Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bulletin of Mathematical Biology ▮ (▮▮▮▮) ▮▮▮–▮▮
Bulletin of Mathematical Biology
www.elsevier.com/locate/ybulm

Drug resistance in an immunological model of HIV-1 infection with impulsive drug effects

R.J. Smith[a], L.M. Wahl[b,*]

[a]*Department of Mathematics and Department of Veterinary Pathobiology, University of Illinois at Urbana-Champaign, IL 61802, USA*
[b]*Department of Applied Mathematics, University of Western Ontario, London, ON, N6A 5B7, Canada*

Received 6 July 2004; accepted 28 October 2004

Abstract

We consider an SIR-type model of immunological behaviour for HIV dynamics, including the effects of reverse transcriptase inhibitors and other drugs which prevent cellular infection. We use impulsive differential equations to model drug behaviour. We classify different regimes according to whether the drug efficacy is negligible, intermediate or high. We consider two strains of the virus: a wild-type strain that can be controlled by both intermediate and high drug concentrations, and a mutant strain that can only be controlled by high drug concentrations. Drug regimes may take trajectories through one, two or all three regimes, depending on the dosage and the dosing schedule. We demonstrate that drug resistance arises at both intermediate and high drug levels. At low drug levels resistance does not emerge, but the total T cell count is proven to be significantly lower than in the disease-free state. 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, both viral sub-populations will be cleared. In the latter case the immune system is maintained by a population of T cells which have absorbed sufficient quantities of the drug to prevent infection by even the drug-resistant strain. We provide estimates of a range of dosages and dosing schedules which would, if physiologically tolerable, theoretically eliminate free virus in this system. Our results predict that to control viral load, decreasing the interval between doses is more effective than increasing the dose.

* Corresponding author. Fax: +1 519 661 3523.
E-mail address: lwahl@uwo.ca (L.M. Wahl).

0092-8240/$30 © 2004 Society for Mathematical Biology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.bulm.2004.10.004

1. Introduction

The emergence of drug resistance is one of the most prevalent reasons for treatment failure in HIV therapy. A large number of mathematical models have been developed to describe the population dynamics of HIV-1 including drug treatment and drug resistance; studies in the last decade include Perelson et al. (1996), de Boer and Boucher (1996), de Jong et al. (1996), Kirschner and Webb (1996), Perelson et al. (1997), de Boer and Perelson (1998), Wein et al. (1998), Wodarz and Nowak (1999), Ribeiro and Bonhoeffer (2000), Wodarz et al. (2000), Wodarz (2001) and Perelson (2002). Historically, such models have focussed on the emergence of drug resistance within an individual during continuous drug therapy (Nowak et al., 1991; McLean and Nowak, 1992; Frost and McLean, 1994; Coffin, 1995; Bonhoeffer and Nowak, 1997; Stilianakis et al., 1997; Nowak et al., 1997; Austin and Anderson, 1999).

In contrast, a handful of studies have modeled the interaction of *changing* drug concentrations with the population dynamics of a pathogen, examining the conditions necessary for the emergence of drug resistance (Kepler and Perelson, 1998; Lipsitch and Levin, 1998; Wahl and Nowak, 2000).

We have recently proposed the use of impulsive differential equations to model dynamic drug concentrations during HIV-1 therapy (Smith and Wahl, 2004). This framework allowed us to capitalize on a fairly sophisticated mathematical literature (Bainov and Simeonov, 1989, 1993, 1995; Lakshmikantham et al., 1989), facilitating our investigation of drug classes with different mechanisms of action.

In the sections that follow we extend this approach to examine the conditions required for the emergence of drug resistance during HIV therapy. We consider drug regimes for classes of drugs that mimic the dynamics of reverse transcriptase inhibitors. Specifically, these are drugs that prevent viral infection of T cells. Such drugs include reverse transcriptase inhibitors (nucleoside, nonnucleoside and nucleotide), fusion inhibitors and integrase inhibitors. The class of such drugs that prevent viral infection will be henceforth referred to as “preventative drugs”.

We assume a single drug regimen (though this regimen may actually consist of a number of different preventative drugs), a wild-type strain of the virus that initially dominates and a drug-resistant strain, which has lower infectivity. Intermediate drug levels will affect the wild-type strain of the virus alone, while high drug levels will affect both strains. Our approach is a novel one, using impulsive differential equations to model drug behaviour and classifying different model regimes according to whether the drug efficacy is negligible, intermediate or high.

The aim of this study is to determine how dosing schedules and concentrations of preventative drugs will facilitate or prevent the emergence of drug resistance. Although the advantage of our formulation is that dynamic changes in drug concentration can be examined using a completely analytical approach, we illustrate many of our results using numerical simulations.

2. The model

2.1. T cells

We would like to examine the various possible fates of a $CD4^+$ T cell in some detail. At any time, a T cell may come into contact with (1) a virion infected with the wild-type virus, (2) a virion infected with the mutant strain of the virus, or (3) the drug. Depending on the level of drug absorbed, the cell may subsequently become immune to neither, one or both strains of the virus. The T cells can thus be classified into five populations, described below in paragraphs (a) through (e) and pictured in Fig. 1A.

(a) Let T_S be the population of susceptible (noninfected) $CD4^+$ T cells. These cells are produced at a constant rate, λ. There are four possible fates for these T cells: they may die at natural death rate d_S; they may (b) become infected with the wild-type virus; (c) they may become infected with the mutant virus; or (d) they may absorb the drug.

(b) We use T_I to denote the population of $CD4^+$ T cells infected with the wild-type virus. These cells produce wild-type infectious virus, V_I, and have a significantly higher death rate, d_I (Ho et al., 1995). Like the healthy cells, these cells may later absorb drug. Since the viral genome has already been transcribed into the host DNA, absorbing the drug has no effect on these infected cells. Thus, the only possible fate for these cells is cell death.

(c) T_Y denotes the cells infected with the mutant virus. These cells produce mutant infectious virus, V_Y, and we assume their death rate is the same as cells infected with the wild-type virus. The drug will also have no effect on these infected cells. We also assume that there is no difference in the average number of infectious virions produced per infectious cell. The only possible fate for these cells is also cell death.

(d) T_{RI} denotes noninfected cells which have absorbed sufficient quantities of the drug so that the wild-type strain is inhibited, but not enough to prevent infection by the mutant strain of the virus. These cells may come into contact with either the wild-type or the mutant strain of the virus or the drug. If the cell comes into contact with a wild-type virus, it cannot become infected, so this has no consequence for the cell. However, if the cell comes into contact with the mutant virus, it will become infected with the mutant strain. If the cell comes into contact with sufficiently high drug concentrations, it can become (e) a T_{RY} cell. If not, the cell will eventually revert back to a susceptible cell when the drug effect wears off, or undergo cell death.

(e) T_{RY} denotes noninfected cells which have absorbed sufficient quantities of the drug so that both strains of the virus are inhibited. Such cells cannot become infected while they remain in this state. These cells will eventually revert back to T_{RI} cells as the drug effect wears off, or undergo cell death.

Wild-type infectious virions, V_I (respectively mutant infectious virions V_Y), are produced by T cells infected with the wild-type virus (respectively the mutant strain of the virus) and are removed by clearance and infection of susceptible cells.

2.2. Drugs

We use $R(t)$ to denote the intracellular concentration of the drug and its active metabolites (Hoggard and Back, 2002). We assume that drugs are taken at (not necessarily fixed)

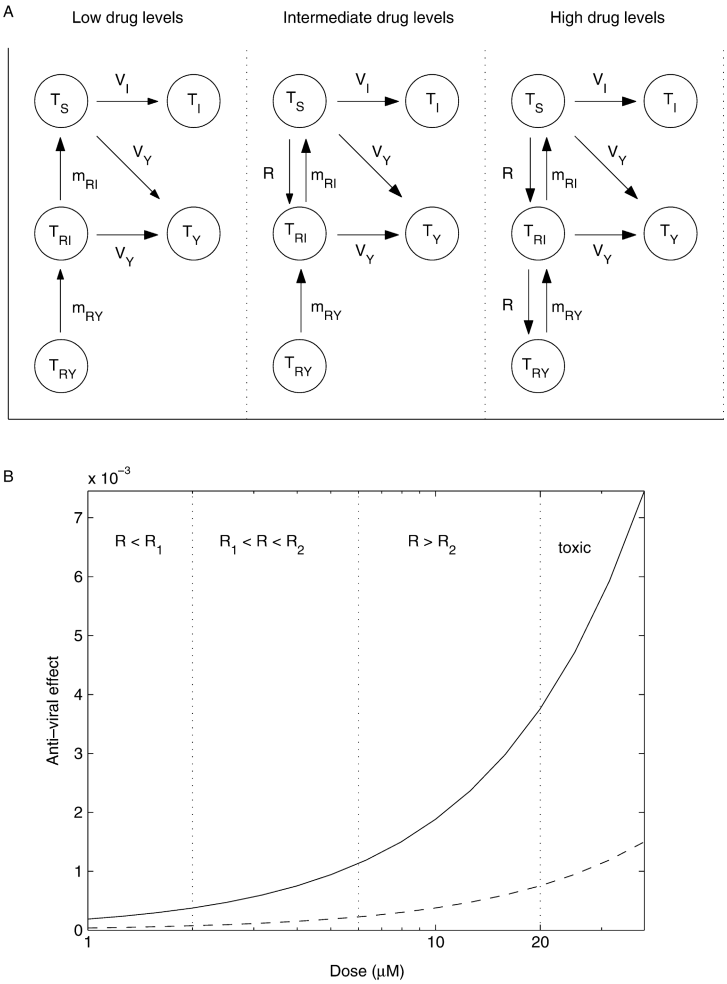

Fig. 1. Three-regime model. A: The five classes of T cells are susceptible (S), infected with the wild type (I), infected with the mutant type (Y), inhibited with intermediate levels of drug (RI) and inhibited with high levels of drug (RY). Each cell may come into contact with a wild type virion, a mutant virion, or the drug. Once infected, cells cannot move into the class of drug-inhibited cells. Cells inhibited with intermediate levels of drug are immune to the wild-type virus while they remain inhibited, but can be infected by the mutant strain. Cells inhibited with high drug levels cannot be infected while they remain in this state. B: Example dose–effect curves for the wild-type (solid curve) and drug-resistant (dashed curve) viral strains. When drug concentration R is less than R_1, the probability that a T cell absorbs sufficient drug to block infection is negligible for both strains. Between thresholds ($R_1 < R < R_2$), only the wild-type strain has a non-negligible probability of being blocked by the drug. For high drug concentrations ($R > R_2$), this probability grows monotonically with dose for both viral strains. IC_{50} values for the reverse transcriptase inhibitor AZT were used in this example. See text for details.

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 $t = t_k$.

According to impulsive theory, we can describe the nature of the impulse at time r_k via the difference equation

$$\Delta R \equiv R(t_k^+) - R(t_k^-) = f(t_k, R(t_k^-)). \tag{2.1}$$

(We refer the interested reader to Bainov and Simeonov (1989, 1993, 1995) and Lakshmikantham et al. (1989) for more details on the theory of impulsive differential equations.)

This technique assumes that the change in intracellular drug concentration immediately after a dose is taken is nearly instantaneous, that is, the time to peak is negligible on the relevant time scale. By neglecting the known dispersion and delay as the drug enters the intracellular space, we overestimate the temporal effects of dosing at intervals. The implications of this assumption will be taken up further in the Discussion. For a fuller treatment of the effects of spatially distinct compartments, see Kepler and Perelson (1998); for a detailed model of the kinetics of drug action, see Austin et al. (1998).

