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Modeling Immune Response to BK Virus Infection and Donor Kidney in Renal Transplant Recipients

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Abstract

In this paper we develop and validate with bootstrapping techniques a mechanistic mathematical model of immune response to both BK virus infection and a donor kidney based on known and hypothesized mechanisms in the literature. The model presented does not capture all the details of the immune response but possesses key features that describe a very complex immunological process. We then estimate model parameters using a least squares approach with a typical set of available clinical data. Sensitivity analysis combined with asymptotic theory is used to determine the number of parameters that can be reliably estimated given the limited number of observations.

Keywords

Renal transplant; human polyomavirus type 1 (BKV); mathematical model; inverse problem; sensitivity analysis

1 Introduction

According to the OPTN/SRTR 2011 Annual Report [15], 17,604 kidney transplants were performed in the United States between 2010 and 2011. Overall, there were 54,599 active candidates on the waiting list for kidney transplants, roughly 3-fold more than those that underwent transplant. These numbers reflect trends consistent with previous years, in which the number of patients waiting for transplants greatly exceeds the availability of organs.

Given these facts, and the fact that as of June 30, 2011, 164,200 adults in the U.S. were surviving with a functioning kidney graft, about twice as many as a decade earlier, optimal care of renal transplant patients is of great importance.

To reduce risk of allograft rejection, the standard of care for renal transplant recipients involves life-long pharmacological immunosuppression, making patients susceptible to opportunistic infections. Specifically, this therapy can render the recipients susceptible to an array of viral pathogens and may also reactivate latent viruses. For some time, human polyomavirus type 1, named “BK virus” (BKV), has been a common pathogen found in kidney transplant patients. BKV is one of the two human polyomaviruses and was first discovered in 1970 in the urine of a kidney transplant patient with the initials B.K. [22]. This double stranded non-enveloped DNA virus with icosahedral capsids asymptotically infects more than 90% of the adult population worldwide and establishes a state of non-replicative infection [25, 26, 29], or latent state. The infection is established in the kidneys and peripheral blood, specifically the renal tubular epithelial and urothelial cells, where replication-permissive cells express the viral capsid proteins followed by virion assembly in the nucleus [16]. This process eventually causes host cell lysis and the release of infectious progeny, deeming BKV replication as cytopathic, leading to a new round of active infection and latency. A high-level of BKV replication in the kidney results in a complication known as PVAN (polyomavirus associated nephropathy) [18, 20, 21, 23]. It is characterized by viral cytopathic changes of renal tubular epithelial cells, with enlarged nuclei, cell rounding, detachment and denudation of basal membranes [32]. Increasing prevalence rates of PVAN (1–10%) have been reported, with allograft dysfunction and loss in greater than 50% of cases [27, 35]. Therefore, PVAN is viewed as one of the leading causes of renal allograft loss in the first two years of transplantation. Unfortunately, there are no available licensed anti-polyoma viral drugs and treatment relies on improving immune function to control BKV replication [18]. Given BKV infection is a significant health threat to immunosuppressed renal transplant patients, patient outcomes might be improved with the use of mathematical modeling to predict the course of the disease in individuals and recommend optimized treatment strategies.

Mathematical modeling is widely used and historically accepted in the physical science and engineering communities as an aid in the understanding of complex phenomena. Specifically, we highlight the use of mathematical modeling with experimental investigations to enhance the understanding of biological processes. The process involves a sequences of steps: (i) empirical observations, experiments and data collection; (ii) formalization of the biological model; (iii) abstraction of mathematical model; (iv) formalization of uncertainty and use of a statistical model; (v) model analysis; (vi) changes in understanding; and (vii) design of new experiments [12]. Given the highly iterative process of mathematical modeling and the recently developed quantitative techniques such as real-time PCR measurements of viral load, flow cytometry of T cell subsets and ELISPOT for virus-specific T cell function, it is feasible to couple mathematical modeling with biological experimentation to investigate the dynamics of viral infection and the cellular immune response.

Here we give a brief review of recent works that use mathematical modeling to investigate viral infection in relation to organ transplant dynamics. Funk et al., in [21], summarized investigations of a retrospective analysis of BKV plasma load in renal transplant recipients undergoing allograft nephrectomy or changes in immunosuppressive regimens. PCR measurements of viral DNA are applied to a standard mathematical model for viral load decay kinetics to estimate the half-life and doubling time of BKV as well as clearance and growth rates [21]. This model addresses purely BKV replication. The next iteration in the modeling process requires the development of dynamic models that account for interactions between viral infection and host cell populations. Funk, Gosert, et al., in [19] extended a 1-compartment model to a 2-compartment model with six state variables describing BKV replication dynamics in renal tubular epithelial cells and in urothelial cells. Estimation of parameters was based on population level clearance, proliferation, etc., rates. The study presented a basic model integrating two replication sites, the kidney and the urinary tract, and derived four variants which incorporated coupled and decoupled dynamics of the two sites. It remains unknown whether the two replicate sites are in fact coupled, however, results in [19] suggest that viral expansion was best explained by models where BKV replication started in the kidney followed by urothelial amplification and then reached an equilibrium amongst both replication sites. The model does not address the response of the immune system to viral infection and donor kidney and little to no statistically-based model validation or calibration as proposed in our efforts here was carried out.

Other viral infections have been studied in relation to organ transplant dynamics. In particular, two models have been developed involving human cytomegalovirus (HCMV) infection. Kepler et al., in [28] developed a dynamic model describing the pathogenesis of primary HCMV infection in immunocompetent and immunosuppressed patients at the cellular/viral mechanistic scale for application to individual clinical data and patient health. The model incorporates dynamics of the viral load, immune response as well as actively-infected, susceptible and latently-infected cells. Results highlight the necessity of longitudinal data for multiple state variables for robust parameter estimation. In addition, Banks et al., in [8] developed a model to describe the immune response to both HCMV viral infection and introduction of a donor kidney in a renal transplant recipient. Dynamics of the viral load, susceptible and infected host cells, the immune response specific to viral infection and the transplant, and creatinine are incorporated into the model. Delineation between the cellular immune response to HCMV infection and the alloreactive immune response to the transplanted kidney as well as the incorporation of creatinine dynamics are vital additions to the dynamic model.

In the present effort, we develop a mathematical model of BKV infection and renal transplant dynamics at the cellular/viral mechanistic scale for application to renal transplant immunosuppressed individual clinical data. Specifically, we adapt dynamics of the HCMV model in [8] to allot for more specific BKV infection features. We eliminate the use of an antiviral treatment term, incorporate the effect of the alloreactive immune response and the presence of susceptible host cells on the clearance of creatinine, and add the effect of susceptible host cells on the concentration of allospecific CD8+ T cells. In contrast to the model in [19], we assume that the two BKV replication sites, the kidney and the urinary tract, are decoupled, focussing on the kidney as the main replication site. This choice was

not only made given the inconclusive literature but the data types available. Examining BKV replication within the urinary tract would require BKV urine data, which is not included in our available data sets. We use a typically available data set to pursue statistically-based model validation or calibration in attempts to discern the specific information content one might expect in such a data set.

The remainder of this paper is organized as follows. In Section 2, we present the biological model for which we base our mathematical model describing the immune response to both BKV infection and the introduction of a donor kidney in a renal transplant patient. An overview of clinical data acquired from collaborators is also given in Section 2. Model calibration and analysis is detailed in Section 3, where we give details regarding a log-transformed system, provide a detailed procedure for sensitivity analysis as well as outline our iterative inverse problem methodology. Details regarding computation of standard errors and confidence intervals are also discussed in Section 3. Results are given and discussed as well. In Section 4 we summarize efforts and conclusions drawn as well as suggest future research efforts.

2 Mathematical model description and data

In this section we describe the dynamics of the viral load V , susceptible H_S and infected H_I host cells, BKV-specific E_V and allospecific E_K effector CD8+ T cells and serum creatinine C with a brief description of the underlying biological model for which we base our mathematical model. Table 1 lists the state variables and Figure 1 diagrams the intracellular dynamics embodied in the model.

Active BKV infection targets both renal tubular epithelial cells and urothelial cells. For this model, however, we focus on one BKV target, the renal tubular epithelial cells. Susceptible host cells, the uninfected kidney tubular epithelial cells, H_S , in the absence of infection, are

assumed to proliferate through the term $\lambda_{HS} \left(1 - \frac{H_S}{\kappa_{HS}}\right) H_S$, indicating that new epithelial cells are derived from proliferation of existing H_S . Proliferation is modeled by logistic dynamics with λ_{HS} being the maximum proliferation rate and κ_{HS} is the cell density at which proliferation shuts off. A loss of susceptible cells, H_S , due to viral infection which occurs by cell-to-cell transmission, is represented by the term $\beta H_S V$. Here we assume that one copy of virion infects one cell. Infected host cells or BKV replicating cells, H_I , lyse due to the cytopathic effect of BK virus, represented by the term $\delta_{HI} H_I$ and produce ρ_V virions before death. In addition, infected host cells are eliminated by the BK-specific effector CD8+ T cells with rate term $\delta_{EH} E_V H_I$. Free virus is naturally cleared at the rate δ_V by the body and a loss of viral concentration is experienced through the infection of susceptible host cells.

