

Short Communication: Dynamic Constraints on the Second Phase Compartment of HIV-Infected Cells

Adam M. Spivak,¹ S. Alireza Rabi,¹ Moira A. McMahon,¹ Liang Shan,¹ Ahmad R. Sedaghat,^{1,*}
Claus O. Wilke,² and Robert F. Siliciano^{1,3}

Abstract

The cells responsible for the second phase decay of HIV-1 viremia following the initiation of antiretroviral therapy have yet to be identified. A dynamic model that considers where drugs act in the virus life cycle places constraints on candidate cell types. In this regard, the rapid drop in viremia in patients starting regimens containing the integrase inhibitor raltegravir is of particular interest. We show here that the time delay between reverse transcription and integration is short in differentiated macrophages, making these cells poor candidates for the second phase compartment under the assumptions of standard models of viral dynamics.

PIONEERING STUDIES OF VIRAL dynamics¹⁻⁵ established that the decay in plasma HIV-1 levels following initiation of treatment depends on the turnover rate of productively infected cells. The classic model assumes that antiretroviral drugs completely stop new infection, revealing the decay rates of previously infected cells. The rapid initial decay in viremia reflects the short half-life (1 day) of productively infected CD4⁺ T lymphoblasts, the primary target cells for HIV-1. When most of these cells have died, a second slower phase becomes apparent, reflecting virus production by another as yet unidentified population of infected cells with a half-life of ~14 days.

Differences in decay rates of viremia in patients starting different regimens can provide insight into the nature of the second phase compartment. A general model developed by Sedaghat *et al.* describes how decay dynamics are influenced by the stage in the virus life cycle at which an antiviral drug acts, termed a stage effect (Fig. 1A).⁶ Assuming full efficacy, drugs acting later after virus entry can produce a more rapid decay rate in viremia than drugs acting earlier in the life cycle. This effect is observed if the sum of the early stage infected cell death rate and the rate of conversion of early stage infected cells to late stage, productively infected cells is less than the decay rate of productively infected cells. If not, decay dynamics may still be altered by the fact that for drugs acting earlier in the life cycle there is a shoulder period before decay begins (Fig. 1B), reflecting the decay of early stage infected cells, either through death or progression to late stages of infection.^{6,7} The length of this shoulder can be estimated as

$1/(\delta_{M1} + k_M)$, where δ_{M1} is the decay rate of the early stage cells and k_M is the rate of conversion to late stage cells.

This model directly reflects the stepwise nature of viral replication, and as long as a fundamental assumption of complete inhibition is met, it can be used to evaluate candidate second phase cell types in situations in which decay dynamics differ with different drug regimens. In treatment-naïve patients, viremia falls below the limit of detection more quickly in the patients starting regimens including the integrase inhibitor raltegravir (RAL) than in patients starting comparable regimens including the reverse transcriptase (RT) inhibitor efavirenz (EFV) in place of RAL.⁸ Murray and colleagues⁹ suggested that the rapid drop in viremia in patients on RAL is due to a smaller second phase compartment in the presence of RAL rather than a change in the first or second phase decay rates. In this situation, the rapid first phase decay continues for longer before virus production from the second phase compartment becomes quantitatively dominant. The end result is that viremia falls below the limit of detection more quickly in patients on RAL.

As is illustrated in Fig. 1B, the lack of a shoulder effect could contribute to lower apparent second phase viremia in patients starting RAL. According to the model described in Fig. 1A, second phase viremia on RAL is suppressed by a factor of $\delta_{M2}/(\delta_{M1} + k_M)$ relative to second phase viremia on EFV, where δ_{M2} is the decay rate of productively infected second phase cells. The derivation of this effect involves considering the limit of the ratio between the model predictions of second phase viremia on RAL and second phase viremia on EFV as

¹Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

²Section of Integrative Biology, Center for Computational Biology and Bioinformatics, and Institute for Cell and Molecular Biology, The University of Texas at Austin, Austin, Texas.

³Howard Hughes Medical Institute, Baltimore, Maryland.

*Current address: Department of Otolaryngology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts.