For simplicity, we assume that drugs are cleared from the body at a constant rate. This has the effect of overestimating the clearance rate, since presumably clearance from the intracellular compartments will be delayed. However, we argue that such an assumption is a reasonable approximation in the absence of more complete knowledge of the mechanics of drug clearance, and further that this assumption ensures that our estimates of overall treatment efficacy are conservative.

To model the effects of a resistance mutation on drug efficacy, we consider an underlying scenario as illustrated in Fig. 1B. 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; drug resistance implies an increase in the IC_{50} concentration of the drug. The y-axis, or "effect" in the dose–effect curve, is related to the probability that a given T cell absorbs sufficient quantities of the drug to prevent viral infection. Thus when $R < R_1$, this probability is negligible for both viral strains. In some region $R_1 < R < R_2$, this probability remains negligible for the drug-resistant virus, but grows monotonically with dose for the wild-type. Similarly when $R > R_2$, the probability of blocking infection is significant for both wild-type and drug-resistant strains, although higher for the wild-type. In all three cases where the probability that infection will be blocked is non-negligible, we assume that this 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). Our model of HIV dynamics therefore consists of three distinct systems in which different drug actions are possible, depending on the drug concentration R (Fig. 1A).

2.3. Combining T cell populations with virus and drugs

For $R < R_1$, the dynamics of virions and T cells are given by:

$$
\begin{aligned}
\frac{dV_I}{dt} &= n_I \omega T_I - d_V V_I - r_I T_S V_I \\
\frac{dV_Y}{dt} &= n_I \omega T_Y - d_V V_Y - r_Y T_S V_Y - r_Y T_{RI} V_Y \\
\frac{dV_{NI}}{dt} &= n_I (1-\omega)(T_I + T_Y) - d_V V_{NI} \\
\frac{dT_S}{dt} &= \lambda - r_I T_S V_I - r_Y T_S V_Y - d_S T_S + m_{RI} T_{RI} \\
\frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I \\
\frac{dT_Y}{dt} &= r_Y T_S V_Y - d_I T_Y + r_Y T_{RI} V_Y \\
\frac{dT_{RI}}{dt} &= -r_Y T_{RI} V_Y - (d_S + m_{RI}) T_{RI} + m_{RY} T_{RY} \\
\frac{dT_{RY}}{dt} &= -(d_S + m_{RY}) T_{RY}
\end{aligned}
\tag{2.2}
$$

for $t \neq t_k$ (see impulsive conditions below).

Here t is time in days, n_I is the number of virions produced per infected cell per day, ω is the fraction of virions produced by an infected T cell which are infectious, d_V is the rate at which free virus is cleared, d_S is the noninfected CD4$^+$ T cell death rate, d_I is the infected CD4$^+$ T cell death rate, r_I is the rate at which wild-type virus infects T cells, and r_Y is the rate at which the drug-resistant virus infects T cells. The constant λ represents a source of susceptible cells, while m_{RI} and m_{RY} are the rates at which the drug is cleared from the intracellular compartment for intermediate and high drug concentrations respectively.

For $R_1 < R < R_2$, the dynamics of virions and T cells are given by:

$$
\begin{aligned}
\frac{dV_I}{dt} &= n_I \omega T_I - d_V V_I - r_I T_S V_I \\
\frac{dV_Y}{dt} &= n_I \omega T_Y - d_V V_Y - r_Y T_S V_Y - r_Y T_{RI} V_Y \\
\frac{dV_{NI}}{dt} &= n_I (1-\omega)(T_I + T_Y) - d_V V_{NI} \\
\frac{dT_S}{dt} &= \lambda - r_I T_S V_I - r_Y T_S V_Y - d_S T_S - r_P T_S R + m_{RI} T_{RI} \\
\frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I \\
\frac{dT_Y}{dt} &= r_Y T_S V_Y - d_I T_Y + r_Y T_{RI} V_Y \\
\frac{dT_{RI}}{dt} &= r_P T_S R - r_Y T_{RI} V_Y - (d_S + m_{RI}) T_{RI} + m_{RY} T_{RY} \\
\frac{dT_{RY}}{dt} &= -(d_S + m_{RY}) T_{RY}
\end{aligned}
\tag{2.3}
$$

for $t \neq t_k$. Here r_P is the rate at which the drug inhibits the wild-type T cells when drug concentrations are intermediate.

For $R > R_2$, the dynamics of virions and T cells are given by:

$$\begin{aligned}
\frac{dV_I}{dt} &= n_I \omega T_I - d_V V_I - r_I T_S V_I \\
\frac{dV_Y}{dt} &= n_I \omega T_Y - d_V V_Y - r_Y T_S V_Y - r_Y T_{RI} V_Y \\
\frac{dV_{NI}}{dt} &= n_I (1-\omega)(T_I + T_Y) - d_V V_{NI} \\
\frac{dT_S}{dt} &= \lambda - r_I T_S V_I - r_Y T_S V_Y - d_S T_S - r_R T_S R + m_{RI} T_{RI} \\
\frac{dT_I}{dt} &= r_I T_S V_I - d_I T_I \\
\frac{dT_Y}{dt} &= r_Y T_S V_Y - d_I T_Y + r_Y T_{RI} V_Y \\
\frac{dT_{RI}}{dt} &= r_R T_S R - r_Y T_{RI} V_Y - (d_S + m_{RI}) T_{RI} + m_{RY} T_{RY} - r_Q T_{RI} R \\
\frac{dT_{RY}}{dt} &= r_Q T_{RI} R - (d_S + m_{RY}) T_{RY}
\end{aligned} \tag{2.4}$$

for $t \neq t_k$. Here r_R and r_Q are the rates at which the drug inhibits the wild-type and drug-resistant T cells, respectively, when drug concentrations are high.

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 addition, the dynamics of the drug, R are given by:

$$\begin{aligned}
&\frac{dR}{dt} = -d_R R \qquad t \neq t_k \\
&\text{with impulsive conditions} \qquad \Delta R = R^i \qquad t = t_k.
\end{aligned} \tag{2.5}$$

Here, d_R is the rate at which the drug is cleared and R^i is the dosage.

Note that, using (2.1), we have

$$R(t_k^+) = R(t_k^-) + R^i. \tag{2.6}$$

The impulse times t_k can be assumed fixed, reflecting regular dosing periods, although we can set t_1 to be significantly large to reflect the fact that drugs are not taken until after the infection has been diagnosed. We will likewise assume that $R(0) = 0$.

Thus (2.2)–(2.4), together with (2.5) describe our three-regime model of impulsive differential equations.

3. Asymptotic behaviour

For each region, we find equilibria and (some) impulsive periodic orbits by equating the non-impulsive derivatives to zero. We then determine the stability of these equilibria and orbits, in order to understand the behaviour of trajectories in each region.

3.1. Region 1: low drug levels

System (2.2) has equilibria

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}) = \left(0, 0, 0, \frac{\lambda}{d_S}, 0, 0, 0, 0\right),$$
$$\left(\bar{V}_I, 0, \frac{n_I(1-\omega)}{d_V}\bar{T}_I, \frac{d_V d_I}{r_I(n_I\omega - d_I)}, \bar{T}_I, 0, 0, 0\right),$$
$$\left(0, \bar{V}_Y, \frac{n_I(1-\omega)}{d_V}\bar{T}_Y, \frac{d_V d_I}{r_Y(n_I\omega - d_I)}, 0, \bar{T}_Y, 0, 0\right),$$

where

$$\bar{V}_I = \frac{\lambda r_I(n_I\omega - d_I) - d_V d_I d_S}{r_I d_V d_I} \qquad \bar{V}_Y = \frac{\lambda r_Y(n_I\omega - d_I) - d_V d_I d_S}{r_Y d_V d_I}$$
$$\bar{T}_I = \frac{\lambda r_I(n_I\omega - d_I) - d_V d_I d_S}{r_I d_I(n_I\omega - d_I)} \qquad \bar{T}_Y = \frac{\lambda r_Y(n_I\omega - d_I) - d_V d_I d_S}{r_Y d_I(n_I\omega - d_I)}.$$

We shall refer to these three equilibria as the disease-free equilibrium, the wild-type equilibrium and the mutant equilibrium, respectively.

The Jacobian matrix for Region 1 (described by (2.2) and (2.5)) is $J_1 = [J_1^{(a)}|J_1^{(b)}]$ where

$$J_1^{(a)} = \begin{bmatrix} -d_V - r_I T_S & 0 & 0 & -r_I V_I \\ 0 & -d_V - r_Y T_S - r_Y T_{RI} & 0 & -r_Y V_Y \\ 0 & 0 & -d_V & 0 \\ -r_I T_S & -r_Y T_S & 0 & -r_I V_I - r_Y V_Y - d_S \\ r_I T_S & 0 & 0 & r_I V_I \\ 0 & r_Y T_S + r_Y T_{RI} & 0 & r_Y V_Y \\ 0 & -r_Y T_{RI} & 0 & 0 \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

$$J_1^{(b)} = \begin{bmatrix} n_I\omega & 0 & 0 & 0 & 0 \\ 0 & n_I\omega & -r_Y V_Y & 0 & 0 \\ n_I(1-\omega) & n_I(1-\omega) & 0 & 0 & 0 \\ 0 & 0 & m_{RI} & 0 & 0 \\ -d_I & 0 & 0 & 0 & 0 \\ 0 & -d_I & r_Y V_Y & 0 & 0 \\ 0 & 0 & -r_Y V_Y - d_S - m_{RI} & m_{RY} & 0 \\ 0 & 0 & 0 & -d_S - m_{RY} & 0 \\ 0 & 0 & 0 & 0 & -d_R \end{bmatrix}.$$

Note that $T_{RI} = T_{RY} = 0$ for all three equilibria. Thus, this matrix has characteristic equation

$$0 = \det(J_1(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, 0, 0, R) - \mu I)$$
$$= (d_V + \mu)(r_Y V_Y + d_S + m_{RI} + \mu)(d_S + m_{RY} + \mu)(d_R + \mu) f_1(\mu),$$

where $f_1(\mu)$ is the determinant of

$$\begin{bmatrix} -d_V - r_I T_S - \mu & 0 & -r_I V_I & n_I\omega & 0 \\ 0 & -d_V - r_Y T_S - \mu & -r_Y V_Y & 0 & n_I\omega \\ -r_I T_S & -r_Y T_S & -r_I V_I - r_Y V_Y - d_S - \mu & 0 & 0 \\ r_I T_S & 0 & r_I V_I & -d_I - \mu & 0 \\ 0 & r_Y T_S & r_Y V_Y & 0 & -d_I - \mu \end{bmatrix}$$

and V_I, V_Y and T_S are equilibrium values.

For the disease-free equilibrium, $V_I = 0$, $V_Y = 0$ and $T_S = \frac{\lambda}{d_S}$, so we have

$$f_1(\mu) = -(d_S + \mu)(\mu^2 + a_1\mu + b_1)(\mu^2 + a_2\mu + b_2)$$

where

$$\begin{aligned} a_1 &= d_V + d_I + \frac{r_I\lambda}{d_S} \\ b_1 &= d_I\left(d_V + \frac{r_I\lambda}{d_S}\right) - \frac{n_I\omega r_I\lambda}{d_S} \\ a_2 &= d_V + d_I + \frac{r_Y\lambda}{d_S} \\ b_2 &= d_I\left(d_V + \frac{r_Y\lambda}{d_S}\right) - \frac{n_I\omega r_Y\lambda}{d_S}. \end{aligned}$$

We have

$$b_2 = d_V d_I + \frac{r_Y\lambda}{d_S}(d_I - n_I\omega) < 0$$

usually, since n_I is large compared to the other constants and ω is not too close to zero. Thus there is an eigenvalue with positive real part. It follows that the disease-free equilibrium of system (2.2), (2.5) is usually unstable.

