Published in final edited form as:

AIDS. 2015 November 28; 29(18): 2419–2426. doi:10.1097/QAD.0000000000000843.

Early HIV RNA decay during raltegravir-containing regimens exhibits two distinct subphases (1a and 1b)

Adriana Andrade¹, Jeremie Guedj², Susan L. Rosenkranz³, Darlene Lu³, John Mellors⁴, Daniel R. Kuritzkes⁵, Alan S. Perelson⁶, and Ruy M. Ribeiro⁶ for the ACTG A5249s protocol team

¹The Johns Hopkins University, Baltimore, MD 21205

²INSERM, IAME, UMR 1137, F-75018 Paris, France and Université Paris Diderot, IAME, UMR 1137, Sorbonne Paris Cité, F-75018 Paris, France

³Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston MA 02115

⁴University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

⁵Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115

⁶Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM 87545

Abstract

Background—We analyzed the early kinetics with integrase inhibitor treatment to gain new insights into viral dynamics.

Methodology—We analyzed data from 39 HIV-1 infected, treatment-naïve, participants; 28 treated with raltegravir (RAL; multiple doses) monotherapy for 9 days, and 11 with RAL 400 mg BID and emtricitabine (200 mg QD)/tenofovir disoproxil fumarate (300 mg QD). Plasma HIV-1 RNA was measured frequently; the data was fitted using a mathematical model of viral dynamics distinguishing between infected cells with unintegrated HIV DNA and productively infected cells. Parameters were estimated using mixed-effect models.

Results—RAL treatment led to a biphasic viral decline with a rapid first phase (1a) lasting ~5 days followed by a slower phase (1b). Phase 1a is attributed to the rapid elimination of productively infected cells. Phase 1b reflects the loss of infected cells with non-integrated provirus due to cell loss and integration of HIV DNA. The half-life of productively infected cells and of infected cells that had completed reverse transcription but had not yet integrated HIV DNA were ~19 h and between 3.6 and 5.8 days, respectively. The effectiveness of RAL in preventing HIV-1 integration was 94% and 99.7%, for the combination therapy and monotherapy groups, respectively.

Conclusion—We found that the first phase of viral decay with RAL therapy was composed of 2 subphases corresponding to the half-lives of infected cells with integrated proviruses and with unintegrated HIV-1 DNA.

INTRODUCTION

Viral kinetic analyses after initiation of antiviral treatment have provided important insights into the biology of different viral infections, such as HIV, hepatitis B and C viruses, and influenza infection [1–6]. In the case of HIV, following treatment initiation with protease and/or reverse transcriptase inhibitors [1], plasma HIV RNA declines in a biphasic manner: a rapid initial viral decline (half-life ~ 1 day) occurs within the first 2 weeks post treatment initiation [7, 8], which is then followed by a slower virus decline, with an average half-life of 14 days, typically until the virus reaches the limit of detection of conventional assays. With more sensitive assays further slower phases of decline were identified [9–11]. Mathematical modeling of this decrease in plasma viremia attributes the first two phases of decline to the decay of two distinct populations of cells: one short lived (e.g., recently infected activated CD4+ T-cells) and the other consisting of long-lived infected cells (e.g., resting T cells, macrophages) [1, 7, 12].

It is unclear whether this pattern of viral decay also applies to newer classes of antiretroviral agents, in particular the integrase strand transfer inhibitors (InSTI) [13–17]. Studies have shown that treatment with raltegravir (RAL), an InSTI, leads to rapid suppression of plasma HIV RNA [18–20]. Two studies in particular have shown that combination antiretroviral therapy (ART) including RAL leads to undetectable plasma HIV-1 RNA faster than regimens with a non-nucleoside reverse transcriptase inhibitor (NNRTI) [18, 19]. The effects of RAL on viral decay have been analyzed using new mathematical models that separate pre- and post-integration steps of the viral lifecycle [12, 21–23]. Results of these modeling analyses showed that the first-phase of viral decay was more profound than those described with antiretroviral agents other than RAL [21]. These findings were subsequently confirmed in the AIDS Clinical Trials Group 5248 (A5248), a prospective, single-arm study to estimate the various phases of viral decay in treatment-naïve participants initiating ART with RAL plus the reverse transcriptase inhibitors tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) [17].

