

ARTICLE

Characterizing Class-Specific Exposure-Viral Load Suppression Response of HIV Antiretrovirals Using A Model-Based Meta-Analysis

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We applied model-based meta-analysis of viral suppression as a function of drug exposure and *in vitro* potency for short-term monotherapy in human immunodeficiency virus type 1 (HIV-1)-infected treatment-naïve patients to set pharmacokinetic targets for development of nonnucleoside reverse transcriptase inhibitors (NNRTIs) and integrase strand transfer inhibitors (INSTIs). We developed class-specific models relating viral load kinetics from monotherapy studies to potency normalized steady-state trough plasma concentrations. These models were integrated with a literature assessment of doses which demonstrated to have long-term efficacy in combination therapy, in order to set steady-state trough concentration targets of 6.17- and 2.15-fold above potency for NNRTIs and INSTIs, respectively. Both the models developed and the pharmacokinetic targets derived can be used to guide compound selection during preclinical development and to predict the dose–response of new antiretrovirals to inform early clinical trial design.

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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

There is a lack of convenient and simple approaches to predict clinical exposure–response (E-R) for novel antiretrovirals (ARVs) in preclinical and early clinical development. It is desirable to leverage the wealth of clinical experience with ARVs to perform a model-based meta-analysis of viral suppression as a function of drug exposure and *in vitro* potency.

WHAT QUESTION DID THIS STUDY ADDRESS?

What is the E-R relationship for class-specific ARVs in HIV-infected treatment-naïve patients administered as short-term monotherapy?

What is the optimal PK targets for ARVs in early development?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE?

Modeling analysis characterized class-specific E-R of ARVs and derived the optimal PK targets.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS?

Our models and the PK targets derived can be used to guide compound selection during preclinical development to predict E-R of new ARVs to inform early clinical trial design. This would remarkably enhance the probability of achieving a conclusive and successful trial outcome.

Human immunodeficiency virus 1 (HIV-1) infection remains a global health challenge. Although more than a dozen antiretroviral (ARV) agents are currently approved for the treatment of HIV, and have proven to be effective for long-term viral suppression, commercial demand for ARVs remains high. In particular, there is a continued need for compounds possessing better safety and tolerability profiles, improved resistance profiles, and properties that simplify drug administration.¹ Given the potential for viral resistance, current standard of care for treatment of HIV includes a backbone of two nucleoside reverse transcriptase inhibitors (NRTIs) with an anchor agent from another class. Integrase inhibitors (INSTIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) are two classes of anchor

agents. Due to the potential for emergence of resistance, the US Food and Drug Administration (FDA) guidance on developing ARVs for treatment of HIV recommends that efficacy of a single ARV only be tested in a short-term (less than 14 days) monotherapy trial.² Achieving significant viral load reduction in these short-duration monotherapy studies is accepted as sufficient evidence of efficacy to justify dose selection of a novel ARV for longer duration combination therapy studies in phase II and III.

To guide development of novel ARVs, particularly during early clinical development, it is critical to have a good understanding of the target drug exposure, which must be achieved clinically to ensure robust long-term viral suppression when given in combination with other ARVs as part of

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the complete regimen. Fang and Jadhav^{3,4} hypothesized and showed evidence to support that *in vivo* clinical potency and exposure–response (E-R) within a class of ARVs is related to *in vitro* potency (i.e., IC_{50}) by a class-specific scaling factor. This suggests that if clinical drug exposures for all ARVs within a class were normalized by their respective *in vitro* IC_{50} measured in a unique, controlled assay, their clinical E-R relationships established in the dose-ranging monotherapy trials would collapse into a single class-specific curve. If this curve were modeled, the model could then be used to predict the viral load suppression in a monotherapy trial as a function of systemic drug exposure and *in vitro* potency for a novel ARV. Given that robust response in a monotherapy trial has been used to select doses for phase II and III testing in longer duration combination therapy trials, we have proposed that the potency normalized exposure associated with the registered dose of approved ARVs could be used to assess how far up the monotherapy E-R curve an ARV must be dosed in order to be successful in long-term combination. An underlying assumption in this extrapolation is that the resistance profile for a novel ARV is similar to or improved relative to existing ARVs.

In this work we applied model-based meta-analysis techniques^{5–8} to evaluate whether or not such a class-specific E-R relationship is apparent within the wealth of existing clinical data for ARVs from the NNRTI and InSTI classes and how this could be used to guide early clinical development. Viral load data from short-term monotherapy trials in HIV-infected treatment-naïve patients and drug exposure data either collected within these trials or from other trials were pooled from multiple internal and external sources. *In vitro* potency (IC_{50}) measures were also collected from an internal controlled assay in the presence of 100% normal human serum. The models developed were used to derive class-specific pharmacokinetic targets to guide compound selection during preclinical development and to predict E-R profiles of new ARVs to inform the design of early clinical trials. These models are expected to enhance the probability of achieving a conclusive and successful trial outcome, and the confidence in advancing a new ARV for further development by facilitating head-to-head simulation with existing or emerging treatment options.

MATERIALS AND METHODS

Analysis data

Three sets of data were collected: viral load change with time in monotherapy trials, associated exposure, and *in vitro* potency.