For the mutant equilibrium, $V_I = 0$, $V_Y = \frac{\lambda r_Y(n_I\omega - d_I) - d_V d_I d_S}{r_Y d_V d_I}$ and $T_S = \frac{d_V d_I}{r_Y(n_I\omega - d_I)}$, so we have

$$f_1(\mu) = (\mu^2 + a_3\mu + b_3)\det\begin{bmatrix} -d_V - r_Y T_S - \mu & -r_Y V_Y & n_I\omega \\ -r_Y T_S & -r_Y V_Y - d_S - \mu & 0 \\ r_Y T_S & r_Y V_Y & -d_I - \mu \end{bmatrix}$$

where

$$\begin{aligned} a_3 &= d_V + d_I + r_I T_S \\ b_3 &= d_V d_I + r_I T_S(d_I - n_I\omega) \\ &= d_V d_I + r_I \frac{d_V d_I}{r_Y(n_I\omega - d_I)}(d_I - n_I\omega) \\ &= \frac{d_V d_I(r_Y - r_I)}{r_Y} \\ &< 0 \end{aligned}$$

since $r_I > r_Y$. Thus there is an eigenvalue with positive real part. It follows that the mutant equilibrium of system (2.2), (2.5) is unstable.

For the wild-type equilibrium, $V_I = \frac{\lambda r_I(n_I\omega - d_I) - d_V d_I d_S}{r_I d_V d_I}$, $V_Y = 0$ and $T_S = \frac{d_V d_I}{r_I(n_I\omega - d_I)}$, so we have

$$f_1(\mu) = (\mu^2 + a_4\mu + b_4)\det\begin{bmatrix} -d_V - r_I T_S - \mu & -r_I V_I & n_I\omega \\ -r_I T_S & -r_I V_I - d_S - \mu & 0 \\ r_I T_S & r_I V_I & -d_I - \mu \end{bmatrix}$$

where

$$\begin{aligned} a_4 &= d_V + d_I + r_Y T_S \\ b_4 &= d_V d_I + r_Y T_S(d_I - n_I\omega) \\ &= d_V d_I + r_Y \frac{d_V d_I}{r_I(n_I\omega - d_I)}(d_I - n_I\omega) \\ &= \frac{d_V d_I(r_I - r_Y)}{r_I} \\ &> 0 \end{aligned}$$

since $r_I > r_Y$. Next,

$$\det\begin{bmatrix} -d_V - r_I T_S - \mu & -r_I V_I & n_I\omega \\ -r_I T_S & -r_I V_I - d_S - \mu & 0 \\ r_I T_S & r_I V_I & -d_I - \mu \end{bmatrix} = -\mu^3 - a_5\mu^2 - b_5\mu - c_5$$

where

$$\begin{aligned} a_5 &= d_V + d_I + d_S + r_I T_S + r_I V_I \\ b_5 &= d_V d_I + d_V d_S + d_I d_S + r_I d_V V_I + r_I d_S T_S + r_I d_I V_I + r_I(d_I - n_I\omega)T_S \\ &= d_V d_I + r_I(n_I\omega - d_I)\left(\frac{\lambda}{d_I} + \frac{\lambda}{d_V} - T_S\right) + r_I d_S T_S \\ &= d_V d_I + r_I(n_I\omega - d_I)\left(\frac{\lambda r_I(n_I\omega - d_I)(d_V + d_I) - d_V^2 d_I^2}{r_I d_V d_I(n_I\omega - d_I)}\right) + r_I d_S T_S \\ &> 0 \end{aligned}$$

usually, since n_I and λ are large compared to the other constants and ω is not too close to zero. Finally,

$$\begin{aligned} c_5 &= d_V d_I d_S + r_I d_V d_I V_I + r_I d_S(d_I - n_I\omega)T_S \\ &= r_I(n_I\omega - d_I)(\lambda - d_S T_S) \\ &> 0 \end{aligned}$$

since $T_S < \frac{\lambda}{d_S}$. Hence, all eigenvalues usually have negative real part. It follows that the wild-type equilibrium is usually stable.

Thus, when there are low or zero drug levels, the wild-type virus dominates, barring any internal periodic orbits or chaotic attractors (but simulations in Section 5 do not show any such behaviour).

3.2. Region 2: intermediate drug levels

In this case, there are no equilibria, due to the impulsive nature of the drugs, which do not reach equilibrium, but rather approach an impulsive periodic orbit, as we shall

show in Section 4. However, we can find impulsive periodic orbits by setting the left hand side of system (2.3) equal to zero. This yields four impulsive periodic orbits: disease-free (extinction of virus and infected cells), wild-type (extinction of mutant), mutant (extinction of wild-type) and an interior periodic orbit (both strains coexist). We denote equilibrium solutions by $\bar{X}$ and impulsive periodic orbits by X^*. In all cases, there is an impulsive periodic orbit R^* satisfying (2.5) and $R_1 < R(t) < R_2$.

The disease-free periodic orbit is in the form

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) = (0, 0, 0, T_S^*, 0, 0, T_{RI}^*, 0, R^*),$$

where

$$T_S^* = \frac{\lambda(d_S + m_{RI})}{d_S(d_S + m_{RI} + r_P R^*)}$$
$$T_{RI}^* = \frac{\lambda r_P R^*}{d_S(d_S + m_{RI} + r_P R^*)}.$$

The wild-type periodic orbit is in the form

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) = (V_I^*, 0, V_{NI}^*, \bar{T}_S, T_I^*, 0, T_{RI}^*, 0, R^*),$$

where

$$V_I^* = \frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{d_S(d_S + m_{RI} + r_P R^*)}{r_I(d_S + m_{RI})}$$
$$V_{NI}^* = \frac{n_I r_I(1-\omega)}{d_V d_I}\bar{T}_S V_I^*$$
$$\bar{T}_S = \frac{d_V d_I}{r_I(n_I\omega - d_I)}$$
$$T_I^* = \frac{r_I}{d_I}\bar{T}_S V_I^*$$
$$T_{RI}^* = \frac{r_P d_V d_I R^*}{r_I(n_I\omega - d_I)(d_S + m_{RI})}.$$

Note that this orbit is only positive when $V_I^* > 0$.

The mutant periodic orbit is in the form

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) = (0, \bar{V}_Y, \bar{V}_{NI}, T_S^*, 0, \bar{T}_Y, T_{RI}^*, 0, R^*),$$

where

$$\bar{V}_Y = \frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{d_S}{r_Y}$$
$$\bar{V}_{NI} = \frac{n_I(1-\omega)}{n_I\omega - d_I}\bar{V}_Y$$
$$T_S^* = \frac{d_V d_I(\lambda r_Y(n_I\omega - d_I) + m_{RI} d_V d_I)}{r_Y(n_I\omega - d_I)[\lambda r_Y(n_I\omega - d_I) + d_V d_I(d_S + m_{RI} + r_P R^*)]}$$
$$\bar{T}_Y = \frac{d_V}{n_I\omega - d_I}\bar{V}_Y$$

$$T_{RI}^* = \frac{d_V d_I}{r_Y(n_I\omega - d_I)} - T_S^*.$$

This orbit is usually positive, since λ and n_I are large compared to the other constants.

The interior periodic orbit is in the form

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) = (V_I^*, V_Y^*, \bar{V}_{NI}, \bar{T}_S, T_I^*, T_Y^*, \bar{T}_{RI}, 0, R^*),$$

where

$$V_I^* = \frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{r_P R^*}{r_I - r_Y} + \frac{m_{RI}}{r_Y}$$

$$V_Y^* = \frac{r_P R^*}{r_I - r_Y} - \frac{d_S + m_{RI}}{r_Y}$$

$$\bar{V}_{NI} = \frac{n_I(1-\omega)}{n_I\omega - d_I}\left[\frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{d_S}{r_Y}\right]$$

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

$$T_I^* = \frac{d_V}{n_I\omega - d_I} V_I^*$$

$$T_Y^* = \frac{d_V}{n_I\omega - d_I} V_Y^*$$

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

Note that the interior periodic orbit is positive only if

$$R_1 > \frac{(d_S + m_{RI})(r_I - r_Y)}{r_P r_Y} \quad \text{and} \quad R_2 < \frac{r_I - r_Y}{r_P}\left(\frac{\lambda(n_I\omega - d_I)}{d_V d_I} + \frac{m_{RI}}{r_Y}\right).$$

However, we expect that $r_I \approx r_Y$ and R_1 is not too close to zero, so the former will usually be true, whereas the latter is usually true since λ and n_I are large compared to the other constants (and in practice λ may be of the same order as $\frac{1}{r_I - r_Y}$). Furthermore,

$$V_I + V_Y = \frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{d_S}{r_Y}.$$

Since λ and n_I are large, we expect this to be positive.

The Jacobian matrix for Region 2 (described by (2.3) and (2.5)) is $J_2 = [J_2^{(a)} | J_2^{(b)}]$ where

$$J_2^{(a)} = \begin{bmatrix} -d_V - r_I T_S & 0 & 0 & -r_I V_I \\ 0 & -d_V - r_Y(T_S + T_{RI}) & 0 & -r_Y V_Y \\ 0 & 0 & -d_V & 0 \\ -r_I T_S & -r_Y T_S & 0 & -r_I V_I - r_Y V_Y - d_S - r_P R \\ r_I T_S & 0 & 0 & r_I V_I \\ 0 & r_Y(T_S + T_{RI}) & 0 & r_Y V_Y \\ 0 & -r_Y T_{RI} & 0 & r_P R \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

$$J_2^{(b)} = \begin{bmatrix} n_I\omega & 0 & 0 & 0 & 0 \\ 0 & n_I\omega & -r_Y V_Y & 0 & 0 \\ n_I(1-\omega) & n_I(1-\omega) & 0 & 0 & 0 \\ 0 & 0 & m_{RI} & 0 & -r_P T_S \\ -d_I & 0 & 0 & 0 & 0 \\ 0 & -d_I & r_Y V_Y & 0 & 0 \\ 0 & 0 & -r_Y V_Y - d_S - m_{RI} & m_{RY} & r_P T_S \\ 0 & 0 & 0 & -d_S - m_{RY} & 0 \\ 0 & 0 & 0 & 0 & -d_R \end{bmatrix}.$$

This matrix has characteristic equation

$$\begin{aligned} 0 &= \det(J_1(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, 0, R) - \mu I) \\ &= -(d_V + \mu)(d_S + m_{RY} + \mu)(d_R + \mu) f_2(\mu), \end{aligned}$$

where $f_2(\mu) = \det[F_2^{(a)} | F_2^{(b)}]$, for

$$F_2^{(a)} = \begin{bmatrix} -d_V - r_I T_S - \mu & 0 & -r_I V_I \\ 0 & -d_V - r_Y(T_S + T_{RI}) - \mu & -r_Y V_Y \\ -r_I T_S & -r_Y T_S & -r_I V_I - r_Y V_Y - d_S - r_P R - \mu \\ r_I T_S & 0 & r_I V_I \\ 0 & r_Y(T_S + T_{RI}) & r_Y V_Y \\ 0 & -r_Y T_{RI} & r_P R \end{bmatrix}$$

$$F_2^{(b)} = \begin{bmatrix} n_I\omega & 0 & 0 \\ 0 & n_I\omega & -r_Y V_Y \\ 0 & 0 & m_{RI} \\ -d_I - \mu & 0 & 0 \\ 0 & -d_I - \mu & r_Y V_Y \\ 0 & 0 & -r_Y V_Y - d_S - m_{RI} - \mu \end{bmatrix}$$

and V_I, V_Y and T_S are equilibrium or impulsive periodic solutions.