Here we developed a model including the pre- and post-integration steps of the HIV-1 lifecycle to analyze participants treated with either RAL monotherapy [20], or RAL combined with the reverse transcriptase inhibitors FTC and TDF (ACTG A5248) [17]. The model we present can be solved analytically and exhibits two early phases of viral decay, called phases 1a and 1b, rather than a single early phase as seen with protease and reverse transcriptase inhibitors used alone or in combination. We use the plasma HIV-1 RNA data from those studies to estimate these slopes and the absolute effectiveness of RAL. This is in contrast to prior modeling work on the effects of protease and reverse transcriptase inhibitors where one could only estimate the effectiveness of one drug regimen relative to another [24, 25].

METHODS

Model

In our model of early viral decline, target cells, T , are infected at rate βTV , proportional to the availability of target cells and free virus. The infection rate constant β takes into

consideration both the rate at which virus meets target cells as well as the rate of reverse transcription. Cells in which reverse transcription has occurred, I_1 , can be lost at rate δ_1 , or can progress to provirus integration at rate k . We note that δ_1 includes not only loss by death of the cell, but also other factors, like degradation of the unintegrated viral DNA. Cells with integrated provirus become productively infected, I_2 , and are lost at rate δ_2 , possibly larger than δ_1 due to viral cytopathic effects. In turn, virus, V , is produced by cells I_2 at rate p per cell and is cleared from the circulation at rate c per virion. A schematic of this model is presented in the left panel of Figure 1. This system can be described by the following equations

$$\begin{aligned}\frac{dI_1}{dt} &= (1 - \eta)\beta VT - \delta_1 I_1 - k(1 - \omega)I_1 \\ \frac{dI_2}{dt} &= k(1 - \omega)I_1 - \delta_2 I_2 \\ \frac{dV}{dt} &= pI_2 - cV\end{aligned}\quad (1)$$

In equation (1), we have also included the effect of reverse transcriptase inhibitors in blocking infection with effectiveness η , where η varies between 0 and 1, with 1 indicating 100% effectiveness; as well as the effect of an integrase inhibitor in blocking integration with effectiveness ω . This model is a simple extension of the basic model of viral infection [1] and similar models have been proposed [12, 23, 26]. Here, as has been done before [1, 6], we assume that the plasma HIV RNA is at set-point before therapy, that the virus and infected cells are in quasi-steady state (i.e., $V \approx p I_2/c$), and, as we focus on the early viral kinetics, we make the standard approximation that the number of target cells remains constant. These assumptions allow us to solve the resulting linear system of equations, and we find that plasma HIV RNA over time is given by

$$V(t) = \frac{V_0 e^{-\frac{(t-\tau)}{2}(k(1-\omega)+\delta_1+\delta_2+\theta)}}{2\theta} \left[\left(e^{\theta(t-\tau)} - 1 \right) (k(1-\omega)+\delta_1+\delta_2(1-2\omega)) + \left(e^{\theta(t-\tau)} + 1 \right) \theta \right], \quad (2)$$

where $\theta = \sqrt{(k(1-\omega)+\delta_1-\delta_2)^2 + 4\delta_2(\delta_1+k)(1-\eta)(1-\omega)}$, V_0 is the initial plasma HIV RNA before therapy, t is the time on therapy and we have allowed for a pharmacological delay, τ , before the effect of therapy is manifested in a change in plasma HIV RNA.

An interesting property of this model is that it predicts two early phases of decay, even though we have not included long-lived infected cells, such as macrophages or resting T cells, as has been done before to study long-term decay characteristics under potent ART [1, 7]. Indeed, analysis of this model indicates that there is an initial phase of viral decay, phase 1a, with slope $\sim \delta_2$; followed by phase 1b with slope $\sim \delta_1 + k(1-\omega)\eta$ (Figure 1, right panel). The possibility of these phases (1a and 1b) for different monotherapy regimens in a more complex model has recently been discussed [23].

Human Participant Data

In order to determine the effect of RAL monotherapy or in combination with TDF/FTC, we analyzed two data sets with frequent plasma HIV RNA sampling from HIV-infected participants treated with this InSTI.

