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Quantifying the Impact of Nevirapine-Based Prophylaxis Strategies To Prevent Mother-to-Child Transmission of HIV-1: a Combined Pharmacokinetic, Pharmacodynamic, and Viral Dynamic Analysis To Predict Clinical Outcomes▽†

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Single-dose nevirapine (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 pregnant Ugandan woman and their newborns who were receiving sd-NVP prophylaxis to prevent mother-to-child HIV-1 transmission. Based on these data, we developed a mathematical model system to quantify the impact of different sd-NVP regimens at delivery and of extended infant NVP prophylaxis (6, 14, 21, 26, 52, 78, and 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 transplacental 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 an overall decline in cost-effectiveness for increasing durations of newborn prophylaxis was indicated. The total number of infections with resistant virus in newborns 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.

HIV-1 infection remains a serious health care problem worldwide. In 2009, approximately 370,000 children became infected with HIV-1 (53). Rates of mother-to-child transmission 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% to 10%), during birth (10% to 20%), and via breastfeeding (10% to 20%) (12).

Administration of single-dose nevirapine (NVP) intrapartum and to newborns significantly reduces transmission of HIV-1 from the mother to the child (23) and is an essential component 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 breastfeeding period (57), which can be as long as 2 years. Clinical trial data on extended newborn NVP prophylaxis are currently available only for durations of 6 weeks and 6 months (3, 10, 38). However, to evaluate the effectiveness of extended newborn NVP prophylaxis, a quantification of the HIV-1 transmission risks after different durations of extended NVP prophylaxis in newborns is required.

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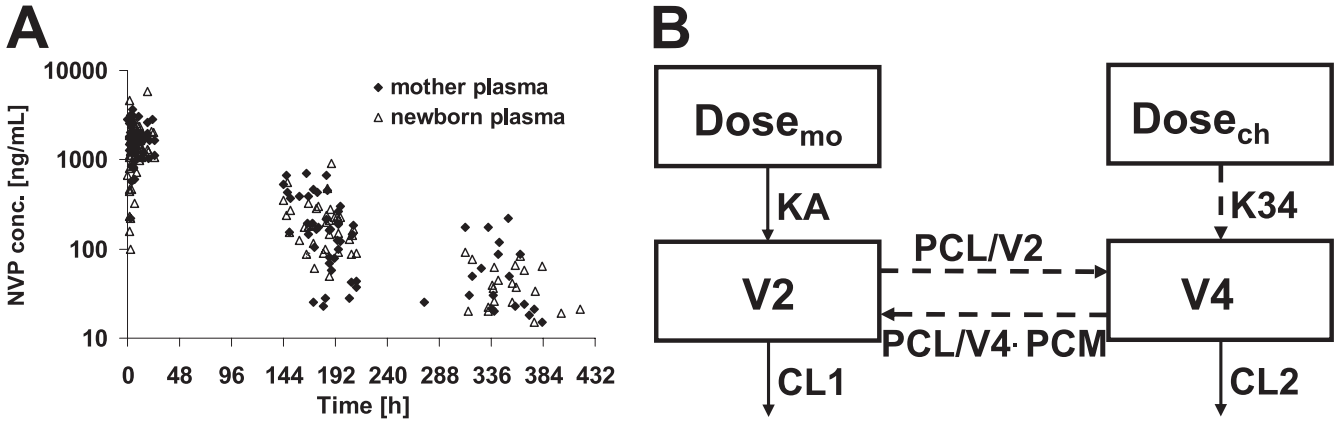

FIG. 1. Final PK model of mother and newborn data. (A) Observed NVP concentrations in plasma samples from HIV-1-infected pregnant women/mothers and newborns sampled at delivery and at week 1 and week 2 after single dose of 200 mg NVP for pregnant women and 2 mg/kg NVP administered to newborns (raw data are from reference 28). (B) Schematic structural model for PK in mothers and newborns. The absorption rate constants for oral doses of mothers and newborns are KA and K34, respectively. *V*2 describes the central volume of distribution for maternal data. *V*4 is the volume of distribution of the peripheral compartment (fetus/newborn compartment). Both compartments were linked by a placental 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 for 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 compartments was described by CL1 and CL2, respectively.

In the present study, NVP plasma data for 62 Ugandan mothers and newborns who took NVP single-dose prophylaxis were simultaneously analyzed in a single integrated population pharmacokinetic (PK) model for both populations; the present work extends a previously published pharmacokinetic study which analyzed mother and newborn NVP concentrations separately (28). The aim of this work was to combine pharmacokinetic and pharmacodynamic (PD) analyses by developing a single mathematical modeling framework. The framework should be used to predict the impacts 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.

MATERIALS AND 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 analysis after the women 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.

The median age and body weight of the pregnant women were 26 years and 56 kg (ranges, 16 to 39 years and 42 to 84 kg), respectively. Newborns had a median body weight of 3.1 kg (range, 2.0 to 3.9 kg). The median time period between NVP intake by pregnant women and birth was 5.1 h (range, 0.3 to 24.8 h). The median time interval between birth and NVP administration to the newborn was 0.9 h (range, 0.1 to 40.6 h), and that between NVP intake by the pregnant women and NVP administration to the newborns was 8.5 h (range, 1.3 to 46 h) (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 (Fig. 1A). NVP concentrations were determined by a validated liquid chromatography (LC)-tandem mass spectrometry method according to the criteria set by the FDA (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, update 1, 2006) was chosen due to the situation of sparse data. The pharmacokinetic model was parameterized in terms of clearance (CL) and volumes 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, 2,000 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 (n = 1,000 simulations), and the statistics of 5th, median, and 95th percentiles were calculated using R, version 2.9.

The schematic structure of the final PK model for maternal and newborn data is presented in Fig. 1B. 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 each fixed to 1.34 h^{-1} due to no available data during the absorption process. Prior published values of absorption rates varied between 0.013 h^{-1} and 3.81 h^{-1} (median, 1.3 h^{-1}) (4, 11, 16, 26).

Maternal plasma concentrations were associated to the central compartment with the volume of distribution *V*2 and fetal/newborn concentrations to the peripheral compartment with the volume of distribution *V*4. After delivery, but before the NVP administration to newborns, significant NVP concentrations were detected in the plasma of newborns. Considering the placenta permeability to NVP (35, 37), we implemented a transplacental exchange of NVP between pregnant woman and fetuses (placental clearance [PCL]) before the time of delivery (dashed lines in Fig. 1B). The ratio of NVP plasma concentrations between fetuses 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 Fig. 2A and B. The dashed lines represent the 5th and 95th model-simulated percentiles, and solid lines represent the model-simulated median of NVP concentrations. The VPC of mother plasma data (Fig. 2A) and newborn plasma data (Fig. 2B) revealed sufficient model predictive performance for the general trend. Overall, the model-predicted