For the disease-free periodic orbit, $V_I = V_Y = 0$, $T_S = \frac{\lambda(d_S + m_{RI})}{d_S(d_S + m_{RI} + r_P R^*)}$ and $T_{RI} = \frac{\lambda r_P R^*}{d_S(d_S + m_{RI} + r_P R^*)}$, so we have

$$f_2(\mu) = (\mu^2 + e_1\mu + g_1)(\mu^2 + e_2\mu + g_2)(\mu^2 + e_3\mu + g_3)$$

where

$$\begin{aligned} e_1 &= 2d_S + m_{RI} + r_P R^* \\ g_1 &= d_S(d_S + m_{RI} + r_P R^*) \\ e_2 &= d_V + d_I + r_I \frac{\lambda(d_S + m_{RI})}{d_S(d_S + m_{RI} + r_P R^*)} \\ g_2 &= d_V d_I + r_I(d_I - n_I\omega) \frac{\lambda(d_S + m_{RI})}{d_S(d_S + m_{RI} + r_P R^*)} \\ e_3 &= d_V + d_I + r_Y \frac{\lambda}{d_S} \\ g_3 &= d_V d_I + r_Y(d_I - n_I\omega) \frac{\lambda}{d_S} < 0 \end{aligned}$$

usually, since n_I is large and ω is not too close to zero. Thus there is an eigenvalue with positive real part. It follows that the disease-free periodic orbit of system (2.3) and (2.5) is usually unstable.

For the mutant orbit, $V_Y = \frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{d_S}{r_Y}$, $T_S + T_{RI} = \frac{d_V d_I}{r_Y(n_I\omega - d_I)}$ and $T_S = \frac{d_V d_I(\lambda r_Y(n_I\omega - d_I) + m_{RI} d_V d_I)}{r_Y(n_I\omega - d_I)[\lambda r_Y(n_I\omega - d_I) + d_V d_I(d_S + m_{RI} + r_P R^*)]}$, so we have

$$f_2(\mu) = (\mu^2 + e_4\mu + g_4)\det M_0$$

where

$$e_4 = d_I + d_V + r_I T_S$$
$$g_4 = d_V d_I - r_I T_S(n_I\omega - d_I)$$
$$M_0 = \begin{bmatrix} -d_V - r_Y(T_S + T_{RI}) + \mu & -r_Y V_Y & n_I\omega & -r_Y V_Y \\ -r_Y T_S & -r_Y V_Y - d_S - r_R R - \mu & 0 & m_{RI} \\ r_Y(T_S + T_{RI}) & r_Y V_Y & -d_I - \mu & r_Y V_Y \\ -r_Y T_{RI} & r_P R & 0 & -r_Y V_Y - d_S - m_{RI} - \mu \end{bmatrix}.$$

We have

$$\begin{aligned} g_4 &= d_V d_I - \frac{r_I d_V d_I(\lambda r_Y(n_I\omega - d_I) + m_{RI} d_V d_I)}{r_Y[\lambda r_Y(n_I\omega - d_I) + d_V d_I(d_S + m_{RI} + r_P R^*)]} \\ &= \frac{d_V d_I[(\lambda r_Y(n_I\omega - d_I) + d_V d_I m_{RI})(r_Y - r_I) + d_V d_I(d_S + r_P R^*) r_Y]}{r_Y[\lambda r_Y(n_I\omega - d_I) + d_V d_I(d_S + m_{RI} + r_P R^*)]} \\ &< 0 \end{aligned}$$

usually, since $r_Y < r_I$ and λ and n_I are large. Thus, the mutant orbit is usually unstable.

Proposition 3.1. *When there are intermediate drug levels, the wild-type and mutant virus will coexist.*

Proof. Suppose the wild-type periodic orbit is stable. Then since it has the property that $\bar{V}_Y = 0$, we must have $\frac{dV_Y(t)}{dt} \le 0$ for an appropriate orbit when t is sufficiently large (note that $V_Y(t)$ is continuous for all t). Suppose $V_Y(\tau) = \epsilon > 0$ for some sufficiently large time τ. Furthermore, for some γ satisfying $0 < \gamma < \frac{d_V}{r_I}$ we have

$$\frac{d_V d_I}{r_I(n_I\omega - d_I)} < T_S(\tau) < \frac{d_V d_I}{r_I(n_I\omega - d_I)} + \gamma$$
$$\frac{r_P d_V d_I R_1}{r_I(n_I\omega - d_I)(d_S + m_{RI})} < T_{RI}(\tau) < \frac{r_P d_V d_I R_2}{r_I(n_I\omega - d_I)(d_S + m_{RI})}$$

since $R_1 < R(\tau) < R_2$. Then we have

$$T_Y(\tau) > \frac{r_Y}{d_I}\left(\frac{d_V d_I}{r_I(n_I\omega - d_I)} + \frac{r_P d_V d_I R_1}{r_I(n_I\omega - d_I)(d_S + m_{RI})}\right)\epsilon.$$

Thus

$$\begin{aligned} \frac{dV_Y(\tau)}{dt} &> n_I\omega r_Y\epsilon\left(\frac{d_V}{r_I(n_I\omega - d_I)} + \frac{r_P d_V R_1}{r_I(n_I\omega - d_I)(d_S + m_{RI})}\right) \\ &\quad - r_Y\epsilon\left(\frac{d_V d_I}{r_I(n_I\omega - d_I)} + \gamma + \frac{r_P d_V d_I R_2}{r_I(n_I\omega - d_I)(d_S + m_{RI})}\right) - d_V\epsilon \end{aligned}$$

$$= \frac{r_Y \epsilon}{r_I(n_I\omega - d_I)(d_S + m_{RI})}[r_P d_V(n_I\omega R_1 - d_I R_2) + (d_V - \gamma r_I)(n_I\omega - d_I)(d_S + m_{RI})] - d_V\epsilon > 0$$

usually, since $n_I\omega \gg \frac{d_I R_2}{R_1} > d_I$ for n_I large and ω not too close to zero. It follows that for τ sufficiently large, $V_Y(\tau)$ is increasing, which is a contradiction. Thus the wild-type periodic orbit is unstable. Since the disease-free and mutant orbits are also unstable, it follows that the mutant and wild-type strains will coexist in Region 2. □

3.3. Region 3: high drug levels

If $r_R \geq r_Q$, system (2.3) has three impulsive periodic orbits: disease free (extinction of virus and infected cells), wild type (extinction of mutant) and mutant (extinction of wild type). In all cases, there is an impulsive periodic orbit R^* satisfying (2.5) and $R(t) > R_2$. If $r_R < r_Q$ then there is also an interior impulsive periodic orbit, but we expect from the dose effect curves that this will not be the case.

The disease-free periodic orbit is in the form

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) = (0, 0, 0, T_S^*, 0, 0, T_{RI}^*, T_{RY}^*, R^*),$$

where

$$T_S^* = \frac{\lambda[(d_S + m_{RY})(d_S + m_{RI}) + d_S r_Q R^*]}{d_S[(d_S + m_{RY})(d_S + m_{RI} + r_R R^*) + r_Q R^*(d_S + r_R R^*)]}$$

$$T_{RI}^* = \frac{\lambda(d_S + m_{RY}) r_R R^*}{d_S[(d_S + m_{RY})(d_S + m_{RI} + r_R R^*) + r_Q R^*(d_S + r_R R^*)]}$$

$$T_{RY}^* = \frac{\lambda r_R r_Q (R^*)^2}{d_S[(d_S + m_{RY})(d_S + m_{RI} + r_R R^*) + r_Q R^*(d_S + r_R R^*)]}.$$

The wild-type periodic orbit is in the form

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) = (V_I^*, 0, V_{NI}^*, \bar{T}_S, T_I^*, 0, T_{RI}^*, T_{RY}^*, R^*),$$

where

$$V_I^* = \frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{d_S}{r_I} - \frac{r_R R^*}{r_I} + \frac{m_{RI}(d_S + m_{RY}) r_R R^*}{r_I[(d_S + m_{RY})(d_S + m_{RI}) + d_S r_Q R^*]}$$

$$V_{NI}^* = \frac{n_I(1-\omega)}{d_V} T_I^*$$

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

$$T_I^* = \frac{r_I}{d_I} \bar{T}_S V_I^*$$

$$T_{RI}^* = \frac{d_V d_I (d_S + m_{RY}) r_R R^*}{r_I(n_I\omega - d_I)[(d_S + m_{RY})(d_S + m_{RI}) + d_S r_Q R^*]}$$

$$T_{RY}^* = \frac{d_V d_I r_R r_Q (R^*)^2}{r_I(n_I\omega - d_I)[(d_S + m_{RY})(d_S + m_{RI}) + d_S r_Q R^*]}.$$

Note that the wild-type orbit only exists if

$$\frac{\lambda r_I(n_I\omega - d_I)}{d_V d_I}\theta - (d_S + r_R R^*)\theta + m_{RI}(d_S + m_{RY})r_R R^* > 0 \tag{3.7}$$

where $\theta = (d_S + m_{RY})(d_S + m_{RI}) + d_S r_Q R^*$. It turns out that this condition is also an important stability condition for the disease-free periodic orbit, as we shall demonstrate shortly.

The mutant periodic orbit is in the form

$$(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) = (0, V_Y^*, V_{NI}^*, \bar{T}_S, 0, T_Y^*, T_{RI}^*, T_{RY}^*, R^*),$$

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

$$\eta(T_S^*)^2 + \left[\zeta(r_R R^* + m_{RI} - \eta) + \lambda\right] T_S^* - \zeta(\lambda + m_{RI}\zeta) = 0$$

for

$$\eta = \frac{d_S r_Q R^*}{d_S + m_{RY}}$$

$$\zeta = \frac{d_V d_I}{r_Y(n_I\omega - d_I)}$$

and where

$$V_Y^* = \frac{\lambda}{r_Y T_S^*} - \frac{d_S}{r_Y} - \frac{r_R R^*}{r_Y} + \frac{m_{RI} T_{RI}^*}{r_Y T_S^*}$$

$$V_{NI}^* = \frac{n_I(1-\omega)}{n_I\omega - d_I} V_Y^*$$

$$T_Y^* = \frac{d_V}{n_I\omega - d_I} V_Y^*$$

$$T_{RI}^* = \frac{d_V d_I}{r_Y(n_I\omega - d_I)} - T_S^*$$

$$T_{RY}^* = \frac{r_Q R^*}{d_S + m_{RY}} T_{RI}^*.$$

This orbit is only positive if $V_Y^* > 0$.