The cellular immune response is the key defense against the BK-viral infection. The terms λ_{EV} and δ_{EV} represent the source and death rates of BK-specific effector CD8+ T cells. The concentration of BK-specific CD8+ T cells increases in response to the presence of free virus through the term $\rho_{EV} E_V$, where ρ_{EV} is a bounded positive increasing function of free virus concentration. Specifically, $\rho_{EV}(V) = (\rho_{EV} V)/(V + \kappa_V)$ is a saturating nonlinearity with

positive constants ρ_{EV} and κ_V . The alloreactive immune response to the transplanted kidney is incorporated into the model via a state variable, E_K , which denotes the effector CD8+ T cells that specifically target the transplant. The source rate for E_K , λ_{EK} , is dependent upon the HLA donor/recipient matching conducted prior to transplantation. Similar to the BK-specific CD8+ T cells, the concentration of allospecific CD8+ T cells increases in response to the presence of susceptible host cells H_S , since BK-virus targets kidney cells, represented by the term $\rho_{EK} E_K$, where $\rho_{EK}(H_S) = (\rho_{EK} H_S)/(H_S + \kappa_{KH})$ with positive constants ρ_{EV} and κ_{KH} . The death rate of E_K is represented by δ_{EK} .

Finally, we discuss the role of creatinine in the model. Creatinine is a waste product in the blood resulting from muscle activity and is removed by the healthy kidney. Therefore, serum creatinine concentration C is used as a surrogate for glomerular filtration rate (GFR), a commonly used index of kidney function [30]. The production rate of C is represented by λ_C and when the kidney is impaired, creatinine is not effectively filtered and its concentration increases. To account for the negative effect of the alloreactive immune response E_K on the kidney and the positive effect of susceptible cells H_S (Recall that the renal allograft is a site of replication. Hence, the concentration of susceptible cells reflects the health of the kidney.), the clearance rate δ_C is defined as follows

$$\delta_C(E_K, H_S) = \frac{\delta_{C0} \kappa_{EK}}{E_K + \kappa_{EK}} \cdot \frac{H_S}{H_S + \kappa_{CH}}.$$

Table 2 lists the parameters used in the model.

Based on the above discussion, the model is given as follows.

$$\dot{H}_S = \lambda_{HS} \left(1 - \frac{H_S}{\kappa_{HS}}\right) H_S - \beta H_S V, \quad (1)$$

$$\dot{H}_I = \beta H_S V - \delta_{HI} H_I - \delta_{EH} E_V H_I, \quad (2)$$

$$\dot{V} = \rho_V \delta_{HI} H_I - \delta_V V - \beta H_S V, \quad (3)$$

$$\dot{E}_V = (1 - \varepsilon_I) [\lambda_{EV} + \rho_{EV}(V) E_V] - \delta_{EV} E_V, \quad (4)$$

$$\dot{E}_K = (1 - \varepsilon_I) [\lambda_{EK} + \rho_{EK}(H_S) E_K] - \delta_{EK} E_K, \quad (5)$$

$$\dot{C} = \lambda_C - \delta_C(E_K, H_S) C. \quad (6)$$

with initial conditions,

$$(H_S(0), H_I(0), V(0), E_V(0), E_K(0), C(0)) = (H_{S0}, H_{I0}, V_0, E_{V0}, E_{K0}, C_0). \quad (7)$$

We note that (1)–(4) describe the immune response to the viral infection coupled with (5) and (6) describing the immune response to the transplanted kidney. Here ε_I represents the efficacy of immunosuppressive drugs and is assumed to be scaled to less than or equal to 1. This variable serves as the controller of the system to achieve balance between under-suppression and over-suppression of the patient's immune system.

2.1 Data

The data for our investigation is from Massachusetts General Hospital (MGH) and our discussions here consists of one patient record, TOS003. The data collection is performed as part of the patient's routine medical care. Visits include pre-transplantation evaluation, day of transplant, and post-transplantation visits. Day of transplant and post-transplantation visits are pertinent for model validation. Record TOS003 describes immunosuppression regimen, renal function recorded in plasma creatine (mg/dL) levels, and infectious complications given in BKV viral plasma loads (in DNA copies/mL). TOS003 associated data consists of eight viral load measurements and sixteen serum creatinine measurements. It should be noted that the recipient was diagnosed with "BK virus reactivation" over the course of the first three months post-transplantation. It was documented that TOS003 was given immunosuppressive treatment upon organ transplantation and monitored accordingly. We note that the efficacy of the immunosuppression is a function of time, given the evidence in the data, however, it is difficult to quantify the efficacy of the immunosuppression from the drug regimen records. The dosage, type, and combination of drugs are changed after each visit. Hence, for simplicity, we assume that ε_I can be approximated by the following piecewise constant function.

$$\varepsilon_I(t) = \begin{cases} \varepsilon_1, & t \in [0, 21], \\ \varepsilon_2, & t \in (21, 60], \\ \varepsilon_3, & t \in (60, 120], \\ \varepsilon_4, & t \in (120, 450], \end{cases} \quad (8)$$

where the time frames are chosen based on the drug regimen record. It is also important to note that we are assuming test results displaying "None detected" equate to the detection limit 20 copies/mL of free virus in the system [21]. In addition, no test quantifying the BK viral load was conducted pre-transplant or the day of transplant, however, due to the seroprevalence of BKV in the population, it is assumed that the patient had a latent infection.

3 Model calibration and analysis

The model contains 6 variables and 29 constant parameters (model parameters and initial conditions). Equations (1)–(6) were first written as a vector system

$$\dot{\bar{\mathbf{x}}} = \bar{\mathbf{g}}(\bar{\mathbf{x}}; \bar{\mathbf{p}}, \bar{\mathbf{x}}_0),$$

$$\bar{\mathbf{x}}(0) = \bar{\mathbf{x}}_0,$$

where $\mathbf{x}^- = [H_S, H_I, V, E_V, E_K, C]^T$ is the vector of model states, $\mathbf{p}^- = [\lambda_{HS}, \beta, \delta_{EH}, \delta_V, \rho_{EV}, \delta_{EV}, \delta_{EK}, \delta_{C0}, \kappa_{HS}, \delta_{HI}, \rho_V, \lambda_{EV}, \kappa_V, \lambda_{EK}, \lambda_C, \kappa_{EK}, \kappa_{CH}, \rho_{EK}, \kappa_{KH}, \varepsilon_1, \varepsilon_2, \varepsilon_3, \varepsilon_4]^T \in \mathbb{R}^{23}$ is the vector of model parameters, and $\mathbf{x}_0^- = [H_{S0}, H_{I0}, V_0, E_{V0}, E_{K0}, C_0]^T \in \mathbb{R}^6$ is the set of initial conditions. Due to the scale difference between model states, we adopted the approach used in [1, 3]: solutions were determined for a log-transformed system. We remark that this is common in the biological literature to use log scale to deal with very large numbers; for example, in HIV studies and other viral infection studies both the viral load level (which can be as high as millions of copies/mL) and the changes in the viral load level can be large and hence they are often reported and analyzed in log scale (e.g., see [24, 31, 33]). Since model parameter values and initial conditions are in different scales, a subset of the model parameters and initial conditions are also log-transformed to the log scale. This log-transformation approach is used to convert all the analyzed quantities roughly to the same scale. It is worth noting that whenever we carry out a log-transformation for a quantity, we only log-transform its corresponding numerical value. In other words, $\log_{10}(x)$ is always understood as a shorthand notation for $\log_{10}((x \text{ [units]})/(1 \text{ [units]}))$ so the argument of log function is really dimensionless. An alternative approach, a common practice in engineering, to deal with the scale difference among analyzed quantities is to normalize them so that the numerical values of the resulting quantities vary between 0 and 1, e.g., $x = (x - x_{\min})/(x_{\max} - x_{\min})$, where x stands for quantity x after normalization and subscripts min and max respectively denote the minimal and maximal values of x . However, for most biological applications the minimal and maximal values of the analyzed quantities are usually unknown and may vary among individuals, which is true for the problem we presented here. Thus, this normalization approach would result in a large number of extra unknown parameters that need to be identified (recall that the model contains 6 model states and 29 constant parameters). Considering the limited number of observations available to us, this approach can provide significant, if not impossible, challenges for the inverse problem investigated below and thus will not be considered in this paper.

