

## Fighting a virus with a virus: a dynamic model for HIV-1 therapy

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### Abstract

A mathematical model examined a potential therapy for controlling viral infections using genetically modified viruses. The control of the infection is an indirect effect of the selective elimination by an engineered virus of infected cells that are the source of the pathogens. Therefore, this engineered virus could greatly compensate for a dysfunctional immune system compromised by AIDS. In vitro studies using engineered viruses have been shown to decrease the HIV-1 load about 1000-fold. However, the efficacy of this potential treatment for reducing the viral load in AIDS patients is unknown. The present model studied the interactions among the HIV-1 virus, its main host cell (activated CD4+ T cells), and a therapeutic engineered virus in an in vivo context; and it examined the conditions for controlling the pathogen. This model predicted a significant drop in the HIV-1 load, but the treatment does not eradicate HIV. A basic estimation using a currently engineered virus indicated an HIV-1 load reduction of 92% and a recovery of host cells to 17% of their normal level. Greater success (98% HIV reduction, 44% host cells recovery) is expected as more competent engineered viruses are designed. These results suggest that therapy using viruses could be an alternative to extend the survival of AIDS patients.

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**Keywords:** HIV-1 therapy; Viral therapy; HIV-1 dynamics; Engineered virus; Hunter-virus

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### 1. Introduction

Current therapies for acquired immunodeficiency syndrome (AIDS) employ inhibitors of the enzymes required for replication of human immunodeficiency virus (HIV-1) to reduce the HIV-1

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load [1,2]. Genetic engineering offers an alternative approach, featuring modification of a viral genome to produce recombinants capable of controlling infections by other viruses [3,4]. This method has been used to modify rhabdoviruses, including the rabies [5] and the vesicular stomatitis viruses (VSV) [6], making them capable of infecting and killing cells previously attacked by HIV-1. The engineered virus codifies the coreceptor pair CD4 and CXCR4 of the host cell membrane and bind specifically to the protein complex gp120/41 of HIV-1 expressed on the surface of infected cells [6]. The engineered virus causes a rapid cytopathic infection. This destruction of infected cells decreased by about 1000-fold HIV-1 load [6]. Since these experiments were conducted in vitro the potential effectiveness of this treatment for reducing the viral load of AIDS patients is unknown. In the present study, we modeled the interactions among HIV-1, its main host cell and an engineered virus in an in vivo context, in order to predict the dynamics of the system and evaluate the potential efficacy of this approach to treatment. Results from the present study demonstrated that this treatment could be effective in reducing individual HIV-1 load.

## 2. Model

Viruses depend on host cells to reproduce. HIV-1 infects many types of cells such as dendritic cells and macrophages, but it preferentially replicates (>90%) in some of the lymphocytes, the activated CD4+ T cells [1,7,8]. The model considered only the main host cell for HIV-1, namely the activated CD4+ T cell. The analysis was based on a simple model of viral dynamics [9], using the following variables: the number of normal or virus-free host cells  $x$ , infected cells  $y$ , and a pathogen virus  $v$  that represents HIV-1. Normal cells are produced at a constant rate  $\lambda$ , and die at rate  $dx$  (Fig. 1). The infection rate is equal to  $\beta xv$ , and infected cells die at a rate  $ay$ , releasing pathogens at a rate  $ky$ , but pathogens are removed from the plasma at rate  $uv$ . The resulting differential equations are:

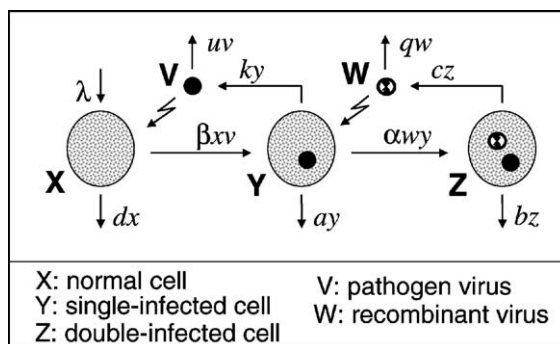


Fig. 1. Model for a double viral infection. Pathogen viral particles  $v$  infect normal cells  $x$ , producing single-infected cells  $y$ ; these are the target of a new infection by the recombinant virus  $w$  that converts them in double-infected cells  $z$ . Double-infected cells are able to produce recombinants but no pathogens. Definitions of parameters are in Table 1.

