
Supporting Information

Argument for using P^2

Retroviruses and lentiviruses incorporate two single strands of mRNA into each capsid. In brief, the need to use P^2 in Eq. 6 arises from this fact that 2 mRNA molecules are incorporated into each virion produced from an infected cell.

Thus, there are 3 possible types of virion that can be produced from an I_D cell:

wild-type HIV-1 virion containing:	$1 V^{RNA}$	+	$1 V^{RNA}$
therapeutic crHIV-1 virion containing:	$1 V_T^{RNA}$	+	$1 V_T^{RNA}$
heterozygous virion containing:	$1 V^{RNA}$	+	$1 V_T^{RNA}$

where:

HIV-1 full-length mRNA	=	V^{RNA}
crHIV-1 full-length mRNA	=	V_T^{RNA}

Thus, there is some loss of resources that must be accounted for when considering the ratio of HIV-1 to crHIV-1 virions produced from an I_D cell. Experiments show that various genetic engineering techniques (removal of the splice sites from the crHIV-1 vector and increasing crHIV-1 mRNA half-life; discussed in the text) can boost the cytoplasmic concentration of crHIV-1 mRNA relative to HIV-1 mRNA. Eq. 6 effectively assumes that P describes the increase in production of V_T^{RNA} relative to V^{RNA} in an I_D cell. So, the proportion of mRNA in an I_D cell by:

$$V^{RNA} + P V_T^{RNA} = 1$$

To determine the proportion of interacting RNA pairs we can assume a binomial distribution and square this equation to obtain:

$$(V^{RNA})^2 + 2 P (V^{RNA}) (V_T^{RNA}) + P^2 (V_T^{RNA})^2 = 1$$

It is clear that the proportion of interacting crHIV-1 RNA pairs is P^2 times the proportion of interacting HIV-1 RNA pairs. Thus, for every HIV-1 virion produced from an I_D cell, P^2 crHIV-1 virions are produced from that same I_D cell. I.e.:

$$V : V_T = 1 : P^2$$

Thus Eqs. 3 & 6 are:

$$\dot{V} = n \delta I + 1 D n \delta' I_D - c V \quad [3]$$

$$\dot{V}_T = P^2 D n \delta' I_D - c V_T \quad [6]$$

(The coefficient of 1 has been added in Eq. 3 for clarification)

But we have not yet accounted for the production of heterozygous virions.

An et al. (1999) found that infectivity of HIV-1 based heterozygous virions is highly diminished due to a block in the life-cycle that is pre-integration but post-entry. They hypothesized that the block occurs during reverse transcription (RT) because the RT enzyme jumps between the 2 heterozygous RNA strands and a type of destructive interference occurs creating a non-viable cccDNA that cannot integrate into the host genome.

Thus, there can be 3 fates for a heterozygous virion produced from an I_D cell upon infection of a T cell:

- 1) The V^{RNA} strand goes through RT and an HIV-1 cccDNA is produced
 \Rightarrow the heterozygote is effectively an HIV-1 virion
- 2) The V_T^{RNA} strand goes through RT and an crHIV-1 cccDNA is produced
 \Rightarrow the heterozygote is effectively a crHIV-1 virion
- 3) A non-viable cccDNA is produced
 \Rightarrow the heterozygote dies

In order to account for heterozygous virions we must examine the middle term $2 P(V^{RNA})(V_T^{RNA})$ which describes the proportion of heterozygous virions produced.

If we the unrealistic assumption that all the heterozygous virions produce HIV-1 cccDNA then we essentially have:

$$V : V_T = (1 + 2 P) : P^2$$

whereas, making the equally unrealistic assumption that all heterozygous virions produce crHIV-1 cccDNA yields:

$$V : V_T = 1 : (2 P + P^2)$$

Since both of these events are likely occurring we can write an interpolation:

$$V : V_T = (1 + 2 P Q) : ((2 P)(L - Q) + P^2)$$

where $L \leq 1$ is the proportion of heterozygous virion that survive while $Q \leq L$ is a proportion of heterozygous virions that produce HIV-1 cccDNA. The results of An et al. (1999), that heterozygous virions are non-viable, imply that L is very small ($L \ll 1 \Rightarrow Q \ll 1$), thus, here we make the simplifying, and conservative, assumption that:

$$V : V_T = 1 : P^2$$

Derivation of $F(\delta, n)$ from in vivo data of G. Funk (2003)

By assuming $\delta' = D \times \delta$ we are assuming that HIV-1 gene products kill the infected cell and that decreased expression of these genes correlates 1:1 with decreased mortality of the infected cell.

Another possibility is that the correlation is not 1:1.

