficient for mothers and newborns and resembled the variability in the observed data.

**HIV-1 Dynamics Model**

In order to quantify the impact of NVP prophylaxis on virus transmission, we adapted the virus dynamics model presented in (55) by discarding the longer lived cell types (representing macrophages and latently infected T-cells), as they do not impact the observed viral dynamics after short course maternal NVP. The utilized model of HIV-1 dynamics and mother-to-child transmission is depicted in Figure 3 A.

Briefly, the mathematical model of virus dynamics and mutation comprises T-cells $T$, free virus $V$, early infected T-cells $T_1$ (after reverse transcription but before viral genomic integration) and productively infected T-cells $T_2$ (after viral genomic integration). The average rate of change of the different T-cell species and the number of viruses is given by the following system of ordinary differential equations:

\[
\frac{dT}{dt} = \dot{\lambda}(t) - T \cdot \delta_T - \sum_i \beta(i, t) \cdot V(i) \cdot T + \sum_i \delta_{\text{PIC}} \cdot T_1(i) 
\]

\[
\frac{dT_1}{dt} = \sum_j p_{j \rightarrow i} \cdot \beta(j, t) \cdot V(j) \cdot T - T_1(i) \cdot (\delta_{T_1} + k_T + \delta_{\text{PIC}}) 
\]

\[
\frac{dT_2}{dt} = k_T \cdot T_1(i) - T_2(i) \cdot \delta_{T_2}
\]
\[
\frac{d}{dt} V(i) = N \cdot T_2(i) - V \cdot CL_V(t)
\] (4)

In summary, free virus \( V \) of strain \( i \) can infect T-cells with infection rate constant \( \beta \), which encompasses all steps from target cell binding, fusion, to reverse transcription, resulting in early infected cells \( T_1 \), which turn into productively infected cells \( T_2 \) by provirus translocation into the nucleus and integration with rate \( k_T \). \( T_2 \) produce new virus \( V \) with the rate constant \( N \) (on average 1000 virions/day/cell (45)). Native, early infected and productively infected T-cells are degraded with rate constants \( \delta_T \), \( \delta_{T_1} \), and \( \delta_{T_2} \), respectively. In early infected cells \( T_1 \) (prior to proviral integration) essential components of the pre-integration complex can be degraded with rate constant \( \delta_{pic} \), returning the cell to an uninfected stage \( T \) (55). Native T-cells are produced with rate constant \( \lambda(t) \) and free virus \( V \) is cleared with rate \( CL_V(t) \) by the immune system. We assumed that the rate constants \( \lambda(t) \) and \( CL_V(t) \) are constant for the HIV-infected mothers, whereas they were considered time-dependent for the newborn, due to immune system development and growth. A derivation of the parameters \( \lambda(t) \) (newborn) and \( CL_V(t) \) (newborn) is provided in the supplementary text S1. All model parameters are displayed in Table 1.

Viral Mutation:

HIV can acquire drug resistance by mutation during the process of reverse transcription (comprised in parameter \( \beta \) in the model). The probability that a specific mutation occurs during the process of reverse transcription has been quantified \textit{ex vivo} to be \( \mu = 2.16 \cdot 10^{-5} \) (per base and reverse transcription process) (30). A single genomic point
mutation inducing a change at the protein level, e.g. position Y181 -> 181C (Y181C) will therefore occur with probability \( \mu \) during reverse transcription, whereas with probability 
\( (1 - \mu) \) this specific mutation will not occur. In our model, 2 specific sites \( L \) are regarded to undergo mutation; resulting in the K103N and the Y181C change in the reverse transcriptase enzyme, respectively. As an example, the probability that the wild type virus \( wt \) will neither be mutated at one of the 2 sites is 
\[ p_{wt \rightarrow wt} = (1 - \mu)^2. \]
The probabilities that precisely one mutation occurs is given by 
\[ p_{wt \rightarrow Y181C} = (1 - \mu) \cdot \mu \] 
and the probability of two specific mutations by 
\[ p_{wt \rightarrow K103N/Y181C} = \mu^2. \]
More generally, the probability that a certain transition by mutation from some strain \( j \) to some strain \( i \) occurs during reverse transcription \( p_{j \rightarrow i} \), is given by:
\[
 p_{j \rightarrow i} = \mu^{h(i,j)} \cdot (1 - \mu)^{L-h(i,j)}, 
\]
where \( h(i,j) \) denotes the hamming distance (the number of differences) between strain \( j \) and strain \( i \). All mutation probabilities for the utilized model are depicted in Figure 3 B.

Coupling of Viral Dynamics with NVP Pharmacokinetics:

The efficacy of the non-nucleoside reverse transcriptase inhibitor NVP \( (1 - \eta(i,t)) \) at time \( t \) against strain \( i \) was implemented using the standard \( E_{\text{max}} \) model with slope parameter \( (44, 47) \):
\[
(1 - \eta(i,t)) = \frac{1}{1 + \left( \frac{C(t)}{IC_{50}(i)} \right)^{h(i)}} 
\]
where \( C(t) \) denotes the NVP concentration at time \( t \) (derived during PK analysis, see above), \( IC_{50}(i) \) denotes the strain-specific fifty percent inhibitory concentration and \( h(i) \)
denotes the strain-specific slope parameter (see Figure 3 C). The strain specific infection rate constant under treatment was given by
\[ \beta(i,t) = (1 - \eta(i,t)) \cdot (1 - s(i)) \cdot \beta(wt, \phi) \cdot SF(t), \]
where \( \beta(wt, \phi) \) denotes the infection rate constant of the wild type \( wt \) in the absence of drug \( \phi \) (given in Table 1) and \( s(i) \), denotes the fitness loss (e.g., loss in the activity of reverse transcriptase) relative to the wild type (shown in Figure 3 C). The scaling factor \( SF(t) \) corrects the infection rate \( \beta \) for the differences in target cell concentration between mother (reference target cell concentration) and uninfected newborn. \( SF(t) \) was considered to be time-dependent for newborns due to immune system development and growth (see Equation S3 and Figure S1 of the supplementary material), whereas it was set to the value of 1 for HIV-1 infected mothers.