The Jacobian matrix for Region 3 (described by (2.4) and (2.5)) is $J_3 = [J_3^{(a)} | J_3^{(b)}]$ where

$$J_3^{(a)} = \begin{bmatrix} -d_V - r_I T_S & 0 & 0 & -r_I V_I \\ 0 & -d_V - r_Y(T_S + T_{RI}) & 0 & -r_Y V_Y \\ 0 & 0 & -d_V & 0 \\ -r_I T_S & -r_Y T_S & 0 & -r_I V_I - r_Y V_Y - d_S - r_R R \\ r_I T_S & 0 & 0 & r_I V_I \\ 0 & r_Y(T_S + T_{RI}) & 0 & r_Y V_Y \\ 0 & -r_Y T_{RI} & 0 & r_R R \\ 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

$$J_3^{(b)} = \begin{bmatrix} n_I\omega & 0 & 0 & 0 & 0 \\ 0 & n_I\omega & -r_Y V_Y & 0 & 0 \\ n_I(1-\omega) & n_I(1-\omega) & 0 & 0 & 0 \\ 0 & 0 & m_{RI} & 0 & -r_R T_S \\ -d_I & 0 & 0 & 0 & 0 \\ 0 & -d_I & r_Y V_Y & 0 & 0 \\ 0 & 0 & -r_Y V_Y - d_S - m_{RI} - r_Q R & m_{RY} & r_R T_S - r_Q T_{RI} \\ 0 & 0 & r_Q R & -d_S - m_{RY} & r_Q T_{RI} \\ 0 & 0 & 0 & 0 & -d_R \end{bmatrix}.$$

This matrix has characteristic equation

$$\begin{aligned} 0 &= \det(J_1(V_I, V_Y, V_{NI}, T_S, T_I, T_Y, T_{RI}, T_{RY}, R) - \mu I) \\ &= (d_V + \mu)(d_R + \mu) f_3(\mu), \end{aligned}$$

where $f_3(\mu) = \det[F_3^{(a)} | F_3^{(b)}]$ for

$$F_3^{(a)} = \begin{bmatrix} -d_V - r_I T_S - \mu & 0 & -r_I V_I \\ 0 & -d_V - r_Y(T_S + T_{RI}) - \mu & -r_Y V_Y \\ -r_I T_S & -r_Y T_S & -r_I V_I - r_Y V_Y - d_S - r_R R - \mu \\ r_I T_S & 0 & r_I V_I \\ 0 & r_Y(T_S + T_{RI}) & r_Y V_Y \\ 0 & -r_Y T_{RI} & r_R R \\ 0 & 0 & 0 \end{bmatrix}$$

$$F_3^{(b)} = \begin{bmatrix} n_I\omega & 0 & 0 & 0 \\ 0 & n_I\omega & -r_Y V_Y & 0 \\ 0 & 0 & m_{RI} & 0 \\ -d_I - \mu & 0 & 0 & 0 \\ 0 & -d_I - \mu & r_Y V_Y & 0 \\ 0 & 0 & -r_Y V_Y - d_S - m_{RI} - r_Q R - \mu & m_{RY} \\ 0 & 0 & r_Q R & -d_S - m_{RY} - \mu \end{bmatrix}$$

and V_I, V_Y and T_S are equilibrium or impulsive periodic solutions.

For the disease-free periodic orbit, $V_I = V_Y = 0$, so we have

$$f_3(\mu) = (\mu^2 + k_1\mu + l_1) \det M_1$$

where

$$\begin{aligned} k_1 &= d_V + d_I + r_I T_S \\ l_1 &= d_V d_I - r_I(n_I\omega - d_I) T_S \end{aligned}$$

and where

$$M_1 = \begin{bmatrix} -d_V - r_Y(T_S + T_{RI}) - \mu & 0 & n_I\omega & 0 & 0 \\ -r_Y T_S & -d_S - r_R R - \mu & 0 & m_{RI} & 0 \\ r_Y(T_S + T_{RI}) & 0 & -d_I - \mu & 0 & 0 \\ -r_Y T_{RI} & r_R R & 0 & -d_S - m_{RI} - r_Q R - \mu & m_{RY} \\ 0 & 0 & 0 & r_Q R & -d_S - m_{RY} - \mu \end{bmatrix}.$$

We have

$$l_1 = d_V d_I - \frac{\lambda r_I(n_I\omega - d_I)[(d_S + m_{RY})(d_S + m_{RI}) + d_S r_Q R^*]}{d_S[(d_S + m_{RY})(d_S + m_{RI} + r_R R^*) + r_Q R^*(d_S + r_R R^*)]}$$

$$= \frac{d_V d_I \left[(d_S + r_R R^*)\theta - m_{RI}(d_S + m_{RY}) r_R R^* - \frac{\lambda r_I (n_I\omega - d_I)}{d_V d_I}\theta\right]}{d_S[(d_S + m_{RY})(d_S + m_{RI} + r_R R^*) + r_Q R^*(d_S + r_R R^*)]}$$
$$< 0$$

if and only if the wild-type orbit exists, by Eq. (3.7). Thus, if the wild-type orbit exists, then there is an eigenvalue with positive real part and hence the disease-free periodic orbit of system (2.4) and (2.5) will be unstable.

For the wild-type periodic orbit, $V_Y = 0$, so we have

$$f_3(\mu) = (\mu^2 + k_2\mu + l_2)\det M_2$$

where

$$k_2 = d_V + d_I + r_Y(T_S + T_{RI})$$
$$l_2 = d_V d_I - r_Y(n_I\omega - d_I)(T_S + T_{RI})$$
$$M_2 = \begin{bmatrix} -d_V - r_I T_S - \mu & -r_I V_I & n_I\omega & 0 & 0 \\ -r_I T_S & -r_I V_I - d_S - r_R R - \mu & 0 & m_{RI} & 0 \\ r_I T_S & r_I V_I & -d_I - \mu & 0 & 0 \\ 0 & r_R R & 0 & -d_S - m_{RI} - r_Q R - \mu & m_{RY} \\ 0 & 0 & 0 & r_Q R & -d_S - m_{RY} \end{bmatrix}.$$

We have

$$l_2 = d_V d_I - r_Y(T_S^* + T_{RI}^*)(n_I\omega - d_I)$$
$$= \frac{d_V d_I[((d_S + m_{RI})(d_S + m_{RY}) + d_S r_Q R^*)(r_I - r_Y) - r_Y(d_S + m_{RY}) r_R R^*]}{r_I[(d_S + m_{RI})(d_S + m_{RY}) + d_S r_Q R^*]}$$
$$< 0$$

for realistic parameters, since $r_I \approx r_Y$. Thus there is an eigenvalue with positive real part. It follows that the wild-type periodic orbit of system (2.4) and (2.5) is usually unstable.

The disease-free orbit will be stable if

$$\frac{\lambda(n_I\omega - d_I)}{d_V d_I} - \frac{d_S}{r_I} - \frac{r_R R^i e^{-d_R\tau}}{r_I(1 - e^{-d_R\tau})} + \frac{m_{RI}(d_S + m_{RY}) r_R R^i}{r_I[(d_S + m_{RY})(d_S + m_{RI})(1 - e^{-d_R\tau}) + d_S r_Q R^i e^{-d_R\tau}]} < 0.$$

Solving for the positive root of the quadratic, the disease-free orbit will be stable if

$$R^i > \frac{(1 - e^{-d_R\tau})}{2a}\left[b + \sqrt{b^2 + 4ac}\right] \tag{3.8}$$

where

$$a = r_R e^{-2d_R\tau} d_S r_Q$$
$$b = \left[\frac{\lambda r_I(n_I\omega - d_I)}{d_V d_I} - d_S\right] d_S r_Q e^{-d_R\tau} - r_R e^{-d_R\tau}(d_S + m_{RY})(d_S + m_{RI}) + m_{RI}(d_S + m_{RY}) r_R$$
$$c = \left[\frac{\lambda r_I(n_I\omega - d_I)}{d_V d_I} - d_S\right](d_S + m_{RY})(d_S + m_{RI}).$$

Since a and c are usually positive, it follows that the right hand side of (3.8) is real and positive. Thus, there is a nonempty region of parameter space where the disease-free orbit will be stable. In particular, for τ large, the condition is approximately $R^i > e^{2d_R\tau} m_{RI}(d_S + m_{RY})/r_Q d_S$. We shall refer to this subset of Region 3 as the region of viral elimination, since viral elimination is possible, although not guaranteed, for these parameter values. See Section 5 for further results on this region.

Note that condition (3.8) is actually a stronger condition than needed to ensure the stability of the disease-free orbit; that is, there may be regions of parameter space for which the disease-free orbit is stable, but condition (3.8) is not met. In contrast, condition (3.7) gives an "if and only if" condition for stability. Unlike Eq. (3.8), however, Eq. (3.7) depends on R^*, the periodic orbit of the drug, which may be difficult to estimate in practice.

3.4. Summary of asymptotic behaviour

In summary for this section, then, we find that at low drug levels resistance does not emerge, and a stable equilibrium is predicted between the wild-type virus, T cells infected by the wild-type virus, and healthy T cells. In contrast at intermediate drug levels, 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 virus, but have negligible effect on the drug-resistant strain.

In the third case, if the summed effect of all preventative drugs reduces replication of *both* the wild-type and drug-resistant viral strains ("high" drug levels), one of two scenarios can occur. Either drug resistance will emerge, or 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 free virus in our model is not equivalent to clearing the infection.) The latter case is possible when condition (3.7) is *not* fulfilled. For realistic parameter values, we can approximate condition (3.7) as $\lambda r_I n_I \omega > r_R R^* d_V d_I$. Thus elimination of free virus is possible if the number of infectious virions produced per infected cell, or the infectivity of these virions, is not too large, or if the periodic orbit of the drug and the drug efficacy are not too small. These conditions make intuitive sense, and the exact magnitude of the parameters required in order for viral elimination to be possible is given by condition (3.7). As mentioned above, (3.8) is an alternative expression for the same condition; the difference is that (3.8) is a stronger condition than strictly necessary, but may be more easily evaluated in practice.

4. Equilibrium T cell counts

In this section, we examine the total T cell count at equilibrium or at the stable periodic orbit(s) predicted for low, intermediate and high drug concentrations.

Suppose the drugs are given at fixed intervals. Let $\tau = t_{k+1} - t_k$ be the dosing interval (for $k \geq 1$). For t satisfying $t_k < t \leq t_{k+1}$, we have

$$R(t) = R(t_k^+)e^{-d_R(t-t_k)}.$$

The impulsive effect means we have a recursion relation at the moments of impulse, given by (2.6). Thus

$$R(t_k^+) = R^i \frac{1 - e^{-kd_R\tau}}{1 - e^{-d_R\tau}} \to \frac{R^i}{1 - e^{-d_R\tau}}$$

as $k \to \infty$.

Note that

$$\begin{aligned} R(t_k^+) - \frac{R^i}{1 - e^{-d_R\tau}} &= R^i \frac{1 - e^{-kd_R\tau}}{1 - e^{-d_R\tau}} - \frac{R^i}{1 - e^{-d_R\tau}} \\ &= -\frac{R^i e^{-kd_R\tau}}{1 - e^{-d_R\tau}} < 0. \end{aligned}$$

However, if $R(0) = \frac{R^i}{1-e^{-d_R\tau}}$, then $R(\tau^-) = \frac{R^i e^{-d_R\tau}}{1-e^{-d_R\tau}}$ and so

$$\begin{aligned} R(\tau^+) &= \frac{R^i e^{-d_R\tau}}{1 - e^{-d_R\tau}} + R^i \\ &= \frac{R^i}{1 - e^{-d_R\tau}}. \end{aligned}$$

It follows that the impulse points $\frac{R^i}{1-e^{-d_R\tau}}$ and $\frac{R^i e^{-d_R\tau}}{1-e^{-d_R\tau}}$ define the ends of a positive impulsive periodic orbit in drug concentration, to which the endpoints of each cycle monotonically increase. In particular, since $R(0) < \frac{R^i}{1-e^{-d_R\tau}}$, it follows that

$$R(t) \le \frac{R^i}{1 - e^{-d_R\tau}} \tag{4.9}$$

for all t. Since the impulsive drug orbits are asymptotically stable, it follows that for any $\epsilon > 0$ there exists t_1 such that

$$R(t) > \frac{R^i e^{-d_R\tau}}{1 - e^{-d_R\tau}} - \epsilon \tag{4.10}$$

for all $t > t_1$.

