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Predicting Hepatotoxicity in Patients Receiving T-DM1 Treatment using ALT Blood Concentrations

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Introduction

During cancer treatments, it is imperative to keep a close eye on the side effects of whatever drug you are using to target the infected cells. For example, many drugs will target cancerous cells by attaching themselves onto different receptors on the membranes of the cells. However, these treatments are not always exact in affecting only the cancerous cells. One of the major side effects of these drugs is that different types of non-cancerous cells are accidentally killed during the process of cancer treatments when the drug latches onto the similar receptors of those cells. For example, when the antibody-drug conjugate trastuzumab emtansine (T-DM1) is administered into the bloodstream it will accidentally target and kill liver cells in the patient's body due to the liver cells' CKAP5 receptors attaching to T-DM1 molecules. When these healthy liver cells die, they release alanine aminotransferase (ALT) as well as aspartate aminotransferase (AST). There is a silver lining to this, however, as these enzymes can be used to track liver cell death. So that raises the question: can we quantify the amount of liver cell damage from T-DM1 treatment by studying the relationship between the dosage of T-DM1 and the concentration of ALT in the bloodstream? We chose to focus on ALT rather than AST due to ALT primarily being released by liver cells, whereas AST can be released by the liver, brain, lungs, and other organs in the body [1]. Thus by modeling the concentration of ALT released by specific dosages of T-DM1, the patients' recorded ALT levels can be referenced to find an optimal dosage to lower ALT levels while still being considered an efficient treatment.

Antibody-drug conjugates (ADCs) are a class of chemotherapeutics that links monoclonal antibodies to a cytotoxic agent via a linker [2]. These therapeutics can target specific cancer cells based on their possession of certain antigens and then release toxins to kill the cell [2]. One such ADC is trastuzumab

emtansine (T-DM1), where trastuzumab (T) is the antibody and emtansine (DM1) is the cytotoxin [3]. DM1 disrupts the polymerization of a key structural element called microtubules [3, 4]. Trastuzumab targets the growth factor receptor HER2, a protein over-expressed in about 20-25 percent of breast cancers [2, 3]. This property makes T-DM1 especially useful in treating breast cancer [2]. When T-DM1 binds with HER2, DM1 is released which disorganizes the microtubules and leads to cell death [2]. However, T-DM1 has been known to impact unintended cells [4]. One significant instance of this is in hepatocytes, or liver cells. When T-DM1 binds with CKAP5, an extracellular protein involved in microtubule stabilization, calcium rushes into the cell [2, 4]. These conditions disrupt the stabilization of microtubules and ultimately cause hepatocyte death [2, 4]. A key marker of hepatotoxicity is the presence of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), enzymes released due to hepatocyte death [2].

Mathematical Model

The model we create will predict the ALT concentration in a blood sample after injecting T-DM1 throughout a 3-week regimen. Our primary objective is to accurately characterize the relationship between a serum concentration of T-DM1 and hepatotoxicity.

A few assumptions are made within our model that helped guide the development process. We assume the patient has no abnormal conditions or previous treatments that would affect ALT levels. The dosage of T-DM1 is the first dosage during treatment. The patient's characteristics, such as age, gender, economic status, prior treatment, etc., do not alter their respective levels of ALT. The total number of hepatocytes at any given time does not affect the rate at which T-DM1 binds to CKAP5. This assumption was made due to the number of healthy hepatocytes vastly outnumbering the concentration of T-DM1 that can bind to those cells. We additionally assume that the birth and natural death rates of hepatocytes do not significantly affect the rate at which T-DM1 binds to CKAP5. However these assumptions will be relaxed and will be discussed later when we introduce the second version of the model. In addition, hepatotoxicity does not contribute to additional hepatocyte death. This means that past hepatocyte death does not affect the rate at which future hepatocytes die. Since we want to focus solely on ALT levels from liver cells, we can lump together the clearance of T-DM1 when the drug either targets cancer cells or when it dissipates in the bloodstream together. In order to model the serum concentration of T-DM1 we will take it to be a known function derived from Figure 1. We fit an exponential function, $A(t)$, to the data. $A(t)$ represents the serum concentration of T-DM1 and has the form:

$$A(t) = A_0 e^{-\alpha t}. \tag{1}$$

We assume that ALT is only released when the cell is completely dead and is not produced simply as a result of cell damage. We are assuming that ALT

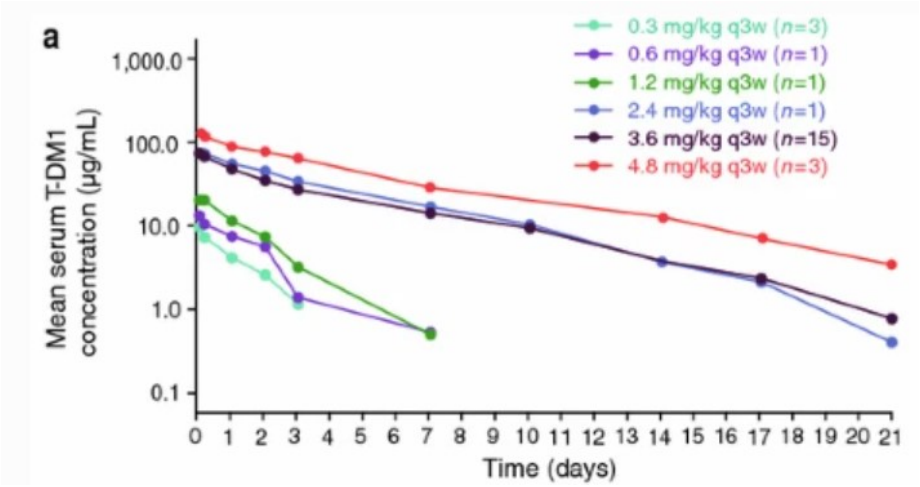


Figure 1: Mean serum T-DM1 concentration by dose level over a 21 day period in μg/ml [3].

is released instantaneously once the liver cell is completely dead even though the process of cell death is not instantaneous. Another important assumption is that there is already ALT that is seen in the bloodstream unrelated to T-DM1 treatments. When the hepatocytes naturally die, they still do release ALT. The total amount of ALT in a patient's bloodstream is approximately 84,000 IU [5]. This is a baseline value, we are interested in the event that this ALT baseline increases above normal due to hepatoxicity. However, it is also possible for ALT to be elevated due to other conditions, such as the brain damage and skeletal muscle diseases [6, 7]. When we look at ALT levels in the body, only looking at ALT that has been released from liver injury is of interest. Since we have already established that the patient has no such conditions that would affect ALT levels, we can safely assume that ALT elevation is coming only from the hepatocyte damage. This model will allow the client to measure toxicity levels based on the dosage of T-DM1 given to the patient. Additionally, another model describing the relationship between dosage of T-DM1 and effectiveness could be established by the client. This other model would give an optimum range of dosages that will maximize the effectiveness at combating the cancerous cells while minimizing the risk of harming the patient.

Initial Condition	Dimension	Unit	Value	Source
A_0	C	μ/ml	Drug Concentration	[3]
B_0	N	Billions of cells	174	[8]
D_0	N	Billions of cells	0	Model assumption
E_0	N	IU	45	[5]
F_0	N	IU	1**	-

Table 1: Initial conditions. "*" denotes that the value was used in similar study of a different but similar drug.

Parameter	Definition	Dimension	Value	Source
α	Rate T-DM1 binds to liver cells	$\frac{1}{T}$	0.1712-0.7124	[3]
β	Rate healthy liver cells become damaged	$\frac{N}{CT}$	0.00237254167	-
σ	Rate damaged liver cells die	$\frac{1}{T}$	5*	[5]
ν	Rate healthy liver cells regenerate	$\frac{1}{T}$	1***	[5]
ξ	Rate healthy liver cells die	$\frac{1}{T}$	$1.8741 \cdot 10^{-4}$	[5]
μ	Clearance rate of ALT into the bloodstream	$\frac{1}{T}$	15.75**	-
ϕ	Clearance rate of ALT out of the bloodstream	$\frac{1}{T}$	0.35	[5]
γ	ALT produced from liver cells	$\frac{C}{N}$	483	[5]
K_{max}	Maximum rate at which T-DM1 causes cell death	$\frac{1}{T}$	0.0003502	-
K_{50}	50% of the drug concentration associated with K_{max}	N	55.33	[3]
B_{ss}	Carrying capacity modifier	-	1.000188***	-

Table 2: Model parameters. "*" denotes that the value was used in similar study of a different but similar drug.

Model v1.

This is our basic compartmental model which will take a T-DM1 dosage and determine the damage done to the liver using ALT serum concentration as a marker. We have established the mean T-DM1 serum concentration as a known function, $A(t)$, as shown in Equation (1). We developed a set of differential equations to model the change in the amount of healthy hepatocytes, the amount of

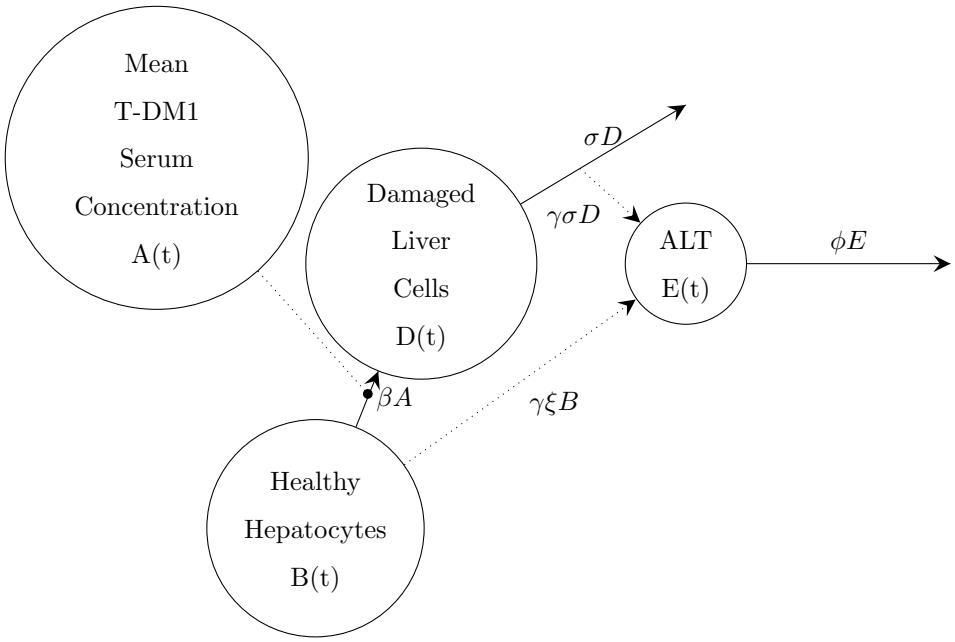


Figure 2: Flow Diagram of Model v1 and v2.

damaged hepatocytes, and the ALT serum concentration using the relationships depicted in Figure 2. For all of our equations, including Equation (1), t is in days.

$$\frac{dB}{dt} = -\beta A \tag{2}$$

$$\frac{dD}{dt} = \beta A - \sigma D \tag{3}$$

$$\frac{dE}{dt} = \gamma\sigma D + \gamma\xi B - \phi E. \tag{4}$$

Equation (2) describes the change in the number of healthy hepatocytes that are affected by T-DM1 over time t. Variable B represents the total number of healthy hepatocytes, and β is rate at which T-DM1 binds with hepatocytes. Equation (3) describes the change in number of damaged hepatocytes at the rate T-DM1 binds to liver cells, β, minus the rate of damaged hepatocyte death, σ. Variable D represents the total number of damaged hepatocytes. Equation (4) describes how the ALT serum concentration is changing over time t, where E is the total number of ALT molecules. $\gamma\sigma D$ describes ALT being produced from T-DM1 effected cells, where σ refers to the rate at which T-DM1 effected hepatocytes die and γ refers to the number of ALT molecules produced from the total number of hepatocytes. $\gamma\xi B$ describes healthy liver cells producing

ALT at a rate $\xi\gamma$ through natural cell death. ϕE describes the clearance rate of ALT. Values for each of the parameters are listed in Table 2.