**Deterministic –Stochastic Hybrid Simulation**

The kinetics of biological systems, in which all reactions occur quasi-continuously over time or involve large numbers of reactants are well approximated by continuous-deterministic simulations (by numerical solution of the systems’ ordinary differential equations). However, the exact kinetics of biological systems which involve rare reaction events with small numbers of reactants are intrinsically stochastic and are therefore only poorly approximated by continuous-deterministic simulation (61). In our modelling framework, the process of HIV-1 transmission denotes such an event in which the outcome is intrinsically stochastic: Either the transmitted virus becomes entirely cleared by the immune system before establishing stable infection \( (V = 0) \), or it succeeds in establishing infection \( (V \) approaches its steady state).
In order to fully regard the intrinsic stochasticity of rare events in the utilized model (such as viral challenges) and to allow efficient simulation of quasi-continuous kinetics, we chose the deterministic-stochastic hybrid simulation approach presented in (1).

*Prediction of HIV-1 Transmission after Intrapartum- and Extended NVP Prophylaxis*

Model simulations were performed starting with the time of the first maternal dose (if no dose was given, with the time of birth) and continued until 2 years postpartum. The number of viruses coming into contact with the newborn during delivery and breastfeeding was modeled as a function of the maternal viral load at the particular time of the respective viral challenge. The intrapartum virus transmission was modeled in terms of a single viral challenge at the time of delivery, while virus transmission via breastfeeding was modeled in terms of repeated viral challenges during the time after delivery until 2 years postpartum. The probability of a viral challenge during breastfeeding was assumed to decrease over time (see Figure S2, supplementary material). Child growth and immune system development were considered simultaneously to the model simulation (see supplementary text S1 and Figure S1, supplementary material). If stable infection of the newborns occurred (defined as total number of viruses $\geq 1 \cdot 10^6$ in the newborn) the respective simulation was stopped and the time between birth and child infection was recorded for subsequent evaluation. The cumulative infection risk 2 years postpartum was assessed by Kaplan-Meier estimates and an intrauterine transmission probability of 5% (12) was added. All model predictions are based on 1000 hybrid deterministic-stochastic simulations to ensure statistic confidence in the results.
We considered four scenarios for sd-NVP: (A: no prophylaxis, B: single postpartum newborn 2 mg/kg NVP dose, C: single intrapartum maternal 200 mg NVP dose and D: intrapartum maternal 200 mg NVP dose plus postpartum newborn 2 mg/kg NVP dose). We took into account the patient characteristics from the Ugandan program for the prevention of mother-to-child transmission discussed above, in particular the individual time intervals between maternal NVP administration and birth; median 5.1 h [range: 0.3-24.8 h] and the time intervals between birth and newborn NVP administration; median 0.9 h [range: 0.1-40.6 h].

For the extended newborn NVP prophylaxis, we first simulated HIV-1 dynamics with maternal intrapartum NVP plus one postpartum newborn NVP dose, as described above, until day 1 after birth, after which we simulated HIV-1 dynamics until 2 years postpartum, following either 6 weeks (SWEN-study (3, 38)), 14-, 21 weeks, 6 months (HPTN 046-study (10)), 52-, 78- or 104 weeks of daily oral 2 mg/kg NVP administration, taking into account the pharmacokinetic characteristics of the population in the program for the prevention of mother-to-child transmission.

Results

Pharmacokinetics of NVP in Pregnant Women/Mothers and Their Newborns

The estimated PK parameters (using the model in Figure 1 B) are presented in Table 2. Mother and newborn data were best described by combined 1-compartment models with first-order absorption and elimination processes. Since the bioavailability of the oral dose was unknown, the estimated PK parameters have to be reported as relative parameters. The relative volume of distribution of mother data, V2/F was estimated to be 90.9 L and
the relative NVP clearance to be 1.22 L/h. Inter-individual variabilities (IIV) were implemented for all structural parameters relating to mother data and estimated to be moderate (34% and 33% coefficient of variation (CV) for V2/F and CL1/F, respectively) but high for KA (160% CV). The placenta clearance PCL/F was estimated to be 111 L/h suggesting a rapid placental transfer. The partition coefficient between NVP concentrations of fetus and pregnant women (PCM) was quantified to be 1.38. The large volume of distribution and low elimination capacity resulted in a long half-life of 52 h for mothers. The relative volume of distribution V4/F for newborns was estimated to be 20.0 L and the relative clearance CL2/F to be 0.21 L/h. The half-life of NVP in newborns was 66 h.

