Pharmacokinetics of Injectable, Long-Acting Nevirapine for HIV Prophylaxis in Breastfeeding Infants

John M. Cortez, Jr.,^a Rafaela Quintero,^a John A. Moss,^b Martin Beliveau,^c Thomas J. Smith,^{a,b} Marc M. Baum^b

Auritec Pharmaceuticals, Inc., Pasadena, California, USA^a; Department of Chemistry, Oak Crest Institute of Science, Pasadena, California, USA^b; Pharsight Consulting Services, Montreal, Quebec, Canada^c

Mother-to-child transmission (MTCT) of HIV-1 remains a global health problem. The World Health Organization (WHO) recommendations advise the administration of a once-daily, oral, prophylactic regimen of the nonnucleoside reverse transcriptase inhibitor nevirapine (NVP) from birth until 4 to 6 weeks of age for infants born to HIV-infected mothers in regions without access to safe and nutritionally adequate alternatives to breast milk. A critical factor driving the successful implementation of the WHO guidelines involves sustaining high adherence to the frequent dosing. With these challenges in mind, we have developed the first injectable, sustained-release NVP formulations with the goal of providing, for 6 weeks or longer, preventative plasma drug levels from a single subcutaneous administration at birth. The long-acting NVP consists of large ($> 50 \mu\text{m}$), monodisperse NVP particles coated with biocompatible polymers that control the drug release kinetics. Two lead formulations exhibiting burst-free, sustained-release kinetics for up to 75 days *in vitro* were developed. Subsequent *in vivo* studies in rats demonstrated no toxicity related to the formulations. Rat plasma NVP concentrations were above the analytical assay's limit of quantification for up to 28 days. Pharmacokinetic analysis of the rat plasma NVP concentration-time data allowed absorption rate constants to be calculated. These data then were used to simulate infant NVP exposure from a single injected dose ($< 200 \text{ mg}$) of our long-acting formulations, demonstrating preliminary feasibility of the technology to maintain safe, preventative NVP plasma levels (0.2 to $3.0 \mu\text{g ml}^{-1}$) for 6 weeks or longer.

Mother-to-child transmission (MTCT) of HIV-1 remains a global health problem (1). In 2008, an estimated 1.4 million HIV-infected women gave birth in low- and middle-income countries, resulting in 430,000 new pediatric infections (2). In breastfeeding populations and in the absence of a prevention strategy, the MTCT rate has been estimated to be 25 to 45% (3). Administration of highly active antiretroviral therapy (HAART) to pregnant HIV-infected women is effective for preventing MTCT (2), but access to HAART is limited in resource-constrained regions, where $> 90\%$ of HIV-1-infected pregnant women reside (2).

HIV prevention programs aim to reduce MTCT to $< 5\%$ using antiretroviral prophylaxis (2). Interventions currently target the peripartum/neonatal period (3). The most widely used regimen is derived from the HIVNET 012 study, which demonstrated that oral administration of the nonnucleoside reverse transcriptase inhibitor (NNRTI) nevirapine (NVP) to the mother at labor onset (a single 200-mg dose) and a 2 mg kg of body weight⁻¹ dose administered to the infant within 72 h of birth reduced transmission by half (4, 5).

A significant risk of vertical transmission of HIV in breastfed infants occurs within the first 6 weeks postpartum (6, 7). As a result, the World Health Organization (WHO) recommendations advise the administration of an antiretroviral prophylactic regimen consisting of once-daily oral NVP (10- to 15-mg dose, depending on birth weight) from birth until 4 to 6 weeks of age for infants born to HIV-infected mothers in regions without access to safe and nutritionally adequate alternatives to breast milk (2).

A critical factor determining the successful implementation of the WHO guidelines of daily NVP administration to breastfeeding infants for 4 to 6 weeks is sustaining high adherence to the frequent dosing. It has been well established across different delivery methods that adherence to therapy is inversely related to the dos-

ing period (8–11). In addition, the amount of health care resources required for a treatment or prevention strategy in the developing world is directly proportional to the required dosing frequency (12, 13). For this reason, much effort has been expended to reduce the dosing frequency in tuberculosis eradication campaigns, where a twice-a-week regimen is considered a significant advance over daily dosing (14). Sustained-release drug delivery holds significant promise as a means of reducing the dosing frequency and thus increasing the effectiveness of HIV prophylaxis with NVP.

There have been a few reports on sustained-release NVP. Chen and colleagues described a subcutaneous NVP implant that exhibited excellent *in vitro-in vivo* correlation in rats and demonstrated the long-term safety and feasibility of the concept of sustained-release NVP (15). However, surgical implantation is a major drawback, especially in resource-limited settings. Additionally, because the implant is visible under the skin, the possibility of stigmatization makes this approach potentially dangerous for the mother and child (16).

Received 17 July 2014 Returned for modification 28 August 2014

Accepted 3 October 2014

Accepted manuscript posted online 13 October 2014

Citation Cortez JM, Jr, Quintero R, Moss JA, Beliveau M, Smith TJ, Baum MM. 2015. Pharmacokinetics of injectable, long-acting nevirapine for HIV prophylaxis in breastfeeding infants. *Antimicrob Agents Chemother* 59:59–66. doi:10.1128/AAC.03906-14.

Address correspondence to Marc M. Baum, m.baum@oak-crest.org.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/AAC.03906-14>.

Copyright © 2015, American Society for Microbiology. All Rights Reserved. doi:10.1128/AAC.03906-14

Here, the first report on injectable sustained-release NVP formulations is presented. The goals of the research were to measure the *in vitro* release profiles of polymer-coated NVP microparticles, to evaluate the pharmacokinetics (PK) of the formulations *in vivo* using a rat model, and to simulate the PK in human neonates. The results demonstrate the preliminary feasibility of the technology for maintaining safe and preventative NVP plasma levels for 6 weeks or longer with a single injectable dose.

MATERIALS AND METHODS

Materials. Nevirapine (NVP) was obtained from Sinobright Import Export Co., Ltd. (China) and was used as received. The identity and purity of the drug substance were confirmed by ^1H nuclear magnetic resonance (NMR) spectroscopy and melting point measurement. Poly(vinyl alcohol) (PVA) with a mean molecular weight (M_w) of 145,000 was obtained from Sigma-Aldrich (St. Louis, MO). Two poly(D,L-lactide) (PLA) polymers were used: M_w of 250,000 (Fluka Chemical Corp., St. Louis, MO) and M_w of 10,000 (Resomer R 202 S; Boehringer-Ingelheim, Petersburg, VA).