Nondimensionalization

Due to our very large initial condition values displayed in Table 1, we nondimensionalized our system of equations. To start, we chose our characteristic time scale to be a fraction of the rate at which hepatocytes die. Each of the variables are scaled by their initial conditions. Each variable's characteristic scale is denoted with a superscript of "*". For example, we set $\tilde{E} = \frac{E}{E^*}$. $\tilde{E}$ is the nondimensional amount of ALT molecules. We decided to scale time by the rate damaged liver cells die as this is the most significant physiological process in our models. We scaled initial conditions for healthy livers cells and ALT molecules in the serum by themselves. In order to avoid a zero in the denominator of the scale for damaged hepatocytes we chose to scale D_0 by B_0.

$$\begin{aligned} t = t^*\tilde{t}, \quad & t^* = \tfrac{1}{\sigma} \\ \mathrm{A} = \mathrm{A}^*\tilde{A} \quad & A^* = A_0 \\ \mathrm{B} = \mathrm{B}^*\tilde{B} \quad & B^* = B_0 \\ D = D^*\tilde{D} \quad & D^* = B_0 \\ \mathrm{E} = \mathrm{E}^*\tilde{E} \quad & E^* = E_0. \end{aligned}$$

To demonstrate how we nondimensionalized, we will take a closer look with Equation 3.

$$\begin{aligned} \frac{dE}{dt} &= \gamma\sigma D + \gamma\xi B - \phi E \\ \frac{d(E^*\tilde{E})}{d(t^*\tilde{t})} &= \gamma\sigma D^*\tilde{D} + \gamma\xi B^*\tilde{B} - \phi E^*\tilde{E} \\ \frac{d\tilde{E}}{d\tilde{t}} &= \frac{t^*}{E^*}(\gamma\sigma D^*\tilde{D} + \gamma\xi B^*\tilde{B} - \phi E^*\tilde{E}) \\ \frac{d\tilde{E}}{d\tilde{t}} &= \frac{\sigma t^* D^*\tilde{D}}{E^*} + \frac{\gamma\xi t^* B^*\tilde{B}}{E^*} - \phi t^*\tilde{E}. \end{aligned}$$

By the nondimensionalization of Equation (3), the scales of each variable are now relative to their initial conditions and therefore dimensionless. We followed this procedure to derive the following nondimensionalized model:

$$\frac{d\tilde{B}}{d\tilde{t}} = -\hat{\beta}\tilde{A}, \qquad \tilde{B}(0) = 1 \tag{5}$$

$$\frac{d\tilde{D}}{d\tilde{t}} = \hat{\beta}\tilde{A} - \tilde{D}, \qquad \tilde{D}(0) = 0. \tag{6}$$

$$\frac{d\tilde{E}}{d\tilde{t}} = \hat{\gamma}\tilde{D} + \hat{\gamma_\xi}\tilde{B} - \hat{\phi}\tilde{E}, \qquad \tilde{E}(0) = 1. \tag{7}$$

The scaled parameters, which are denoted with a "ˆ", are defined in Table 3. For the remainder of the paper, when referring to version 1 of our model it will be this nondimensional model.

Scaled Parameter	Value
$\hat{\alpha}$	$\frac{\alpha}{\sigma}$
$\hat{\beta}$	$\frac{\beta A_0}{\sigma B_0}$
$\hat{\gamma}$	$\frac{\gamma B_0}{E_0}$
$\hat{\gamma_\xi}$	$\frac{\xi\gamma B_0}{\sigma E_0}$
$\hat{\phi}$	$\frac{\phi}{\sigma}$
$\hat{K_{max}}$	$K_{max}A_0^\omega$
$\hat{K_{50}}$	σK_{50}^ω
$\hat{A_0}$	σA_0^ω
$\hat{\nu}$	$\frac{\nu}{\sigma}$
$\hat{\xi}$	$\frac{\xi}{\sigma}$
$\hat{\mu}$	$\frac{\mu F_0}{\sigma B_0}$
$\hat{\mu F_0}$	$\frac{\mu F_0}{\sigma E_0}$

Table 3: Scaled Parameters from nondimensionalization.

Model v2.

For this version of our model, we introduce a Hill function to encode the magnitude of T-DM1's effect on the liver. This model allows further control over the extent of T-DM1's affect on the liver. As before, $A(t)$ is a known function, given in Equation 1 describing serum T-DM1 concentration over time t in days. We developed the following differential equations to model the change in amount of healthy hepatocytes, the amount of damaged hepatocytes, and ALT serum concentration using the relationships depicted in Figure 2:

$$\frac{dB}{dt} = \frac{-K_{max}A^\omega}{K_{50}^\omega + A^\omega}B \tag{8}$$

$$\frac{dD}{dt} = \frac{K_{max}A^\omega}{K_{50}^\omega + A^\omega}B - \sigma D \tag{9}$$

$$\frac{dE}{dt} = \gamma\sigma D + \gamma\xi B - \phi E. \tag{10}$$

Equations (8) and (9) make use of a Hill function, which is the primary difference between model 1 and 2. K_{max} denotes the maximum rate T-DM1 damages hepatocytes and K_{50} denotes to the serum T-DM1 concentration associated with 50% of K_{max}. ω is the Hill coefficient which is used to encode the degree of binding cooperativity between T-DM1 and CKAP5.
The second key difference between this model and out first version pertains to our assumptions. Here we are relaxing the assumption that the total number of hepatocytes does not significantly impact the rate of cell damage. Equation (8) encodes the change in the number of healthy hepatocytes, B, over t days. The Hill function term encodes the magnitude of the affect of T-DM1 on the liver. And reflecting our relaxed assumption, Equation (8) multiplies this magnitude by the number of healthy hepatocytes. Equation (9) encodes the change in damaged hepatocytes, D. This is encoded by adding the the number of damaged cells as determined in Equation (8) and subtracting the number of dead cells, σD, at the rate damaged cells die, σ. Equation (10) remains the same as Equation (4) from version 1 of our model.

Nondimensionalization

To, again, minimize the size of our initial conditions, displayed in Table 1, we nondimensionalized the model. The procedure described in version 1's nondimensionalization is followed. All choices of notation and scale are identical. As a result, we have the following nondimensional model:

$$\frac{d\tilde{B}}{d\tilde{t}} = \frac{-\hat{K_{max}}\tilde{A}^{\omega}}{\hat{K_{50}} + \hat{A}_0\tilde{A}^{\omega}}\tilde{B}, \qquad \tilde{B}(0) = 1 \tag{11}$$

$$\frac{d\tilde{D}}{d\tilde{t}} = \frac{\hat{K_{max}}\tilde{A}^{\omega}}{\hat{K_{50}} + \hat{A}_0\tilde{A}^{\omega}}\tilde{B} - \tilde{D}, \qquad \tilde{D}(0) = 0 \tag{12}$$

$$\frac{d\tilde{E}}{d\tilde{t}} = \hat{\gamma}\tilde{D} + \hat{\gamma_{\xi}}\tilde{B} - \hat{\phi}\tilde{E}, \qquad \tilde{E}(0) = 1. \tag{13}$$

Table 3 defines our scaled parameters. For the remainder of the paper, when referring to version 2 of our model it will be this nondimensional model.

Model v3.

In order to add further complexity, by considering hepatocyte birth and death, we introduce this new model. Thus, we can gain a better understanding of the system as a whole. Figure 3 illustrates these two new relationships.

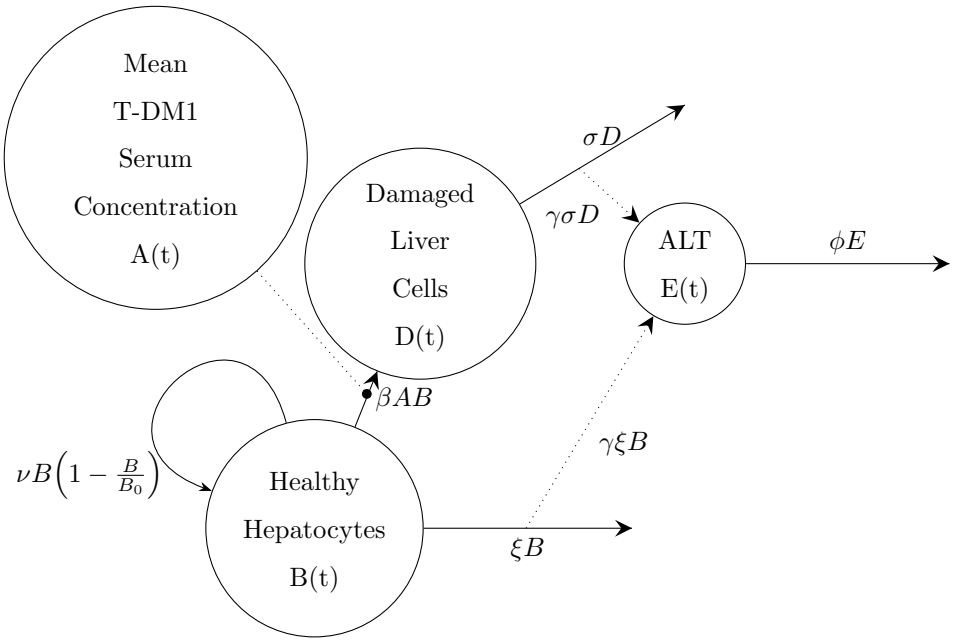


Figure 3: Flow Diagram of Model v3.

