A Mathematical Analysis of Rebound in a Target-Mediated Drug Disposition Model. I. Without Feedback

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Abstract

We consider the possibility of free receptor (antigen/cytokine) levels rebounding to higher than the baseline level after one or more applications of an antibody drug using a target-mediated drug disposition model. Using geometry and dynamical systems analysis, we show that rebound will occur if and only if the elimination rate of the drug-receptor product is slower than the elimination rates of the drug and of the receptor. We also analyse the magnitude of rebound through approximations and simulations and demonstrate that it increases if the drug dose increases or if the difference between the elimination rate of the drug-receptor product and the minimum of the elimination rates of the drug and of the receptor increases.

1 Introduction

An interesting property of protein-therapeutic antibody interactions is the potential of the antibody to change the distribution and clearance pathways of the target protein. This could, in turn, result in modified concentrations of the target protein in blood and in other organs. For example, binding and neutralisation of cytokines such as interleukins by blocking/neutralising antibodies causes an increase in the total levels of the cytokine (Meno-Tetang and Lowe, 2005). This increase is due to the blockade of the clearance pathways for the cytokine - the large drug-target complex cannot be cleared through the kidneys and since the complex cannot bind to the target, target-mediated clearance is also impaired. This complexation process may also have other less well-understood consequences such as the potential for release of the target from the complex at a later time, and distribution of the target to tissues and organs due to the longer plasma half-life and potential release of target in the tissues.

In this paper, we will investigate the phenomenon of receptor rebound, i.e., a post-dose rise in receptor levels to higher than pre-dose (baseline). The increase of the antigen - "antigen rebound" - has been sparsely studied and only anecdotal reports exist in the literature. For example, rebound symptoms have been reported on cessation of anti-tumour necrosis factor (TNF) therapies (Bravo Vergal et al, 2007) and a corresponding increase in TNF levels have been demonstrated to occur in patients (Bhatia and Kast, 2007). Similarly, treatment with an anti-IL6 antibody has also been shown to increase total IL6 activity (Klein et al, 1995) and an increase in tumour size on cessation of VEGF treatment has also been reported (Cacheux et al, 2008). Apart from the pharmacokinetic (PK) interaction described previously, there might be multiple other reasons for the rebound in target levels after cessation of treatment with an antibody - an increase in the production rate of the target antigen due to homeostatic feedback and residual bio-activity of the antibody-target complex, magnified by the vastly higher levels of the complex are two apparent reasons.

The potential for PK interaction between the target protein and antibody to result in rebound in free antigen levels is only now being appreciated (Stefanini et al, 2010; Krippendorff and Huisinga,

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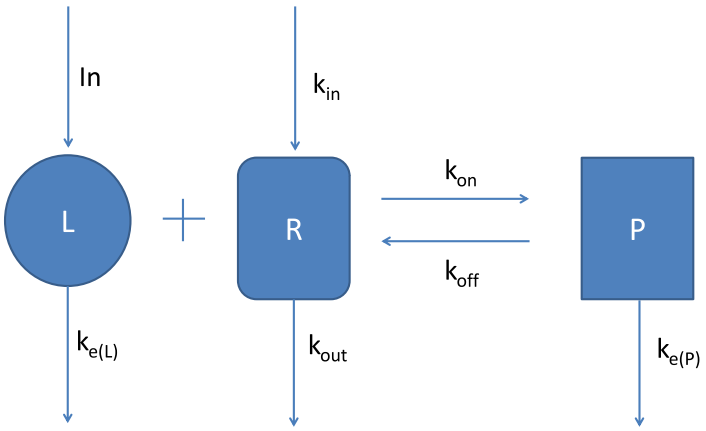

Figure 1: The TMDD reaction mechanism

2009). Stefanini et al (2010) use a semi-physiological pharmacokinetic model to hypothesise that the observed increase in VEGF levels after bevacizumab dosing may be due to the diffusion properties of the drug, VEGF, and the complex across various tissues. Krippendorff and Huisinga (2009) provide analytical proof that ligand accumulation occurs when its clearance pathway is blocked, as with a standard blocking/neutralising antibody approach. However, a systematic evaluation of the physiological conditions and target/antibody PKPD properties that could result in the occurrence of antigen rebound is not yet available. Such analysis could be critical in designing antibody therapies and dosing regimen that are unlikely to result in antigen rebound and, therefore, maximise the therapeutic potential of the target.

We consider when a simple protein-antibody interaction results in rebound in protein levels in a target-mediated drug disposition (TMDD) model, the same one as we considered previously (Aston et al, 2011) to predict potency. Defining receptor rebound as the post-dose rise in receptor levels to higher than pre-dose (baseline), we show that, under the assumptions of the basic TMDD model, rebound will happen if and only if the elimination rate of the target-drug complex is slower than both the elimination rate of the drug and the elimination rate of the target.

In Section 2, we describe the TMDD model that we will work with and state some important properties of the model, including global asymptotic stability of the baseline state. In Section 3, we derive precise conditions on the parameters for the existence and non-existence of rebound in this model. Section 4 contains a discussion of the results and some conclusions. The proofs of the properties in Section 2 are presented in the Appendix.

2 The TMDD Model

We consider a one-compartment TMDD model based on the original work by Levy (1994) and Mager and Jusko (2001) where the drug ligand L binds reversibly with the receptor R to form a receptor-ligand complex P as shown in Fig. 1. The TMDD model assumes a mechanism-based reaction to explain the drug-receptor interaction. The parameters of the model are the binding rate constants k_{on} and k_{off}, the receptor turnover and elimination rates k_{in} and k_{out}, and the elimination rates of the ligand and complex $k_{\text{e(L)}}$ and $k_{\text{e(P)}}$. The drug ligand L, receptor R, and receptor-ligand complex P are all concentrations. The system is assumed to be initially at steady state, into which a single bolus infusion L_0 of the ligand into the central (plasma) compartment is made (represented in Fig. 1 by 'In'). The differential equations that comprise the mathematical model for this system are given by

$$\frac{dL}{dt} = -k_{\text{e(L)}}L - k_{\text{on}}LR + k_{\text{off}}P \tag{1}$$

$$\frac{dR}{dt} = k_{\text{in}} - k_{\text{out}}R - k_{\text{on}}LR + k_{\text{off}}P \tag{2}$$

$$\frac{dP}{dt} = k_{\text{on}} LR - k_{\text{off}} P - k_{\text{e(P)}} P \tag{3}$$

A steady state of this system is given by $L = P = 0$, $R = k_{\text{in}}/k_{\text{out}}$. Adding the bolus injection L_0 gives the initial conditions

$$L(0) = L_0, \qquad R(0) = R_0 = \frac{k_{\text{in}}}{k_{\text{out}}}, \qquad P(0) = 0.$$

We non-dimensionalise these equations (in the same way as used by Peletier and Gabrielsson (2009)) by defining the dimensionless variables

$$x = \frac{L}{L_0}, \quad y = \frac{R}{R_0}, \quad z = \frac{P}{R_0}, \quad \tau = k_{\text{on}} R_0 t.$$

In terms of these non-dimensional quantities, equations (1)–(3) become

$$\dot{x} = -k_1 x - xy + \mu k_2 z \tag{4}$$

$$\dot{y} = k_3(1 - y) - \frac{xy}{\mu} + k_2 z \tag{5}$$

$$\dot{z} = \frac{xy}{\mu} - (k_2 + k_4)\, z \tag{6}$$

where dot denotes differentiation with respect to τ, with initial conditions

$$x(0) = 1, \qquad y(0) = 1, \qquad z(0) = 0,$$

and the dimensionless parameters are defined by

$$\mu = \frac{R_0}{L_0}, \quad k_1 = \frac{k_{\text{e(L)}}}{k_{\text{on}} R_0}, \quad k_2 = \frac{k_{\text{off}}}{k_{\text{on}} R_0}, \quad k_3 = \frac{k_{\text{in}}}{k_{\text{on}} R_0^2} = \frac{k_{\text{out}}}{k_{\text{on}} R_0}, \quad k_4 = \frac{k_{\text{e(P)}}}{k_{\text{on}} R_0}.$$

Clearly, this choice of non-dimensionalisation requires that $k_{\text{on}} \neq 0$ and $k_{\text{in}} \neq 0$ (so that $R_0 \neq 0$). We note that all parameters must be non-negative due to their physical meaning, and additionally we will assume that they are in fact all strictly positive. The three variables x, y, and z are related to physical quantities and so must also be non-negative. After the ligand is added to the system in its baseline state (corresponding to $(x, y, z) = (0, 1, 0)$), initially the receptor level decreases, but after a while it goes up again and returns to its baseline value. Rebound occurs if in the return to the baseline value, the receptor level increases to values above the baseline.

To support the rebound analysis, in this section we will show that the TMDD model has indeed an invariant positive octant $x, y, z \geq 0$ (as required by their physical meaning); that there is a unique steady state (the baseline state) in the positive octant, which is a global attractor. We also derive the local behaviour near the baseline value and show some properties of the total amount of drugs and the total amount of receptor. The proofs are straightforward and can be found in Appendix A.

Lemma 2.1 *[Peletier and Gabrielsson (2012b)] Assuming that $k_{\text{on}} > 0$ and $k_{\text{in}} > 0$, the octant of $\mathbf{R}^3$ defined by $x, y, z \geq 0$ is invariant under the flow of the equations (4)–(6) and the y-axis is an invariant line.*
In the original variables: the octant $L, R, P \geq 0$ is invariant under the flow of the equations (1)–(3) and the receptor axis is an invariant line, i.e., if there is initially no ligand and product, then this situation persists for all time.