Formulation of the NVP long-acting 1 (LA-1) microparticle suspension. Nevirapine powder (90% of particles of $<5\ \mu\text{m}$) was compacted at 0.62 GPa, resulting in the formation of “hyalinized” wafers that were glassy, translucent, and friable and approximately $150\ \mu\text{m}$ thick. The wafers were gently pressed through a standard (U.S. standard ASTM E 11-61 specification) number 45 sieve ($355\text{-}\mu\text{m}$ mesh opening) with a glass rod and collected on a number 50 sieve ($300\text{-}\mu\text{m}$ mesh opening) as rough cubes. The particles were dip coated with a 1% (wt/vol) PVA solution in deionized water five successive times, heat treated at 150°C for 1 h, spray coated with a 5% (wt/vol) PLA (M_w of 250,000) solution in dichloromethane-ethyl acetate (1:1, vol/vol) five successive times and dip coated in a 1% (wt/vol) PLA and PVA solution five times, followed by another five coats of PLA. The polymer content of the formulation was measured by quantitative ^1H NMR (qNMR) spectroscopy using the following peaks: δ_{H} , 7.0 to 7.3 ppm (NVP), 5.0 to 5.3 ppm (PLA), and 4.4 to 4.7 ppm (PVA). The particle size distribution was measured by laser diffraction with a model LS100Q (Beckman-Coulter, Brea, CA) dynamic light-scattering analyzer and use of a Fraunhofer optical model. The thickness of the polymer membrane was calculated based on these measurements, assuming that the shape is an idealized cuboidal, the diameter measured by laser diffraction equals the space diagonal of the cube, and the densities of the drug and polymers are equal.

Formulation of the NVP long-acting 2 (LA-2) microparticle suspension. Nevirapine powder was recrystallized from reagent alcohol to afford cuboidal crystals that were filtered *in vacuo* and collected on a sieve stack between a number 60 sieve ($250\text{-}\mu\text{m}$ mesh opening) and a number 80 sieve ($180\text{-}\mu\text{m}$ mesh opening). The particles were coated using a model MFL01 Micro Flo-Coater (Vector Corporation, Marion, IA) fluidized bed system and a 5% (wt/vol) PLA (M_w of 10,000) solution in dichloromethane-ethyl acetate (1:1, vol/vol). The PLA content of the formulation was determined by qNMR spectroscopy as described above. The particle size distribution and polymer membrane thickness were determined as above.

***In vitro* release kinetics measurements.** To each of six vessels (1 liter) in a USP type II dissolution system, deionized water (500 ml) and the NVP test formulation (ca. 2 mg) were added, and the suspension was stirred at 30 rpm and ambient temperature (26 to 30°C). Aliquots (3 ml) were removed from the vessels at predetermined time points and replaced with deionized water. The samples were analyzed by UV spectroscopy to determine the NVP concentration ($\lambda_{\text{max}} = 215\ \text{nm}$). The NVP release half-life ($t_{1/2}$) was calculated by fitting the kinetics data to a single exponential decay equation model, and the absorption rate constant (K) was calculated as $\ln 2/t_{1/2}$.

Animals. Two animal studies were carried out at Charles River Laboratories (Shrewsbury, MA) under approved internal institutional animal care and use committee protocols and standard guidelines according to

the Guide for the Care and Use of Laboratory Animals (DHEW no. NIH 86-23). Study 1 (JNN00006, March 2006) evaluated the PK and toxicity of subcutaneous NVP long-acting 1 (NVP LA-1) in Sprague-Dawley rats ($n = 4$, mean weight of $0.33\ \text{kg}$). Study 2 (JNN00030, May 2009) evaluated the PK and toxicity of subcutaneous NVP LA-2 in Sprague-Dawley rats ($n = 10$, mean weight of $0.21\ \text{kg}$). All animals were housed under standard conditions and had *ad libitum* access to water and a standard laboratory diet. Euthanasia (carbon dioxide asphyxiation followed by thoracotomy) was performed in accordance with accepted American Veterinary Medical Association (AVMA) guidelines.

NVP LA administration and sampling. Doses were administered once via subcutaneous injection: study 1, $60\ \text{mg}$ NVP LA-1 suspension in $1\ \text{ml}$ saline injected via a 14-G needle; study 2, NVP LA-2 suspension, weight-adjusted dose of $36\ \text{mg}\ \text{kg}^{-1}$ (dose concentration, $12\ \text{mg}\ \text{ml}^{-1}$ in 1% [wt/wt] carboxymethylcellulose) injected via an 18-G needle. Blood samples were collected at predetermined time points: for study 1 on days 1 (1 and 6 h postdose), 2, 3, 4, 6, 10, 14, and 28; for study 2 on days -7, 1 (1, 3, 7, and 12 h postdose), 2, 3, 7, 14, 21, and 28. Blood samples ($0.5\ \text{ml}$) were collected from the jugular vein into tubes containing K_2EDTA and stored on wet ice before being processed for plasma. Plasma samples were stored and transported at -70°C .

Assessment of toxicity. The toxicity was evaluated by monitoring clinical observations, cageside observations, and body weight. A complete gross necropsy was conducted on all animals. The necropsy included examination of the carcass and musculoskeletal system, all external surfaces and orifices, the cranial cavity and external surface of the brain, and all thoracic, abdominal, and pelvic cavities with their associated organs and tissues. All abnormalities were described and recorded. The tissues from the administration site were examined *in situ*, dissected free, and fixed in 10% neutral buffered formalin or other suitable fixative. The tissue specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The tissue specimens were read and interpreted by a veterinary pathologist with training and experience in laboratory animal pathology.

Analysis of NVP in rat plasma. Plasma samples from both studies were analyzed at the Charles River Laboratories using the bioanalytical methods described below. Both had a lower limit of quantitation (LLOQ) of $1\ \text{ng}\ \text{ml}^{-1}$ NVP.