We have only modified the equation for the change in healthy hepatocytes for this model.

$$\frac{dB}{dt} = \frac{-K_{max}A^{\omega}}{K_{50}^{\omega}+A^{\omega}}B + \left(\nu\left(1-\frac{B}{B_0 B_{ss}}\right)-\xi\right)B \tag{14}$$

$$\frac{dD}{dt} = \frac{K_{max}A^{\omega}}{K_{50}^{\omega}+A^{\omega}}B - \sigma D \tag{15}$$

$$\frac{dE}{dt} = \gamma\sigma D + \gamma\xi B - \phi E. \tag{16}$$

The new term in Equation (14) is the number of healthy hepatocytes multiplied by the difference in birth and death rates of those hepatocytes. The birth rate term incorporates a carrying capacity equivalent to the initial value of the healthy hepatocytes, as listed in Table 2. There is only new parameters called ν which denotes the regeneration rate of healthy hepatocytes.

Nondimensionalization

The same procedure used earlier to nondimensionalize model 1 and 2 is used here. All notation and characteristic time scale choices remain the same. We now have the following nondimensional model:

$$\frac{d\tilde{B}}{d\tilde{t}} = \frac{-\hat{K_{max}}\tilde{A}^{\omega}}{\hat{K_{50}} + \hat{A}_0\tilde{A}^{\omega}}\tilde{B} + (\hat{\nu}(1 - \frac{\tilde{B}}{B_{ss}}) - \hat{\xi})\tilde{B}, \qquad \tilde{B}(0) = 1 \tag{17}$$

$$\frac{d\tilde{D}}{d\tilde{t}} = \frac{\hat{K_{max}}\tilde{A}^{\omega}}{\hat{K_{50}} + \hat{A}_0\tilde{A}^{\omega}}\tilde{B} - \tilde{D}, \qquad \tilde{D}(0) = 0 \tag{18}$$

$$\frac{d\tilde{E}}{d\tilde{t}} = \hat{\gamma}\tilde{D} + \hat{\gamma_\xi}\tilde{B} - \hat{\phi}\tilde{E}, \qquad \tilde{E}(0) = 1. \tag{19}$$

Table 3 defines our scaled parameters. For the remainder of the paper, when referring to version 3 of our model it will be this nondimensional model.

Model v4.

For this version of the model, we further develop the physiology of our system. Figure 4 shows an additional compartment in our model, this represents a delay in the movement of ALT. This represents ALT existing in the liver after being released from damaged cells and then that ALT moving from the liver into the bloodstream.

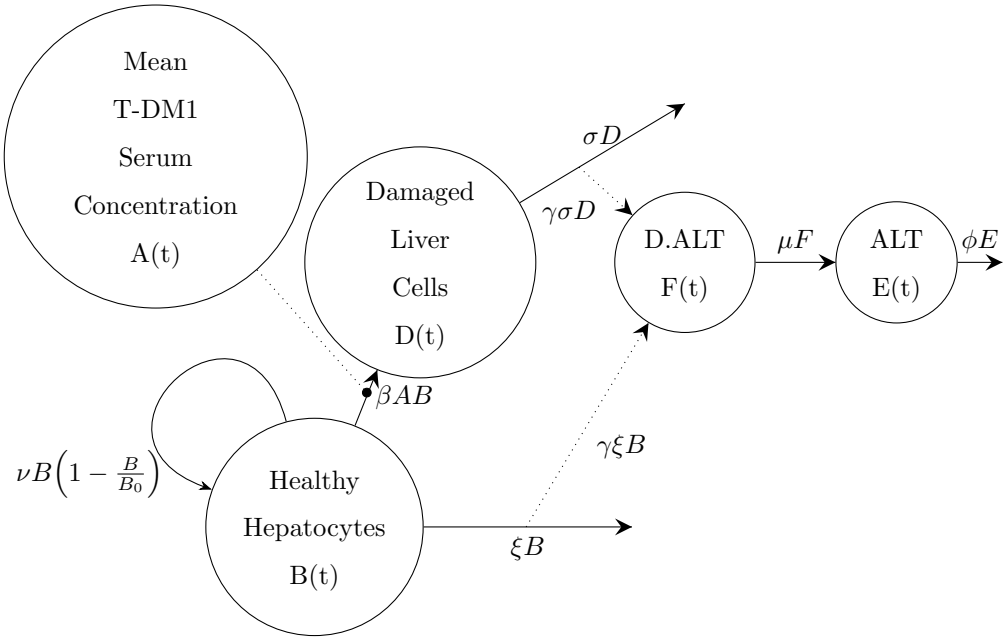


Figure 4: Flow Diagram of Model v4.

The equations for healthy and damaged hepatocytes remain the same as in version 3 of our model. We have introduced an additional equation for ALT in the liver and altered our equation for ALT in the bloodstream.

$$\frac{dB}{dt} = \frac{-K_{max}A^{\omega}}{K_{50}^{\omega} + A^{\omega}}B + B\left(\nu\left(1 - \frac{B}{B_0 B_{ss}}\right) - \xi\right) \tag{20}$$

$$\frac{dD}{dt} = \frac{K_{max}A^{\omega}}{K_{50}^{\omega} + A^{\omega}}B - \sigma D \tag{21}$$

$$\frac{dF}{dt} = \gamma\sigma D + \gamma\xi B - \mu F \tag{22}$$

$$\frac{dE}{dt} = \mu F - \phi E. \tag{23}$$

Equation (22) is the change in ALT in the liver. This includes ALT from T-DM1 affected cells and ALT from normal cell death and damage. The last term in this equation denotes the clearance of ALT from the liver into the bloodstream at rate μ. Equation (23) is the change in ALT concentration in the bloodstream. This includes the difference between the ALT cleared from the liver into the bloodstream and the ALT cleared from the bloodstream at rate ϕ.

Nondimensionalization

Equation (23) adds the following characteristic scale to our nondimensionalization procedure:

$$F = F^* \tilde{F}, \quad F^* = F_0.$$

We now have the following nondimensional model:

$$\frac{d\tilde{B}}{d\tilde{t}} = \frac{-\hat{K_{max}}\tilde{A}^{\omega}}{\hat{K_{50}} + \hat{A}_0\tilde{A}^{\omega}}\tilde{B} + (\hat{\nu}(1 - \frac{\tilde{B}}{B_{ss}}) - \hat{\xi})\tilde{B}, \qquad \tilde{B}(0) = 1 \tag{24}$$

$$\frac{d\tilde{D}}{d\tilde{t}} = \frac{\hat{K_{max}}\tilde{A}^{\omega}}{\hat{K_{50}} + \hat{A}_0\tilde{A}^{\omega}}\tilde{B} - \tilde{D}, \qquad \tilde{D}(0) = 0 \tag{25}$$

$$\frac{d\tilde{F}}{d\tilde{t}} = \hat{\gamma}\tilde{D} + \hat{\gamma_\xi}\tilde{B} - \hat{\mu}\tilde{F}, \qquad \tilde{F}(0) = 1 \tag{26}$$

$$\frac{d\tilde{E}}{d\tilde{t}} = \hat{\mu}\tilde{F} - \hat{\phi}\tilde{E}, \qquad \tilde{E}(0) = 1. \tag{27}$$

Table 3 defines our scaled parameters, with the following exceptions:

$$\hat{\gamma} = \frac{\gamma B_0}{F_0}$$
$$\hat{\gamma_\xi} = \frac{\gamma\xi B_0}{\sigma F_0}$$
$$\hat{\phi} = \frac{\phi E_0}{\sigma B_0}.$$

For the remainder of the paper, when referring to version 4 of our model it will be this nondimensional model.

Parameterization

Mean Serum Concentration

The parameters for Equation (1) are fit according to the data displayed in Figure 1 using the curve fit function from the Scipy library in Python. The curve was fit using the curvefit function in the library which looks to minimize the sum of the squared residuals. The results are displayed in Table 4.

Dosing Regimen	Number of Data Points	$A_0 \pm$ standard error	$\alpha \pm$ standard error
.3 mg/kg	4	11.29081 ± 1.0907	0.8224 ± 0.1309
.6 mg/kg	6	14.2862 ± 2.2042	0.5743 ± 0.0922
1.2 mg/kg	6	22.4405 ± 0.9311	0.5797 ± 0.0249
2.4 mg/kg	9	69.4441 ± 1.2836	0.2036 ± 0.0048
3.6 mg/kg	9	76.5372 ± 4.6088	0.3023 ± 0.0402
4.8 mg/kg	9	110.6609 ± 2.0688	0.1685 ± 0.0041

Table 4: Parameters for $A(t)$ using mean serum concentration data in Figure 1 for 6 dosages of T-DM1.

We also found it useful to fit the data in Figure 5, in particular the data associated with the serum T-DM1. This was done in the same manner as before. The results are displayed in Table 5 and Figure 6.

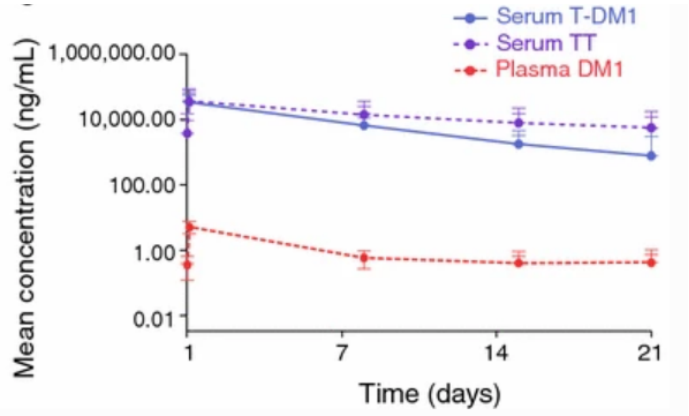


Figure 5: Mean concentration of three metrics related to T-DM1, combining three phase two studies [3].

Equation Units	Number of Data Points	$A_0 \pm$ standard error	$\alpha \pm$ standard error
ng/mL	4	46511.1720 ± 763.3310	0.2349 ± 0.0092

Table 5: Parameter values for $A(t)$ using mean serum T-DM1 concentration data from Figure 5.