The residual variability was best described using separate proportional error models for maternal and newborn data, respectively. The proportional error was moderate (27% CV) for mother and higher (49% CV) for newborn data. The precision of the estimated PK parameters was sufficient with RSE < 20.5% for fixed-effects and RSE < 33% for random-effects parameters. The goodness of the final PK model was demonstrated by GOF plots for observed versus model-predicted NVP concentrations. Overall data spread around the line of identity suggesting adequate goodness of the PK model (see supplementary Figure S3). For model evaluation, the final PK parameter estimates were compared to the median and the 95% confidence interval obtained from the 1668 successful bootstrap runs (83.4%), see Table 2. For the fixed- and random-effects parameters, the bootstrap medians were very similar to the original model parameter values. All of them were within the 95% confidence interval, indicating an accurate and precise description of the NVP data of both populations by the PK model.
HIV-1 Transmission Risk under various NVP Single Dose Prophylaxis Scenarios

During the program for prevention of mother-to-child transmission in Uganda (28), NVP was administered once to pregnant women during labor and once to newborns shortly after delivery, with the aim of lowering the transmission probability of HIV-1 from mother-to-child. The results and model-predicted HIV-1 transmission probabilities under the four sd-NVP prophylaxis scenarios are illustrated in Figure 4 (A: no NVP prophylaxis, B: single postpartum newborn NVP dose, C: single intrapartum maternal NVP dose and D: intrapartum maternal NVP dose plus postpartum newborn NVP dose). The model-predicted transmission risks agreed very well with published data from various trials (3, 19, 27, 34, 38, 49-50, 60). Without prophylaxis, the estimated HIV-1 transmission probability after 2 years was 35.8 ± 2.9% (Figure 4 A). A single postpartum newborn dose reduced the transmission probability to 31.6 ± 2.7% (Figure 4 B) whereas a single intrapartum maternal dose lowered the transmission probability substantially to 19.1 ± 2.2% (Figure 4 C). The combination of maternal and newborn doses reduced the transmission probability to 19.7 ± 2.2% (Figure 4 D), which is insignificantly different from a single maternal dose alone. The intrapartum infection risk (typically assessed 2 weeks after birth) was 18 ± 2.4%, 12.3 ± 2%, 0.1 ± 0.1% and 0.1 ± 0.1% for the four investigated regimens. From the shape of the curves in Figure 4 A-D it can also be seen that the subsequent risk of HIV-1 transmission (mainly through breastfeeding) is highest during the first 200 days after birth.
Mechanism of Prevention of Intrapartum HIV-1 Transm ission by Maternal NVP Prophylaxis

Our data indicate that maternal NVP single dose alone decreases the transmission risk of HIV-1 substantially compared to newborn NVP dose alone (compare Figure 4 B with Figure 4 C). Hence, we elucidated the mechanisms by which the maternal NVP dose lowers HIV-1 transmission probabilities.

The dynamics of viral load decay in the HIV-1 infected mother after the maternal NVP dose are shown in Figure 5 A. The viral load declined by less than a factor of two during the first 30 h after single dose NVP. However, the newborn was born within a range of 0.3-24.8 h, median: 5.1 h (28) (dashed horizontal bar and the open circle in Figure 5 A), indicating that the maternal dose had little or no effect on the number of virions that come in contact with the newborn during intrapartum virus challenge.

In Figure 5 B the concentrations of NVP in a representative newborn from the PK investigation are depicted at the time of delivery (intrapartum challenge). Since NVP is known to cross the placenta (35, 37) and this process was quantified by us (PCL, PCM, see above), a fraction of the maternal NVP concentration was present in the newborn at the time of delivery, where it is able to prevent HIV from infecting cells (Figure 5 B) by lowering the infection rate \( \beta \) (see Methods section).

Predictors for Selection and Persistence of NVP-resistant HIV-1 Strains in Mothers after NVP Single Dose Administration

Previous studies reported that a single dose of NVP can already select drug resistant viral strains in the HIV-1 infected mothers (17, 22), compromising subsequent maternal treatment success (8, 25, 29) and potentially promoting the transmission of NVP.
resistant strains to the child during subsequent breastfeeding. We wanted to assess predictors for the selection of drug resistant strains in HIV-1 infected mothers, which might subsequently lead to the transmission of resistant virus to the breastfed child. Our model predictions revealed a strong correlation between the individual half-life of NVP in mothers and the duration in which NVP-resistant strains dominated the viral population in the HIV-1 infected mothers after a single intrapartum maternal NVP dose, see Figure 6 A (spearman’s rank correlation coefficient $r^2_S = 0.98$). The model-predicted dynamics of resistance appearance and fading for some representative mothers are shown in Figure 6 B-E. Our model predictions indicate that depending on the individual pharmacokinetics of NVP, NVP-resistant strains become selected and might subsequently dominate the virus population until NVP will be eliminated and resistant virus will be outgrown by the wild type (see Figure 6 B-E) once again. This has important implications on the probability that resistance is transmitted from mother-to-child and on the success of subsequent extended newborn NVP prophylaxis.

In supplementary text S2, we derived equations that clarify the relation between individual NVP concentrations $C$ and resistance selection. Using these equations, it is possible to compute the minimum NVP concentration that favors the selection of a resistant strain over the wild type virus. For mutant K103N, Y181C and the double mutant (K103N/Y181C) the determined minimum concentrations that favor their selection are 6.56 ng/mL, 17.7 ng/mL and 21.6 ng/mL, respectively, based on the phenotypic parameters used in this work ($IC_{50}^{(wt)}, s^{(res)}$ and $h(i)$).

This indicates, that single-point mutations are already selected at concentrations below the $IC_{50}$ value of the wild type (22 ng/mL (47)), which can persist in the plasma of the mother for several weeks after sd-NVP, depending on the individual pharmacokinetic
NVP concentration-time profile. More importantly, if transmission of HIV-1 from mother-to-child occurs during the particular time frame when the resistant virus dominates, it will likely involve resistant virus and therefore lead to resistance spread.