Using PubMed, a search was performed to identify clinical efficacy trials for NNRTIs and InSTIs. The cutoff date for the retrieval of publications was 30 June 2014. Trials were included if they were randomized controlled monotherapy trials in HIV-1-infected treatment-naïve patients ≥ 17 years old with treatment duration ≤ 14 days. Medical data available in approval packages from the FDA and European Medicines Agency (EMA) were also evaluated as well as meeting proceedings from key conferences. Merck (Kenilworth, NJ) internal databases were explored for unpublished information. The primary efficacy end point was the viral load change from baseline. The information on potential explanatory variables

was also collected for each trial to evaluate their potential impact on trial results, e.g., baseline viral load.

Exposure information was collected in monotherapy studies when reported, including steady-state trough concentration (C_{trough}), maximum concentration (C_{max}), and area under the curve during dosing interval (AUC_T). For compounds with no exposure reported in monotherapy trials, exposure levels were obtained from pharmacokinetic study results in HIV patients at a matching dose level/regimen from either published articles or drug labels.

In vitro potency measures were obtained from a standardized single-cycle HIV infection assay using wildtype virus in the presence of 100% normal human serum.⁹ The inflection point (e.g., IC_{50} , referred to as “IP” in this assay and throughout the remainder of the article) was recorded as the *in vitro* potency measurement. Compounds with no reliable IP potency data were excluded from the analysis.

Additional information about trial search and data collection can be found in **Supplementary Material 1**.

Modeling analysis

The meta-analysis was performed using a trial-specific random-effect logistic regression approach with the nonlinear mixed-effects function (glms) provided in the nlme package (v. 3.1) in software R (v. 2.14.2 [www.r-project.org]). This approach would appropriately account for random or known, trial-to-trial differences in the patient populations so that an accurate comparison across trials could be made.^{5–8} Observations were weighted appropriately by the square root of the number of subjects, reflecting an increased confidence in observations in trials with larger sample size.

The following model was used to characterize the viral load change from baseline for each drug class:

$$VL = E_0 + E_{\text{drug}} + \eta + \varepsilon \quad (1)$$

where VL is the patient's \log_{10} viral load change from baseline; E_0 is the nonparametric placebo effect, which takes a different value for each study–time combination for each trial.^{6,7} E_{drug} is the drug effect (see Eq. 2, below); η is the trial-specific random effect assumed normally distributed with mean 0 and variance ω^2/N (N is the sample size); ε reflects the random residual error, assumed normally distributed with mean 0 and variance σ^2/N .

A sigmoidal E_{max} model was used to characterize the E-R relationship for each drug class:

$$E_{\text{drug}} = \frac{E_{\text{max}} * C^\gamma}{EC_{50}^\gamma + C^\gamma} \quad (2)$$

where E_{max} is the maximal drug effect relative to placebo; C is the exposure (e.g., trough concentration) normalized by *in vitro* potency; EC_{50} is the potency normalized exposure to achieve 50% of E_{max} ; and γ is the optional Hill coefficient ($\gamma = 1$ for simple E_{max} model).

The apparent time dependence of drug effect was modeled through E_{max} parametrization by:⁸

$$E_{\text{max},t} = E_{\text{max,drug}} * (1 - e^{-K_{\text{on}} * t}) \quad (3)$$

where $E_{\max,t}$ is the maximal drug effect at time t ; $E_{\max,\text{drug}}$ is the overall maximal drug effect; and K_{on} is the onset rate constant. Alternative time-dependent parametrization of E_{\max} was attempted but with no sufficient improvement in the model fit. The time dependence of E_{\max} reflects the maximum viral load drop relative to placebo that could be expected at a given time posttreatment initiation given the underlying rate-limiting aspects of viral dynamics.

Several exposure metrics were assessed during the analysis, including steady-state C_{trough} , C_{max} , and AUC_{τ} . The impacts of covariates on model parameters (E_{\max} , EC_{50} , γ , and K_{on}) were carefully evaluated, including baseline viral load, demographic characteristics of age, race, body weight, etc. These covariates were selected based on their potential clinical relevance and the data availability.

Analysis models were deemed appropriate if convergence was achieved and the standard error of parameter estimates were obtained. Model selection was based on a log-likelihood ratio test ($P < 0.05$) and the scientific plausibility of the model.

Simulation

Simulations were performed using the final E-R models to derive the class-specific pharmacokinetic target, e.g., the minimum efficacious steady-state exposure (e.g., C_{trough}) normalized by *in vitro* potency (IP). For each drug class, simulations were conducted for the assessed average E-R relationship and its associated uncertainty for viral load suppression as a function of the steady-state $C_{\text{trough}}/\text{IP}$ ratio. The predictive distribution of the E-R profiles was derived by sampling 10,000 sets of model parameters from the variance-covariance matrix of the parameter estimates. For each set of the parameters, the E-R relationship was calculated for a typical trial (i.e., trial-specific random effect and random residual error being zero, reflecting population mean). The 90% uncertainty interval was taken between the 5th and 95th percentile of the predictive distribution. The simulated E-R profiles for each trial were then used to evaluate the percent of maximal viral inhibition associated with the $C_{\text{trough}}/\text{IP}$ ratios at the registered doses of approved drugs, or doses intended for registration for drugs in development. The underlying assumption is that ARVs at the marketed doses in each assessed drug class (i.e., NNRTI or InSTI) are generally associated with similar and acceptable long-term efficacy when coadministered with two NRTIs. Additionally, the assumption made is that the resistance profile and the between-subject pharmacokinetic variability of a novel ARV are similar to or lower than that achieved with existing drugs within a class. The $C_{\text{trough}}/\text{IP}$ ratio for which the 50th percentile of the model simulations achieves the target level of viral inhibition was then set as the pharmacokinetic target for that drug class.