$$\begin{aligned}
\dot{x} &= \lambda - dx - \beta xv, \\
\dot{y} &= \beta xv - ay, \\
\dot{v} &= ky - uv.
\end{aligned} \tag{1}$$

We then added a second virus, the recombinant (genetically modified) virus, whose density is  $w$ . The recombinant infects cells previously infected by the pathogen, turning them at a rate  $\alpha wy$  into double-infected cells, whose density is  $z$ . Recombinants are removed at rate  $qw$ . The double-infected cells die at rate  $bz$  and release recombinants at rate  $cz$ . The equations for the full system are:

$$\begin{aligned}
\dot{x} &= \lambda - dx - \beta xv, \\
\dot{y} &= \beta xv - ay - \alpha wy, \\
\dot{z} &= \alpha wy - bz, \\
\dot{v} &= ky - uv, \\
\dot{w} &= cz - qw.
\end{aligned} \tag{2}$$

### 3. Analytical results

The model has three steady states as shown below:

(a) Equilibrium with an absence of viruses:

$$(\widehat{x}_1, \widehat{y}_1, \widehat{z}_1, \widehat{v}_1, \widehat{w}_1) = (\lambda/d, 0, 0, 0, 0). \tag{3}$$

(b) Equilibrium with the pathogen:

$$(\widehat{x}_2, \widehat{y}_2, \widehat{z}_2, \widehat{v}_2, \widehat{w}_2) = \left( \frac{au}{\beta k}, \frac{\lambda}{a} - \frac{du}{\beta k}, 0, \frac{\lambda k}{au} - \frac{d}{\beta}, 0 \right). \tag{4}$$

(c) Equilibrium with both viruses:

$$\begin{aligned}
\widehat{x}_3 &= \lambda / (d + \beta bkq / (\alpha cu)), \\
\widehat{y}_3 &= bq / (\alpha c), \\
\widehat{z}_3 &= (q/c) \widehat{w}_3, \\
\widehat{v}_3 &= bkq / (\alpha cu), \\
\widehat{w}_3 &= (\alpha \beta \lambda ck - \beta abkq - \alpha acdu) / (\alpha (\beta bkq + \alpha cdu)).
\end{aligned} \tag{5}$$

The steady state with the pathogen present (Eq. (4)) is possible when the equilibrium density of the pathogen is greater than zero ( $\widehat{v}_2 > 0$ ), and this leads to a condition for invasion of the pathogen:  $R_v = \beta \lambda k / adu > 1$  [9]. This describes the ratio of growth and death rates for the pathogen pathway (Fig. 1). For the third equilibrium to exist (Eq. (5)), the density of the recombinant virus must be greater than zero ( $\widehat{w}_3 > 0$ ), and this leads to the condition  $R_w$ :

$$R_w = (\alpha\lambda c/abq)(R_v - 1)/R_v > 1. \quad (6)$$

$R_w$  depends on the product of the growth-rates-to-death-rates ratio and the net growth of the pathogen,  $(R_v - 1)/R_v$ . Appendix A shows that  $R_w$  describes the condition for invasion of the recombinant by accounting for the number of secondary infections produced by one cell infected with the recombinant. So if  $R_w$  is larger than 1 indicates that at the start of the infection each recombinant-infected cell produces more than one newly recombinant-infected cell, resulting in an increase in this cell population.

Thus the infection with the pathogen progresses when  $R_v > 1$ , leading to a steady state (Eq. (4)). In this situation, the normal host cell density  $x$  decreases in favor of the infected population  $y$ , and the level of pathogens  $v$  is very high. The immune system response may decrease viral density by direct attack or through infected cells. However, this response appears some weeks to months after infection and would presumably only be temporary since the immune system is severely compromised at later stages of the disease [10], and therefore, is ignored here. This model better describes the initial and latest stage of HIV-1 infection (see [9,11] for an immune model for a