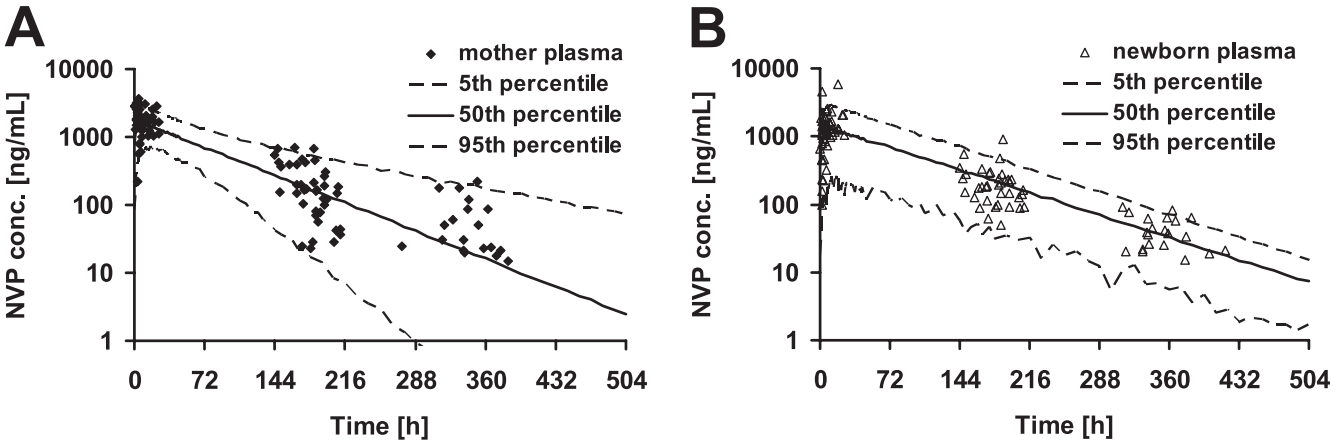

FIG. 2. VPCs of the final PK model of mother and newborn data. VPCs of the observed NVP concentrations in maternal plasma (A) and in newborn plasma (B) over time, 5th and 95th percentiles of model simulations, and model-simulated medians are shown.

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 previously (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 administration. The utilized model of HIV-1 dynamics and mother-to-child transmission is depicted in Fig. 3A.

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:

$$\frac{dT}{dt} = \lambda(t) - T \cdot \delta_T - \sum_i \beta(i,t) \cdot V(i) \cdot T + \sum_i \delta_{PIC} \cdot T_1(i) \quad (1)$$

$$\frac{dT_1(i)}{dt} = \sum_j p_{j \to i} \cdot \beta(j,t) \cdot V(j) \cdot T - T_1(i) \cdot (\delta_{T_1} + k_T + \delta_{PIC}) \quad (2)$$

$$\frac{dT_2(i)}{dt} = k_T \cdot T_1(i) - T_2(i) \cdot \delta_{T_2} \quad (3)$$

$$\frac{dV(i)}{dt} = N \cdot T_2(i) - V(i) \cdot CL_V(t) \quad (4)$$

In summary, free virus V of strain i can infect T cells with an infection rate constant of β, which encompasses all steps from target cell binding via 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 at rate k_T. T_2 produce new virus V_i with the rate constant N(on average 1,000 virions/day/cell [45]). Native, early infected, and productively infected T cells are degraded with rate constants of δ_T, δ_{T1}, and δ_{T2}, respectively. In early infected cells T_1 (prior to proviral integration), essential components of the preintegration complex can be degraded with a rate constant of δ_{PIC}, returning the cell to an uninfected stage T (55). Native T cells are produced with a rate constant of $\lambda(t)$, and free virus V_i is cleared at 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 newborns due to immune system development and growth. A derivation of the parameters $\lambda(t)$ (newborn) and $CL_V(t)$ (newborn) is provided in supplemental text S1. All model parameters are displayed in Table 1.

(i) Viral mutation. HIV can acquire drug resistance by mutation during the process of reverse transcription (comprised in parameter β in the model). The probability that a specific mutation occurs during the process of reverse transcription has been quantified *ex vivo* to be $\mu = 2.16 \times 10^{-5}$(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 μ 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 changes in the reverse transcriptase enzyme. As an example, the probability that the wild-type virus *wt* will not be mutated at one of the 2 sites is $p_{wt \to wt} = (1 - \mu)^2$. The probability that precisely one mutation occurs is given by $p_{wt \to Y181C} = (1 - \mu) \cdot \mu$ and the probability of two specific mutations by $p_{wt \to K103N/Y181C} = \mu^2$. More generally, the probability that a certain transition by mutation from some strain j to some strain i occurs during reverse transcription ($p_{j \to i}$) is given by

$$p_{j \to i} = \mu^{h(i,j)} \cdot (1 - \mu)^{L - h(i,j)} \quad (5)$$

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 Fig. 3B.

(ii) Coupling of viral dynamics with NVP pharmacokinetics. The efficacy of the nonnucleoside reverse transcriptase inhibitor NVP $[1 - \eta(i, t)]$ at time t against strain i was implemented using the standard E_{max} model with slope parameter (44, 47):

$$1 - \eta(i,t) = \frac{1}{1 + \left(\frac{C(t)}{IC_{50}(i)}\right)^{h(i)}} \quad (6)$$

where $C(t)$ denotes the NVP concentration at time t (derived during PK analysis; see above), $IC_{50}(i)$ denotes the strain-specific 50% inhibitory concentration, and $h(i)$ denotes the strain-specific slope parameter (Fig. 3C). 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 ϕ (given in Table 1) and $s(i)$ denotes the fitness loss (e.g., loss of reverse transcriptase activity) relative to the wild type (shown in Fig. 3C). The scaling factor $SF(t)$ corrects the infection rate β 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 Fig. S1 in the supplemental 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 quasicontinuously 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 modeling framework, the process of HIV-1 transmission is 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 quasicontinuous kinetics, we