G. Funk (2003) analyzed drug treatment data from 40 HIV-1 positive patients and showed that the burst size (n) correlates with cell death rate (δ). They determined the correlation parameters for δ vs. $n\delta$ via nonlinear regression to data:

$$\delta = a \times n + b$$

where $a = 4 \times 10^{-4}$ and $b = 0.21$

(Funk's other parameter values were 10 fold greater than ours so we take $a = 4 \times 10^{-3}$ and $b = 0.021$)

In the expanded crHIV-1 gene therapy model, the death rate for an I_D cell is $\delta' = F(\delta, n) \times \delta$ and the HIV-1 burst size from I_D cells is Dn .

Thus:

$$\delta' = a \times Dn + b$$

or

$$F(\delta, n) = \frac{aDn + b}{\delta}$$

Using $\delta' = F(\delta, n) \times \delta$ (i.e. $\delta' = aDn + b$) in place of $\delta' = D \times \delta$ does not visibly alter the results, since δ' cancels out of all steady state equations, except I_D (as explained in the next section).

δ' cancels out of all steady state equations (except I_D)

The steady state equations for \dot{I}_D , \dot{V} and \dot{V}_T (the only equations in which $F(\delta, n)$ appears) are:

$$\overline{I}_D = \frac{1}{\delta'} k \overline{V} \overline{I}_T$$

$$\overline{V} = \frac{1}{c} (n \delta \overline{I} + D n \delta' \overline{I}_D)$$

$$\overline{V}_T = \frac{1}{c} (P D n \delta' \overline{I}_D)$$

It is clear that δ' appears in the numerator in \overline{V}_T and \overline{V} equations and in the denominator in the \overline{I}_D equation. Thus, δ' cancels out of the term $\delta' \overline{I}_D$.

So, δ' appears only in the steady state equations for I_D and not in the \overline{V}_T or \overline{V} equations. Furthermore, since I_D does not explicitly appear in any other equations we can be assured that δ' does not appear in the steady states on any other equation in Eqs. **1-6**.

Accounting for viral loss due to infection

Basic Model (with viral loss due to infection)

$$\dot{T} = \lambda - dT - kVT$$

$$\dot{I} = kVT - \mu I$$

$$\dot{V} = n\delta I - cV - kVT$$

The term in bold (viral loss due to infection) is omitted from the Basic Model because it is a low probability event (k is small); this omission is the standard, accepted form of the Basic Model.

Also, inclusion of this term does not change the results at all.

crHIV-1 model (with viral loss due to infection)

$$\dot{T} = \lambda - dT - kVT - kV_T T$$

$$\dot{I} = kVT - \mu I$$

$$\dot{I}_T = kV_T T - dI_T - kVI_T$$

$$\dot{I}_D = kVI_T - \delta' I_D$$

$$\dot{V} = n\delta I + Dn\delta' I_D - cV - kVI_T - kVT$$

$$\dot{V}_T = P^2 Dn\delta' I_D - cV_T - kV_T T$$

Including $kV_T T$ and kVT make absolutely no difference in the results while including kVI_T makes only a small quantitative, but no qualitative change in the result.

crHIV-1 super-infection of I_T cells

HIV-1 *nef* downregulates CD4 thus not allowing for HIV-1 superinfection.

But crHIV-1 can infect a cell multiple times since it does not encode *nef*.

For simplicity, we have limited our consideration to the case of crHIV-1 dual infection (i.e. superinfection of 2 crHIV-1).

We consider 2 variations of super-infection:

Model 2 where superinfection produces a different species of dually infected cell, a cell that has altered P and D values.

Model 1 (superinfection leads to I_D cells)

