

Resistance to Protease Inhibitors in a Model of HIV-1 Infection with Impulsive Drug Effects

Rachelle E. Miron · Robert J. Smith?

Received: 13 April 2013 / Accepted: 2 September 2013 / Published online: 5 November 2013
© Society for Mathematical Biology 2013

Abstract *Background* The emergence of drug resistance is one of the most prevalent reasons for treatment failure in HIV therapy. This has severe implications for the cost of treatment, survival and quality of life.

Methods We use mathematical modelling to describe the interaction between T cells, HIV-1 and protease inhibitors. We use impulsive differential equations to examine the effects of different levels of protease inhibitors in a T cell. We classify three different regimes according to whether the drug efficacy is low, intermediate or high. The model includes two strains: the wild-type strain, which initially dominates in the absence of drugs, and the mutant strain, which is the less efficient competitor, but has more resistance to the drugs.

Results Drug regimes may take trajectories through one, two or all three regimes, depending on the dosage and the dosing schedule. Stability analysis shows that resistance does not emerge at low drug levels. At intermediate drug levels, drug resistance is guaranteed to emerge. At high drug levels, either the drug-resistant strain will dominate or, in the absence of longer-lived reservoirs of infected cells, a region exists where viral elimination could theoretically occur. We provide estimates of a range of dosages and dosing schedules where the trajectories lie either solely within a region or cross multiple regions.

Conclusion Under specific circumstances, if the drug level is physiologically tolerable, elimination of free virus is theoretically possible. This forms the basis for theoretical control using combination therapy and for understanding the effects of partial adherence.

R.E. Miron · R.J. Smith? (✉)

Department of Mathematics, The University of Ottawa, 585 King Edward Ave,
Ottawa ON K1N 6N5, Canada
e-mail: rsmith43@uottawa.ca

R.J. Smith?

Faculty of Medicine, The University of Ottawa, 585 King Edward Ave, Ottawa ON K1N 6N5,
Canada

Keywords Resistance to protease inhibitors · Impulsive differential equations

1 Introduction

The effects of drug resistance have altered the history of disease progression (Pillay et al. 2006). Drug resistance can emerge with lack of adherence to any strict drug therapy (Janeway et al. 2006). Mutation development occurs quickly at a rate of approximately 3×10^{-5} per nucleotide base cycle of replication (Janeway et al. 2006). Since HIV is highly variable, it rapidly develops resistance to antiretroviral drugs (Janeway et al. 2006). This results in the spread of a different form of HIV, so that antiretroviral therapy can no longer control the virus (Wheeler et al. 2010).

Many mathematical models have been developed to describe drug resistance (Shiri and Welte 2008; Shi et al. 2008; Huang et al. 2010; von Wyl et al. 2012; Wagner and Blower 2012; Heye et al. 2012), but such models have focussed on the emergence of drug resistance during continuous therapy (Wu et al. 2007; Rong et al. 2007a, 2007b; Mohanty and Dixit 2008; Bhunu et al. 2009; Smith? and Aggarwala 2009; Kitayimbwa et al. 2013; Rosenbloom et al. 2012). A more recent tool to model drug dynamics is impulsive differential equations. Smith? and Wahl (2004) used impulsive differential equations to model the interaction between cell/virus dynamics and reverse transcriptase inhibitors, integrase inhibitors and fusion inhibitors. Smith? and Wahl (2005) also used impulsive differential equations to model drug resistance by considering immunological behaviour for HIV dynamics, including the effects of reverse transcriptase inhibitors and other drugs that prevent cellular infection. Smith? (2006) answered the question of determining how many doses may be skipped before HIV treatment response is adversely affected by the emergence of drug-resistance using impulsive differential equations. Krakovska and Wahl (2007) developed a model that predicts optimal treatment regimens, and used this model, coupled with impulsive differential equations, to investigate the effects of adherence. Lou and Smith? (2011) developed a mathematical model that describes the binding of the virus to T cells in the presence of the fusion inhibitor enfuvirtide using impulsive differential equations. Lou et al. (2012) used impulsive differential equations to develop a rigorous approach to analyze the threshold behaviours of nonlinear virus dynamics models with impulsive drug effects and to examine the feasibility of virus clearance.

The other major class of antiretroviral drugs used to treat HIV-positive patients are protease inhibitors (PIs). PIs aim to stop the viral protease, which cleaves polyproteins to produce the virion proteins and viral enzymes (Janeway et al. 2006). This decreases the number of infectious virions that bud from the infected CD4⁺ T cells and increases the number of non-infectious virions, which cannot infect other susceptible T cells (Janeway et al. 2006). Modelling PIs is significantly different to modelling reverse transcriptase inhibitors, integrase inhibitors and fusion inhibitors, since cells still become infected and still cause budding even with drug present. Here, we examine the conditions required for the emergence of drug resistance to protease inhibitors during HIV-1 drug therapy.

We consider two strains: the wild-type strain, which initially dominates in the absence of drugs, and the mutant strain, which is the less efficient competitor, but has

more resistance to the drugs. At an intermediate level of drug, the drug will affect the wild-type strain alone, whereas a high level of drug will control both strains (Rong et al. 2007b; Kepler and Perelson 1998). We describe three models for each drug level and use impulsive differential equations to model the drug dynamics flowing between each model.

This paper is organised as follows. In Sect. 2, we present the model describing the interactions between the $CD4^+$ T cells, the wild-type and mutant virus, and the drugs. In Sect. 3, we fix the drug level as a constant and find the equilibrium points, as well as the stability, for all three regions. In Sect. 4, numerical simulations are performed to show the effects of varying drug levels. We conclude with a discussion.

2 The Model

2.1 T Cells

We use nine state variables describing $CD4^+$ T cells in a variety of stages during infection and while on drugs. The variable T_S describes susceptible $CD4^+$ T cells, whereas T_I and T_Y describe cells infected by wild-type or mutant virus, respectively. Once an intermediate level of drug has entered the cells, we have three new classes. T_{PN} describes the susceptible cells that have an intermediate level of drug. T_{PI} and T_{PY} describe the cells that are infected with the wild-type or mutant strain, respectively, and that also have an intermediate level of drug. As more drug is taken, a $CD4^+$ T cell is inhibited with a high level of drug. We have three such cell types: susceptible cells inhibited with high drug levels, T_{PPN} ; cells infected with the wild-type strain, T_{PPI} ; and cells infected with the mutant strain, T_{PPY} . We also describe the virions by V_I and V_Y for the wild-type and mutant strains, respectively. Non-infectious virus is denoted by V_N . The interaction between the cells and virus can be seen in Fig. 1, and a description of the state variables is listed in Table 1. When low drug levels are present, there is not enough drug to inhibit the T cells from being infected by either the wild-type or the mutant strains. When intermediate drug levels are present, there is not enough drug in the T cells to inhibit the drug-resistant strain from producing infectious virus, but cells infected with the wild-type strain will only produce non-infectious virus. When high drug levels are present, T cells are unable to produce infectious virus, regardless of whether they are infected with the wild-type or mutant strain.

2.2 Drugs

Similar to Smith? and Wahl (2004), we use $P(t)$ to denote the intracellular concentration of the drug and its active metabolites. We assume that drugs are taken at (not necessarily fixed) times t_k . The effect of the drugs is assumed to be instantaneous, resulting in a system of impulsive differential equations, whereby solutions are continuous for $t \neq t_k$ (satisfying the associated system of ordinary differential equations) and undergo an instantaneous change in state when $t = t_k$. This can be assumed since the time during which the drug is absorbed is smaller than the time during which the drug is cleared (Smith? and Schwartz 2008).

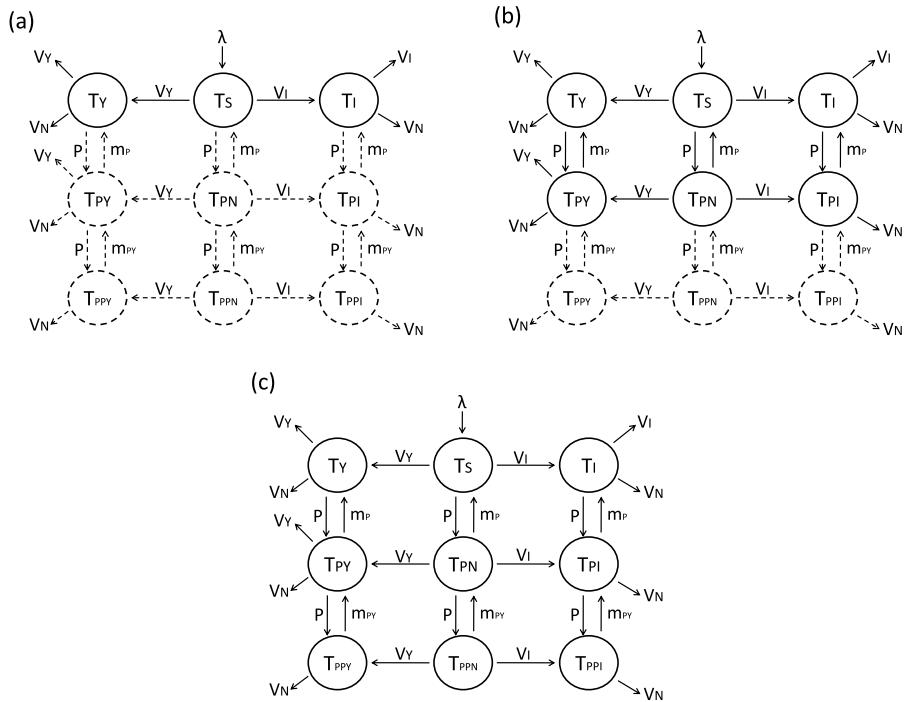


Fig. 1 The model. (a) The model for Region 1. T_S , T_I and T_Y are the susceptible, infected with wild-type strain and infected with mutant strain T cells, respectively. V_I and V_Y are the wild-type and mutant strains, respectively. λ is the rate of new T cells produced. In Region 1, there is not enough drug to inhibit the T cells from creating either wild-type or mutant virus. (b) The model for Region 2. T_{PN} , T_{PI} and T_{PY} are the susceptible, infected with wild-type strain and infected with mutant strain T cells, respectively, at intermediate drug levels. P is the drug concentration and m_P is the clearance rate. In Region 2, there is not enough drug in the T cells to inhibit the mutant strain from producing infectious virions, but the wild-type strain can be controlled, meaning it will only produce non-infectious virions, V_N . (c) The model for Region 3. T_{PPN} , T_{PI} and T_{PY} are the susceptible, infected with wild-type strain and infected with mutant strain T cells, respectively, at high drug levels. In Region 3, there are sufficient levels of drug in the T cells to inhibit infectious viral budding of either strain

According to impulsive theory found in the mathematical literature (Lakshmikantham et al. 1989; Bainov and Simeonov 1989, 1993, 1995), we can describe the nature of the impulse at time t_k via a difference equation

$$\Delta P \equiv P(t_k^+) - P(t_k^-) = f(t_k, P(t_k^-)), \quad (1)$$

where $f(t, P)$ is a mapping of the solution before the impulse, $P(t_k^-)$, to after the impulse effect, $P(t_k^+)$.

To model the effects of resistance mutation on drug efficacy, we consider the dose-effect curve illustrated in Fig. 2. Here, the solid line shows a dose-effect curve for the wild-type virus, while the dashed line shows the same curve for a drug-resistant strain with 10-fold resistance; drug resistance implies an increase in the half-maximal inhibitory concentration IC_{50} of the drug. The axis, or effect, in the dose-effect curve is the probability that a given T cell absorbs sufficient quantities of the drug to pre-

Table 1 Definition and units of parameters/state variables

| Parameter/State variable | Units | Description |
|--------------------------|--|---|
| n_I | day ⁻¹ | Number of virions produced by a susceptible CD4 ⁺ T cell |
| ω | | Proportion of infectious virions produced |
| r_I | day ⁻¹ | Infection rate of CD4 ⁺ T cells with wild-type virus |
| r_Y | day ⁻¹ | Infection rate of CD4 ⁺ T cells with mutant virus |
| d_V | day ⁻¹ | Death rate for the virus |
| d_S | day ⁻¹ | Death rate for the susceptible CD4 ⁺ T cells |
| d_I | day ⁻¹ | Death rate for the infected CD4 ⁺ T cells |
| r_P | μM^{-1} day ⁻¹ | Rate at which drug inhibits CD4 ⁺ T cells when drug concentrations are intermediate |
| r_{PP} | μM^{-1} day ⁻¹ | Rate at which drug inhibits CD4 ⁺ T cells when drug concentrations are high |
| d_P | day ⁻¹ | Drug clearance rate |
| λ | cells μL^{-1} day ⁻¹ | Production rate of CD4 ⁺ T cells |
| m_P | day ⁻¹ | Clearance rate of the drug from an intermediate inhibited cell |
| m_{PP} | day ⁻¹ | Clearance rate of the drug from a highly inhibited cell |
| P_1 | μM | Region 1 threshold |
| P_2 | μM | Region 2 threshold |
| τ | day | Dosing interval |
| P^i | μM | Drug dosage |
| V_I | virus μM^{-1} | Wild-type virus |
| V_Y | virus μM^{-1} | Mutant virus |
| V_N | virus μM^{-1} | Non-infectious virus |
| T_S | cells μM^{-1} | Susceptible CD4 ⁺ T cells |
| T_I | cells μM^{-1} | CD4 ⁺ T cells infected by the wild-type virus |
| T_Y | cells μM^{-1} | CD4 ⁺ T cells infected by the mutant virus |
| T_{PN} | cells μM^{-1} | Susceptible CD4 ⁺ T cells with an intermediate level of drug |
| T_{PI} | cells μM^{-1} | CD4 ⁺ T cells infected by the wild-type strain also with an intermediate level of drug |

vent the viral protease from being created in order to stop viral budding. Thus, when $P < P_1$ (Region 1), this probability is negligible for both viral strains. In some region $P_1 < P < P_2$ (Region 2), this probability remains negligible for the drug-resistant virus, but grows monotonically with dose for the wild-type. Similarly, when $P > P_2$ (Region 3), the probability of blocking the viral protease is significant for both wild-type and drug-resistant strains, although higher for the wild-type. In all three cases where the probability that the viral protease will be blocked is non-negligible, we assume that the probability grows linearly with increasing dose, although at different rates for different strains and regions (note that the dose-effect curves in these regimes are much closer to linear than suggested by this semilog plot). The Region 1 and Region 2 thresholds (P_1 and P_2 , respectively) are calculated similarly to Miron and Smith? (2010). Our model of HIV dynamics, therefore, consists of three distinct

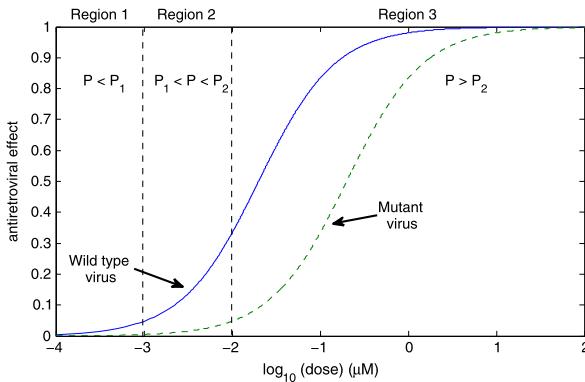


Fig. 2 Example of dose-effect curves for the wild-type (solid curve) and 10-fold resistance (dashed curve) virus strains. When drug concentrations are in Region 1, the amount of drug absorbed is insufficient to control either the wild-type or mutant strain. When the drug concentrations are in Region 2, drug absorbed can block the wild-type strain, but the resistant strain still emerges. When the drug concentration is in Region 3, both virus strains are controlled. This example is for the protease inhibitor ritonavir

systems in which different drug actions are possible, depending on the drug concentration P (see Fig. 1).