In study 1, thawed plasma sample aliquots ($25\ \mu\text{l}$) were transferred to 96-well plates followed by the addition of internal standard (IS) solution ($0.25\ \mu\text{g}\ \text{ml}^{-1}$ glyburide in acetonitrile, $100\ \mu\text{l}$). The samples were mixed by vortex agitation, followed by centrifugation (5 min), and transferred to a new plate where they were dried under a nitrogen stream. The samples were reconstituted in $100\ \mu\text{l}$ 10% (vol/vol) acetonitrile in water prior to analysis. The concentration of NVP was determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a high-performance liquid chromatography (HPLC) system consisting of series 200 micropumps (PerkinElmer, Shelton, CT) interfaced to an API 5000 mass spectrometer (Applied Biosystems, Concord, ON, Canada) using a Thermo Scientific (Waltham, MA) Hypersil Gold column (2.1 by $20\ \text{mm}$; $3\ \mu\text{m}$) controlled at 40°C as the stationary phase. The following gradient program was used: 1.0-min ramp from 95:5 A/B (A, 1.0% [vol/vol] formic acid in water; B, 1.0% [vol/vol] formic acid in acetonitrile) to 5:95 A/B, resulting in a total run time of 1.9 min. The measured transition ions (m/z) under the atmospheric pressure chemical ionization (APCI) positive ionization mode were as follows: NVP, parent, 267.1 atomic mass units (amu), and product, 226.1 amu; glyburide (IS), parent, 494.2 amu, and product, 369.1 amu.

In study 2, thawed plasma sample aliquots ($25\ \mu\text{l}$) were transferred to 96-well plates followed by the addition of internal standard solution ($0.10\ \mu\text{g}\ \text{ml}^{-1}$ reserpine in acetonitrile, $100\ \mu\text{l}$). The samples were mixed by vortex agitation, followed by centrifugation (5 min), and transferred to a new plate, and Milli-Q water ($50\ \mu\text{l}$) was added to all samples. The concentration of NVP was determined by LC-MS/MS using an HPLC system consisting of series 200 micropumps (PerkinElmer) interfaced to an API

4000 mass spectrometer (Applied Biosystems) using a Waters (Waltham, MA) X-Terra C₁₈ column (2.1 by 30 mm; 3.5 μm) at ambient temperature as the stationary phase. The following gradient program was used: 0.5-min ramp from 95:5 A/B (A, 1.0% [vol/vol] formic acid in water; B, 1.0% [vol/vol] formic acid in acetonitrile) to 5:95 A/B, resulting in a total run time of 2.1 min. The measured transition ions (*m/z*) under the TurboIon-Spray (TIS) positive ionization mode were as follows: NVP, parent, 267.1 amu, and product, 226.1 amu; reserpine (IS), parent, 609.1 amu, and product, 195.2 amu.

Pharmacokinetic analysis software. The population PK modeling and simulations were performed using Phoenix NLME software (version 6.3.0.395; Pharsight Corporation, Sunnyvale, CA). Data set preparation, exploration, and visualization of the data were performed using Microsoft Excel, Phoenix, or GraphPad Prism (version 6.02; GraphPad Software, Inc.).

Population PK data set construction. A population PK data set was constructed based on data collected in rats. The data set included the nominal times of NVP dose administration and of blood sampling collection. For study 1, it was assumed that 75% of the dose was received by the animal, i.e., 45 mg (135 mg kg⁻¹), due to injection inefficiencies. For study 2, it was assumed that 80% of the intended dose was received, i.e., 6.7 mg (29 mg kg⁻¹). Concentrations below the lower limit of quantification (BLLOQ) of the assay were set as missing.

Population PK model development. The population PK analysis was performed in a stepwise manner by modeling plasma drug concentration data following subcutaneous administration in rats of the long-acting NVP. Prior to performance of modeling and simulations, a visual inspection of the mean and individual concentration-time profiles was performed to identify possible outliers (subjects and concentrations) and candidate structural PK models. A basic structural PK model was used for fitting the plasma drug concentration versus time data obtained from preclinical studies. The goodness of fit (GOF) was evaluated using diagnostic plots and related statistical estimators.

Structural PK model buildup. Various compartmental PK models were constructed to assess the PK. For example, 1- and 2-compartment models with linear disposition were compared. In general, the models consisted of the following: description of the relationships between plasma NVP concentrations and time and the residual component describing uncertainty in the state of knowledge about the critical model components. The population PK model had the following form:

$$C_{p,ij} = C(D_i, t_j, \theta_i) + \varepsilon_{ij} \quad (1)$$

$$\theta_i = (\theta_{i1}, \dots, \theta_{im})$$

where $C_{p,ij}$ is the concentration at the j th time (t_j) for animal i , D_i represents the dosing history for animal i , θ_i is the vector of m model parameters for animal i , and ε_{ij} is the random error associated with the j th concentration for animal i .

Due to the low number of rats ($n = 4$ or $n = 10$) available for each formulation, no between-subject variability (BSV) was added in the PK model although various residual error models were evaluated. Residual variability was nominally assumed to have an additive component and a component proportional to the prediction:

$$y_{ij} = \hat{y}_{ij} \times (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \quad (2)$$

where y_{ij} and $\hat{y}_{ij}$ represent the j th observed and predicted plasma drug concentrations for the i th participant, and ε is the random residual variability. Each ε (ε_1 and ε_2) is normally distributed with mean 0 and variance σ^2 . This model can be simplified to an additive only model ($\varepsilon_1 = 0$) or proportional only model ($\varepsilon_2 = 0$).

The robustness of the NVP PK parameters was evaluated using graphical tools and statistical estimators (see Fig. S1 and S2 in the supplemental material). Model evaluation and selection of the structural model were based on (i) the model acceptability according to the pharmacology and physiology of the drug; (ii) the statistical standard criteria of GOF, such as the log-likelihood difference between rival models (e.g., a decrease in the

TABLE 1 Physical characteristics of long-acting NVP formulations used in animal studies

Physical characteristic	Results for formulation:	
	NVP LA-1	NVP LA-2
Appearance	Orange-brown	Off white
Median diameter, d_p (μm)	411	239
Skewness	-3.63	-4.26
Kurtosis	16.6	23.8
PVA content (% wt/wt)	4	0
PLA content (% wt/wt)	25	9
Membrane thickness (μm)	13	2
Dissolution half-life ($t_{1/2}$) (days)	3.15 (2.96–3.36) ^a	18.26 (16.33–20.70)
Dissolution rate constant (K) (day ⁻¹)	0.220 (0.201–0.234)	0.0380 (0.0335–0.0424)

^a 95% confidence intervals are in parentheses.

minimum objective function value, -2LL); and (iii) the pertinent graphical representations of GOF (e.g., fitted and observed concentrations versus time and residuals versus time) and precision of the parameter estimates. The naive pooled approach was used.