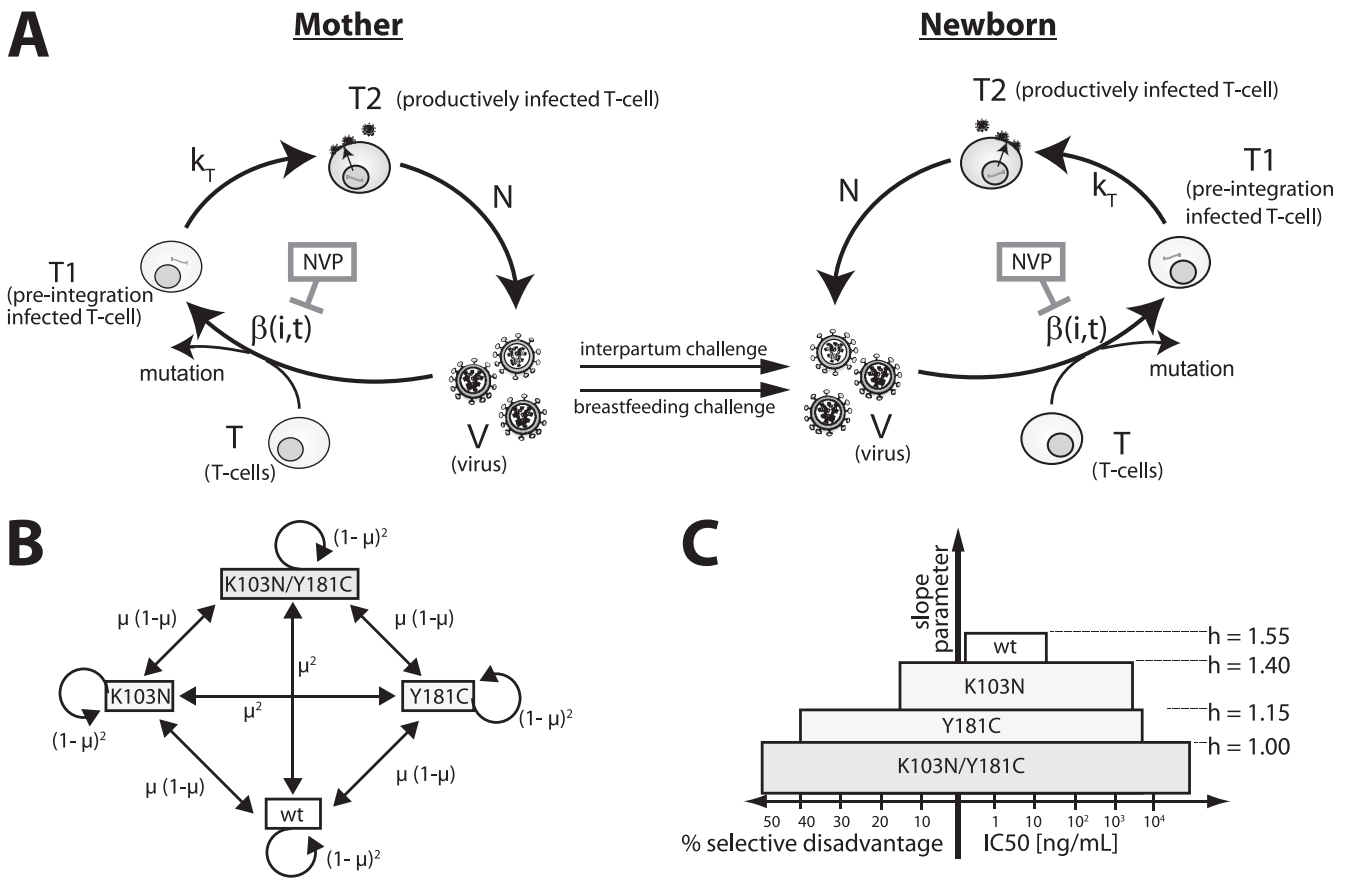

FIG. 3. Mathematical model of virus dynamics, mutation, and transmission. (A) Life cycle models of HIV-1 in mothers and newborns and their interconnection via intrapartum and breastfeeding challenge. Free virus can infect T cells with an infection rate constant β, which encompasses all steps from target cell binding via fusion to reverse transcription. Early infected T cells (after reverse transcription but prior to provirus integration) become transformed into productively infected cells T_2 after provirus translocation into the nucleus and integration with rate k_T. Productively infected T cells T_2 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 (see Fig. S2 in the supplemental 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}, whereas the left extension indicates their fitness loss and the height of the bars indicates the slope parameter. The IC_{50} values were 22 ng/ml (47), 2,168 ng/ml, 8,671 ng/ml (44), and >11,500 ng/ml for the wild type and the K103N, Y181C, and K103N/Y181C mutants, respectively. The selective disadvantages with respect to the wild type were 12.5%, 40%, and 52.5% for the K103N, Y181C, the double mutants, respectively (32). The slope parameters were 1.55, 1.40, 1.15, and 1.0 for the wild type and the K103N, Y181C, and K103N/Y181C mutants (44, 47), respectively.

TABLE 1. Virus dynamics parameters

Parameter	Value[a]	Reference
k_T	0.35	62
δ_{T2}	1	31
N	1000	45
μ	2.16×10^5	30
δ_T, δ_{T1}	0.02	45
β(wt, ϕ)	8×10^{-12}	45
δ_{PIC}	0.35	55
λ (newborn)	See equation S2 in the supplemental material	
CL_V (newborn)	See equation S4 in the supplemental material	
λ (mother)	2.86×10^{7}[b]	57
CL_V (mother)	23	31

[a] All units are 1/day, except the point mutation probability μ in (1/reverse transcriptions/base), the infection rate constant β(wt, ϕ) (1/virions/day), and the T-cell production λ (cells/day/kg of body weight).

[b] The maternal zero-order T-cell production of 2×10^9 (56) was divided by the weight (70 kg) of the patients described in reference 56 to yield this value.

chose the deterministic-stochastic hybrid simulation approach presented in reference 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 (or, 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 Fig. S2 in the supplemental material). Child growth and immune system development were considered simultaneously with the model simulation (see supplemental text S1 and Fig. S1 in the supplementary material). If stable infection of the newborns occurred (defined as a total number of $\geq 1 \times 10^6$ viruses 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 at 2 years postpartum was assessed by Kaplan-Meier estimates, and an intrauterine transmission probability of 5% (12) was

TABLE 2. Population PK estimates of NVP in the final combined PK model for mothers and newborns, including results of the bootstrap

Model parameter	Unit	Population estimate	RSE,[a] %	Bootstrap[b] median	95% confidence interval (2.5th and 97.5th percentiles)
Fixed effects					
KA	h^{-1}	1.34 fixed			
*V*2/F	Liters	90.9	5.85	89.6	75.4–101.4
CL1/F	Liters · h^{-1}	1.22	6.33	1.20	1.01–1.38
*V*4/F	Liters	20.0	18.6	20.0	9.92–37.2
CL2/F	Liters · h^{-1}	0.21	16.1	0.21	0.11–0.38
K34	h^{-1}	1.34 fixed			
PCL/F	Liters · h^{-1}	111.0	20.5	99.7	5.90–463.8
PCM		1.38	7.68	1.36	1.10–1.61
Random effects					
Interindividual variability					
ωKA	% CV	159.7	30.3	150.5	51.9–209.8
ωCL1/F	% CV	32.9	25.7	31.8	23.1–40.5
ω*V*2/F	% CV	34.1	33.1	33.1	20.7–43.4
Residual variability					
σ proportional (mothers)	% CV	27.2	10.6	26.8	19.4–32.1
σ proportional (newborns)	% CV	49.1	11.0	48.1	38.9–59.0

[a] RSE, relative standard error (standard error divided by population estimate × 100; for the random-effects parameters, RSE is related to the corresponding variance scale).
[b] $n = 1668$.

added. All model predictions are based on 1,000 hybrid deterministic-stochastic simulations to ensure statistical confidence in the results.

We considered four scenarios for single-dose 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 to 24.8 h]) and the time intervals between birth and newborn NVP administration (median, 0.9 h [range, 0.1 to 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 weeks, 21 weeks, or 6 months (HPTN 046 study [10]), or 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 Fig. 1B) 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, *V*2/F, was estimated to be 90.9 liters and the relative NVP clearance to be 1.22 liters/h. Interindividual variabilities (IIV) were implemented for all structural parameters relating to mother data and were estimated to be moderate (coefficients of variation [CV] of 34% and 33% for V2/F and CL1/F, respectively) but were high for KA (CV, 160%). The placenta clearance PCL/F was estimated to be 111 liters/h, suggesting a rapid placental transfer. The partition coefficient between NVP concentrations in fetuses 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 and the relative clearance CL2/F to be 0.21 liter/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. The proportional error was moderate (CV, 27%) for mother data and higher (CV, 49%) for newborn data. The precision of the estimated PK parameters was sufficient, with RSE of <20.5% for fixed-effects parameters and <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 the data spread around the line of identity, suggesting adequate goodness of the PK model (see Fig. S3 in the supplemental material). For model evaluation, the final PK parameter estimates were compared to the median and the 95% confidence interval obtained from the 1,668 successful bootstrap runs (83.4%) (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 probability of transmission of HIV-1 from mother to child. The results and model-predicted HIV-1 transmission probabilities under the four single-dose NVP prophylaxis scenarios are illustrated in Fig. 4 (Fig. 4A, no NVP prophylaxis, Fig. 4B, single postpartum newborn NVP dose; Fig. 4C, single intrapartum maternal NVP dose; and Fig. 4D, intrapartum maternal NVP dose plus postpartum newborn NVP dose). The model-pre-