$$\dot{T} = \lambda - dT - kVT - kV_T T$$

$$\dot{I} = kVT - \mu I$$

$$\dot{I}_T = kV_T T - dI_T - kVI_T - kV_T I_T$$

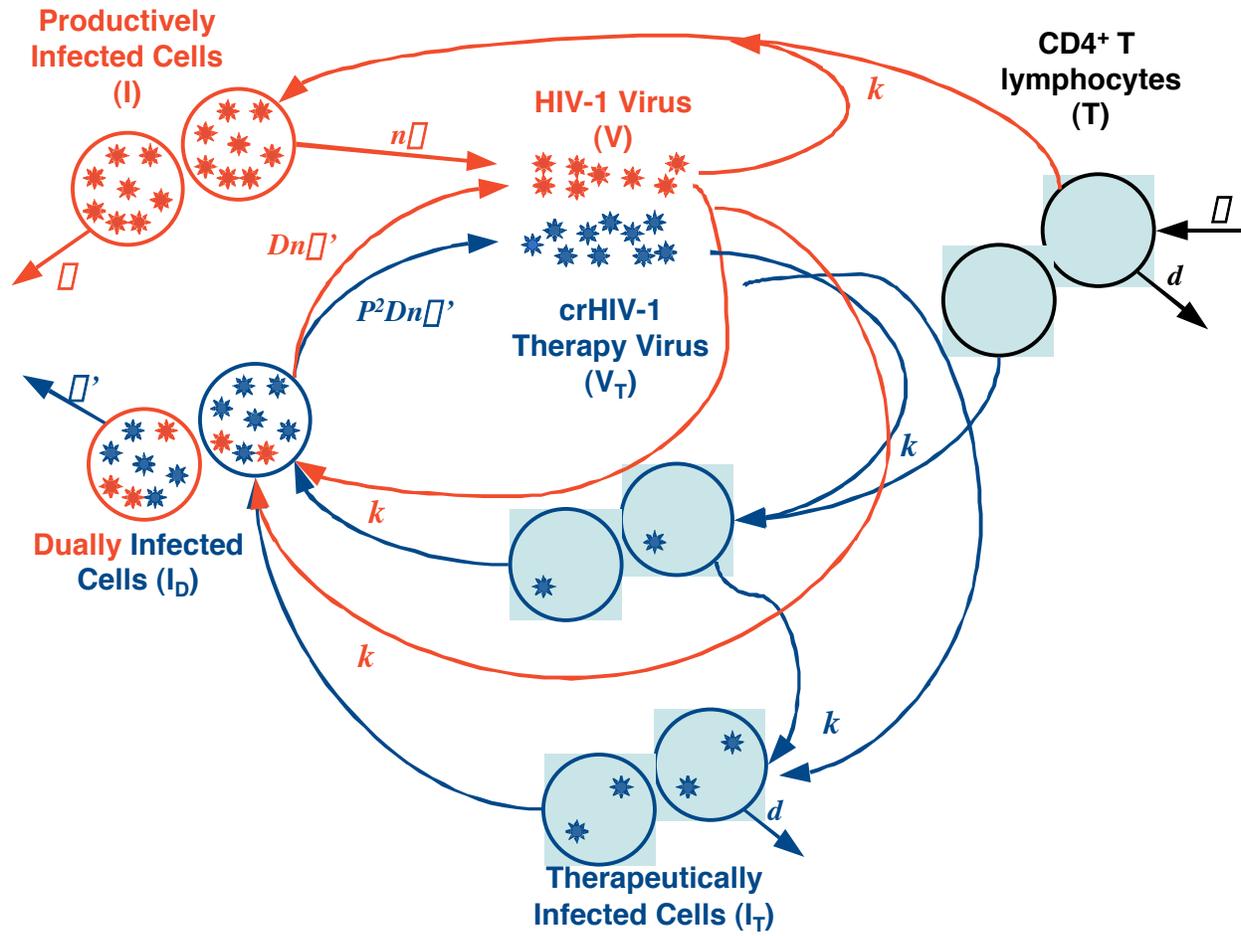
$$\dot{I}_{T2} = kV_T I_T - dI_{T2} - kVI_{T2}$$

$$\dot{I}_D = kVI_T + kVI_{T2} - \delta' I_D$$

$$\dot{V} = n\delta I + Dn\delta' I_D - cV$$

$$\dot{V}_T = P^2 Dn\delta' I_D - cV_T$$

The schematic below describes Model 1.



Results from superinfection Model 1 are identical to Eqs. 1-6.

Model 2

(superinfection produces a different species of

dually infected cell, a cell that has altered P and D values)