Next we consider steady state solutions of the non-dimensional equations (4)–(6). We show that the steady state corresponding to $L = P = 0$ and $R = R_0$ is the only physically relevant steady state and is a global attractor.

Lemma 2.2 *In the region of* $\mathbf{R}^3$ *defined by* $x, y, z \geq 0$*, equations (4)–(6) have a unique steady state, given by*

$$x = 0, \quad y = 1, \quad z = 0. \tag{7}$$

In the original variables: the baseline state $L = P = 0$*,* $R = R_0$*, is a unique steady state in the positive invariant octant* $L, R, P \geq 0$*.*

It is possible to prove a global stability result for this steady state. Before we get to this, we first mention a few other global properties of the dynamical system which can be found for example in Peletier and Gabrielsson (2012a,b, 2009). The total amount of ligand is given by $L + P$, hence its non-dimensional version is $u = x + \mu z$. From the differential equation for $u(\tau)$, it follows easily that the total amount of ligand is always decreasing and converges to 0 for time τ going to infinity. The total amount of receptor is $R + P$ and its non-dimensional version is $v = y + z$. Initially the non-dimensional total amount of receptor is $v(0) = 1$. As shown by Peletier and Gabrielsson (2012b, 2009), if $k_3 \geq k_4$, i.e., the elimination rate of the receptor is faster than or equal to the elimination rate of the product, then the total amount of receptor stays above or at its initial value. On the other hand, if the elimination rate of the receptor is slower than or equal to the elimination rate of the product, then the total amount of receptor stays below or at its initial value.

Lemma 2.3 *[Peletier and Gabrielsson (2012a,b, 2009)] Assume that* $k_1 > 0$ $(k_{\mathrm{e(L)}} > 0)$ *or* $k_4 > 0$ $(k_{\mathrm{e(P)}} > 0)$*. Define the non-dimensional form of the total amount of ligand* $u = x + \mu z$ *and the non-dimensional form of the total amount of receptor* $v = y + z$*. For all time* $\tau \geq 0$*:*

- *The non-dimensional total amount of ligand* $u(\tau)$ *is monotonic decreasing in time and decays to* 0 *for* $\tau \to \infty$ *and hence the total amount of ligand* $L(t) + P(t)$ *is monotonic decreasing in time and decays to* 0 *for* $t \to \infty$*;*

- *The non-dimensional total amount of receptor* $v(\tau)$ *always stays above, below or at its initial value* $v(0) = 1$ *depending on the relative magnitude of* k_3 *and* k_4*. In particular, for all* $\tau \geq 0$

$$v(\tau) \quad \begin{cases} \leq 1, & \text{if} \quad k_3 < k_4; \\ = 1, & \text{if} \quad k_3 = k_4; \\ \geq 1, & \text{if} \quad k_3 > k_4. \end{cases}$$

  In terms of the original variables, the total amount of receptor $R(t) + P(t)$ *always stays above, below or at the baseline value* R_0 *depending on the relative magnitude of the elimination rates* $k_{\mathrm{out}}(k_3)$ *and* $k_{\mathrm{e(P)}}(k_4)$*.*

A consequence of this lemma is that if it is measured at some moment that the total amount of receptor is above the baseline level, then the elimination rate of the receptor must be faster than the elimination rate of the product, i.e., $k_3 > k_4$. This is a sufficient and necessary condition.

Corollary 2.4 *Assume that* $k_{\mathrm{e(L)}} > 0$ $(k_1 > 0)$ *or* $k_{\mathrm{e(P)}} > 0$ $(k_4 > 0)$*. The elimination rate of the receptor is faster than the elimination rate of the product if and only if the maximal total receptor level is larger than the receptor baseline:*

$$\max_{t>0} \left(R(t) + P(t)\right) > R_0 \Leftrightarrow k_{\mathrm{out}} > k_{\mathrm{e(P)}} \quad \text{or} \quad \max_{\tau>0} v(\tau) > 1 \Leftrightarrow k_3 > k_4.$$

With the previous observations, we can show the global stability of the asymptotic state.

Theorem 2.5 *The steady state (7) of the equations (4)–(6), and hence the baseline state* $L = P = 0$*,* $R = R_0$ *of equations (1)–(3), is globally asymptotically stable.*

There is an intuitive physical explanation of this result. In the proof, we consider the non-dimensional form of the total amount of ligand u. Since we are assuming that, after the initial injection, there is no further input of ligand into the system, but that the ligand and the product are being eliminated (at rates $k_{e(L)}$ and $k_{e(P)}$ respectively), then clearly the total ligand must decrease over time, as proved. Once the ligand and product have all been eliminated, the receptor must return to the steady state value from which it started. The formal proof can be found in Appendix A.

The behaviour close to the globally stable fixed point is dominated by the linearisation about the fixed point and hence is determined by its eigenvalues and eigenvectors. To find those, we consider the Jacobian of equations (4)–(6) evaluated at the steady state solution (7)

$$J_0 = \begin{bmatrix} -(k_1+1) & 0 & \mu k_2 \\ -\frac{1}{\mu} & -k_3 & k_2 \\ \frac{1}{\mu} & 0 & -(k_2+k_4) \end{bmatrix}. \tag{8}$$

The matrix J_0 has eigenvalues

$$\begin{aligned} \lambda_1 &= \frac{1}{2}\left(-(1+k_1+k_2+k_4) + \sqrt{(1+k_1+k_2+k_4)^2 - 4(k_1k_2+k_1k_4+k_4)}\right) && (9)\\ &= \frac{1}{2}\left(-(1+k_1+k_2+k_4) + \sqrt{(1+k_1-k_2-k_4)^2 + 4k_2}\right) && (10)\\ \lambda_2 &= \frac{1}{2}\left(-(1+k_1+k_2+k_4) - \sqrt{(1+k_1+k_2+k_4)^2 - 4(k_1k_2+k_1k_4+k_4)}\right) && (11)\\ &= \frac{1}{2}\left(-(1+k_1+k_2+k_4) - \sqrt{(1+k_1-k_2-k_4)^2 + 4k_2}\right) && (12)\\ \lambda_3 &= -k_3 && (13) \end{aligned}$$

with corresponding eigenvectors

$$v_i = (\mu(\lambda_i+k_3)(k_2+k_4+\lambda_i), -(\lambda_i+k_4), \lambda_i+k_3), \quad i=1,2, \quad \text{and} \quad v_3 = (0,1,0). \tag{14}$$

The definition of the eigenvalues gives that λ_1 and λ_2 are always real (since the term under the square root is always positive) and that $\lambda_2 < \lambda_1$. Clearly $\lambda_2, \lambda_3 < 0$ and, using (9), it is easily verified that $\lambda_1 < 0$ also, since all of the non-dimensional constants are positive. Furthermore, if $\lambda_1 = -k_3 = \lambda_3$, then also $v_1 = v_3$. Hence in this case there is a degenerate eigenvalue with geometric multiplicity 1 and algebraic multiplicity 2. These properties of the eigenvalues give the local stability of the steady state solution (7).

Lemma 2.6 *The steady state (7) of the equations (4)–(6), and hence the baseline state $L = P = 0$, $R = R_0$ of equations (1)–(3), is linearly asymptotically stable.*

Below we give some more bounds on the eigenvalues, depending on the parameters k_1–k_4. These bounds play a crucial role in the analysis of the rebound.

Lemma 2.7 *For all values of $k_1, k_2, k_4 > 0$*

$$\lambda_2 < -(k_2+k_4) < \lambda_1 < 0. \tag{15}$$

Lemma 2.8 *For all values of $k_2, k_4 > 0$, the eigenvalue λ_1 is a monotonic decreasing function of k_1. Also,*

$$\begin{aligned} &\textit{if} \;\; k_1 > k_4 \;\; \textit{then} \;\; k_4 < -\lambda_1 < k_1 \\ &\textit{if} \;\; k_1 = k_4 \;\; \textit{then} \;\; -\lambda_1 = k_1 \\ &\textit{if} \;\; k_1 < k_4 \;\; \textit{then} \;\; k_1 < -\lambda_1 < k_4 \end{aligned}$$

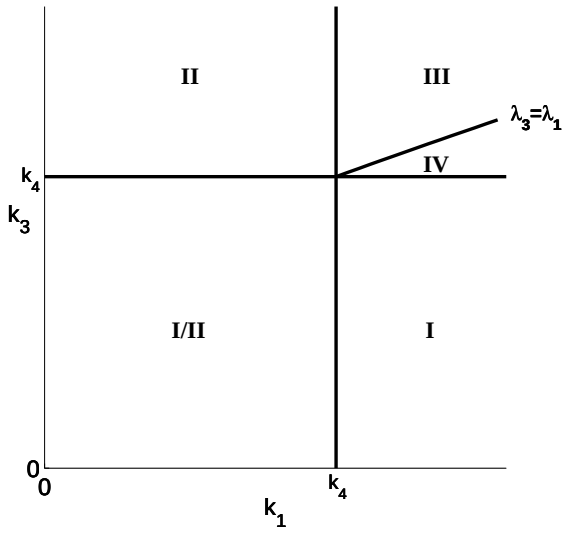

Figure 2: For k_2 and k_4 fixed, the (k_1, k_3) parameter plane is divided up by the lines $k_1 = k_4$, $k_3 = k_4$ and the curve $k_3 = -\lambda_1$ ($\lambda_3 = \lambda_1$) for $k_1 \geq k_4$.