2.3 Combining T Cell Populations with Virus and Drugs

The dynamics of the $CD4^+$ T cells and virus can be modelled by the following set of ordinary differential equations:

$$\begin{aligned}
 \frac{dV_I}{dt} &= n_I \omega T_I - d_V V_I - r_I T_S V_I - r_I T_{PN} V_I - r_I T_{PPN} V_I \\
 \frac{dV_Y}{dt} &= n_I \omega (T_Y + T_{PY}) - d_V V_Y - r_Y T_S V_Y - r_Y T_{PN} V_Y - r_Y T_{PPN} V_Y \\
 \frac{dV_N}{dt} &= n_I (1 - \omega) (T_I + T_Y + T_{PY}) + n_I (T_{PI} + T_{PPI} + T_{PPY}) - d_V V_N \\
 \frac{dT_S}{dt} &= \lambda - r_I T_S V_I - r_Y T_S V_Y - d_S T_S - \alpha_1 r_P P T_S + m_P T_{PN} \\
 \frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I - \alpha_1 r_P P T_I + m_P T_{PI} \\
 \frac{dT_Y}{dt} &= r_Y T_S V_Y - d_I T_Y - \alpha_1 r_P P T_Y + m_P T_{PY} \\
 \frac{dT_{PN}}{dt} &= \alpha_1 r_P P T_S - m_P T_{PN} - r_Y T_{PN} V_Y - r_I T_{PN} V_I - d_S T_{PN} \\
 &\quad - \alpha_2 r_{PP} P T_{PN} + m_{PP} T_{PPN} \\
 \frac{dT_{PI}}{dt} &= r_I T_{PN} V_I + \alpha_1 r_P P T_I - m_P T_{PI} - \alpha_2 r_{PP} P T_{PI} + m_{PP} T_{PPI} - d_I T_{PI}
 \end{aligned} \tag{2}$$

$$\begin{aligned}
 \frac{dT_{PY}}{dt} &= r_Y T_{PN} V_Y + \alpha_1 r_P P T_Y - m_P T_{PY} - \alpha_2 r_{PP} P T_{PY} + m_{PP} T_{PPY} - d_I T_{PY} \\
 \frac{dT_{PPN}}{dt} &= \alpha_2 r_{PP} P T_{PN} - m_{PP} T_{PPN} - r_Y T_{PPN} V_Y - r_I T_{PPN} V_I - d_S T_{PPN} \\
 \frac{dT_{PPI}}{dt} &= r_I T_{PPN} V_I + \alpha_2 r_{PP} P T_{PI} - m_{PP} T_{PPI} - d_I T_{PPI} \\
 \frac{dT_{PPY}}{dt} &= r_Y T_{PPN} V_Y + \alpha_2 r_{PP} P T_{PY} - m_{PP} T_{PPY} - d_I T_{PPY},
 \end{aligned}$$

for $t \neq t_k$ (see impulsive conditions below). This model varies between the three regions mentioned in Sect. 2.2 by simply changing α_1 and α_2 . In Region 1, $\alpha_1 = 0$ and $\alpha_2 = 0$. In Region 2, $\alpha_1 = 1$ and $\alpha_2 = 0$. In Region 3, $\alpha_1 = 1$ and $\alpha_2 = 1$.

Here, t is the time in days, n_I is the number of virions produced per day, ω is the proportion of infectious virions produced from an infected CD4⁺ T cell, and r_I and r_Y are the infection rates of CD4⁺ T cells with wild-type or mutant virus, respectively. The constant λ is the rate at which new susceptible CD4⁺ T cells are produced, while the death rates are denoted by d_V , d_S and d_I for the virus, the susceptible and infected CD4⁺ T cells, respectively. We denote by r_P the rate at which drug inhibits the T cells when drug concentrations are intermediate and the drug concentration is denoted by P . Note that, once the wild-type virus infects T_{PN} , the new infected cell only produces non-infectious virus. The rate m_P is the clearance rate of the drug from an intermediate inhibited cell. The rate at which drug inhibits the T cells when drug concentrations are high is denoted by r_{PP} . The rate m_{PP} is the clearance rate of the drug from a highly inhibited cell to an intermediate inhibited cell.

All death rates, rates of infection and λ are assumed to be positive. We assume $0 \ll \omega \leq 1$ and $r_I > r_Y$ (i.e., the wild-type is the more infectious strain of the virus). Furthermore, $d_S < d_I < d_V$ (Ho et al. 1995).

In order to analyze this model, we consider each region separately and look at sub-models of system (2).

Consider Region 1 ($\alpha_1 = 0$ and $\alpha_2 = 0$). We assume here that the primary difference between wild-type and mutant virus is the rate of infection and that, in the absence of drugs, the wild-type strain is the better competitor. Also, there is not enough drug to inhibit the T cells from being infected by either the wild-type or the mutant strains. In this case, $\frac{dT_{PPN}}{dt}$ is negative, meaning T_{PPN} decays to zero. This also means T_{PN} , T_{PI} , T_{PY} , T_{PPI} and T_{PPY} decay to zero. This then forces all terms in the first six equations of system (2) including T_{PN} , T_{PI} , T_{PY} , T_{PPN} , T_{PPI} and T_{PPY} to decay to zero. Thus our subsystem excludes all the terms including T_{PN} , T_{PI} , T_{PY} , T_{PPN} , T_{PPI} and T_{PPY} .

The same can be concluded for the subsystem for Region 2 ($\alpha_1 = 1$ and $\alpha_2 = 0$). Again $\frac{dT_{PPN}}{dt}$ is negative, meaning T_{PPN} decays to zero. This also means T_{PPI} and T_{PPY} decay to zero. This then forces all terms in the first nine equations of system (2) including T_{PPN} , T_{PPI} and T_{PPY} to decay to zero. Thus, our subsystem excludes all the terms including T_{PPN} , T_{PPI} and T_{PPY} .

In summary, we have the following subregions of system (2), for $t \neq t_k$.

For $P < P_1$ (Region 1, Fig. 1a), the dynamics of the CD4⁺ T cells and virions are given by

$$\begin{aligned}\frac{dV_I}{dt} &= n_I \omega T_I - d_V V_I - r_I T_S V_I \\ \frac{dV_Y}{dt} &= n_I \omega T_Y - d_V V_Y - r_Y T_S V_Y \\ \frac{dV_N}{dt} &= n_I (1 - \omega) (T_I + T_Y) - d_V V_N \\ \frac{dT_S}{dt} &= \lambda - r_I T_S V_I - r_Y T_S V_Y - d_S T_S \\ \frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I \\ \frac{dT_Y}{dt} &= r_Y T_S V_Y - d_I T_Y.\end{aligned}\tag{3}$$

For $P_1 < P < P_2$ (Region 2, Fig. 1b), the dynamics of the CD4⁺ T cells and virions are given by

$$\begin{aligned}\frac{dV_I}{dt} &= n_I \omega T_I - d_V V_I - r_I T_S V_I - r_I T_{PN} V_I \\ \frac{dV_Y}{dt} &= n_I \omega (T_Y + T_{PY}) - d_V V_Y - r_Y T_S V_Y - r_Y T_{PN} V_Y \\ \frac{dV_N}{dt} &= n_I (1 - \omega) (T_I + T_Y + T_{PY}) + n_I T_{PI} - d_V V_N \\ \frac{dT_S}{dt} &= \lambda - r_I T_S V_I - r_Y T_S V_Y - d_S T_S - r_P P T_S + m_P T_{PN} \\ \frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I - r_P P T_I + m_P T_{PI} \\ \frac{dT_Y}{dt} &= r_Y T_S V_Y - d_I T_Y - r_P P T_Y + m_P T_{PY} \\ \frac{dT_{PN}}{dt} &= r_P P T_S - m_P T_{PN} - r_Y T_{PN} V_Y - r_I T_{PN} V_I - d_S T_{PN} \\ \frac{dT_{PI}}{dt} &= r_P P T_I - m_P T_{PI} + r_I T_{PN} V_I - d_I T_{PI} \\ \frac{dT_{PY}}{dt} &= r_P P T_Y - m_P T_{PY} + r_Y T_{PN} V_Y - d_I T_{PY}.\end{aligned}\tag{4}$$

For $P > P_2$ (Region 3, Fig. 1c), the dynamics of the CD4⁺ T cells and virions are given by

$$\frac{dV_I}{dt} = n_I \omega T_I - d_V V_I - r_I T_S V_I - r_I T_{PN} V_I - r_I T_{PPN} V_I$$

$$\begin{aligned}
\frac{dV_Y}{dt} &= n_I \omega (T_Y + T_{PY}) - d_V V_Y - r_Y T_S V_Y - r_Y T_{PN} V_Y - r_Y T_{PPN} V_Y \\
\frac{dV_N}{dt} &= n_I (1 - \omega) (T_I + T_Y + T_{PY}) + n_I (T_{PI} + T_{PPI} + T_{PPY}) - d_V V_N \\
\frac{dT_S}{dt} &= \lambda - r_I T_S V_I - r_Y T_S V_Y - d_S T_S - r_P P T_S + m_P T_{PN} \\
\frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I - r_P P T_I + m_P T_{PI} \\
\frac{dT_Y}{dt} &= r_Y T_S V_Y - d_I T_Y - r_P P T_Y + m_P T_{PY} \\
\frac{dT_{PN}}{dt} &= r_P P T_S - m_P T_{PN} - r_Y T_{PN} V_Y - r_I T_{PN} V_I - d_S T_{PN} \\
&\quad - r_{PP} P T_{PN} + m_{PP} T_{PPN} \\
\frac{dT_{PI}}{dt} &= r_I T_{PN} V_I + r_P P T_I - m_P T_{PI} - r_{PP} P T_{PI} + m_{PP} T_{PPI} - d_I T_{PI} \\
\frac{dT_{PY}}{dt} &= r_Y T_{PN} V_Y + r_P P T_Y - m_P T_{PY} - r_{PP} P T_{PY} + m_{PP} T_{PPY} - d_I T_{PY} \\
\frac{dT_{PPN}}{dt} &= r_{PP} P T_{PN} - m_{PP} T_{PPN} - r_Y T_{PPN} V_Y - r_I T_{PPN} V_I - d_S T_{PPN} \\
\frac{dT_{PPI}}{dt} &= r_I T_{PPN} V_I + r_{PP} P T_{PI} - m_{PP} T_{PPI} - d_I T_{PPI} \\
\frac{dT_{PPY}}{dt} &= r_Y T_{PPN} V_Y + r_{PP} P T_{PY} - m_{PP} T_{PPY} - d_I T_{PPY}.
\end{aligned} \tag{5}$$

The dynamics of the drug are modelled using impulsive differential equations. The exponential decay can be written as a differential equation, where $P(t)$ is the drug concentration. The dynamics of the drug are

$$\begin{aligned}
\frac{dP}{dt} &= -d_P P & t \neq t_k \\
\Delta P &= P^i & t = t_k.
\end{aligned} \tag{6}$$

The rate at which the drug is cleared is d_P and P^i is the dosage. Assuming a drug is taken at time t_k , by the definition of an impulsive effect, we have

$$P(t_k^+) = P(t_k^-) + P^i. \tag{7}$$

Here we assume that $P(0) = 0$.

Thus, (3)–(5), together with (6) describe our three-regime model of impulsive differential equations. A list and description of all the parameters and state variables can be found in Table 1.

3 Analysis

3.1 Asymptotic Behaviour

In order to interpret system (2), we analyze each region separately. We investigate the stability of each equilibrium by fixing the drug concentration level as a constant in order to approximate the effect of the impulsive periodic orbit. Region 1 has no effect from the drug concentration, but the analysis of Regions 2 and 3 include P^* as the representative value of drug levels. Section 4 demonstrates numerically the effects of changing the drug concentration levels (including the impulse condition).

3.1.1 Region 1: Low Drug Levels

System (3) has three equilibrium points.

Disease-Free Equilibrium The disease-free equilibrium is

$$(V_I, V_Y, V_N, T_S, T_I, T_Y) = \left(0, 0, 0, \frac{\lambda}{d_S}, 0, 0 \right).$$

The basic reproductive number of Region 1, $R_{0,1}$, is computed using the next-generation method (Diekmann et al. 1990; van den Driessche and Watmough 2002) at the disease-free equilibrium. Using the same notation as van den Driessche and Watmough (2002), we find

$$F = \begin{pmatrix} 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ r_I \bar{T}_S & 0 & 0 & 0 \\ 0 & r_Y \bar{T}_S & 0 & 0 \end{pmatrix}$$

$$V = \begin{pmatrix} d_V + r_I \bar{T}_S & 0 & -n_I \omega & 0 \\ 0 & d_V + r_Y \bar{T}_S & 0 & -n_I \omega \\ 0 & 0 & d_I & 0 \\ 0 & 0 & 0 & d_I \end{pmatrix},$$

where F is non-negative and V is a non-singular M-matrix. Then $R_{0,1}$ is the spectral radius of the FV^{-1} matrix. Thus,

$$R_{0,1} = \max\{R_{0,1,a}, R_{0,1,b}\}$$

$$= \max\left\{ \frac{r_I \lambda n_I \omega}{d_I(d_V d_S + r_I \lambda)}, \frac{r_Y \lambda n_I \omega}{d_I(d_V d_S + r_Y \lambda)} \right\}.$$

Note that $R_{0,1,a} > R_{0,1,b}$ since $r_I > r_Y$.