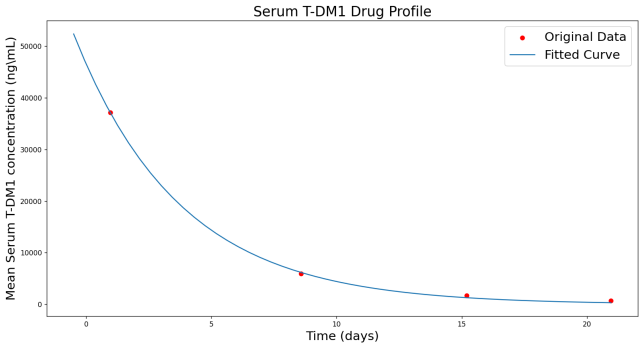


Figure 6: The curve fit to data where the original data is the red points on the graph and the blue smooth line is the function fit

Parameter Verification

We determined β by examining the properties of the results for model version 1. From Equation (33),

$$\begin{aligned} \frac{\hat{\gamma_\xi}\hat{\beta}}{\hat{\alpha}} - \hat{\gamma_\xi} &< 0 \\ \frac{\hat{\gamma_\xi}\hat{\beta}}{\hat{\alpha}} &< \hat{\gamma_\xi} \\ \hat{\beta} &< \hat{\alpha}. \end{aligned}$$

So we take minimum $\hat{\alpha}$, using the minimum α value from Table 4, and determine the upper bound for $\hat{\beta}$ to be 0.0337. Thus,

$$\beta < 0.1685.$$

Furthermore, using the data described in Figure 7a, we chose the value of β to be such that the results for the elevation of ALT molecules to be by a factor of 2 at its maximum. This was done by testing a range of β values in MATLAB. We approximated,

$$\beta = 0.00237254167.$$

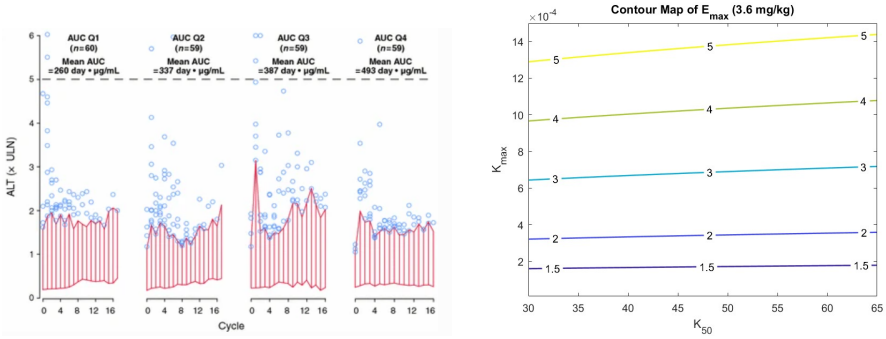
Parameter values for Equation (4) were verified by observing the steady states of our model. We set the drug concentration and time to 0, as well as each derivative. We get the following relationship as a result:

$$\hat{\gamma_\xi} = \hat{\phi}.$$

By substituting in known parameters into this equation, we were able to verify this relationship between γ, ϕ, and ξ.

Hill Function Parameters

In order to assign values to K_{max} and K_{50} we used Figure 7a. This figure shows that the ALT elevation in the bloodstream was between 0 and 2 times the initial value for the ninety-fifth percentile of data points collected for a 3.6 mg/kg dose [3]. We used this as a bound for the maximum in the solution for the ALT blood levels. In MATLAB, we created a range of values for each of the parameters and solved version 2 of the model for each range. As a result of this process, we obtained Figure 7b. Figure 7b shows that K_{50} did not have much of an effect on the ALT in the bloodstream. So we chose K_{50} to be 50% of A_0 associated with the largest dose from Table 4, which is 55.33 μg/kg. Using the maximum value of 2 for ALT blood levels and taking K_{50} to be 55.33, we used Figure 7b to find that the value of K_{max} was 0.0003502.



(a) ALT blood concentration for 3.6 mg/kg T-DM1 dosage over a 3-week regime. The red bars are 95% confidence intervals and the blue circles are patients outside of the confidence interval [3].

(b) A contour map of K_{max}, K_{50} and maximum ALT blood level solutions. K_{max} is varied between 10^{-5} and .0015 for 50 points. K_{50} is varied between 30 and 65 for 50 points.

Figure 7: ALT blood concentration confidence intervals and contour map.

Results

Model 1 could be solved analytically, while Models 2, 3 and 4 had to be solved numerically. The nondimensionalized form of each of these models were used

in the solution in order to minimize error. However, we did provide solutions in terms of dimensional numbers as well in order to better contextualize our results. The procedure to solve Model 1 is described below. For the numerical solutions, MATLAB's ode45 integrator was used. Each set of results displayed in Figures 8, 9, 10, and 12 were created using the parameters in Table 2, initial conditions in Table 1, and the varied values for initial drug concentration and its respective rate of decay displayed in Table 4. Each graph shows the respective solutions for six different dosages of T-DM1 over a 21 day regimen. We have also included baseline results representing the system with no dosage of T-DM1.

Model v1.

We begin the solution of this model by solving Equation (1):

$$\tilde{A}(\tilde{t}) = e^{-\hat{\alpha}\tilde{t}}. \tag{28}$$

By substitution, Equation (2) becomes:

$$\frac{d\tilde{B}}{d\tilde{t}} = -\hat{\beta}e^{-\hat{\alpha}\tilde{t}}.$$

Integrating and solving with our initial condition, $\tilde{B}(0) = 1$, the solution is:

$$\tilde{B}(\tilde{t}) = \frac{\hat{\beta}}{\hat{\alpha}}(e^{-\hat{\alpha}\tilde{t}} - 1) + 1. \tag{29}$$

By substitution, Equation (3) becomes:

$$\frac{d\tilde{D}}{d\tilde{t}} = \hat{\beta}e^{\frac{-\alpha\tilde{t}}{\sigma}} - \tilde{D}.$$

Solving with initial condition $\tilde{D}(0) = 0$:

$$\tilde{D}(\tilde{t}) = \frac{\hat{\beta}}{(1-\hat{\alpha})}(e^{-\hat{\alpha}\tilde{t}} - e^{-\tilde{t}}). \tag{30}$$

By substitution, Equation (4) becomes:

$$\frac{d\tilde{E}}{d\tilde{t}} = \frac{\hat{\gamma}\hat{\beta}}{1-\hat{\alpha}}(e^{-\hat{\alpha}\tilde{t}} - e^{-\tilde{t}}) + \frac{\hat{\gamma_\xi}\hat{\beta}}{\hat{\alpha}}(e^{-\hat{\alpha}\tilde{t}} - 1) + \hat{\gamma_\xi} - \hat{\phi}\tilde{E}.$$

Rearranging the terms to match exponential terms:

$$\frac{d}{d\tilde{t}}[e^{\hat{\phi}\tilde{t}}\tilde{E}] = \left[\frac{\hat{\gamma}\hat{\beta}}{1-\hat{\alpha}} + \frac{\hat{\gamma_\xi}\hat{\beta}}{\hat{\alpha}}\right]e^{\phi-\hat{\alpha}\tilde{t}} - \frac{\hat{\gamma}\hat{\beta}}{1-\hat{\alpha}}e^{(\hat{\phi}-1)\tilde{t}} - \left(\frac{\hat{\gamma_\xi}\hat{\beta}}{\hat{\alpha}} - \hat{\gamma_\xi}\right)e^{\hat{\phi}\tilde{t}}.$$

We introduce parameters C_1, C_2, and C_3, and define them to be:

$$C_1 = \frac{\hat{\gamma}\hat{\beta}}{1-\hat{\alpha}} + \frac{\hat{\gamma_\xi}\hat{\beta}}{\hat{\alpha}}, \tag{31}$$

$$C_2 = \frac{\hat{\gamma}\hat{\beta}}{1-\hat{\alpha}}, \tag{32}$$

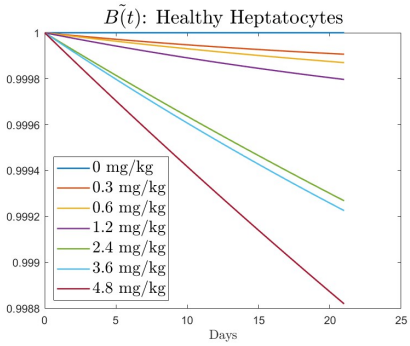
$$C_3 = \frac{\hat{\gamma}_\xi \hat{\beta}}{\hat{\alpha}} - \hat{\gamma}_\xi. \tag{33}$$

Integrating the equation:

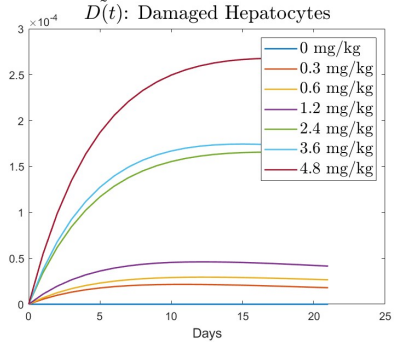
$$\tilde{E}(\tilde{t}) = \frac{C_1}{\hat{\phi}-\hat{\alpha}} e^{-\hat{\alpha}\tilde{t}} - \frac{C_2}{\hat{\phi}-1} e^{-\tilde{t}} - \frac{C_3}{\hat{\phi}} + Ce^{-\hat{\phi}\tilde{t}}, \quad C_3 < 0 \tag{34}$$

and solving for C with the initial condition $\tilde{E}(0) = 1$

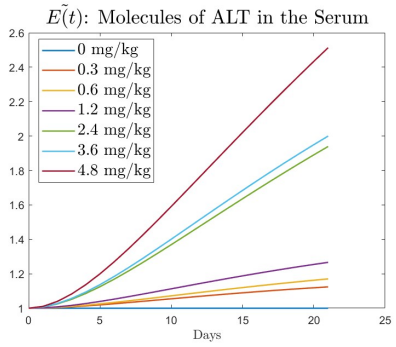
$$C = 1 - \frac{C_1}{\hat{\phi}-\hat{\alpha}} + \frac{C_2}{\hat{\phi}-1} + \frac{C_3}{\hat{\phi}}, \quad C_3 < 0.$$



(a) Healthy hepatocytes.



(b) Damaged hepatocytes.



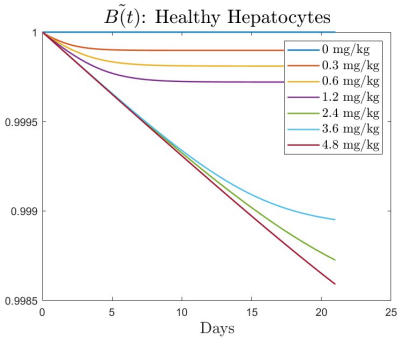
(c) ALT molecules.