Table 1  
Interpretation and value of the parameters

Parameter	Definition	Value (day <sup>-1</sup> )	Comments
$d$	Death rate of host cell <sup>a</sup>	0.01	Average life span of CD4+T cell is two years, so $d = 0.0014$ [28]. From modeling, $d = 0.01$ [19].
$\lambda$	Production rate of host cell	2 cell/mm <sup>3</sup>	Density of total CD4+ T cell in the blood in healthy human is $X' = 1000$ cell/mm <sup>3</sup> [12]. Assumed equilibrium, their production $\lambda'$ equal loss $\lambda' = X'd = 10$ . Assumed that a fraction $\tau = 0.2$ of new generated cells are activated $\lambda = \lambda'\tau = 2$ [12].
$\beta$	Infection rate of host cell by HIV-1	0.004 mm <sup>3</sup> /vir	Estimated indirectly as a small value that preserves both infections. For single infection $\beta = 0.00027$ [12], $\beta = 0.00065$ [19], $\beta = 0.0035$ [29], upper bound $\beta = 0.007$ [30].
$a$	Death rate of HIV-1 infected cell	0.33	Based on life span of HIV-1 infected cells of three days [12]. Other estimates: $a = 0.49$ [18], $a = 0.39$ [19].
$\alpha$	Infection rate by recombinant	0.004 mm <sup>3</sup> /vir	Estimated indirectly as a small value that preserves the double infection. Assumed identical to $\beta$ .
$b$	Death rate of double-infected cell	2	Based on observations of virus release within 8 h of infection before lysis [6].
$k$	HIV-1 production rate by a cell	50 vir/cell	$k = n_1 a$ . $n_1$ is total number of infectious HIV-1 produced by a cell: $n_1 \sim 140$ [31], $n_1 = 180$ [1].
$u$	Removal rate of HIV-1	2	Based on life span of 1/2 day [12]. Another value, $u = 3$ [18,19].
$c$	Production rate of recombinant by a double-infected cell	2000 vir/cell	$c = n_2 b$ . $n_2$ is total number of infectious re-combinant produced by a double-infected cell. In vitro total number of recombinants per cell is $\sim 3333$ [6]. Assumed $n_2 = 1000$ .
$q$	Removal rate of recombinant	2	Assumed identical to $u$ .

<sup>a</sup> Host cells refer to activated CD4+ T cells.

single virus; Eq. (1) describes a target-cell limited model where the sharp declines in virus load following infection results by limitation in target cells and does not involve an immune response [9,12]). For the recombinant infection to succeed, it is necessary that  $R_w > 1$ , leading to the equilibrium in Eq. (5). The stability of this equilibrium is described in the following section. Notice that host cells are generated in the presence of pathogens, thus it was assumed that at the time of infection a proportion  $p = \tau$  of the existing CD4+ T cells were activated [12]. It results in a host cell density at infection equal to its equilibrium value without viruses  $\hat{x}_1$  (Eq. (3), Table 1 for  $\lambda$ ).

#### 4. Numerical results

The temporal dynamics of the model was extensively studied by numerical simulations using a routine for stiff ordinary differential equations from the software MATLAB 6. The model was evaluated for the HIV-1 and the VSV-recombinant infections; and most of the values for the parameters were based on the literature (Table 1). In the absence of data, infection and removal rates for the recombinant ( $\alpha, q$ ) were assumed identical to their equivalents in the pathogen.  $\beta$  was set small enough to allow for the development of both infections. The parameters  $b$  and  $c$  were set to magnitudes estimated under in vitro conditions.

Fig. 2(a) shows the results for a single infection with the pathogen. The population of the pathogen rapidly increased showing a peak towards the end of the first week and stabilizing within four weeks after infection. Simultaneously, the host cells were severely depleted by the infection, stabilizing around two orders of magnitude lower than their initial level. These patterns are comparable to previous results [9,12]. Timing and size of pathogen peak depended on initial conditions for host cell number and pathogen load. Broad variation for initial pathogen load ( $10^{-6}$ – $10^2$  vir/mm<sup>3</sup>) only moved by a few days the peak timing, but varying initial cell number from 200 to 10 cell/mm<sup>3</sup> delayed by two weeks the peak and decreased 5.5-fold its size. The small value of 10 for the number of initial host cells corresponds to average values in healthy individuals [13]. The larger value follows Ref. [12] and was chosen on the basis that HIV-1 increase T cell activation. The initial condition does not change the equilibrium. Fig. 2(b) shows the full model with the double infection. In addition to the dynamics of the pathogen infection, the recombinant virus generates double-infected cells. A few days after the recombinant infection, the pathogen load decayed by 12-fold and then generated damped oscillations around the equilibrium. At the same time, the normal cells increased by one order of magnitude during the first week, and stabilized slowly to equilibrium. The normal cell trajectory oscillated slightly toward its equilibrium, in comparison to the major fluctuations of other elements of the system. Fig. 2(c) also shows the infection with both viruses, but it simulates a higher infection rate for the recombinant. This higher infection rate significantly increased the normal cells and decreased the pathogen, stabilizing the system more rapidly. The recovery of normal cells for the double infection using the default values was to 17% of the density in individuals without pathogen ( $\hat{x}_3 = 33$  cell/mm<sup>3</sup> respecting  $\hat{x}_1 = 200$  cell/mm<sup>3</sup>, Fig. 2(b)). However, when the recombinant infection rate exceeded that of the pathogen, such as  $\alpha = 4\beta$  (Fig. 2(c)), the recovery of normal cells increased up to 44% ( $\hat{x}_3 = 89$  cell/mm<sup>3</sup>).