The first data set was obtained from 28 HIV-infected participants treated with RAL monotherapy for 9 days, from a previously published dose ranging study [20]. Participants received 100, 200, 400, or 600 mg RAL twice daily, and had plasma HIV RNA measured before the first dose, 6 and 12 hours post-dose, and on days 1, 2, 3, 4, 7, 9. Plasma was assayed for HIV-1 RNA using the Amplicor HIV-1 Monitor Assay, version 1.5 with a limit of quantification (LOQ) = 400 copies/ml; if the result was below that level, the UltraSensitive HIV-1 Monitor Assay (LOQ = 50 copies/ml) was used (Roche Molecular Diagnostics, Alameda, CA) [20]. We treated plasma HIV RNA data below the limits of quantification as censored at the corresponding values.

The second set of data was obtained from 11 HIV-infected participants treated with a combination of FTC/TDF 200 mg/300 mg daily plus RAL 400 mg twice daily enrolled in an intensive viral dynamics substudy (A5249s) of ACTG protocol A5248 [17]. Plasma HIV-1 RNA was measured immediately prior to administration of the first dose (0 hr), and at 2, 4, 6, 12, 18, 24, 30, 36, 42, and 48 hours and days 3, 4, 7, and 10 and 14 after treatment initiation. For consistency with the first data set, only data through day 10 was used in the current analyses. Plasma was assayed for HIV-1 RNA using the Amplicor HIV-1 Monitor, version 1.5, UltraSensitive protocol (LOQ=50 copies/mL; Roche Molecular Systems, Branchburg, NJ).

Both clinical studies were approved by the respective Institutional Review Boards and all participants provided written informed consent [17, 20].

Statistical Analyses

Comparisons of variables (e.g., baseline plasma HIV RNA) between the two treatment groups were performed using the Wilcoxon test. Significance was considered at the $\alpha=0.05$ level.

Data Fitting—Participant plasma HIV RNA data was fit using non-linear mixed-effects. In this approach, each individual parameter is written as $\mu_i = \theta e^{\phi_i}$, where θ represents the mean value of the parameter in the population, and the random term ϕ_i , chosen from a Gaussian distribution with mean 0 and standard deviation α , accounts for the inter-individual variability. The inclusion of such a random part for the parameters was tested using a likelihood ratio test. Given that antiviral effectiveness was high and very close to 1, the estimation of individual effectiveness parameters was stabilized using a logistic transformation. As is usually done, we assumed an additive independent error on the logarithm of the plasma HIV RNA data with standard error σ .

Equation (2) was fitted to the data to estimate the parameters using an extension of the Stochastic Approximation Expectation-Maximization algorithm to handle data below the LOQ, using a censored likelihood [27, 28], implemented in the software MONOLIX 4.2.2 (www.lixoft.eu).

Covariate Selection—After the best viral kinetic model was selected, we examined the influence of RAL monotherapy vs. combination therapy on the parameters. For each model parameter separately, we compared participant-specific estimates between the two treatment

groups using the Wilcoxon test. All the parameters for which the treatment group (monotherapy vs. combination therapy) had a P-value < 0.1 during the individual screening were tested in the mixed-effects model. The effect of treatment in each parameter was introduced successively from the one having the lowest P-value (the most significant) to the one having the largest P-value (the least significant). Each model covariate was tested separately using a likelihood ratio test and was kept in the final model if the P-value was < 0.05 . For RAL monotherapy, we repeated this procedure to test dose as a covariate.

Random Effect Covariance Structure Selection—After the covariate model was selected, the correlation between the empirical Bayes estimates of the parameters was tested using partial Spearman correlation. All correlations associated with a P-value < 0.1 were included in the model and were tested using a likelihood ratio test. However, none of these correlations were significant in the final model.

RESULTS

The two groups of HIV-infected participants had similar baseline plasma HIV RNA values (median 4.8 [range 3.59 to 5.59] and 4.74 [range 4.42 to 5.79] $\log_{10}$ copies/ml, for monotherapy and combination therapy, respectively; $p=0.82$). At ~10 days (9 to 11 days), the plasma HIV RNA was still similar between the two groups (2.60 vs. 2.47 $\log_{10}$ copies/ml, $p=0.60$), and the relative decay from baseline was not significantly different ($p=0.77$).