3 Rebound

Assuming that the rate constants are strictly positive and that $k_{\text{out}} \ll k_{\text{on}} L_0$ (hence k_{on} large or L_0 large compared to k_{out}), the dynamics of the receptor can be described as follows: After the injection of the ligand, the receptor initially reduces rapidly (Aston et al, 2011; Peletier and Gabrielsson, 2012a,b) and then gradually increases on a slower time scale and finally returns back to the steady state. Rebound occurs if the receptor levels y ever exceeds the steady state value $y = 1$. We observe that the existence of rebound is essentially a local property, since we only need to find one point or a small region where $y > 1$. However, the non-existence of rebound is a global property, since in this case we require $y \leq 1$ for all $t > 0$.

To analyse the rebound problem, we use the global properties for the (nondimensional) total amount of receptor v and the estimates on the eigenvalues from the previous section. We fix k_2 and k_4 and divide the (k_1, k_3) parameter plane up into different regions using the lines $k_1 = k_4$, $k_3 = k_4$ and the curve $k_3 = -\lambda_1$ (or equivalently $\lambda_3 = \lambda_1$) for $k_1 \geq k_4$. We noted in the previous section that $\lambda_1 < 0$ and so the curve $k_3 = -\lambda_1$ clearly gives positive values of k_3. From Lemma 2.8, it follows that $-\lambda_1$ is a monotonically increasing function of k_1 and so the curve $k_3 = -\lambda_1$ is also a monotonically increasing function of k_1.

The relative position of λ_1 and λ_3 $(= -k_3)$ determines which eigenvalue is the least negative one and hence dominates the asymptotic behaviour near the fixed point. Lemma 2.8 gives that when $k_1 = k_4$, then $\lambda_1 = -k_4$ and so the curve $k_3 = -\lambda_1$ also passes through the intersection point of the lines $k_3 = k_4$ and $k_4 = k_1$. This scenario is shown in Fig. 2. As $\lambda_3 = -k_3$, it follows immediately that above the curve $k_3 = -\lambda_1$, we have $\lambda_3 < \lambda_1 < 0$ and below this curve the opposite holds, i.e. $\lambda_1 < \lambda_3 < 0$.

Below we analyse each region. For regions I and II, we will show that there is a trapping region beneath the plane $R = R_0$ such that the solution must stay in this region. Hence the receptor level cannot exceed the baseline in these regions. For regions III and IV, we consider the asymptotic approach to the baseline state and show that this approach is always from above the plane $R = R_0$. This implies that the receptor level will exceed the baseline at some moment in time. So altogether we prove the following theorem.

Theorem 3.1 *Assuming that all parameters k_1–k_4 are strictly positive, rebound occurs in equations (4)–(6) if and only if*

$$k_4 < k_1 \quad \textit{and} \quad k_4 < k_3.$$

Thus if k_2 and k_4 are kept fixed, then rebound happens if and only if the ratios k_1/k_4 and k_3/k_4 are both larger than 1.

Converting back to the dimensional parameters, we obtain the following result.

Corollary 3.2 *Assume that all rate constants are strictly positive and define rebound as the phenomenon that the receptor level rises above its baseline after an initial decrease due a drug dose, i.e., $R(t) > R_0$ for some $t > 0$. Rebound occurs in equations (1)–(3) if and only if the elimination rate of the product is slower than the elimination rates of both the ligand and the receptor, or in a formula*

$$k_{\mathrm{e(P)}} < k_{\mathrm{e(L)}} \quad \textit{and} \quad k_{\mathrm{e(P)}} < k_{\mathrm{out}}.$$

Illustrative example

To illustrate Theorem 3.1 and Corollary 3.2, we simulate the dynamics (time course) of the drug (ligand), receptor and ligand-receptor complex, see Fig. 3. We fix the parameters $k_{\mathrm{off}} = 1\,\mathrm{day}^{-1}$ and $k_{\mathrm{on}} = 1\,\mathrm{nM}^{-1}\mathrm{day}^{-1}$ and set $R_0 = 1\,\mathrm{nM}$; this implies that k_2 is fixed with $k_2 = 1$. These values are motivated by the IgE mAb omalizumab case study by Agoram et al (2007), where $k_{\mathrm{off}} = 0.9\,\mathrm{day}^{-1}$, $k_{\mathrm{on}} = 0.6\,\mathrm{nM}^{-1}\mathrm{day}^{-1}$ and $R_0 = 2.7\,\mathrm{nM}$. We have taken the sligthly modified values to facilitate comparison between those values and the value of the other parameters.

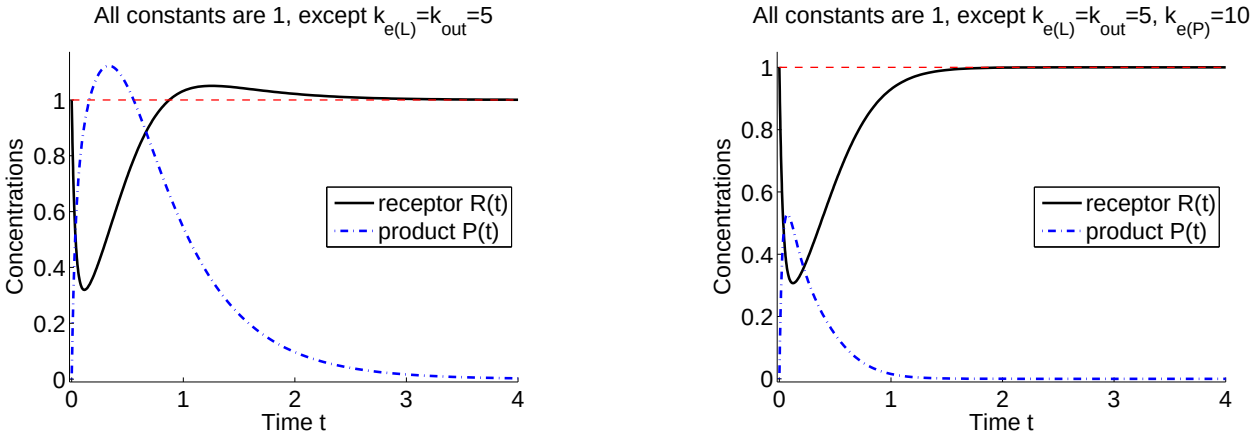

Figure 3: Time course of the receptor and product levels with $k_{\mathrm{e(P)}}$ varying. We have taken $k_{\mathrm{e(L)}} = k_{\mathrm{out}} = 5\,\mathrm{day}^{-1}$; $k_{\mathrm{off}} = 1\,\mathrm{day}^{-1}$; $k_{\mathrm{on}} = 1\,\mathrm{nM}^{-1}\mathrm{day}^{-1}$; $R_0 = 1\,\mathrm{nM}$, $L_0 = 25\,\mathrm{nM}$. On the left we have $k_{\mathrm{e(P)}} = 1\,\mathrm{day}^{-1}$; on the right $k_{\mathrm{e(P)}} = 10\,\mathrm{day}^{-1}$. The receptor baseline is denoted by the dashed (red in colour version) line. On the left, the maximal receptor level $R_{\max}$ is 1.05 nM, so in this case the rebound gives a 5% increase above baseline.

We start with fixing $k_{\mathrm{e(L)}} = k_{\mathrm{out}} = 5\,\mathrm{day}^{-1}$, hence $k_1 = k_3 = 5$ and investigate changing $k_{\mathrm{e(P)}}$ from inside the rebound region ($k_{\mathrm{e(P)}} = 1\,\mathrm{day}^{-1}$) to outside ($k_{\mathrm{e(P)}} = 10\,\mathrm{day}^{-1}$). We take $L_0 = 25\,\mathrm{nM}$. In both cases, after the drug is added, there is a very fast drop in receptor levels and a rise in product levels. After this fast initial phase, the product decreases again to zero, while the receptor increases. On the left ($k_{\mathrm{e(P)}} = 1\,\mathrm{day}^{-1}$, i.e., inside the rebound region), the receptor goes above the baseline before returning to the baseline and hence there is a 5% rebound. On the right ($k_{\mathrm{e(P)}} = 10\,\mathrm{day}^{-1}$), the receptor returns to its baseline value while staying below it all the time.

The magnitude of the rebound is influenced by the initial amount of drug L_0. This is illustrated in Fig. 4, where we use $k_{\mathrm{e(P)}} = 1\,\mathrm{day}^{-1}$. The smaller the drug dose L_0, the smaller the rebound, but the efficacy of the drug is also less as can be seen from the increase in the minimal receptor values.

The value of the product elimination rate $k_{\mathrm{e(P)}}$ also influences the magnitude of the rebound, the smaller $k_{\mathrm{e(P)}}$, the larger the rebound, with the maximal saturation at $k_{\mathrm{e(P)}} = 0$. This is illustrated in Fig. 5, where we use $L_0 = 1000\,\mathrm{nM}$ to illustrate that the rebound can become quite significant for a large dose and small elimination rate of the product $k_{\mathrm{e(P)}}$. More details about the magnitude of the

	L_0					
	1000	100	25	10	5	2
$R_{\max}$	1.12	1.08	1.05	1.03	1.02	1.01
$R_{\min}$	0.01	0.08	0.32	0.58	0.75	0.89

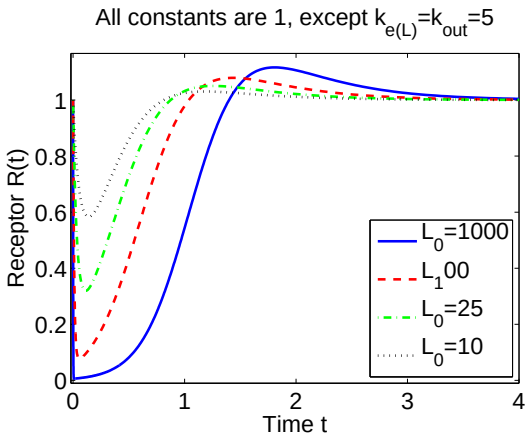

Figure 4: The drug dose L_0 affects the magnitude of rebound and the efficacy of the drug. We use $k_{\mathrm{e(P)}} = 1\,\mathrm{day}^{-1}$ and the baseline value is $R_0 = 1\,\mathrm{nM}$.