Theorem 3.1 *The disease-free equilibrium is locally asymptotically stable in Region 1 if $R_{0,1} < 1$ and unstable if $R_{0,1} > 1$.*

Proof The Jacobian matrix for Region 1 is

$$J = \begin{bmatrix} -d_V - r_I T_S & 0 & 0 & -r_I V_I & n_I \omega & 0 \\ 0 & -d_V - r_Y T_S & 0 & -r_Y V_Y & 0 & n_I \omega \\ 0 & 0 & -d_V & 0 & n_I(1 - \omega) & n_I(1 - \omega) \\ -r_I T_S & -r_Y T_S & 0 & -r_I V_I - r_Y V_Y - d_S & 0 & 0 \\ r_I T_S & 0 & 0 & r_I V_I & -d_I & 0 \\ 0 & r_Y T_S & 0 & r_Y V_Y & 0 & -d_I \end{bmatrix}.$$

For the disease-free equilibrium, $\bar{V}_I = \bar{V}_Y = 0$ and $\bar{T}_S = \frac{\lambda}{d_S}$, so the characteristic polynomial is

$$\begin{aligned} 0 &= \det(J(\bar{V}_I, \bar{V}_Y, \bar{V}_N, \bar{T}_S, \bar{T}_I, \bar{T}_Y) - \mu I_6) \\ &= (-d_V - \mu)(-d_S - \mu)[\mu^2 + a_1\mu + b_1][\mu^2 + a_2\mu + b_2], \end{aligned} \quad (8)$$

where

$$\begin{aligned} a_1 &= d_V + r_I \bar{T}_S + d_I \\ b_1 &= d_I d_V + d_I r_Y \bar{T}_S + n_I \omega r_Y \bar{T}_S \\ a_2 &= d_V + r_Y \bar{T}_S + d_I \\ b_2 &= d_I d_V + d_I r_I \bar{T}_S - n_I \omega r_I \bar{T}_S \\ &= \frac{d_I d_V d_S + r_I \lambda d_I}{d_S} (1 - R_{0,1,a}). \end{aligned}$$

We have $b_2 > 0$ if $R_{0,1,a} < 1$. By the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006), the Jacobian matrix has all eigenvalues with negative real part. Thus the disease-free equilibrium is locally asymptotically stable when $R_{0,1} < 1$ and unstable when $R_{0,1} > 1$. \square

Endemic Equilibria The wild-type equilibrium is

$$(V_I, V_Y, V_N, T_S, T_I, T_Y) = (\bar{V}_I, 0, \bar{V}_N, \bar{T}_S, \bar{T}_I, 0),$$

where

$$\begin{aligned} \bar{V}_I &= \frac{d_S d_V + r_I \lambda}{r_I d_V} (R_{0,1,a} - 1) \\ \bar{V}_N &= \frac{n_I(1 - \omega)(d_S d_V + r_I \lambda)}{r_I d_V (n_I \omega - d_I)} (R_{0,1,a} - 1) \\ \bar{T}_S &= \frac{\lambda d_V}{(d_S d_V + r_I \lambda)(R_{0,1,a} - 1) + d_S} \\ \bar{T}_I &= \frac{d_S d_V + r_I \lambda}{r_I (n_I \omega - d_I)} (R_{0,1,a} - 1). \end{aligned}$$

The mutant equilibrium is

$$(V_I, V_Y, V_N, T_S, T_I, T_Y) = (0, \bar{V}_Y, \bar{V}_N, \bar{T}_S, 0, \bar{T}_Y),$$

where

$$\begin{aligned}\bar{V}_Y &= \frac{d_S d_V + r_Y \lambda}{r_Y d_V} (R_{0,1,b} - 1) \\ \bar{V}_N &= \frac{n_I (1 - \omega) (d_S d_V + r_Y \lambda)}{r_Y d_V (n_I \omega - d_I)} (R_{0,1,b} - 1) \\ \bar{T}_S &= \frac{\lambda d_V}{(d_S d_V + r_Y \lambda) (R_{0,1,b} - 1) + d_S} \\ \bar{T}_Y &= \frac{d_S d_V + r_Y \lambda}{r_Y (n_I \omega - d_I)} (R_{0,1,b} - 1).\end{aligned}$$

The wild-type equilibrium exists if $R_{0,1,a} > 1$, and the mutant equilibrium exists if $R_{0,1,b} > 1$.

Theorem 3.2 *When the endemic equilibria exist ($R_{0,1,a} > 1$ and/or $R_{0,1,b} > 1$), the wild-type equilibrium is locally asymptotically stable and the mutant equilibrium is unstable.*

Proof For the wild-type equilibrium, $\bar{V}_Y = 0$, the characteristic polynomial is

$$\begin{aligned}0 &= \det(J(\bar{V}_I, \bar{V}_Y, \bar{V}_N, \bar{T}_S, \bar{T}_I, \bar{T}_Y) - \mu I_6) \\ &= (d_V + \mu) [\mu^2 + a_1 \mu + b_1] [\mu^3 + a_2 \mu^2 + b_2 \mu + c_2],\end{aligned}$$

where

$$\begin{aligned}a_1 &= d_V + r_Y \bar{T}_S + d_I \\ b_1 &= \frac{d_I d_V (r_I - r_Y)}{r_I} \\ a_2 &= r_I \bar{V}_I + d_S + d_I + d_V + r_I \bar{T}_S \\ b_2 &= (d_V + r_I \bar{T}_S) d_S + (r_I \bar{V}_I + d_S) d_I + d_V r_I \bar{V}_I \\ c_2 &= (d_I d_V + r_I \bar{T}_S d_I - n_I \omega r_I \bar{T}_S) (r_I \bar{V}_I + d_S) - r_I \bar{T}_S (r_I \bar{V}_I d_I - r_I \bar{V}_I n_I \omega) \\ &= d_I (d_S d_V + r_I \lambda) (R_{0,1,a} - 1).\end{aligned}$$

Since $r_I > r_Y$, we have $a_1, b_1 > 0$ meaning the quadratic equation $\mu^2 + a_1 \mu + b_1$ has no roots with non-negative real parts. We have $a_2, b_2, c_2 > 0$ and $a_2 b_2 - c_2 > 0$ if $R_{0,1,a} > 1$. By the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006), the Jacobian matrix has all eigenvalues with negative real part if $R_{0,1} > 1$, meaning the wild-type equilibrium is locally asymptotically stable whenever it exists.

For the mutant equilibrium, $\bar{V}_I = 0$, the characteristic polynomial is

$$\begin{aligned} 0 &= \det(J(\bar{V}_I, \bar{V}_Y, \bar{V}_N, \bar{T}_S, \bar{T}_I, \bar{T}_Y) - \mu I_6) \\ &= (d_V + \mu)[\mu^2 + a_1\mu + b_1][\mu^3 + a_2\mu^2 + b_2\mu + c_2], \end{aligned}$$

where

$$a_1 = d_V + r_I \bar{T}_S + d_I$$

$$b_1 = \frac{d_I d_V (r_Y - r_I)}{r_Y}$$

$$a_2 = r_Y \bar{V}_Y + d_S + d_I + d_V + r_Y \bar{T}_S$$

$$b_2 = (d_V + r_Y \bar{T}_S) d_S + (r_Y \bar{V}_Y + d_S) d_I + d_V r_Y \bar{V}_Y$$

$$\begin{aligned} c_2 &= (d_I d_V + r_Y \bar{T}_S d_I - n_Y \omega r_Y \bar{T}_S) (r_Y \bar{V}_Y + d_S) - r_Y \bar{T}_S (r_Y \bar{V}_Y d_I - r_Y \bar{V}_Y n_Y \omega) \\ &= (d_S d_V d_I + r_Y \lambda) (R_{0,1,b} - 1). \end{aligned}$$

Since the mutant equilibrium has the same characteristic polynomial as the wild-type equilibrium, except that r_I and \bar{V}_I are interchanged with r_Y and \bar{V}_Y , we have $a_1 > 0$ and $b_1 < 0$ since $r_Y - r_I < 0$. By the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006), the Jacobian matrix has an eigenvalue with positive real part if $R_{0,1} > 1$, meaning the mutant equilibrium is unstable. \square

We can also show that no interior equilibria exist for realistic parameters in Region 1. We assumed that $r_I > r_Y$ since the mutant virus is less infectious.

Theorem 3.3 *For system (3), if $r_I \neq r_Y$, there are no interior equilibria.*

Proof Let $r_I \neq r_Y$. For an interior equilibrium, we have $V_I \neq 0$ and $V_Y \neq 0$. Setting the right-hand side of system (3) to zero, we get, from the fifth and sixth equations of system (3),

$$T_I = \frac{r_I}{d_I} T_S V_I \quad (9)$$

$$T_Y = \frac{r_Y}{d_I} T_S V_Y. \quad (10)$$

Setting the first equation of system (3) equal to zero, and substituting T_I as in Eq. (9), we get

$$r_I T_S V_I \frac{n_I \omega - d_I}{d_I} - d_V V_I = 0.$$

Since $V_I \neq 0$, we have

$$T_S = \frac{d_V d_I}{r_I (n_I \omega - d_I)}. \quad (11)$$

Similarly, setting the second equation of system (3) equal to zero, and substituting T_Y as in Eq. (10), we get

$$T_S = \frac{d_V d_I}{r_Y(n_I \omega - d_I)}, \quad (12)$$

since $V_Y \neq 0$.

This implies that $r_I = r_Y$, which is a contradiction. \square

In summary, if $R_{0,1} < 1$, the disease-free equilibrium is locally asymptotically stable in Region 1, and if $R_{0,1} > 1$, the wild-type equilibrium is locally asymptotically stable and the disease-free and mutant equilibria are unstable in Region 1. Also, in Region 1, there are no interior equilibria.

3.1.2 Region 2: Intermediate Drug Levels

In this case, the drug concentration level affects the outcome of the system. We denote the equilibrium solutions by \bar{X} as those not affected by the drug dynamics (Region 1), and denote the equilibria by X^* as those that are affected by the drug dynamics (Regions 2 and 3). System (4) has four equilibria. In all cases, we fix P^* constant such that $P_1 < P^* < P_2$, where P_1 and P_2 are the Region 1 and Region 2 thresholds, respectively.

Disease-Free Equilibrium The disease-free equilibrium is

$$(V_I, V_Y, V_N, T_S, T_I, T_Y, T_{PN}, T_{PI}, T_{PY}, P) = (0, 0, 0, T_S^*, 0, 0, T_{PN}^*, 0, 0, P^*),$$

where

$$T_S^* = \frac{\lambda}{d_S + r_P P^*} + \frac{m_P T_{PN}^*}{d_S + r_P P^*}$$

$$T_{PN}^* = \frac{\lambda r_P P^*}{d_S(m_P + d_S) + d_S r_P P^*}.$$

The basic reproductive number in Region 2, $R_{0,2}$, is computed as before using the next-generation method and is given by

$$R_{0,2} = \max\{R_{0,2,a}, R_{0,2,b}\},$$

where

$$R_{0,2,a} = \frac{n_I \omega (r_Y T_S^* + r_Y T_{PN}^*)}{d_I (d_V + r_Y T_S^* + r_Y T_{PN}^*)}$$

$$R_{0,2,b} = \frac{n_I \omega (m_P (r_I T_S^* + r_I T_{PN}^*) + d_I r_I T_S^*)}{d_I (d_I + r_P P^* + m_P) (r_I T_S^* + r_I T_{PN}^* + d_V)}.$$

Theorem 3.4 *The disease-free equilibrium is locally asymptotically stable in Region 2 if $R_{0,2} < 1$ and unstable if $R_{0,2} > 1$.*

Proof The Jacobian matrix for Region 2 is $J = [J_1|J_2]$, where

$$J_1 = \begin{bmatrix} -d_V - r_I T_S - r_I T_{PN} & 0 & 0 & -r_I V_I \\ 0 & -d_V - r_Y T_S - r_Y T_{PN} & 0 & -r_Y V_Y \\ 0 & 0 & -d_V & 0 \\ -r_I T_S & -r_Y T_S & 0 & -r_I V_I - r_Y V_Y - d_S - r_P P \\ r_I T_S & 0 & 0 & r_I V_I \\ 0 & r_Y T_S & 0 & r_Y V_Y \\ -r_I T_{PN} & -r_Y T_{PN} & 0 & r_P P \\ r_I T_{PN} & 0 & 0 & 0 \\ 0 & r_Y T_{PN} & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

$$J_2 = \begin{bmatrix} n_I \omega & 0 & -r_I V_I & 0 & 0 & 0 \\ 0 & n_I \omega & -r_Y V_Y & 0 & n_I \omega & 0 \\ n_I(1 - \omega) & n_I(1 - \omega) & 0 & n_I & n_I(1 - \omega) & 0 \\ 0 & 0 & m_P & 0 & 0 & -r_P T_S \\ -d_I - r_P P & 0 & 0 & m_P & 0 & -r_P T_I \\ 0 & -d_I - r_P P & 0 & 0 & m_P & -r_P T_Y \\ 0 & 0 & -m_P - r_Y V_Y - r_I V_I - d_S & 0 & 0 & r_P T_S \\ r_P P & 0 & r_I V_I & -m_P - d_I & 0 & r_P T_I \\ 0 & r_P P & r_Y V_Y & 0 & -m_P - d_I & r_P T_Y \\ 0 & 0 & 0 & 0 & 0 & -d_P \end{bmatrix}.$$

For the disease-free equilibrium, $V_I^* = V_Y^* = 0$, so the characteristic polynomial is

$$0 = \det(J(V_I^*, V_Y^*, V_N^*, T_S^*, T_I^*, T_Y^*, T_{PN}^*, T_{PI}^*, T_{PY}^*, P^*) - \mu I_{10}) \\ = (d_V + \mu)(d_P + \mu)[\mu^3 + a_1\mu^2 + b_1\mu + c_1]f(\mu),$$

where

$$a_1 = m_P + d_I + d_V + r_I T_S^* + r_I T_{PN}^* + d_I + r_P P^* \\ b_1 = (d_I + r_P P^*)(d_V + r_I T_S^* + r_I T_{PN}^*) \\ + (m_P + d_I)(d_V + r_I T_S^* + r_I T_{PN}^* + d_I + r_P P^*) - m_P r_P P^* - n_I \omega r_I T_S^* \\ c_1 = d_I(r_I T_{PN}^* + r_I T_S^* + d_V)(d_I + r_P P^* + m_P)(1 - R_{0,2,b})$$

and where

$$f(\mu) = \det \begin{bmatrix} -d_V - r_Y T_S - r_Y T_{PN} & 0 & n_I \omega & 0 & n_I \omega \\ -r_Y T_S & -d_S - r_P P & 0 & m_P & 0 \\ r_Y T_S & 0 & -d_I - r_P P^* & 0 & m_P \\ -r_Y T_{PN} & r_P P & 0 & -m_P - d_S & 0 \\ r_Y T_{PN} & 0 & r_P P & 0 & -m_P - d_I \end{bmatrix}.$$

The third-order polynomial $\mu^3 + a_1\mu^2 + b_1\mu + c_1$ has $a_1, c_1 > 0$ and $a_1 b_1 - c_1 > 0$ if $R_{0,2,b} < 1$. Thus, by the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006), the third-order polynomial has all eigenvalues with negative real part.