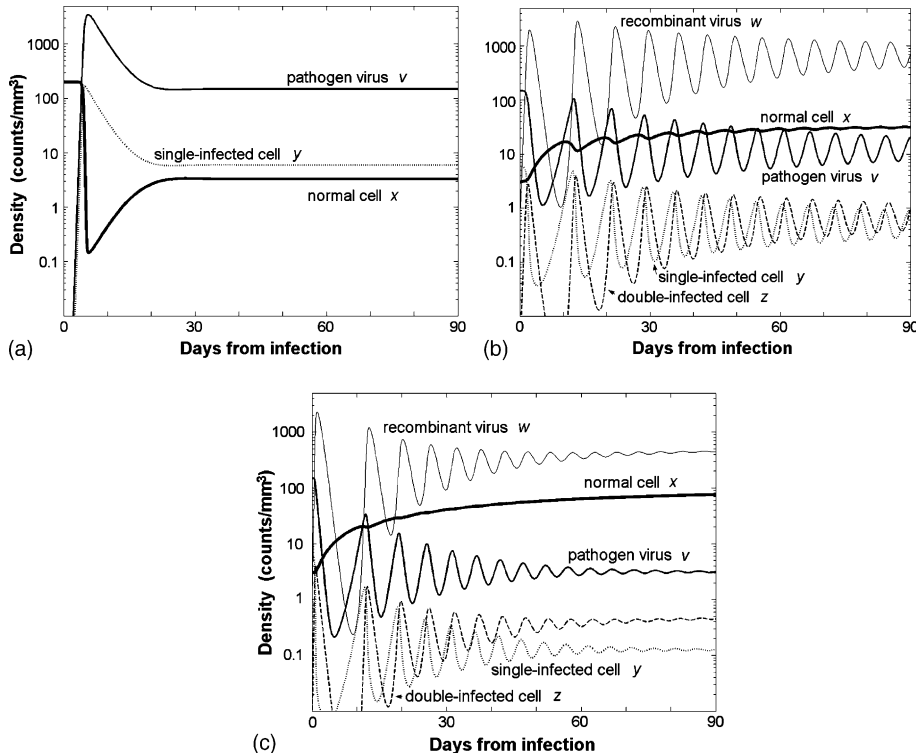


Fig. 2. Simulations. (a) The single-infection system. A pathogen virus  $v$  infects a host cell  $x$ , generating single-infected cell  $y$ . Initial condition for the host cells was  $x(0) = 200$ , and the pathogen invaded with  $v(0) = 10^{-6}$  (a few hundred viruses in the whole body). Quantities corresponded to  $1 \text{ mm}^3$  in the peripheral blood. The values of the parameters were from Table 1. (b) System with the double infection, and with equal rates of infection for both viruses ( $\alpha = \beta$ ). In addition to the pathogen infection, a recombinant virus  $w$  infects a single-infected cell  $y$ , generating a double-infected cell  $z$ . Equilibrium densities from (a) were the initial conditions [ $x(0) = 3$ ,  $y(0) = 6$ ,  $v(0) = 149$ ], and the recombinant invades with  $w(0) = 1$ . The production rate of recombinants was  $c = 2000$ . (c) Alternative system with the double infection with  $c = 2000$ , but  $\alpha = 4\beta$ .

The equilibrium for the system using the default values exhibited negative eigenvalues, indicating that it was locally stable to small perturbations. These eigenvalues were obtained using the software *Mathematica*. Since we are interested in understanding global stability, we performed numerous simulations to infer the macrostability of the equilibrium. The simulations considered different initial conditions for the invasion of the recombinant ranging from  $w(0) = 10^{-6}$ –100 counts/mm<sup>3</sup>. All initial conditions in this range converged to the same equilibrium, suggesting that it was globally stable. Further, the trajectories to the equilibrium from different initial conditions converged rapidly a few days after infection. These results suggest that all doses of the recombinant at the time of the treatment may have the same effect in controlling the pathogen.