	$k_{\mathrm{e(P)}}$				
	1	0.1	0.01	0.001	0
$R_{\max}$	1.12	1.38	1.42	1.43	1.43
$R_{\min}$	0.06	0.06	0.06	0.06	0.06

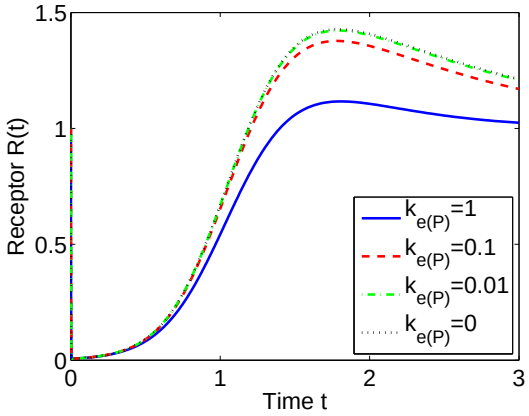

Figure 5: The product elimination rate $k_{\mathrm{e(P)}}$ affects the magnitude of rebound, but has less effect on the efficacy of the drug with this dose. We use $L_0 = 1000\,\mathrm{nM}$, the baseline value is $R_0 = 1\,\mathrm{nM}$ and the other constants are $k_{\mathrm{e(L)}} = k_{\mathrm{out}} = 5\,\mathrm{day}^{-1}$.

rebound can be found in Section 3.7.

After presenting this example, we now prove Theorem 3.1 by analysing each region in turn.

3.1 Region I: $k_3 \leq k_4$ $(k_{\mathrm{out}} \leq k_{\mathrm{e(P)}})$

Using the total amount of receptor variable v, we can define a simple trapping region which implies that rebound cannot occur in this region.

Theorem 3.3 *There is no rebound when $k_3 \leq k_4$.*

Proof From Lemma 2.3 it follows that in this region the non-dimensional total amount of receptor satisfies $v(\tau) \leq 1$ for all $\tau \geq 0$. As $y = v - z$ and $z \geq 0$, this implies immediately that $y(\tau) \leq 1$ for all $\tau \geq 0$. So we can immediately conclude that there is no rebound in this region. □

We note that it follows from Lemma 2.3 that when $k_3 = k_4$ the flow for the full equations is restricted to the invariant plane $v = y + z = 1$. The trajectory in this invariant plane is shown in Fig. 6. This case was considered in detail by Peletier and Gabrielsson (2009).

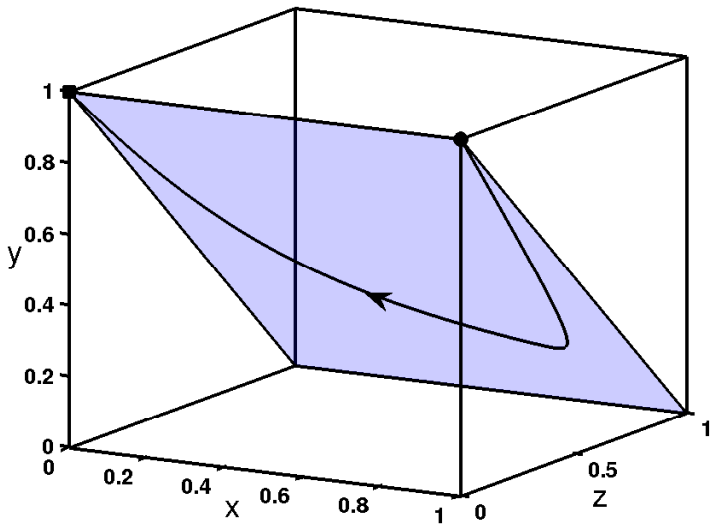

Figure 6: The trajectory starting with $y(0) = 1 = x(0)$, $z(0) = 0$ in the invariant plane $y + z = 1$ when $k_3 = k_4$. The starting point (initial condition) is denoted by the solid circle, the asymptotic steady state by the solid square.

3.2 Region II: $k_1 \leq k_4$ $(k_{\mathrm{e(L)}} \leq k_{\mathrm{e(P)}})$

To analyse region II, we define a trapping region in the (x, y, z)-phase space, with the $y = 1$ plane being one boundary, which ensures that no rebound can happen.

Theorem 3.4 *Rebound does not occur if $k_1 \leq k_4$.*

Proof We define a wedge shaped region in phase space by the conditions

$$0 \leq y \leq 1, \quad \mu k_2 z \leq x \leq 1 - \mu z, \quad z \geq 0. \tag{16}$$

This region, which is shown in Fig. 7, is bounded by the planes

$$y = 0, \quad y = 1, \quad x = \mu k_2 z, \quad x = 1 - \mu z, \quad z = 0. \tag{17}$$

Note that as the non-dimensional total amount of ligand is given by $u = x + \mu z$, the condition $\mu k_2 z \leq x \leq 1 - \mu z$ is equivalent to $\mu(k_2 + 1)z \leq u \leq 1$.

At time $\tau = 0$ the trajectory is at a corner point of this region and we will show that initially it moves into the wedge. Furthermore, we will show that any trajectory that is inside this wedge cannot escape, and so the wedge is a trapping region. Since one of the planes that defines this region is $y = 1$, it follows that this plane cannot be crossed and so there can be no rebound.

The initial condition of interest, namely $I = (x(0), y(0), z(0)) = (1, 1, 0)$, lies on a corner point of the region defined by (16) at the intersection of the three planes $y = 1$, $x = 1 - \mu z$ $(u = 1)$ and $z = 0$. Using the notation $\dot{y}|_I$ to denote the time derivative of $y(t)$ evaluated at the initial condition I, and similarly for $\dot{u}|_I$ and $\dot{z}|_I$, it is easily verified that

$$\dot{y}|_I = -\frac{1}{\mu} < 0, \quad \dot{u}|_I = -k_1 < 0, \quad \dot{z}|_I = \frac{1}{\mu} > 0$$

and so the trajectory will initially move from the corner point to inside the wedge.

To prove that the wedge is a trapping region, we will show that at the bounding planes the vector field is pointing inwards or is tangent to the bounding plane. In case of tangencies, we also have to check that this does not allow the trajectory to escape. To verify that the vector field is pointing inwards at an edge, we will show that it is pointing in the direction of the inward directed normal to each of the planes that form the edge. We now consider each of the planes given in (17) in turn.

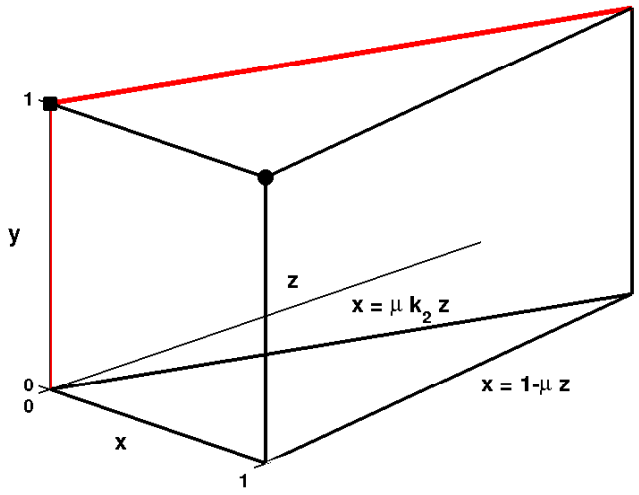

Figure 7: The wedge shaped region defined by (16) which is a trapping region for parameter values in region II. The starting point (initial condition) is denoted by the solid circle, the asymptotic steady state by the solid square. The vector field is directed inside this wedge, except for the bold (red in colour version) lines where the vector field is potentially tangent.

- The bounding plane $y = 0$.
  We already considered this plane in Lemma 2.1 when we considered the invariance of the octant with x, y and z non-negative. We have seen that the vector field on this plane is always pointing into the octant.

- The bounding plane $y = 1$.
  Using (5) we note that
  $$\dot{y}|_{y=1} = \frac{-x + \mu k_2 z}{\mu}.$$
  It follows from the definition of our region in (16) that $-x + \mu k_2 z \leq 0$. Thus, when $x > \mu k_2 z$, then $\dot{y}|_{y=1} < 0$ and the vector field points into the trapping region on the interior of this plane. At the edge of the intersection of the planes $y = 1$ and $x = \mu k_2 z$, the vector field is tangent to the plane and we will consider this special case below.

- The bounding plane $x = \mu k_2 z$.
  The inward pointing normal to this plane is $(1, 0, -\mu k_2)$. For $y < 1$ and $z > 0$ the inner product of the vector field with this normal is
  $$\begin{aligned} \dot{x} - \mu k_2 \dot{z}|_{x=\mu k_2 x} &= -k_1 x - (1 + k_2)xy + \mu k_2 (1 + k_2 + k_4) z|_{x=\mu k_2 x} \\ &= \mu k_2 (1 + k_2)(1 - y)z + \mu k_2 (k_4 - k_1) z > 0. \end{aligned} \tag{18}$$
  as $k_1 \leq k_4$. Hence the vector field at the interior of this plane and at the edges with $y = 0$ and $x = 1 - \mu z$ is directed to the inside of the trapping region. At the intersection of this plane with the planes $z = 0$ and $y = 1$ the vector field is tangent and we will consider these cases below.