Let,

$$x_i = \log_{10}(\bar{x}_i), i=1, 2, 3, 4, 5,$$

$$x_6 = \bar{x}_6,$$

$$x_{0i} = \log_{10}(\bar{x}_{0i}), i=1, 2, 3, 4, 5,$$

$$x_{06} = \bar{x}_{06},$$

$$p_j = \log_{10}(\bar{p}_j), j=1, 2, \dots, 19,$$

$$p_j = \bar{p}_j, j=20, 21, 22, 23.$$

Then we have the system

$$\dot{\mathbf{x}} = \mathbf{g}(\mathbf{x}; \mathbf{p}, \mathbf{x}_0), \mathbf{x}(0) = \mathbf{x}_0, \quad (9)$$

where $\mathbf{g} = (g_1, g_2, \dots, g_6)^T$ is given by

$$g_i(\mathbf{x}; \mathbf{p}, \mathbf{x}_0) = \frac{10^{-x_i}}{\ln(10)} \bar{g}_i(\bar{\mathbf{x}}; \bar{\mathbf{p}}, \bar{\mathbf{x}}_0), i=1, 2, \dots, 5,$$

$$g_6(\mathbf{x}; \mathbf{p}, \mathbf{x}_0) = \bar{g}_6(\bar{\mathbf{x}}; \bar{\mathbf{p}}, \bar{\mathbf{x}}_0).$$

We remark that by using the above log-transformed system (9) one can resolve a problem of states becoming unrealistically negative in solving model (1)–(6) due to round-off error: nonnegative solutions of this model should stay so throughout numerical simulation. This log-transformation approach also enables the changes in model states due to the changes in parameters to be more measurable. In addition, this enables more easily formulation of a reasonable stopping criterion for the inverse problem and can also make the associated optimization algorithm converge faster (vastly different magnitudes of analyzed quantities increase condition numbers and hence the associated optimization algorithm may require more iterations to converge). From a statistical point of view, log-transformation is also a standard technique to render the observations more nearly normally distributed, and it is also commonly used to render heteroscedastic measurement errors more homoscedastic (e.g., see [13, 14, 37]) so that the resulting inverse problem is easier to implement (in ordinary least squares approaches as opposed to generalized least squares approaches, e.g., see [11, 37] for details).

3.1 Forward simulations

Forward simulations were carried out by numerically solving the log transformed version of model equations (9) in Matlab using the ODE variable order variable step-size solver `ode15s` over a time course of 450 days. Parameter values used are listed in Table 3. The values of these parameters were either derived from published experimental studies or through simulation to acquire a benchmark value. For those parameters whose values can be found or derived from the literature, specifics are detailed below.

1. The parameter ρ_V , which quantifies the number of virions released by BKV-infected cells, was taken to be $\rho_V \approx 6000$ copies/cell [19, 20, 21, 36].
2. The measured BK viral half-life was found to be $t_{\frac{1}{2}} \approx 1.1\text{hrs} - 9\text{days}$. This implies that the total clearance rate of BK is from 0.077 to 15.1232 per day. In the simulations, we set $\delta_V = 0.4$ per day [19, 20].

3.2 Sensitivity analysis

In practice, one may be in a situation of estimating a large number of unknown parameters with a limited data set. Such a situation is true in our case. To alleviate some of this difficulty, sensitivity analysis has been widely used in inverse problem investigations [2, 3, 4, 5, 6, 12, 34]. This process identifies the model parameters and initial conditions to which the model outputs are most sensitive. Specifically, sensitivity analysis provides insight into how changes in the parameters can affect the model output. In addition, this framework can be used to construct confidence intervals for parameter estimates using asymptotic properties of estimators.

To compute the sensitivities of model outputs to the model parameters and initial conditions, one proceeds to determine the sensitivity of each model state x_i to each parameter p_j and initial condition x_{0j} . These are defined as the derivatives of the model states with respect to the parameters, $\frac{\partial x_i}{\partial p_j}$ and $\frac{\partial x_i}{\partial x_{0j}}$, which satisfy in our case

$$\frac{d}{dt} \frac{\partial x_i}{\partial p_j} = \sum_{l=1}^6 \frac{\partial g_i}{\partial x_l} \frac{\partial x_l}{\partial p_j} + \frac{\partial g_i}{\partial p_j},$$

$$\frac{\partial x_i}{\partial p_j}(0) = 0, i=1, 2, \dots, 6, j=1, 2, \dots, 23, \quad (10)$$

$$\frac{d}{dt} \frac{\partial x_i}{\partial x_{0j}} = \sum_{l=1}^6 \frac{\partial g_i}{\partial x_l} \frac{\partial x_l}{\partial x_{0j}},$$

$$\frac{\partial x_i}{\partial x_{0j}}(0) = \delta_{ij}, i=1, 2, \dots, 6, j=1, 2, \dots, 6, \quad (11)$$

where

$$\delta_{ij} = \begin{cases} 1, & i=j, \\ 0, & i \neq j. \end{cases}$$

The sensitivities $\frac{\partial \mathbf{x}}{\partial \mathbf{p}}$ and $\frac{\partial \mathbf{x}}{\partial \mathbf{x}_0}$ can be calculated by solving (9), (10), (11) simultaneously in

Matlab using `ode15s` where the derivatives $\frac{\partial \mathbf{g}}{\partial \mathbf{x}}$, $\frac{\partial \mathbf{g}}{\partial \mathbf{p}}$ are obtained through automatic differentiation (AD) using `myAD` and `tssolve` in Matlab. In our case, we have data for both the free virus, V , and serum creatinine, C , states. Therefore, we only need to compute the sensitivities corresponding to states x_i , $i = 3, 6$. Initial conditions and model parameter values used are listed in Table 3. Results of the sensitivity analysis informed us as to which

parameters were to be estimated. These findings along with the corresponding confidence intervals for these estimated parameters are described below.

It is worth emphasizing that sensitivity coefficients obtained above are for the log-transformed system (9). We note that in the case where $x_i = \bar{x}_i$, $p_j = \log_{10}(\bar{p}_j)$ and $x_{0j} = \log_{10}(\bar{x}_{0j})$ we have

$$\frac{\partial x_i}{\partial p_j} = \frac{\partial x_i}{\partial \bar{x}_i} \frac{\partial \bar{x}_i}{\partial \bar{p}_j} \frac{\partial \bar{p}_j}{\partial p_j} = \log(10) \bar{p}_j \frac{\partial \bar{x}_i}{\partial \bar{p}_j}, \quad \frac{\partial x_i}{\partial x_{0j}} = \frac{\partial x_i}{\partial \bar{x}_i} \frac{\partial \bar{x}_i}{\partial \bar{x}_{0j}} \frac{\partial \bar{x}_{0j}}{\partial x_{0j}} = \log(10) \bar{x}_{0j} \frac{\partial \bar{x}_i}{\partial \bar{x}_{0j}}.$$

Hence, the sensitivity coefficients $\frac{\partial x_i}{\partial p_j}$ and $\frac{\partial x_i}{\partial x_{0j}}$ obtained here are really the so-called relative sensitivity coefficients in [34]. We also note that in the case where $x_i = \log_{10}(\bar{x}_i)$, $p_j = \log_{10}(\bar{p}_j)$ and $x_{0j} = \log_{10}(\bar{x}_{0j})$ we have

$$\frac{\partial x_i}{\partial p_j} = \frac{\partial x_i}{\partial \bar{x}_i} \frac{\partial \bar{x}_i}{\partial \bar{p}_j} \frac{\partial \bar{p}_j}{\partial p_j} = \frac{\bar{p}_j}{\bar{x}_i} \frac{\partial \bar{x}_i}{\partial \bar{p}_j}, \quad \frac{\partial x_i}{\partial x_{0j}} = \frac{\partial x_i}{\partial \bar{x}_i} \frac{\partial \bar{x}_i}{\partial \bar{x}_{0j}} \frac{\partial \bar{x}_{0j}}{\partial x_{0j}} = \frac{\bar{x}_{0j}}{\bar{x}_i} \frac{\partial \bar{x}_i}{\partial \bar{x}_{0j}}.$$

Hence, the sensitivity coefficients $\frac{\partial x_i}{\partial p_j}$ and $\frac{\partial x_i}{\partial x_{0j}}$ obtained here are really the so-called dimensionless sensitivity coefficients in [34]. For more information on the relative sensitivity coefficients and dimensionless sensitivity coefficients, we refer the interested reader to [34].