$$\dot{T} = \lambda - dT - kVT - kV_T T$$

$$\dot{I} = kVT - \mu I$$

$$\dot{I}_T = kV_T T - dI_T - kVI_T - kV_T I_T$$

$$\dot{I}_{T2} = kV_T I_T - dI_{T2} - kVI_{T2}$$

$$\dot{I}_D = kVI_T - \delta' I_D$$

$$\dot{I}_{D2} = kVI_{T2} - \delta'' I_{D2}$$

$$\dot{V} = n\delta I + Dn\delta' I_D + D_2 n\delta'' I_{D2} - cV$$

$$\dot{V}_T = P^2 Dn\delta' I_D + (2P)^2 D_2 n\delta'' I_{D2} - cV_T$$

since there are 2 copies of the crHIV-1 genome in I_{D2} cells

$$P \rightarrow 2P$$

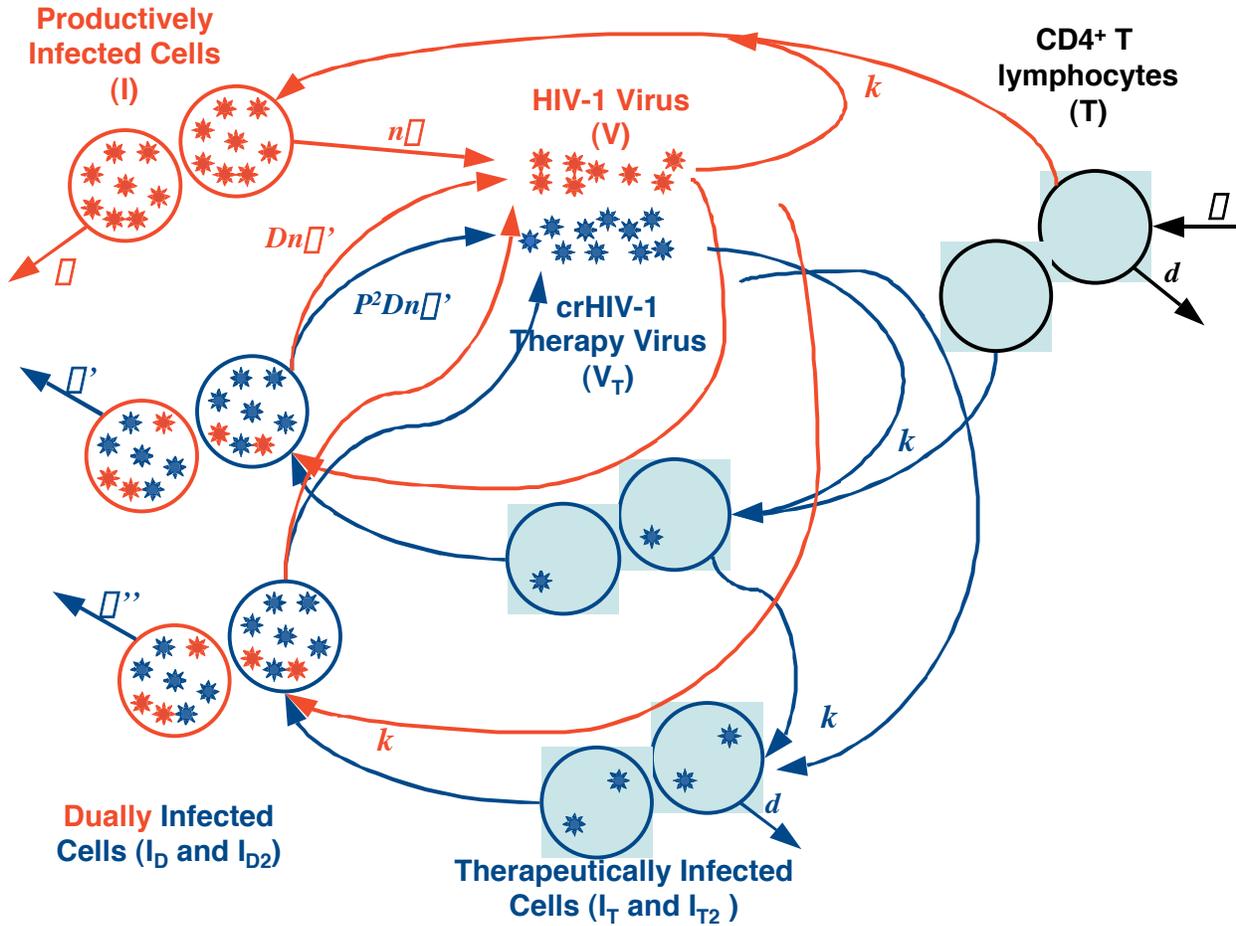
and

$$D \rightarrow D_2$$

where

$$D \leq D_2 \leq 2D \quad \text{and} \quad \delta'' = D_2 \mu$$

The schematic below describes Model 2.



Results from superinfection Model 2 show significantly improved HIV-1 set point reduction compared to Eqs. 1-6.

Thus, crHIV-1 superinfection, which is likely to occur, improves therapy and Eqs. 1-6 appear to be a lower limit of therapeutic efficacy.

Derivation of R_0^T

The basic reproductive ratio, R_0 , is defined as the number of secondary infections from a single infected cell, assuming that virtually all cells are uninfected (i.e. $\dot{T} = \lambda - dT \Rightarrow \bar{T} = \frac{\lambda}{d}$).

A simple derivation of R_0 from the basic model of HIV-1 in vivo dynamics is as follows:

$$R_0 = \text{target cell density} \times \text{burst size} \times \text{infection rate constant} \times \text{average lifetime of the virus}$$

$$= \frac{\lambda}{d} \times n \times k \times \frac{1}{c}$$

For the crHIV-1 gene therapy model we defined a new R_0 for the crHIV-1 virus, R_0^T , defined as the number of secondary crHIV-1 infections obtained from a single crHIV-1 infection assuming

that virtually all cells are uninfected by crHIV-1 but that the system is at steady state for HIV-1 infection (i.e. steady state for the basic model of HIV-1 in vivo dynamics:

$$\bar{T} = \frac{c}{kn}, \quad \bar{V} = \frac{n\lambda}{c} - \frac{d}{k}, \quad \bar{I} = \frac{\lambda}{\delta} - \frac{cd}{\delta kn}.$$

When $R_0^T \geq R_0$ crHIV-1 infection will persist, when $R_0^T < R_0$ crHIV-1 infection will die.