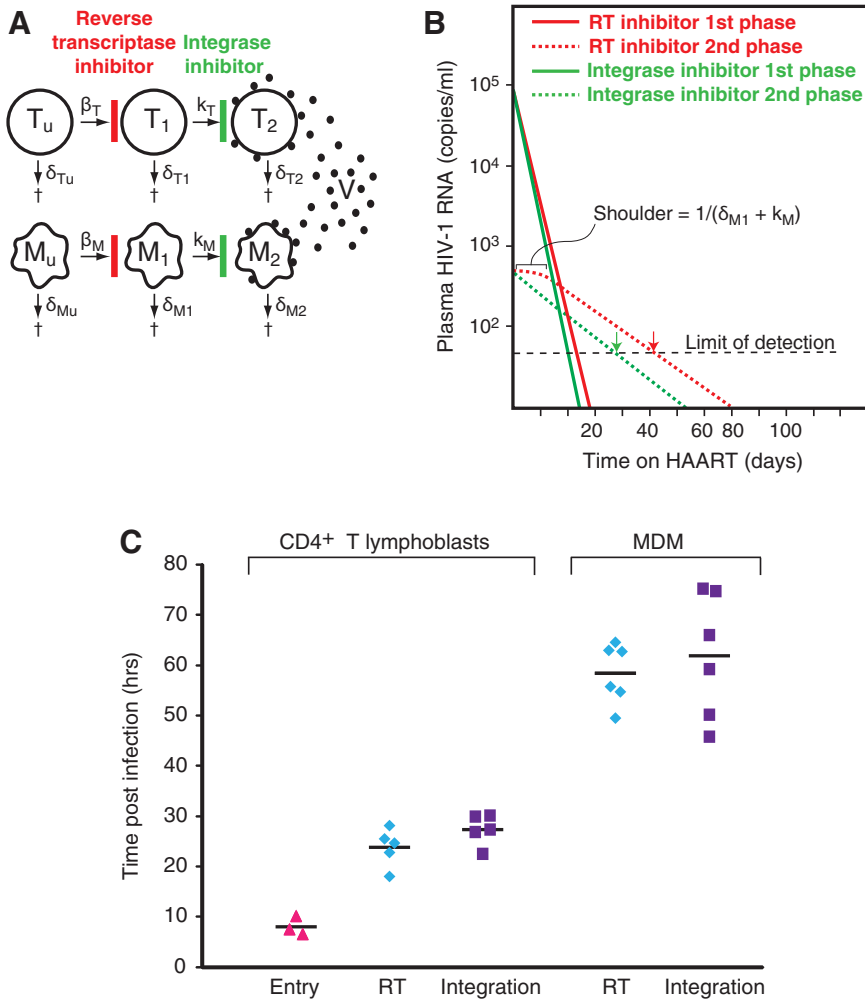


FIG. 1. (A) A model of HIV-1 dynamics reflecting the action of RT inhibitors and integrase inhibitors. The model is based on the work of Sedaghat *et al.*⁶ and envisions first (T) and second (M) phase compartments, each consisting of uninfected cells (T_u , M_u), early stage infected cells (T_1 , M_1), and late stage infected cells (T_2 , M_2). For this analysis, we consider early stage infected cells to be cells in which the virus has completed reverse transcription but not integration. The conversion of uninfected cells to early stage infected cells occurs at a rate that depends on the concentrations of uninfected cells and free virus and the rate constants β_T and β_M and is blocked by RT inhibitors. Late stage cells are cells in which integration has occurred. The conversion of early to late stage infected cells is governed by the rate constants k_T and k_M and is blocked by integrase inhibitors. Each cell population decays at a characteristic rate (δ). The accumulation of a large number of M_1 cells can in principle explain the more rapid decay observed in patients on RAL. (B) Decay dynamics are predicted by the model shown in (A) in patients on RT inhibitor-based (red lines) or integrase inhibitor-based (green lines) regimens. First phase decay (solid lines) and second phase decay (dotted lines) are shown for each regimen. In patients on RT inhibitor-based regimens, there is a shoulder representing the time it takes for M_1 cells to convert to M_2 cells. The