Equations describing the allometric scaling of interspecies PK parameters are based on the premise that physical or physiological parameters in species vary as a function of body weight (17, 18). Body weight was included *de facto*, based on the need for allometric functions on clearances (CL and CL_d) and volumes of distribution (central [V_c] and peripheral [V_p]) for simulations in humans. The following power function was used:

$$\theta_i = \theta_{\text{Typical}} \cdot \left(\frac{\text{Cov}_i}{\text{Cov}_{\text{reference value}}} \right)^{\theta_{\text{eff}}} \quad (3)$$

where θ_i is the value of the parameter for the i th individual, θ_{Typical} is the typical value of the PK parameter for an individual having the covariate equal to the covariate reference value, Cov_i is the value of the covariate for the individual, $\text{Cov}_{\text{reference value}}$ is the median of the covariate in the animals, and θ_{eff} is the value of the effect of the covariate on the parameter. The nominal covariate effect was -0.25 on absorption rate, 1 on volumes, and 0.75 on clearance (19).

Human simulations. Infant parameters for the human simulations were compiled from recent clinical studies in Uganda examining the population PK of single-dose NVP in breastfeeding infants of HIV-positive mothers (20, 21). The newborns' weights ranged from 2.0 to 3.9 kg, and the following systemic parameters were determined from population PK estimates: $V/\text{bioavailability}$ (F), 20.0 liters; CL/F , 0.21 liters h⁻¹; and $t_{1/2}$, 66 h. These systemic parameters were combined with presystemic rat parameters, essentially the absorption rate constant, k_a , in order to simulate human exposure. Simulations were performed using a naive-pooled approach to estimate the mean plasma drug concentrations in humans.

Statistical analysis. Data set group statistics (medians and standard deviations [SD]) and plots were carried out using GraphPad Prism.

RESULTS

Physical characteristics of the long-acting NVP formulations.

The physical characteristics of the sustained-release microparticle NVP formulations are presented in Table 1 and Fig. 1. The orange-brown color of NVP LA-1 (Table 1) is the result of dehydration of the PVA backbone during thermal processing, leading to conjugated double bonds (22, 23). *In vitro* cumulative release profiles (Fig. 2; Table 1) from both formulations exhibited burst-free, sustained release for up to 75 days and could be fit to a simple exponential decay model.

Toxicity. No adverse events related to the test material were noted during the course of the studies. Overall, no significant ab-

FIG 1 Particle size distribution histograms, as volume distributions (V), for NVP LA-1 (A) and NVP LA-2 (B), measured by laser diffraction using a Fraunhofer optical model.

normalities were observed. The animals showed no failure to eat or drink, no failure to groom, and no weight loss. There was no clinical evidence of inflammation at the injection sites. In general, inflammation within the skin sections examined was uncommon and confined to the subpannicular fascia.

Of the nine skin sections examined in study 1, only four sections (i.e., both middle and caudal injection sites of two animals [ID 02 and 03]) contained evidence of cellular infiltrate/inflammation. Within primarily the subpannicular fascia, there were scattered to regional, and sometimes perivascular, minimal mild chronic inflammation (i.e., admixture of histiocytes/macrophages, lymphocytes, and/or plasma cells) that was typically admixed with widespread variable numbers of foamy macrophages with a granular to fibrillar cytoplasmic content. Also noted in the subpannicular fascia of two animals (ID 02 and 03) was a foreign body reaction characterized by aggregates of multinucleated giant cells and macrophages, associated with fragments of a basophilic polymorphic granular to smooth material interpreted as residual capsular material from the test article. This mild chronic inflammatory reaction is consistent with other reports in the literature of responses to the injection of other approved drug delivery systems based on polylactic acid polymers (24).

In study 2, one animal (ID 03) had abrasions on both shoulders and scabs on the right shoulder and abdomen. No visible lesions were observed on tissues and organs collected after necropsy, including the administration site.

Pharmacokinetics of sustained-release NVP in rats. Rat plasma NVP concentration versus time plots following a single subcutaneous dose are shown in Fig. 3. These data were fit to compartmental PK models in order to assess the absorption rate of

each formulation. Pharmacokinetic parameters are presented in Table 2.

Human simulation. Human (2.0 and 3.9 kg body weight) simulations of plasma NVP concentration versus time plots following a single subcutaneous dose are shown in Fig. 4. The bioavailable fraction was assumed to be 100% in these simulations.

DISCUSSION

The primary objectives of the current study were to develop sustained-release injectable formulations of NVP, measure the PK of the formulations in rats, and use the PK data to simulate the PK of the formulations in human infants. The results are discussed below with an emphasis on HIV prophylaxis in breastfeeding infants.

Long-acting parenteral antiretroviral therapy and HIV prophylaxis. Daily dosing is burdensome to the user, leading to concomitant adherence-related issues in HIV prevention and the treatment of HIV/AIDS. As a result, long-acting antiretroviral therapy (LA-ART) and long-acting preexposure prophylaxis (LA-PrEP) have emerged recently as alternative regimens (25–27). These efforts primarily have focused on nanomilled formulations of the antiretroviral (ARV) drugs, allowing them to be administered parenterally as injections (25, 26). The ARV drugs atazanavir and ritonavir, nanoformulated in poloxamer 188 excipient by high-pressure homogenization, were evaluated preclinically as LA-ART candidates in humanized mouse (28) and nonhuman primate models (29). Parenteral administration of the integrase strand-transfer inhibitor GSK1265744 (GSK744) fully protected rhesus macaques against repeated low-dose intrarectal challenges of SHIV162P3 (30). Monthly or bimonthly injections of long-acting nanoparticulate formulations of GSK744 and the non-

FIG 2 Cumulative *in vitro* release kinetics (mean $\pm$ SD, $n = 6$) of long-acting NVP formulations. The gray line corresponds to the fit result using an exponential decay equation model. (A) NVP LA-1, $R^2 = 0.9739$; (B) NVP LA-2, $R^2 = 0.8815$.

FIG 3 Rat plasma NVP concentration (median $\pm$ SD) versus time plots (NVP LA-1, study 1 [A]; NVP LA-2, study 2 [B]) following a single subcutaneous injection of a particle suspension. The gray dotted line indicates the LLOQ of 1 ng mL^{-1} .

nucleoside reverse transcriptase inhibitor rilpivirine (RPV) are being investigated clinically as possible regimens for HIV therapy (25, 27, 31).