In order to derive the new R_0^T we segment the infection process into 2 components:

- 1) crHIV-1 infection of T cells
and
- 2) HIV-1 infection of I_T cells.

The 1st component will yield roughly the density of I_T target cells for the 2nd component.

1)

target cell density for crHIV-1 \times crHIV-1 burst size \times crHIV-1 infectivity \times crHIV-1 lifetime

$$\begin{aligned} \left(\bar{T} = \frac{c}{kn}\right) & \times P^2 D n & \times & k & \times & \frac{1}{c} \\ & = P^2 D \delta' \end{aligned}$$

Essentially this is the λ value for the I_T cell population.

This is the I_T "target" cell density except that we have not accounted for the death rate of I_T cells (these cells die at a per cell rate of d). We now account for the average lifetime of I_T cells to find the actual target cell density:

$$\left(P^2 D \delta' \times \frac{1}{d}\right)$$

2)

I_T "target" cell density \times HIV-1 virus density \times HIV-1 infectivity

$$\begin{aligned} \left(P^2 D \delta' \times \frac{1}{d}\right) & \times \bar{V} = \frac{n\lambda}{c} - \frac{d}{k} & \times & k \\ & = P^2 D \left(\frac{n\lambda k}{dc} - 1\right) \\ & = P^2 D (R_0 - 1) & [\zeta] \end{aligned}$$

But, in order, for crHIV-1 to persist we require $R_0^T \geq R_0$ or $\frac{R_0^T}{R_0} \geq 1$.

(This can be explained by considering the predator-prey model of 2 predator species competing for the same prey. Except in special cases, the principle of competitive exclusion applies, the species with the greater R_0 outcompetes or excludes the other predator species. Here, in our crHIV-1 model, if $R_0 > R_0^T$ then HIV-1 outcompetes crHIV-1 and excludes crHIV-1. But if the opposite is true $R_0^T > R_0$ then crHIV-1 is not excluded

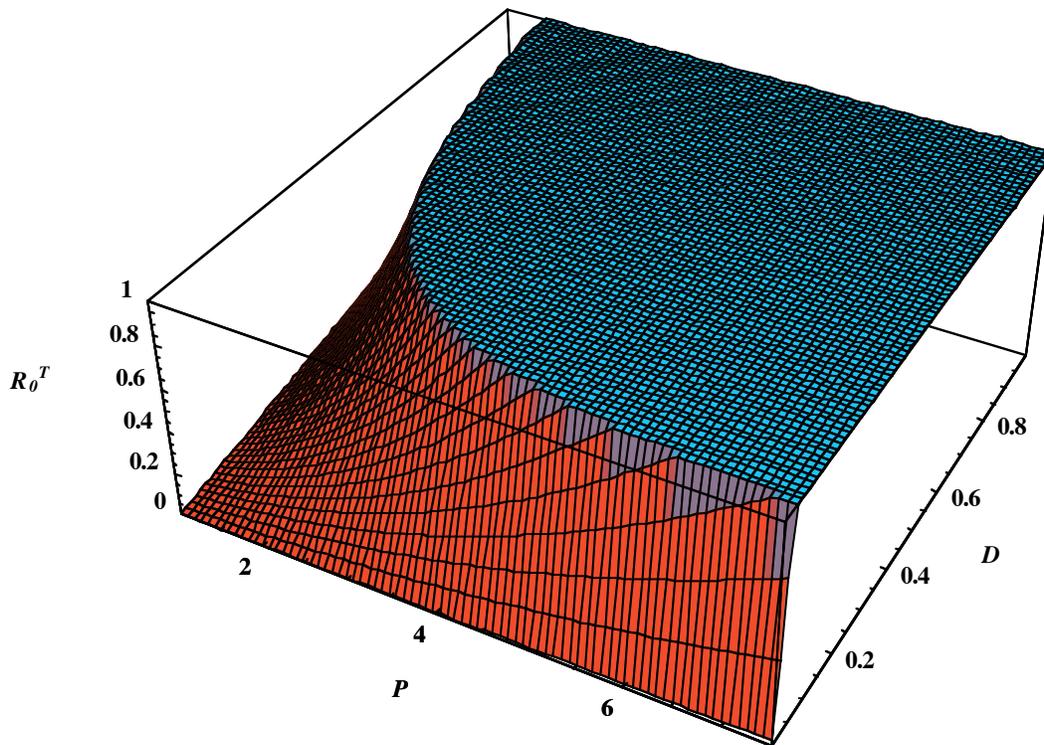
but will never outcompete or exclude HIV-1 since crHIV-1 replication depends on HIV-1).