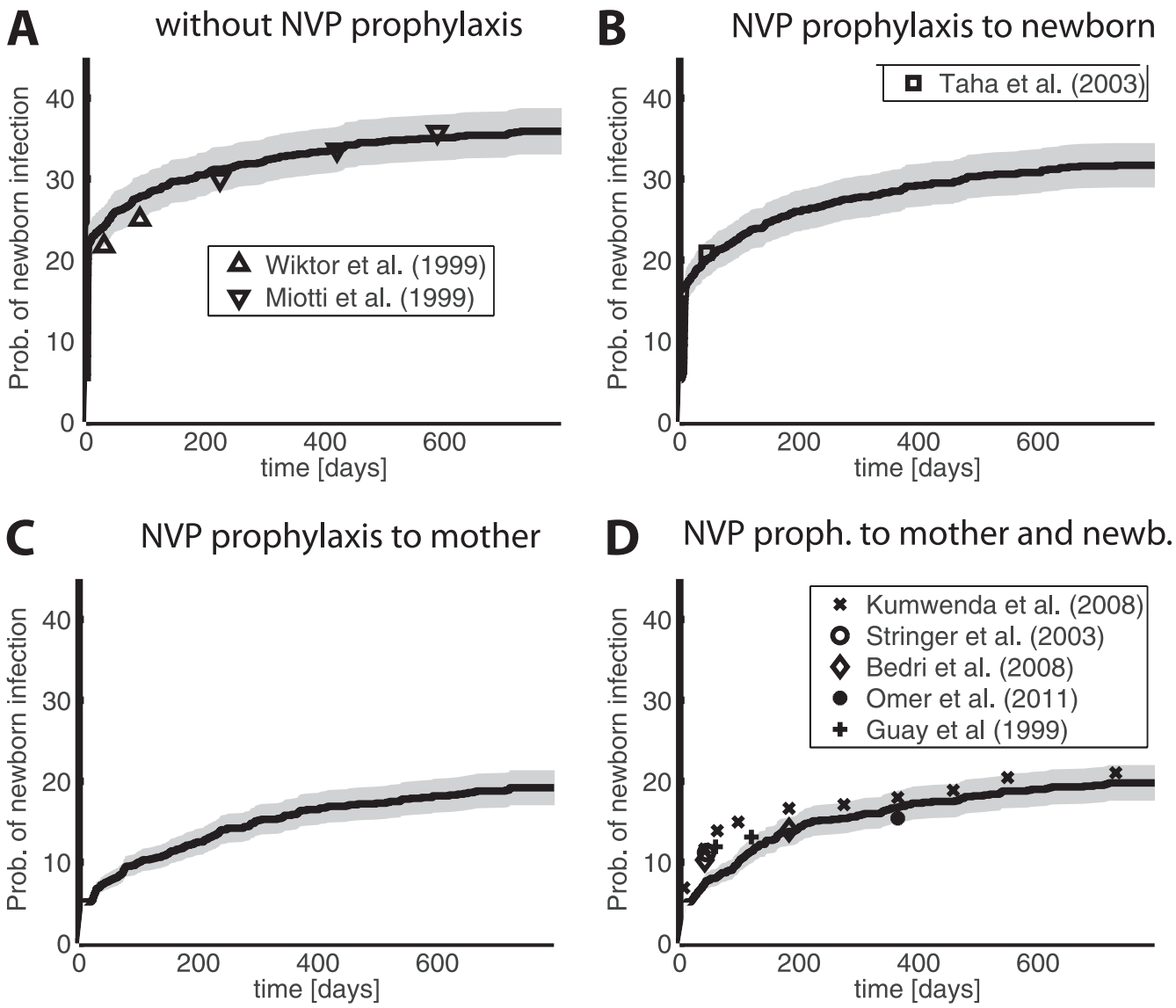

FIG. 4. Cumulative HIV-1 transmission risk under various NVP single-dose prophylaxis strategies. Solid lines denote the Kaplan-Meier estimates of the model-predicted cumulative probability of infection, whereas light gray areas represent the confidence range for the model predictions. (A) No NVP is given (data are from references 60 and 34). (B) A single postpartum NVP dose (2 mg/kg) is given to the newborn within 72 h after birth (data are from reference 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 (data are from references 3, 19, 27, 38, and 49). In all simulations, an intrauterine transmission probability of 5% (12) was assumed.

dicted 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% (Fig. 4A). A single postpartum newborn dose reduced the transmission probability to 31.6% ± 2.7% (Fig. 4B), whereas a single intrapartum maternal dose lowered the transmission probability substantially to 19.1% ± 2.2% (Fig. 4C). The combination of maternal and newborn doses reduced the transmission probability to 19.7% ± 2.2% (Fig. 4D), which is insignificantly different from the results for a single maternal dose alone. The intrapartum infection risks (typically assessed 2 weeks after birth) were 18% ± 2.4%, 12.3% ± 2%, 0.1% ± 0.1%, and 0.1 ± 0.1% for the four investigated regimens. From the shapes of the curves in Fig. 4A to 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 transmission by maternal NVP prophylaxis. Our data indicate that a single maternal NVP dose alone decreases the risk of transmission of HIV-1 substantially compared to a newborn NVP dose alone (compare Fig. 4B and C). Hence, we elucidated the mechanisms by which the maternal NVP dose lowers HIV-1 transmission probabilities.

The dynamics of viral load decay in an HIV-1-infected mother after the maternal NVP dose are shown in Fig. 5A. 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

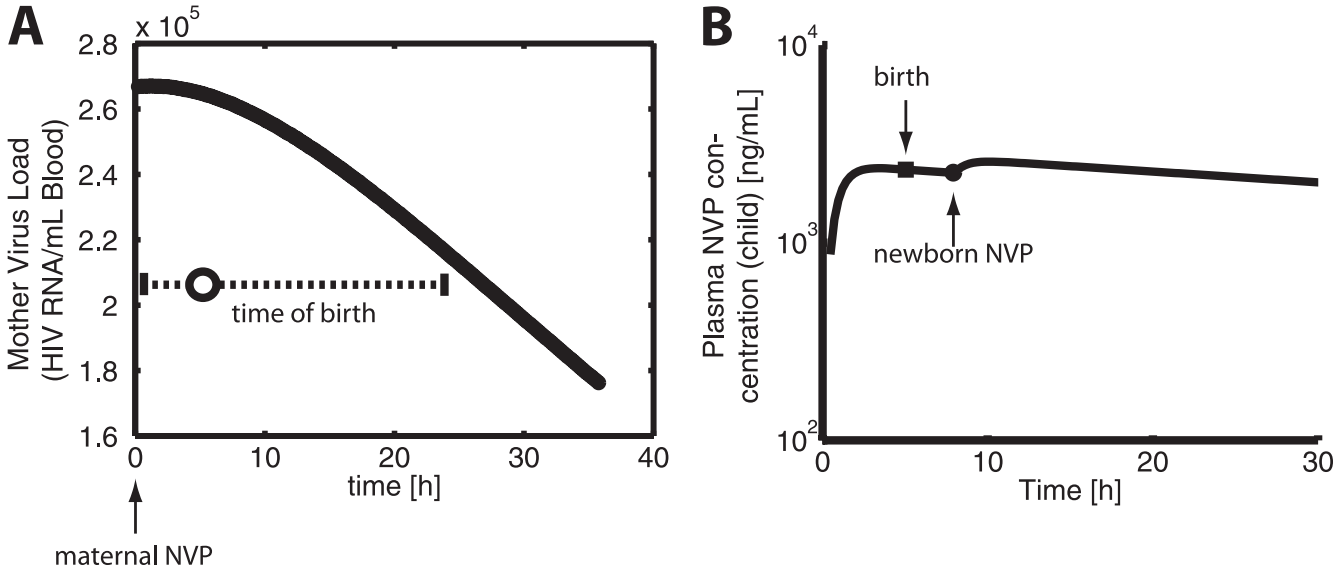

FIG. 5. (A) Viral load (thick line) during the first 30 h in the plasma of HIV-1-infected pregnant women/mothers after a single intrapartum dose of NVP in relation to the time of delivery (the circle denotes the median time of delivery [see Materials and Methods], and the dashed bar denotes the range).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