HIV prophylaxis using long-acting NVP formulations. Parenteral long-acting ARV regimens require infrequent dosing (e.g., monthly) to be suitable for HIV PrEP (25). In the case of NVP for prevention of HIV infection in breastfeeding infants, a single dose should provide prophylactic systemic drug exposure for 6 weeks, in accordance with the WHO guidelines (2). The dosing frequency of ARV nanoformulations is determined by the aqueous solubility of the drug, the antiviral potency, and the systemic clearance kinetics. These criteria severely limit the number of FDA-approved ARV agents suitable for reformulation as nanoparticles (25). Surface-modified NVP nanosuspensions were developed for organ-targeted delivery and evaluated in the rat model (32). Bare and surface-modified NVP nanoparticles did not maintain detectable plasma levels beyond 24 h, suggesting that an alternative drug delivery approach is required.

A novel approach to sustained delivery of ARV agents. The long-acting parenteral depot drug delivery system described here differs significantly from the more widely used nanoparticle formulations and microspheres produced by emulsion-solvent evaporation, consisting of a drug encapsulated within a biodegradable polymer matrix. Our approach involves large ($>50\text{-}\mu\text{m}$) particles

consisting of solid drug cores coated with thin, biocompatible polymer membranes (Table 1). The dissolution by surface action of a solid particle suspended in liquid medium can be described in terms of the Noyes-Whitney equation (33), where the rate of dissolution is directly proportional to the particle surface area. Large-particle depots also greatly increase the drug payload compared to that for the more common small-particle ($<10 \text{ }\mu\text{m}$) formulations. For example, a micronized drug particle of $7 \text{ }\mu\text{m}$ in diameter with a $5\text{-}\mu\text{m}$ coating has a drug loading of only 10% (34), whereas our NVP LA-1 is made up of $411\text{-}\mu\text{m}$ particles with a $13\text{-}\mu\text{m}$ -thick coating, affording a drug loading of 71% (Table 1).

Both NVP LA formulations consist predominantly of large particles (median d_p , $>200 \text{ }\mu\text{m}$ for both formulations) (Table 1) with high monodispersity (Fig. 1). This observation is supported by the degree of observed kurtosis (Table 1), suggesting a leptokurtic distribution where the size of the majority of particles is close to the mean size. The negative skewness values (Table 1) suggest a deviation from idealized symmetry, with tailing at the fine particle sizes.

The relatively small membrane thickness in both candidates ensures that transmembrane flux determines the kinetics of dissolution. While both formulations displayed first-order dissolution kinetics *in vitro*, NVP LA-1 released its drug payload considerably faster than NVP LA-2. The half-life of release, $t_{1/2}$, of NVP LA-2 was 5.7 times higher than that for NVP LA-1 (Table 1). This is likely due to a more even coating of the blocking polymer (PLA) in NVP LA-2 obtained by the fluidized bed coating, rather than the manual spray coating used for NVP LA-1.

Our formulation strategy results in the absence of a burst in initial release (Fig. 2), a nontrivial finding, as all sustained-release parenteral suspensions currently marketed have significant burst effects (35, 36). The absence of a concentration burst makes the novel drug delivery strategy described here translational to other therapeutic arenas where controlled drug release within a target concentration window is important for favorable pharmacodynamic outcomes.

Pharmacokinetics of sustained-release NVP in rats. PK parameters from both studies are summarized in Table 2. It should be noted that the values of V and CL derived from the models (Table 2) do not correspond to “true” systemic and peripheral parameters as would be obtained following intravenous (i.v.) dosing. Rather, these values represent formulation-dependent parameters influenced by the so-called “flip-flop” phenomena (37) that arise because the absorption kinetics of the sustained release formulations are slower than the corresponding systemic elimina-

TABLE 2 Summary of key pharmacokinetic parameter values for the long-acting NVP formulations in Sprague-Dawley rats

Parameter ^a	Estimate (% RSE ^b) for:	
	Study 1, NVP LA-1 ($n = 4$, mean wt 0.33 kg)	Study 2, NVP LA-1 ($n = 10$, mean wt 0.21 kg)
K_a (day^{-1})	1.89 (26.1)	15.4 (41.8)
$t_{1/2,a}$ (days)	0.367 (0.0266)	0.0450 (0.0166)
V_c/F (liters)	8.66 (17.9)	74.0 (16.2)
V_p/F (liters)	14.3 (NA ^c)	NA (NA)
CL/F (liters day^{-1})	3.43 (11.3)	48.2 (47.2) ^d
CL_p/F (liters day^{-1})	0.541 (35.8)	NA (NA)
Error	± 28.6 (40.4) $\mu\text{g liter}^{-1}$ ± 50.1 (32.4) %	± 79.0 (9.4) %

^a K_a , absorption rate; $t_{1/2,a}$, absorption half-life; V_c , central volume of distribution; V_p , peripheral volume of distribution; CL , clearance; CL_p , peripheral clearance; F , bioavailability.

^b RSE, relative standard error.

^c NA, not available.

^d Represents intrinsic clearance (V_m/K_m), where V_m is the maximal metabolic rate and K_m is the Michaelis-Menten constant.

FIG 4 Human plasma NVP simulation versus time plots following a single subcutaneous injection of a long-acting NVP particle suspension. (A) NVP LA-1, 200-mg dose, 2.0-kg body weight; (B) NVP LA-1, 200-mg dose, 3.9-kg body weight; (C) NVP LA-2, 60-mg dose, 2.0-kg body weight; (D), NVP LA-2, 60-mg dose, 3.9-kg body weight.

tion. The absence of suitable i.v. NVP rat PK data precluded co-modeling to force the systemic and peripheral values of V and CL and subsequently determine physically meaningful absorption rate constant (K_a) values for both formulations. A modeling approach therefore was adopted that optimized K_a , V , and CL to best fit the observed profiles, with respect to the maximum concentration of drug in serum (C_{max}) and the time to maximum concentration of drug in serum (T_{max}); therefore, the PK parameters for both formulations shown in Table 2 cannot be compared directly. While these values may not represent physical reality, they were useful as a framework for human simulation and to establish quantitative estimates for lead prioritization.