Computing the determinant of $f(\mu)$, we get

$$\det[f(\mu)] = [\mu^2 + a_2\mu + b_2][\mu^3 + a_3\mu^2 + b_3\mu + c_3],$$

where

$$a_2 = 2d_S + r_P P^* + m_P$$

$$b_2 = d_S r_P P^* + d_S(m_P + d_S)$$

$$a_3 = r_P P^* + 2d_I + d_V + r_Y T_S^* + r_Y T_{PN}^* + m_P$$

$$b_3 = -m_P r_P P^* - n_I \omega r_Y (T_S^* + T_{PN}^*) + (r_P P^* + d_I)(d_V + r_Y T_S^* + r_Y T_{PN}^*)$$

$$+ (m_P + d_I)(r_P P^* + d_I + d_V + r_Y T_S^* + r_Y T_{PN}^*)$$

$$c_3 = (d_V + r_Y T_S^* + r_Y T_{PN}^*)[-m_P r_P P^* + (m_P + d_I)(d_I + r_P P^*)]$$

$$- n_I \omega [r_Y T_S^*(m_P + d_I) + m_P r_Y T_{PN}^* + r_Y T_S^* r_P P^* + r_Y T_{PN}^*(d_I + r_P P^*)]$$

$$= d_I(r_Y T_{PN}^* + r_Y T_S^* + d_V)(d_I + r_P P^* + m_P)(1 - R_{0,2,a}).$$

We have $a_3, c_3 > 0$ and $a_3 b_3 - c_3 > 0$ if $R_{0,2,b} < 1$. Hence, by the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006), the Jacobian matrix has all eigenvalues with negative real part meaning the disease-free equilibrium is locally asymptotically stable when $R_{0,2} < 1$ and unstable when $R_{0,2} > 1$. \square

Endemic Equilibria The wild-type equilibrium is

$$(V_I, V_Y, V_N, T_S, T_I, T_Y, T_{PN}, T_{PI}, T_{PY}, P) \\ = (V_I^*, 0, V_N^*, T_S^*, T_I^*, 0, T_{PN}^*, T_{PI}^*, 0, P^*),$$

where V_I^* is the positive root of the quadratic equation

$$\frac{\alpha_1}{\xi_1} V_I^{*2} + \frac{\beta_1}{\xi_1} V_I^* + \gamma_1 = 0,$$

for

$$\xi_1 = \frac{d_V m_P (r_P P^* + d_I)}{(r_P P^* + d_I)[(m_P - d_I)(r_P P^* + d_I) - r_P P^* + d_I]}$$

$$\alpha_1 = r_I^2 (d_I + r_P P^*)$$

$$\beta_1 = r_I (d_I + r_P P^*) (m_P + d_S - m_P r_P P^*) + r_I (r_I \lambda - d_V r_I \lambda)$$

$$+ (m_P + r_P P^*) (d_I + r_P P^*) - r_I^2 \xi_1 r_P P^* \lambda$$

$$\gamma_1 = r_I r_P P^* \lambda \left(\frac{1}{\xi_1} - m_P - d_S + r_P P^* + d_I \right)$$

$$+ \frac{m_P + r_P P^*}{\xi_1} [r_I \lambda - d_V r_I \lambda + (d_I + r_P P^*) (m_P + d_S - m_P r_P P^*)]$$

and where

$$\begin{aligned}
 V_N^* &= \frac{n_I(1-\omega)T_I^* + n_I T_{PI}^*}{d_V} \\
 T_S^* &= \frac{\lambda + m_P T_{PN}^*}{r_I V_I^* + d_S + r_P P^*} \\
 T_I^* &= \frac{d_V V_I^* + r_I T_S^* V_I^* + r_I T_{PN}^* V_I^*}{n_I \omega} \\
 T_{PN}^* &= \frac{r_P T_S^* P^*}{m_P + r_I V_I^* + d_S} \\
 T_{PI}^* &= \frac{r_P P^* T_I^* + r_I T_{PN}^* V_I^*}{m_P + d_I}.
 \end{aligned}$$

The mutant equilibrium is

$$\begin{aligned}
 (V_I, V_Y, V_N, T_S, T_I, T_Y, T_{PN}, T_{PI}, T_{PY}, P) \\
 = (0, V_Y^*, V_N^*, T_S^*, 0, T_I^*, T_{PN}^*, 0, T_{PY}^*, P^*),
 \end{aligned}$$

where V_Y^* is the positive root of the quadratic equation

$$\frac{\alpha_2}{\xi_2} V_Y^{*2} + \frac{\beta_2}{\xi_2} V_Y^* + \gamma_2 = 0,$$

for

$$\begin{aligned}
 \xi_2 &= \frac{(d_I + r_P P^*)(m_P + d_I) + m_P r_P P^*}{n_I \omega (d_I + r_P P^* + m_P)(m_P + d_I)} \\
 \alpha_2 &= r_Y \\
 \beta_2 &= d_S + r_P P^* - \xi r_Y (m_P + d_I) \\
 \gamma_2 &= -\xi d_V (m_P + d_I) (d_S + r_P P^*) - r_Y \lambda (m_P + d_I) + r_Y \lambda
 \end{aligned}$$

and where

$$\begin{aligned}
 V_N^* &= \frac{n_I(1-\omega)T_Y^* + n_I(1-\omega)T_{PY}^*}{d_V} \\
 T_S^* &= \frac{\lambda + m_P T_{PN}^*}{r_Y V_Y^* + d_S + r_P P^*} \\
 T_Y^* &= \frac{r_Y V_Y^* T_S^* + m_P T_{PY}^*}{d_I + r_P P^*} \\
 T_{PN}^* &= \frac{r_P P^* T_S^*}{d_S + r_Y V_Y^* + m_P} \\
 T_{PY}^* &= \frac{r_P T_Y^* P^* + r_Y T_{PN}^* V_Y^*}{m_P + d_I}.
 \end{aligned}$$

The interior equilibrium is

$$(V_I, V_Y, V_N, T_S, T_I, T_Y, T_{PN}, T_{PI}, T_{PY}, P) \\ = (V_I^*, V_Y^*, V_N^*, T_S^*, T_I^*, T_Y^*, T_{PN}^*, T_{PI}^*, T_{PY}^*, P^*),$$

where V_Y^* is the positive root of the quadratic equation

$$\delta_1 V_Y^{*2} + \delta_2 V_Y^* + (\alpha_1 + \alpha_2) = 0,$$

for

$$\begin{aligned} \alpha_1 &= r_Y \lambda \left[\left(n_I \omega - (d_I + r_P P^*) + \eta_1 r_P P^* \right) \left(m_P + d_S + \frac{\eta_3 - \eta_2}{a - b} \right) \right. \\ &\quad \left. + r_P P^* \eta_1 (r_P P^* + 1) (d_I + r_P P^*) \right] \\ \alpha_2 &= (\eta_1 + r_P P^* d_V - d_V (d_I + r_P P^*)) \left[\left(\frac{\eta_3 - \eta_2}{a - b} + d_S + r_P P^* \right) \right. \\ &\quad \times \left. \left(m_P + \frac{\eta_3 - \eta_2}{a - b} + d_S \right) - r_P P^* m_P \right] \\ \delta_1 &= r_Y^2 \left(\frac{2b}{b - a} \right)^2 (\eta_1 + r_P P^* d_V - d_V (d_I + r_P P^*)) \\ \delta_2 &= r_Y^2 \lambda \left(\frac{2a}{a - b} \right)^2 (\eta_1 r_P P^* - r_P P^* - d_I + n_I \omega) \\ \eta_1 &= \frac{n_I \omega m_P + n_I \omega (d_I + r_P P^*)}{m + P + d_I + n_I \omega r_P P^*} \\ \eta_2 &= \frac{r_Y}{\eta_1 r_P P^* d_V - d_V (d_I + r_P P^*)} \left[r_P P^* (\eta_1 (r_P P^* + 1) (d_I + r_P P^*)) \right. \\ &\quad \left. + (n_I \omega - (d_I + r_P P^*) + \eta_1 r_P P^*) (m_P + d_S) \right] \\ \eta_3 &= \frac{r_I (m_P + d_I)}{d_V (m_P + d_I) - r_P P^* d_V} \left[r_P P^* \left(\frac{r_P P^* + n_I \omega}{m_P + d_I} - (d_I + r_P P^*) \right) \right. \\ &\quad \left. + \left(n_I \omega - (d_I + r_P P^*) + \frac{r_P P^*}{m_P + d_I} \right) (m_P + d_S) \right] \\ a &= \frac{r_Y}{\eta_1 r_P P^* d_V - d_V (d_I + r_P P^*)} [n_I \omega - d_I - r_P P^* - \eta_1 r_P P^*] \\ b &= \frac{r_I (m_P + d_I)}{r_P P^* d_V - d_V (m_P + d_I)} \left[n_I \omega - d_I - r_P P^* + \frac{r_P P^*}{m_P + d_I} \right], \end{aligned}$$

and where

$$V_I^* = \frac{n_I \omega T_I^*}{d_V + r_I T_S^* + r_I T_{PN}^*}$$

$$\begin{aligned}
V_N^* &= \frac{n_I(1-\omega)(T_I^* + T_Y^* + T_{PY}^*) + n_I T_{PI}^*}{d_V} \\
T_S^* &= \frac{\lambda(m_P + r_Y V_Y^* + r_I V_I^* + d_S)}{(r_Y V_Y^* + r_I V_I^* + d_S + r_P P^*)(r_Y V_Y^* + r_I V_I^* + d_S) + m_P(r_Y V_Y^* + r_I V_I^* + d_S)} \\
T_I^* &= \frac{V_I^*(d_V + r_I T_S^* + r_I T_{PN}^*)}{n_I \omega} \\
T_Y^* &= \frac{r_Y V_Y^* T_S^* + m_P T_{PY}^*}{d_I + r_P P^*} \\
T_{PN}^* &= \frac{r_P P^* T_S^*}{m_P + r_Y V_Y^* + r_I V_I^* + d_S} \\
T_{PI}^* &= \frac{r_P P^* T_I + r_I T_{PN}^* V_I^*}{m_P + d_I} \\
T_{PY}^* &= \frac{r_P P^* V_Y^*(d_V + r_Y T_S^* + r_Y T_{PN}^*) + r_Y T_{PN}^* V_Y^*}{m_P + d_I + n_I \omega r_P P^*}.
\end{aligned}$$

Theorem 3.5 When the endemic equilibria exist and we have that $R_{0,2,a} > 1$ and $R_{0,2,b} > 1$, the wild-type and resistant strains will co-exist in Region 2.

Proof For the wild-type equilibrium, $V_Y^* = 0$, the characteristic polynomial is

$$\begin{aligned}
0 &= \det(J(V_I^*, V_Y^*, V_N^*, T_S^*, T_I^*, T_Y^*, T_{PN}^*, T_{PI}^*, T_{PY}^*, P^*) - \mu I_{10}) \\
&= (-d_V - \mu)(-d_P - \mu)[\mu^3 + a\mu^2 + b\mu + c]f(\mu),
\end{aligned}$$

where

$$f(\mu) = \det \begin{bmatrix} -d_V - r_I T_S^* - r_I T_{PN}^* & -r_I V_I^* & n_I \omega \\ -r_I T_S^* & -r_I V_I^* - d_S - r_P P^* & 0 \\ r_I T_S^* & r_I V_I^* & -d_I - r_P P^* \\ -r_I T_{PN}^* & r_P P^* & 0 \\ r_I T_{PN}^* & 0 & r_P P^* \\ & -r_I V_I^* & 0 \\ & m_P & 0 \\ & 0 & m_P \\ -m_P - d_S - r_I V_I^* & 0 & \\ r_I V_I^* & -m_P - d_I & \end{bmatrix}$$

and where

$$\begin{aligned}
a &= 2d_I + r_P P^* + m_P + d_V + r_Y T_S^* + r_Y T_{PN}^* \\
b &= (d_V + r_Y T_S^* + r_Y T_{PN}^*)[(m_P + d_I) + (d_I + r_P P^*)] + (d_I + r_P P^*)(m_P + d_I) \\
&\quad - r_Y T_S^* n_I \omega + m_P r_P P^* - r_Y T_{PN}^* n_I \omega \\
c &= -n_I \omega r_Y T_S^* (m_P + d_I) + (d_V + r_Y T_S^* + r_Y T_{PN}^*)(m_P d_I + d_I^2 + d_I r_P P^*)
\end{aligned}$$

$$\begin{aligned}
& -r_P P^* n_I \omega r_Y T_S^* - r_Y T_{PN}^* n_I \omega m_P - r_Y T_{PN}^* n_I \omega (d_I + r_P P^*) \\
& = d_I (r_Y T_{PN}^* + r_Y T_S^* + d_V) (d_I + r_P P^* + m_P) (1 - R_{0,2,a}).
\end{aligned}$$

We have $a > 0$ and $c < 0$ if $R_{0,2,a} > 1$. Hence, by the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006), the Jacobian matrix has an eigenvalue with a positive real part and thus the wild-type equilibrium is unstable.

For the mutant equilibrium, $V_I^* = 0$, the characteristic polynomial is

$$\begin{aligned}
0 & = \det(J(V_I^*, V_Y^*, V_N^*, T_S^*, T_I^*, T_Y^*, T_{PN}^*, T_{PI}^*, T_{PY}^*, P^*)) - \mu I_{10} \\
& = (-d_V - \mu)(-d_P - \mu)[\mu^3 + a\mu^2 + b\mu + c]f(\mu),
\end{aligned}$$

where

$$f(\mu) = \det \begin{bmatrix} -d_V - r_I T_S - r_Y T_{PN} & -r_Y V_Y & n_I \omega \\ -r_Y T_S & -r_Y V_Y - d_S - r_P P & 0 \\ r_Y T_S & r_Y V_Y & -d_I - r_P P \\ -r_Y T_{PN} & r_P P & 0 \\ r_Y T_{PN} & 0 & r_P P \\ & -r_Y V_Y & n_I \omega \\ & m_P & 0 \\ & 0 & m_P \\ m_P - d_S - r_Y V_Y & 0 & \\ r_Y V_Y & -m_P - d_I & \end{bmatrix}$$

and where

$$\begin{aligned}
a & = 2d_I + r_P P^* + m_P + d_V + r_I T_S^* + r_I T_{PN}^* \\
b & = (d_V + r_I T_S^* + r_I T_{PN}^*)[(m_P + d_I) + (d_I + r_P P^*)] + (d_I + r_P P^*)(m_P + d_I) \\
& \quad - r_I T_S^* n_I \omega - m_P r_P P^* \\
c & = -n_I \omega r_I T_S^* (m_P + d_I) + (d_V + r_I T_S^* + r_I T_{PN}^*)(m_P + d_I)(d_I + r_P P^*) \\
& \quad - r_P P^* m_P (d_V + r_I T_S^* + r_I T_{PN}^*) - m_P n_I \omega r_I T_{PN}^* \\
& = d_I (r_I T_{PN}^* + r_I T_S^* + d_V) (d_I + r_P P^* + m_P) (1 - R_{0,2,b}).
\end{aligned}$$

In this case, $a > 0$ and $c < 0$ if $R_{0,2,b} > 1$. It follows from the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006) that the wild-type equilibrium has an eigenvalue with a positive real part and is thus unstable.

Since the disease-free, wild-type and mutant equilibria are all locally unstable, this implies that the wild-type and resistant strains will co-exist. \square

In summary, if $R_{0,2} < 1$, the disease-free equilibrium is locally asymptotically stable in Region 2, and if $R_{0,2} > 1$, the wild-type and resistant strains will co-exist in Region 2. However, it should be noted that it is not necessarily the interior orbit that trajectories approach. There may be other interior periodic orbits or more complex

behaviour in which both strains co-exist. Nevertheless, in this region, the mutant is not eliminated.

3.1.3 Region 3: High Drug Levels

If $r_P \geq r_{PP}$, system (5) has three equilibria: disease free (extinction of virus and infected cells), wild type (extinction of mutant) and mutant (extinction of wild type). In all three cases, there is a P^* such that $P^* > P_2$. If $r_P < r_{PP}$, then there is also an interior equilibrium, but we expect from the dose-effect curves that this will not be the case (Bilello et al. 1996).