Thus, we divide Eq. ζ above by R_0 and define R_0^T as:

$$R_0^T = P^2 D \left(1 - \frac{1}{R_0}\right)$$

When $R_0^T \geq 1$ crHIV-1 infection will persist in vivo, otherwise it will die out.

Below R_0^T was plotted relative to parameters P and D . The graph is cut-off at 1 on the z-axis, in order to emphasize the transition $R_0^T > 1$. The red and blue shading is added in order compare R_0^T to regimes where crHIV-1 is either unstable or stable. The red region corresponds to a regime where all eigenvalues of the Jacobian for Eqs.1-6 are negative for the solution to the Basic Model—thus only HIV-1 is stable (crHIV-1 is unstable and in fact a non-physical solution). The blue region corresponds to a regime where all eigenvalues of the Jacobian for Eqs.1-6 are negative for the solution to the expanded model—thus crHIV-1 is stable (the Basic Model solution is unstable). The purple spikes are a numerical anomaly and are irrelevant. The transcritical bifurcation (transition from red to blue) corresponds with $R_0^T = 1$. The regime where crHIV-1 is stable (blue) corresponds to regime $R_0^T > 1$.



Stability Analysis (Jacobian)

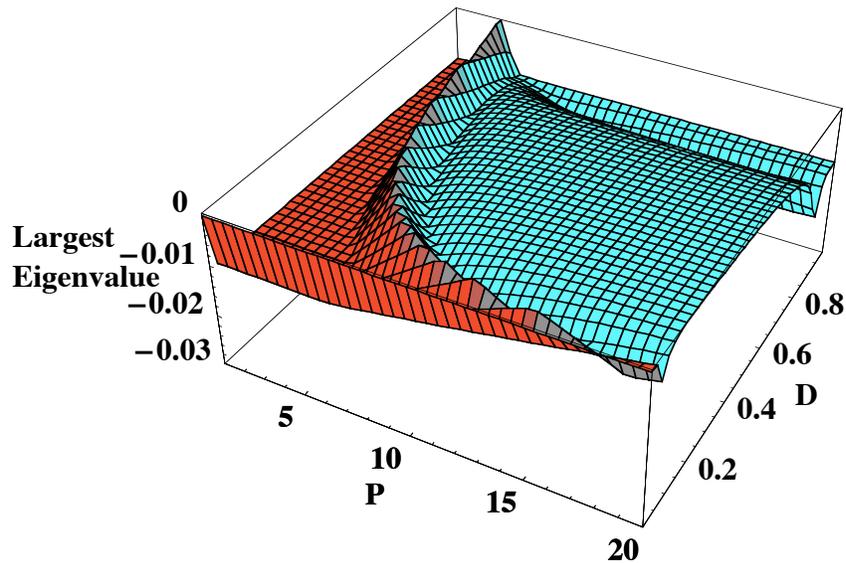
The Jacobian for Eqs. 1-6 has the form:

$$\begin{pmatrix} -d - \bar{V}k - \bar{V}_T k & 0 & -\bar{T}k & 0 & 0 & -\bar{T}k \\ \bar{V}k & -\delta & \bar{T}k & 0 & 0 & 0 \\ 0 & n\delta & -c & 0 & n\mathcal{D}\delta\delta' & 0 \\ \bar{V}_T k & 0 & -\bar{T}_T k & -d - \bar{V}k & 0 & \bar{T}k \\ 0 & 0 & \bar{T}_T k & \bar{V}k & -\delta\delta' & 0 \\ 0 & 0 & 0 & 0 & n\mathcal{D}\mathcal{P}^2\delta\delta' & -c \end{pmatrix}$$

where bar above the state variable denotes the steady state value for that state variable.

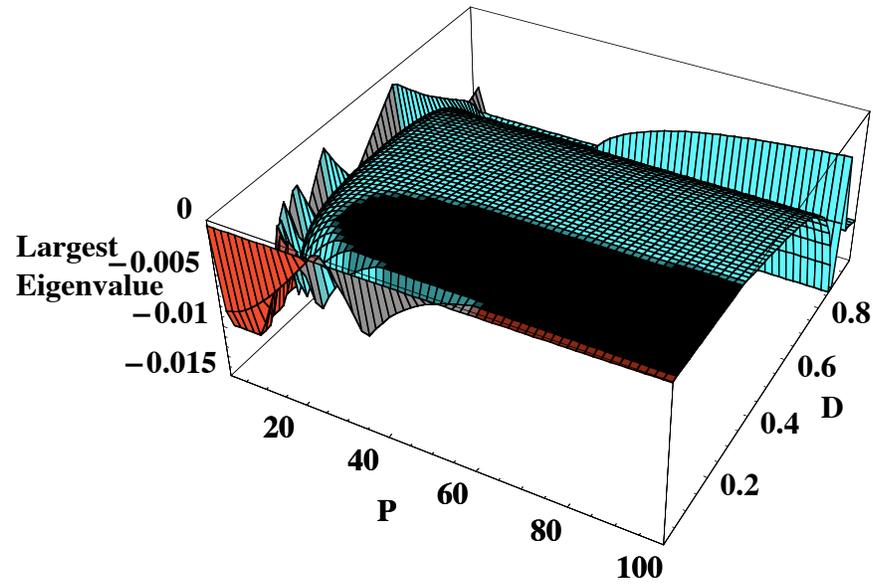
Below are graphs of the largest Eigenvalue of the Jacobian for the Basic Model steady state (i.e. only HIV-1 present) as well as the steady state when the crHIV-1 solution persists.