- The bounding plane $x = 1 - \mu z$
  We have already seen that this is equivalent to $u = 1$. Using the dynamics for u, we see that the inner product of the u-vector field with the inward pointing normal at this plane is
  $$-\dot{u}|_{u=1} = k_1 u + \mu(k_4 - k_1) z|_{u=1} = k_1 + \mu(k_4 - k_1) z.$$
  As $0 < k_1 \leq k_4$ and $z \geq 0$, we clearly have $-\dot{u}|_{u=1} > 0$ and so the normal derivative is strictly positive and the vector field is therefore directed inside the trapping region.

- The bounding plane $z = 0$
  We already considered this plane in Lemma 2.1 when we considered the invariance of the octant with x, y and z non-negative. We have seen that for $x > 0$ the vector field on this plane is always pointing into the octant. The special case of the y-axis is considered below.

In the above discussion, there were several cases where the normal derivative vanished along an edge of the region, at which two planes meet. We now consider these edges in more detail. Before doing this however, we note that the corner point of the region defined by $z = 0$, $y = 1$ is the final steady state that all trajectories converge to (by Theorem 2.5) and at this point, the derivatives of all three variables vanish. So when we consider the various edges, we can exclude this corner point from consideration. The two edges that we have to consider are as follows:

- The edge $x = \mu z$, $y = 1$.
  When $k_1 < k_4$, we see from (18) that at this edge the vector field is tangent to the plane $y = 1$ and points inwards along the plane $x = \mu x$ if $z > 0$. Thus, the vector field is directed away from the edge and so no trajectory can approach this edge.

  If $k_1 = k_4$, we see that the vector field is tangent to the edge as it is tangent to both planes. So this edge is invariant under the flow, with the trajectory converging to the steady state along this line. Thus, it is not possible for a trajectory to escape the trapping region along this line.

- The edge $x = 0$, $z = 0$, hence the y-axis.
  We have already seen that this axis is invariant under the flow (Lemma 2.1), with the trajectory moving up the axis towards the steady state at $y = 1$. Thus, this edge cannot be crossed.

Combining all of the above results, it follows that the vector field at the interior of the bounding planes is always pointing into the wedge and that at the edges it is either pointing inwards or if it is tangent to an edge, an orbit cannot approach this edge. So we can conclude that if a trajectory starts in the wedge shaped region defined by (16), then it cannot escape from this region, and so the wedge is a trapping region for the flow.

As mentioned previously, one of the boundary planes of the trapping region (16) is $y = 1$ and so there can be no rebound. □

Clearly there is some overlap between this region and region I, but the conclusion that rebound does not occur is consistent in the overlapping region.

3.3 Region III: $k_1 > k_4$ $(k_{e(L)} > k_{e(P)})$, $k_3 > -\lambda_1$

We show that there is rebound in this case by considering the linearised manifold from the steady state in the direction associated with the slowest converging eigenvalue which is λ_1.

Theorem 3.5 *Rebound occurs if $k_1 > k_4$ and $k_3 > -\lambda_1$.*

Proof It is clear from (9) and (11) that $\lambda_2 < \lambda_1$ and since we are also assuming that $-\lambda_3 = k_3 > -\lambda_1$, then $\lambda_3 < \lambda_1$ and so the eigenvalue that is closest to zero is λ_1, which has corresponding eigenvector v_1 (see (14)). The one-dimensional linearised manifold from the steady state in the direction of the eigenvector v_1 is therefore

$$\begin{bmatrix} x \\ y \\ z \end{bmatrix} = \begin{bmatrix} 0 \\ 1 \\ 0 \end{bmatrix} + \alpha \begin{bmatrix} \mu(k_2 + k_4 + \lambda_1)(\lambda_1 + k_3) \\ -(\lambda_1 + k_4) \\ \lambda_1 + k_3 \end{bmatrix}. \tag{19}$$

We are only interested in the positive octant, hence x and z must be non-negative quantities. This will only occur if $\alpha \geq 0$, since the first and third entries in the eigenvector are positive, using Lemma 2.7 and since $\lambda_1 + k_3 > 0$.

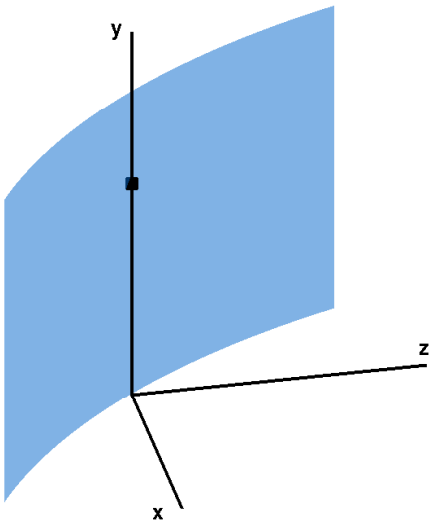

Figure 8: A sketch of the two-dimensional manifold in a neighbourhood of the steady state (indicated by the solid square) which is tangent to the plane generated by the eigenvectors v_2 and v_3.

From Lemma 2.8, we see that along this manifold, y exceeds the steady state value $y = 1$ since $k_1 > k_4$. Now generically, almost all trajectories will approach the steady state tangent to the linearised manifold (19), since this is associated with the eigenvalue λ_1 which is closest to zero. If this is the case then y must approach the steady state value $y = 1$ from above, so that rebound occurs.

Of course, it is always possible that the generic condition stated above does not hold for some initial conditions. If this were the case, then the trajectory would approach the steady state tangent to one of the other linearised manifolds generated by the eigenvectors v_2 or v_3. We now show that this is not possible.

A given trajectory would not approach the steady state tangent to the linearised manifold (19) only if the initial condition happened to lie on the global two-dimensional stable manifold which is tangent to the plane generated by the eigenvectors v_2 and v_3 at the steady state. Finding global two-dimensional manifolds is not usually possible, and so this condition would seem difficult to check. However, we will show that the two-dimensional tangent plane associated with the eigenvector directions v_2 and v_3 lies outside the positive octant, except for the y-axis. This will allow us to infer results concerning the global two-dimensional manifold from this.

We note from Lemma 2.7 that the first entry in the eigenvector v_2 is negative and so the first and third entries of the eigenvector have opposite sign. The one-dimensional linearised manifold associated with this eigenvector can be obtained by replacing λ_1 with λ_2 in (19). Since the first and third entries of v_2 are of opposite sign, it follows that along the linearised manifold, we will have $xz \leq 0$ so that precisely one of the variables x and z is always negative (except at the steady state). Clearly, with the exception of the steady state itself, this manifold is outside the phase space for our equations, since we require all the variables to be non-negative.

We note that the linearised manifold associated with the eigenvector v_3 is simply the y-axis. As noted in Lemma 2.1, the y-axis is in fact invariant under the flow and so this axis is also the global one-dimensional stable manifold associated with the eigenvalue λ_3.

We can now describe the form of the global two-dimensional stable manifold associated with the eigenvectors v_2 and v_3. Since the y-axis is the global one-dimensional stable manifold in the direction of v_3 and the linearised manifold in the v_2 direction has $xz \leq 0$, it follows that the global two-dimensional stable manifold will include the y-axis, but that in the neighbourhood of this axis, the manifold will be outside of the phase space of the problem (see Fig. 8). Thus, the only physically relevant trajectories that approach the steady state on this manifold are the trajectories on the y-axis. Our initial condition is not on the y-axis and so our trajectory must approach the steady state tangent to the eigenvector v_1. Thus the condition $k_1 > k_4$ is sufficient to ensure that rebound does occur. □

3.4 Region IV: $k_4 < k_3 < -\lambda_1$ ($k_{\text{out}} > k_{\text{e(P)}}$)

The second inequality in these conditions is equivalent to $\lambda_1 < \lambda_3$, hence λ_3 is the slowest eigenvalue. As in the previous region, we will show that there is rebound in this case by considering the manifold associated with this slowest eigenvalue.

Theorem 3.6 *Rebound occurs if* $k_4 < k_3 < -\lambda_1$.

Proof Our assumptions imply that $\lambda_1 < -k_3 = \lambda_3$ and so the eigenvalue closest to zero in this case is λ_3. Thus, most orbits will approach the steady state tangent to the y-axis. With regard to rebound, the important question is whether on this axis they approach the point $y = 1$ from above or from below.

From Lemma 2.3, we see that in this region we have $v(t) \geq 1$ for all $t \geq 0$. The plane $v = 1$ meets the y-axis at the steady state $y = 1$, $x = z = 0$. Clearly the only part of the y-axis that is above the plane $v = 1$ consists of the part where $y > 1$ (as $z = 0$ at the y-axis). Thus, when $k_3 > k_4$, generically almost all orbits will approach $y = 1$ from above, resulting in rebound.

As in the proof of Theorem 3.5, we must also consider the possibility that an orbit might approach tangent to one of the other eigenvectors for a particular choice of parameter values. The one-dimensional linearised manifold in the direction of the eigenvector v_2 is outside of the phase space for the problem, using a similar proof to that given in the proof of Theorem 3.5, and so it is not possible for a physically relevant trajectory to approach the steady state tangent to this one-dimensional manifold.