Disease-Free Equilibrium The disease-free equilibrium is

$$(V_I, V_Y, V_N, T_S, T_I, T_Y, T_{PN}, T_{PI}, T_{PY}, T_{PPN}, T_{PPI}, T_{PPY}, P) \\ = (0, 0, 0, T_S^*, 0, 0, T_{PN}^*, 0, 0, T_{PPN}^*, 0, 0, P^*),$$

where

$$T_S^* = \frac{\lambda + m_P T_{PN}^*}{d_S + r_P P^*} \\ T_{PN}^* = \frac{r_P P^* T_S^* + m_{PP} T_{PPN}^*}{m_P + d_S + r_{PP} P^*} \\ T_{PPN}^* = \frac{r_{PP} P^* T_{PN}^*}{m_{PP} + d_S}.$$

The basic reproductive number in Region 3, $R_{0,3}$, is computed as before using the next-generation method and is given by

$$R_{0,3} = \max\{R_{0,3,a}, R_{0,3,b}\} \quad (13)$$

where

$$R_{0,3,a} = n_I \omega \left((d_I + r_P P^* + m_P) (m_{PP} (r_Y T_S^* + r_Y T_{PN}^* + r_Y T_{PPN}^*) \right. \\ \left. + d_I (r_Y T_S^* + r_Y T_{PN}^*)) + r_Y T_S^* d_I r_{PP} P^* \right) \\ \times (d_I (d_V + r_Y T_S^* + r_Y T_{PN}^* + r_Y T_{PPN}^*) (d_I (d_I + r_P P^* \\ + m_P + r_{PP} P^* + m_{PP}) + m_P m_{PP} + r_P P^* m_{PP} + r_P P^* r_{PP} P^*))^{-1}$$

$$R_{0,3,b} = n_I \omega \left((d_I + m_P) (m_P (r_I T_S^* + r_I T_{PN}^*) + d_I r_I T_S^*) \right. \\ \left. + d_I r_{PP} P^* r_I T_S^* + m_P m_{PP} r_I T_{PPN}^* \right) \\ \times (d_I (d_V + r_I T_S^* + r_I T_{PN}^* + r_I T_{PPN}^*) (d_I (d_I + r_P P^* \\ + m_P + r_{PP} P^* + m_{PP}) + m_P m_{PP} + r_P P^* m_{PP} + r_P P^* r_{PP} P^*))^{-1}.$$

The Jacobian matrix for Region 3 is $J = [J_1|J_2|J_3|J_4|J_5]$, where

$$\begin{aligned}
J_1 = & \begin{bmatrix} -d_V - r_I T_S - r_I T_{PN} - r_I T_{PPN} & 0 \\ 0 & -d_V - r_Y T_S - r_Y T_{PN} - r_Y T_{PPN} \\ 0 & 0 \\ -r_I T_S & -r_Y T_S \\ r_I T_S & 0 \\ 0 & r_Y T_S \\ -r_I T_{PN} & -r_Y T_{PN} \\ r_I T_{PN} & 0 \\ 0 & r_Y T_{PN} \\ r_I T_{PPN} & 0 \\ 0 & r_Y T_{PPN} \\ 0 & 0 \end{bmatrix} \\
J_2 = & \begin{bmatrix} 0 & -r_I V_I & n_I \omega & 0 \\ 0 & -r_Y V_Y & 0 & n_I \omega \\ -d_V & 0 & n_I(1 - \omega) & n_I(1 - \omega) \\ 0 & -r_I V_I - r_Y V_Y - d_S - r_P P & 0 & 0 \\ 0 & r_I V_I & -d_I - r_P P & 0 \\ 0 & r_Y V_Y & 0 & -d_I - r_P P \\ 0 & r_P P & 0 & 0 \\ 0 & 0 & r_P P & 0 \\ 0 & 0 & 0 & r_P P \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix} \\
J_3 = & \begin{bmatrix} -r_I V_I & 0 \\ -r_Y V_Y & 0 \\ 0 & n_I \\ m_P & 0 \\ 0 & m_P \\ 0 & 0 \\ -m_P - r_Y V_Y - r_I V_I - d_S - r_{PP} P & 0 \\ r_I V_I & -m_P - d_I - r_{PP} P \\ r_Y V_Y & 0 \\ r_{PP} P & 0 \\ 0 & r_{PP} P \\ 0 & 0 \\ 0 & 0 \end{bmatrix}
\end{aligned}$$

$$J_4 = \begin{bmatrix} 0 & -r_I V_I & 0 \\ n_I \omega & -r_Y V_Y & 0 \\ n_I(1-\omega) & 0 & n_I \\ 0 & 0 & 0 \\ 0 & 0 & 0 \\ m_P & 0 & 0 \\ 0 & m_{PP} & 0 \\ 0 & 0 & m_{PP} \\ -m_P - d_I - r_{PP} P & 0 & 0 \\ 0 & -m_{PP} - r_I V_I - r_Y V_Y - d_S & 0 \\ 0 & r_I V_I & -m_{PP} - d_I \\ r_{PP} P & r_Y V_Y & 0 \\ 0 & 0 & 0 \end{bmatrix}$$

$$J_5 = \begin{bmatrix} 0 & 0 \\ 0 & 0 \\ n_I & 0 \\ 0 & -r_P T_S \\ 0 & -r_P T_I \\ 0 & -r_P T_Y \\ 0 & r_P T_S - r_{PP} T_{PN} \\ 0 & r_P T_I - r_{PP} T_{PI} \\ m_{PP} & r_P T_Y - r_{PP} T_{PY} \\ 0 & r_{PP} T_{PN} \\ 0 & r_{PP} T_{PI} \\ -m_{PP} - d_I & r_{PP} T_{PY} \\ 0 & -d_P \end{bmatrix}.$$

The Region of Viral Elimination We now investigate the conditions under which the disease-free equilibrium becomes stable. We will consider a subset of Region 3, called Region 4, where P^* is sufficiently large so that the disease-free equilibrium is asymptotically stable. We shall refer to this subset of Region 3 as the region of viral elimination. Rearranging terms in T_{PN}^* from the disease-free equilibrium, we get

$$T_{PN}^* = \frac{\lambda r_P P^* (m_{PP} + d_S)}{(d_S^2 + d_S r_{PP} P^* + d_S m_{PP})(d_S + r_P P^*) + d_S m_P (m_{PP} + d_S)}.$$

If we divide the numerator and the denominator by P^{*2} and take $P^* \rightarrow \infty$, we get

$$T_{PN}^* \rightarrow 0$$

in Region 4. Since $T_{PN}^* \rightarrow 0$ and $P^* \rightarrow \infty$, this implies that $T_S^* \rightarrow 0$. Rearranging terms in T_{PPN}^* from the disease-free equilibrium, we get

$$T_{PPN}^* = \frac{\lambda r_P P^* r_P P^{*2}}{(d_S^2 + d_S r_{PP} P^* + d_S m_{PP})(d_S + r_P P^*) + d_S m_P (m_{PP} + d_S)}.$$

Again, if we divide the numerator and the denominator by P^{*2} and take $P^{*} \rightarrow \infty$, we get

$$T_{PPN}^* \rightarrow \frac{\lambda}{d_S}.$$

Thus, in Region 4, when $P^{*} \rightarrow \infty$, the disease-free equilibrium has $T_S^* \rightarrow 0$, $T_{PN}^* \rightarrow 0$ and $T_{PPN}^* \rightarrow \frac{\lambda}{d_S}$.

The basic reproductive number for Region 4 is such that $R_{0,3} < 1$. When $P^{*} \rightarrow \infty$, we see that $R_{0,3}$ defined by (13) equals zero meaning in the limit of $P^{*} \rightarrow \infty$, the basic reproductive number certainly drops below unity.

Theorem 3.6 *The disease-free equilibrium is locally asymptotically stable when $P^{*} \rightarrow \infty$.*

Proof Computing the Jacobian in Region 4 for the disease-free equilibrium, $V_I^* = V_Y^* = 0$, we get the characteristic polynomial

$$\begin{aligned} 0 &= \det(J(V_I^*, V_Y^*, V_N^*, T_S^*, T_I^*, T_Y^*, T_{PN}^*, T_{PI}^*, T_{PY}^*, T_{PPN}^*, T_{PPI}^*, T_{PPY}^*, P^*)) \\ &\quad - \mu I_{13}) \\ &= (-d_P - \mu)(-d_V - \mu)(\mu^7 + a_1\mu^6 + a_2\mu^3 + a_3\mu^4 + a_4\mu^3 + a_5\mu^2 \\ &\quad + a_6\mu + a_7)f(\mu) \end{aligned}$$

where

$$f(\mu) = \det \begin{bmatrix} -d_V - r_Y T_{PPN} - \mu & n_I \omega & 0 \\ 0 & -d_I - r_P P - \mu & 0 \\ 0 & r_P P & 0 \\ r_Y T_{PPN} & 0 & n_I \omega \\ & m_P & 0 \\ & -m_P - r_{PP} P - d_I - \mu & m_{PP} \\ & r_{PP} P & -m_{PP} - d_I - \mu \end{bmatrix}.$$

When taking $P^{*} \rightarrow \infty$, the seventh-order polynomial can be reduced to a third-order characteristic polynomial $\mu^3 + b_1\mu^2 + b_2\mu + b_3$, where

$$\begin{aligned} b_1 &= 2m_{PP} + d_S + d_I + d_V + r_I T_{PPN}^* \\ b_2 &= m_{PP}(d_S + d_V + r_I T_{PPN}^*) + (d_I + m_{PP})(d_V + r_I T_{PPN}^*) \\ &\quad + d_S(d_I + m_{PP} + d_V + r_I T_{PPN}^*) \\ b_3 &= m_{PP}d_S(d_V + r_I T_{PPN}^*) + d_S(d_I + m_{PP})(d_V + r_I T_{PPN}^*). \end{aligned}$$

By the Routh–Hurwitz conditions (Truccolo et al. 2003; Allen 2006), the third-order polynomial has all roots with negative real part if $b_1, b_3 > 0$ and $b_1 b_2 > b_3$. The

Routh–Hurwitz conditions are always satisfied when $P^* \rightarrow \infty$, meaning the third-order polynomial has all eigenvalues with a negative real part.

Computing the determinant of $f(\mu)$ and taking $P^* \rightarrow \infty$, the fourth-order polynomial can be reduced to a second-order characteristic polynomial $\mu^2 + a\mu + b$, where

$$a = d_V + r_Y T_{PPN}^* + d_I$$

$$b = (d_V + r_Y T_{PPN}^*)d_I.$$

By the Routh–Hurwitz condition (Truccolo et al. 2003; Allen 2006), $f(\mu)$ has all roots with negative real part. Therefore, the disease-free equilibrium is locally asymptotically stable in Region 4 for $P^* \rightarrow \infty$. \square

Note that elimination of free virus for this model is not equivalent to clearing the infection. See the Discussion for more details.

It can also be shown that the wild-type equilibrium in Region 4 gives

$$V_I^* = -\left(\frac{r_I \lambda + d_V d_S}{d_V r_I}\right)$$

$$V_N^* = \frac{n_I(1-\omega)T_I^* + n_I(T_{PI}^* + T_{PPI}^*)}{d_V}$$

$$T_S^* = \frac{\lambda + m_P T_{PN}^*}{r_I V_I^* + d_S + r_P P^*}$$

$$T_{PN}^* = \frac{r_P P^* T_S^* + m_{PP} T_{PPN}^*}{m_P + r_I V_I^* + d_S + r_{PP} P^*}$$

$$T_{PPN}^* = \frac{r_{PP} P^* T_{PN}^*}{m_{PP} + r_I V_I^* + d_S}$$

$$T_I^* = \frac{r_I V_I^* T_S^* + m_P T_{PI}^*}{d_I + r_P P^*}$$

$$T_{PI}^* = \frac{r_I V_I^* T_{PN}^* + r_P P^* T_I^* + m_{PP} T_{PPI}^*}{m_P + r_{PP} P^* + d_I}$$

$$T_{PPI}^* = \frac{r_I V_I^* T_{PPN}^* + r_{PP} P^* T_{PI}^*}{m_{PP} + d_I}$$

and the drug-resistant equilibrium in Region 4 gives

$$V_Y^* = -\left(\frac{r_Y \lambda (r_{PP} + d_S(r_P + r_{PP})) + d_S d_V r_{PP}}{r_Y^2 \lambda (r_P + r_{PP}) + d_V r_Y r_{PP}}\right)$$

$$V_N^* = \frac{n_I(1-\omega)(T_Y^* + T_{PY}^*) + n_I T_{PPY}^*}{d_V}$$

$$T_S^* = \frac{\lambda + m_P T_{PN}^*}{r_Y V_Y^* + d_S + r_P P^*}$$

$$\begin{aligned}
T_{PN}^* &= \frac{r_P P^* T_S^* + m_{PP} T_{PPN}^*}{m_P + r_Y V_Y^* + d_S + r_{PP} P^*} \\
T_{PPN}^* &= \frac{r_{PP} P^* T_{PN}^*}{m_{PP} + r_Y V_Y^* + d_S} \\
T_Y^* &= \frac{r_Y V_Y^* T_S^* + m_P T_{PY}^*}{d_I + r_P P^*} \\
T_{PY}^* &= \frac{r_Y V_Y^* T_{PN}^* + r_P P^* T_Y^* + m_{PP} T_{PPY}^*}{m_P + r_{PP} P^* + d_I} \\
T_{PPY}^* &= \frac{r_Y V_Y^* T_{PPN}^* + r_{PP} P^* T_{PY}^*}{m_{PP} + d_I}.
\end{aligned}$$

These are biologically meaningless in Region 4 since V_I^* and V_Y^* are negative.

In summary, if $P^* \rightarrow \infty$, the disease-free equilibrium is locally asymptotically stable and viral elimination is theoretically possible.

3.1.4 Summary of Asymptotic Behaviour

In summary for this section, we find that, at low drug levels (Region 1), resistance does not emerge and thus the wild-type strain dominates. In contrast, at intermediate drug levels (Region 2), drug resistance is guaranteed to emerge. Recall that we have defined intermediate drug levels as the regime in which the drugs significantly inhibit replication of the wild-type strain but have negligible effect on the drug-resistant strain.

For high drug levels (Regions 3), there exists a region (Region 4) where both populations of free virus will be driven to extinction. (We note that our model does not consider longer-lived reservoirs of virus, such as latent T cells, and thus elimination of a free virus in our model is not equivalent to clearing infection.) Thus, if the drug level is very large, virus elimination is theoretically possible.

3.2 Equilibrium T Cell Counts

In this section, we examine the total uninfected T cell count at the stable equilibrium predicted in low, intermediate and high drug concentrations.

For low drug levels ($P < P_1$), we know from Sect. 3.1.1 that the wild-type virus dominates. The total uninfected T cell count in Region 1 is

$$\bar{T}_S = \frac{d_I d_V}{r_I (n_I \omega - d_I)}.$$

Since n_I is large, \bar{T}_S is small. Thus there are low levels of uninfected T cells in Region 1.