3.3 Parameter estimation

The observed amount of free virus (DNA) in the blood is represented by $\bar{y}_i^1$, with corresponding measured time point t_i^1 , $i = 1, 2, \dots, n_1$ and $\bar{y}_i^2$ is the observed amount of serum creatinine at time point t_i^2 , $i = 1, 2, \dots, n_2$. We define $y_i^1 = \log_{10}(\bar{y}_i^1)$, $i = 1, 2, \dots, n_1$ and $y_i^2 = \bar{y}_i^2$, $i = 1, 2, \dots, n_2$. Following standard inverse problem procedures [11, 12, 13, 14, 34, 37], we define random variables Y_i^1 and Y_i^2 and formulate the statistical model as follows:

$$Y_i^1 = f_1(t_i^1; \mathbf{q}_0) + \varepsilon_i^1, \quad i = 1, 2, \dots, n_1, \quad (12)$$

$$Y_i^2 = f_2(t_i^2; \mathbf{q}_0) + \varepsilon_i^2, \quad i = 1, 2, \dots, n_2,$$

where $n_1 = 8$ and $n_2 = 16$. Here $f_1(t_i^1; \mathbf{q}_0) = x_3(t_i^1; \mathbf{q}_0)$ and $f_2(t_i^2; \mathbf{q}_0) = x_6(t_i^2; \mathbf{q}_0)$, where $\mathbf{q}_0 \in \mathbb{R}^K$ denotes the hypothesized “true values” of the model parameters and initial conditions that need to be estimated (K is a positive integer and denotes the number of model parameters and initial conditions to be estimated). The observation errors ε_i^1 , $i = 1, 2, \dots, n_1$ and ε_i^2 , $i = 1, 2, \dots, n_2$ are assumed to be independent and identically distributed (*i.i.d*) with

zero mean and constant variance σ_0^2 . Therefore, $\mathbf{q}_0$ can be properly estimated by using an ordinary least squares (OLS) technique

$$\hat{\mathbf{q}} = \underset{\mathbf{q} \in \mathcal{Q}}{\operatorname{argmin}} \left[\sum_{i=1}^{n_1} |f_1(t_i^1; \mathbf{q}) - y_i^1|^2 + \sum_{i=1}^{n_2} |f_2(t_i^2; \mathbf{q}) - y_i^2|^2 \right], \quad (13)$$

where $\mathcal{Q}$ is some compact set of admissible values in $\mathbb{R}^\kappa$.

Note that we have a large number of parameters (29 parameters) with little experimental data ($n_1 + n_2 = 24$ time observations). Hence, in attempts to produce reliable estimates, the parameter estimation was implemented in an iterative process similar to that used in Adams et al., [1], Banks et al., [3]. This process entailed identifying the most influential or sensitive parameter subset at each step. Quantification of influence is determined by sensitivity rankings based on the quantities

$$S_V(q_j) = \sqrt{\sum_{k=1}^{n_1} \left(\frac{\partial x_3(t_k^1)}{\partial q_j} \right)^2}, \quad j=1, 2, \dots, \kappa,$$

$$S_C(q_j) = \sqrt{\sum_{k=1}^{n_2} \left(\frac{\partial x_6(t_k^2)}{\partial q_j} \right)^2}, \quad j=1, 2, \dots, \kappa. \quad (14)$$

Again, κ denotes the number of model parameters and initial conditions to be estimated.

Initially, we computed sensitivity rankings for all parameters with respect to the viral load and creatinine state variables using (14), where parameters values are chosen as those given in Table 3. Our findings are given in Table 6 Appendix A. Given these results in Table 6, we identified the parameters that are the most influential in the viral load state variables and the most influential in the creatinine state variables. We subsequently designed an iterative inverse problem algorithm that utilizes the sensitivity rank information to propose parameters sets that we wish to estimate. All sensitivity ranking results for the following algorithm are found in the tables in Appendix A.

Parameters were estimated using the Matlab routine `lsqnonlin`. It is important to note that the use of overbar notation when referencing model parameters and initial conditions refers to the original scale. This is done to simplify notation when reporting estimated and fixed parameters within the algorithm. We remind the reader that all simulations were conducted using the log-transformed system with log-scaled parameter values as previously discussed.

Step 1: We fixed model parameters and initial conditions $q_{U1}^- = [\lambda_{EV}, \lambda_{EK}, \kappa_{EK}, \kappa_{CH}, \kappa_{KH}, H_{S0}, H_{I0}, V_0, E_{K0}]^T \in \mathbb{R}^9$ using corresponding values found in Table 3 based on sensitivity rankings in Table 6. Next, we estimated the remaining parameters $q_{E1}^- = [\lambda_{HS}, \beta, \delta_{EH}, \delta_V, \rho_{EV}, \delta_{EV}, \delta_{EK}, \delta_{C0}, \kappa_{HS}, \delta_{HI}, \rho_V, \kappa_V, \lambda_C, \rho_{EK}, \varepsilon_1, \varepsilon_2, \varepsilon_3, \varepsilon_4, E_{V0}, C_0]^T \in \mathbb{R}^{20}$ with initial values found in Table 3 and obtained q_{E1}^- using the OLS procedure in

(13). Results $\theta_{opt}^1 = [q_{U1}, \hat{q}_{E1}]^T \in \mathbb{R}^{29}$ are found in Table 10. We will refer to this experiment as “parameters estimation # 1”.

Step 2: We performed a sensitivity analysis for the 20 estimated parameters in Step 1 using corresponding values in θ_{opt}^1 . The resulting sensitivity rankings are presented in Table 8. We then fixed an additional 5 parameters chosen from these 20 parameters based on the sensitivity rankings. That is, we fixed 14 model parameters and initial conditions $q_{U2} = [q_{U1}, \delta_{EH}, \kappa_V, \varepsilon_1, E_{V0}, C_0]^T \in \mathbb{R}^{14}$ using corresponding values found in θ_{opt}^1 . We estimated the remaining parameters $q_{E2} = [\lambda_{HS}, \beta, \delta_V, \rho_{EV}, \delta_{EV}, \delta_{EK}, \delta_{C0}, \kappa_{HS}, \delta_{HI}, \rho_V, \lambda_C, \rho_{EK}, \varepsilon_2, \varepsilon_3, \varepsilon_4]^T \in \mathbb{R}^{15}$ with initial values found in θ_{opt}^1 and obtained $\hat{q}_{E2}$ using OLS procedure. Results $\theta_{opt}^2 = [q_{U2}, \hat{q}_{E2}]^T \in \mathbb{R}^{29}$ are found in Table 12. We will refer to this experiment as “parameters estimation # 2”.

Step 3: We performed a sensitivity analysis for the 15 estimated parameters in Step 2 using corresponding values in θ_{opt}^2 . The resulting sensitivity rankings are presented in Table 9. We then fixed an additional 5 parameters chosen from these 15 parameters based on the sensitivity rankings. That is, we fixed 19 model parameters and initial conditions $q_{U3} = [q_{U2}, \lambda_{HS}, \delta_{C0}, \delta_{HI}, \lambda_C, \varepsilon_2]^T \in \mathbb{R}^{19}$ using corresponding values found in θ_{opt}^2 . We then estimated the remaining parameters $q_{E3} = [\beta, \delta_V, \rho_{EV}, \delta_{EV}, \delta_{EK}, \kappa_{HS}, \rho_V, \rho_{EK}, \varepsilon_3, \varepsilon_4]^T \in \mathbb{R}^{10}$ with initial values found in θ_{opt}^2 and obtained $\hat{q}_{E3}$ using the OLS procedure. Results $\theta_{opt}^3 = [q_{U3}, \hat{q}_{E3}]^T \in \mathbb{R}^{29}$ are found in Table 14. We will refer to this experiment as “parameters estimation # 3”.

Step 4: We performed a sensitivity analysis for the 10 estimated parameters in Step 3 using corresponding values in θ_{opt}^3 . The resulting sensitivity rankings are presented in Table 7. We then fixed an additional 5 parameters chosen from these 10 parameters based on the sensitivity rankings. That is, we fixed 24 model parameters and initial conditions $q_{U4} = [q_{U3}, \delta_V, \kappa_{HS}, \rho_V, \varepsilon_3, \varepsilon_4]^T \in \mathbb{R}^{24}$ using corresponding values found in θ_{opt}^3 . Next, we estimated the remaining parameters $q_{E4} = [\beta, \rho_{EV}, \delta_{EV}, \delta_{EK}, \rho_{EK}]^T \in \mathbb{R}^5$ with initial values found in θ_{opt}^3 and obtained $\hat{q}_{E4}$ using the OLS procedure. Results $\theta_{opt}^4 = [q_{U4}, \hat{q}_{E4}]^T \in \mathbb{R}^{29}$ are found in Table 4. We will refer to this experiment as “parameters estimation # 4”.

Next, we outline a method to quantify the uncertainty in our parameter estimations. Two methods that have been widely used in the literature to quantify uncertainty in parameter estimates are asymptotic theory and bootstrapping. Both have been investigated and compared in Banks et al. [7] for problems with different form and level of noise. It was found that asymptotic theory is always faster computationally than bootstrapping and there is no clear advantage in using bootstrapping versus asymptotic theory when the constant variance using OLS is assumed. For these reasons, we will use asymptotic theory [4, 37] to quantify the uncertainty in our parameter estimations.