RESULTS

Data used for the analysis

Table 1 provides an overview of the trials included in the analysis. Viral load and pharmacokinetic data from 14 trials (nine NNRTIs and five InSTIs) were included. One NNRTI of interest, delavirdine, was excluded from the analysis as there was no reliable estimate of *in vitro* potency using the HIV

replication assay. All 14 studies were randomized, double-blind, multiple-dose, parallel-group, placebo-controlled trials. Among these studies, i) one study did not report viral load response for the placebo arm¹⁰ (imputed median values from available placebo arms were used for the analysis); ii) two studies did not report patient exposure^{10,15} (exposure levels derived from the FDA prescribing information²² or reported in other clinical pharmacokinetic studies²³ were used for the analysis); iii) one study reported combined viral load reduction results from a mixed patient population including treatment naïve and treatment-experienced subjects (used as it was for the analysis);²⁰ and iv) two studies were unpublished Merck-sponsored trials (i.e., MK-1 and MK-2).

Exposure-viral load drop response characterization

For both NNRTIs and InSTIs, the reductions in viral load during short-term monotherapies were well described by E_{\max} models. C_{trough} (normalized by *in vitro* potency, $C_{\text{trough}}/\text{IP}$) was used as the exposure metric in the final model, consistent with the existing clinical understanding. C_{max} and AUC_{τ} were also tested as alternative independent variables in the model but not selected, as there were no apparent improvements in model performances as indicated by visual inspection of the goodness-of-fit plots and/or by the statistical analysis (data not shown). Based on the underlying hypothesis that all compounds within a given class would have comparable E-R relationships when adjusted for potency, a single E_{\max} (maximum effect), EC_{50} ($C_{\text{trough}}/\text{IP}$ at which half of the maximal effect was achieved), Hill factor (shape), and onset rate constant (K_{on}) when C_{trough} normalized by *in vitro* potency were estimated for each class.

The parameters of the final E-R models are summarized in **Table 2**. All parameters were well estimated with a relative standard error less than 25%. A Hill factor of 1.78 was identified for NNRTI, while Hill factor could not be reliably estimated for InSTIs and so was fixed to 1. Maximum drug effect (E_{\max}) tended to increase nonlinearly with baseline viral load for NNRTIs, while baseline viral load levels had no statistically significant effect on the E-R of InSTIs.

Performances of the final E-R models were assessed through superposition of the observed data and the model fits. **Figure 1** shows the fits for the viral load change from baseline vs. potency normalized C_{trough} for NNRTIs at Day 7 (**Figure 1a**) and InSTIs at Day 10 (**Figure 1b**), respectively. The figures indicate that the pooled E-R data were in general adequately described by the E-R models developed for each class. An overlay of the fitted viral load change time course profiles vs. observations stratified by compounds, studies, and drug classes is presented in **Figure 2**, showing that the final E-R models were able to characterize the time course of viral load suppressions reasonably well across all studied drugs and dose regimens within each class. Of note, placebo profiles seem to be overpredicted for some trials (e.g., nevirapine), which might be due to the overall limited sample size and high data variability. Alternative placebo parameterizations were attempted but with no significant improvement in model performance. Additional goodness-of-fit plots can be found in **Supplementary Material 2**, which also indicates that the final model predictions were in good agreement with the observations.