**Extended NVP Prophylaxis Strategies to Prevent HIV-1 Transmission via Breastfeeding**

We explored the impact of extended newborn NVP prophylaxis on HIV-1 transmission risk in order to evaluate whether these may, similar to pre-exposure viral prophylaxis, decrease the probability that viral challenges lead to infection in breastfed infants. In addition to the maternal dose, we analyzed the impact of 6 weeks (SWEN-study, [17-18]), 14-, 21 weeks, 6 month (HPTN 046-study, [19]), 52-, 78- or 104 weeks of extended newborn NVP 2 mg/kg dosing, on the transmission risk of HIV-1. The predictions for 6 weeks and 6 months extended newborn NVP are displayed in Figure 7 A and Figure 7 B, respectively, together with clinical data from the SWEN-study (3, 38) (6 weeks extended NVP) and the HPTN 046 trial (10) (6 month extended NVP). The agreement between predicted- and observed transmission probabilities was very good. The cumulative HIV-1 transmission risk 2 years postpartum, in the case of 6-, 14-, 21 weeks, 6 month, 52-, 78- or 104 weeks of extended NVP dosing were 19.6% ± 2.1%, 15.8% ± 1.9%, 15.5% ± 1.9%, 15.8% ± 1.9%, 11.8% ± 1.6%, 10.4% ± 1.4% and 8.5% ± 1.1%, respectively (see Figure 7 C): All except the 6 weeks extended NVP regimen significantly reduced HIV-1 transmission during 2 years postpartum, compared to intrapartum single dose maternal/newborn NVP (cross-tab $\chi^2$ test, p < 0.05, respectively). Notably, the 52- and 104 weeks regimens reduced the risk of transmission by further 50% and 60% compared to intrapartum maternal/newborn NVP dose alone.
The reduction of HIV-1 transmission per week extended NVP was 0.02%, 0.28%, 0.20%, 0.14%, 0.15%, 0.12% and 0.10% for the 6-, 14-, 21 weeks, 6 months, 52-, 78- or 104 weeks regimens, respectively.

**Probability of Transmitting Resistant Virus during Extended NVP Prophylaxis**

The proportion of infections with NVP-resistant virus among the newborns that became infected $P(\text{res.|inf.})_{0-2y}$ for the entire evaluation period (2 years postpartum) was 23.1%, 25.7%, 33.0%, 30.3%, 49.3%, 60.0% and 100%, respectively, in the 6-, 14-, 21 weeks, 6 months, 52-, 78- or 104 weeks extended NVP regimens (it was 22.3% in the single dose intrapartum maternal- plus postpartum newborn regimen), neglecting intrauterine infection. The proportion of infections with NVP-resistant virus among the infected newborns during weeks 0-6 and > 6 weeks postpartum were $P(\text{res.|inf.})_{0-6w} = 100\%$ and $P(\text{res.|inf.})_{>6w} = 16.9\%$, respectively in the 6 weeks extended NVP regimen, which is in good agreement with published data from the SWEN study ($P(\text{res.|inf.})_{0-6w} = 92\%$ and $P(\text{res.|inf.})_{>6w} = 15\%$, respectively (36)). For a single maternal and newborn NVP dose, the conditional probabilities were $P(\text{res.|inf.})_{0-6w} = 73.9\%$ and $P(\text{res.|inf.})_{>6w} = 12.8\%$, which overestimates the transmission of resistant strains during week 0-6, but agrees well published data on resistance transmission after 6 weeks ($P(\text{res.|inf.})_{0-6w} = 38\%$ and $P(\text{res.|inf.})_{>6w} = 15\%$, respectively (36)). The total number of infants infected with resistant virus during the breastfeeding period $P(\text{res.|inf.}) \cdot P(\text{inf.})$ was not significantly different in any extended newborn NVP regimen (3.38%, 2.77%, 3.47%, 3.27%, 3.35%, 3.24% and 3.50%, respectively in the 6-, 14-, 21 weeks, 6 month, 52-, 78- or 104 weeks regimens).
regimens, neglecting intrauterine infection) and was very similar to the single dose intrapartum maternal- plus postpartum newborn regimen (3.28%). Our results indicated that extended NVP only allows infection with resistant virus during the duration of its administration. Our predictions also indicated that all infections with resistant virus occurred before 200 days postpartum in agreement with resistance domination in the breastfeeding mothers (shown in Figure 6).