For low drug levels ($R < R_1$), we know from Section 3.1 that the wild-type virus dominates. Furthermore, since n_I is large, $\bar{T}_S$ is small. Thus the immune system is maintained primarily by T_I cells when the drug concentrations are low. The total T cell count at the stable (wild-type) equilibrium is

$$\bar{T}_S + \bar{T}_I = \frac{\lambda}{d_I} + \frac{d_V(d_I - d_S)}{r_I(n_I\omega - d_I)}. \tag{4.11}$$

Since $d_S < d_I$, this will be significantly smaller than $\frac{\lambda}{d_S}$, the T cell count in the disease-free state (see Smith and Wahl, 2004).

For intermediate drug levels ($R_1 < R < R_2$), we use the results of Section 3.2 to show that the total T cell count for the interior periodic orbit is

$$\bar{T}_S + T_I^* + T_Y^* + \bar{T}_{RI} = \frac{\lambda}{d_I} + \frac{d_V(d_I - d_S)}{r_Y(n_I\omega - d_I)}. \tag{4.12}$$

The same comments as above apply to (4.12). However, it should be noted that we have not shown that the interior orbit is necessarily the orbit to which trajectories approach.

There may be other interior periodic orbits, or more complex behaviour in which both strains coexist.

For high drug levels, we examine the effect on the T cell count as the dosing intervals shrink to zero, or as the doses increase to infinity. This implies that trajectories will ultimately reside in Region 3, since drug concentrations will eventually accumulate beyond the cut-off value R_2.

To evaluate these limits, we will frequently use the following straightforward lemma:

Lemma 4.1. *Suppose x is a variable satisfying*

$$x'(t) < c - q(\phi)x(t)$$

where c is a constant and $q(\phi)$ is independent of x and t. Then

(a) *If $x(0) < \frac{c}{q(\phi)}$ it follows that*

$$x(t) < \frac{c}{q(\phi)}$$

for all t.

(b) *If $x(0) < \frac{c}{q(\phi)}$ and $\lim_{\phi\to 0} q(\phi) = \infty$ it follows that*

$$x(t) \to 0$$

as $\phi \to 0$ for all t.

(See Smith and Wahl (2004) for a proof.)

Remark. Lemma 4.1 also holds if the inequalities are reversed.

We assume initial conditions to reflect the very earliest stages of the virus, with no drug effects initially. Thus

$$\begin{aligned} &0 < V_Y(0) \ll V_I(0) \ll \frac{n_I\lambda}{d_S d_V} \\ &0 \ll T_S(0) \le \frac{\lambda}{d_S} \end{aligned} \tag{4.13}$$

and $T_I(0) = T_Y(0) = T_{RI}(0) = T_{RY}(0) = R(0) = 0$.

Note that for all models, we have, using Lemma 4.1,

$$\begin{aligned} T_S' + T_I' + T_Y' + T_{RI}' + T_{RY}' &= \lambda - d_S(T_S + T_{RI} + T_{RY}) - d_I(T_I + T_Y) \\ &\le \lambda - d_S(T_S + T_{RI} + T_{RY} + T_I + T_Y) \\ T_S + T_{RI} + T_{RY} + T_I + T_Y &\le \frac{\lambda}{d_S}. \end{aligned} \tag{4.14}$$

Lemma 4.2. *For Region 3, $V_j < \frac{n_I\lambda}{d_S d_V}$ for $j = I, Y$, and*

$$\begin{aligned} T_S &> \frac{\lambda d_S d_V(1 - \mathrm{e}^{-d_R\tau})}{(2r_I n_I\lambda + d_S^2 d_V)(1 - \mathrm{e}^{-d_R\tau}) + r_R R^i d_S d_V} \\ &\to 0 \end{aligned}$$

as $\tau \to 0$ or $R^i \to \infty$.

Proof. From model (2.4) and using Lemma 4.1 and (4.14), we have

$$\begin{aligned} V_j' &< \frac{n_I\lambda}{d_S} - d_V V_j \\ V_j &< \frac{n_I\lambda}{d_S d_V}. \end{aligned}$$

Using this result, (4.10), Lemma 4.1, (4.13), (4.14) and the fact that $r_I > r_Y$, we have,

$$\begin{aligned} T_S' &> \lambda - 2r_I\frac{n_I\lambda}{d_S d_V}T_S - d_S T_S - r_R T_S\frac{R^i}{1-\mathrm{e}^{-d_R\tau}} \\ T_S &> \frac{\lambda d_S d_V(1-\mathrm{e}^{-d_R\tau})}{(2r_I n_I\lambda + d_S^2 d_V)(1-\mathrm{e}^{-d_R\tau}) + r_R R^i d_S d_V} \\ &\equiv \alpha(\tau) \to 0 \end{aligned}$$

as $\tau \to 0$ or $R^i \to \infty$. □

Theorem 4.1. *As $t \to \infty$ and either $\tau \to 0$, or $R^i \to \infty$, $T_S, T_I, T_Y, T_{RI} \to 0$ and $T_{RY} \to T_{RY}^{(\infty)}$ in Region 3, where $T_{RY}^{(\infty)}$ satisfies*

$$\frac{\lambda}{d_S + m_{RY}} \le T_{RY}^{(\infty)} \le \frac{\lambda}{d_S}. \tag{4.15}$$

Proof. From model (2.4) and using (4.10) and Lemma 4.1 we have, for $t > t_1$,

$$\begin{aligned} T_S' &< \lambda - d_S T_S - \frac{r_R R^i \mathrm{e}^{-d_R\tau}}{1-\mathrm{e}^{-d_R\tau}}T_S + \epsilon r_R T_S + \frac{\lambda m_{RI}}{d_S} \\ T_S &< \frac{\lambda(d_S+m_{RI})(1-\mathrm{e}^{-d_R\tau})}{d_S\left[r_R R^i \mathrm{e}^{-d_R\tau} + (d_S-\epsilon r_R)(1-\mathrm{e}^{-d_R\tau})\right]} \\ &\quad - \left(\frac{\lambda(d_S+m_{RI})(1-\mathrm{e}^{-d_R\tau})}{d_S\left[r_R R^i \mathrm{e}^{-d_R\tau} + (d_S-\epsilon r_R)(1-\mathrm{e}^{-d_R\tau})\right]} - T_S(0)\right) \\ &\quad \times \exp\left[-\frac{\lambda(d_S+m_{RI})(1-\mathrm{e}^{-d_R\tau})t}{d_S\left[r_R R^i \mathrm{e}^{-d_R\tau} + (d_S-\epsilon r_R)(1-\mathrm{e}^{-d_R\tau})\right]}\right] \\ &\equiv \beta(t,\tau) \\ &\to 0 \end{aligned}$$

as $t \to \infty$ and either $\tau \to 0$ or $R^i \to \infty$. Next,

$$\begin{aligned} T_I' &< \frac{r_I\beta(t,\tau)\lambda n_I}{d_S d_V} - d_I T_I \\ T_I &< \frac{r_I\beta(t,\tau)\lambda n_I}{d_S d_I d_V} \\ &\to 0 \end{aligned}$$

as $t \to \infty$ and $\tau \to 0$.

The right hand side of (4.15) follows from (4.14). From model (2.4), and using (4.9), (4.10), Lemmas 4.1 and 4.2, for any $\epsilon > 0$, there exists t_1 such that

$$\begin{aligned} T'_{RI} &> \frac{\alpha(\tau) r_R R^i \mathrm{e}^{-d_R\tau}}{1-\mathrm{e}^{-d_R\tau}} - r_R\epsilon\alpha(\tau) - \left(\frac{\lambda n_I r_Y}{d_S d_V} + d_S + m_{RI} + \frac{r_Q R^i}{1-\mathrm{e}^{-d_R\tau}}\right) T_{RI} \\ T_{RI} &> \frac{\alpha(\tau) r_R R^i \mathrm{e}^{-d_R\tau} - r_R\epsilon\alpha(\tau)(1-\mathrm{e}^{-d_R\tau})}{\left(\frac{\lambda n_I r_Y}{d_S d_V} + d_S + m_{RI}\right)(1-\mathrm{e}^{-d_R\tau}) + r_Q R^i} \\ &\equiv \gamma(\tau). \end{aligned}$$

Then if ϵ is sufficiently small, we have

$$\begin{aligned} T'_{RY} &> \left(\frac{r_Q R^i \mathrm{e}^{-d_R\tau}}{1-\mathrm{e}^{-d_R\tau}} - r_Q\epsilon\right)\gamma(\tau) - (d_S + m_{RY}) T_{RY} \\ T_{RY} &> \frac{\alpha(\tau) r_Q r_R (R^i)^2 \mathrm{e}^{-2d_R\tau} - (1-\mathrm{e}^{-d_R\tau})\epsilon\alpha(\tau) r_Q r_R [2R^i \mathrm{e}^{-d_R\tau} - \epsilon(1-\mathrm{e}^{-d_R\tau})]}{(d_S + m_{RY})\left[\left(\frac{\lambda n_I r_Y}{d_S d_V} + d_S + m_{RI}\right)(1-\mathrm{e}^{-d_R\tau}) + r_Q R^i\right](1-\mathrm{e}^{-d_R\tau})}. \end{aligned}$$

As $\tau \to 0$, we have

$$\begin{aligned} &\alpha(\tau) \to 0 \\ &\frac{\alpha(\tau)}{1-\mathrm{e}^{-d_R\tau}} \to \frac{\lambda}{r_R R^i}. \end{aligned}$$

Thus,

$$T_{RY}^{(\infty)} \geq \frac{\lambda}{d_S + m_{RY}}.$$

Using (4.9) and (4.10), Lemma 4.1 and (4.14), for ϵ sufficiently small, there exists t_2 such that

$$\begin{aligned} T'_{RI} &< \frac{\beta(t,\tau) r_R R^i}{1-\mathrm{e}^{-d_R\tau}} - (d_S + m_{RI}) T_{RI} + \frac{\lambda m_{RY}}{d_S} - \frac{r_Q R^i \mathrm{e}^{-d_R\tau}}{1-\mathrm{e}^{-d_R\tau}} T_{RI} + r_Q\epsilon T_{RI} \\ T_{RI} &< \frac{\beta(t,\tau) r_R R^i + \frac{\lambda m_{RY}}{d_S}(1-\mathrm{e}^{-d_R\tau})}{(d_S + m_{RI} - r_Q\epsilon)(1-\mathrm{e}^{-d_R\tau}) + r_Q R^i \mathrm{e}^{-d_R\tau}} \\ &\equiv \delta(t,\tau) \\ &\to 0 \end{aligned}$$

as $t \to \infty$ and either $\tau \to 0$ or $R^i \to \infty$.

Finally, using Lemmas 4.1 and 4.2, we have

$$\begin{aligned} T'_Y &= r_Y(T_S + T_{RI}) V_Y - d_I T_Y \\ &< r_Y(\beta(t,\tau) + \delta(t,\tau)) \frac{\lambda n_I}{d_S d_V} - d_I T_Y \\ T_Y &< r_Y(\beta(t,\tau) + \delta(t,\tau)) \frac{\lambda n_I}{d_I d_S d_V} \\ &\to 0 \end{aligned}$$

as $t \to \infty$ and either $\tau \to 0$ or $R^i \to \infty$. □

4.1. Summary of T cell results

In this section we have shown that although drug resistance does not emerge at low drug levels, the total T cell count will be significantly lower than T cell counts in the disease-free state.

At intermediate drug levels, the total T cell count will not be very different from the T cell count at low drug levels (compare Eqs. (4.11) and (4.12)).