Table 1 Overview of studies included in the analysis

Drugs (trade name)	Abbreviation	Vendor	Approved dose regimen	MW	<i>In vitro</i> potency* (nM)	Active arm	Sample size/arm	Duration (day)	C_{trough} /potency at approved dose	Trial reference
<i>NNRTI (nucleoside reverse transcriptase inhibitors)</i>										
Doravirine	NA	Merck	In development	425.8	12	25/200 mg QD	6	7	NA	Anderson M ¹⁰
Efavirenz (Sustiva TM)	EFV	Bristol-Myers Squibb	600 mg QD	315.7	30	200 mg QD	21	14	187	Bacheler LT ¹¹
Etravirine (Intencep TM)	ETR	Tibotec	200 mg BID	435.3	67	0/900 mg BID	12	7	10.2	Gruzdev B ¹²
IDX899	NA	Idenix	Discontinued	413.8	617	30/100/200/400/800 mg QD	6-8	7	NA	Zala C ¹³
Lersivirine	LRV	ViiV	Discontinued	310.4	14	10/30/100/500 mg BID, 100/500/750 mg QD	6	7	NA	Fätkenheuer G ¹⁴
MK-1	NA	Merck	Discontinued	435.3	64	40/150 mg QD	6	7	NA	Internal Report 2010
Nevirapine (Viramune TM)	NVP	Boehringer Ingelheim	200 mg QD x 14 days and 200 mg BID after 14 days	266.9	246	200 mg QD	6	14	34.6	Havir DV ¹⁵
RDEA806	NA	Ardea	Discontinued	595.9	236	400 mg BID, 600/800/1000 mg QD	9	7	NA	Moyle G ¹⁶
Rilpivirine (Edurant TM)	RPV	Tibotec	25 mg QD	366.4	56	25/50/100/150 mg QD	9	7	3.89	Goebel F ¹⁷
<i>InSTI (Integrase Strand Transfer Inhibitor)</i>										
Cabotegravir	NA	GlaxoSmithKline	In development	403.4	1,304	5/30 mg QD	8	10	NA	Spreen W ¹⁸
Dolutegravir (Tivicay TM)	DTG	GlaxoSmithKline	50 mg QD	419	170	2/10/50 mg QD	7-10	10	15.6	Min S ¹⁹
Elvitegravir (Stribild TM , combination)	EVG	Gilead	150 mg QD	447.9	149	800 mg QD, 200/400/800 mg BID	6	10	6.72	DeJesus E ²⁰
MK-2	NA	Merck	Discontinued	503.5	30	50/200/800 mg QD, 25/100 mg BID	2	10	NA	Internal Report 2012
Raltegravir (Isentress TM)	RAL	Merck	400 mg BID	444.4	88	100/200/400/600 mg BID	6-8	10	1.61	Markowitz M ²¹

*obtained from a standardized single-cycle HIV infection assay using wildtype virus in the presence of 100% normal human serum. BID, twice a day; QD, once a day; MW, molecular weight; NA, not available.

Table 2 Parameter estimates for final models

Parameter	Estimate (RSE%)	
	NNRTI	InSTI
E_{max} (\log_{10} copies/mL)	-3.81 (14.4)	-3.20 (8.21)
Baseline effect on E_{max}^a	1.81 (21.5)	-
EC_{50} (Unitless)	0.458 (11.3)	0.113 (21.3)
γ	1.78 (21.2)	1 Fixed
K_{on} (1/day)	0.0986 (22.4)	0.129 (14.1)

E_{max} , maximum drug effect; EC_{50} , *in vitro* potency normalized steady-state trough concentration to reach 50% maximum drug effect; γ , Hill factor; K_{on} , onset rate constant; RSE%, relative standard error %; NNRTI, nonnucleoside reverse transcriptase inhibitors; InSTI, integrase strand transfer inhibitor.

^aFor NNRTI: $E_{max} = (-3.81) \cdot (\text{Baseline}/4.55)^{1.81}$; For InSTI, $E_{max} = 3.20$.

Baseline viral load was a significant covariate on E_{max} for NNRTIs. That is, for a higher baseline viral load, a greater change from baseline vs. placebo at Day 7 was achieved for NNRTIs. As shown in **Supplementary Material 3**, ID3899 had the highest baseline viral load, which may have contributed to its greater Day 7 responses, while doravirine and MK-1 had baseline viral loads on the lower end and their responses tended to be smaller despite a relatively higher C_{trough}/IP . Simulations (**Figure 3**) suggest that for a viral load baseline of 5.5 (\log_{10} copies/mL), the maximum viral load change from baseline vs. placebo in NNRTIs would be ~2.3-fold greater when compared with a viral load baseline of 3.5. It should be noted that while baseline viral load had a significant effect on maximum drug effect of NNRTIs, it should not affect the estimation of pharmacokinetic target as the target profile was assessed as percent maximum viral inhibition (as described below). No significant impact of baseline on viral load response was found for InSTIs. This could be attributed to the relatively lower number of studies used for the analysis: a total of five studies for InSTIs vs. nine for NNRTIs.

Class-specific pharmacokinetic target determination

The final E-R model was used to simulate viral load change from baseline as a function of *in vitro* potency normalized trough concentrations (C_{trough}/IP) to assess class-specific pharmacokinetic targets. Simulations of Day 7 viral load change from baseline vs. C_{trough}/IP at marketed doses showed that the long-term efficacy of NNRTIs in combination therapy trials was associated with doses that achieved $\geq 99\%$ of maximal viral inhibition (E_{max}) at Day 7 in monotherapy. The C_{trough}/IP ratio was 6.17 when the model-predicted median response was $\sim 0.99 \cdot E_{max}$ (**Figure 4**). In contrast, long-term efficacy of InSTIs in combination therapy trials was associated with doses resulting in $\geq 95\%$ of maximal viral inhibition (E_{max}) at Day 10 in monotherapy. The C_{trough}/IP was 2.15 when the model-predicted median response was $\sim 0.95 \cdot E_{max}$.