We calculated standard errors and confidence intervals [11, 12] in order to quantify the uncertainty in parameter estimates. To compute these values, we must define some terms. Recall the statistical model defined in (12). Let

$$\mathcal{F}(\mathbf{q}) = (f_1(t_1^1; \mathbf{q}), f_1(t_2^1; \mathbf{q}), \dots, f_1(t_{n_1}^1; \mathbf{q}), f_2(t_1^2; \mathbf{q}), f_2(t_2^2; \mathbf{q}), \dots, f_2(t_{n_2}^2; \mathbf{q}))^T.$$

Then the sensitivity matrix $\chi(\mathbf{q})$ is an $(n_1 + n_2) \times \kappa$ matrix, where $N = n_1 + n_2$ is the total number of viral and creatinine data points and κ is the number of estimated parameters, with its (i, j) th element being defined as

$$\chi_{ij}(\mathbf{q}) = \frac{\partial \mathcal{F}_i(\mathbf{q})}{\partial q_j}, i=1, 2, \dots, n_1+n_2, j=1, 2, \dots, \kappa, \quad (15)$$

where $\mathcal{F}_i$ is the i th element of $\mathcal{F}$, and q_j is the j th element of $\mathbf{q}$. Given the data $\{y_i\}_{i=1}^N = \{y_1^1, y_2^1, \dots, y_{n_1}^1, y_1^2, y_2^2, \dots, y_{n_2}^2\}$ and the resulting parameter estimate $\hat{\mathbf{q}}$, the variance σ_0^2 can be approximated by

$$\sigma_0^2 \approx \hat{\sigma}^2 = \frac{1}{N - \kappa} \sum_{j=1}^N [y_j - \mathcal{F}_j(\hat{\mathbf{q}})]^2. \quad (16)$$

With these values, we can calculate

$$\hat{\Sigma}(\hat{\mathbf{q}}) = \hat{\sigma}^2 [\chi(\hat{\mathbf{q}})^T \chi(\hat{\mathbf{q}})]^{-1}. \quad (17)$$

This matrix is known as the covariance matrix and is used to compute the standard errors for each element of $\hat{\mathbf{q}}$ given by

$$SE_k(\hat{\mathbf{q}}) = \sqrt{\hat{\Sigma}_{kk}(\hat{\mathbf{q}})}, k=1, 2, \dots, \kappa. \quad (18)$$

Hence, the $100(1 - \alpha)\%$ confidence intervals are given by

$$[\hat{q}_k - t_{1-\alpha/2} SE_k(\hat{\mathbf{q}}), \hat{q}_k + t_{1-\alpha/2} SE_k(\hat{\mathbf{q}})]. \quad (19)$$

We determine $t_{1-\alpha/2}$ by $\text{Prob}\{T \leq t_{1-\alpha/2}\} = \alpha/2$, where T has a student's t distribution $t^{N-\kappa}$ with $N - \kappa$ degrees of freedom.

For the following results, we chose $\alpha = 0.05$. Results obtained for parameter estimation steps #1, #2, #3 are presented in Appendix B. We observe that parameter estimations # 1–3 produced good fits to the data (as seen in Figures 4–6). However, there is substantial uncertainty in at least some components of the estimates for all three cases (see Tables 11, 13, 15). The reliability of parameter estimates depends on the number of parameters estimated. Specifically, $\max_i |\gamma_i|$ increases as κ increases, where γ_i denotes the absolute ratio of the standard error to the corresponding estimate for the i th estimated parameter. This is in agreement with common understanding that for a fixed number of observations, the

estimation accuracy decreases as the number of parameters increases [11]. Further discussions of these results are found in Appendix B.

Results corresponding to parameter estimation # 4 associated with estimating 5 parameters are given in Figure 2, Table 4, and Table 5. We observe from Figure 2 that we obtain good fits to the data, where the model outputs are obtained using parameter values given in Table 4. Table 5 illustrates the estimate, the standard error, the 95% confidence intervals and the absolute ratio of the standard error to the corresponding estimate (denoted by γ). From Table 5, we can see that $\gamma_i < 0.2$, $i = 1, 2, \dots, 5$. These parameter estimates for β , ρ_{EV} , δ_{EV} , δ_{EK} , ρ_{EK} are reliable. Therefore by Figure 2 and Table 5 we may infer that the goodness-to-fit is reasonable.

The next step is then to validate our model with these 5 free parameters. There are a number of methods in the literature that can be used for model validation (e.g., see [17, 38] and the reference therein). These include data-splitting methods, cross-validation methods and bootstrapping methods. Specifically, both data-splitting methods and cross-validation methods use part of the data (training data) for parameter estimation and the rest of the data (test data) for validation. However bootstrapping involves repeatedly fitting the model using the bootstrapping samples and evaluating the performance of the resulting fitted model with the original data. In other words, bootstrapping samples are used as training data and the original data is used as test data. We thus see that bootstrapping method uses the entire data set for parameter estimation and hence it is very efficient and is especially useful for the case where one has a limited number of observations or even with a single (one patient only) clinical data set. Recall that the sample size of our single patient data set is small. Hence, bootstrapping is chosen to validate our model.

Let $J(\mathbf{q}; \{y_j\}_{j=1}^N) = \frac{1}{N} \left(\sum_{j=1}^N |y_j - \mathcal{F}_j(\mathbf{q})|^2 \right)$, where $\{y_j\}_{j=1}^N = \{y_1^1, y_2^1, \dots, y_{n_1}^1, y_1^2, y_2^2, \dots, y_{n_2}^2\}$, $\mathcal{F}(\mathbf{q}) = (f_1(t_1^1; \mathbf{q}), f_1(t_2^1; \mathbf{q}), \dots, f_1(t_{n_1}^1; \mathbf{q}), f_2(t_1^2; \mathbf{q}), f_2(t_2^2; \mathbf{q}), \dots, f_2(t_{n_2}^2; \mathbf{q}))^T$ and $N = n_1 + n_2$. The procedures for using bootstrapping to calculate the predication error are given as follows.

1. Using the estimate $\hat{\mathbf{q}}$ obtained in the parameter estimation #4 to calculate

$$J(\hat{\mathbf{q}}; \{y_j\}_{j=1}^N).$$

2. a. Define the standardized residuals

$$\bar{r}_j = \sqrt{\frac{N}{N - \kappa_\theta}} (y_j - \mathcal{F}_j(\hat{\mathbf{q}})), j=1, 2, 3, \dots, N.$$

Set $m = 1$.

- b. Create a bootstrapping sample of size N using random sampling with replacement from $\{\bar{r}_1, \dots, \bar{r}_N\}$ to form a bootstrapping sample

$$\{r_1^{(m)}, \dots, r_N^{(m)}\}.$$

- c. Create bootstrap sample points

$$y_j^{(m)} = \mathcal{F}_j(\hat{\mathbf{q}}) + r_j^{(m)}, j=1, 2, 3, \dots, N.$$

- d. Obtain a new estimate $\mathbf{q}^{(m)}$ from the bootstrapping sample $\{y_j^{(m)}\}_{j=1}^N$ using OLS. Calculate $J(\hat{\mathbf{q}}^{(m)}; \{y_j^{(m)}\}_{j=1}^N)$ and $J(\hat{\mathbf{q}}^{(m)}; \{y_j\}_{j=1}^N)$.
- e. Set $m = m+1$ and repeat steps (b)–(d) until $m > M$ (e.g., typically $M = 200$ as in our calculations below).

3. Calculate the estimate of optimism (a measure of how much worse our model fits to the new data compared to the training data)

$$O = \frac{1}{M} \left(\sum_{m=1}^M J(\hat{\mathbf{q}}^{(m)}; \{y_j\}_{j=1}^N) - \sum_{m=1}^M J(\hat{\mathbf{q}}^{(m)}; \{y_j^{(m)}\}_{j=1}^N) \right).$$

4. Calculate the optimism adjusted predication error as $J(\hat{\mathbf{q}}; \{y_j\}_{j=1}^N) + O$.

Using the above procedures, we found that the optimism is 3.2667×10^{-4} and the optimism adjusted predication error is 3.4248×10^{-3} . This implies that our model with 5 free parameters is reasonably validated.

To gain some information on the dynamics of other model states (that is, H_S , H_I , E_V and E_K), we plotted these states versus time in Figure 3, where the results were obtained with parameter values given in Table 4. From this figure, we observe that the number of infected cells (indicated in the left panel) increases at the very beginning and then decreases until it settles down to a small value. This agrees with the viral load data shown in Figure 2 as the only source for production of new virus is from the infected cells. We also observe that the number of BK-specific CD8+ T cells E_V (shown in the right panel of Figure 3) increases at the beginning in response to the presence of large amount of free BK virus and then decreases sharply when the viral load decreases to a small level. The trend of increasing-decreasing-increasing of allospecific CD8+ T cells E_K (indicated in the right panel of Figure 3) perfectly explains the pattern shown in the serum creatinine data (see the right panel of Figure 2). This is because E_K has a negative effect on the health of the kidney and the damage to the kidney results in increases in the serum creatinine level (as explained in Section 2).