At high drug levels, the total T cell count will be less than *or equal to* cell counts in the disease-free state, and will be dominated by the population T_{RY}, those cells that have absorbed sufficient drugs to prevent infection by either viral strain. Interestingly, all other types of T cells will be driven to zero as drug concentrations increase.

5. Numerical simulations

Putting together the results from Sections 3 and 4, we demonstrate some of the likely behaviour, given that drug concentrations will likely move through all three regions. Intuitively, we expect that when $R(t)$ is high, the mutant strain of the virus should dominate, but in relatively low numbers. Then as $R(t)$ falls to intermediate levels, the wild-type strain can coexist with the mutant. When $R(t)$ becomes low, the wild-type virus gains control. Depending on the amount of time the drug spends in each region (if any), trajectories will likely oscillate, with either coexistence, one or the other strain gaining dominance, or the drugs eliminating both strains.

After the transient behaviour has settled into a periodic orbit, we will have

$$\frac{R^i e^{-d_R\tau}}{1-e^{-d_R\tau}} \leq R(t) \leq \frac{R^i}{1-e^{-d_R\tau}}.$$

It follows that trajectories will remain solely in Region 1 if

$$0 < R^i < R_1(1-e^{-d_R\tau}),$$

whereas trajectories will be outside Region 1 if

$$R^i > R_1 e^{d_R\tau}(1-e^{-d_R\tau}).$$

Trajectories will remain solely within Region 2 if

$$R_1 e^{d_R\tau}(1-e^{-d_R\tau}) < R^i < R_2(1-e^{-d_R\tau})$$

whereas trajectories will be outside Region 2 if either

$$R^i < R_1(1-e^{-d_R\tau}) \qquad \text{or} \qquad R^i > R_2 e^{d_R\tau}(1-e^{-d_R\tau}).$$

Finally, trajectories will remain solely within Region 3 if

$$R^i > R_2 e^{d_R\tau}(1-e^{-d_R\tau})$$

whereas trajectories will be outside Region 3 if

$$R^i < R_2(1-e^{-d_R\tau}).$$

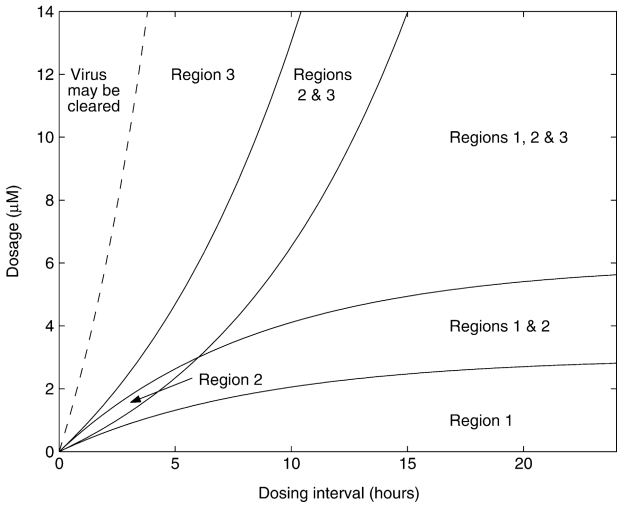

Fig. 2. The possible combinations of regions that trajectories of drug concentrations may traverse, for given dosages and dosing intervals. Parameters used were $n_I = 262.5$ day^{-1}, $\omega = 0.8$, $r_I = 0.02$ day^{-1}, $r_Y = 0.01$ day^{-1}, $d_V = 3$ day^{-1}, $d_S = 0.1$ day^{-1}, $d_I = 0.5$ day^{-1}, $r_P = r_R = 40$ μM^{-1} day^{-1}, $r_Q = 10.4$ μM^{-1} day^{-1}, $d_R = 24\log(2)/6$ day^{-1}, $\lambda = 180$ cells μL^{-1} day^{-1}, $m_{RI} = m_{RY} = 24\log(2)/8$ day^{-1}, $R_1 = 3$ μM and $R_2 = 6$ μM. These parameters are similar to those described in greater detail in Smith and Wahl (2004); in particular the value of r_R assumes that trough concentrations are approximately sufficient to inhibit viral replication in all T cells at some point during their lifetime. The values of r_P and r_Q were estimated from r_R by comparing best-fit linear slopes in the dose–effect curves of Fig. 1B. We illustrate the case when the drug in use has a 6 h half-life in plasma, and an 8 h intracellular half-life.

Fig. 2 demonstrates which regions drug concentration trajectories will visit, for various combinations of dosing interval and dose. The curves plotted are

$$R^i = R_1(1 - e^{-d_R\tau})$$
$$R^i = R_1 e^{d_R\tau}(1 - e^{-d_R\tau})$$
$$R^i = R_2(1 - e^{-d_R\tau})$$
$$R^i = R_2 e^{d_R\tau}(1 - e^{-d_R\tau})$$

and the dotted curve is the curve given by equality in Eq. (3.8). Parameters are chosen from the literature and are similar to those described more fully in Smith and Wahl (2004) (see figure legend).

Figs. 3 and 4 illustrate the different cases found in Fig. 2. In all cases, parameters are as given in the legend to Fig. 2, with only the dosing interval τ and the dosage R^i varied. The figures give phase-plane plots of the populations of cells infected by the wild-type or drug-resistant viral strains. The insets in Fig. 3 show the total population of all classes of T cells; uninfected cell classes are shown to the left of the vertical line and infected cell classes to the right.

Fig. 3A demonstrates the behaviour when trajectories remain solely in Region 1. In this case there is no drug-resistant strain of the virus. T cells infected with the wild-type strain dominate, with all other T cells approaching zero. This demonstrates (4.11).

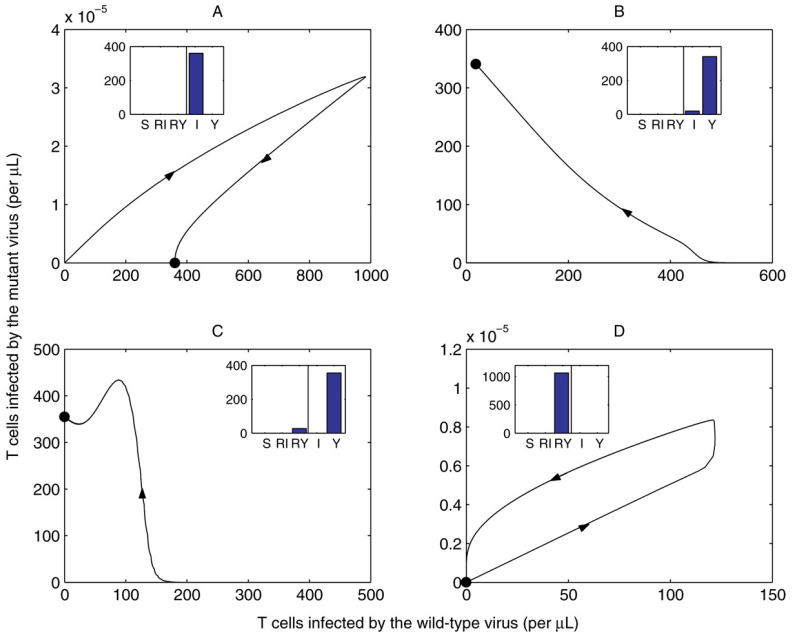

Fig. 3. Infected cell populations when trajectories of drug concentration remain solely within a region. Values of T_I and T_Y were estimated by numerical integration of system (2.2), (2.3) or (2.4) using a fourth/fifth order Runge–Kutta algorithm. Initial conditions were $V_I(0) = 500$, $T_S(0) = 1000$ and $V_Y(0) = 5 \times 10^{-5}$; all other initial populations were set to zero. All parameters except the dose and dosing interval are as for Fig. 2. The inset shows the size of each T cell population at the final time (shown as a solid circle on the phase-plane plot); three populations on the left are uninfected T cells, two populations on the right are infected cells. A: Region 1 ($\tau = 12$ h, $R^i = 1$ μM). In this case there is no 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 ($\tau = 2$ h, $R^i = 1$ μM). In this case both strains of the virus coexist. T cells infected with the mutant strain are approximately nine times as numerous as T cells infected with the wild-type strain. C: Region 3, where the dosing intervals and dosages are not too extreme ($\tau = 6$ h, $R^i = 8$ μM). In this case there are large numbers of T cells infected by the drug-resistant viral strain, and a small population of uninfected T cells inhibited with high drug levels. D: Region of viral elimination ($\tau = 0.3$ h, $R^i = 20$ μM). In this case both strains of the virus are eliminated. Uninfected T cells inhibited with high drug levels dominate, with all other T cells approaching zero. Furthermore, the total T cell count is similar to that of the uninfected immune system.

Fig. 3B demonstrates the behaviour when trajectories remain solely in Region 2. In this case both strains of the virus coexist. T cells infected with the mutant strain are approximately nine times as numerous as T cells infected with the wild-type strain. This demonstrates (4.12).

Fig. 3C demonstrates the behaviour when trajectories remain solely in Region 3, but where the dosing intervals and dosages are not too extreme. In this case there are high numbers of T cells infected by the drug-resistant strain, and a small population of uninfected T cells inhibited with high drug concentrations.

Fig. 3D demonstrates the behaviour when trajectories remain solely within the region of viral elimination. In this case both strains of the virus are eliminated. Uninfected T cells

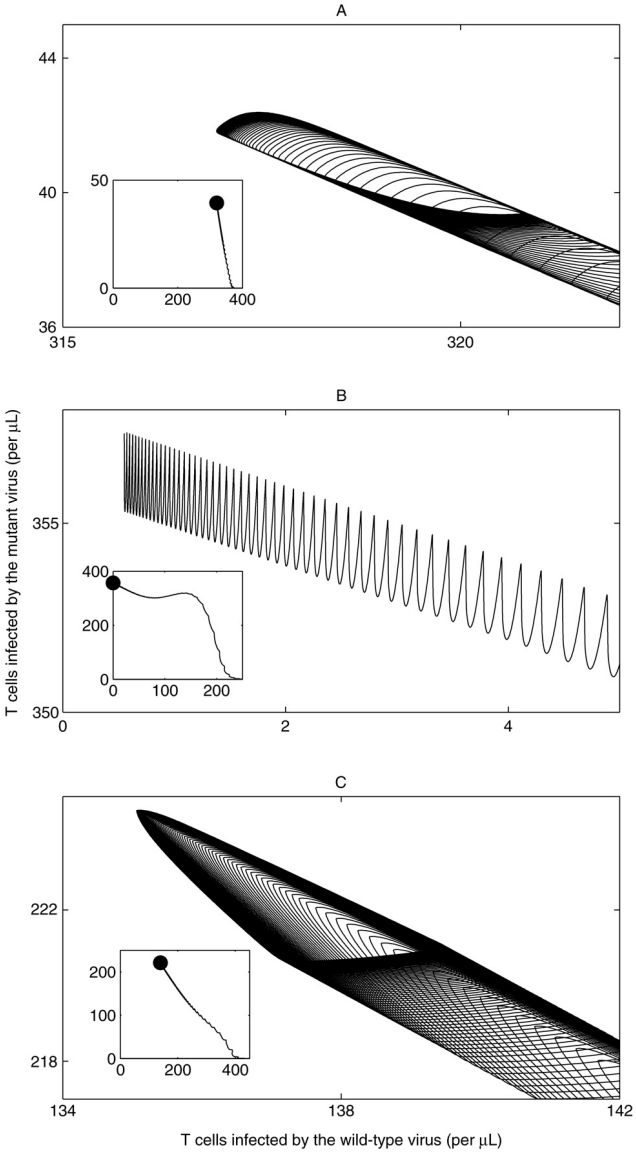

Fig. 4. The behaviour when trajectories of drug concentration move between regions. All parameters except the dose and dosing interval are as for Fig. 2. The main panels illustrate the periodic orbits obtained between cells infected by the wild-type and drug-resistant viral strains. The inset shows the overall phase-plane behaviour with a solid circle illustrating the final time; periodic orbits are not visible on this scale. A: Regions 1 and 2 ($\tau = 12$ h, $R^i = 3$ μM). In this case both strains of the virus coexist. T cells infected with the wild-type strain are approximately eight times as numerous as T cells infected with the mutant strain. B: Regions 2 and 3 ($\tau = 8$ h, $R^i = 8$ μM). In this case the mutant strain dominates, driving the wild-type virus toward extinction. Note that there are also low numbers of uninfected T cells inhibited with high drug levels (not shown). C: Regions 1, 2 and 3 ($\tau = 12$ h, $R^i = 6$ μM). In this case both strains of the virus coexist.

inhibited with high drug concentrations dominate, with all other T cells approaching zero. Furthermore, the total T cell count is similar to that of the uninfected immune system. This demonstrates Theorem 4.1. We note that for the dose–effect curves illustrated in Fig. 1B (modelled after the reverse transcriptase inhibitor AZT) the dose required to eliminate both the wild-type and drug-resistant virus would not be physiologically tolerable.