Overall, a two-compartment model with linear absorption and linear elimination adequately described the study 1 formulation, while a one-compartment model with linear absorption and saturable elimination adequately described the study 2 formulation. Parameter precision and residual error were lower for the study 1 formulation (see Fig. S1 and S2 in the supplemental material), reflecting the better fit of the model to these formulation data. The subcutaneous bioavailability, F , was assumed to be 100% for both formulations based on previous studies in rats (38). The rate of absorption for study 2 was greater than that for study 1, indicating that the peak levels were achieved quickly for the study 2 formulation. Bearing the above caveats regarding direct comparison of PK parameters across the two formulations in mind, we determined that the *in vivo* absorption rate constant (K_a) of NVP LA-2 was 8 times that of NVP LA-1; i.e., NVP LA-1 had a larger (slower) release absorption half-life, $t_{1/2, a}$ (Table 2). This difference cannot be explained in terms of the bioavailability, F , as this would shift the concentrations in the vertical direction (Fig. 3) but would not change the shape of the drug time course profile. The absolute NVP level (C_{max} and area under the curve [AUC]) was lower for study 2, however, due to the lower dose, higher volume, and clearance and/or different bioavailability.

Implications for HIV prevention in breastfeeding infants.

The sustained-release NVP formulations described here are intended primarily for HIV-1 prevention of MTCT, not HAART regimens. Prophylactic efficacy in breastfeeding neonates of HIV-positive mothers requires sustained infant NVP blood exposure that significantly exceeds the 90% inhibitory concentration (IC_{90}) value of the drug (39, 40), although the protective concentration threshold is unknown. The IC_{90} for NVP has been reported for different HIV strains: wild-type HXB2, $40 \pm 16 \text{ ng mL}^{-1}$ ($150 \pm 59 \text{ nM}$); wild-type NL4-3, $32 \pm 14 \text{ ng mL}^{-1}$ ($120 \pm 52 \text{ nM}$) (41). HIVNET 023 was a phase 1/2 open-label randomized trial designed to determine the safety and minimum dosing schedule of NVP for maintaining a trough plasma drug concentration of $>100 \text{ ng mL}^{-1}$ among breastfeeding infants for 24 weeks (42). Based on these values and newborn NVP plasma drug data from clinical studies (20, 21, 43), a conservative lower prophylactic NVP infant plasma drug concentration of $0.2 \mu\text{g mL}^{-1}$ (5 times the IC_{90}) is targeted here. Steady-state NVP plasma levels of $3 \mu\text{g mL}^{-1}$ are generally considered safe and represent a therapeutic target (44, 45). Based on this analysis, it is proposed that a long-acting NVP formulation should maintain infant drug plasma levels between 0.2 and $3.0 \mu\text{g mL}^{-1}$.

Combining the presystemic absorption rate constants obtained from rats, which provide estimates of the rate-limiting release from the microparticles, with the systemic parameters of NVP following oral administration in infants, the performance of these formulations in pediatric subjects at the proposed dose regimen can be determined via simulation.

Simulations showed that both formulations administered via subcutaneous injection at less than the daily oral adult 200-mg NVP dose would maintain NVP plasma levels in the above target range, or longer, in infants. The NVP LA-1 simulation (Fig. 4A and B) predicts plasma NVP levels dropping to ca. $0.2 \mu\text{g mL}^{-1}$ within a few weeks after dosing. When administered as a 60-mg dose, NVP LA-2 is pre-

dicted to maintain NVP plasma levels in the target 0.2 to 3.0 $\mu\text{g ml}^{-1}$ range for 90 days or longer (Fig. 4C and D).

Based on these encouraging results, we have applied for and received (25 November 2009) orphan drug designation for NVP in the “prevention of HIV infection in pediatric patients under the age of 16 years” (http://www.accessdata.fda.gov/scripts/opdlisting/oopd/OOPD_Results_2.cfm?Index_Number=293409).

In conclusion, long-acting NVP has translational potential as a candidate regimen for HIV prophylaxis in breastfeeding infants in resource-constrained regions. Sustained-release NVP delivery and distribution might improve drug adherence and reduce transmission compared to regimens with daily dosing. This innovative approach is in alignment with World Health Organization guidelines.

ACKNOWLEDGMENTS

The project described here was supported by grants R43HD050146 and R44HD050146 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Child Health and Human Development or the National Institutes of Health.