4 Concluding remarks and future research effort

Our efforts here to develop a mathematical model with statistically based model validation can be properly viewed as an algorithmic approach to determining the information content in a specific experimental data set. That is, we illustrate one approach to determining how many and which model parameters can be estimated with confidence from a given data set. In this context we have developed a mechanistic mathematical model to describe the immune response to both BKV infection and a donor kidney, and have estimated model

parameters and initial conditions using the clinical data. Due to the large number of unknown parameters and limited number of observations, one is unable to reliably estimate all the parameters. To alleviate this difficulty, we employed sensitivity analysis combined with asymptotic theory of estimators to determine the number of parameters that can be reliably estimated. Numerical results show that we were able to reliably estimate five parameters with the given eight viral load data points and sixteen creatinine data points. This clearly demonstrates that one requires more informative longitudinal data, particularly observations on other model variables, in order to fully validate the model. In this context an immediate future effort would be to use optimal design methods (e.g., [9, 10]) to determine when an experimenter should take measurements and what model variables to measure. The obtained results should then be used to guide the future data collection efforts.

Once we obtain sufficient amount of informative longitudinal data to reliably estimate all the unknown parameters, another immediate future effort is to quantify uncertainties of model response, which is due to uncertainty in parameter estimators. Based on the asymptotic theory, we know that parameter estimators are multivariate normally distributed. Thus, this would involve uncertainty quantification of the system of ordinary differential equations (9) driven by a multivariate normally distributed random vector (referred to as a random differential equation). To do this, one could use the fact that the probability density function of solutions to random differential equations is described by the integral of the solution to a hyperbolic partial differential equation with integrator being the cumulative distribution function of a multivariate normally distributed random vector (see [11, Section 7.3.2.2] for details). An alternative approach to do this is to use Monte Carlo methods or other numerical methods such as stochastic Galerkin methods and stochastic collocation methods (e.g., see [11, Section 7.3] and the references therein for details).

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Appendix A

Results corresponding to the sensitivity analysis conducted during Step 1- Step 4 of the iterative inverse problem algorithm are given in Table 6, Table 7, Table 8, and Table 9.

Appendix B

Results corresponding to parameter estimation # 1 associated with estimating 20 parameters are found in Figure 4, Table 10, and Table 11. We can see from Figure 4 that the estimate θ_{opt}^1 produced relatively good fits for both the viral and serum creatinine data. However, we observe from Table 11 that the ratio of standard errors to the corresponding estimate, γ , is huge for all parameters except C_0 . This means that the estimates for all the parameters except C_0 are not reliable. One possible explanation for this result is the size of the condition number of the Fisher information, $\chi(\mathbf{q})^T \chi(\mathbf{q})$. In this particular case, it is 2.0604×10^{17} , obtained using Matlab function `cond`. This is considerably large and suggests that the matrix

is very close to being singular. This presents an issue when calculating the standard errors, since the inverse of the Fisher matrix is required.

Results corresponding to parameter estimation # 2 associated with estimating 15 parameters are found in Figure 5, Table 10, and Table 13. Figure 5 also shows relatively good fits for both the viral and serum creatinine data. We observe, however, from Table 13 that γ is large for parameters λ_{HS} , β , ρ_{EV} , δ_{EV} , δ_{EK} , δ_{C0} , κ_{HS} , δ_{HI} , ρ_V , ρ_{EK} , ε_2 , ε_3 , ε_4 . Yet, $1 < \gamma < 2$ for λ_{HS} , δ_{C0} and $0.9 < \gamma < 1$ for λ_C . We note that $\max_i |\gamma_i| = 1074.3$, which is much less than that corresponding to parameter estimation # 1. This suggests that there is less uncertainty in the estimates obtained compared to the previous case. It is important to note that the condition number of the Fisher matrix is 1.6620×10^{11} and has decreased.

Results corresponding to parameter estimation # 3 associated with estimating 10 parameters are given in Figure 6, Table 14 and Table 15. Figure 6 shows relatively good fits for both the viral and serum creatinine data. However, we observe from Table 15 that γ is large for parameter δ_V , $1 < \gamma < 4$ for parameters ρ_{EV} , δ_{EV} , ρ_V and $0.4 < \gamma < 1$ for parameters β , δ_{EK} , κ_{HS} , ρ_{EK} , ε_3 , ε_4 . Note that $\max_i |\gamma_i| = 25.924$, which is much less than that corresponding to parameter estimation # 1, 2. This means that we have less uncertainty in parameter estimates compared to that of the other two cases. Similar to the previous two cases, the condition number of the Fisher matrix is 1.9733×10^8 and has decreased.

Figure 1.
BKV model.

Figure 2.

Using θ_{opt}^4 in Table 4 for parameter estimation # 4, (Left) Plot of model solution and viral load data on $[0, 450]$. (Right) Plot of model solution and serum creatinine data on $[0, 450]$.

Figure 3. Model solution obtained with parameter values given in Table 4: (left) results for H_S and H_I , (right) results for E_V and E_K .

Figure 4.

Using θ_{opt}^1 in Table 10 for parameter estimation # 1, (Left) Plot of model solution and viral load data on $[0, 450]$. (Right) Plot of model solution and serum creatinine data on $[0, 450]$.

Figure 5.

Using θ_{opt}^2 in Table 12 for parameter estimation # 2, (Left) Plot of model solution and viral load data on $[0, 450]$. (Right) Plot of model solution and serum creatinine data on $[0, 450]$.

Figure 6.

Using θ_{opt}^3 in Table 14 for parameter estimation # 3, (Left) Plot of model solution and viral load data on $[0, 450]$. (Right) Plot of model solution and serum creatinine data on $[0, 450]$.

Table 1

Description of state variables.

State	Description	Units
H_S	concentration of susceptible host cells	cells/mL
H_I	concentration of infected host cells	cells/mL
V	concentration of free BKV	copies/mL
E_V	concentration of BKV-specific CD8+ T cells	cells/mL
E_K	concentration of allospecific CD8+ T cells that target kidney	cells/mL
C	concentration of serum creatinine	mg/dL

Table 2

Model parameters and their corresponding descriptions.

Parameter	Description	Unit
λ_{HS}	proliferation rate for H_S	1/day
κ_V	saturation constant	copies/mL
κ_{HS}	saturation constant	cells/mL
λ_{EK}	source rate of E_K	cells/(mL·day)
β	infection rate of H_S by V	mL/(copies·day)
δ_{EK}	death rate of E_K	1/day
δ_{HI}	death rate of H_I by V	1/day
λ_C	production rate for C	mg/(dL·day)
ρ_V	# virions produced by H_I before death	copies/cell
δ_{C0}	maximum clearance rate for C	1/day
δ_{EH}	elimination rate of H_I by E_V	mL/(cells·day)
κ_{EK}	saturation constant	cells/mL
δ_V	natural clearance rate of V	1/day
κ_{CH}	saturation constant	cells/mL
λ_{EV}	source rate of E_V	cells/(mL·day)
ρ_{EK}^-	maximum proliferation rate for E_K	1/day
δ_{EV}	death rate of E_V	1/day
κ_{KH}	saturation constant	cells/mL
ρ_{EV}^-	maximum proliferation rate for E_V	1/day
ε_I	efficacy of immunosuppressive drugs	

Table 3

Initial guess for parameter values $\theta = (\mathbf{p}, \bar{\mathbf{x}}_0)$ used in the parameter estimation simulations.

Parameter	Value	Parameter	Value
λ_{HS}	0.03 per day	κ_{HS}	1×10^3 cells/mL
β	8×10^{-8} mL/(copies·day)	δ_{HI}	0.08 per day
δ_{EH}	1.5×10^{-3} mL/(cells·day)	ρ_V	6×10^3 copies/cell
δ_V	0.4 per day	λ_{EV}	1×10^{-3} cells/(mL·day)
ρ_{EV}	0.3 per day	κ_V	100 copies/mL
δ_{EV}	0.1 per day	λ_{EK}	2×10^{-3} cells/(mL·day)
δ_{EK}	0.1 per day	λ_C	6×10^{-3} mg/(dL·day)
δ_{C0}	0.01 per day	κ_{EK}	0.2 cells/mL
κ_{CH}	10 cells/mL	ρ_{EK}	0.2 per day
κ_{KH}	85 cells/mL	ε_1	0.1
ε_2	0.38	ε_3	0.6
ε_4	0.3	H_{S0}	5×10^3 cells/mL
H_{I0}	60 cells/mL	V_0	5×10^4 copies/mL
E_{V0}	0.04 cells/mL	E_{K0}	0.4 cells/mL
C_0	1.07 mg/dL		

Table 4

Parameter estimation # 4 results for TOS003 viral load and serum creatinine data on [0, 450]. Top half of the table gives fixed parameters whereas the bottom half of the table displays the estimated parameters.