Red = HIV-1 stable, Blue = crHIV-1 Therapy Virus stable, Black = Unstable



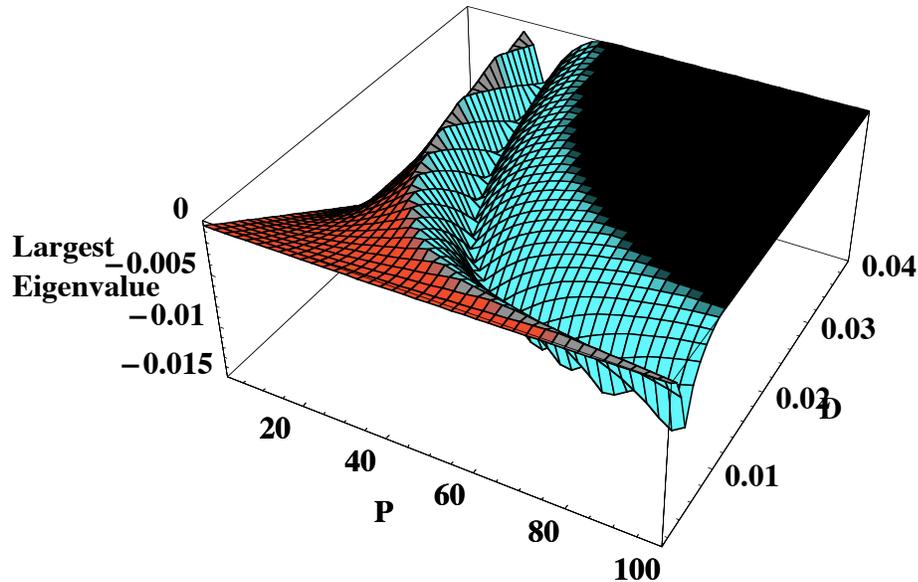
If P is increased there is a Hopf bifurcation and a region of instability arises (black region):

Red = HIV-1 stable, Blue = crHIV-1 Therapy Virus stable, Black = Unstable



A subregion of the plot ($D < 0.05$) explains the transitions between different stable regions and between stable and unstable regions:

Red = HIV-1 stable, Blue = crHIV-1 Therapy Virus stable, Black = Unstable



Distributed Delay Model

Here we incorporated distributed delays (box-car method) into Eqs. 1-6. Using the equations below were performed simulations in Berkeley Madonna Diff. Eq. solving software (<http://www.berkeleymadonna.com/>) to obtain Fig. 3.

We incorporate 2 delays here. The viral eclipse phase describes the delay between HIV-1 infecting a target cell and converting this cell to a productively infected cell ($T \rightarrow I \rightarrow I_m$, where I_m describes cells producing HIV-1). In addition there is another viral eclipse phase after HIV-1 infects a crHIV-1 therapeutically infected cell. This crHIV-1 eclipse phase is the delay that between HIV-1 infecting an I_T cell and that cell becoming an I_D cell that produces both HIV-1 and crHIV-1 ($I_T \rightarrow I_D \rightarrow I_{D_m}$, where I_{D_m} describes cells producing HIV-1 and crHIV-1). We assume for simplicity that both of these viral eclipse phases (delays) are equivalent (so the delay for $T \rightarrow I \rightarrow I_m$ is equal to the delay for $I_T \rightarrow I_D \rightarrow I_{D_m}$). For simplicity we assume that any delay between crHIV-1 infecting the cell and that cell becoming a therapeutically infected cell ($T \rightarrow I_T$) is relatively short compared to the HIV-1 viral eclipse phase.