within a range of 0.3 to 24.8 h (median, 5.1 h) (28) (dashed line and circle in Fig. 5A), 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 Fig. 5B the concentrations of NVP in a representative newborn from the PK investigation at the time of delivery are depicted (intrapartum challenge). Since NVP is known to cross the placenta (35, 37) and this process was quantified by us (PCL and 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 (Fig. 5B) by lowering the infection rate β (see Materials and Methods).

Predictors for selection and persistence of NVP-resistant HIV-1 strains in mothers after administration of a single NVP dose. Previous studies reported that a single dose of NVP can already select drug-resistant viral strains in 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 for which NVP-resistant strains dominated the viral population in the HIV-1-infected mothers after a single intrapartum maternal NVP dose (Fig. 6A) (Spearman's rank correlation coefficient $r_S^2 = 0.98$). The model-predicted dynamics of appearance and fading of resistance for some representative mothers are shown in Fig. 6B to 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 is eliminated and resistant virus is outgrown by the wild type (Fig. 6B to E) once again. This has important implications for the probability that resistance is transmitted from mother to child and for the success of subsequent extended newborn NVP prophylaxis.

In supplemental text S2, we derive equations that clarify the relationship 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 the K103N and Y181C mutants 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} of the wild type (22 ng/ml [47]), which can persist in the plasma of the mother for several weeks after single-dose 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 this may, similarly to preexposure 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 weeks, 21 weeks, and 6 months (HPTN 046 study, [19]), or 52, 78, or 104 weeks of extended newborn NVP (2 mg/kg) dosing on the risk of transmission of HIV-1. The predictions for 6 weeks and 6 months of extended newborn NVP administration are displayed in Fig. 7A and B, respectively, together with clinical data from the SWEN study (3, 38)

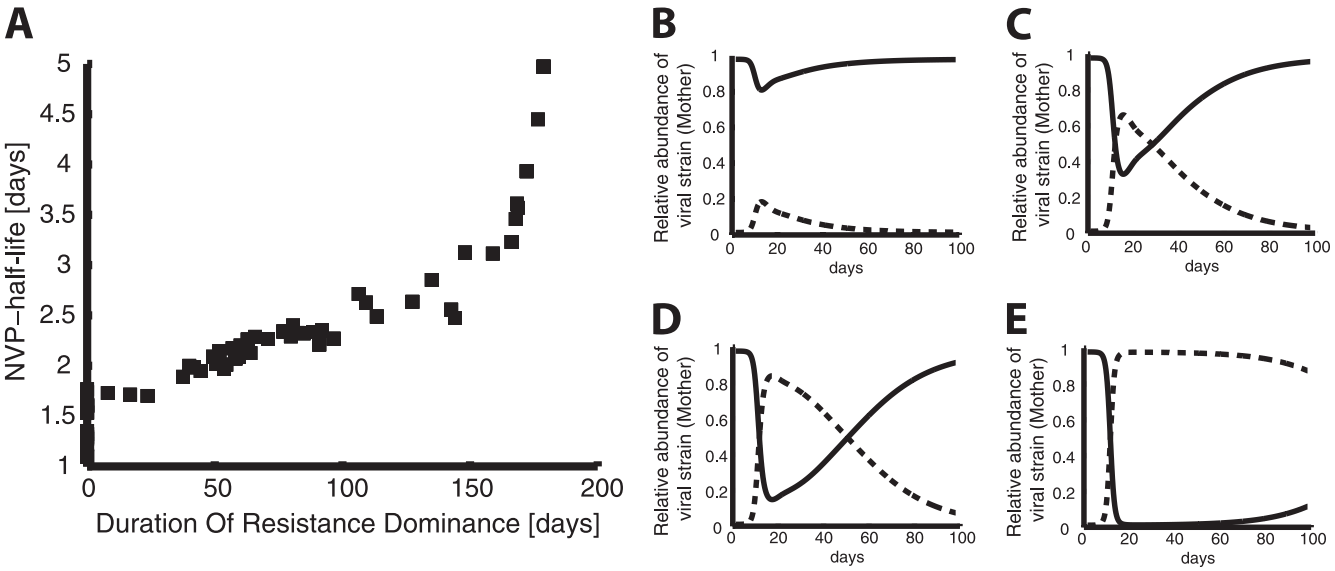

FIG. 6. Predicted correlation between NVP elimination and persistence of NVP resistance in HIV-1-positive mothers after a single dose of NVP. (A) Correlation of individual NVP half-life and predicted duration for which NVP resistance dominates the viral population in mothers. (B to E) Examples of resistance appearance and fading in distinct representative HIV-1-positive mothers after single-dose NVP administration at the onset of labor. Solid lines, relative wild-type abundance; dashed lines, relative abundance of NVP-resistant strains. The respective half-lives of NVP in the distinct representative mothers were 1.3, 1.7, 2, and 2.6 days for panels B to E.

(6 weeks extended NVP) and the HPTN 046 trial (10) (6 months extended NVP). The agreement between predicted and observed transmission probabilities was very good. The cumulative HIV-1 transmission risks at 2 years postpartum in the cases of 6 weeks, 14 weeks, 21 weeks, 6 months, 52 weeks, 78 weeks, and 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 (Fig. 7C): All except the 6-week extended NVP regimen significantly reduced HIV-1 transmission during 2 years postpartum compared to intrapartum single-dose maternal/newborn NVP (cross-tab χ^2 test, $P < 0.05$). Notably, the 52- and 104-week regimens reduced the risk of transmission by a further 50% and 60% compared to intrapartum maternal/newborn