Parameter	θ_{opt}^4	Parameter	θ_{opt}^4
$\log_{10} \lambda_{HS}$	-1.5205	$\log_{10} \delta_{EH}$	-2.7500
$\log_{10} \delta_V$	-0.4295	$\log_{10} \delta_{C0}$	-1.8520
$\log_{10} \kappa_{HS}$	3.0111	$\log_{10} \delta_{HI}$	-1.0704
$\log_{10} \rho_V$	3.6327	$\log_{10} \lambda_{EV}$	-3.0000
$\log_{10} \kappa_V$	2.2569	$\log_{10} \lambda_{EK}$	-2.6990
$\log_{10} \lambda_C$	-2.1805	$\log_{10} \kappa_{EK}$	-0.6990
$\log_{10} \kappa_{CH}$	1.0000	$\log_{10} \kappa_{KH}$	1.9294
ε_1	0.1009	ε_2	0.3658
ε_3	0.5999	ε_4	0.3649
$\log_{10} H_{S0}$	3.6990	$\log_{10} H_{S0}$	1.7782
$\log_{10} V_0$	4.6990	$\log_{10} E_{V0}$	-1.4234
$\log_{10} E_{K0}$	-0.3979	C_0	0.6763
$\log_{10} \beta$	-7.0674	$\log_{10} \rho_{EV}$	-0.6006
$\log_{10} \delta_{EV}$	-0.9636	$\log_{10} \delta_{EK}$	-0.9945
$\log_{10} \rho_{EK}$	-0.7853		

Table 5

Parameter estimates, asymptotic standard errors, confidence intervals, and the ratios γ of standard error to the corresponding estimate for parameter estimation # 4.

Parameter	Estimate	SE	95% CI	γ
$\log_{10} \beta$	-7.0674	8.0555×10^{-3}	[-7.0813, -7.0534]	1.1398×10^{-3}
$\log_{10} \rho_{EV}$	-0.6006	0.034668	[-0.6605, -0.5406]	0.057728
$\log_{10} \delta_{EV}$	-0.9636	0.044640	[-1.0407, -0.8864]	0.046328
$\log_{10} \delta_{EK}$	-0.9945	0.128860	[-1.2173, -0.7717]	0.129580
$\log_{10} \rho_{EK}$	-0.7853	0.140580	[-1.0283, -0.5422]	0.179020

Table 6

Sensitivity rankings for 29 estimated parameters.

S_V	Parameter	S_C	Parameter
0	δ_{EK}	1.8375×10^{-8}	κ_V
0	δ_{C0}	2.5563×10^{-6}	λ_{EV}
0	λ_{EK}	6.1953×10^{-5}	κ_{CH}
0	λ_C	1.4577×10^{-4}	E_{V0}
0	κ_{EK}	1.8694×10^{-4}	δ_{EH}
0	κ_{CH}	1.9313×10^{-4}	λ_{HS}
0	ρ_{EK}	5.6584×10^{-4}	V_0
0	κ_{KH}	7.3820×10^{-4}	H_{S0}
0	E_{K0}	1.9130×10^{-3}	H_{I0}
0	C_0	4.1888×10^{-3}	δ_{EV}
4.2652×10^{-3}	V_0	4.4437×10^{-3}	λ_{EK}
0.016519	H_{I0}	0.011330	δ_{HI}
0.025134	H_{S0}	0.019960	ρ_{EV}
0.025148	λ_{EV}	0.063921	δ_V
0.087472	λ_{HS}	0.071157	ρ_V
1.4476	ε_4	0.084435	β
5.3296	κ_V	0.14688	κ_{HS}
14.003	E_{V0}	0.34333	κ_{EK}
15.359	δ_{HI}	0.42737	E_{K0}
18.007	δ_{EH}	0.51850	κ_{EK}
131.04	ε_1	0.68038	δ_{C0}
280.99	δ_V	1.2403	ε_4
282.13	ρ_V	1.3561	ε_1
292.00	ε_3	3.1602	C_0
296.00	κ_{HS}	3.5369	ε_2
300.73	β	5.1086	ε_3
446.59	ε_2	49.962	λ_C
1433.4	δ_{EV}	59.907	δ_{EK}
4680.9	ρ_{EV}	75.318	ρ_{EK}

Table 7

Sensitivity rankings for 10 estimated parameters.

S_V	Parameter	S_C	Parameter
0	δ_{EK}	0.011400	δ_{EK}
0	$\bar{\rho}_{EK}$	0.024951	$\bar{\rho}_{EK}$
1.0137	δ_V	0.30254	δ_V
40.538	ε_3	0.34492	ρ_V
171.61	ε_4	0.38744	β
175.47	κ_{HS}	0.45276	κ_{HS}
205.94	ρ_V	1.8521	ε_3
235.60	β	50.789	ε_4
624.14	δ_{EV}	135.23	$\bar{\rho}_{EK}$
996.73	$\bar{\rho}_{EV}$	157.37	δ_{EK}

Table 8

Sensitivity rankings for 20 estimated parameters.

S_V	Parameter	S_C	Parameter
0	δ_{EK}	1.4997×10^{-7}	κ_V
0	δ_{C0}	1.2963×10^{-4}	E_{V0}
0	λ_C	1.9144×10^{-4}	δ_{EH}
0	ρ_{EK}^-	0.012164	δ_{EV}
0	C_0	0.026468	ρ_{EV}^-
0.66447	E_{V0}	0.029674	δ_{HI}
1.0155	δ_{EH}	0.040367	λ_{HS}
4.6235	ε_1	0.32979	δ_V
5.7545	κ_V	0.37520	ρ_V
12.076	λ_{HS}	0.42114	β
15.216	δ_{HI}	0.48602	κ_{HS}
17.445	ε_2	0.64429	ε_1
40.518	ε_3	1.5687	ε_2
173.58	ε_4	1.9628	ε_3
175.34	κ_{HS}	2.2956	C_0
183.78	δ_V	7.8627	δ_{C0}
205.82	ρ_V	9.0800	λ_C
235.35	β	53.932	ε_4
639.54	δ_{EV}	145.34	ρ_{EK}^-
1022.7	ρ_{EV}^-	168.46	δ_{EK}

Table 9

Sensitivity rankings for 15 estimated parameters.

S_V	Parameter	S_C	Parameter
0	δ_{EK}	0.0097914	δ_{EV}
0	δ_{C0}	0.021305	ρ_{EV}
0	λ_C	0.026802	δ_{HI}
0	ρ_{EK}	0.027472	λ_{HS}
10.683	λ_{HS}	0.26025	δ_V
13.489	δ_{HI}	0.29872	ρ_V
18.399	ε_2	0.33683	β
42.884	ε_3	0.37132	κ_{HS}
168.53	κ_{HS}	1.5572	ε_2
174.92	δ_V	1.7727	ε_3
186.84	ε_4	7.6471	δ_{C0}
195.44	ρ_V	8.2445	λ_C
223.38	β	42.258	ε_4
688.21	δ_{EV}	110.23	ρ_{EK}
1104.2	ρ_{EV}	130.27	δ_{EK}

Table 10

Parameter estimation # 1 results for TOS003 viral load and serum creatinine data on [0, 450]. Top half of the table gives fixed parameters whereas the bottom half of the table displays the estimated parameters.

Parameter	θ_{opt}^1	Parameter	θ_{opt}^1
$\log_{10} \lambda_{EV}$	-3.0000	$\log_{10} \lambda_{EK}$	-2.6990
$\log_{10} \kappa_{EK}$	-0.69897	$\log_{10} \kappa_{CH}$	1.0000
$\log_{10} \kappa_{KH}$	1.9294	$\log_{10} H_{S0}$	3.6990
$\log_{10} H_{S0}$	1.7782	$\log_{10} V_0$	4.6990
$\log_{10} E_{K0}$	-0.39794		
$\log_{10} \lambda_{HS}$	-1.5291	$\log_{10} \beta$	-7.0633
$\log_{10} \delta_{EH}$	-2.7500	$\log_{10} \delta_V$	-0.43203
$\log_{10} \rho_{EV}$	-0.60674	$\log_{10} \delta_{EV}$	-0.96806
$\log_{10} \delta_{EK}$	-0.99357	$\log_{10} \delta_{C0}$	-1.8907
$\log_{10} \kappa_{HS}$	3.0032	$\log_{10} \delta_{HI}$	-1.0681
$\log_{10} \rho_V$	3.6349	$\log_{10} \kappa_V$	2.2569
$\log_{10} \lambda_C$	-2.1994	$\log_{10} \rho_{EK}$	-0.78374
ε_1	0.10085	ε_2	0.36754
ε_3	0.60763	ε_4	0.35925
$\log_{10} E_{V0}$	-1.4234	C_0	0.67629

Table 11

Parameter estimates, asymptotic standard errors, confidence intervals, and the ratios γ of standard error to the corresponding estimate for parameter estimation # 1.