We next analyzed if the treatment data with RAL monotherapy showed evidence of two phases of viral decay, by comparing fits of a simple heuristic double exponential function with a single exponential function. The double exponential fitted the data much better ($p < 0.00005$ using a likelihood ratio test). We then fit the analytical solution of our model, equation (2), to the monotherapy data alone. The absence of reverse transcriptase inhibitors implies $\eta=0$ and leads to a simpler equation with fewer parameters. These fits (Figure S1 in Supplementary Information) confirmed that there were two phases of viral decay, 1a and 1b, early on (before day 10) and provided estimates of the loss rates of cells pre-integration, i.e., $\delta_1 \approx 0.1/\text{day}$ and post-integration, i.e. $\delta_2 \approx 1.0/\text{day}$. The use of a mixed-effects model also allowed us to rigorously test the effect of dose on the viral decay and the effectiveness of RAL in blocking integration, ω . We found that dose was not a significant covariate for effectiveness ($p=0.11$). This may be the case because, in this trial, trough concentrations of RAL were above the IC95 (inhibitory concentration for 95% of infections in 50% human serum) at all doses [20].

We next fitted the two data sets together. Analysis of the model shows that i) the predicted plasma HIV RNA is largely insensitive to variations in the effectiveness of RT inhibitors, η , for $\eta > 0.9$; ii) there is a trade-off between estimates of k , the transition rate from reverse transcription to integration of proviral DNA, and the RAL effectiveness, ω . These identifiability limitations were taken into account by fixing η , at $\eta=0.95$ for the combination therapy and $\eta=0$ for monotherapy; and trying different fixed values for k , based on previous work in the literature. Mohammadi *et al.* [29] performed a detailed *in vitro* study of the lifecycle of a cell infected with HIV. In that study (figure 1 and supplementary material OF

[29]), the authors showed that the median time of transition from reverse transcription to proviral integration is ~9 h. Murray *et al.* [26] analyzed the durations of phases of the HIV lifecycle based on the delays observed in plasma HIV RNA decline during monotherapy with drugs from different classes, which inhibit distinct steps of the lifecycle. They estimated that it took ~5 h from reverse transcription to integration. From these times (t), we can calculate $k=\ln(2)/t$, obtaining that this rate varies between 1.8 day^{-1} [29] and 3.3 day^{-1} [23, 26].

In Figure 2, we show the median predicted decays for the two studies. Some representative individual fits are shown in Figure 3 (all fits are shown in Supplementary Information). The fits confirm that there is a double decay (phase 1a and phase 1b) in early infection (before day 10) in both monotherapy and combination therapy. We then tested the effect of monotherapy vs. combination therapy as a covariate for ω , τ , δ_1 and δ_2 . The effect of treatment group was highly significant when we allowed for a different value of RAL effectiveness, ω , for the monotherapy and the combination therapy studies, with the former being systematically larger than the latter ($p<0.00005$). For example, Table 1, when we fix $k=2.6 \text{ day}^{-1}$, we found $\tau=11 \text{ h}$, $\delta_1=0.15 \text{ day}^{-1}$, $\delta_2=0.89 \text{ day}^{-1}$, $\omega=0.997$ (monotherapy) and $\omega=0.940$ (combination therapy), with the difference in effectiveness being significant, $p<0.00005$. If we fix k at 1.8 day^{-1} or 3.3 day^{-1} , our estimates vary less than 2% for δ_2 . The estimates of ω also show minor variations (Table 1). When $k=1.8 \text{ day}^{-1}$, ω for monotherapy and combination therapy are 0.995 and 0.910, respectively; and for $k=3.3 \text{ day}^{-1}$, the estimates are 0.997 and 0.939, respectively, which are essentially unchanged from the estimates with $k=2.6 \text{ day}^{-1}$. However, for $k=1.8 \text{ day}^{-1}$, we estimate $\delta_1=0.19 \text{ day}^{-1}$, whereas for $k=3.3 \text{ day}^{-1}$, we estimate $\delta_1=0.12 \text{ day}^{-1}$.