Since most parameter values can only be roughly estimated, we evaluated the model's sensitivity to changes in these magnitudes (Table 2, rows 1–26). Most of the new values result from multiplying and dividing the default magnitude by 2, and may represent extreme values. The equilibrium densities (Table 2, columns 3–6) were obtained from Eqs. (3)–(5). For comparison the

Table 2

Effect of parameter values on the density of normal cell  $x_i$  pathogen  $v_i$  and on the stability of the system

Row	Parameter values	$\widehat{x}_2$ (% $\widehat{x}_1$ )	$\widehat{x}_3$ (% $\widehat{x}_1$ )	$\widehat{v}_2$	$\widehat{v}_3$ (% $\widehat{v}_2$ )	Oscillations ( $t = 200$ )	
						$x_3$	$v_3$
1	Default (Table 1)	3 (2)	33 (17)	149	12 (8)	–	$12 \pm 1$
2	$\lambda = 1.0, d = 0.005$	3 (2)	18 (9)	74	12 (17)	$15 \pm 1$	$23 \pm 20$
3*	$\lambda = 4.0, d = 0.02$	3 (2)	57 (29)	298	12 (4)	–	–
4	$\lambda = 1.0$	3 (3)	17 (17)	73	12 (17)	$13 \pm 1$	$24 \pm 21$
5*	$\lambda = 4.0$	3 (1)	66 (17)	300	12 (4)	–	–
6	$d = 0.005$	3 (1)	36 (9)	150	12 (8)	–	$12 \pm 1$
7	$d = 0.02$	3 (3)	29 (29)	147	12 (9)	28	$12 \pm 4$
8	$\beta = 0.002, \alpha = \beta$	7 (3)	33 (17)	146	25 (17)	$27 \pm 3$	$50 \pm 45$
9	$\beta = 0.008, \alpha = \beta$	2 (1)	33 (17)	150	6 (4)	–	–
10*	$\beta = 0.002, \alpha = 4\beta$	7 (3)	89 (44)	150	6 (4)	–	–
11*	$\beta = 0.004, \alpha = 4\beta$	3 (2)	88 (44)	149	3 (2)	–	–
12*	$a = 0.165, k = 25$	3 (2)	57 (29)	149	6 (4)	56	$7 \pm 2$
13	$a = 0.66, k = 100$	3 (2)	18 (9)	149	25 (17)	–	$25 \pm 1$
14	$b = 1.0, c = 1000$	3 (2)	33 (17)	149	12 (8)	$27 \pm 3$	$27 \pm 25$
15	$b = 4.0, c = 4000$	3 (2)	33 (17)	149	12 (8)	–	–
16	$k = 25$	7 (3)	57 (29)	73	6 (9)	56	$7 \pm 2$
17	$k = 100$	2 (1)	18 (9)	300	25 (8)	–	$25 \pm 1$
18	$c = 1000$	3 (2)	18 (9)	149	25 (17)	$15 \pm 2$	$45 \pm 39$
19*	$c = 4000$	3 (2)	57 (29)	149	6 (4)	–	–
20	$u = 1.0, q = 1.0$	2 (1)	33 (17)	300	12 (4)	–	–
21	$u = 4.0, q = 4.0$	7 (3)	33 (17)	73	12 (17)	$18 \pm 8$	$80 \pm 79$
22	$u = 1.0$	2 (1)	18 (9)	300	25 (8)	–	–
23	$u = 4.0$	7 (3)	57 (29)	73	6 (9)	$23 \pm 11$	$95 \pm 94$
24*	$q = 0.5$	3 (2)	89 (44)	149	3 (2)	$81 \pm 1$	$5 \pm 4$
25*	$q = 1.0$	3 (2)	57 (29)	149	6 (4)	56	$6 \pm 3$
26	$q = 4.0$	3 (2)	18 (9)	149	25 (17)	–	$25 \pm 1$
27*	Advanced	3 (2)	188 (94)	149	0.16 (0.1)	165	$0.16 \pm 0.06$

Sub-index 1 refers to a system with no virus, the 2 with the pathogen but not the recombinant, and the 3 with both viruses. The columns 3–6 describe densities of equilibria from Eqs. (3)–(5). The density of normal cells as a percentage at the time of the pathogen infection (% $\widehat{x}_1$ ), and the density of the pathogen as a percentage in single-infected individuals (% $\widehat{v}_2$ ) are in parentheses. ‘Oscillations’ describes fluctuations in densities through day 200 after infection obtained from simulations. ‘–’ means identical to equilibrium values. \* indicates cases consistent with a high recuperation of normal cells, reduction of pathogen, and stability of the system. ‘Advanced’ considered very competent values for the recombinant parameters:  $\alpha = 4\beta$ ,  $c = 10,000$  and  $q = 0.5$ .