DISCUSSION

Short-term monotherapy trials for ARV drugs, as predictors of long-term viral inhibition outcomes in combination therapy, are critical to assess the clinical E-R for a specific drug and to inform dose selection for long-term combination therapy trials. This work presents a model-based meta-analysis

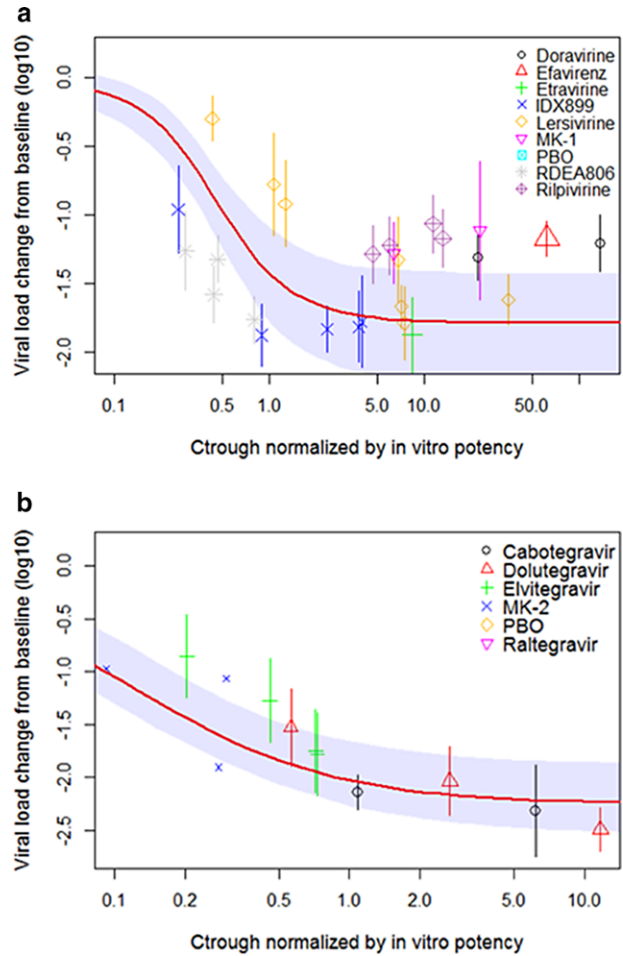


Figure 1 Fits of the exposure-response model for viral load change from baseline from all monotherapy trials for each drug class (a, NNRTI, b, InSTI). The symbols and bars represent the observed mean and the 90% confidence interval. The solid line and shaded area represent the model predicted viral load inhibition and the 90% confidence interval. Exposures presented are steady-state trough concentration normalized by *in vitro* potency from a single-cycle HIV infection assay using wildtype virus in the presence of 100% human serum. Note confidence interval is not presented for compound of MK-2 due to its small sample size (2/arm) in the monotherapy trial. PBO, placebo; NNRTI, nonnucleoside reverse transcriptase inhibitor; InSTI, integrase strand transfer inhibitor.

characterizing E-R of ARVs in HIV-infected treatment-naive patients during short-term monotherapy. Viral load declines during monotherapy could be well described as a function of potency-normalized C_{trough} with a common E-R model structure within a given ARV class. As indicated in **Figures 1** and **2**, ARVs have tended to be studied in monotherapy at doses that are associated with a E-R well up the plateau of the E-R curves (i.e. maximum level of efficacy response). The apparent existence of a class-specific E-R relationship allowed us to simultaneously analyze all relevant and available data from clinical monotherapy trials using model-based meta-analysis techniques. This greatly enhanced the precision of parameter estimates, particularly around EC_{50} , where limited clinical data have been collected. In this work, C_{trough}