For intermediate drug levels ($P_1 < P < P_2$), we use the results of Sect. 3.1.2 to show the total uninfected T cell count for the interior equilibrium is

$$T_S^* + T_{PN}^* = T_S^* + \frac{r_P P^* T_S^*}{m_P + d_S}$$

$$\begin{aligned}
&= T_S^* \left(1 + \frac{r_p P^*}{m_P + d_S} \right) \\
&= T_S^* (1 + \epsilon),
\end{aligned}$$

where ϵ is small since m_P is large compared to P^* in Region 2. For realistic parameters in the intermediate drug levels (see Table 1), the total number of uninfected T cells is only slightly larger than for low drug levels.

For high drug levels ($P > P_2$), we examine the effect on the total uninfected T cell count as the dosing interval shrinks to zero, or as the doses increase to infinity, which we have defined as Region 4. The total number of uninfected T cells in Region 4 approaches

$$T_S^* + T_{PN}^* + T_{PPN}^* \rightarrow \frac{\lambda}{d_S},$$

where $T_S^* \rightarrow 0$, $T_{PN}^* \rightarrow 0$ and $T_{PPN}^* \rightarrow \frac{\lambda}{d_S}$ when $P^* \rightarrow \infty$ (described in Sect. 3.1.3). Thus, the total number of uninfected T cells in Region 4 is identical to the disease-free state as $P^* \rightarrow \infty$. This implies that, if the drug levels are sufficiently high, the number of infected T cells approaches that of the uninfected patient.

Note that we do not explicitly have the total number of uninfected T cells in Region 3. Based on numerical simulations shown in the inset of Fig. 4c, the total uninfected T cell count is not as high as in Region 4.

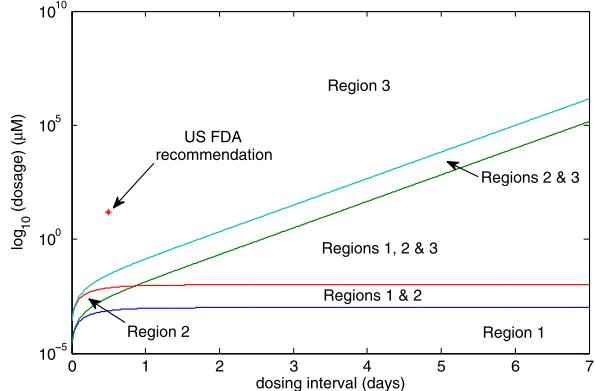
4 Including Impulses

We demonstrate the effects of varying the drug concentration levels by including an impulse (Eq. (7)). Adding this perturbation will cause drug concentration levels to fluctuate between regions. The endpoints of the impulsive periodic orbit are used in order to bound the orbit to stay within a region meaning the representative value P^* stays within a specific region. The endpoints are also used in order to show the effects of trajectories crossing multiple regions; this does not apply if P^* is constant.

4.1 Region Thresholds

We demonstrate the various T cell, virus, and drug behaviours given that the drug concentrations may move through all four regions. Based on the results of stability with P constant in Sect. 3, we expect that, when including the impulse condition and $P(t)$ is low, the wild-type strain of the virus should dominate. When $P(t)$ increases (attaining intermediate levels), the wild-type strain can co-exist with the mutant. When $P(t)$ becomes high, the mutant strain will dominate unless $P(t)$ is very high, where we expect viral elimination. Depending on the amount of time the drug spends in each region (if any), trajectories will likely oscillate, with either co-existence, one or the other strain gaining dominance, or the drugs eliminating both strains.

Fig. 3 The possible combinations of regions that drug concentrations may traverse, for given dosages and dosing intervals. All parameters can be found in Table 1. This example is for the protease inhibitor ritonavir. The asterisk is the FDA-approved dosage and dosing interval for ritonavir. Note the log scale on the y-axis



It was shown in Miron and Smith? (2010), assuming perfect adherence that the impulsive periodic orbit has endpoints

$$P(t_n^-) \rightarrow \frac{P^i e^{-d_P \tau}}{1 - e^{-d_P \tau}}$$

and

$$P(t_n^+) \rightarrow \frac{P^i}{1 - e^{-d_P \tau}},$$

as $n \rightarrow \infty$. Here P^i is the dosage, d_P is the rate at which the drug is cleared and $\tau = t_{k+1} - t_k$ is the (fixed) time between doses for perfect adherence.

It follows that trajectories will remain solely in Region 1 if

$$0 < P^i < P_1(1 - e^{-d_P \tau}).$$

Trajectories will remain solely in Region 2 if

$$P_1 e^{d_P \tau} (1 - e^{-d_P \tau}) < P^i < P_2 (1 - e^{-d_P \tau}).$$

Finally, trajectories will remain solely in Region 3 if

$$P^i > P_2 e^{d_P \tau} (1 - e^{-d_P \tau}).$$

4.2 Numerical Simulations

We now illustrate the dynamics of drug concentrations fluctuating between different regions. Figure 3 demonstrates the regions drug concentration trajectories will visit, for various combinations of dosing interval and dose. The curves plotted are

$$P^i = P_1 (1 - e^{-d_P \tau})$$

$$P^i = P_1 e^{d_P \tau} (1 - e^{-d_P \tau})$$

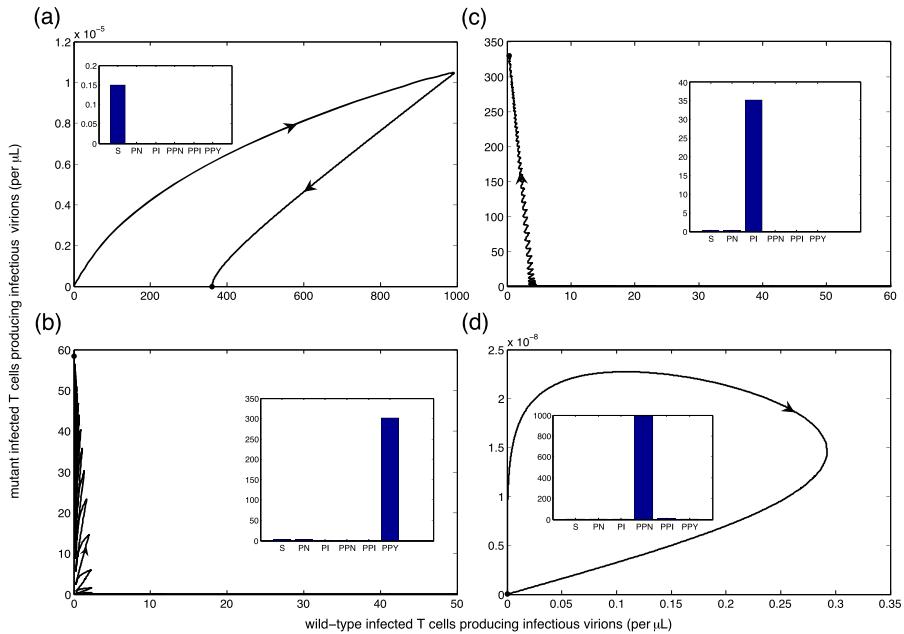


Fig. 4 Infected T cell populations when trajectories of drug concentration remain solely within a region. Values of T_I and T_Y were estimated by numerical integration of systems (3), (4) and (5). The *inset* shows the total number of uninfected T cells, and T cells unable to produce infectious virus. Note that the threshold for Region 2 is adjusted in (b) as mentioned in Sect. 4. (a) Region 1 ($\tau = 1$, $P^i = 10^{-4}$). In this case, there are no cells infected with the drug-resistant strain of the virus. T cells infected with the wild-type strain dominate, with all other T cells approaching zero. (b) Region 2 ($\tau = 0.1$, $P^i = 0.5$). In this case, both strains of the virus co-exist. There are much higher levels of mutant virus than wild-type virus strains. (c) Region 3, where the dosing intervals and dosages are not too extreme ($\tau = 0.1$, $P^i = 4$). In this case, there are large amounts of the drug-resistant strain, and there is a large population of infected T cells with the drug-resistant strain with high drug levels. (d) Region 4, the region of viral elimination ($\tau = 0.0001$, $P^i = 30$). In this case, both strains of the virus are eliminated. Uninfected T cells with the drug-resistant strain with high drug levels dominate, with all other T cells approaching zero

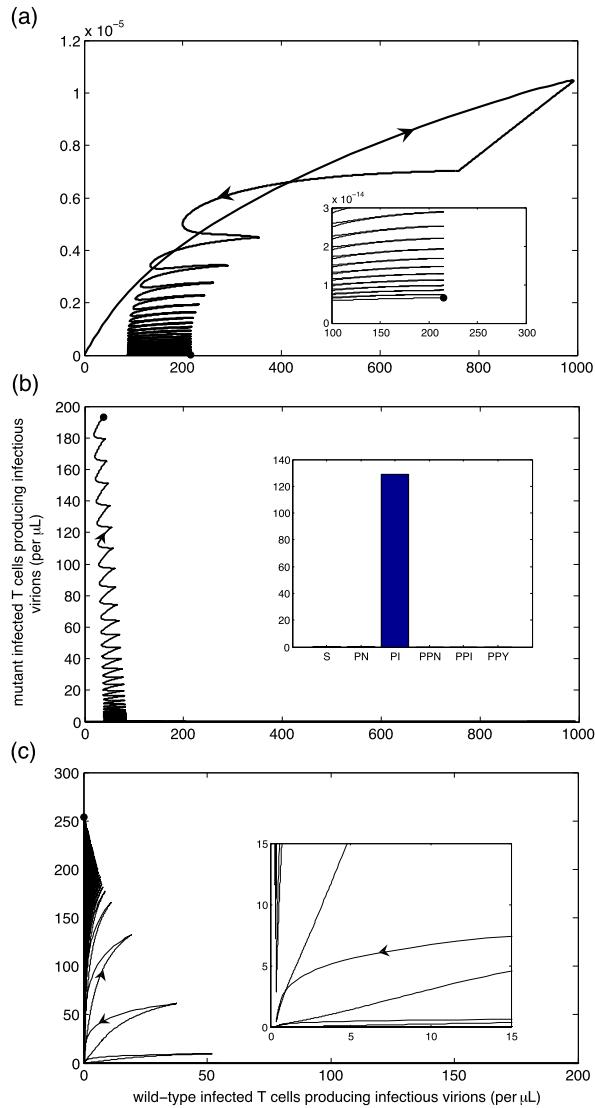
$$P^i = P_2(1 - e^{-d_P \tau})$$

$$P^i = P_2 e^{d_P \tau} (1 - e^{-d_P \tau}).$$

The asterisk in Fig. 3 is the FDA-approved dosage and dosing interval for the protease inhibitor ritonavir. The recommended dose lies in Region 3.

Figures 4 and 5 illustrate phase-plane plots of the populations of cells able to produce the wild-type or drug-resistant viral strains. Figure 4 illustrates the drug concentrations remaining in one specific region whereas Fig. 5 illustrates the drug concentrations when two or more regions are crossed. In all cases, parameters and initial conditions are as in Table 2, with only the dosing interval τ and the dosage P^i varied. The insets in Fig. 4 show the total number of uninfected T cells or T cells that only produce non-infectious virions. It should be noted that the results from Fig. 4 are similar if P is fixed or if it is oscillating, since the endpoints of the periodic orbit

Fig. 5 The behaviour when trajectories of drug concentrations cross multiple regions. All parameters except the dose and dosing interval are as for Fig. 4. Note that the threshold for Region 2 is adjusted in Figs. 5 and 5b as mentioned in Sect. 4. The main panels illustrate the dynamics between T cells able to produce wild-type and mutant virions. (a) Regions 1 and 2 ($\tau = 1$, $P^i = 0.3$). In this case, both strains of the virus co-exist. T cells able to produce the wild-type strain are significantly more numerous than T cells able to produce the mutant strain. The inset shows a closer look at the final time (shown as a solid circle on the main panels). (b) Regions 2 and 3 ($\tau = 0.5$, $P^i = 0.6$). In this case, there is also co-existence, but the T cells able to produce the mutant strain dominate. The inset shows the high number of wild-type infected T cells unable to produce infectious virions. (c) Regions 1, 2, 3, and 4 ($\tau = 1.5$, $P^i = 3$). In this case, both strains of the virus co-exist, but the number of T cells able to produce the mutant strain is significantly higher. The inset shows that we do not have viral elimination. Note that the values chosen for the dosing interval and dosage allow trajectories to enter Region 4, but viral elimination does not occur since trajectories do not remain in Region 4



remain solely within a region. Figure 5 occurs only if P oscillates between two or more regions.

In Region 1, the wild-type virus dominates. Initially, both strains of the virus can infect cells (initial increase in Fig. 4a), but if the system stays in Region 1, the wild-type strain will out-compete the mutant strain. The end result is only wild-type-infected cells (Fig. 4a) and low amounts of susceptible T cells (inset of Fig. 4a).

If the drug forces the system to stay within Region 2, the mutant strain is the better competitor since it faces no evolutionary pressure, unlike the wild-type. The distance between Region 1 and Region 2 is so small that it is likely that the drug will

Table 2 Range of parameters

| Parameter/ State variable | Range | Initial/Sample value | References |
|---------------------------|--|----------------------|--|
| $n_I \omega$ | 10^2 – 10^4 | $10^5 \times 0.01$ | Perelson et al. (1996), Smith? and Wahl (2004, 2005) |
| r_I | 0.001–0.1 | 0.01 | Smith? and Aggarwala (2009), Smith? and Wahl (2004) |
| r_Y | 0.0003–0.03 | 0.0032 | Smith? and Wahl (2005) |
| d_V | 1–5 | 3 | Smith? and Wahl (2005), Perelson et al. (1993); De Boer et al. (2010) |
| d_S | 0.002–0.2 | 0.02 | Smith? and Aggarwala (2009), Smith? and Wahl (2004, 2005), Perelson et al. (1993), De Boer et al. (2010) |
| d_I | 0.05–1 | 0.5 | Smith? and Aggarwala (2009), Smith? and Wahl (2004, 2005), Perelson et al. (1993), De Boer et al. (2010) |
| r_P | 30–50 | 40 | Smith? and Wahl (2005) |
| r_{PP} | 8–13 | 10.4 | Smith? and Wahl (2005) |
| λ | 100–250 | 180 | Smith? and Wahl (2005) |
| m_P | 1–4 | $24 \log(2)/6.2$ | Smith? and Wahl (2005) |
| m_{PP} | 1–4 | $24 \log(2)/6.2$ | Smith? and Wahl (2005) |
| P^i | **R2: 10^{-2} – 10^{-1} **R3: 10–40 **R4: 40–100 | (varied) | |
| d_P | | 24 $\log(2)/6.2$ | Smith? and Wahl (2005) |
| P_1 | | 10^{-3} | Section 2.2 |
| P_2 | | 10^{-2} | Section 2.2 |
| τ | | (varied) | |
| V_I | | 500 | Smith? and Wahl (2005) |
| V_Y | | 5×10^{-5} | Smith? and Wahl (2005) |
| V_N | | 0 | Smith? and Wahl (2005) |
| T_S | | 1000 | Smith? and Wahl (2005) |
| T_I | | 0 | Smith? and Wahl (2005) |
| T_Y | | 0 | Smith? and Wahl (2005) |
| T_{PN} | | 0 | Smith? and Wahl (2005) |
| T_{PI} | | 0 | Smith? and Wahl (2005) |
| T_{PY} | | 0 | Smith? and Wahl (2005) |
| T_{PPN} | | 0 | Smith? and Wahl (2005) |
| T_{PPI} | | 0 | Smith? and Wahl (2005) |
| T_{PPY} | | 0 | Smith? and Wahl (2005) |
| P | | 0 | Smith? and Wahl (2005) |

** R2, R3, and R4 denote Region 2, Region 3, and Region 4

never remain solely in Region 2. For Fig. 4b, we increased the Region 2 threshold to $P_2 = 10^1$ in order to show numerically the effect of staying in Region 2. The end result is co-existence of the wild-type and mutant infected cells with high amounts of

T cells able to produce resistant virus, and low amounts of uninfected T cells (inset of Fig. 4b).