Discussion

Short-course NVP prophylaxis is still widely used in resource-constrained settings to prevent mother-to-child transmission of HIV-1. Since pregnant women and their newborns represent particular subpopulations, plasma of mothers and newborns were sampled for PK investigation during a Ugandan program for the prevention of mother-to-child transmission, which comprised sd-NVP to pregnant women and newborns each. For PK analysis of the NVP data, a combined population PK model was developed and subsequently incorporated into pharmacodynamic (PD) investigations. We found, in agreement with similar studies (4, 11, 13, 28), that a one-compartment model with first-order absorption and elimination processes was sufficient to describe the pharmacokinetics of NVP in pregnant women/mothers and newborns. Based on our previously published separated PK models for pregnant women/mothers and newborns (28), we developed a combined PK model in the present work that simultaneously analyzed the NVP concentrations of pregnant women/mothers and newborns. Before delivery, the PK model constituted the structure of a two-compartment model, where the central- and peripheral compartments were linked to the pregnant women/mothers and the fetus, respectively. Utilizing this model structure, we were able to estimate the
plasma/placenta transfer of NVP as newborns presented measureable NVP plasma concentrations before receiving their own NVP dose. After delivery the combined PK model for pregnant women/mothers and fetus was separated into two one-compartment models for mothers and newborns, respectively. All PK parameters were precisely estimated as shown by small relative standard errors. The estimated relative volume of distribution in mothers was very high (V2/F = 91 L) and in excellent agreement with previously published values (range: 77-106 L) (4, 13, 26, 37). Maternal NVP elimination capacity was low (CL1/F = 1.22 L/h) and within the range of previously published values (1.23-1.42 L/h) (4, 11, 37). The calculated half-life of NVP in mothers was 52 h, being also within the range of previously published values 43-61 h (4, 11, 37). The half-life in newborns (66 h) was slightly longer than the published value of 47 h (37), but considerably shorter than the value of 110 h, observed in (4). However, in the previous study (4), newborn plasma was only sampled over a very short interval (0-50 h), whereas data in our investigation was sampled over a considerably longer period of time (0-420 h), allowing to more accurately determine the elimination of NVP in newborns. The evaluation of the final combined PK model by GOF plots and VPC demonstrated appropriateness and sufficient predictive performance. Hence, the PK model could be used as an input for further PD investigations. In order to simultaneously analyze the impact of NVP pharmacokinetics on HIV-1 acquisition in the newborn, we developed a PK-coupled stochastic HIV-1 dynamics model. Models for HIV-1 dynamics in asymptomatically infected individuals are rather established (reviewed in (40)). Few in silico studies have linked viral dynamics to pharmacokinetics (15, 21, 43), modeled the impact of pharmacokinetics on the emergence of drug resistance (54), or considered the dynamics of HIV-1 infection (51-52). However, all these aspects, which concurrently
occur in vivo, have, to the authors’ knowledge, never been addressed simultaneously by mathematical modeling. In this study, we combined all these aspects in a single model. Furthermore, our model considers many aspects of child growth, immune system development and the characteristics of viral challenge during delivery and breastfeeding, which have been validated with external data (see Figure S1, supplementary material). Although no parameter adjustments for the HIV-1 dynamics model have been performed, model-predicted HIV-1 transmission rates under various NVP-based treatment scenarios were in excellent agreement with data from nine independent studies (see Figure 4 and Figure 7), confirming the validity of the chosen approach. Throughout this work, a reduced virus dynamics model was used, which is suited to accurately predict viral load decay in HIV-1 infected individuals following single dose administration of NVP and to predict the subsequent risk of child infection. In the case of multiple dose maternal drug administration, we recommend to use a model that can capture all phases of viral load decline, e.g. (55). In the present analysis we did not focus on viral load dynamics after the infection of the child, but rather focused on the infection risk (respective simulations were stopped, if newborn infection occurred). For accurately analyzing viral load dynamics in infected children, we also recommend to use more elaborated viral dynamics models, e.g. (55).

Our predictions indicated a significant impact of maternal NVP administration on the reduction of HIV-1 transmission to the newborn (see Figure 4 C). An analysis of the HIV-1 dynamics in the pregnant women between the period of NVP administration and delivery indicated that the effect of maternal NVP on intrapartum transmission was not due to a reduction in the number of virus particles potentially coming into contact with the newborn during delivery, since viral load decayed only by less than a factor of two
during the first 30 h after NVP administration (see Figure 5 A). This model-derived result is confirmed by clinically observed delays in virus load decline for NVP monotherapy (24-48 h (20)). Likewise, delays in the onset of viral decay have been observed in the case of ritonavir monotherapy (~30 h (41)) and under highly active antiretroviral therapy (HAART) (~18 h (31)). We therefore conclude that a maternal dose, administered at the onset of labor, may hardly have an impact of the number of viruses that come into contact with the newborn during delivery. Instead, the PK analysis coupled to the virus dynamics model, revealed that the main effect of the maternal dose is to provide potentially protective NVP concentrations via trans-placenta transport to the newborn at the moment of virus contact during delivery (see Figure 5 B), subsequently preventing HIV-1 infection. These finding were confirmed by rapid NVP exchange through the placenta (as indicated by the exchange parameters PCL, PCM in Table 2 and the almost identical time points of maximum concentrations ($t_{\text{max}}$) values in maternal and newborn plasma and cord blood (4)). This mechanism of HIV-1 transmission prevention provided by the maternal single dosing is highly similar to a pre-exposure prophylaxis, which has recently demonstrated high potential in reducing HIV-1 transmission in the context of sexual HIV-1 transmission (18). This particular mechanism of HIV-1 prevention by maternal sd-NVP has important implications for the timing of the maternal dose: Since trans-placental exchange is rapid (4), the newborn’s NVP concentrations during delivery would offer maximal protective effect at $t_{\text{max,mother}}$ of 3.5 h [range: 3.0-4.1 h] (calculated from individual PK parameter estimates). While NVP is absorbed rapidly (9), HIV-1 prevention by the maternal dose is likely suboptimal before $t_{\text{max,mother}}$. The protective effect however lasts for relatively long periods of time, since NVP is slowly
eliminated (4, 9, 28) (see also Table 2). This indicates that the maternal NVP administration at the onset of labor might be most effective, if feasible.