length of this shoulder is given by $1/(\delta_{M1} + k_M)$. Note that this shoulder can be obscured by first phase viremia. The time required for viremia to fall below the limit of detection for each regimen is shown with a colored arrow. (C) Experimental measurements of the time required for completion of the fusion, reverse transcription, and integrase reactions in primary $CD4^+$ T lymphoblasts and MDM. $CD4^+$ T lymphoblasts were generated by culturing peripheral blood mononuclear cells (PBMCs) from multiple healthy blood donors for 72 h in the presence of phytohemagglutinin, followed by negative selection to isolate $CD4^+$ cells. To prepare MDM, monocytes isolated from PBMCs by positive selection with $CD14^+$ microbeads (Miltenyi Biotec) were cultured in the presence of 1 ng/ml M-CSF (R&D Systems) for 7 days. Cells were infected with GFP-expressing HIV-1 pseudoviruses capable of a single round of viral replication as previously described.^{10,11,19} Maximally inhibitory concentrations of the antiretroviral drugs enfuvirtide, EFV, and RAL were added at various times after infection to block fusion, reverse transcription, and integration, respectively. For MDM infections, the virus was pseudotyped vesicular stomatitis virus G protein instead of an X4 HIV-1 envelope, and thus enfuvirtide could not be used. The progressive loss of inhibition with later addition of drug allowed calculation of the weighted average time for completion of each process.

time increases.^{6,7} Essentially the reduced second phase viremia on RAL reflects the fact that second phase decay at rate δ_{M2} begins as soon as an RAL-based regimen is started while decay is delayed by the shoulder period equal to $1/(\delta_{M1} + k_M)$ in patients starting an EFV-based regimen. By this analysis, the observed 70% reduction in second phase viremia⁹ can be completely explained by a stage effect if the transition from early to late stages of infection in second phase cells (k_M) is slow ($t_{1/2} > 6$ days), so that infected cells can accumulate in an EFV-resistant, RAL-susceptible state (M_1 cells, Fig. 1A).

Based on susceptibility to infection, turnover rate, and resistance to HIV-1 cytopathic effects, the macrophage represents a reasonable candidate for the second phase compartment. To determine whether terminally differentiated

macrophages meet the kinetic criteria described above, we measured k_M in monocyte-derived macrophages (MDM) using time of addition experiments.¹⁰ The completion of reverse transcription was slower in MDM than in $CD4^+$ T lymphoblasts (Fig. 1C). However, the interval between completion of reverse transcription, measured by loss of EFV-mediated inhibition, and completion of integration, measured by loss of RAL-mediated inhibition, was short in both cell types (~ 0.14 days). If this interval is an accurate reflection of the transition from M_1 to M_2 cells, then k_M for macrophages is approximately 7.14 day^{-1} . Thus for reasonable values of δ_{M1} ($0.69\text{--}0.05 \text{ day}^{-1}$),^{7,10} the shoulder is short (3.1–3.3 h), and the reduction in second phase viremia on RAL due to the stage effect is small (0.6–0.7%).

If the fundamental assumption of viral dynamics models is correct (complete or near complete inhibition of ongoing replication) and if macrophages derived from monocytes by *in vitro* differentiation accurately model infection *in vivo*, then differentiated macrophages do not meet the kinetic criteria for the second phase compartment. It is possible that the fundamental assumption is not satisfied and that the virus continues to replicate in patients on HAART. However, this assumption is strongly supported by recent pharmacodynamic¹¹ and treatment intensification studies,¹² and thus alternatives for the second phase compartment should be considered. The second phase may reflect infection of monocytes, which differentiate into macrophages after leaving the circulation. Integration is delayed in monocytes, occurring days not hours after integration.¹³ In resting CD4⁺ T cells, integration may be delayed by a block in nuclear import.^{9,10,14–18} Both cell types thus represent candidates for the second phase compartment. Interestingly, the length of the shoulder (Fig. 1B) is also influenced by the decay of early stage infected cells (δ_{M1}). In *in vitro* studies,¹⁰ the decay rate of un-integrated viral genomes in resting CD4⁺ T cells is too rapid ($t_{1/2} = 1$ day) to be consistent with the observed second phase decay. However, *in vivo* studies of the loss of recoverable virus from resting CD4⁺ T cells following initiation of therapy suggest a slower decay.¹⁷

These results highlight the importance of definitively identifying the cells responsible for the second phase decay. While macrophages have often been considered as the source, the measured kinetics of replication in differentiated macrophages are not consistent with predictions from standard models of viral dynamics, and thus other cell types should be considered.

Acknowledgments

This work was supported by the Doris Duke Charitable Foundation and by NIH Grants AI51178 and AI081600. A.S. was supported by NIH Grants T32 AI07247 and T32 AI007291.

Author Disclosure Statement

No competing financial interests exist.