REFERENCES

1. Joint United Nations Programme on HIV/AIDS. 2013. Global report: UNAIDS report on the global AIDS epidemic 2013. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland.
2. World Health Organization. 2010. Antiretroviral drugs for treating pregnant women and preventing HIV infection in infants: recommendations for a public health approach. World Health Organization, Geneva, Switzerland.
3. De Cock KM, Fowler MG, Mercier E, de Vincenzi I, Saba J, Hoff E, Alnwick DJ, Rogers M, Shaffer N. 2000. Prevention of mother-to-child HIV transmission in resource-poor countries: translating research into policy and practice. *JAMA* 283:1175–1182. <http://dx.doi.org/10.1001/jama.283.9.1175>.
4. Guay LA, Musoke P, Fleming T, Bagenda D, Allen M, Nakabiito C, Sherman J, Bakaki P, Ducar C, Deseyve M, Emel L, Mirochnick M, Fowler MG, Mofenson L, Miotti P, Dransfield K, Bray D, Mmiro F, Jackson JB. 1999. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVMET 012 randomised trial. *Lancet* 354:795–802. [http://dx.doi.org/10.1016/S0140-6736\(99\)80008-7](http://dx.doi.org/10.1016/S0140-6736(99)80008-7).
5. Jackson JB, Musoke P, Fleming T, Guay LA, Bagenda D, Allen M, Nakabiito C, Sherman J, Bakaki P, Owor M, Ducar C, Deseyve M, Mwatha A, Emel L, Duefield C, Mirochnick M, Fowler MG, Mofenson L, Miotti P, Gigliotti M, Bray D, Mmiro F. 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. [http://dx.doi.org/10.1016/S0140-6736\(03\)14341-3](http://dx.doi.org/10.1016/S0140-6736(03)14341-3).
6. Miotti PG, Taha TET, Kumwenda NI, Broadhead R, Mtimaivalye LAR, Van der Hoeven L, Chipangwi JD, Liomba G, Biggar RJ. 1999. HIV transmission through breastfeeding—a study in Malawi. *JAMA* 282:744–749. <http://dx.doi.org/10.1001/jama.282.8.744>.
7. Nduati R, John G, Mbori-Ngacha D, Richardson B, Overbaugh J, Mwatha A, Ndinya-Achola J, Bwayo J, Onyango FE, Hughes J, Kreiss J. 2000. Effect of breastfeeding and formula feeding on transmission of HIV-1—a randomized clinical trial. *JAMA* 283:1167–1174. <http://dx.doi.org/10.1001/jama.283.9.1167>.
8. Kruse W, Eggertkruse W, Rampmaier J, Runnebaum B, Weber E. 1991. Dosage frequency and drug compliance behavior—a comparative study on compliance with a medication to be taken twice or 4 times daily. *Eur J Clin Pharmacol* 41:589–592. <http://dx.doi.org/10.1007/BF00314990>.
9. Serksen S, West J. 2002. Implantable, polymeric systems for modulated drug delivery. *Adv Drug Deliv Rev* 54:1225–1235. [http://dx.doi.org/10.1016/S0169-409X\(02\)00090-X](http://dx.doi.org/10.1016/S0169-409X(02)00090-X).
10. Kutilek VD, Sheeter DA, Elder JH, Torbett BE. 2003. Is resistance futile? *Curr Drug Targets Infect Disord* 3:295–309. <http://dx.doi.org/10.2174/1568005033481079>.
11. Yeaw J, Benner JS, Walt JG, Sian S, Smith DB. 2009. Comparing adherence and persistence across 6 chronic medication classes. *J Manag Care Pharm* 15:728–740.
12. Schieber G, Maeda A. 1999. Health care financing and delivery in developing countries. *Health Aff (Millwood)* 18:193–205. <http://dx.doi.org/10.1377/hlthaff.18.3.193>.
13. Narasimhan V, Brown H, Pablos Mendez A, Adams O, Dussault G, Elzinga G, Nordstrom A, Habte D, Jacobs M, Solimano G, Sewankambo N, Wibulpolprasert S, Evans T, Chen L. 2004. Responding to the global human resources crisis. *Lancet* 363:1469–1472. [http://dx.doi.org/10.1016/S0140-6736\(04\)16108-4](http://dx.doi.org/10.1016/S0140-6736(04)16108-4).
14. Benator D, Bhattacharya M, Bozeman L, Burman W, Cantazaro A, Chaisson R, Gordin F, Horsburgh CR, Horton J, Khan A, Lahart C, Metchock B, Pachucki C, Stanton L, Vernon A, Villarino ME, Wang YC, Weiner M, Weis S. 2002. Rifapentine and isoniazid once a week versus rifampicin and isoniazid twice a week for treatment of drug-susceptible pulmonary tuberculosis in HIV-negative patients: a randomised clinical trial. *Lancet* 360:528–534. [http://dx.doi.org/10.1016/S0140-6736\(02\)09742-8](http://dx.doi.org/10.1016/S0140-6736(02)09742-8).
15. Chen J, Walters K, Ashton P. 2005. Correlation of in vitro-in vivo release rates for sustained release nevirapine implants in rats. *J Control Release* 101:357–358.
16. Rankin WW, Brennan S, Schell E, Laviwa J, Rankin SH. 2005. The stigma of being HIV-positive in Africa. *PLoS Med* 2:e247. <http://dx.doi.org/10.1371/journal.pmed.0020247>.
17. West GB, Brown JH. 2005. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. *J Exp Biol* 208:1575–1592. <http://dx.doi.org/10.1242/jeb.01589>.
18. Sharma V, McNeill JH. 2009. To scale or not to scale: the principles of dose extrapolation. *Br J Pharmacol* 157:907–921. <http://dx.doi.org/10.1111/j.1476-5381.2009.00267.x>.
19. Gabrielson J, Weiner D. 2006. Pharmacokinetic and pharmacodynamic data analysis: concepts and applications, 4th ed. Swedish Pharmaceutical Press, Stockholm, Sweden.
20. Frank M, von Kleist M, Kunz A, Harms G, Schutte C, Kloft C. 2011. 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. *Antimicrob Agents Chemother* 55:5529–5540. <http://dx.doi.org/10.1128/AAC.00741-11>.
21. Frank M, Harms G, Kunz A, Kloft C. 2013. Population pharmacokinetic analysis of a nevirapine-based HIV-1 prevention of mother-to-child transmission program in Uganda to assess the impact of different dosing regimens for newborns. *J Clin Pharmacol* 53:294–304. <http://dx.doi.org/10.1177/0091270012448397>.
22. Byron PR, Dalby RN. 1987. Effects of heat treatment on the permeability of polyvinyl alcohol films to a hydrophilic solute. *J Pharm Sci* 76:65–67. <http://dx.doi.org/10.1002/jps.2600760118>.
23. Petrova NV, Evtushenko AM, Chikhacheva IP, Zubov VP, Kubrakova IV. 2005. Effect of microwave irradiation on the cross-linking of polyvinyl alcohol. *Russ J Appl Chem* 78:1158–1161. <http://dx.doi.org/10.1007/s11167-005-0470-1>.
24. Hyon SH. 2000. Biodegradable poly(lactic acid) microspheres for drug delivery systems. *Yonsei Med J* 41:720–734. <http://dx.doi.org/10.3349/ymj.2000.41.6.720>.
25. Spreen WR, Margolis DA, Pottage JC. 2013. Long-acting injectable antiretrovirals for HIV treatment and prevention. *Curr Opin HIV AIDS* 8:565–571. <http://dx.doi.org/10.1097/COH.000000000000002>.
26. Williams J, Sayles HR, Meza JL, Sayre P, Sandkovsky U, Gendelman HE, Flexner C, Swindells S. 2013. Long-acting parenteral nanoformulated antiretroviral therapy: interest and attitudes of HIV-infected patients. *Nanomedicine* 8:1807–1813. <http://dx.doi.org/10.2217/nnm.12.214>.
27. Dolgin E. 2014. Long-acting HIV drugs advanced to overcome adherence challenge. *Nat Med* 20:323–324. <http://dx.doi.org/10.1038/nm0414-323>.
28. Dash PK, Gendelman HE, Roy U, Balkundi S, Alnouti Y, Mosley RL, Gelbard HA, McMillan J, Gorantla S, Poluektova LY. 2012. Long-acting nanoformulated antiretroviral therapy elicits potent antiretroviral and neuroprotective responses in HIV-1-infected humanized mice. *AIDS* 26:2135–2144. <http://dx.doi.org/10.1097/QAD.0b013e328357f5ad>.