Figure 8: Nondimensionalized Plots for Model 1 over 21 days for 6 doses.

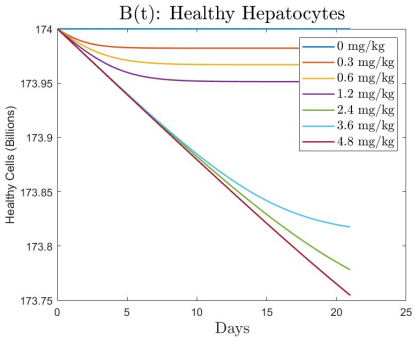
Figure 8 displays the solutions of Model 1. This model is the most basic of the four in terms of complexity, it also upholds the assumption that the number of healthy hepatocytes does not affect the future rate that T-DM1 damages hepatocytes. As a result, the number of healthy hepatocytes decreases exponentially, as displayed in Figure 8a. The damaged hepatocytes increase quickly in the beginning of treatment, and reach a steady state that the count remains at, as shown in Figure 8b. Finally, the number of ALT molecules increases fairly close to linearly throughout the 21 day regimen, as shown in Figure 8c. The ALT does not return back to the initial value.

Model v2.

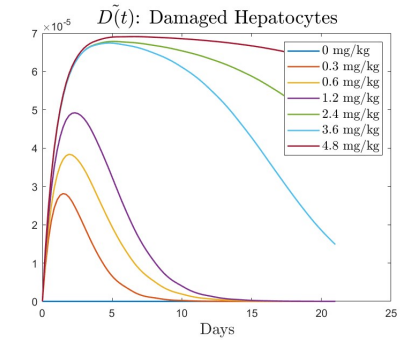
Figure 9 displays the results for Model 2. This model incorporated the Hill function in order to account for the magnitude of T-DM1 damage. The effect of this can mostly be seen manifest as steady states in each of the solutions. Unlike the results from Model 1, as in Figure 8, the healthy hepatocytes eventually do stop decreasing, and the amount of ALT molecules stop increasing. As for the results for the damaged hepatocytes, Figure 8b shows the damaged hepatocytes stop increasing which is also seen in Figure 9c. However, the damaged hepatocytes do decrease back to zero in the Model 2, unlike in Model 1. This is due to relaxation of our assumption in Model 2 that the number of healthy hepatocytes does not affect the rate that future hepatocytes become damaged.



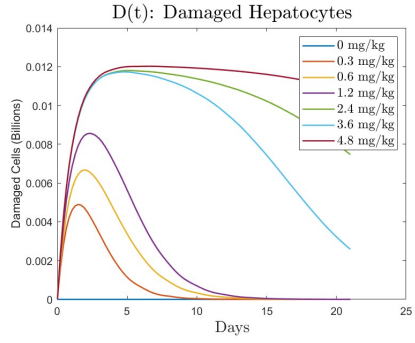
(a) Healthy hepatocytes.



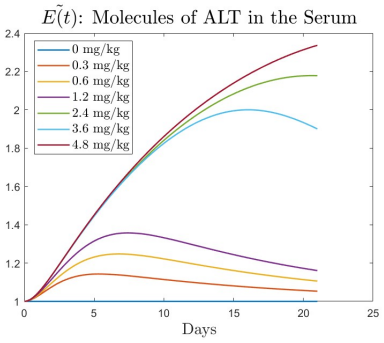
(b) Healthy hepatocytes.



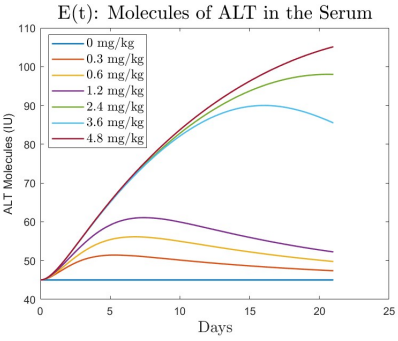
(c) Damaged hepatocytes.



(d) Damaged hepatocytes.



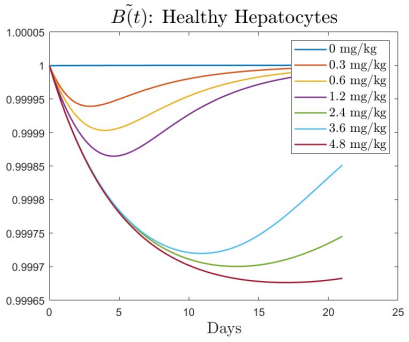
(e) ALT molecules.



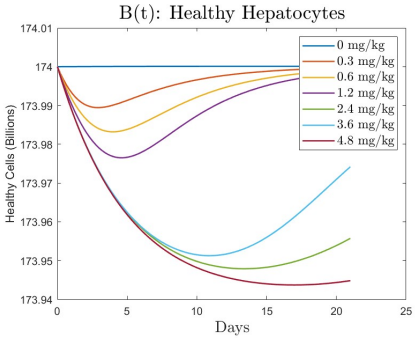
(f) ALT Molecules.

Figure 9: Nondimensional and dimensional Plots for Model 2 over 21 days for 6 doses.

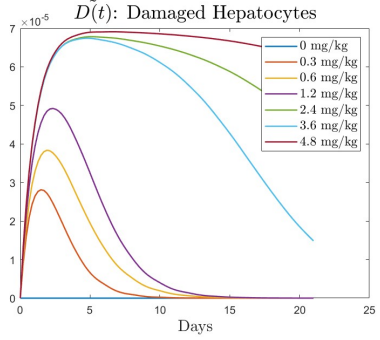
Model v3.



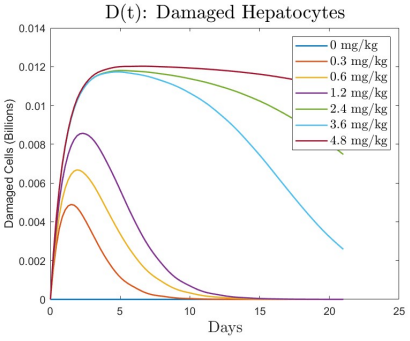
(a) Healthy hepatocytes.



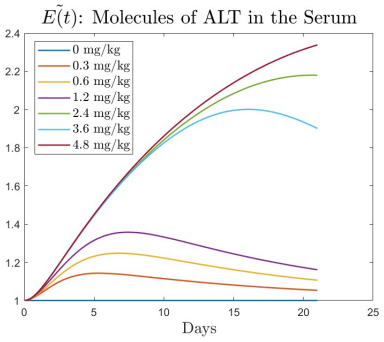
(b) Healthy hepatocytes.



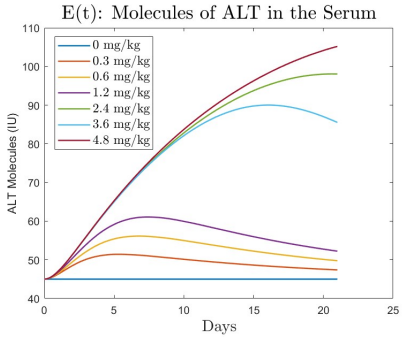
(c) Damaged hepatocytes.



(d) Damaged hepatocytes.



(e) ALT molecules.



(f) ALT molecules.

Figure 10: Nondimensional and dimensional plots for Model 3 over 21 days for 6 doses.

Model 3 introduces hepatocyte regeneration and their natural decay for healthy hepatocytes. This addition significantly changed the results for healthy hep-

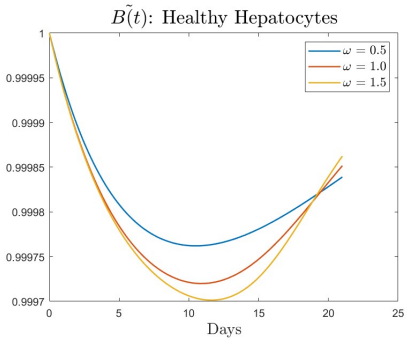
atocytes, shown in Figure 10a. Each solution rapidly decreases initially until the regeneration rate outpaces the rate healthy hepatocytes are damaged. At which point, the healthy hepatocyte count begins increase back towards initial amount. The modifications to this model did not however change the solutions for damaged hepatocytes and ALT serum molecules, shown in Figures 10c, and 10e, in comparison to Model 2.

Observing the Nature of ω in the Hill Function

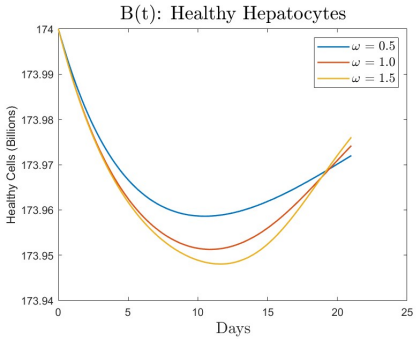
For all the solutions to the different models, we assumed the Hill coefficient, ω, to be equal to 1 when finding the results for the varying dosages. When ω is equal to 1, the system undergoes noncooperative binding (NB), which is completely independent binding. This means that the binding between T-DM1 and CKAP5 is not affected by whether or not binding has already occurred in the system. However, when $\omega > 1$, this represents positive cooperative binding (PCB), where when T-DM1 has bonded to CKAP5, the affinity of binding in the future increases, making binding occur much more frequently over time. When $\omega < 1$, it represents negative cooperative binding (NCB). This is essentially the opposite of positive cooperative binding where when T-DM1 has bonded to the CKAP5, its affinity for binding decreases over time. Thus, we can see what different values of ω would do to our models while also using these ideas to verify the Hill function and check to see if it is behaving properly.

We decided to look at how varying the values of ω would change the solutions to $B(t)$, $D(t)$, and $E(t)$ with a specific dosage. Three values were chosen with $\omega_1 = 0.5$, $\omega_2 = 1.0$, and $\omega_3 = 1.5$ with our third model, with natural birth and death parameters, used to observe the behavior of the varying Hill coefficients. When looking at Figure 11a, we see that ω_1 binds to less hepatocytes than ω_2. NCB causes the binding of the T-DM1 to CKAP5 to happen less frequently over time, which we see in the results where there are healthy cells being attacked by T-DM1. The exact opposite is occurring for ω_3, where PCB is causing more cells to be targeted earlier on into the treatment, causing a much more dramatic decrease in healthy liver cells. Also, an interesting observation of the different Hill coefficients is that the higher the Hill coefficient value is, the faster the system will return to the original carrying capacity, even though it has causes a greater magnitude of healthy liver cells to become damaged.