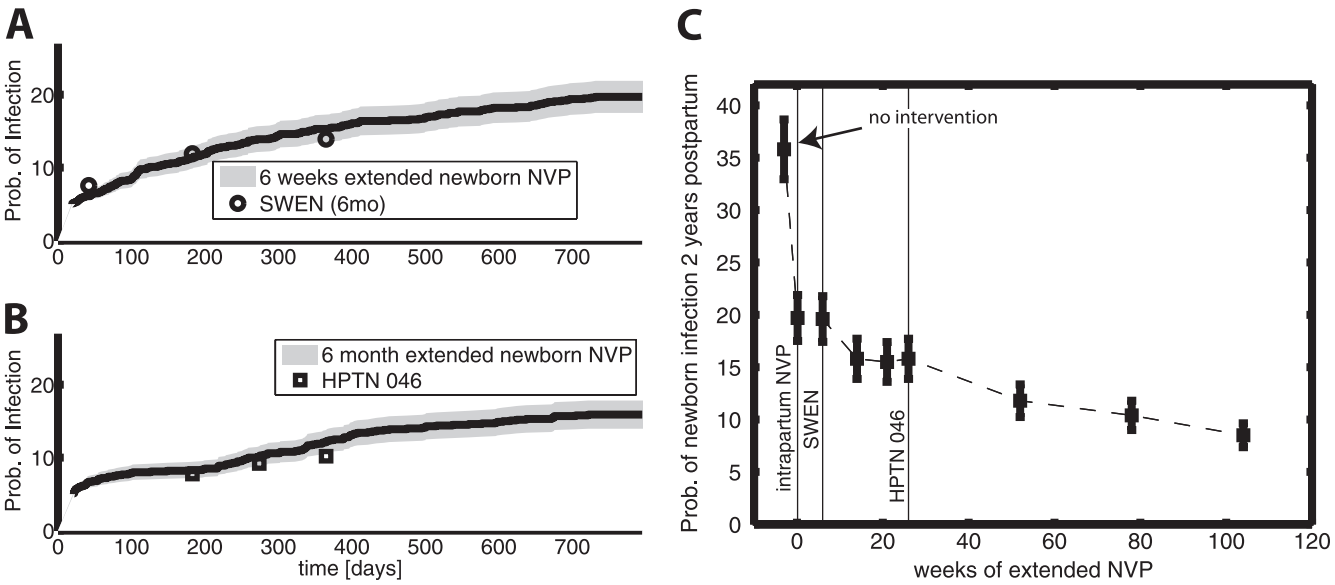

FIG. 7. HIV-1 transmission risk in the case of extended newborn NVP dosing. (A) Predicted transmission risk after 6 weeks of extended NVP treatment (solid line) and confidence range (light gray area) together with clinical data from the SWEN study (3, 38) (circles). (B) Predicted transmission risk after 6 months of extended NVP treatment (solid line) and confidence range (light gray area) together with clinical data from the HPTN 046 study (10) (squares). The intrauterine transmission risk was assumed to be 5% (12). (C) Predicted transmission risk after 2 years in the cases of no prophylaxis, a single maternal and newborn NVP dose, or 6 weeks, 14 weeks, 21 weeks, 6 months, 52 weeks, 78 weeks, or 104 weeks of extended newborn NVP treatment in addition to a single intrapartum maternal NVP dose.

NVP dosing alone. The reductions of HIV-1 transmission per week of extended NVP were 0.02%, 0.28%, 0.20%, 0.14%, 0.15%, 0.12%, and 0.10% for the 6-week, 14-week, 21-week, 6-month, 52-week, 78-week, and 104-week regimens, respectively.

Probability of transmitting resistant virus during extended NVP prophylaxis. The proportions of infections with NVP-resistant virus among the newborns who became infected [$P(\text{res.}|\text{inf.})_{0\text{-}2y}$] for the entire evaluation period (2 years postpartum) were 23.1%, 25.7%, 33.0%, 30.3%, 49.3%, 60.0%, and 100%, respectively, in the 6-week, 14-week, 21-week, 6-month, 52-week, 78-week, and 104-week extended NVP regimens (it was 22.3% in the single-dose intrapartum maternal plus postpartum newborn regimen), neglecting intrauterine infection. The proportions of infections with NVP-resistant virus among the infected newborns during weeks 0 to 6 and >6 weeks postpartum were $P(\text{res.}|\text{inf.})_{0\text{-}6w} = 100\%$ and $P(\text{res.}|\text{inf.})_{>6w} = 16.9\%$, respectively, in the 6-week extended NVP regimen, which is in good agreement with published data from the SWEN study [$P(\text{res.}|\text{inf.})_{0\text{-}6w} = 92\%$ and $P(\text{res.}|\text{inf.})_{>6w} = 15\%$, respectively (36)]. For a single maternal and newborn NVP dose, the conditional probabilities were $P(\text{res.}|\text{inf.})_{0\text{-}6w} = 73.9\%$ and $P(\text{res.}|\text{inf.})_{>6w} = 12.8\%$, which overestimates the transmission of resistant strains during weeks 0 to 6 but agrees well with published data on resistance transmission after 6 weeks [$P(\text{res.}|\text{inf.})_{0\text{-}6w} = 38\%$ and $P(\text{res.}|\text{inf.})_{>6w} = 15\%$, respectively (36)]. The total number of infants infected with resistant virus during the breastfeeding period [$P(\text{res.}|\text{inf.}) \cdot P(\text{inf.})$] 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-week, 14-week, 21-week, 6-month, 52-week, 78-week, and 104-week regimens, neglecting intrauterine infection) and was very similar to that in the single dose intrapartum maternal plus postpartum newborn regimen (3.28%). Our results indicated that extended NVP allows infection with resistant virus only 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 (Fig. 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 was sampled for PK investigation during a Ugandan program for the prevention of mother-to-child transmission, which comprised single-dose NVP given to pregnant women and newborns. 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 separate 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 in 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 fetuses, 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 fetuses 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 liters) and in excellent agreement with previously published values (range, 77 to 106 liters) (4, 13, 26, 37). The maternal NVP elimination capacity was low (CL1/F = 1.22 liters/h) and within the range of previously published values (1.23 to 1.42 liters/h) (4, 11, 37). The calculated half-life of NVP in mothers was 52 h, which was also within the range of previously published values (43 to 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 reported in reference 4. However, in the previous study (4), newborn plasma was sampled only over a very short interval (0 to 50 h), whereas data in our investigation were sampled over a considerably longer period of time (0 to 420 h), allowing more accurate determination of 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 reference 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 occur concurrently *in vivo*, have to our 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 Fig. S1 in the supplemental 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 (Fig. 4 and Fig. 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 the use of a model that can capture all phases of viral load decline (see, e.g., reference 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 (the respective simulations were stopped if newborn infection occurred). For accurately analyzing viral load dynamics in infected children, we also recommend the use of more elaborate viral dynamics models (see, e.g., reference 55).

Our predictions indicated a significant impact of maternal NVP administration on the reduction of HIV-1 transmission to the newborn (Fig. 4C). 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 (Fig. 5A). This model-derived result is confirmed by clinically observed delays in virus load decline for NVP monotherapy (24 to 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 with the virus dynamics model revealed that the main effect of the maternal dose is to provide potentially protective NVP concentrations via transplacental transport to the newborn at the moment of virus contact during delivery (Fig. 5B), subsequently preventing HIV-1 infection. These finding were confirmed by rapid NVP exchange through the placenta (as indicated by the exchange parameters PCL and PCM in Table 2 and the almost identical time points of maximum concentration [t_{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 preexposure prophylaxis, which has recently demonstrated a high potential to reduce HIV-1 transmission in the context of sexual HIV-1 transmission (18). This particular mechanism of HIV-1 prevention by maternal single-dose NVP has important implications for the timing of the maternal dose: since transplacental exchange is rapid (4), the newborn's NVP concentrations during delivery would offer maximal protective effect at t_{max} (mother) of 3.5 h [range, 3.0 to 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_{max} (mother). The protective effect, however, lasts for relatively long periods of time, since NVP is slowly eliminated (4, 9, 28) (Table 2). This indicates that maternal NVP administration at the onset of labor, if feasible, might be most effective.