DISCUSSION

We have analyzed in detail the early dynamics of plasma HIV RNA decay with RAL monotherapy and in combination with RT inhibitors. From a biological point of view, the virus assayed in the periphery is mostly being produced in lymphoid tissues by populations of (i) newly infected activated CD4 T-cells, (ii) recently activated latently infected CD4 T-cells, and (iii) long lived productively infected cells (such as macrophages). The latter two populations will not be affected by the InSTI or RTI therapy studied here, as these drugs block *de novo* infection. In the model, we assume that these latter populations contribute relatively little to the early decay observed, since we only analyze the first 10 days. Thus, the first phase of viral decay mostly represents the loss of newly infected cells by the blunting of productive infection and/or cell death. This assumption is borne out because the model that we propose fits the early viral loss decay data quite well across all patients (fits in Supplementary Information). We found a two-phase decay (phases 1a and 1b) during the first ~10 days of treatment. This profile is different from that predicted by early models used to analyze protease and RT inhibitor-based therapy in HIV infection [1, 6, 7]. In our model, phase 1a is due to a rapid loss of productively infected cells that contained integrated provirus at the start of treatment. On the other hand, phase 1b is driven by continuous slow decay of cells that complete reverse transcription and integration (*i.e.* it is due to $\omega<1$) (Figure 1, right panel).

Interestingly, ART, using a combination of four reverse transcriptase and protease inhibitors may also lead to a 2-phase decay early on, as seen in figure 1 of Markowitz *et al.* [24]. However in that study, this was not explored and the second phase of decay was simply attributed to the loss of long-lived infected cells [24]. In our model of the effects of RAL-based therapy, the inclusion of long-lived infected cells would give rise to an additional phase of decay as long as the loss rate of these long-lived infected cells was noticeably less than the loss rate of cells with an integrated provirus (i.e., I_2 cells). It seems unlikely that monotherapy with RAL would lead to the same mechanistic explanation for the two-phase decay, but we cannot exclude it completely and we are conducting further analyses of this point that will be reported elsewhere.

The estimation of the loss rate, δ_1 , of infected cells before integration of HIV, I_1 , was somewhat unstable, because it depended on our choice of the integration rate, k . However, it was always larger than 0.1 day^{-1} , corresponding to a half-life shorter than 7 days; and for our highest estimate of δ_1 , the half-life of I_1 is 4 days. Both of these estimates are substantially shorter than the expected half-life of uninfected CD4+ T-cells [30–33] and of long-lived infected cells [7]. It is interesting to speculate as to whether the higher loss rate may be mediated by an immune response recognizing infected cells before they are productively infected [34–38]. In this context, we should note that Spivak *et al.*'s [22] *in vitro* results indicate that the integration time is only $\sim 3.4 \text{ h}$, which would correspond to $k=5.0 \text{ day}^{-1}$. Using this value of k , our estimate of δ_1 would be even lower. Another important aspect is that these estimates of k are valid for cells that will ultimately become productively infected, as opposed to cells where integration may be aborted.

One limitation of our analyses is that the monotherapy and combination therapy datasets were obtained from different studies. This means that some of the protocols used were different, as detailed in the methods section. However it is unlikely that there will be further trials with RAL monotherapy. To be able to use this unique dataset and to mitigate issues with different protocols, we applied a mixed-effects fitting approach, which allows for systematic differences in the model parameters for the two studies. Interestingly we did not find any differences between the parameters, except for the efficacy of RAL.

Previous quantitative analyses of RAL therapy over 8 weeks or more suggested that the first- phase of viral decay was longer than in efavirenz (EFV)-based regimens [17, 21]. One study using the same participants as the combination therapy trial discussed here found that the first- phase decay was longer and slower in RAL-based vs. EFV-based combination therapy [17]. Our study extends that previous work, because that analysis did not use a mechanistic model of viral dynamics. Thus, we now report that what was previously described as a slower first-phase corresponds to two phases. Other modeling analyses of RAL therapy considered that the effectiveness of drug was 100% (i.e., $\omega=1$) [12, 23], which in our model would imply a single first-phase of decay. We note that Gilmore *et al.* [23], also using $\omega=1$, suggested the possibility of a multi-slope first-phase based on a different model, where their slopes would correspond to different processes in the viral life-cycle.