cells and the pathogen density are also described in terms of percentage change in response to the infections. As the result of the pathogen infection, the density of normal cells declined to 1–3% of the amount in individuals without pathogen. After the addition of the recombinant, the normal cell density increased up to 8–44% of the density in an individual without pathogen, while the pathogen declined 83–98% of the density in an infected individual. In the best case considered, the pathogen was reduced to a very low density, but it was not exterminated by this therapy. Results from simulations indicated that those equilibria may be reached at different times, depending on the stability of the system, as well, that they may represent average values for low amplitude limit

cycles. The last two columns of Table 2 resulted from simulations and described fluctuations in density through day 200 after infection. By that time amplitudes were generally stabilized. The large oscillations were limit cycles, and their frequencies and amplitudes were determined by the parameter values, and not on the initial conditions [14]. Limit cycles were associated with low efficiency in the control of the pathogens. Table 2 (rows 1–26) indicates that a high recovery of normal cells (29–44%), elimination of pathogens (96–98%) and stability of the system are favored by large host cell and recombinant production rates ( $\lambda, c$ ), and larger infection rate for the recombinant ( $\alpha > \beta$ ). The parameter values ‘Advanced’ at the bottom of Table 2 considered simultaneously competent values for several recombinant parameters. This set of values predicted a 99.9% elimination of the pathogens. This 1000-fold reduction of pathogens may represent the upper limit for the efficiency of this therapy.

The parameters production  $c$  and infection rates  $\alpha$  of the recombinant virus could be manipulated genetically [15]. Appendix B illustrated the magnitude of these parameters for a recovery up to 40% of normal host cells. This level of cell recovery required that the product of the infection and production rates of the recombinant ( $\alpha c$ ) must be at least 133 times larger than the pathogen ( $\alpha c / \beta k \geq 133$ ). Fig. 3 described in detail the positive effect of increasing only the recombinant production rate  $c$  on the efficiency of this therapy. It showed that increasing  $c$  increased normal cells, decreased pathogens, and stabilized the system from limit cycles. At lower production rates ( $c < 2000$ , and default values), the system was characterized by oscillation in densities, and lower efficiencies in cell recovery and pathogen elimination. At intermediate production rates ( $c \sim 2000$ –10,000) the efficiency increases significantly with  $c$ , with cell recovery ranging from 17% to 50% and pathogen elimination from 92% to 98%. For larger production rates (extreme values), cell recovery and pathogen elimination levels progressed slowly with  $c$ .

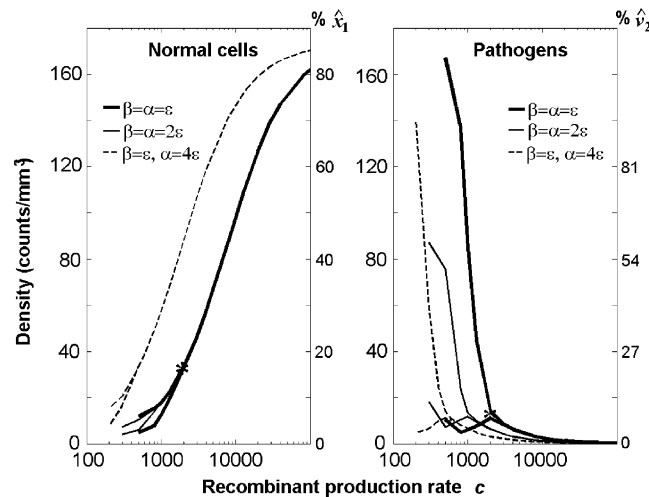


Fig. 3. Effect of the recombinant production rate ( $c$ ), and the infection rates of pathogen and recombinant ( $\alpha, \beta$ ) on the densities of normal cells ( $x$ ) and pathogens ( $v$ ) at day 200 after infection. Bifurcated lines indicate upper and lower bounds of limit cycles found at some densities.  $\epsilon = 0.004$ ; initial conditions and other parameters not specified were as Fig. 2(b). \* indicated default values. Right vertical axes describe density in terms of recovery of normal cells ( $\% \hat{x}_1$ ), and remnant of pathogen percentages ( $\% \hat{v}_2$ ).