A single dose of NVP can select drug resistant viral strains in the HIV-infected mothers (17, 22) (see Figure 6) and lead to transmission of NVP resistant strains to the child (e.g. via breastfeeding). Pooled estimates showed that 36% (19–76%) of women have detectable NVP resistance mutations 6–8 weeks after exposure to a single dose of NVP (2). Our model slightly overestimated resistance development in the mothers after receiving a single intrapartum NVP dose (62% and 70% at week 8 if the detection limit for resistance was 50% and 20% respectively). This overestimation can be partially explained by the use of a simplified model of resistance development in our computational study, which ignores the genetic background on which resistance develops; e.g. if resistance develops on some viral strain, which is particularly unfit, then the resistance is less likely to be selected, see parameter $s_{\text{res}}$ in Equation (7). Instead, in order to reduce the complexity of our mathematical model (and to reduce the computational cost), we assumed that all susceptible viral strains were as fit as the wild type and therefore all viral strains that develop a particular mutation (K103N, Y181C and K103N/Y181C) were only assigned a fitness loss that comes from the resistance mutation and not from the genetic background of the founder strain. In future, more realistic and computationally feasible solutions for this problem should be developed. Nevertheless, our estimates of resistance transmission to the newborns/infants were in good agreement with clinical data from the SWEN study (36).

Our model predictions suggested a correlation between the individual half-life of NVP in mothers and the duration in which NVP-resistant strains dominated the viral population in the HIV-1 infected mothers after a single intrapartum maternal NVP dose. Selection of
resistant strains could be explained mathematically (see supplementary material) and minimum concentrations for the selection of NVP-resistant strains were derived. Combining the pharmacokinetic analysis of individual pharmacokinetics with the model of HIV-1 dynamics and transmission, we predicted that transmission of NVP-resistant strains would occur during the first 200 days after single dose maternal NVP, in line with the time frame in which resistant strains likely dominate the viral population (Figure 6). Figure 6 A-B suggest that NVP resistance might not become selected in mothers after single dose administration, if individual NVP elimination is fast enough (short NVP half-life). This indicates that resistance selection and subsequent resistance transmission to the child via breastfeeding could be reduced if drugs were administered to the mothers, which, in contrast to NVP, exhibit a very short half-life (e.g. zidovudine). However, we also showed that NVP effectively prevents intrapartum HIV transmission by being transferred across the placenta to the child, so that any drug which might replace maternal single dose NVP should also be able to cross the placenta in order to effectively protect the child from infection during the birth process (see Figure 5). Adding drugs to the maternal sd-NVP is another effective approach to reduce resistance selection in the HIV-1 infected mothers and to further lower intrapartum transmission rates (5, 7, 33), potentially by increasing the genetic barrier to resistance selection. A thorough understanding of the underlying mechanisms, however, is still lacking and mathematical models including combinations of drugs for elucidation remain to be developed in future.

Currently, two main strategies are pursued in order to reduce subsequent HIV-1 transmission via breastfeeding: (i) maternal ART or (ii) extended newborn NVP prophylaxis. Maternal ART has been shown to reduce HIV-1 transmission via
breastfeeding, by lowering maternal viral load to less than 400 copies per mL (14, 46), but long-term drug treatment might not be available in resource-limited settings. Extended newborn NVP administration has been suggested to reduce the transmission risk of HIV-1 by postpartum breastfeeding and might be the regimen of choice in extremely resource-limited settings for reasons of cost-effectiveness compared to maternal ART (57). In Figure 7, we analyzed the impact of 6-, 14-, 21 weeks, 6 month, 52-, 78- or 104 weeks extended newborn NVP on the transmission risk of HIV-1. Our data agrees very well with published data from the SWEN-study (3, 38) (6 weeks extended NVP) and the HPTN 049-study (10) (6 month extended NVP). Although a reduction of the HIV-1 transmission risk at 1 year postpartum was reported in the SWEN-study (6 weeks extended NVP), this reduction was not significantly different from single dose intrapartum maternal and newborn NVP dose alone (13.9% vs. 15.4%, \( p = 0.33 \); including 5% intrauterine transmission probability) (38). Our results support this finding: The estimated transmission probability 1 year postpartum was 15.3 ± 1.9% and 16.8 ± 2% \( p = 0.28 \) (including 5% intrauterine transmission probability), respectively for 6 weeks extended NVP and single dose intrapartum maternal and newborn NVP dose. At 2 years postpartum a significant reduction in the HIV-1 transmission could be achieved for all investigated extended NVP regimens, except the 6 weeks extended NVP regimen, in comparison to single dose intrapartum maternal and newborn NVP dose alone. The cost-effectiveness however decreases with increasing length of extended NVP as reflected by the reduction of HIV-1 transmission per week of extended newborn NVP. This indicates that although substantial further decrease of HIV-1 transmission could be achieved by extended NVP regimens which cover most of the breastfeeding period, shorter periods of extended NVP might be more feasible in (extremely) resource-limited
settings with regard to cost-effectiveness. Our estimates of resistance transmission to the newborns were in good agreement with clinical data from the SWEN-study (36). Overall, our results indicated an increase in the proportion of infections with resistant virus for longer durations of extended NVP prophylaxis. However, the total number of newborns who become infected with resistant virus was not increased by any of the extended NVP prophylaxis regimens compared to NVP single dose; mainly because extended NVP simultaneously minimizes the transmission probability.

Summarized, we have developed a coupled in vitro/in vivo pharmacokinetic-pharmacodynamic model to assess the effects of distinct NVP prophylaxis regimens on the prevention of mother-to-child transmission of HIV-1 and resistance formation. Our model shows very good predictive performance compared to data from clinical studies. The model may be adapted to predict the outcome of other drug interventions and could therefore be used as a supportive tool to improve HIV-1 prevention, maximize cost-effectiveness and reduce risk of resistance selection when novel studies are planned.