A single dose of NVP can select drug-resistant viral strains in HIV-infected mothers (17, 22) (Fig. 6) and lead to transmission of NVP-resistant strains to the child (e.g., via breastfeeding). Pooled estimates showed that 36% (19 to 76%) of women have detectable NVP resistance mutations at 6 to 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(res) in Equation S7 in the supplemental material]. 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 assigned a fitness loss that comes only from the resistance mutation and not from the genetic background of the founder strain. In the future, more realistic and computationally feasible solutions for this problem should be developed. Nevertheless, our estimates of transmission of resistance 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 for 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 the supplemental 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 a single maternal dose of NVP, in line with the time frame in which resistant strains likely dominate the viral population (Fig. 6). Figure 6 A and 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 (Fig. 5). Adding other drugs to the maternal single-dose NVP is another effective approach to reduce resistance selection in 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 remain to be developed in the future.

Currently, two main strategies are pursued in order to reduce 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 the 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 risk of transmission 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 Fig. 7, we analyze the impact of 6-week, 14-week, 21-week, 6-month, 52-week, 78-week, or 104-week extended newborn NVP treatment on the risk of transmission of HIV-1. Our data agree very well with published data from the SWEN study (3, 38) (6 weeks extended NVP) and the HPTN 049 study (10) (6 months 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 that with single-dose intrapartum maternal and newborn NVP alone (13.9% versus 15.4%; $P = 0.33$ [including 5% intrauterine transmission probability]) (38). Our results support this finding. The estimated transmission probabilities at 1 year postpartum were 15.3% ± 1.9% and 16.8% ± 2% ($P = 0.28$) (including 5% intrauterine transmission probability), respectively, for 6 weeks of extended NVP and for single-dose intrapartum maternal and newborn NVP. 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 alone. The cost-effectiveness, however, decreases with increasing length of extended NVP treatment as reflected by the reduction of HIV-1 transmission per week of extended newborn NVP treatment. This indicates that although substantial further decreases of HIV-1 transmission could be achieved by extended NVP regimens which cover most of the breastfeeding period, shorter periods of extended NVP treatment might be more feasible in (extremely) resource-limited settings with regard to cost-effectiveness. Our estimates of transmission of resistance 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 single-dose NVP, mainly because extended NVP simultaneously minimizes the transmission probability.

In summary, 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 outcomes of other drug interventions and could therefore be used as a supportive tool to improve HIV-1 prevention, maximize cost-effectiveness, and reduce the risk of resistance selection when novel studies are planned.

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REFERENCES

1. **Alfonsi, A., E. Cances, G. Turinici, B. Ventura, and W. Huisinga.** 2005. Exact simulation of hybrid stochastic and deterministic models for biochemical systems. ESAIM Proc. **14:**1–23.
2. **Arrive, E., et al.** 2007. Prevalence of resistance to nevirapine in mothers and children after single-dose exposure to prevent vertical transmission of HIV-1: a meta-analysis. Int. J. Epidemiol. **36:**1009–1021.
3. **Bedri, A., et al.** 2008. Extended-dose nevirapine to 6 weeks of age for infants to prevent HIV transmission via breastfeeding in Ethiopia, India, and Uganda: an analysis of three randomised controlled trials. Lancet **372:**300–313.
4. **Benaboud, S., et al.** 2011. Population pharmacokinetics of nevirapine in HIV-1-infected pregnant women and their neonates. Antimicrob. Agents Chemother. **55:**331–337.
5. **Chaix, M. L., et al.** 2006. Low risk of nevirapine resistance mutations in the prevention of mother-to-child transmission of HIV-1: Agence Nationale de Recherches sur le SIDA Ditrame Plus, Abidjan, Cote d'Ivoire. J. Infect. Dis. **193:**482–487.
6. **Chasela, C. S., et al.** 2010. Maternal or infant antiretroviral drugs to reduce HIV-1 transmission. N. Engl. J. Med. **362:**2271–2281.
7. **Chi, B. H., et al.** 2007. Single-dose tenofovir and emtricitabine for reduction of viral resistance to non-nucleoside reverse transcriptase inhibitor drugs in women given intrapartum nevirapine for perinatal HIV prevention: an open-label randomised trial. Lancet **370:**1698–1705.
8. **Coffie, P. A., et al.** 2008. Maternal 12-month response to antiretroviral therapy following prevention of mother-to-child transmission of HIV type 1, Ivory Coast, 2003-2006. Clin. Infect. Dis. **46:**611–621.
9. **Cooper, C. L., and R. P. van Heeswijk.** 2007. Once-daily nevirapine dosing: a pharmacokinetics, efficacy and safety review. HIV Med. **8:**1–7.
10. **Coovadia, H., et al.** 2011. HPTN: efficacy of extended daily infant NVP through age 6 month compared to 6 weeks for postnatal PMTCT of HIV through breastfeeding, abstr. 123LB. Abstr. Conf. Retroviruses Opportun. Infect. http://www.retroconference.org/2011/Abstracts/42412.htm.
11. **Cressey, T. R., et al.** 2005. Persistence of nevirapine exposure during the postpartum period after intrapartum single-dose nevirapine in addition to zidovudine prophylaxis for the prevention of mother-to-child transmission of HIV-1. J. Acquir. Immune Defic. Syndr. **38:**283–288.
12. **De Cock, K. M., et al.** 2000. Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. JAMA **283:**1175–1182.
13. **de Maat, M. M., et al.** 2002. Population pharmacokinetics of nevirapine in an unselected cohort of HIV-1-infected individuals. Br. J. Clin. Pharmacol. **54:**378–385.
14. **de Vincenzi, I.** 2011. Triple antiretroviral compared with zidovudine and single-dose nevirapine prophylaxis during pregnancy and breastfeeding for prevention of mother-to-child transmission of HIV-1 (Kesho Bora study): a randomised controlled trial. Lancet Infect. Dis. **11:**171–180.
15. **Dixit, N. M., and A. S. Perelson.** 2004. Complex patterns of viral load decay under antiretroviral therapy: influence of pharmacokinetics and intracellular delay. J. Theor. Biol. **226:**95–109.
16. **Elsherbiny, D., et al.** 2009. Population pharmacokinetics of nevirapine in combination with rifampicin-based short course chemotherapy in HIV- and tuberculosis-infected South African patients. Eur. J. Clin. Pharmacol. **65:**71–80.
17. **Eshleman, S. H., et al.** 2001. Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET 012). AIDS **15:**1951–1957.
18. **Grant, R. M., et al.** 2010. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N. Engl. J. Med. **363:**2587–2599.
19. **Guay, L. A., et al.** 1999. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. Lancet **354:**795–802.
20. **Havlir, D. V., S. Eastman, A. Gamst, and D. D. Richman.** 1996. Nevirapine-resistant human immunodeficiency virus: kinetics of replication and estimated prevalence in untreated patients. J. Virol. **70:**7894–7899.
21. **Huang, Y., S. L. Rosenkranz, and H. Wu.** 2003. Modeling HIV dynamics and antiviral response with consideration of time-varying drug exposures, adherence and phenotypic sensitivity. Math Biosci. **184:**165–186.
22. **Jackson, J. B., et al.** 2000. Identification of the K103N resistance mutation in Ugandan women receiving nevirapine to prevent HIV-1 vertical transmission. AIDS **14:**F111–F115.
23. **Jackson, J. B., et al.** 2003. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: 18-month follow-up of the HIVNET 012 randomised trial. Lancet **362:**859–868.
24. **John-Stewart, G., et al.** 2004. Breast-feeding and transmission of HIV-1. J. Acquir. Immune Defic. Syndr. **35:**196–202.
25. **Jourdain, G., et al.** 2004. Intrapartum exposure to nevirapine and subsequent maternal responses to nevirapine-based antiretroviral therapy. N. Engl. J. Med. **351:**229–240.
26. **Kappelhoff, B. S., et al.** 2005. Nevirapine and efavirenz pharmacokinetics and covariate analysis in the 2NN study. Antivir. Ther. **10:**145–155.
27. **Kumwenda, N. I., et al.** 2008. Extended antiretroviral prophylaxis to reduce breast-milk HIV-1 transmission. N. Engl. J. Med. **359:**119–129.
28. **Kunz, A., et al.** 2009. Persistence of nevirapine in breast milk and plasma of