Parameter	Estimate	SE	95% CI	γ
$\log_{10} \lambda_{HS}$	-1.5291	321.79	[-745.52, 742.46]	210.45
$\log_{10} \beta$	-7.0633	530.68	$[-1.2340 \times 10^3, 1.2199 \times 10^3]$	75.131
$\log_{10} \delta_{EH}$	-2.7500	7.7607×10^5	$[-1.7943 \times 10^6, 1.7943 \times 10^6]$	2.8221×10^5
$\log_{10} \delta_V$	-0.4320	6.7490×10^3	$[-1.5604 \times 10^4, 1.5603 \times 10^4]$	1.5622×10^4
$\log_{10} \rho_{EV}$	-0.6067	997.13	$[-2.3060 \times 10^3, 2.3047 \times 10^3]$	1.6434×10^3
$\log_{10} \delta_{EV}$	-0.9681	2.0163×10^3	$[-4.6627 \times 10^3, 4.6608 \times 10^3]$	2.0828×10^3
$\log_{10} \delta_{EK}$	-0.9936	2.5211×10^3	$[-5.8298 \times 10^3, 5.8278 \times 10^3]$	2.5374×10^3
$\log_{10} \delta_{C0}$	-1.8907	254.42	[-590.10, 586.32]	134.56
$\log_{10} \kappa_{HS}$	3.0032	1.7893×10^3	$[-4.1339 \times 10^3, 4.1399 \times 10^3]$	595.81
$\log_{10} \delta_{HI}$	-1.0681	2.2293×10^3	$[-5.1553 \times 10^3, 5.1531 \times 10^3]$	2.0871×10^3
$\log_{10} \rho_V$	3.6349	8.7049×10^3	$[-2.0122 \times 10^4, 2.0129 \times 10^4]$	2.3948×10^3
$\log_{10} \kappa_V$	2.2569	5.6586×10^3	$[-1.3080 \times 10^4, 1.3085 \times 10^4]$	2.5073×10^3
$\log_{10} \lambda_C$	-2.1994	121.88	[-284.00, 279.60]	55.416
$\log_{10} \rho_{EK}$	-0.7837	2.4214×10^3	$[-5.5990 \times 10^3, 5.5974 \times 10^3]$	3.0895×10^3
ε_1	0.1009	1.5278×10^3	$[-3.5321 \times 10^3, 3.5323 \times 10^3]$	1.5148×10^4
ε_2	0.3675	128.05	[-295.69, 296.43]	348.40
ε_3	0.6076	1.6913×10^3	$[-3.9097 \times 10^3, 3.9109 \times 10^3]$	2.7835×10^3
ε_4	0.3593	142.45	[-328.99, 329.71]	396.53
$\log_{10} E_{V0}$	-1.4234	9.3880×10^5	$[-2.1705 \times 10^6, 2.1705 \times 10^6]$	6.5953×10^5
C_0	0.6763	0.16971	[0.28393, 1.0687]	0.25094

Table 12

Parameter estimation # 2 results for TOS003 viral load and serum creatinine data on [0, 450]. Top half of the table gives fixed parameters whereas the bottom half of the table displays the estimated parameters.

Parameter	θ_{opt}^2	Parameter	θ_{opt}^2
$\log_{10} \delta_{EH}$	-2.7500	$\log_{10} \lambda_{EV}$	-3.0000
$\log_{10} \kappa_V$	2.2569	$\log_{10} \lambda_{EK}$	-2.6990
$\log_{10} \kappa_{EK}$	-0.69897	$\log_{10} \kappa_{CH}$	1.0000
$\log_{10} \kappa_{KH}$	1.9294	ε_1	0.10085
$\log_{10} H_{S0}$	3.6990	$\log_{10} H_{S0}$	1.7782
$\log_{10} V_0$	4.6990	$\log_{10} E_{V0}$	-1.4234
$\log_{10} E_{K0}$	-0.39794	C_0	0.67629
$\log_{10} \lambda_{HS}$	-1.5205	$\log_{10} \beta$	-7.0671
$\log_{10} \delta_V$	-0.43233	$\log_{10} \bar{\rho}_{EV}$	-0.60429
$\log_{10} \delta_{EV}$	-0.96680	$\log_{10} \delta_{EK}$	-0.99302
$\log_{10} \delta_{C0}$	-1.8520	$\log_{10} \kappa_{HS}$	3.0060
$\log_{10} \delta_{HI}$	-1.0704	$\log_{10} \rho_V$	3.6344
$\log_{10} \lambda_C$	-2.1805	$\log_{10} \bar{\rho}_{EK}$	-0.78540
ε_2	0.36575	ε_3	0.61164
ε_4	0.36374		

Table 13

Parameter estimates, asymptotic standard errors, confidence intervals, and the ratios γ of standard error to the corresponding estimate for parameter estimation # 2.

Parameter	Estimate	SE	95% CI	γ
$\log_{10} \lambda_{HS}$	-1.5205	2.8635	[-6.7692, 3.7282]	1.8832
$\log_{10} \beta$	-7.0671	64.938	[-126.10, 111.96]	9.1888
$\log_{10} \delta_V$	-0.4323	464.46	[-851.79, 850.92]	1074.3
$\log_{10} \rho_{EV}$	-0.6043	53.623	[-98.895, 97.686]	88.737
$\log_{10} \delta_{EV}$	-0.9668	79.892	[-147.41, 145.48]	82.636
$\log_{10} \delta_{EK}$	-0.9930	36.222	[-67.388, 65.402]	36.477
$\log_{10} \delta_{C0}$	-1.8520	3.7012	[-8.6362, 4.9323]	1.9985
$\log_{10} \kappa_{HS}$	3.0060	32.931	[-57.357, 63.369]	10.955
$\log_{10} \delta_{HI}$	-1.0704	74.871	[-138.31, 13.617]	69.944
$\log_{10} \rho_V$	3.6344	550.96	[-1006.3, 1013.5]	151.60
$\log_{10} \lambda_C$	-2.1805	2.0517	[-5.9412, 1.5802]	0.9409
$\log_{10} \rho_{EK}$	-0.7854	26.666	[-49.664, 48.093]	33.952
ε_2	0.3657	10.447	[-18.784, 19.516]	28.564
ε_3	0.6116	30.913	[-56.052, 57.275]	50.541
ε_4	0.3637	14.389	[-26.011, 26.739]	39.559

Table 14

Parameter estimation # 3 results for TOS003 viral load and serum creatinine data on [0, 450]. Top half of the table gives fixed parameters whereas the bottom half of the table displays the estimated parameters.

Parameter	θ_{opt}^3	Parameter	θ_{opt}^3
$\log_{10} \lambda_{HS}$	-1.5205	$\log_{10} \delta_{EH}$	-2.7500
$\log_{10} \delta_{CO}$	-1.8520	$\log_{10} \delta_{HI}$	-1.0704
$\log_{10} \lambda_{EV}$	-3.0000	$\log_{10} \kappa_V$	2.2569
$\log_{10} \lambda_{EK}$	-2.6990	$\log_{10} \lambda_C$	-2.1805
$\log_{10} \kappa_{EK}$	-0.6990	$\log_{10} \kappa_{CH}$	1.0000
$\log_{10} \kappa_{KH}$	1.9294	ε_1	0.1009
ε_2	0.3658	$\log_{10} H_{S0}$	3.6990
$\log_{10} H_{S0}$	1.7782	$\log_{10} V_0$	4.6990
$\log_{10} E_{V0}$	-1.4234	$\log_{10} E_{K0}$	-0.3979
C_0	0.6763		
$\log_{10} \beta$	-7.0675	$\log_{10} \delta_V$	-0.4295
$\log_{10} \rho_{EV}$	-0.6005	$\log_{10} \delta_{EV}$	-0.9635
$\log_{10} \delta_{EK}$	-0.9945	$\log_{10} \kappa_{HS}$	3.0111
$\log_{10} \rho_V$	3.6327	$\log_{10} \rho_{EK}$	-0.7850
ε_3	0.5999	ε_4	0.3649

Table 15

Parameter estimates, asymptotic standard errors, confidence intervals, and the ratios γ of standard error to the corresponding estimate for parameter estimation # 3.

Parameter	Estimate	SE	95% CI	γ
$\log_{10} \beta$	-7.0675	3.1628	[-12.637, -1.4978]	0.44751
$\log_{10} \delta_V$	-0.4295	11.135	[-20.038, 19.179]	25.924
$\log_{10} \rho_{EV}$	-0.6005	1.3981	[-3.0626, 1.8617]	2.3285
$\log_{10} \delta_{EV}$	-0.9635	1.9509	[-4.3990, 2.4721]	2.0249
$\log_{10} \delta_{EK}$	-0.9945	0.41297	[-1.7218, -0.26729]	0.41524
$\log_{10} \kappa_{HS}$	3.0111	1.7573	[-0.083412, 6.1057]	0.58359
$\log_{10} \rho_V$	3.6327	12.5245	[-18.423, 25.688]	3.4477
$\log_{10} \rho_{EK}$	-0.7850	0.40087	[-1.4910, -0.079107]	0.51064
ε_3	0.5999	0.59392	[-0.44597, 1.6458]	0.9900
ε_4	0.3649	0.22157	[-0.025269, 0.75510]	0.60718