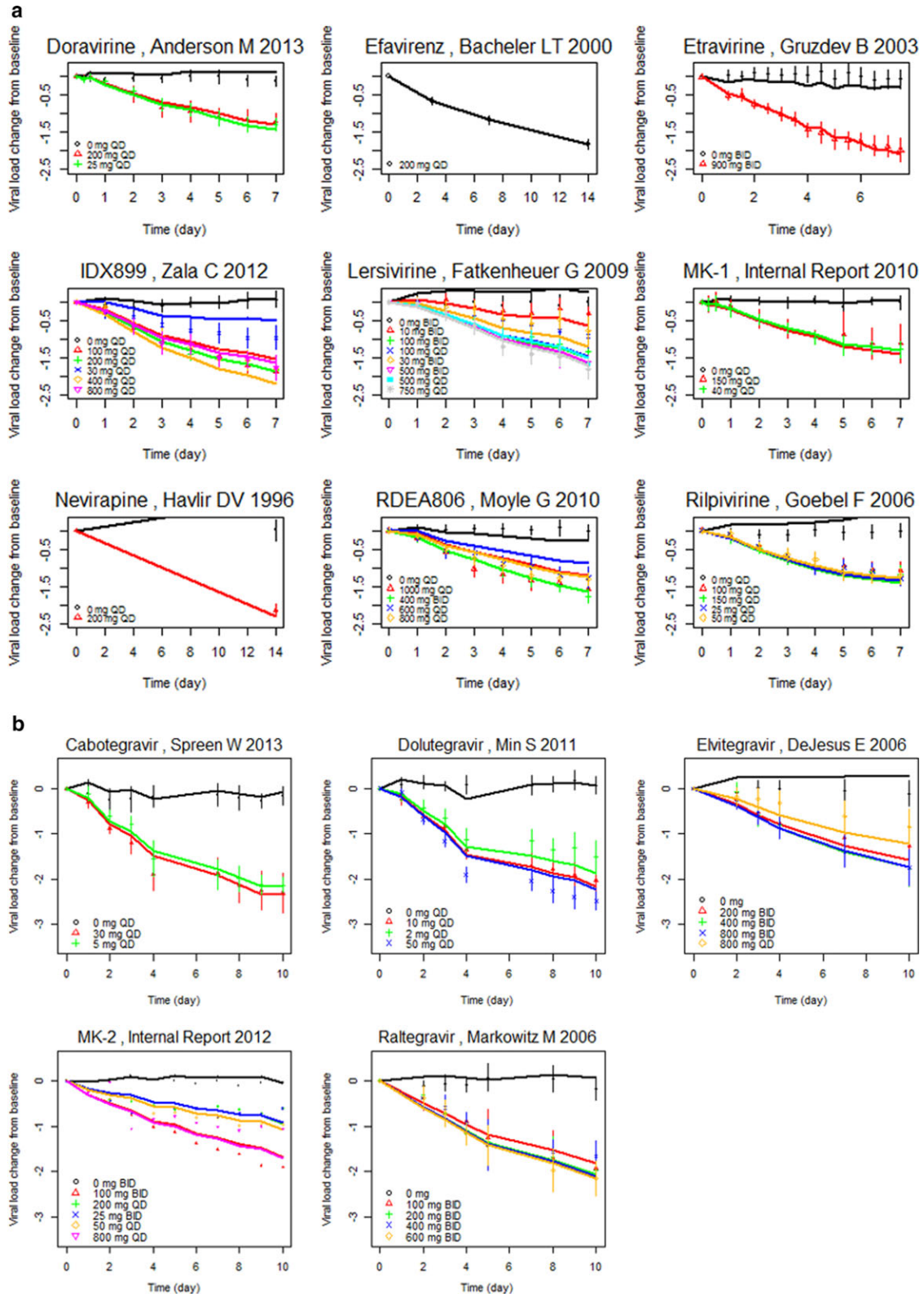


Figure 2 Fits of viral load response-time courses for individual drug from all studies (**a**, NNRTI; **b**, InSTI). The symbols and bars represent the observed mean and the 90% confidence interval, stratified by study, arm, and treatment duration (days). The solid line represents the model predicted viral load drop-time course. Note confidence interval is not presented for MK-2 due to its small sample size (2/arm) in the monotherapy trial. Data presented were \log_{10} viral load change from baseline. BID, twice a day; QD, once a day; NNRTI, nonnucleoside reverse transcriptase inhibitor; InSTI, integrase strand transfer inhibitor.

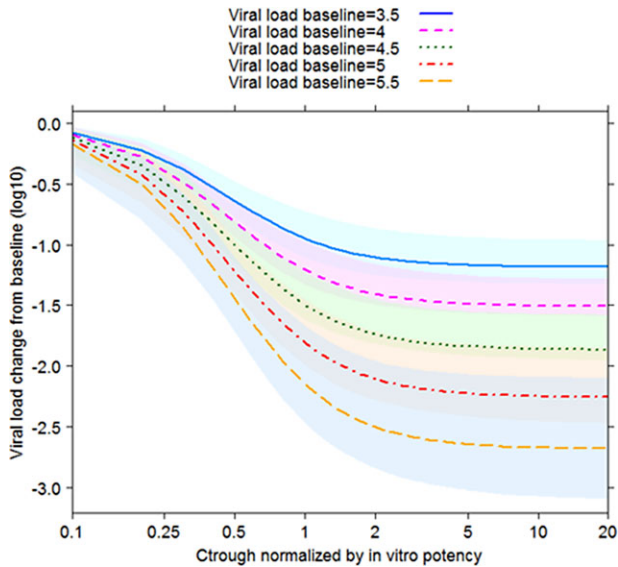


Figure 3 Simulated exposure–response profiles for NNRTIs indicating impact of baseline viral load. The lines and the shaded areas represent the predicted mean and the 90% confidence interval for the viral load change from baseline as a function of baseline (3.5 to 5.0 in log₁₀ copies/mL) following 7 days monotherapy treatment. Exposures presented are steady-state trough concentration normalized by *in vitro* potency from a single-cycle HIV infection assay using wildtype virus in the presence of 100% human serum. C_{trough} , trough concentration; NNRTI, nonnucleoside reverse transcriptase inhibitor; InSTI, integrase strand transfer inhibitor.

was used as the measure of drug exposure given that analysis using either AUC_{τ} or C_{max} as the independent variable in place of C_{trough} did not improve the model performance. In addition, achieving minimal effective C_{trough} has been considered critical to improve the virological efficacy for anti-HIV-1 agents.²⁴ It should be noted that in the absence of clinical dose-fractionation studies, there is typically a strong correlation between pharmacokinetic parameters within a given compound; therefore, the selection of C_{trough} as the independent variable should not be overinterpreted. The study designs included in the analysis were insufficient to support an assessment of the measure of exposure metric most correlated with efficacy.