Thus, we neglect this $T \rightarrow I_T$ delay in the model below.

$$\begin{aligned}
\dot{T} &= \lambda - dT - kVT - kV_T T \\
\dot{I}_1 &= kVT - bI_1 \\
\dot{I}_2 &= b(I_1 - I_2) \\
&\dots \\
\dot{I}_m &= bI_{m-1} - \delta I_m \\
\dot{I}_T &= kTV_T - dI_T - kVI_T \\
\dot{I}_{D_1} &= kV_T I_T - bI_{D_1} \\
\dot{I}_{D_2} &= b(I_{D_1} - I_{D_2}) \\
&\dots \\
\dot{I}_{D_m} &= bI_{D_{m-1}} - \delta' I_{D_m} \\
\dot{V} &= n\delta I_m + Dn\delta' I_{D_m} - cV \\
\dot{V}_T &= P^2 Dn\delta' I_{D_m} - cV_T
\end{aligned}$$

(in the simulation used to produce Fig. 3

$b = 16$ and $m = 30$ was used, all other parameters are as in Table 1)

Expanding other Basic Model architectures to incorporate a crHIV-1 gene therapy virus

■ Incorporating an immune response

The Basic Model does not account for immune processes (such as CD8+ cytotoxic T lymphocytes) controlling viral load by encountering HIV-1 infected cells and killing them. Immune recognition occurs because HIV-1 proteins and protein fragments are presented on the membrane of infected cells.

Here we incorporate an immune response into the crHIV-1 model.

Immune cells (Z) kill HIV-1 productively infected cells at a per cell rate κ and kill productive I_D cells at per cell rate κ' . Immune cells also die at a rate δ_Z .

Cells infected only with crHIV-1 are not recognized by immune cells because they do not express HIV-1 proteins that can be presented on the cell membrane.

$$\begin{aligned}
\dot{T} &= \lambda - dT - kVT - kV_T T \\
\dot{I}_1 &= kVT - bI_1 \\
\dot{I}_2 &= b(I_1 - I_2) \\
&\dots \\
\dot{I}_m &= bI_{m-1} - \delta_0 I_m - \kappa Z I_m \\
\dot{I}_T &= kTV_T - dI_T - kVI_T \\
\dot{I}_{D_1} &= kVT - bI_{D_1} \\
\dot{I}_{D_2} &= b(I_{D_1} - I_{D_2}) \\
&\dots \\
\dot{I}_{D_m} &= bI_{D_{m-1}} - \delta_0' I_{D_m} - \kappa' Z I_{D_m} \\
\dot{V} &= n\delta I_m + Dn\delta' I_{D_m} - cV \\
\dot{V}_T &= P^2 Dn\delta' I_{D_m} - cV_T
\end{aligned}$$

for completeness we tried a variety of functions to describe how Z cells are activated (α)

$$\begin{aligned}
\dot{Z} &= \alpha I_m - \delta_Z Z \\
\text{or} \\
\dot{Z} &= \alpha (I_m + I_{D_m}) - \delta_Z Z \\
\text{or} \\
\dot{Z} &= \alpha Z (I_m + I_{D_m}) - \delta_Z Z \\
\text{or} \\
\dot{Z} &= \alpha - \delta_Z Z
\end{aligned}$$

(as above we used $b = 16$, $m = 30$, α and κ were calculated so that steady state was the initial condition for the simulations)

None of these models affect the qualitative behavior of the crHIV-1 gene therapy model because crHIV-1 converts the susceptible cell population T into a different reservoir of

crHIV-1 transduced cells. Thus, the crHIV-1 effect on HIV-1 can be roughly mimicked by decreasing λ or increasing d in the Basic Model.

■ Models with logistic T cell growth

Since the robust qualitative affect of crHIV-1 introduction depends upon the growth characteristics of the T reservoir (i.e. parameters λ and d), we explored the effect of altering this growth rate. Logistic growth is commonly used in biological models to describe population growth in a more realistic manner than constant or exponential growth, since it assumes a limitation of resources and limits population growth above a set threshold value. Logistic growth has been considered in the context of the Basic Model (Nelson and Perelson, 1999), but is usually assumed to have a small affect and is thus ignored. We explored two forms of logistic growth, for T cells, into the Basic Model: a form that limits growth when only T cells approach the threshold value T_{\max} and a form that limits growth when $T + I_T$ approaches the threshold T_{\max} . T_{\max} was assumed to be between 2000 and 4000 cells/ μ l of blood. We performed dynamic simulations using the distributed delay model above since algebraic steady state analysis was not practical. The equations are as above in the distributed delay section except that the T equation is as follows:

$$\dot{T} = \lambda + rT \left(1 - \frac{T}{T_{\max}}\right) - dT - kVT - kV_T T$$

or

$$\dot{T} = \lambda + rT \left(1 - \frac{T+I_T}{T_{\max}}\right) - dT - kVT - kV_T T$$

We tested an array of r values between 0.001 – 0.1 and an array of T_{\max} values between 2000 – 4000 cells/ μ L.

The decrease in HIV-1 set point was not qualitatively affected by either of these alterations to the model.

Figures using other parameter values ($c = 3.0$)

The Figures below are identical for Fig. 2 except that steady states were calculated using $c = 3.0 \text{ day}^{-1}$ instead of $c = 30.0 \text{ day}^{-1}$.

All other parameter values are as in Table I