The remaining case is related to the eigenvector v_1. The one-dimensional linearised manifold in the direction of v_1 is given by (19) and so we must consider whether it is possible that a trajectory could approach the steady state tangent to this manifold. From (19), we note that along this linearised manifold we have

$$\begin{aligned} v \;=\; y + z \;&=\; 1 - \alpha(\lambda_1 + k_4) + \alpha(\lambda_1 + k_3) \\ &=\; 1 + \alpha(k_3 - k_4). \end{aligned} \tag{20}$$

In this case, we must restrict to $\alpha \leq 0$ to ensure that $x, z \geq 0$. We also note from the assumptions of the Theorem that $k_3 - k_4 > 0$, and so we see that along this linearised manifold we have $v < 1$ for $\alpha < 0$. Since we have already noted that our trajectory must satisfy $v \geq 1$ in this case, clearly it cannot approach the steady state tangent to this one-dimensional linearised manifold, since it lies below the $v = 1$ plane.

Thus, we conclude that all trajectories must approach the steady state tangent to the y-axis and from above, and so rebound must occur under these conditions. □

3.5 The boundary between regions III and IV ($\lambda_1 = \lambda_3$)

The boundary between the regions III and IV is the line $\lambda_3 = \lambda_1$ (or equivalently $\lambda_1 + k_3 = 0$), with $k_1 > k_4$ and $k_3 > k_4$. Along this curve, the Jacobian matrix (8) has a repeated eigenvalue ($\lambda_1 = \lambda_3$) and the eigenvector v_1 coincides with the eigenvector v_3 (see (14)), and so there is only one eigenvector in this case, as would be expected for a multiple eigenvalue. As the curve $\lambda_3 = \lambda_1$ is crossed from above to below (with $k_4 < k_1$), i.e. from region III to region IV, the trajectory initially approaches the steady state tangent to the eigenvector v_1. As the curve $\lambda_1 = \lambda_3$ is reached, the eigenvectors v_1 and v_3 coincide and are parallel to the y-axis. Once the curve has been crossed the trajectories then approach the steady state tangent to v_3, and so there is a smooth transfer between these two cases as the line is crossed. In each open region, we used an asymptotic argument to conclude that the trajectory with the given initial condition has to approach via the eigenvector associated with the least negative eigenvalue and from above the plane $y = 1$. We have not yet considered the boundary, although it can be expected on grounds of continuity that the scenario will be very similar. This is indeed the case as is shown below.

Theorem 3.7 *Rebound occurs if $k_4 < k_3 = -\lambda_1$.*

Proof As $\lambda_1 = \lambda_3$ and $\lambda_2 < \lambda_1$, the least negative eigenvalue is a double one and hence a two-dimensional stable manifold is associated with it. The linearised two-dimensional manifold can be expressed in terms of the eigenvector ($v_1 = v_3 = (0, 1, 0)$) and the generalised eigenvector v_g, which is defined by

$$(J_0 - \lambda_1 I)v_g = v_1.$$

The solution of this equation is

$$v_g = (\mu k_2, 0, \lambda_1 + k_1 + 1)\,/[k_2(\lambda_1 + k_1)].$$

Note that $\lambda_1 + k_1 > 0$ as $k_1 > k_4$ (see Lemma 2.8) and so this vector is well-defined and points into the positive octant of the phase space.

The solutions on the linearised manifold are linear combinations of $e^{\lambda_1 t}(v_g + tv_1)$ and $e^{\lambda_1 t}v_1$ (Jordan and Smith, 2007). Asymptotically all solutions in the two-dimensional linearised manifold align with the eigenvector v_1 direction, which is the y-axis. This property carries over to all solutions on the global nonlinear two-dimensional manifold associated with the repeated eigenvalue $\lambda_1 = \lambda_3$. The eigenvalue λ_2 is more negative than λ_1 and we have seen in the previous two sections that its stable one-dimensional manifold is outside the positive octant (except for the steady state). So all trajectories from inside the octant will asymptotically align with the y-axis.

Since we are assuming that $k_3 > k_4$, we see from Lemma 2.3 that the total receptor v satisfies $v(\tau) \geq 1$ for all $\tau \geq 0$. As in the proof of Theorem 3.6, the only part of the y-axis that is above the plane $v = 1$ is the part with $y > 1$, and so all orbits must approach the steady state $y = 1$ from above, resulting in rebound in this case as claimed. □

3.6 Multiple dosing

Thus far, we have considered only a single bolus injection. We now consider the possibility of rebound when there are multiple bolus doses. The rebound phenomenon that we have been studying occurs as the receptor level is returning to its steady state. It might be conjectured that our rebound results also hold whether there is a single bolus injection initially, or multiple bolus injections. Clearly, multiple doses will keep the receptor level low for a longer period, but the process of returning to the steady state could well be similar to the single dose case. We now show that this is indeed the case.

Theorem 3.8 *If the system (1)–(3) is subject to an arbitrary (but finite) number of bolus doses of the ligand of arbitrary (but finite) amounts at any desired points in time, then the conditions of Corollary 3.2 still apply for determining whether or not rebound will occur.*

Proof In terms of the non-dimensional equations (4)–(6), an additional injection L_1 of the ligand at a time $t_1 > 0$ corresponds to a discontinuous increase in the variable x such that

$$x(t_1^+) = x(t_1^-) + \frac{L_1}{L_0}.$$

Further bolus doses would correspond to additional discontinuous increases in x. We now consider each of the Theorems in the four regions in turn and consider the effect of such changes in x on the result.

In Theorem 3.3 (region I), the proof that there is no rebound lies in the fact that the trajectory always has $v \leq 1$. The changes made to x from the additional doses have no effect on the value of $v = y + z$, and so the trajectory will continue to satisfy the condition $v \leq 1$ when there are multiple doses, and so there is again no rebound.

In the proof of Theorem 3.4 (region II), an additional dose may have the effect of moving the trajectory outside of the trapping region that we defined. In particular, the variable $u = x + \mu z$ may

exceed the boundary value of $u = 1$ when x is increased. However, we note that we can increase the size of the trapping region by moving this boundary plane to $u = c$ for any $c > 1$. In this case, we have

$$\dot{u}|_{u=c} = -k_1 c - \mu(k_4 - k_1)z$$

and since in this case we are assuming that $k_1 \leq k_4$ and $z \geq 0$, we still have $\dot{u}|_{u=c} < 0$ for any $c > 1$. Thus, provided that the total amount of dosing is finite, we can always find a value of c such that the trajectory with multiple dosing stays inside the trapping region and so, as previously, there can be no rebound in this case.

In Theorems 3.5, 3.6 and 3.7 (regions III, IV and their boundary), the existence of rebound is proved by considering the approach to the steady state in the phase space. The additional doses have no effect on this process. An additional argument used is the fact that the trajectory satisfies $v \geq 1$. As in the previous case, additional doses have no effect on v. So altogether we can conclude that the results still hold and that there will be rebound in this region. □

3.7 Magnitude of the rebound

As can be seen from the diagrams in Figs 3–5 for the example in the introduction of this section, the dynamics has two phases. An initial fast phase in which the receptor levels drop very sharply and a more gradual phase in which the receptor levels recover and rebound if the elimination rate of the product is small. To see these phases more clearly, we rewrite the system in terms of the variables $u = x+\mu z$, the non-dimensional form of the total amount of ligand, and $v = y+z$, the non-dimensional form of the total amount of receptor. We introduced these variables previously in Lemma 2.3. In these variables, together with y, the equations (4)-(6) become

$$\begin{aligned}
\dot{u} &= -k_1\, u + \mu\,(k_1 - k_4)\,(v - y) && (21)\\
\dot{v} &= k_3\,(1 - y) - k_4\,(v - y) && (22)\\
\mu\dot{y} &= -y\,(u - \mu(v - y)) + \mu\, k_3\,(1 - y) + \mu\, k_2\,(v - y) && (23)
\end{aligned}$$

Since $\mu = R_0/L_0$ represents the baseline receptor concentration divided by the initial drug concentration, this is usually quite a small number. As shown by Aston et al (2011), by going to the fast time $\tau = t/\mu$, initially the total drug concentration u and the total receptor concentration v will stay approximately constant, while the receptor quickly adapts such that the right hand side of the $\dot{y}$ equation nearly vanishes, i.e.,

$$-y\,(u - \mu(v - y)) + \mu\, k_3\,(1 - y) + \mu\, k_2\,(v - y) = \mathcal{O}(\mu). \tag{24}$$

More about this fast-slow process and the minimal receptor value, can be found in the paper by Aston et al (2011).