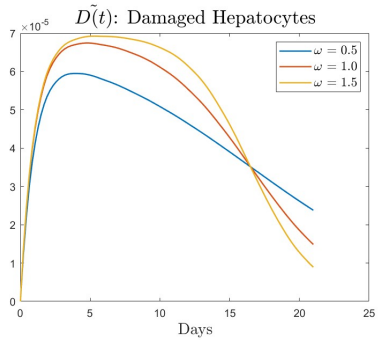
In Figure 11c, we still see the consequences of the different types of cooperation, with ω_3 having a larger magnitude of damaged cells. The majority of the damaged cells occur during the first few days, but after it reaches its apex, there is a sharp decline in damaged cells in the system. This correlates to the "healing period" in Figure 11a, where they begin to approach the carrying capacity. However, with ω_1, the total damaged cells is much lower than ω_3, but due to the slope after the apex, it shows that there is still binding going on much later than both ω_2 and ω_3. Then finally for Figure 11e, the rates at which ALT is produced is what was to be expected with the trends that are shown in Figure 11c, more damaged hepatocytes will produce higher levels of ALT.



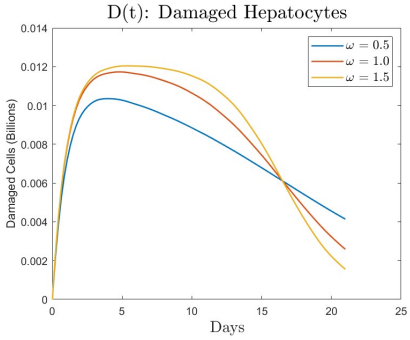
(a) Healthy hepatocytes with varying ω.



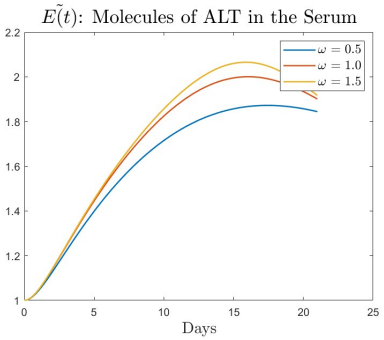
(b) Healthy hepatocytes with varying ω.



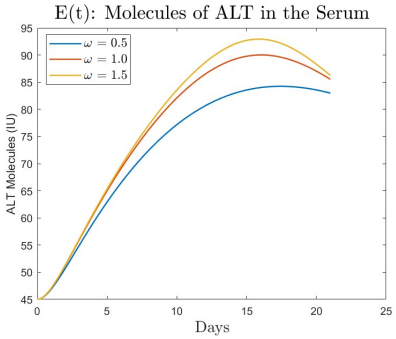
(c) Damaged hepatocytes with varying ω.



(d) Damaged hepatocytes with varying ω.



(e) ALT molecules with varying ω.



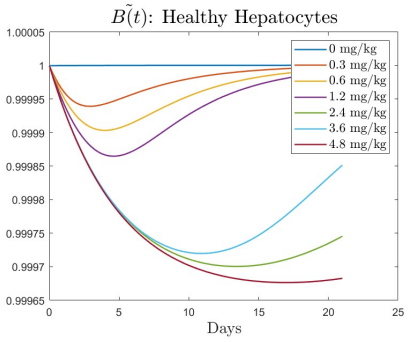
(f) ALT molecules with varying ω.

Figure 11: Nondimensional and dimensional plots for model 3 over 21 days for 6 doses.

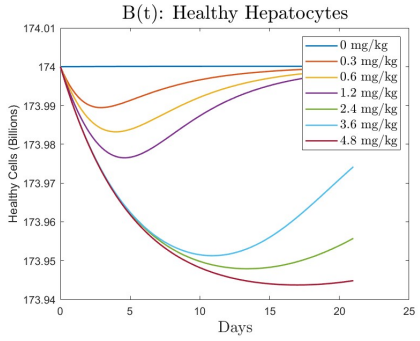
Model v4.

Figure 12 displays the solutions for Model 4. This variation includes the modification for healthy hepatocyte birth and death, this affected the results in the

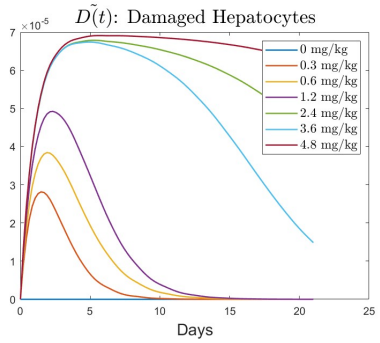
same manner as described for Model 3. In addition, this model includes the addition of a delay function seen in Figure 12e. This delay refers to a period for which ALT molecules are within the liver and have not yet entered the bloodstream. The delayed ALT graph in Figure 12e shows ALT that has been produced, but is not measurable in the serum. Due to how the models were nondimensionalized, this figure follows a very close path to previous models ALT solutions, as seen in figure 9e. This is expected, as for ALT levels to be stable in the control, the rate at which ALT enters the serum must be the same as it exiting, thus our previous ALT equation and our delay equation equal each other. Thus as seen in the previous figures, the ALT delay for the lower doses plateau quickly and slowly fall back to 1, while the larger doses quickly climb, and slowly taper off near the 21 day mark.



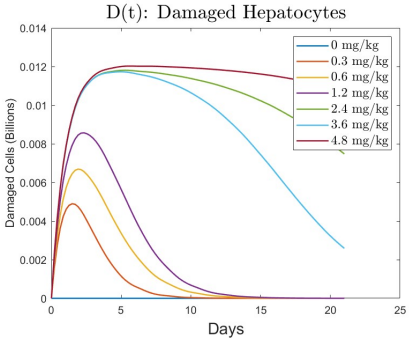
(a) Healthy hepatocytes.



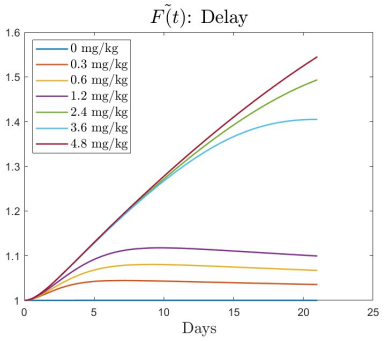
(b) Healthy hepatocytes.



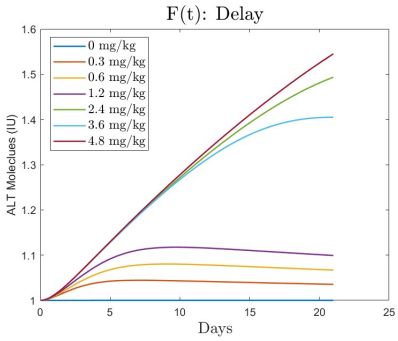
(c) Damaged hepatocytes.



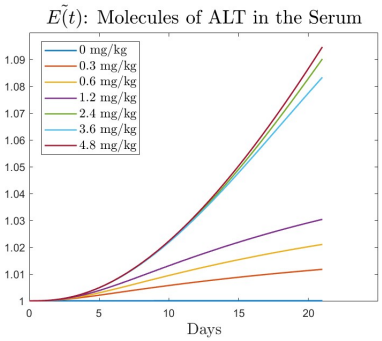
(d) Damaged hepatocytes.



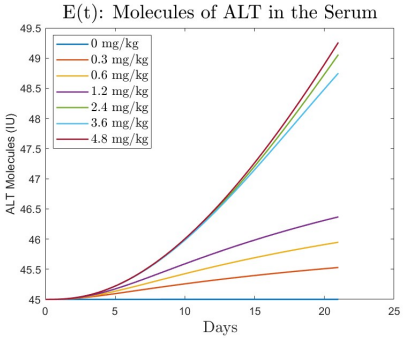
(e) ALT molecules in the liver.



(f) ALT molecules in the liver.



(g) ALT molecules in the serum.



(h) ALT molecules in the serum.

Figure 12: Nondimensional and dimensional plots for Model 4 over 21 days for 6 doses.

The new function for ALT seen in Figure 12g shows a decrease in maximum levels compared to our previous models as well as a slower increase of ALT overall. This is expected as we specifically delayed the increase of ALT in the serum with our equations. The control stays at 1 as no dosage was given, and as such nothing has changed over time. Since we knew that the control should not move, the delay had to have the same entrance rate as the clearance rate of ALT, being 0.35. The three lower dosages are tightly packed staying much lower than the three higher dosages, which are also tightly packed. Expanded past what is shown, we see that all of the ALT levels slowly make their way back to 1, and that the maximums are about half of previous figures.

Summary of All Model Versions

There are some notable similarities between the results for each of the models. Firstly, for each of the solutions, you can see a behavior that is similar to a behavior displayed in Figure 1. That is that the higher three dosages of T-DM1 cluster closer together and the lower three dosages of T-DM1 cluster closer together. There is a notable unexpected behavior associated with the 2.4 mg/kg

dosage. In general, as displayed in Figure 1, a higher dosage of T-DM1 will be associated with a relatively higher serum T-DM1 concentration with respect to a lower dosage throughout a 21 day period. However, the 2.4 mg/kg dosage is associated with a higher serum T-DM1 concentration for 10 our of the 21 days relative to the 3.6 mg/kg dosage. This anomaly can be observed in all of our results.

Figures 9c, 10c, and 12c show the results for damaged hepatocytes in Models 2, 3, and 4. These results are exactly the same. This is as expected since the way damaged hepatocytes are calculated is the same in each of these models. These figures show that the damaged hepatocytes quickly increase at the beginning of treatment, level off at a peak, and then return towards zero as death rate of these cells overtakes the rate at which new hepatocytes are being damaged. As expected, the controls show that with no T-DM1, the damaged hepatocytes stay at zero.

Figures 10a, and 12a also display the same results for healthy hepatocytes for Models 3 and 4. This is also expected as each of the models introduce birth and death rates of hepatocytes and use the same differential equation to describe the population of healthy hepatocytes. Due to the regeneration of the hepatocytes, we see that the three lower dosages are going back to the starting cell count, which was set as the carrying capacity. The three higher dosages took a while longer to bounce back, but seem to be quickly returning to the carrying capacity after the initial dose. The controls here shows that without a dose, the body stays at the carrying capacity of healthy hepatocytes.

Discussion

We have developed four distinct models, with varying levels of complexity. This was in an effort to assess the relevant physiology that should be taken into account when determining the extent to which T-DM1 causes liver injury. We, in addition, sought to develop a model that adequately described the existing data pertaining to ALT blood levels related to T-DM1 treatment.