Acknowledgements

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Legends

Figure 1: Final PK model of mother and newborn data. A: Observed NVP concentrations in the plasma of HIV-1 infected pregnant women/mothers (filled diamonds) and in the plasma of newborns (open triangles) sampled over three time periods: delivery, week 1 and week 2 after single dose of 200 mg NVP for pregnant women and 2 mg/kg NVP administered to newborns (modified from (28)). B: Schematic structural model for the PK of mothers and newborns. The absorption rate constant for oral dose of mothers and newborns are KA and K34, respectively. V2 describes the central volume of distribution for maternal data. V4 equals the volume of distribution of the peripheral compartment (fetus/newborn compartment). Both compartments were linked by placenta clearance (PCL) term before delivery. All dashed lines highlight time-dependent processes while solid lines present continuous processes over the entire investigational period. The partition coefficient fetus to pregnant women (PCM) denotes the ratio between NVP concentrations in the fetus and maternal NVP concentrations before delivery and at quasi steady state. NVP elimination from the central and the peripheral compartment was described by CL1 and CL2, respectively.

Figure 2: VPC of the final PK model of mother and newborn data. A and B: VPC of the observed NVP concentrations in maternal plasma (black diamonds, A) and in newborn plasma (open triangles, B) over time and 5th and 95th percentiles of model simulations and model-simulated median (dashed- and solid lines).

Figure 3: Mathematical model of virus dynamics, mutation and transmission. A: Life-cycle models of HIV-1 in mothers and newborns and their interconnection via intrapartum- and breastfeeding challenge. Free virus can infect T-cells with infection rate
constant β, which encompasses all steps from target cell binding, fusion, to reverse transcription. Early infected T-cells (after reverse transcription but prior to pro-virus integration) become transformed into productively infected cells T2, after pro-virus translocation into the nucleus and integration with rate $k_T$. Productively infected T-cells T2 produce new virus V with rate N. Mutation occurs during the process of reverse transcription (embodied in parameter β). NVP inhibits reverse transcription and therefore affects parameter β in our model. All parameter values are listed in Table 1. Intrapartum viral challenge occurs during delivery, whereas breastfeeding viral challenges occur repeatedly after birth until the age of 2 years, according to the breastfeeding frequency (Figure S2, supplementary material). B: Mutational graph showing the transition probabilities $p_{j\to i}$ between the four virus strains (wild type wt and 3 mutants K103N, Y181C and K103N/Y181C) considered here. C: Phenotypic attributes of the four mutants. The extension of the bars to the right illustrates their IC$_{50}$ value, whereas the left extension indicates their fitness loss and the hight of the bars indicates the slope parameter. The IC$_{50}$-values were 22 ng/mL (47), 2168 ng/mL, 8671 ng/mL (44) and $>$11500 ng/mL for wt, the K103N, the Y181C mutation and the double mutant K103N/Y181C, respectively. The selective disadvantage s with respect to the wild type was 12.5%, 40% and 52.5% for the K103N, the Y181C mutation and the double mutant (32). The slope parameters were 1.55, 1.40, 1.15 and 1.0 for wt, the K103N, the Y181C mutation and the double mutant K103N/Y181C (44, 47), respectively.
triangles denote data from (60) and (34)); B: a single postpartum NVP dose (2 mg/kg) is
given to the newborn within 72 h after birth (squares denote data from (50)); C: a single
intrapartum NVP dose (200 mg) is given to the mother at the onset of labor; D: a single
intrapartum NVP dose (200 mg) and a single postpartum newborn dose (2 mg/kg) were
administered (crosses, open circles, diamonds, filled circles and plus signs denote data
from (3, 19, 27, 38, 49)). In all simulations, an intrauterine transmission probability of 5%
(12) was assumed.

Figure 5: A: Viral load (thick line) during the first 30 h in the plasma of HIV-1 infected
pregnant women/mothers after a single intrapartum dose NVP in relation to the time of
delivery (open circle denotes the median time of delivery, see Methods section and
dashed horizontal bar denotes the range). B: NVP concentration in a representative
newborn from the PK-investigation before- during- and after birth (solid line). The black
square and the black circle indicate the time of birth and the time of the newborn NVP
single oral dose in the representative newborn, respectively.

Figure 6: Predicted correlation between NVP elimination and persistence of NVP
resistance in HIV-1 positive mothers after a single dose of NVP. A: Correlation of
individual NVP half-life and predicted duration in which NVP resistance dominated the
viral population in mothers. B-E: Examples of resistance appearance and fading in
distinct, representative HIV-1 positive mothers after single dose NVP administration at
the onset of labor. Solid line: relative wild type abundance, dashed line: relative
abundance of NVP resistant strains. The respective half-life of NVP in the distinct
representative mothers was 1.3, 1.7, 2 and 2.6 days for panels B-E.
Figure 7: HIV-1 transmission risk in the case of extended newborn NVP dosing.