References

- Wei X, Ghosh SK, Taylor ME, Johnson VA, Emini EA, Deutsch P, *et al.*: Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;373:117–122.
- Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, and Markowitz M: Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123–126.
- Perelson AS, Neumann AU, Markowitz M, Leonard JM, and Ho DD: HIV-1 dynamics in vivo: Virion clearance rate, infected cell life-span, and viral generation time. *Science* 1996;271:1582–1586.
- Perelson AS, Essunger P, Cao Y, Vesanen M, Hurley A, Saksela K, *et al.*: Decay characteristics of HIV-1-infected compartments during combination therapy. *Nature* 1997;387:188–191.
- Wodarz D and Nowak MA: Mathematical models of HIV pathogenesis and treatment. *Bioessays* 2002;24:1178–1187.
- Sedaghat AR, Dinoso JB, Shen L, Wilke CO, and Siliciano RF: Decay dynamics of HIV-1 depend on the inhibited stages of the viral life cycle. *Proc Natl Acad Sci USA* 2008;105:4832–4837.
- Sedaghat AR, Siliciano RF, and Wilke CO: Constraints on the dominant mechanism for HIV viral dynamics in patients on raltegravir. *Antiviral Ther* 2009;14:263–271.
- Markowitz M, Nguyen BY, Gotuzzo E, Mendo F, Ratana-suwan W, Kovacs C, *et al.*: Rapid and durable antiretroviral effect of the HIV-1 integrase inhibitor raltegravir as part of combination therapy in treatment-naive patients with HIV-1 infection: Results of a 48-week controlled study. *J Acquir Immune Defic Syndr* 2007;46:125–133.
- Murray JM, Emery S, Kelleher AD, Law M, Chen J, Hazuda DJ, *et al.*: Antiretroviral therapy with the integrase inhibitor raltegravir alters decay kinetics of HIV, significantly reducing the second phase. *AIDS* 2007;21:2315–2321.
- Zhou Y, Zhang H, Siliciano JD, and Siliciano RF: Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4⁺ T cells. *J Virol* 2005;79:2199–2210.
- Shen L, Peterson S, Sedaghat AR, McMahon MA, Callender M, Zhang H, *et al.*: Dose-response curve sets class-specific limits on inhibitory potential of anti-HIV drugs. *Nat Med* 2008;14:762–766.
- Dinoso JB, Kim SY, Wiegand AM, Palmer SE, Gange SJ, Cranmer L, *et al.*: Treatment intensification does not reduce residual HIV-1 viremia in patients on highly active antiretroviral therapy. *Proc Natl Acad Sci USA* 2009;106:9403–9408.
- Arfi V, Riviere L, Jarrosson-Wuilleme L, Goujon C, Rigal D, Darlix JL, *et al.*: Characterization of the early steps of infection of primary blood monocytes by human immunodeficiency virus type 1. *J Virol* 2008;82:6557–6565.
- Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, and Chen IS: HIV-1 entry into quiescent primary lymphocytes: Molecular analysis reveals a labile, latent viral structure. *Cell* 1990;61:213–222.
- Bukrinsky MI, Stanwick TL, Dempsey MP, and Stevenson M: Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* 1991;254:423–427.
- Spina CA, Guatelli JC, and Richman DD: Establishment of a stable, inducible form of human immunodeficiency virus type 1 DNA in quiescent CD4 lymphocytes in vitro. *J Virol* 1995;69:2977–2988.
- Blankson JN, Finzi D, Pierson TC, Sabundayo BP, Chadwick K, Margolick JB, *et al.*: Biphasic decay of latently infected CD4⁺ T cells in acute human immunodeficiency virus type 1 infection. *J Infect Dis* 2000;182:1636–1642.
- Bukrinsky MI, Sharova N, Dempsey MP, Stanwick TL, Bukrinskaya AG, Haggerty S, *et al.*: Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. *Proc Natl Acad Sci USA* 1992;89:6580–6584.
- Zhang H, Zhou Y, Alcock C, Kiefer T, Monie D, Siliciano J, *et al.*: Novel single-cell-level phenotypic assay for residual drug susceptibility and reduced replication capacity of drug-resistant human immunodeficiency virus type 1. *J Virol* 2004;78:1718–1729.

Address correspondence to:

Robert F. Siliciano
Department of Medicine
Johns Hopkins University School of Medicine
Baltimore, Maryland 21218

E-mail: rsiliciano@jhmi.edu