Fig. 3 also showed that when the infection rate is larger for the recombinant ( $\alpha > \beta$ ) these results are similar but of a larger magnitude.

## 5. Discussion

A mathematical model studied a potential therapy for controlling HIV infection that uses an engineered virus to selectively eliminate infected cells. Results indicated that this viral-therapy of using genetically modified viruses can be effective in reducing the HIV-1 load in patients. The model predicted a 92% reduction of HIV load, and recovery of host cells to 17% of their normal level. This is a basic estimation that used data from a currently available engineered virus [6] and reasonable assumptions for some parameter values. The model also indicated a larger success as more competent engineered viruses are designed (98% HIV reduction and a host cells recovery to 44% of their normal level). Viral-therapy, however, may be less efficient than anti-viral drugs in reducing HIV-1 load, since anti-viral drugs may reduce the viral load to below the limit of detection (0.02–0.5 counts/mm<sup>3</sup>) [8]. Viral-therapy has a secondary effect, a high recombinant load that is considered innocuous in principle [6]. Nevertheless, viral-therapy may have several advantages over present conventional treatment, since it is expected no toxicity, no negative side effects, and no evolution of resistance (Appendix C). An additional advantage is the simplicity of treatment; in simulations and in in vitro experiments [6] the engineered virus remains in the system once introduced, indicating that only one treatment may be required. These advantages could make this viral-therapy an alternative to extend the survival of AIDS patients.

Our results were consistent with those of Schnell et al. [6], however, in their in vitro study the drop in the HIV-1 level after the recombinant infection was larger ( $\sim 0.6\%$ ) but comparable with the best cases simulated here (Table 2). Also, oscillations in HIV density after infection with the recombinant were observed experimentally [6].

The present research provides a qualitative understanding of a simple model involving a hunter-virus to control a pathogen. Further research on viral-therapy may incorporate more realistic assumptions concerning the population dynamics of the entire population of CD4+ T cells; this would include the cellular cycle involving naïve, memory and activated CD4+ cells. It also may consider the impact of recovered CD4+ cells on the restoration of the immune system. Such a study may provide a more practical translation to a clinical situation. It is expected a synergistic interaction between the hunter-virus and the immune response controlling HIV-1 will occur. The engineered virus encodes only envelope human proteins and thus would not induce production of neutralizing antibodies. However, its internal proteins could induce a cytotoxic T cell response, but such responses might help in clearing HIV infection by rapidly killing cells infected with HIV-1 and the engineered virus [6]. This model does not consider the pathogen-recombinant interaction, which may generate hybrid viruses capable of attacking normal or infected cells. Such an interaction was reported as being extremely rare [6]. The simulated infections exhibited temporal dynamic behaviors varying from broad limit cycles to rapid equilibrium convergence. Large production and infection rates for the engineered virus favored equilibrium convergence and a major reduction of HIV-1 load. Thus, viral engineers may look to improve those parameter values in hunter-viruses. More quantitative predictions require detailed measuring of the therapeutic infection parameters such as rates of infectivity, production and

elimination of the engineered virus. The construction of the recombinant caused an important reduction in the efficiency of budding viral particles [6]. Since genetically engineered VSV has crucial medical applications including an alive-vaccine [16] research has been conducted to determine critical modifications in the VSV membrane for virus assembly [15]. This may allow for the design of more efficient recombinants.

A comparable model used a defective interfering virus (DIV) as therapy for AIDS [17]. Similar to our recombinant virus, DIV only replicates in a host cell coinfecting with HIV. The proposed DIV was assumed to obstruct HIV replication by two interfering mechanisms: slowing the assembly of HIV-1, and producing HIV-1 with reduced infectivity (HIV\*). Therefore, the model considered two pathways for double-infected cells: (a) undergo lysis producing HIV, HIV\* and DIV; or (b) stay alive 'cured' producing HIV\* and DIV. In both pathways coinfecting cells experience large removal rates. This model predicted, however, that DIV survival on a peripheral blood HIV-1 infection is unlikely. Our mechanism is simpler because the double-infected cells are killed by the rapid cytopathic infection of the recombinant producing only recombinants. This assumption was based on the recombinant replication speed. Within 3 h after infection the recombinant would shut off cell protein synthesis and within 8 h would produce particles that bud from the cell prior to lysis [6], while the life span of a HIV-1 infected cell is 2–3 days [12,18,19].