If a large enough amount of drug is taken to enter and remain solely in Region 3, there are higher numbers of mutant-infected cells than wild-type-infected cells (Fig. 4c). There is also an increase in the number of mutant-infected T cells inhibited with high drug concentrations (inset of Fig. 4c).

If P is very large such that the system reaches Region 4, there are a large number of uninfected T cells with high drug concentrations (inset of Fig. 4d) and all the T cells able to produce infectious virions are eliminated (Fig. 4d). Note that Figs. 4a, 4c and 4d use the same parameters except the dosing interval and the dosage.

More than likely, the drug will allow the system to cross multiple regions. Figure 5 illustrates the dynamics when trajectories cross more than one region.

Figure 5a shows that initially both the wild-type and mutant virus infect susceptible T cells; the curve mimics that of remaining solely in Region 1. Once the drug fluctuates between Regions 1 and 2, the mutant strain is the better competitor in Region 2, but the wild-type strain is better in Region 1. This can be seen by the impulses shown in Fig. 5a. The impulses are better shown by the sharp edges in the inset of Fig. 5a where we have discontinuities in the derivatives. The end result is co-existence between strains but mostly all wild-type-infected T cells, meaning the system behaves similarly to Region 1. Again, we increase the Region 2 threshold to $P_2 = 10^1$ in order to show numerically the effect of staying below the Region 2 threshold since the distance between the Region 2 and 3 thresholds is so small.

When the system fluctuates between Regions 2 and 3, we observe impulses in both the wild-type and mutant-infected T cells (Fig. 5b). The end result is a large number of mutant-infected T cells. Here, again, we increase the distance between the Regions 1 and 2 thresholds in order to show the results of crossing between Regions 2 and 3. We have co-existence, but mostly the mutant strain dominates. The inset of Fig. 5b shows the increase in wild-type-infected T cells unable to produce infectious virions.

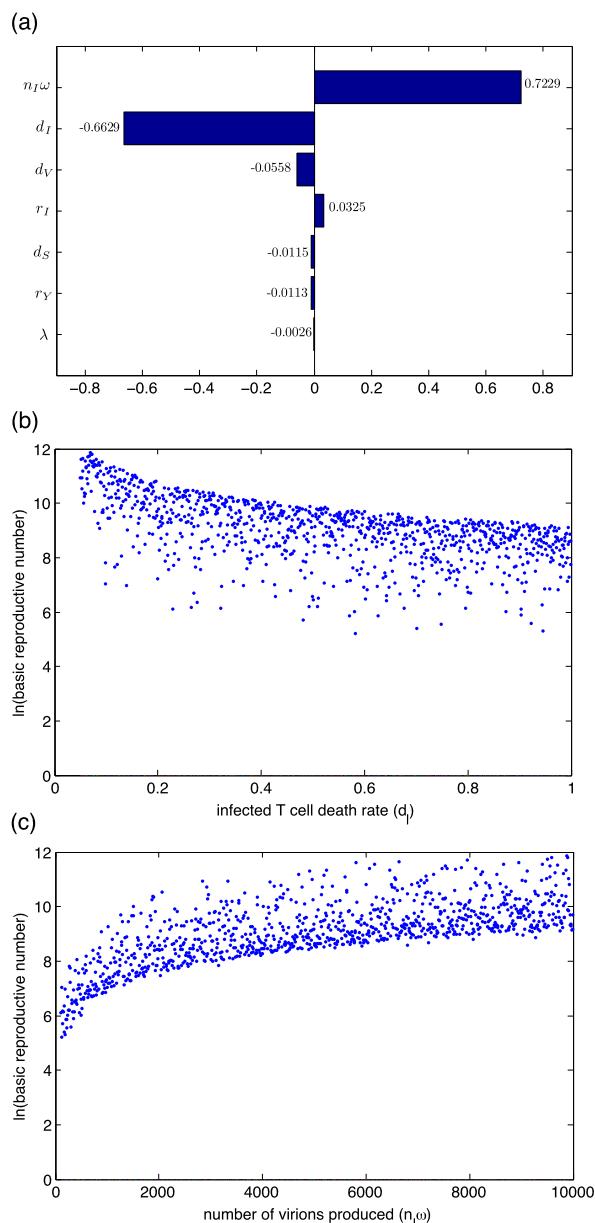
When the drug forces the system to move between Regions 1, 2 and 3, we have near-viral elimination each time the system jumps into high levels of Region 3 (Fig. 5c). The inset of Fig. 5c shows how the trajectories enter Region 4 by causing the infected T cells to approach zero. Then an impulse occurs, after which the mutant and wild-type strains increase, causing fluctuations in the infected T cells and moving away from viral elimination. As a result, there is co-existence but many more T cells infected by the mutant strain.

In summary, if we include a fluctuation of drug levels, the mutant either dominates or co-exists in all cases. Whenever Region 2 is entered, the mutant gains a rapid advantage.

4.3 Sensitivity to Variations

Since parameters may fluctuate, we explore the sensitivity of R_0 to the parameter values using Latin Hypercube Sampling (LHS). LHS is a statistical sampling method that allows for an efficient analysis of parameter variations across simultaneous uncertainty ranges in each parameter (Blower and Dowlatabadi 1994;

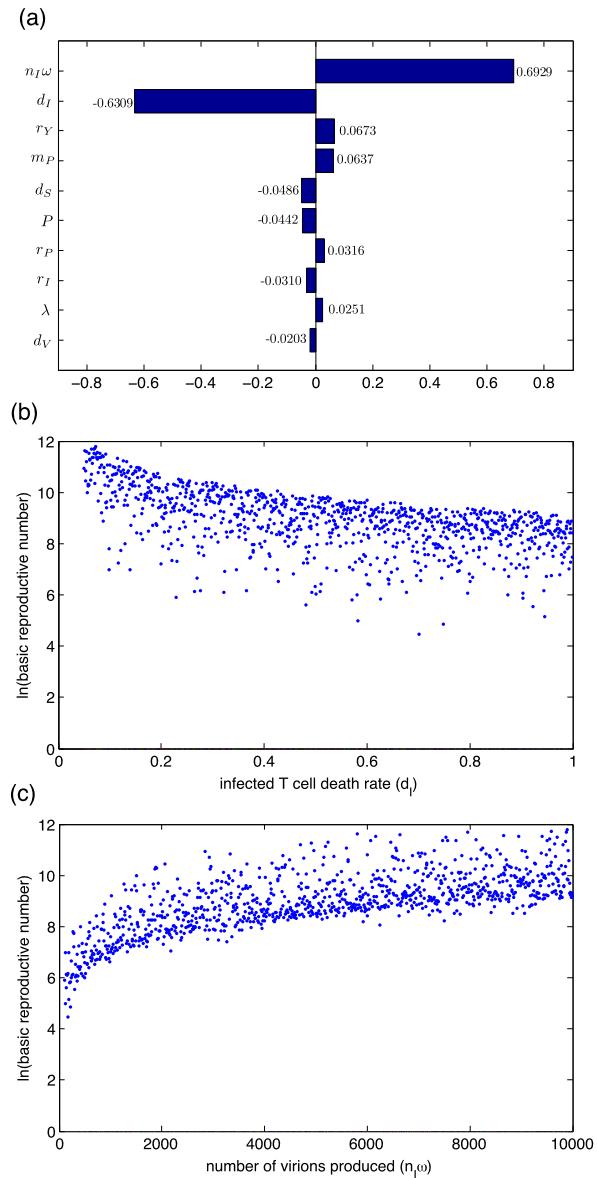
Fig. 6 Sensitivity analysis for Region 1. (a) Partial rank correlation coefficients for R_0 for all parameters. (b) The effect of the death rate for the infected CD4 $^{+}$ T cells d_I on R_0 . (c) The effect of the number of infectious virions produced per day from an infected CD4 $^{+}$ T cell $n_I\omega$ on R_0



Stein 1985); partial rank correlation coefficients rank the coefficients by the degree of influence each has on the outcome, regardless of whether that influence increases or decreases the effect.

Figures 6a, 7a, 8a and 9a show the partial rank correlation coefficient sensitivity analysis for 1000 runs. All relevant parameters are varied against R_0 throughout the ranges given in Table 2. In all regions, R_0 is the most sensitive to the death rate for

Fig. 7 Sensitivity analysis for Region 2. (a) Partial rank correlation coefficients for R_0 for all parameters. (b) The effect of the death rate for the infected CD4 $^{+}$ T cells d_I on R_0 . (c) The effect of the number of infectious virions produced per day from an infected CD4 $^{+}$ T cell $n_I\omega$ on R_0



the infected CD4 $^{+}$ T cells, d_I ; the effect of d_I on R_0 can also be seen in Figs. 6b, 7b, 8b and 9b for Regions 1, 2, 3 and 4, respectively. In Regions 1, 2 and 3, R_0 is also sensitive to the number of infectious virions produced per day from an infected CD4 $^{+}$ T cell, $n_I\omega$; the effect of $n_I\omega$ on R_0 can also be seen in Figs. 6c, 7c and 8c for Regions 1, 2 and 3, respectively. In Region 3, R_0 is also sensitive to the infection rate of susceptible CD4 $^{+}$ T cells with mutant virus, r_Y ; the effect of r_Y on R_0 in Region 3 can also be seen in Fig. 8d. In Region 4, R_0 is sensitive to the clearance rate of the drug from a highly inhibited cell, m_{PP} , and the drug dosage, P^* ; the effect

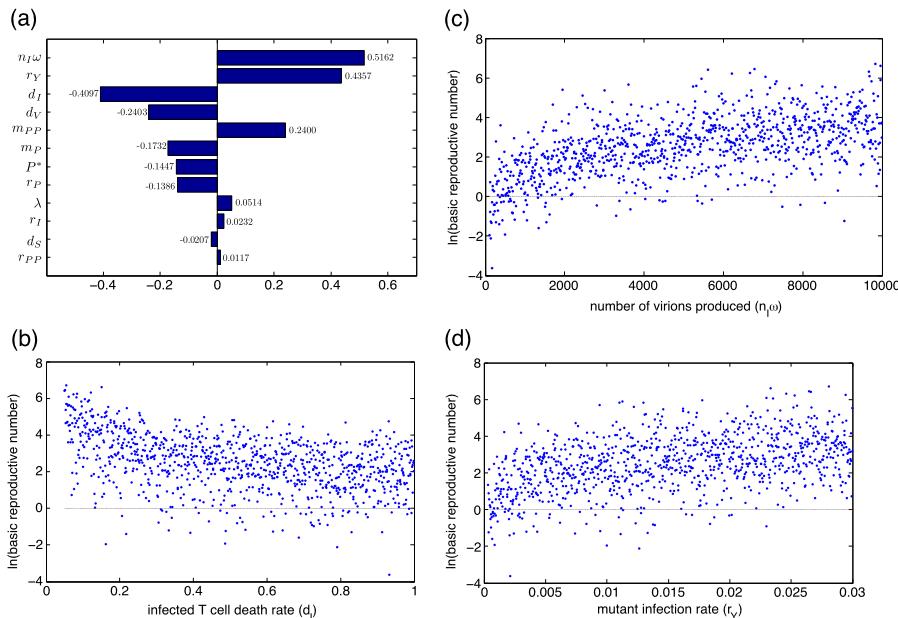


Fig. 8 Sensitivity analysis for Region 3. (a) Partial rank correlation coefficients for R_0 for all parameters. (b) The effect of the death rate for the infected CD4 $^{+}$ T cells d_I on R_0 . (c) The effect of the number of infectious virions produced per day from an infected CD4 $^{+}$ T cell $n_I\omega$ on R_0 . (d) The effect of the infection rate of susceptible CD4 $^{+}$ T cells with mutant virus r_Y , on R_0

of m_{PP} and P^* on R_0 in Region 4 can also be seen in Figs. 9c and 9d, respectively. Figures 6, 7 and 8b, c and d were run using a Monte Carlo simulation with parameters drawn using LHS. The LHS of all remaining parameters not seen in Figs. 6, 7, 8 and 9 are approximately uniformly scattered. Varying $n_I\omega$ and d_I does not reduce R_0 below 1 in Regions 1 or 2. In Region 3, however, there are values for which $R_0 < 1$, corresponding to the region of viral elimination. As P^* gets very large, varying the parameters does make R_0 increase beyond unity; R_0 is always below unity in Region 4. Note that the difference between variations in P^* and the impulse effects are that P^* varies in an orderly way, whereas the impulse occurs at a fixed, regular time. Thus, if P^* varies slightly, it may not have a big effect on R_0 since the impulse may be the cause of the change.

5 Discussion

Drug therapy is crucial to the well-being and survival of HIV-infected patients. The strict drug regimens are often difficult to follow due to major side effects and pill fatigue. We have shown the different effects of having low, intermediate and high levels of drug concentration in a cell, and shown that, in all cases, entering Region 2 causes the mutant to gain rapid advantage.

Initially, we showed the effects on the wild-type and mutant virus populations when drug concentrations in a cell are constant at either low, intermediate or high

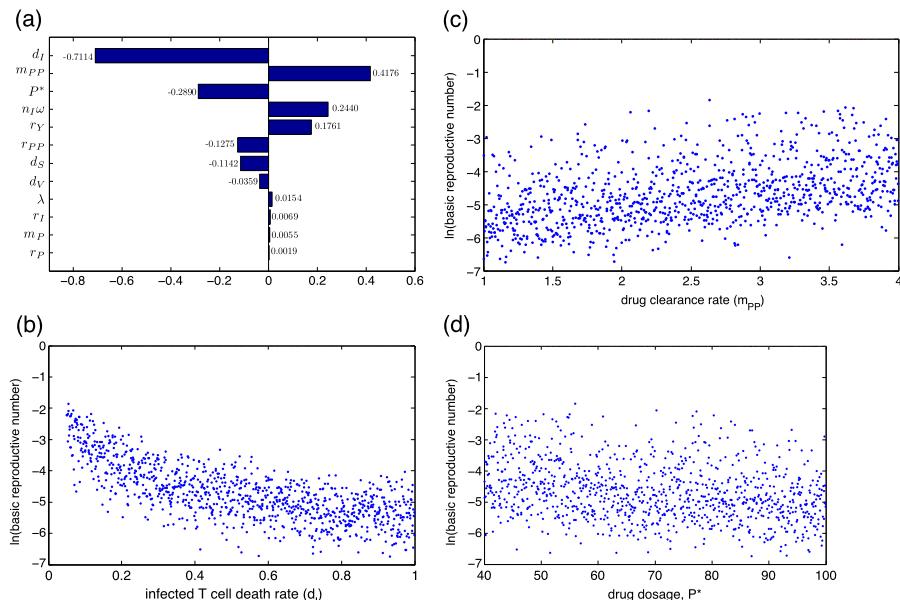


Fig. 9 Sensitivity analysis for Region 4. (a) Partial rank correlation coefficients for R_0 for all parameters. (b) The effect of the death rate for the infected CD4⁺ T cells d_I on R_0 . (c) The effect of the clearance rate of the drug from a highly inhibited cell m_{PP} on R_0 . (d) The effect of the drug dosage P on R_0

levels. We conclude that, if the drug level is at an intermediate or high state, then drug resistance will emerge. We also showed that there is a theoretical region of free-virus elimination.