Fig. 4 plots similar results for situations in which the drug concentration moves between regions over time. Here we plot the periodic orbits observed in each case; the overall phase-plane behaviour is shown in the inset. Fig. 4A demonstrates the behaviour when trajectories move between Regions 1 and 2. In this case both strains of the virus coexist. Fig. 4B demonstrates the behaviour when trajectories move between Regions 2 and 3. In this case the mutant strain dominates, driving the wild-type viral strain toward extinction. We also observed a small population of uninfected T cells inhibited with high drug concentrations (not shown). Fig. 4C demonstrates the behaviour when trajectories move between all three regions. In this case both strains of the virus coexist once again.

6. Discussion

We consider an SIR-type model of immunological behaviour for HIV dynamics, including drugs that act in a manner similar to reverse transcriptase inhibitors, that is, they prevent the virus from transcribing its genome onto the host T cell DNA. Our model provides a novel approach to the question of drug resistance, using impulsive differential equations to model drug behaviour and classifying different model regimes according to whether the drug efficacy is negligible, intermediate or high. We consider two strains of the virus: a wild-type strain that is susceptible to both intermediate and high drug concentrations, and a mutant strain that is only susceptible to high drug concentrations.

This three-regime model is based on an underlying model of the appropriate dose–effect curves (Fig. 1B): at low doses, we assume that T cells will not absorb sufficient drug to counter either strain of the virus. Similarly, T cells which have absorbed sufficient drug to combat the wild-type strain of the virus may have inadequate drug concentration to counter the mutant strain, given that mutations often produce a 5- to 50-fold resistance to the drug in question. Only when the drug concentrations are sufficiently high will both strains of the virus be prevented from transcribing their RNA into the T cell DNA.

We find that impulsive differential equations are an ideal method of approaching such a scheme. This framework allows us to model dynamic changes in drug efficacy using a completely analytical approach. It should be noted, however, that there are several shortcomings to the model. First, we are assuming the drug effects are instantaneous, that is, that the “time to peak” of the drug is negligible on the time scale under consideration. Our results are therefore inaccurate for dosing intervals that shrink to a few hours or less (for example the bottom left corner of Fig. 2). Furthermore, even for longer dosing intervals, dispersion and delay as the drug enters the intracellular space may affect our conclusions. We are encouraged that the intracellular dynamics of some HIV pharmaceuticals are becoming available in recent literature (Hoggard and Back, 2002), and look forward to examining these effects in more detail in future work.

Our model predicts that if drug concentrations are uniformly low, drug resistance will not emerge, but the total T cell count is guaranteed to be significantly lower than in the disease-free state. In the "intermediate" dose range, we prove that drug resistance will emerge. At these drug levels, defined to be doses at which the drugs significantly inhibit replication of the wild-type virus, but have negligible effect on the drug-resistant strain, the total T cell count will be similar to T cell counts at low drug levels. Thus this intermediate range is the worst of both worlds; T cell counts and viral loads are similar to scenarios without drugs, but drug-resistant mutants are now successfully competing with the wild-type.

At high drug concentrations, either drug resistance will emerge, or the free viral population of both strains will be eliminated. As noted previously, elimination of free virus in our model is not equivalent to clearing the infection, since we have not explicitly considered longer-lived reservoirs of virus, such as latent T cells. Nonetheless the latter case is clearly optimal, and is possible if the dosing intervals and dosages are chosen from the region of viral elimination (Fig. 2), described by Eq. (3.8). In this case, we have also proven that as the dosing interval shrinks or the dosage increases, the population of T cells inhibited with high drug levels will approach T cell counts in the uninfected immune system, while all other classes of T cells will approach zero. Although drug toxicities may limit the extent to which this optimum can be approached, it is encouraging to prove that such a scenario is even theoretically possible for reverse transcriptase inhibitors and other "preventative" drugs. We hypothesize that this will *not* be possible for protease inhibitors (Smith and Wahl, 2004), and hope to incorporate this second class of drugs in future work.

In practice, a realistic dosing schedule may take trajectories through one, two or all three regimes. For example drug concentrations may start off high, decrease through intermediate levels and finally reach low levels before the next dose is taken. We illustrate some likely behaviours in Figs. 3 and 4. We also demonstrate that the relationship between the dosage and the dosing intervals will completely determine which region(s) trajectories will remain in over time (Fig. 2). Unfortunately, only when drug concentrations remain uniformly high will the uninfected T cell count be close to the disease-free state. Interestingly, Fig. 2 also predicts that in practice, decreasing the dosing interval for a fixed dosage is more likely to control the virus than increasing the dosage for a fixed dosing interval.

Finally, our model assumes that each and every dose is taken; we hope to examine the complex interplay of drug resistance and adherence (Wahl and Nowak, 2000; Huang et al., 2003) in future work.

References

Austin, D.J., Anderson, R.M., 1999. Studies of antibiotic resistance within the patient, hospitals and the community using simple mathematical models. Philos. Trans. R. Soc. Lond. B 354, 721–738.

Austin, D.J., White, N.J., Anderson, R.M., 1998. The dynamics of drug action on the within-host population growth of infectious agents: melding pharmacokinetics with pathogen population dynamics. J. Theor. Biol. 194, 313–339.

Bainov, D.D., Simeonov, P.S., 1989. Systems with Impulsive Effect. Ellis Horwood Ltd, Chichester.

Bainov, D.D., Simeonov, P.S., 1993. Impulsive Differential Equations: Periodic Solutions and Applications. Longman Scientific and Technical, Burnt Mill.

Bainov, D.D., Simeonov, P.S., 1995. Impulsive Differential Equations: Asymptotic Properties of the Solutions. World Scientific, Singapore.

Bonhoeffer, S., Nowak, M.A., 1997. Pre-existence and emergence of drug resistance in HIV-1 infection. Proc. R. Soc. Lond. B 264, 631–637.

Coffin, J.M., 1995. HIV population dynamics in vivo: implications for genetic variation, pathogenesis and therapy. Science 267, 483–489.

de Boer, R.J., Boucher, C.A., 1996. Anti-CD4 therapy for AIDs suggested by mathematical models. Proc. R. Soc. Lond. B 263, 899–905.

de Boer, R.J., Perelson, A.S., 1998. Target cell limited and immune control models of HIV infections: a comparison. J. Theor. Biol. 190, 201–214.

de Jong, M.D., Veenstra, J., Stilianakis, N.I., Schuurman, R., Lange, J.M., de Boer, R.J., Boucher, C.A., 1996. Host-parasite dynamics and outgrowth of virus containing a single K70R amino acid change in reverse transcriptase are responsible for the loss of HIV-1 RNA load suppression by zidovudine. Proc. Natl. Acad. Sci. 93 (11), 5501–5506.

Frost, S.D.W., McLean, A.R., 1994. Germinal centre destruction as a major pathway of HIV pathogenesis. J. AIDS 7, 236–244.

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

Hoggard, P.G., Back, D.J., 2002. Intracellular pharmacology of nucleoside analogues and protease inhibitors: role of transport molecules. Curr. Opin. Infect. Dis. 15, 3–8.

Huang, Y., Rosenkranz, S.L., Wu, H., 2003. Modeling HIV dynamics and antiviral response with consideration of time-varying drug exposures, adherence and phenotypic sensitivity. Math. Biosci. 184, 165–186.

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

Kirschner, D.E., Webb, G.F., 1996. A model for the treatment strategy in the chemotherapy of AIDS. Bull. Math. Biol. 58, 367–391.

Lakshmikantham, V., Bainov, D.D., Simeonov, P.S., 1989. Theory of Impulsive Differential Equations. World Scientific, Singapore.

Lipsitch, M., Levin, B.R., 1998. The population dynamics of tuberculosis chemotherapy: mathematical models of the roles of noncompliance and bacterial heterogeneity in the evolution of drug resistance. Int. J. Tuberc. Lung Dis. 2, 187–199.

McLean, A.R., Nowak, M.A., 1992. Competition between zidovudine-sensitive and zidovudine-resistant strains of HIV. AIDS 6, 71–79.

Nowak, M.A., Anderson, R.M., McLean, A.R., Wolfs, T.F., Goudsmit, J., May, R.M., 1991. Antigenic diversity thresholds and the development of AIDS. Science 254, 963–969.

Nowak, M.A., Bonhoeffer, S., Shaw, G.M., May, R.M., 1997. Anti-viral drug treatment: dynamics of resistance in free virus and infected cell populations. J. Theor. Biol. 184, 203–217.

Perelson, A.S., 2002. Modelling viral and immune system dynamics. Nat. Rev.: Immunol. 2, 28–36.

Perelson, A.S., Essunger, P., Cao, Y., Vesanen, M., Hurley, A., Saksela, K., Markowitz, M., Ho, D.D., 1997. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 387, 188–191.

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.

Ribeiro, R.M., Bonhoeffer, S., 2000. Production of resistant HIV mutants during antiretroviral therapy. Proc. Natl. Acad. Sci. USA 97, 7681–7686.

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 (5), 1259–1283.

Stilianakis, N.I., Boucher, C.A., de Jong, M.D., Van Leeuwen, R., Schuurman, R., de Boer, R.J., 1997. Clinical data sets on human immunodeficiency virus type 1 reverse transcriptase resistant mutants explained by a mathematical model. J. Virol. 71, 161–168.

Wahl, L.M., Nowak, M.A., 2000. Adherence and drug resistance: predictions for therapy outcome. Proc. R. Soc. Lond. B 267, 835–843.

Wein, L.M., D'Amato, R.M., Perelson, A.S., 1998. Mathematical considerations of antiretroviral therapy aimed at HIV-1 eradication or maintenance of low viral loads. J. Theor. Biol. 192, 81–98.

Wodarz, D., 2001. Helper-dependent vs. helper-independent CTL responses in HIV infection: implications for drug therapy and resistance. J. Theor. Biol. 213, 447–459.

Wodarz, D., Nowak, M.A., 1999. Specific therapy regimes could lead to long-term immunological control of HIV. Proc. Natl. Acad. Sci. USA 96, 14464–14469.

Wodarz, D., Page, K., Arnaout, R., Thomsen, A.R., Lifson, J.D., Nowak, M.A., 2000. A new theory of cytotoxic T-lymphocyte memory: implications for HIV treatment. Philos. Trans. R. Soc. Lond. B 355, 329–343.