Model 1 was our simplest, it was helpful as a baseline for adding complexity to our system. However, it did not accurately describe the existing data. Figure 8c shows an increase in ALT levels by a factor of 2 for a 3.6 mg/kg dose. While this falls within the range of data described in Figure 7a, the shape of its curve does not match our expectations [3]. Model 1 does not depict a steady state or a decrease in ALT elevation, though ALT does clear from the system and Figure 8b shows the amount of damaged hepatocytes stops growing in roughly the first 10 days of treatment. Thus, the results of this model are impractical. The remainder of the models do satisfy this condition of keeping the increase in ALT molecules in the blood for a 3.6 mg/kg dose, or lower, between a factor of 0 and 2. The remaining models also more closely align with our expectations for the behavior of ALT in the blood.

The only significant difference between Models 2 and 3 is pertaining to results for the number of healthy hepatocytes. Model 3 does create a more realistic description of how healthy hepatocytes are damaged quickly initially, but

regeneration eventually overcomes the damage caused by T-DM1 as it leaves the body, as displayed in Figure 10a. However, this complexity is not necessarily as relevant as complexity developed in the results for ALT molecules in the blood. Thus, Models 2 and 3 have similar levels of value to our problem.

Model 4 does affect the ALT levels detected in the blood. Figure 12g shows a much slower and lower estimated increase in ALT molecules. For a 3.6 mg/kg dose, Model 4 estimate about a 8% increase in ALT levels, and the curve appeared as if the level would increase past a 21 day period. This value falls in the range of reasonable values as discussed earlier. This model does also include the more physiologically accurate description of healthy hepatocytes as in Model 3. Model 2 and 4 could be viewed as upper and lower bounds of the range of responses patients could have to T-DM1 treatment.

A significant limitation of our work is that each model describes just one definite response a patient could have. This is unrealistic, there is a range of responses patients may have to T-DM1 treatment. Just for one dosage level, 3.6 mg/kg, patients had an ALT serum level increase anywhere between a factor of 0 and 6, the 95th percentile being under a factor of 2 [3]. For context, an increase by a factor of 5 is considered a grade 3, or severe, liver event [3]. While our models serve as bounds for this range of reactions, they do not exhaustively describe the probabilities associated with a well tolerated or fatal level of liver damage. This limiting characteristic could be improved would be to consider K_{max} to be a patient-specific parameter. This would allow for varying levels of magnitude of T-DM1 damage.

Conclusion

In predicting hepatotoxicity in patients that have received TDM-1 treatment, we decided on using ALT measurements as our main indicator. As such our model takes in a concentration of TDM-1 and using clinical data of ALT levels at specific concentrations, allowing us to find not only the ALT levels, but also see how much of the liver gets are damaged over the course of the treatment.

Our models can be simplified down to three, and later in model version 4, four compartments that signify different aspects of ALT production after a patient is given TDM-1. The model has a solid backbone of how these compartments interact with each other. In addition, most parameters within the model have clinical backing which allows us to be confident that besides a few parameters the model is correct.

Our later models show a rather quick recovery and low amounts of cell death within the liver for lower dosages. For higher dosages, as in previous models, the liver damage is much higher mathematically speaking, however it still accounts for a rather small amount of the liver. As such, our models depicts a rather weak correlation between TDM-1 and liver damage.

The models themselves are only a basic framework of what happens inside of the body. As such, there are many mechanisms that are not included within the models, such as previous damage or resistance to the drug over time. In addition, some of the parameters used don't have clinical trial backing and as

such we cannot attest to their validity past the final result being close to a clinical trial. In that sense, more data backing the parameters, as well as data supporting the final conclusion will be helpful in finalizing any errors within the model.

Acknowledgment

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Appendix

The following script encodes the solutions for Model version 1 and then plots the solutions for 6 dosages of T-DM1.

```
%% Model 1 solutions and plot

beta = 0.00237254167;
sigma = 5;
xi = 1.8741*10^-4;
phi = 0.35;
gamma = 483;
B_0 = 174;
%D_0 = 0;
E_0 = 45;

% A(t) parameters and dosages
P = [10^-10 .3 .6 1.2 2.4 3.6 4.8; 10^-10 11.29 14.29 22.44 69.44 76.54 110.66; 10^-10 .82 .57
.58 .2 .3 .17];
[m,n] = size(P);

for i = 1:n
    %B(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    alpha = p(3);
    t = [0:21];
    plot(t, eq(alpha, A_0, beta, sigma, xi, phi, gamma, t, B_0, E_0),'Linewidth',1.3);
    hold on
end

title('$$\hat{B(t)}$$: Healthy Heptatocytes', 'FontSize', 18, 'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg', '3.6 mg/kg',
'4.8 mg/kg', 'FontSize', 15,'Interpreter','Latex')
xlabel('Days', 'FontSize',12,'Interpreter','Latex')
figure

for i = 1:n
    %D(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    alpha = p(3);
    t = [0:21];
    plot(t, ew(alpha, A_0, beta, sigma, xi, phi, gamma, t, B_0, E_0),'Linewidth',1.3);
    hold on
end

title('$$\hat{D(t)}$$: Damaged Hepatocytes', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg',
'4.8 mg/kg', 'FontSize', 15,'Interpreter','Latex')
xlabel('Days', 'FontSize',12)
figure

for i = 1:n
```

```
    %E(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    %alpha = p(3);
    t = [0:21];
    plot(t, e(alpha, A_0, beta, sigma, xi, phi, gamma, t, B_0, E_0),'Linewidth',1.3);
    hold on
end

title('$$\hat{E(t)}$$: Molecules of ALT in the Serum', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg',
'4.8 mg/kg', 'FontSize', 15, 'Interpreter','Latex')
xlabel('Days', 'FontSize',12, 'Interpreter','Latex')

%This is B(t)
function etq = eq(alpha, A_0, beta, sigma, xi, phi, gamma, t, B_0, E_0)
    t = t/sigma;
    beta_h = (beta*A_0)/(sigma*B_0);
    gamma_h = (gamma*B_0)/E_0;
    %xi_h = (xi*B_0)/(sigma*E_0);
    %D_h = D_0/B_0;
    phi_h = phi/sigma;
    xigamma_h = xi*gamma*B_0/(sigma*E_0);
    alpha_h = alpha/sigma;

    if (beta_h > (alpha/sigma))
        disp("beta_h > alpha/sigma");
    end
    d1 = exp((-alpha/sigma)*t);

    etq = ((beta_h/alpha_h)*(d1-1))+1;
end

%This is D(t)
function etw = ew(alpha, A_0, beta, sigma, xi, phi, gamma, t, B_0, E_0)
    t = t/sigma;
    beta_h = (beta*A_0)/(sigma*B_0);
    gamma_h = (gamma*B_0)/E_0;
    %xi_h = (xi*B_0)/(sigma*E_0);
    %D_h = D_0/B_0;
    phi_h = phi/sigma;
    xigamma_h = xi*gamma*B_0/(sigma*E_0);

    c1 = ((sigma*beta_h)/(sigma-alpha));
    d1 = exp((-alpha/sigma)*t);
    c2 = ((-((sigma*beta_h)/(sigma-alpha))));
    d2 = exp(-t);

    etw = c1*d1 + c2*d2;

    if (beta_h > (alpha/sigma))
        disp("beta_h > alpha/sigma");
    end
end

%This is for E(t)
```

```
function et = e(alpha, A_0, beta, sigma, xi, phi, gamma, t, B_0, E_0)
    t = t/sigma;
    beta_h = (beta*A_0)/(sigma*B_0);
    gamma_h = (gamma*B_0)/E_0;
    xi_h = (xi*B_0)/(sigma*E_0);
    %D_h = D_0/B_0;
    phi_h = phi/sigma;
    xigamma_h = xi*gamma*B_0/(sigma*E_0);

    c1 = ((gamma_h*sigma*beta_h)/(sigma-alpha)) + ((xigamma_h*sigma*beta_h)/alpha);
    d1 = exp((-alpha/sigma)*t);
    c2 = ((gamma_h*sigma*beta_h/(sigma-alpha)));
    d2 = exp(-t);
    c3 = ((xigamma_h*sigma*beta_h)/alpha) - xigamma_h;

    c = 1 - (c1/(phi_h-(alpha/sigma))) + c2/(phi_h-1) + c3/(phi_h);
    et = (c1/(phi_h-(alpha/sigma)))*d1 - (c2/(phi_h-1))*d2 - c3/(phi_h) + c*exp(-phi_h*t);



    if (beta_h > (alpha/sigma))
        disp("beta_h > alpha/sigma");
    end

end
```