We are interested in the maximal y value, so we would like to keep the y variable in our slow system. The slow manifold relation (24) suggests the following coordinate transformation

$$y = \mu\, Y \quad \text{and} \quad u = \frac{1}{Y}\,(k_2 v + k_3) + \mu\, w,$$

leading to the equations

$$\begin{aligned}
\mu\dot{w} &= -\frac{1}{Y}\,(w\, F_1(v, \mu Y) - (v - \mu Y)\,(F_1(v, \mu Y) - F_2(\mu Y)) + F_3(v)) && (25)\\
\dot{v} &= k_3\,(1 - \mu Y) - k_4\,(v - \mu Y) && (26)\\
\dot{Y} &= -Y\,(w - v + \mu Y + k_2 + k_3) && (27)
\end{aligned}$$

with $F_1(v, y) = k_1 y + k_2 v + k_3$, $F_2(y) = k_2(k_3 - k_4) + k_4 y$ and $F_3(v) = k_2 v(k_1 + k_2) + k_3(k_1 + 2k_2 + k_3)$. In these equations, it is now the w variable that changes rapidly, while v and Y change more slowly for

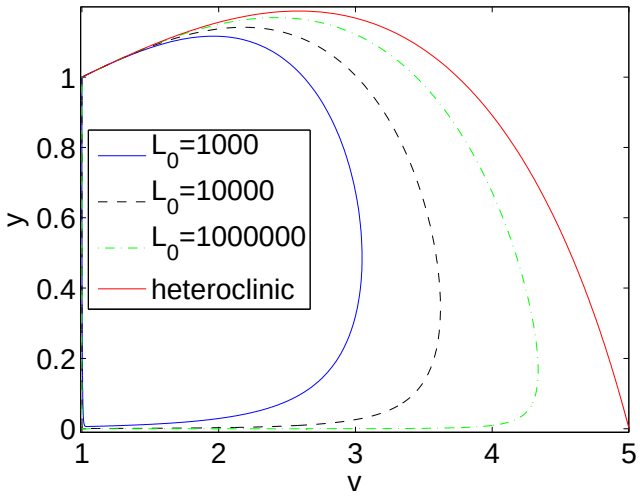

Figure 9: Projection of the heteroclinic orbit and solutions curves for various small values of $\mu = \frac{R_0}{L_0}$. We use $R_0 = 1$, $k_2 = 1 = k_4$ and $k_1 = k_3 = 5$.

small μ. In the fast time variable $\tau = t/\mu$, there is an invariant, normally hyperbolic two dimensional manifold at $\mu = 0$, given by $w = v - \frac{F_2(0)v+F_3(v)}{F_1(v,0)}$. Thus for μ small and Y bounded (i.e., y small), Fenichel's theory (Fenichel, 1979) gives the existence of a two-dimensional invariant manifold

$$w = W_{\text{man}}(v, \mu Y; \mu) = v - \mu Y - \frac{F_2(\mu Y)(v - \mu Y) + F_3(v)}{F_1(v, \mu Y)} + \mathcal{O}(\mu).$$

As $F_1(v, \mu Y) > k_3$ for all μY and v, it follows immediately that this invariant manifold is attracting for $Y > 0$. In Section 2 it is shown that the system has a global attractor which, in the new coordinates, is given by $(w, v, \mu Y) = (-k_2 - k_3, 1, 1)$. Thus by continuing the flow of the invariant manifold $w = W_{\text{man}}(v, \mu Y; \mu)$ it will include this attractor.

Furthermore, in the (w, v, y) dynamics there is another steady state at

$$(w, v, \mu Y) = \left(W_{\text{man}} \left(\frac{k_3}{k_4}, 0; \mu \right), \frac{k_3}{k_4}, 0 \right) \tag{28}$$

which is on the invariant manifold (note that in the original (x, y, z) coordinates this point corresponds to $x \sim \infty$ (as $u \sim \frac{1}{y} \to \infty$)). The eigenvalues of the Jacobian evaluated at this steady state on the invariant manifold are $\lambda_1 = k_1$ and $\lambda_2 = -k_4$ and so it is a saddle point. Hence the heteroclinic connection between this steady state and the global attractor is on the (extended) invariant manifold too.

Remark We note that (27) implies that the plane at $Y = 0$ is invariant, but on the other hand (25) is singular at $Y = 0$. So for $\mu \neq 0$, the steady state (28) is the only intersection of the invariant manifold with the plane $Y = 0$. For all other points on the invariant manifold $Y > 0$. For $\mu = 0$, the curve $Y = 0$, $w = W_{\text{man}}(v, 0; 0)$ is on the invariant manifold and this curve is the stable manifold of the fixed point (28).

The invariant manifold cannot be crossed by any solution, thus if a solution is initially under the manifold, it will stay under the manifold for all time. At the end of the fast phase, our solution will have approached the invariant manifold (from below) near the point $(w, v, \mu Y) = W_{\text{man}}(1, 0; 0) \approx (-(k_1 + k_2 + k_3 - 1 + \frac{k_2(k_3-k_4)}{k_2+k_3}), 1, 0)$. In the (v, y) projection, this point is to the left of the fixed point as $k_3 > k_4$ (a necessary condition for rebound). The invariant manifold is attracting, so it can be expected that in the (v, y) projection the orbit will stay under the heteroclinic connection. This

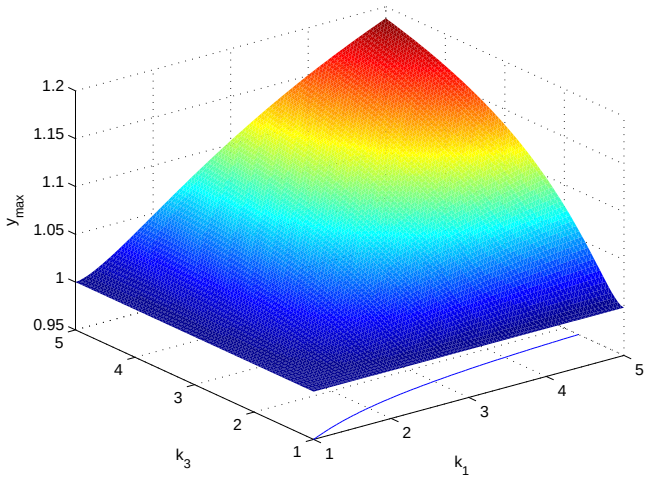

Figure 10: The maximum value of y on the heteroclinic orbit in the rebound region with $R_0 = 1$ and $k_2 = 1 = k_4$. The line in the k_1–k_3 plane is the boundary between regions III and IV. Region IV is near to the k_1 axis.

is illustrated in Fig. 9. Then the maximal y value on the heteroclinic orbit is an upper bound on the magnitude of the rebound. In Fig. 10, we computed this upper bound for $k_{e(P)}(= k_4) = 1$ and $k_{e(P)}(= k_1)$ and $k_{out}(= k_3)$ varying in regions III and IV. The surface is smooth near this boundary and crossing the boundary seems to have little significant effect on the maximum. Clearly, the magnitude of the rebound increases if either the elimination rate of the ligand ($k_{e(L)} \equiv k_1$) is increased or the elimination rate of the free receptor ($k_{out} \equiv k_3$) is increased.

We note that the highest value on the surface shown in Fig. 10 corresponds to the parameter values considered in our earlier example. The maximum rebound according to this upper bound is $y_{max} = 1.18798$. It can be seen from the table in Fig. 4 that the magnitude of rebound is increasing as L_0 increases towards this upper bound.

4 Discussion and Conclusions

We have considered in detail a simple TMDD model and have derived precise conditions for the existence or non-existence of antigen rebound under circumstances described by the model. The key result (Theorem 3.1 and Corollary 3.2) states that rebound occurs if and only if the elimination rate of the product is slower than the elimination rates of both the ligand and the receptor, for one or more bolus doses. To explain this intuitively, we note that the initial fast phase after the administration of the ligand corresponds mainly to the reaction between the ligand and the receptor which forms the product. However, if the resulting product is not removed quickly enough, then the reverse reaction will occur where the product dissociates back into its component parts. Thus, the receptor is being replenished at a rate k_{in} and additional receptor is being formed from this dissociation. The combination of these effects gives the excess receptor which results in the rebound. A further corollary of these results is that the rate of reaction between the ligand and the receptor and the rate of dissociation of the product play no role in determining the existence or non-existence of rebound in this model.

In the development of antibodies, it can be a problem to monitor the free receptor (antigen, cytokines) levels. The total amount of receptor is often used as a substitute to measure drug activity (Haringman et al, 2006; Vugmeister et al, 2009), although Ait-Oudhia et al (2012) recently showed how free antigen levels could be derived from a model and linked to clinical outcomes. From Corollary 2.4, it can be seen that if the total receptor level gets above the receptor baseline then the

elimination rate of the receptor must be faster than the elimination rate of the product. Thus total receptor level getting above the receptor baseline is a necessary condition for rebound, but it is not sufficient. If the elimination rate of the drug is slower than the elimination rate of the product, there will be no antigen rebound, even though the total receptor level is always above or at baseline if the elimination rate of the receptor is faster than the elimination rate of the product.

We have also analysed the magnitude of antigen rebound in the simple TMDD model. For a given baseline receptor level, larger antigen rebound is predicted for higher doses of the administered ligand or for slower complex elimination rates. This is intuitive, since with higher doses larger amounts of complexes are formed and with longer complex elimination times, there is a higher concentration gradient driving dissociation of the complex. Another observation is that the faster the elimination rate of either the ligand ($k_{\mathrm{e(L)}}$) or the receptor (k_{out}), the larger the rebound.