Leveraging existing monotherapy data to predict clinical E–R for novel ARVs was explored using mechanism-based HIV viral dynamics disease models to derive class-specific *in vitro*–*in vivo* IC_{50} scaling factors for several classes of ARVs including NNRTIs and InSTIs.^{3,4} The work of Fang *et al.* focused on a single drug within each class to derive the class-specific scaling factor, and then applied this scaling factor to another drug from the same class as a test case. In contrast, our work simultaneously estimates the E–R relationship across all drugs within a class (**Table 2**), which greatly enhances the model robustness and confidence in application of the model within that class. Furthermore, the precision of our parameter estimates is strengthened by the use of potency measures obtained from a same assay in the presence of 100% human serum—greatly reducing the uncertainties associated with interassay differences and protein binding corrections. It should be appreciated that our model is

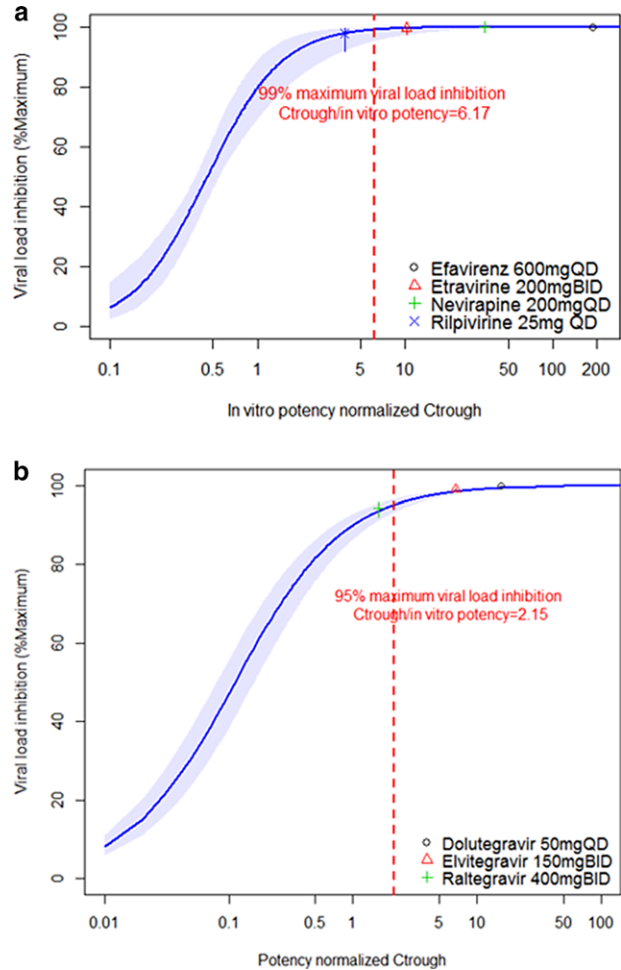


Figure 4 Simulated exposure–response profiles for pharmacokinetic target determination (**a**, NNRTI; **b**, InSTI). The solid lines and the shaded areas represent the predicted mean and the 90% confidence interval for the viral load inhibition as percent of maximum effect. Dashed lines represent the identified *in vitro* potency normalized trough concentrations (6.17 for NNRTI and 2.15 for InSTI) as pharmacokinetic target. Symbols represent simulated percent maximum effect at marketed dose levels for approved drugs. Simulations were conducted in 10,000 trials with typical subject with treatment duration of 7 days for NNRTI and 10 days for InSTIs. Exposures presented are steady-state C_{trough} normalized by *in vitro* potency from a single-cycle HIV infection assay using wildtype virus in the presence of 100% human serum. BID, twice a day; QD, once a day; C_{trough} , trough concentration; NNRTI, nonnucleoside reverse transcriptase inhibitor; InSTI, integrase strand transfer inhibitor.

relatively simple and easy to implement, yet provides comparable, if not better, predictability in viral load suppression profiles following short-term monotherapy when compared with the mechanism-based viral dynamics disease models. Furthermore, our model can be readily transformed into a graphic interface for rapid simulations, a preferred attribute for investigators who are not experts in pharmacometrics.

Hill factors (γ) were used in the E–R models to account for differences in the steepness of the drug inhibitor effect. The Hill factors were assumed to be ARV class-specific, as observed in *in vitro* HIV replication assays.^{9,25,26} In these

assays, it was found that ARVs exhibited different slopes that are characteristic of the class: NNRTIs exhibit slopes of ~ 1.7 , InSTIs and NRTIs have slopes close to 1, and proteinase inhibitors (PIs) exhibit the largest and most variable slopes (1.8 to 4.5). Interestingly, the model-estimated Hill factors for a clinical drug effect of NNRTI (1.78) and InSTI (data did not support estimation of a Hill factor other than 1.0) in this analysis were almost identical to the slope estimations from *in vitro* HIV replication assays. This suggests a good *in vitro*–*in vivo* translation of E–R and enhances the confidence in class-specific Hill factor estimates.