Included is the MATLAB code used to numerically solve model version 1, 2, 3, and 4. This includes a hill_call which calls the hill_M2, hill_M3, and hill_M4 functions. This code was also used to compute the varying omega results as seen in 11. Directions for this are included in the code. In order to create those graphs we used Model 3. In addition this code can be used to create out dimensional plots by uncommenting the appropriate plot calls and ylabel calls for each solution.

```
%% hill_call
%% Numerical Solution - This code calls function hill_solve then uses ode45 to
%% Nondimensional solutions and plot
%% Dimensional plots are commented out

% Hill function parameters
K_m = .0003502;
K_5 = 55.33;
om = 1;

% Parameters
sig = 5;
gam = 483;
phi = .35;
xi = 1.8741*10^-4;
nu = 1;
mu = 15.75;

% Initial Conditions
B_0 = 1;
D_0 = 0;
```

```
E_0 = 1;
F_0 = 1;

% B_D = 174;
% E_D = 45;


tspan = linspace(0,21);
S0 = [B_0;(D_0/B_0);E_0];
S1 = [B_0;(D_0/B_0);E_0;F_0];


% A(t) parameters and dosages
P = [0 .3 .6 1.2 2.4 3.6 4.8;0 11.29 14.29 22.44 69.44 76.54 110.66;0 .82 .57 .58 .2 .3 .17];
[m,n] = size(P);

% Model Version 2
figure('Name', 'Model 2')
for i = 1:n
    % B(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    [t,H] = ode45(@(t,s) hill_M2(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a),tspan,S0);
    A = A_0*exp(-a*t);
    plot(t, H(:,1),'Linewidth',1.3)
    % plot(t, B_D*H(:,1),'Linewidth',1.3)
    hold on
end
title('$$\hat{B(t)}$$: Healthy Hepatocytes', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg', '0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize', 15,'Interpreter','Latex')
% ylabel('Healthy Cells (Billions)')


figure('Name', 'Model 2')
for i = 1:n
    % D(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    [t,H] = ode45(@(t,s) hill_M2(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a),tspan,S0);
    A = A_0*exp(-a*t);
    plot(t,H(:,2),'Linewidth',1.3)
    % plot(t,B_D*H(:,2),'Linewidth',1.3)
    hold on
end
title('$$\hat{D(t)}$$: Damaged Hepatocytes', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize',12,'Interpreter','Latex')
xlabel('Days', 'FontSize', 15,'Interpreter','Latex')
% ylabel('Damaged Cells (Billions)')

figure('Name', 'Model 2')
```

```
for i = 1:n
    %E(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    [t,H] = ode45(@(t,s) hill_M2(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a),tspan,S0);
    A = A_0*exp(-a*t);
    plot(t,H(:,3),'Linewidth',1.3)
    % plot(t,E_D*H(:,3),'Linewidth',1.3)
    hold on
end
title('$$\hat{E(t)}$$: Molecules of ALT in the Serum', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize',15,'Interpreter','Latex')
% ylabel('ALT Molecules (IU)')

% Model version 3
% To recreate omega varation results:
% Uncomment fixed A_0 and a, comment out dose specific A_0, a, and p.
% Change n to be equal to 3
% Uncomment new om and the appropriate legend

figure('Name', 'Model 3')
% om = [0.5, 1, 1.5]
for i = 1:n
    % B(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    % A_0 = 76.54;
    %a = .3;
    [t,H] = ode45(@(t,s) hill_m3(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a,nu),tspan,S0);
    A = A_0*exp(-a*t);
    plot(t,H(:,1),'-','Linewidth',1.3)
    % plot(t,B_D*H(:,1),'-','Linewidth',1.3)
    hold on
end
title('$$\hat{B(t)}$$: Healthy Hepatocytes', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg', '0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize', 15,'Interpreter','Latex')
%legend('   = 0.5','   = 1.0','   = 1.5', 'FontSize', 12)
% ylabel('Healthy Cells (Billions)')

figure('Name', 'Model 3')
for i = 1:n
    % D(t)
    p = P(:,i);
    dose = p(1);
    %dose = 3.6;
    A_0 = p(2);
    %A_0 = 76.54;
    a = p(3);
    %a = .3;
```

```
    [t,H] = ode45(@(t,s) hill_m3(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a,nu),tspan,S0);
    A = A_0*exp(-a*t);
    plot(t,H(:,2),'-','Linewidth',1.3)
    % plot(t,B_D*H(:,2),'-','Linewidth',1.3)
    hold on
end
title('$$\hat{D(t)}$$: Damaged Hepatocytes', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize',12,'Interpreter','Latex')
xlabel('Days', 'FontSize', 15,'Interpreter','Latex')
%legend('   = 0.5','   = 1.0','   = 1.5', 'FontSize', 12)
% ylabel('Damaged Cells (Billions)')

figure('Name', 'Model 3')
for i = 1:n
    %E(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    %A_0 = 76.54;
    %a = .3;
    [t,H] = ode45(@(t,s) hill_m3(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a,nu),tspan,S0);
    A = A_0*exp(-a*t);
    plot(t,H(:,3),'-','Linewidth',1.3)
    % plot(t,E_D*H(:,3),'-','Linewidth',1.3)
    hold on
end
title('$$\hat{E(t)}$$: Molecules of ALT in the Serum', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize',15,'Interpreter','Latex')
%legend('   = 0.5','   = 1.0','   = 1.5', 'FontSize', 12)
%ylabel('ALT Molecules (IU)')


% Model Version 4
om = 1;
figure('Name', 'Model 4')
for i = 1:n
    % B(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    [t,H] = ode45(@(t,s) hill_m4(t,s,K_m,K_5,om,sig,gam,xi,phi,nu,mu,A_0,a),tspan,S1);
    A = A_0*exp(-a*t);
    plot(t,H(:,1),'Linewidth',1.3)
    %plot(t,B_D*H(:,1),'Linewidth',1.3)
    hold on
end
title('$$\hat{B(t)}$$: Healthy Hepatocytes', 'FontSize', 18, 'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize', 15,'Interpreter','Latex')
%ylabel('Healthy Cells (Billions)')
```

```
figure('Name', 'Model 4')
for i = 1:n
    % D(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    [t,H] = ode45(@(t,s) hill_m4(t,s,K_m,K_5,om,sig,gam,xi,phi,nu,mu,A_0,a),tspan,S1);
    A = A_0*exp(-a*t);
    plot(t,H(:,2),'Linewidth',1.3)
    %plot(t,B_D*H(:,2),'Linewidth',1.3)
    hold on
end
title('$$\hat{D(t)}$$: Damaged Hepatocytes', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize', 15)
%ylabel('Damaged Cells (Billions)')

figure('Name', 'Model 4')
for i = 1:n
    %F(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    [t,H] = ode45(@(t,s) hill_BDm4(t,s,K_m,K_5,om,sig,gam,xi,phi,nu,mu,A_0,a),tspan,S1);
    A = A_0*exp(-a*t);
    % nondim and dim are the same for F(t)
    plot(t,H(:,3),'Linewidth',1.3)
    hold on
end
title('$$\hat{F(t)}$$: Delay', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize',15,'Interpreter','Latex')
%ylabel('ALT Moleclues (IU)')

figure('Name', 'Model 4')
for i = 1:n
    %E(t)
    p = P(:,i);
    dose = p(1);
    A_0 = p(2);
    a = p(3);
    [t,H] = ode45(@(t,s) hill_m4(t,s,K_m,K_5,om,sig,gam,xi,phi,nu,mu,A_0,a),tspan,S1);
    A = A_0*exp(-a*t);
    plot(t,H(:,4),'Linewidth',1.3)
    %plot(t,E_D*H(:,4),'Linewidth',1.3)
    hold on
end
title('$$\hat{E(t)}$$: Molecules of ALT in the Serum', 'FontSize', 18,'Interpreter','Latex')
legend('0 mg/kg','0.3 mg/kg','0.6 mg/kg','1.2 mg/kg','2.4 mg/kg','3.6 mg/kg','4.8 mg/kg',
'FontSize', 12,'Interpreter','Latex')
xlabel('Days', 'FontSize',15,'Interpreter','Latex')
%ylabel('ALT Molecules (IU)')
```

The following three functions set up the differential equations for Model 2, 3,

and 4 respectively. Each is called on by hill_call in order to produce our results.

```
function dydt = hill_M2(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a)
%ODE45
B = s(1);
D = s(2);
E = s(3);
B_0 = 174;
E_0 = 45;
A = A_0*exp(-a*t);
dydt = zeros(3,1);
dydt(1) = -K_m*(A*A_0).^(om)/(sig*(K_5^(om) + (A*A_0).^om))*B;
dydt(2) = K_m*(A*A_0).^(om)/(sig*(K_5^(om) + (A*A_0).^om))*B - D;
dydt(3) = (gam*D*B_0)/E_0 + (xi*gam*B*B_0)/(sig*E_0) - (phi*E)/(sig);
end
```

```
function dydt = hill_m3(t,s,K_m,K_5,om,sig,gam,xi,phi,A_0,a,v)
%ODE45
B = s(1);
D = s(2);
E = s(3);
B_SS = 1.000188;
B_0 = 174;
E_0 = 45;
A = A_0*exp(-a*t);
dydt = zeros(3,1);
dydt(1) = (B/sig)*((-K_m*(A*A_0).^(om))/(K_5^(om) + (A*A_0).^om)) + (v*(1-(B/B_SS))-xi)*(B/sig);
dydt(2) = (K_m*(A*A_0).^(om)/(sig*(K_5^(om) + (A*A_0).^om)))*B - D;
dydt(3) = (gam*D*B_0)/E_0 + (gam*xi*B*B_0)/(sig*E_0) - (phi*E)/sig;
end
```

```
function dydt = hill_m4(t,s,K_m,K_5,om,sig,gam,xi,phi,nu,mu,A_0,a)
%ODE45
B = s(1);
D = s(2);
F = s(3);
E = s(4);
B_ss = 1.000188;
B_0 = 174;
E_0 = 45;
F_0 = 1;
A = A_0*exp(-a*t);
dydt = zeros(3,1);
dydt(1) = -K_m*(A*A_0).^(om)/(sig*(K_5^(om) + (A*A_0).^om))*B + B*(nu*(1-(B/B_ss))-xi)/(sig);
dydt(2) = K_m*(A*A_0).^(om)/(sig*(K_5^(om) + (A*A_0).^om))*B - D;
dydt(3) = (gam*D)/F_0 + (xi*B*gam)/(sig*F_0) - (mu*F*F_0)/(sig*B_ss*B_0);
dydt(4) = (mu*F*F_0)/(sig*B_ss*B_0)-(phi*E*E_0)/(sig*B_ss*B_0);
end
```

The following MATLAB code also calls on the hill_M2 function. This code varies values of K_{max} and K_{50} and then solves version 2 of our model. The max of the $E(t)$ solution is then calculated and generates the contour graph displayed in Figure 7b.

```
%% Determining hill function parameters
%initializing range for K_5 and K_m
n = 50;
k_m = linspace(1*10^-5,.0015,n);
k_5 = linspace(30,65,n);
[K_5, K_m] = meshgrid(k_5, k_m);
om = 1;
% Parameters
sig = 5;
gam = 483;
phi = .35;
nu = 1;
mu = .2;
% Initial Conditions
B_0 = 1;
D_0 = 0;
E_0 = 1;
F_0 = 1;
xi = 1.8741*10^-4;

tspan = linspace(0,21);
S0 = [B_0;(D_0/B_0);E_0];
% A(t) parameters and dosages
%P = [.3 .6 1.2 2.4 3.6 4.8; 11.29 14.29 22.44 69.44 76.54 110.66; .82 .57 .58 .2 .3 .17];
%[m,n] = size(P);
A_0 = 76.54;
a = .3;

for i = 1:n
    for j = 1:n
        [t,H] = ode45(@(t,s) hill_M2(t,s,K_m(i,j),K_5(i,j),om,sig,gam,xi,phi,A_0,a),tspan,S0);
        E(i,j) = max(H(:,3));
        %E_n(i,j) = max(H_n(:,4));
    end
end
V = [1.5 ,2, 3, 4, 5];
%surf(K_5,K_m, E)
contour(K_5,K_m,E, V, 'ShowText', 'on','Linewidth',1.3)
xlabel('K_5_0')
ylabel('K_m_a_x')
title('Contour Map of E_m_a_x (3.6 mg/kg)')

%zlabel('E max')
```