Potent treatment, in the absence of protease inhibitors, allowed us to directly estimate the effectiveness of RAL in blocking proviral integration, estimated as 94% and 99.7% in

combination therapy and monotherapy, respectively. This is in contrast to other viral dynamics studies of HIV treatment, which only allow estimation of relative efficacies [24]; and more similar to the situation in HCV infection, where estimation of the absolute effectiveness of different treatment regimens is possible [39]. Here, we estimated very high effectiveness for RAL, especially in the monotherapy trial, which is likely due to the concentrations of the drug achieved in the plasma being well above the IC₉₅ [20]. In the combination therapy study, the effectiveness estimated for RAL in blocking the integration step ($\omega=0.94$) was still high, but lower than for RAL monotherapy ($\omega=0.997$). This is somewhat contradictory to *in vitro* data that shows that this treatment combination acts in synergy to boost the overall effect of treatment [40]. We note, however, that here we are estimating the per cell effectiveness of drug in blocking a given step of the HIV-1 lifecycle. Overall the dynamics of the model are quite different in the two groups (combination ART with RAL vs. RAL monotherapy) (Fig. 2). We predict that from ~15 days onwards after the start of treatment, the plasma HIV RNA demonstrates a greater decrease from baseline in the combination treatment (not shown). This is consistent with the overall effect of therapy being more potent with the combination of antiretroviral agents.

In conclusion, we have shown that HIV-1 therapy with an InSTI leads to a pattern of viral decay unlike what has been seen before with treatment regimens including protease inhibitors, in that the first-phase of decay is composed of two sub-phases. This allowed us to estimate the absolute effectiveness of RAL and the decay rate of infected cells before proviral integration.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge Merck Sharp & Dohme for providing part of the data analyzed in this work.

This work was funded by National Institutes of Health grants R01-AI104373, R01-AI028433 and R01-OD011095, as well as NIH grants to the AIDS Clinical Trials Group (UM1 AI068636, UM1 AI068634). Portions of this work were performed under the auspices of the U.S. Department of Energy under contract DE-AC52-06NA25396.

The content of this manuscript is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or of Merck Sharp & Dohme.

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Figure 1. Schematics of the model definition (left panel) and expected viral load decay profile (right panel).

Figure 2. Predicted median decay of plasma HIV RNA with RAL combination therapy (solid line) and RAL monotherapy (dashed line)

The median parameters estimated from model (2) fitted to the data were used to plot the figure. Plasma HIV RNA is represented as the logarithm of decay from baseline. Parameters are: $V_0=4.64 \log_{10}$ copies/ml, $\tau=0.47$ day, $\delta_1=0.14/\text{day}$, $\delta_2=0.89/\text{day}$, $\omega=0.94$, $\eta=0.95$ (combination therapy) and $V_0=4.90 \log_{10}$ copies/ml, $\tau=0.47$ day, $\delta_1=0.17/\text{day}$, $\delta_2=0.96/\text{day}$, $\omega=0.997$, $\eta=0$ (monotherapy). In both cases, $k=2.6/\text{day}$ was kept fixed.

Figure 3. Representative fits of the model to individual participant data

The top row represents three HIV-infected participants treated with RAL monotherapy, and the bottom row three participants on RAL combination therapy. All individual fits and estimated parameters (Table S1) are shown in the supplementary material.

Table 1

Estimated parameters*

The values of the parameters estimated by fitting Equation (2) to the data sets for different assumptions of the value of k , the rate or proviral integration. The standard errors of the estimates are indicated within brackets. Units for k , δ_1 and δ_2 are day^{-1} , while τ is expressed in days.

	ω	δ_1	δ_2	τ	V_0	
	<i>Mono</i>	<i>Comb</i>				
$k=1.8$	0.995 (0.001)	0.910 (0.034)	0.19 (0.04)	0.91 (0.05)	0.46 (0.06)	4.7 (0.08)
$k=2.6$	0.997 (0.001)	0.940 (0.027)	0.15 (0.04)	0.89 (0.04)	0.47 (0.06)	4.6 (0.08)
$k=3.3$	0.997 (0.001)	0.939 (0.025)	0.12 (0.04)	0.89 (0.04)	0.44 (0.06)	4.7 (0.08)

* ω – efficacy of RAL; δ_1 – loss rate of cells with reverse transcribed, but not integrated HIV-1 DNA provirus; δ_2 – loss rate of cells producing viruses; τ – delay until the effect of therapy is observed; V_0 – baseline viral load.