One of the critical contributions of this work is to determine class-specific pharmacokinetic target (i.e., minimum efficacious exposure), a key go/no-go criterion throughout the discovery and the early clinical development for novel ARVs. It is built on the idea that for a new ARV, i) the E–R relationship can be predicted using established *in vitro*–*in vivo* linkage derived from previously studied drugs within the same class, and ii) a minimum efficacious exposure should be associated with a similar extent of viral load suppression when compared with the approved drugs at marketed dose levels. An ideal pharmacokinetic target should bridge information between the expected human exposure and the *in vitro* potency. By retrospectively applying *in vitro* potency normalized trough concentrations at approved dose levels, a $\sim 99\%$ and a $\sim 95\%$ of maximum viral load reductions (maximum effects estimated at infinite exposure at Day 7 or Day 10 for NNRTI and InSTI, respectively) were identified for NNRTI and InSTI, respectively. To achieve a similar extent in viral load suppression, C_{trough}/IP ratios would be ≥ 6.17 for NNRTI and ≥ 2.15 for InSTI (Figure 4). Merck is using these targets to select new anti-HIV molecules and design proof-of-concept studies.

Comparing the two ARV classes, there was generally a less consistent trend of increased viral load reduction with higher potency normalized exposure (C_{trough}/IP) in NNRTI vs. InSTI (Figure 1). Although some of this variability could be accounted for by differences in baseline viral load, which was a significant covariate in the NNRTI model, it cannot be ruled out that some compounds may have inherent differences in the E–R shape. Internal work (Supplementary Material 4) has been unsuccessful in identifying apparent source of these differences which could be used to strengthen this work. As there is no scientifically compelling reason to exclude any of the compounds, we have retained all compounds in the final model with the acknowledgment of the apparent high degree in data variability. It should be noted that our model-based meta-analysis approach would be of value in setting pharmacokinetic targets for novel compounds despite these limitations, particularly given that the alternative approach would be to arbitrarily select a target that is some multiple of an *in vitro* IC_{50} . It is interesting to note that the approved doses of NNRTIs are associated with $\sim 99\%$ of maximum inhibition in this monotherapy analysis, while InSTIs are associated with $\sim 95\%$ maximal inhibition. Both drug classes demonstrate efficacy in long-term combination therapy, with InSTIs appeared to have improved clinical efficacy.^{27,28} Of note, a high degree of viral load suppression in InSTI class is consistent with the observations in raltegravir,²⁶ where a 45-nM trough has been indicated as the threshold value below which a higher risk of treatment

failure occurred. Viral dynamics simulation using an established model for InSTIs and *in vivo* estimates of raltegravir IC_{50} suggests that the associated maximum viral load drop in a monotherapy trial for a $C_{\text{trough}} < 45$ nM would be $\sim 73\%$ of E_{max} .²⁹ These findings emphasize the importance of achieving high degree of viral load inhibition in a monotherapy study to ensure successful long-term combination therapy. New ARVs are expected to demonstrate the same high level of viral load suppression to maintain therapeutic benefit.

One limitation of the model is around the potential for resistance. Because it is unlikely that resistance will arise during the short duration of monotherapy trials,² the assumption was made that the model-estimated EC_{50} values are for wild-type virus. Doses that have been shown to have robust efficacy in long-term combination therapy in phase II and III trials would be expected to achieve sufficient drug concentrations to prevent resistance in the presence of two NRTIs. By evaluating the C_{trough}/IP ratios associated with doses that are registered and/or have shown robust efficacy in long-term combination studies, our analysis implicitly accounts for resistant mutants. However, compounds that have a poorer resistance profile or greater pharmacokinetic variability relative to historical drugs may require a higher C_{trough}/IP target.

In conclusion, class-specific E–R models were developed for NNRTI and InSTI ARVs by retrospectively analyzing combined data sets from the early-phase short-term monotherapy trials and *in vitro* potency measurements. In addition, class-specific pharmacokinetic targets expected to be associated with full efficacy in long-term combination therapy were established. For NNRTI and InSTI, the projected targets would be steady-state trough concentrations of 6.17- and 2.15-fold above potency, respectively, which were associated with $\sim 99\%$ and 95% maximum viral load drops in a monotherapy study, respectively, as observed at marketed dose levels of approved drugs in each class. These class-specific E–R models and pharmacokinetic targets can be applied to predict E–R for new ARVs within that class to facilitate selection of compounds based on the predicted human dose required to achieve the target. It is also of value to inform dose selection for short-duration monotherapy proof-of-concept studies such that both the maximum suppression of viral load as well as submaximal viral reduction are evaluated. A well-designed monotherapy study lays the foundation for developing key pharmacokinetic/pharmacodynamic models for a given compound that will be used throughout later phases of ARV development. Moreover, the approach used in this analysis may be similarly applied to other antimicrobial areas such as hepatitis C virus infection that share similar drug development processes and dose selection rationale and for which *in vitro* potency has been shown to be highly associated with clinical end points.

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analyzed the data; J.A.G. and M-T.L. contributed new reagents/analytical tools.

Conflict of Interest. Y.X., Y-F.L., D.Z., M.D., E.T, M.L.R., J.A.G, M-T.L., and W.A. are or were employees of Merck Sharp & Dohme Corp., at the time of the study, and may own stock and/or stock options of the company. J.G. has no conflict of interests.

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