mothers and their children after single-dose administration. J. Antimicrob. Chemother. **63:**170–177.
29. **Lockman, S., et al.** 2007. Response to antiretroviral therapy after a single, peripartum dose of nevirapine. N. Engl. J. Med. **356:**135–147.
30. **Mansky, L. M., and H. M. Temin.** 1995. Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. J. Virol. **69:**5087–5094.
31. **Markowitz, M., et al.** 2003. A novel antiviral intervention results in more accurate assessment of human immunodeficiency virus type 1 replication dynamics and T-cell decay in vivo. J. Virol. **77:**5037–5038.
32. **Martinez-Picado, J., and M. A. Martinez.** 2008. HIV-1 reverse transcriptase inhibitor resistance mutations and fitness: a view from the clinic and ex vivo. Virus Res. **134:**104–123.
33. **McIntyre, J. A., et al.** 2009. Efficacy of short-course AZT plus 3TC to reduce nevirapine resistance in the prevention of mother-to-child HIV transmission: a randomized clinical trial. PLoS Med. **6:**e1000172.
34. **Miotti, P. G., et al.** 1999. HIV transmission through breastfeeding: a study in Malawi. JAMA **282:**744–749.
35. **Mirochnick, M., et al.** 1998. Pharmacokinetics of nevirapine in human immunodeficiency virus type 1-infected pregnant women and their neonates. Pediatric AIDS Clinical Trials Group Protocol 250 Team. J. Infect. Dis. **178:**368–374.
36. **Moorthy, A., et al.** 2009. Nevirapine resistance and breast-milk HIV transmission: effects of single and extended-dose nevirapine prophylaxis in subtype C HIV-infected infants. PLoS One **4:**e4096.
37. **Musoke, P., et al.** 1999. A phase I/II study of the safety and pharmacokinetics of nevirapine in HIV-1-infected pregnant Ugandan women and their neonates (HIVNET 006). AIDS **13:**479–486.
38. **Omer, S. B.** 2011. Twelve-month follow-up of six week extended dose nevirapine randomized controlled trials: differential impact of extended-dose nevirapine on mother-to-child transmission and infant death by maternal CD4 cell count. AIDS **25:**767–776.
39. **Palumbo, P., et al.** 2010. Antiretroviral treatment for children with peripartum nevirapine exposure. N. Engl. J. Med. **363:**1510–1520.
40. **Perelson, A. S.** 2002. Modelling viral and immune system dynamics. Nat. Rev. Immunol. **2:**28–36.
41. **Perelson, A. S., A. U. Neumann, M. Markowitz, J. M. Leonard, and D. D. Ho.** 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science **271:**1582–1586.
42. **Petra Study Team.** 2002. Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa, and Uganda (Petra study): a randomised, double-blind, placebo-controlled trial. Lancet **359:**1178–1186.
43. **Rosario, M. C., P. Jacqmin, P. Dorr, E. van der Ryst, and C. Hitchcock.** 2005. A pharmacokinetic-pharmacodynamic disease model to predict in vivo antiviral activity of maraviroc. Clin. Pharmacol. Ther. **78:**508–519.
44. **Sampah, M. E. S., L. Shen, B. L. Jilek, and R. F. Siliciano.** 2011. Dose-response curve slope is a missing dimension in the analysis of HIV-1 drug resistance. Proc. Natl. Acad. Sci. U. S. A. **108:**7613–7618.
45. **Sedaghat, A. R., J. B. Dinoso, L. Shen, C. O. Wilke, and R. F. Siliciano.** 2008. Decay dynamics of HIV-1 depend on the inhibited stages of the viral life cycle. Proc. Natl. Acad. Sci. U. S. A. **105:**4832–4837.
46. **Shapiro, R. L., et al.** 2010. Antiretroviral regimens in pregnancy and breastfeeding in Botswana. N. Engl. J. Med. **362:**2282–2294.
47. **Shen, L., et al.** 2008. Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. Nat. Med. **14:**762–766.
48. **Stocker, H., et al.** 2004. Nevirapine significantly reduces the levels of racemic methadone and (R)-methadone in human immunodeficiency virus-infected patients. Antimicrob. Agents Chemother. **48:**4148–4153.
49. **Stringer, J. S., et al.** 2003. Timing of the maternal drug dose and risk of perinatal HIV transmission in the setting of intrapartum and neonatal single-dose nevirapine. AIDS **17:**1659–1665.
50. **Taha, T. E., et al.** 2003. Short postexposure prophylaxis in newborn babies to reduce mother-to-child transmission of HIV-1: NVAZ randomised clinical trial. Lancet **362:**1171–1177.
51. **Tuckwell, H. C., and E. Le Corfec.** 1998. A stochastic model for early HIV-1 population dynamics. J. Theor. Biol. **195:**451–463.
52. **Tuckwell, H. C., P. D. Shipman, and A. S. Perelson.** 2008. The probability of HIV infection in a new host and its reduction with microbicides. Math Biosci. **214:**81–86.
53. **UNAIDS.** 2010. UNAIDS report on the global AIDS epidemic. http://www.unaids.org/globalreport/Global_report.htm.
54. **von Kleist, M., and W. Huisinga.** 2009. Pharmacokinetic-pharmacodynamic relationship of NRTIs and its connection to viral escape: an example based on zidovudine. Eur. J. Pharm. Sci. **36:**532–543.
55. **von Kleist, M., S. Menz, and W. Huisinga.** 2010. Drug-class specific impact of antivirals on the reproductive capacity of HIV. PLoS Comput. Biol. **6:**e1000720.
56. **Wei, X., et al.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature **373:**117–122.
57. **WHO.** 2010. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: towards universal access. http://www.who.int/hiv/pub/mtct/antiretroviral2010/en/index.html.
58. **WHO.** 2006. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: towards universal access. http://www.who.int/hiv/pub/mtct/antiretroviral/en/index.html.
59. **WHO.** 2010. Guidelines on HIV and infant feeding. http://www.who.int/child_adolescent_health/documents/9789241599535/en/index.html.
60. **Wiktor, S. Z., et al.** 1999. Short-course oral zidovudine for prevention of mother-to-child transmission of HIV-1 in Abidjan, Cote d'Ivoire: a randomised trial. Lancet **353:**781–785.
61. **Wilkinson, D. J.** 2006. Stochastic modelling in systems biology. Chapman & Hall/CRC, Boca Raton, FL.
62. **Zhou, Y., H. Zhang, J. D. Siliciano, and R. F. Siliciano.** 2005. Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4+ T cells. J. Virol. **79:**2199–2210.