Reported data indicate that for most blocking/neutralising biologics, mostly monoclonal antibodies, the complex is eliminated at the same rate as the antibody (e.g. Meno-Tetang and Lowe (2005)). However, in some cases, the complex is eliminated faster than the antibody due to factors such as interference of antigen binding on antibody salvage mechanisms (e.g. Lowe et al (2009); Chan et al (2009)). There are very few examples of the complex being eliminated slower than both ligand and receptor. One such example is that of an anti-IL13 antibody (Vugmeister et al, 2009), where the condition required for antigen rebound was observed in naive cynomolgus monkeys. Another report (Munafo et al, 2007) also suggests that the required condition may have been satisfied. However, in both these cases, no rebound was reported in preclinical or clinical trials. It is possible that the rebound, if it did occur, might have been of too small a magnitude to be of any consequence. Based on our simulations with the model reported by Vugmeister et al (2009), the magnitude of rebound would have been over 250%. However, we suspect a typographical error in the reported model. Using the corrected model (where we replaced a term involving the difference of the concentration of free IL-13 minus the concentrations of bound IL-13, i.e. $C_{\mathrm{IL-13}} - C_{\mathrm{Ab-(IL-13)}} - C_{\mathrm{Ab-(IL-13)_2}}$, by the concentration of free IL-13 $C_{\mathrm{IL-13}}$), the magnitude of the rebound is less than 1% and the graphs are similar to the ones given in the paper by Vugmeister et al (2009). Binding to cell-surface receptors typically results in accelerated turnover of the antibody and the complex (e.g. Ng et al (2006); Wang et al (2012)). There are no reports known to the authors where binding to cell-surface receptor leads to formation of complexes that are cleared slower than the receptor or ligand. The rarity, experimental detectability and potential lack of clinical consequences, as detailed above might be one of the reasons this phenomenon has not received a lot of attention so far. However, it should be noted that many smaller biologic modalities such as Fab fragments and Fc fusion proteins have much shorter half-lives than mAbs ($\sim$1–2 days for example). Therefore, binding to antigen has the potential to create a complex with longer half-life than either receptor or ligand. Furthermore, many preclinical studies are typically done with Fabs and scFvs, so the required conditions for rebound may occur in those cases. Our analysis can provide a framework for predicting rebound and/or interpreting unexpected results under these conditions.

The analysis presented above is limited by the fact that the simplest system of interaction between a ligand and a receptor is described and analysed for rebound. Other factors such as tissue re-distribution and homeostatic feedback may play an important and potentially greater role in antigen rebound and are beyond the scope of this analysis. They will be considered in our subsequent paper (Aston et al, 2012), in which we adapt this model to allow feedback in the production of the target protein, so that when the protein levels are reduced, the production rate increases. In spite of its limitations, the analysis presented above has highlighted the important factors that govern occurrence of antigen rebound in such systems and the need to understand these interactions prior to design of therapies targeting them. The analysis has also highlighted the value added by fundamental quantitative analysis in the design of biologic therapies.

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A Proofs of Invariance and Stability Results

Proof of Lemma 2.1 The phase space is bounded by the three planes $x = 0$, $y = 0$ and $z = 0$. If the vector field given by the equations (4)–(6) at these planes is directed inside or is tangent to the octant, then the given region is invariant (Smith, 1995).

Taking the inner product of the vector field with the inward pointing normal at the plane $x = 0$, we see from (4) that

$$\dot{x}|_{x=0} = \mu k_2 z$$

and so $\dot{x}|_{x=0} \geq 0$ when $z \geq 0$. Hence the vector field is directed inside the octant at the plane $x = 0$.

Similarly, from (5), we obtain

$$\dot{y}|_{y=0} = k_3 + k_2 z$$

and so $\dot{y}|_{y=0} > 0$ when $z \geq 0$. In the same vein, from (6), we obtain

$$\dot{z}|_{z=0} = \frac{xy}{\mu}$$

and so $\dot{z}|_{z=0} \geq 0$ when $x, y \geq 0$.

Note that at the x-axis ($y = z = 0$), the vector field is tangent to the plane $z = 0$, and is pointing inwards as $\dot{y}|_{y=z=0} = k_3$. At the y-axis ($x = z = 0$), we have $\dot{x} = 0$ and $\dot{z} = 0$, hence x and z will stay zero for all time and the y-axis is invariant. On the y-axis, $\dot{y} > 0$ if $y < 1$ and $\dot{y} < 0$ is $y > 1$. Hence all orbits on the y-axis tend to the stable steady state at $y = 1$.

Thus, we can conclude that any trajectory with $x(0), y(0), z(0) \geq 0$ must satisfy $x(t), y(t), z(t) \geq 0$ for all $t > 0$. □

Proof of Lemma 2.2 There are two steady state solutions of equations (4)–(6), one of which is given by (7). The other solution is not in our phase space, and hence is not physically relevant, as the values of x and y are negative. □

Proof of Corollary 2.4 By negating Lemma 2.3, it follows immediately that if the maximal $v(\tau)$ value is above 1, then $k_3 > k_4$. And Lemma 2.3 implies that if $k_3 > k_4$, then $v(\tau) \geq 1$ for all τ. We only have to show that $v(\tau)$ cannot be equal to 1 for all time. This follows immediately with a contradiction argument. Assume that $k_3 > k_4$ and $v(\tau) = 1$ for all $\tau \geq 1$. Then the v-dynamics (22) gives for all $\tau \geq 0$ that $0 = (k_3 - k_4)z$, hence $z(\tau) = 0$. Then (6) implies that $0 = \frac{xy}{\mu}$ for all $\tau \geq 0$. However at $\tau = 0$, we have $\frac{xy}{\mu} = \frac{1}{\mu}$. This is a contradiction, thus the assumption that $v \equiv 1$ is false. □

Proof of Theorem 2.5 In Lemma 2.3 we have shown that $\lim_{\tau\to\infty} u(\tau) = 0$. Since $u = x + \mu z$ and x and z are non-negative functions, we conclude that $\lim_{\tau\to\infty} x(\tau) = \lim_{t\to\infty} z(t) = 0$ also. Hence, every trajectory will converge to a neighbourhood of the y-axis. We have also seen in Lemma 2.1 that the y-axis is invariant and that on this axis, all orbits tend to the stable steady state at $y = 1$. So intuitively it is clear that all trajectories will converge to the steady state (7) as $\tau \to \infty$. To make this formal, we consider the y-dynamics in a neighbourhood of the y-axis.

Let $\varepsilon > 0$. Since $\lim_{\tau\to\infty} x(\tau) = \lim_{\tau\to\infty} z(\tau) = 0$, then there is some $T > 0$ such that for $\tau > T$, we have

$$0 \leq x(\tau) \leq \varepsilon\mu k_3 \quad \text{and} \quad 0 \leq z(\tau) \leq \varepsilon k_3/k_2.$$

Thus with (5), we get for $\tau > T$,

$$k_3\left[1 - (1+\varepsilon)y)\right] \leq \dot{y} \leq k_3\left[1 + \varepsilon - y\right].$$

In other words, $\frac{d}{d\tau}\left[e^{k_3(1+\varepsilon)\tau}\left(y-\frac{1}{1+\varepsilon}\right)\right]\geq 0$ and $\frac{d}{d\tau}\left[e^{k_3\tau}\left(y-(1+\varepsilon)\right)\right]\leq 0$ and we can conclude that for $\tau \geq T$

$$-\frac{\varepsilon}{1+\varepsilon}+e^{-k_3(1+\varepsilon)(\tau-T)}\left(y(T)-\frac{1}{1+\varepsilon}\right)\leq y(\tau)-1\leq \varepsilon+e^{-k_3(\tau-T)}\left(y(T)-(1+\varepsilon)\right)$$

Hence by choosing ε sufficiently small and for all $\tau > T$ sufficiently large, we can make $|y(\tau)-1|$ arbitrarily small. So we conclude that $y(\tau)\to 1$ for $\tau\to\infty$. □

Proof of Lemma 2.7 This observation follows quickly from the expressions for the eigenvalues λ_1 and λ_2. Indeed, from (10) we find that

$$k_2+k_4+\lambda_1=\frac{1}{2}\left(-(1+k_1-k_2-k_4)+\sqrt{(1+k_1-k_2-k_4)^2+4k_2}\right)>0,$$

since $k_2>0$ and hence the square root term is larger than the magnitude of the first term. Similarly, using (12) we get

$$k_2+k_4+\lambda_2=\frac{1}{2}\left(-(1+k_1-k_2-k_4)-\sqrt{(1+k_1-k_2-k_4)^2+4k_2}\right)<0. \quad \square$$

Proof of Lemma 2.8 Let $k_2, k_4 > 0$ be fixed. Differentiating the expression for λ_1, (10), with respect to k_1 gives

$$\frac{\partial\lambda_1}{\partial k_1}=-\frac{1}{2}\left(1-\frac{1+k_1-k_2-k_4}{\sqrt{(1+k_1-k_2-k_4)^2+4k_2}}\right).$$

The second term in the brackets is less than one in modulus (since $k_2>0$), and so this derivative is always negative. Thus, λ_1 is a monotonically decreasing function of k_1.

To derive the stated bounds on $-\lambda_1$ that involve k_4, we note that from (9) we have

$$\lambda_1+k_4=\frac{1}{2}\left(-(1+k_1+k_2-k_4)+\sqrt{(1+k_1+k_2-k_4)^2+4(k_4-k_1)k_2}\right). \tag{29}$$

When $k_1<k_4$, the square root term in (29) always dominates the first term, and so $\lambda_1+k_4>0$. When $k_1=k_4$, we have

$$\lambda_1+k_4=\frac{1}{2}\left(-(1+k_2)+|1+k_2|\right)=0.$$

Finally, when $k_1>k_4$, we note that $1+k_1+k_2-k_4>0$ and in this case, the first term in (29) dominates the square root term and so we find that $\lambda_1+k_4<0$.

Similarly, for the bounds on $-\lambda_1$ involving k_1, from (9) we obtain

$$\lambda_1+k_1=\frac{1}{2}\left(-(1-k_1+k_2+k_4)+\sqrt{(1-k_1+k_2+k_4)^2+4(k_1-k_4)}\right).$$

The sign of $\lambda+k_1$ is then obtained in the same way as for the sign of $\lambda+k_4$ above. □

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