Therapy using viruses has been employed to treat bacterial diseases [20–25], and has been found to be more efficient than antibiotics because of the continuous replication of the virus compared to the rapid decay of antibiotics [23]. Beneficial effects of a virus on viral diseases have also been reported where the hepatitis G virus that coinfects HIV patients increases their survival [26,27]. Results of the present study are important not only in relation to therapies against AIDS but also for other diseases for which these biological devices may be developed.

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## Appendix A. Condition for the recombinant invasion (basic reproductive ratio)

From Fig. 1, each double-infected cell  $Z$  produces recombinants at a rate  $c$  during a mean life  $1/b$ , resulting in a 'net production of recombinants'  $c/b$ . These recombinants  $W$  infect pathogen-infected cells  $Y$  at an infection rate per recombinant of  $\alpha_Y$  (Eq. (2)). Since the mean life time for a recombinant is  $1/q$  the 'net number of infections per recombinant' is  $\alpha_Y/q$ . Then by multiplying the net production of recombinants by the net number of infections, results in the number of secondary infections or basic reproductive ratio [32] for the recombinant:

$$R_w = \alpha y c / b q. \quad (\text{A.1})$$

At the beginning of the recombinant infection the density of pathogen-infected cells  $y$  is given by Eq. (4). By replacing this density into (A.1) gives

$$R_w = \frac{\alpha c}{b q} \left( \frac{\lambda}{a} - \frac{d u}{\beta k} \right). \quad (\text{A.2})$$

By taking  $\lambda/a$  as a common factor off the parentheses in (A.2), the basic reproductive ratio of the recombinant becomes

$$R_w = \frac{\alpha \lambda c}{a b q} \left( 1 - \frac{a d u}{\beta \lambda k} \right). \quad (\text{A.3})$$

The quotient inside the parentheses of (A.3) is the reciprocal of  $R_v$ .

## Appendix B. Extent of recovery of normal host cells

An efficient therapy must also increase normal cell density allowing the recuperation of the immune system. The recovery of normal cells after the recombinant infection is determined by the ratio  $r = \beta b k q / \alpha c u$ . This ratio differentiates the amount of normal cells in a system without viruses ( $\bar{x}_1 = \lambda/d$ , Eq. (3)), from one with both viruses ( $\bar{x}_3 = \lambda/(d+r)$ , Eq. (5)), and it must be small to recover abundant cells. Successful therapy may be indicated by achieving a normal-cell level of at least 40% in a non-infected individual (assuming that activated and total CD4+ T cells vary proportionally, this percentage may represent the presymptomatic stage of AIDS [1]). A recovery of cells up to 40% is reached for  $r \leq 1.5d$ . By considering the default values for  $b$ ,  $q$ ,  $u$ , and  $d$  (Table 1), this ratio is reduced to  $\alpha c / \beta k \geq 133$  which requires a recombinant with a product of production and infection rates 133 larger than the corresponding product for the pathogen.

## Appendix C

### C.1. Could HIV evolve resistance to the recombinant?

For an HIV-1 infection, the HIV-1 envelope protein gp120/41 must bind to receptors at the cell surface, and then HIV-1 fuses its envelope with the membrane of the cell. Later, the recombinant attacks HIV-1-infected cells by binding to the gp120/41 expressed on the cell surface. Consequently, a HIV-1 mutation for resistance to the recombinant infection would require the loss of HIV's ability to bind the cell receptors [6], and this loss of HIV infectivity would therefore not be selected.

### C.2. What toxicity and side effects has incorporating a virus into the human body?

The recombinant was derived from the VSV that causes a non-lethal disease in farm animals consisting of blister-like bumps on the hoofs and tongue. Occasionally, humans become infected

with VSV, and most individuals have no symptoms, but those who become ill usually have a mild flu-like disease [6]. Since the VSV recombinant no longer has its normal coat protein it cannot cause infection in livestock or healthy humans.

Further, lytic bacteriophages – small viruses that are harmless for humans but lethal for their bacterial host – have been used since the early 20th century to cure human bacterial infections [20–22,24,25]. During this period, these phages have been administered extensively in Eastern Europe and the former Soviet Union to treat a broad list of individual and epidemic infections, and there are no reports of serious complications. From a clinical standpoint, the phages appear to be innocuous [25]. Viruses have further potential assisting in prevention and treatment of human infections. For example, Alive Vaccines will use viruses expressing given foreign proteins to induce specific immune responses [16]. Gene Therapy has used viral vectors to transfer genetic material to target cells [33]. Phages are expected to be commercialized in the USA to treat multidrug resistant bacterial infections.

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