A: Predicted transmission risk after 6 weeks extended NVP treatment (solid line) and confidence range (light-grey area) together with clinical data from the SWEN-study (3, 38) (open circles). B: Predicted transmission risk after 6 month extended NVP treatment (solid line) and confidence range (light-grey area) together with clinical data from the HPTN 046-study (10) (open squares). The intrauterine transmission risk was assumed to be 5% (12). C: Predicted transmission risk after 2 years, in the case of no prophylaxis, a single dose maternal and newborn NVP dose, 6-, 14-, 21 weeks, 6 month, 52-, 78- or 104 weeks of extended newborn NVP in addition to a single intrapartum maternal NVP dose.
Table 1: Virus dynamics parameters. All units in [1/day], except the point mutation probability $\mu$ in [1/rev. transcr./base], the infection rate constant $\beta_{(wt, \phi)}$ in [1/virions/day] and the T-cell production $\lambda$ [cells/day/kg body weight].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_T$</td>
<td>0.35</td>
<td>(62)</td>
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<tr>
<td>$\delta_{T_2}$</td>
<td>1</td>
<td>(31)</td>
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<tr>
<td>$N$</td>
<td>1000</td>
<td>(45)</td>
</tr>
<tr>
<td>$\mu$</td>
<td>$2.16 \cdot 10^5$</td>
<td>(30)</td>
</tr>
<tr>
<td>$\delta_T, \delta_{T_1}$</td>
<td>0.02</td>
<td>(45)</td>
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<tr>
<td>$\beta_{(wt, \phi)}$</td>
<td>$8 \cdot 10^{-12}$</td>
<td>(45)</td>
</tr>
<tr>
<td>$\delta_{PIC}$</td>
<td>0.35</td>
<td>(55)</td>
</tr>
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<td>$\lambda$ (newborn)</td>
<td>Eq. S2$^S$</td>
<td></td>
</tr>
<tr>
<td>$CL_V$ (newborn)</td>
<td>Eq. S4$^S$</td>
<td></td>
</tr>
<tr>
<td>$\lambda$ (mother)</td>
<td>$2.86 \cdot 10^7$</td>
<td>(57)</td>
</tr>
<tr>
<td>$CL_V$ (mother)</td>
<td>23</td>
<td>(31)</td>
</tr>
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</table>

$^S$The maternal zero-order T-cell production of $2 \cdot 10^9$ (56) was divided by the weight (70 kg) of the patients in (56), to yield the parameter stated in the table. $^S$ see supplementary text S1.
Table 2: Population PK estimates of NVP of the final combined PK model for mothers and newborns including results of the bootstrap.

<table>
<thead>
<tr>
<th>Model parameters</th>
<th>Units</th>
<th>Population estimates</th>
<th>RSE(^a), %</th>
<th>Bootstrap (^b) median</th>
<th>95% Confidence interval (2.5(^{th}) and 97.5(^{th}) percentile)</th>
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<tbody>
<tr>
<td><strong>FIXED EFFECTS</strong></td>
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<tr>
<td>KA</td>
<td>[h(^{-1})]</td>
<td>1.34 fixed</td>
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<tr>
<td>V2/F</td>
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<td>90.9</td>
<td>5.85</td>
<td>89.6</td>
<td>75.4 – 101.4</td>
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<td>CL1/F</td>
<td>[L·h(^{-1})]</td>
<td>1.22</td>
<td>6.33</td>
<td>1.20</td>
<td>1.01 – 1.38</td>
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<tr>
<td>V4/F</td>
<td>[L]</td>
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<td>18.6</td>
<td>20.0</td>
<td>9.92 – 37.2</td>
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<tr>
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<td>16.1</td>
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<td>[L·h(^{-1})]</td>
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<td>20.5</td>
<td>99.7</td>
<td>5.90 – 463.8</td>
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<td>7.68</td>
<td>1.36</td>
<td>1.10 – 1.61</td>
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<td>Interindividual Variability</td>
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<tr>
<td>(\omega)KA</td>
<td>[% CV]</td>
<td>159.7</td>
<td>30.3</td>
<td>150.5</td>
<td>51.9 – 209.8</td>
</tr>
<tr>
<td>(\omega)CL1/F</td>
<td>[% CV]</td>
<td>32.9</td>
<td>25.7</td>
<td>31.8</td>
<td>23.1 – 40.5</td>
</tr>
<tr>
<td>(\omega)V2/F</td>
<td>[% CV]</td>
<td>34.1</td>
<td>33.1</td>
<td>33.1</td>
<td>20.7 – 43.4</td>
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<td>Residual Variability</td>
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<tr>
<td>(\sigma) proportional (mothers)</td>
<td>[% CV]</td>
<td>27.2</td>
<td>10.6</td>
<td>26.8</td>
<td>19.4 – 32.1</td>
</tr>
<tr>
<td>(\sigma) proportional (newborns)</td>
<td>[% CV]</td>
<td>49.1</td>
<td>11.0</td>
<td>48.1</td>
<td>38.9 – 59.0</td>
</tr>
</tbody>
</table>

\(^a\) Relative standard error (standard error divided by population estimate · 100; for the random effects parameters RSE is related to the corresponding variance scale).

\(^b\) n=1668
**A**

Mother

T1 (pre-integration infected T-cell) → kT → T (productively infected T-cell) → N (virus) → β(i,t) → mutation → T (T-cells) → V (virus) → interpartum challenge → T1 (pre-integration infected T-cell)

Newborn

T2 (productively infected T-cell) → kT → N (virus) → β(i,t) → mutation → V (virus) → breastfeeding challenge → T (T-cells)

**B**

K103N/Y181C → (1-μ)^2 → μ (1-μ) → K103N → (1-μ)^2 → μ (1-μ) → Y181C → (1-μ)^2 → μ (1-μ) → wt → (1-μ)^2 → μ (1-μ)

**C**

Slope parameter:
- h = 1.55
- h = 1.40
- h = 1.15
- h = 1.00

% selective disadvantage vs. IC50 [ng/mL]

- wt
- K103N
- Y181C
- K103N/Y181C
A. Without NVP prophylaxis

B. NVP prophylaxis to newborn

C. NVP prophylaxis to mother

D. NVP prophylaxis to mother and newborn

- Kumwenda et al. (2008)
- Stringer et al. (2003)
- Bedri et al. (2008)
- Omer et al. (2011)
- Guay et al. (1999)
- Taha et al. (2003)
- Wiktor et al. (1999)
- Miotti et al. (1999)