29. Gautam N, Roy U, Balkundi S, Puligujja P, Guo DW, Smith N, Liu XM, Lamberty B, Morsey B, Fox HS, McMillan J, Gendelman HE, Alnouti Y. 2013. Preclinical pharmacokinetics and tissue distribution of long-acting nanoformulated antiretroviral therapy. *Antimicrob Agents Chemother* 57:3110–3120. <http://dx.doi.org/10.1128/AAC.00267-13>.
30. Andrews CD, Spreen WR, Mohri H, Moss L, Ford S, Gettie A, Russell-Lodrigue K, Bohm RP, Cheng-Mayer C, Hong Z, Markowitz M, Ho DD. 2014. Long-acting integrase inhibitor protects macaques from intrarectal simian/human immunodeficiency virus. *Science* 343:1151–1154. <http://dx.doi.org/10.1126/science.1248707>.
31. Jackson AG, Else LJ, Mesquita PM, Egan D, Back DJ, Karolia Z, Ringner-Nackter L, Higgs CJ, Herold BC, Gazzard BG, Boffito M. 2014. A compartmental pharmacokinetic evaluation of long-acting rilpivirine in HIV negative volunteers for pre-exposure prophylaxis (PrEP). *Clin Pharmacol Ther* 96:314–323. <http://dx.doi.org/10.1038/clpt.2014.118>.
32. Shegokar R, Singh KK. 2011. Surface modified nevirapine nanosuspensions for viral reservoir targeting: *in vitro* and *in vivo* evaluation. *Int J Pharm* 421:341–352. <http://dx.doi.org/10.1016/j.ijpharm.2011.09.041>.
33. Costa P, Manuel J, Lobo S. 2001. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci* 13:123–133. [http://dx.doi.org/10.1016/S0928-0987\(01\)00095-1](http://dx.doi.org/10.1016/S0928-0987(01)00095-1).
34. Ansel H, Allen LV, Popovich NG. 1999. *Pharmaceutical dosage forms and drug delivery systems*, 7th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
35. Sartor O. 2003. Eligard: leuprolide acetate in a novel sustained-release delivery system. *Urology* 61:25–31. [http://dx.doi.org/10.1016/S0090-4295\(02\)02396-8](http://dx.doi.org/10.1016/S0090-4295(02)02396-8).
36. Harrison TS, Goa KL. 2004. Long-acting risperidone: a review of its use in schizophrenia. *CNS Drugs* 18:113–132. <http://dx.doi.org/10.2165/00023210-200418020-00005>.
37. Zhou HH. 2003. Pharmacokinetic strategies in deciphering atypical drug absorption profiles. *J Clin Pharmacol* 43:211–227. <http://dx.doi.org/10.1177/0091270002250613>.
38. Usach I, Peris JE. 2011. Bioavailability of nevirapine in rats after oral and subcutaneous administration, *in vivo* absorption from gastrointestinal segments and effect of bile on its absorption from duodenum. *Int J Pharm* 419:186–191. <http://dx.doi.org/10.1016/j.ijpharm.2011.07.047>.
39. Shen L, Peterson S, Sedaghat AR, McMahon MA, Callender M, Zhang HL, Zhou Y, Pitt E, Anderson KS, Acosta EP, Siliciano RF. 2008. Dose-response curve slope sets class-specific limits on inhibitory potential of anti-HIV drugs. *Nat Med* 14:762–766. <http://dx.doi.org/10.1038/nm1777>.
40. McMahon MA, Shen L, Siliciano RF. 2009. New approaches for quantitating the inhibition of HIV-1 replication by antiviral drugs *in vitro* and *in vivo*. *Curr Opin Infect Dis* 22:574–582. <http://dx.doi.org/10.1097/QCO.0b013e328332c54d>.
41. Bachelier L, Jeffrey S, Hanna G, D'Aquila R, Wallace L, Logue K, Cordova B, Hertogs K, Larder B, Buckery R, Baker D, Gallagher K, Scarnati H, Tritch R, Rizzo C. 2001. Genotypic correlates of phenotypic resistance to efavirenz in virus isolates from patients failing nonnucleoside reverse transcriptase inhibitor therapy. *J Virol* 75:4999–5008. <http://dx.doi.org/10.1128/JVI.75.11.4999-5008.2001>.
42. Shetty AK, Coovadia HM, Mirochnick MM, Maldonado Y, Mofenson LM, Eshleman SH, Fleming T, Emel L, George K, Katzenstein DA, Wells J, Maponga CC, Mwatha A, Jones SA, Abdool Karim SS, Bassett MT. 2003. Safety and trough concentrations of nevirapine prophylaxis given daily, twice weekly, or weekly in breast-feeding infants from birth to 6 months. *J Acquir Immune Defic Syndr* 34:482–490. <http://dx.doi.org/10.1097/00126334-200312150-00006>.
43. Musoke P, Guay LA, Bagenda D, Mirochnick M, Nakabiito C, Fleming T, Elliott T, Horton S, Dransfield K, Pav JW, Murarka A, Allen M, Fowler MG, Mofenson L, Hom D, Mmiro F, Jackson JB. 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. <http://dx.doi.org/10.1097/00002030-199903110-00006>.
44. Leth FV, Kappelhoff BS, Johnson D, Losso MH, Boron-Kaczmarek A, Saag MS, Livrozet JM, Hall DB, Leith J, Huitema ADR, Wit FW, Beijnen JH, Lange JMA, 2NN Study Group. 2006. Pharmacokinetic parameters of nevirapine and efavirenz in relation to antiretroviral efficacy. *AIDS Res Hum Retroviruses* 22:232–239. <http://dx.doi.org/10.1089/aid.2006.22.232>.
45. Gathe J, Andrade-Villanueva J, Santiago S, Horban A, Nelson M, Cahn P, Bogner J, Spencer D, Podzamczar D, Yong CL, Nguyen T, Zhang W, Drulak M, Quinson AM. 2011. Efficacy and safety of nevirapine extended-release once daily versus nevirapine immediate-release twice-daily in treatment-naïve HIV-1-infected patients. *Antiviral Ther* 16:759–769. <http://dx.doi.org/10.3851/IMP1803>.