The number of uninfected T cells was also calculated in each region for a constant drug concentration. This is important because a higher number of uninfected T cells means a greater chance of controlling the virus and fighting off opportunistic infections. We showed that the total uninfected T cell count in Region 4 approaches that of the disease-free state. We also showed that Region 2 not only has low levels of uninfected T cells, it also has a large number of T cells able to produce the mutant strain. Similar results apply numerically when drug-concentration levels vary.

Numerical simulations showed the effects of varying the drug-concentration levels. This perturbation, included in the impulse, allows us to examine the effects of varying the drug-concentration levels either solely within a region or across multiple regions. Entering Region 2 depends on the drug dosage and the dosing interval. We have shown that T cells able to produce the mutant strain will dominate if trajectories enter Region 2. This will cause drug resistance and drug failure. It was also shown that crossing all 3 regions results in a high number of T cells able to produce the mutant strain. We have also shown that Region 1 has a high number of T cells able to produce the wild-type strain and a low number of T cells able to produce the mutant strain. Thus it is better for trajectories to remain in Region 1 than in Region 2 since the wild-type strain can be controlled by antiretroviral therapy.

The recommended dosage and dosing interval for one of the FDA-approved protease inhibitors, ritonavir, is shown in Fig. 3. If taken with perfect adherence, the

recommended dosage and dosing interval would remain in Region 3, causing the mutant strain to remain very low. However, if the drug is not administered at the recommended time (twice a day for ritonavir), then trajectories would fall into Region 2 (by moving to the right in Fig. 3) where a rapid outbreak of mutant virus would occur. This can also be seen from Fig. 4. The high amount of mutant-infected T cells inhibited with high drug levels in Region 3 (inset of Fig. 4c) would rapidly become T cells that are able to produce infectious mutant virions when the drug level lowers and enters Region 2. Thus adherence is crucial to avoid the development of resistance.

Both a high dosage and small dosing interval are necessary in order to avoid drug resistance and theoretically attain viral elimination. Although drug toxicities may limit the extent to which this optimum can be approached, such a scenario is theoretically possible for protease inhibitors. One limitation is that the model does not include certain viral reservoirs such as latently infected cells or reproductive reservoirs. These reservoirs would cause the virus to persist even when in Region 4. Thus elimination of free virus in our model is not equivalent to clearing the infection. A further limitation is that we restricted P^* to be constant in the analysis. Similar results can numerically be shown for varying drug levels. Future work would be to develop and compute stability analysis with varying drug levels in order to theoretically show the same results that are shown numerically in the Figs. 4 and 5. Another limitation is that the system only includes protease inhibitors. In reality, most patients take triple drug cocktails including protease inhibitors and reverse transcriptase inhibitors, fusion inhibitors and/or integrase inhibitors. This would also change the dynamics of the system. Combination therapy can down-regulate the effectiveness of certain drugs, meaning their half-life and IC_{50} values could change. Future work would be to examine the effects of combination therapy using impulsive differential equations.

It should be noted that our model considers monotherapy for a single protease inhibitor (or, equivalently, an aggregate of multiple protease inhibitors with the same treatment cycle). Although monotherapy is not recommended, it is often used in the developing world, especially where economics make combination therapy impossible (Okero et al. 2003). Monotherapy is sometimes used when other treatments have failed, or used after prolonged viral suppression. Bierman et al. (2009) and Calza and Manfredi (2012) showed that patients with prolonged viral suppression on highly active antiretroviral therapy (HAART) can successfully be treated with protease inhibitor monotherapy. Pillay et al. (2010) showed that a boosted protease inhibitor monotherapy following a 24 week second-line induction was associated with an increase in low level viraemia, although generally in the absence of PI resistance. Furthermore, understanding monotherapy is a useful precursor for developing complex models of combination therapy.

It is theoretically possible to eliminate free virus in this system if the drug concentration level is very high. The total uninfected T cell count in Region 4 is similar to that of the disease-free state. It was shown numerically that the results for constant drug concentration are similar to varying drug concentrations. Thus high dosage and low interval time between doses can theoretically lead to an elimination of free virus and a disease-free uninfected T cell count.

Acknowledgements R.E.M. is funded by an Ontario Graduate Scholarship. R.J.S. is supported by an NSERC Discovery Grant, an Early Researcher Award and funding from MITACS.

References

Allen, L. J. S. (2006). *Introduction to mathematical biology* (pp. 150–151). Upper Saddle River: Pearson Education.

Bainov, D. D., & Simeonov, P. S. (1989). *Systems with impulsive effect*. Chichester: Ellis Horwood Ltd.

Bainov, D. D., & Simeonov, P. S. (1993). *Impulsive differential equations: periodic solutions and applications*. Burnt Mill: Longman Scientific and Technical.

Bainov, D. D., & Simeonov, P. S. (1995). *Impulsive differential equations: asymptotic properties of the solutions*. Singapore: World Scientific.

Bhunu, C. P., Garira, W., & Magombedze, G. (2009). Mathematical analysis of a two strain HIV/AIDS model with antiretroviral treatment. *Acta Biotheor.*, 57(3), 361–381.

Bierman, W. F., van Agtmael, M. A., Nijhuis, M., Danner, S. A., & Boucher, C. A. (2009). HIV monotherapy with ritonavir-boosted protease inhibitors: a systematic review. *AIDS*, 23(3), 279–291.

Bilello, J. A., Bilello, P. A., Stellrecht, K., Leonard, J., Norbeck, D. W., Kempf, D. J., Robins, T., & Drusano, G. L. (1996). Human serum α_1 acid glycoprotein reduces uptake, intracellular concentration, and antiviral activity of a-80987, and inhibitor of the human immunodeficiency virus type-1 protease. *Antimicrob. Agents Chemother.*, 40(6), 1491–1497.

Blower, S. M., & Dowlatabadi, H. (1994). Sensitivity and uncertainty analysis of complex models of disease transmission: an HIV model, as an example. *Int. Stat. Rev.*, 2, 229–243.

Calza, L., & Manfredi, R. (2012). Protease inhibitor monotherapy as maintenance regimen in patients with HIV infection. *Curr. HIV Res.*, 10(8), 661–672.

De Boer, R. J., Ribeiro, R. M., & Perelson, A. S. (2010). Current estimates for HIV-1 production imply rapid viral clearance in lymphoid tissues. *PLoS Comput. Biol.*, 6(9), e1000906.

Diekmann, O., Heesterbeek, J. A. P., & Metz, J. A. (1990). On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. *J. Math. Biol.*, 28(4), 365.

Hale, J. K. (1969). *Ordinary differential equations*. New York: Wiley.

Heye, T. B., Tadesse, B. T., & Yalow, A. W. (2012). Predictors of treatment failure and time to detection and switching in HIV-infected Ethiopian children receiving first line anti-retroviral therapy. *BMC Infect. Dis.*, 12(1).

Ho, D. D., Neuwann, A. U., Perelson, A. S., Chen, W., Leonard, J. M., & Markowitz, M. (1995). Rapid turnover of plasma viraions and CD4 lymphocytes in HIV-1 infection. *Nature*, 373(6510), 123–126.

Huang, Y., Wu, H., & Acosta, E. P. (2010). Hierarchical Bayesian inference for HIV dynamic differential equation models incorporating multiple treatment factors. *Biom. J.*, 52(4), 470–486.

Janeway, C., Travers, P., Walport, M., & Shlomchik, M. (2006). *Immunobiology: the immune system in health and disease* (6th ed., pp. 461–510). London: Garland Science, Taylor & Francis.

Kepler, T. B., & Perelson, A. S. (1998). Drug concentration heterogeneity facilitates the evolution of drug resistance. *Proc. Natl. Acad. Sci. USA*, 95, 11514–11519.

Kitayimbwa, J. M., Mugisha, J. Y., & Saenz, R. A. (2013). The role of backward mutations on the within-host dynamics of HIV-1. *J. Math. Biol.*, 67(5), 1111–1139.

Krakovska, O., & Wahl, L. M. (2007). Optimal drug treatment regimens for HIV depend on adherence. *J. Theor. Biol.*, 246(3), 499–509.

Lakshmikantham, V., Bainov, D. D., & Simeonov, P. S. (1989). *Theory of impulsive differential equations*. Singapore: World Scientific.

Lou, J., & Smith?, R. J. (2011). Modelling the effects of adherence to the HIV fusion inhibitor enfuvirtide. *J. Theor. Biol.*, 268(1), 1–13.

Lou, J., Lou, Y., & Wu, J. (2012). Threshold virus dynamics with impulsive antiretroviral drug effects. *J. Math. Biol.*, 65(4), 623–652.

Miron, R. M., & Smith?, R. J. (2010). Modelling imperfect adherence to HIV induction therapy. *BMC Infect. Dis.*, 10(6).

Mohanty, U., & Dixit, N. M. (2008). Mechanism-based model of the pharmacokinetics of enfuvirtide, an HIV fusion inhibitor. *J. Theor. Biol.*, 251(3), 541–551.

Okero, F. A., Aceng, E., Madraa, E., Namagala, E., & Serutoke, J. (2003). Scaling up antiretroviral therapy: experience in Uganda. Perspectives and practice in antiretroviral treatment. Case Study. World Health Organization and the Republic of Uganda.

Perelson, A. S., Kirschner, D. E., & De Boer, R. (1993). Dynamics of HIV infection of CD4⁺ T cells. *Math. Biosci.*, 114, 81–125.

Perelson, A. S., Neumann, A. U., Markowitz, M., Leonard, J. M., & Ho, D. D. (1996). HIV-1 dynamics in vivo: virion clearance rate, infected cell lifespan, and viral generation time. *Science*, 271, 1582–1585.

Pillay, D., Bhaskaran, K., Jurriaans, S., Prins, M., Masquelier, B., Dabis, F., Gifford, R., Nielsen, C., Pedersen, C., Balotta, C., Rezza, G., Ortiz, M., de Mendoza, C., Kucherer, C., Poggensee, G., Gill, J., & Porter, K. (2006). The impact of transmitted drug resistance on the natural history of HIV infection and response to first-line therapy. *AIDS*, 20, 21–28.

Pillay, D., Goodall, R., Gilks, C. F., Yirrell, D., Gibb, D., Spyer, M., Kaleebu, P., Munderi, P., Kityo, C., McCormick, A., Nkalubo, J., Lyagoba, F., Chirara, M., & Hakim, J., DART (2010). Virological findings from the SARA trial: boosted PI monotherapy as maintenance second-line ART in Africa. *J. Int. AIDS Soc.*, 13(Suppl 4), O20.

Rong, L., Feng, Z., & Perelson, A. S. (2007b). Emergence of HIV-1 drug resistance during antiretroviral treatment. *Bull. Math. Biol.*, 69(6), 2027–2060.

Rong, L., Gilchrist, M. A., Feng, Z., & Perelson, A. S. (2007a). Modeling within-host HIV-1 dynamics and the evolution of drug resistance: trade-offs between viral enzyme function and drug susceptibility. *J. Theor. Biol.*, 247(4), 804–818.

Rosenbloom, D. I., Hill, A. L., Rabi, S. A., Siliciano, R. F., & Nowak, M. A. (2012). Antiretroviral dynamics determines HIV evolution and predicts therapy outcome. *Nat. Med.* 18(9), 1378–1385.

Shi, V., Tridane, A., & Kuang, Y. (2008). A viral load-based cellular automata approach to modeling HIV dynamics and drug treatment. *J. Theor. Biol.*, 253(1), 24–35.

Shiri, T., & Welte, A. (2008). Transient antiretroviral therapy selecting for common HIV-1 mutations substantially accelerates the appearance of rare mutations. *Theor. Biol. Med. Model.*, 5, 25.

Smith?, R. J. (2006). Adherence to antiretroviral HIV drugs: how many doses can you miss before resistance emerges? *Proc. R. Soc. Lond. Ser. B, Biol. Sci.*, 273(1586), 617–624.

Smith?, R. J., & Aggarwala, B. D. (2009). Can the viral reservoir of latently infected CD4⁺ T cells be eradicated with antiretroviral HIV drugs? *J. Math. Biol.*, 59(5), 697–715.

Smith?, R. J., & Schwartz, E. J. (2008). Predicting the potential impact of a cytotoxic T-lymphocyte HIV vaccine: how often should you vaccinate and how strong should the vaccine be? *Math. Biosci.*, 212, 180–187.

Smith?, R. J., & Wahl, L. M. (2004). Distinct effects of protease and reverse transcriptase inhibition in an immunological model of HIV-1 infection with impulsive drug effects. *Bull. Math. Biol.*, 66, 1259–1283.

Smith?, R. J., & Wahl, L. M. (2005). Drug resistance in an immunological model of HIV-1 infection with impulsive drug effects. *Bull. Math. Biol.*, 67, 783–813.

Stein, M. (1985). The use of LHS for variance reduction in simulations with many random parameters. *IBM Report RC11166* (#50265) 52385, mathematics, 46 pp.

Truccolo, W. A., Rangarajan, G., Chen, Y., & Ding, M. (2003). Analysing stability of equilibrium points in neutral networks: a general approach. *Neutral Netw.*, 16, 1453–1460.

van den Driessche, P., & Watmough, J. (2002). Reproduction numbers and sub-threshold endemic equilibria for compartmental models of disease transmission. *Math. Biosci.*, 180, 29.

von Wyl, V., Cambiano, V., Jordan, M. R., Bertagnolio, S., Miners, A., Pillay, D., Lundgren, J., & Phillips, A. N. (2012). Cost-effectiveness of tenofovir instead of zidovudine for use in first-line antiretroviral therapy in settings without virological monitoring. *PLoS One*, 7(8).

Wagner, B. G., & Blower, S. (2012). Universal access to HIV treatment versus universal ‘test and treat’: transmission, drug resistance & treatment costs. *PLoS One*, 7(9).

Wheeler, W. H., Ziebell, R. A., Zabina, H., Pieniazek, D., Prejean, J., Bodnar, U. R., Mahle, K. C., Heneine, W., Johnson, J. A., & Hall, H. I. (2010). Prevalence of transmitted drug resistance associated mutations and HIV-1 subtypes in new HIV-1 diagnoses, U.S.-2006. *AIDS*, 24(8), 1203–1212.

Wu, J., Yan, P., & Archibald, C. (2007). Modelling the evolution of drug resistance in the presence of antiviral drugs. *BMC Public Health*, 7, 300.