Modelling Tumor growth incorporating the effects of necrosis and the effect of Bevacizumab.

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Overview

1. Introduction to the biological background, model and the experimental data.
2. Mathematical Analysis.
3. Parameter estimation and data fitting.
4. Model predictions and hypothesis for the biological problem.
5. Discussion: The limitations of proposed models and future work.
6. Coding

Introduction: Biological Background

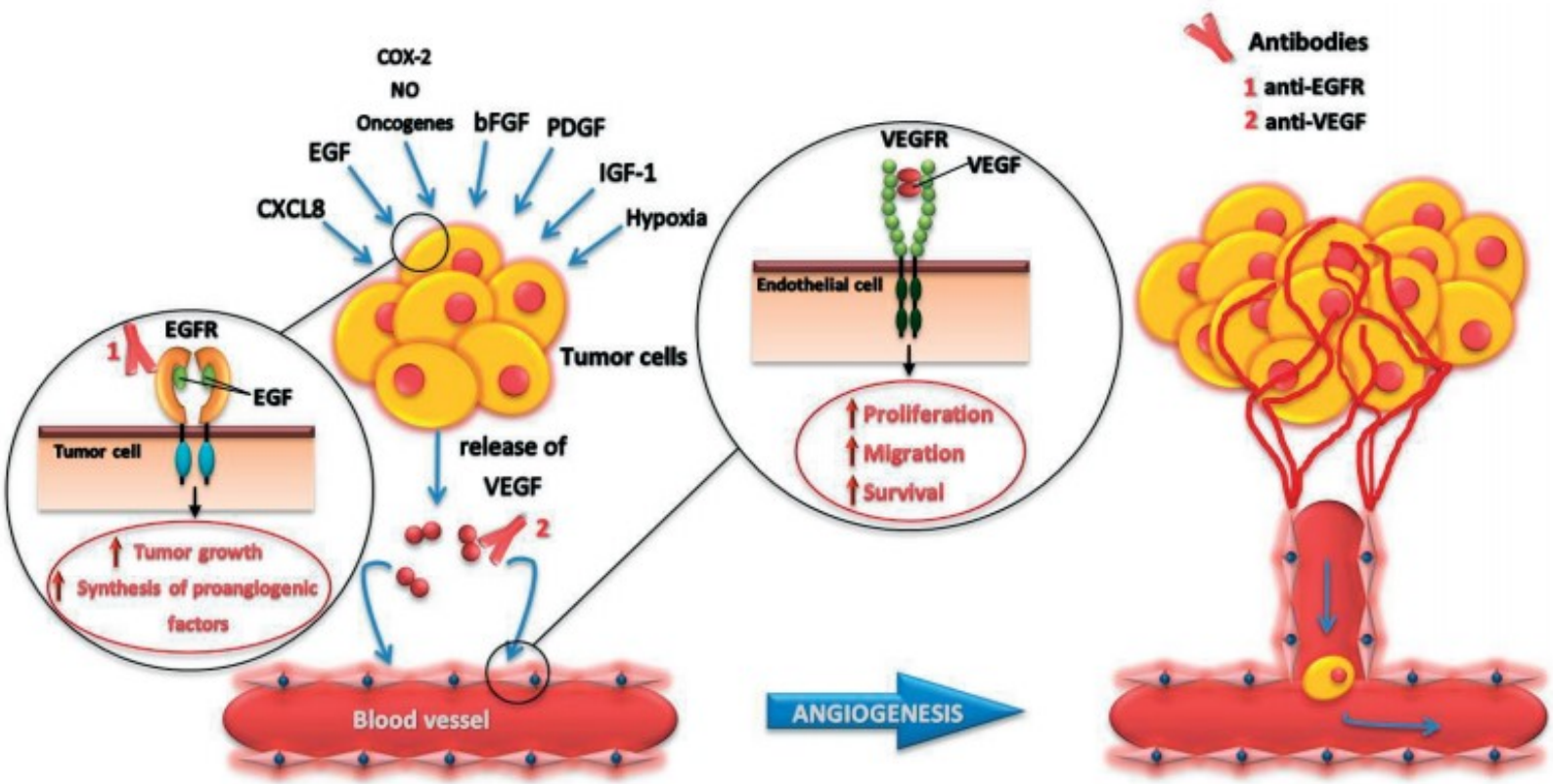


Figure 1: Angiogensis induced by the VEGF proteins secreted by the tumor cells (Figure taken from [5]).

- Angiogenesis is the formation of new blood vessels required for the growth of tumors.
- VEGF (Vascular endothelial growth factor) is a signalling protein produced by cells that stimulates formation of blood vessels.
- The VEGF binds to the corresponding receptors on the surface of the endothelial cells hence inducing angiogenesis. Bevacizumab selectively binds to VEGF therefore inhibiting the binding of VEGF to cell surface receptors [1].
- Cancers are always composed of active (proliferating cells) and inactive tumor cells. There are also specialised cells which are temporarily inactive.
- These quiescent cells can metastasize and remain in dormant state for a long time.

The Experiment

- The C38 colon adenocarcinoma was subcutaneously implanted on two groups of mice (group 1 with mice C1-C5 and group 2 with mice E1-E9)[7].
- Two kinds of therapy was given to the two groups. The first group (C1-C5) received an injection of 10 mg/Kg dosage of Bevacizumab at the beginning of the treatment.
- The second group (E1-E9) received a continuous dosage of 1/18 mg/Kg injection of Bevacizumab for 18 days.
- The data of the tumor volume was collected for 18 days for the two groups of mice.
- C38 colon adenocarcinoma typically do not metastasize due to subcutaneous location [7]. In modelling the tumor growth only the necrotic tumor cells and proliferating tumor cells are considered.

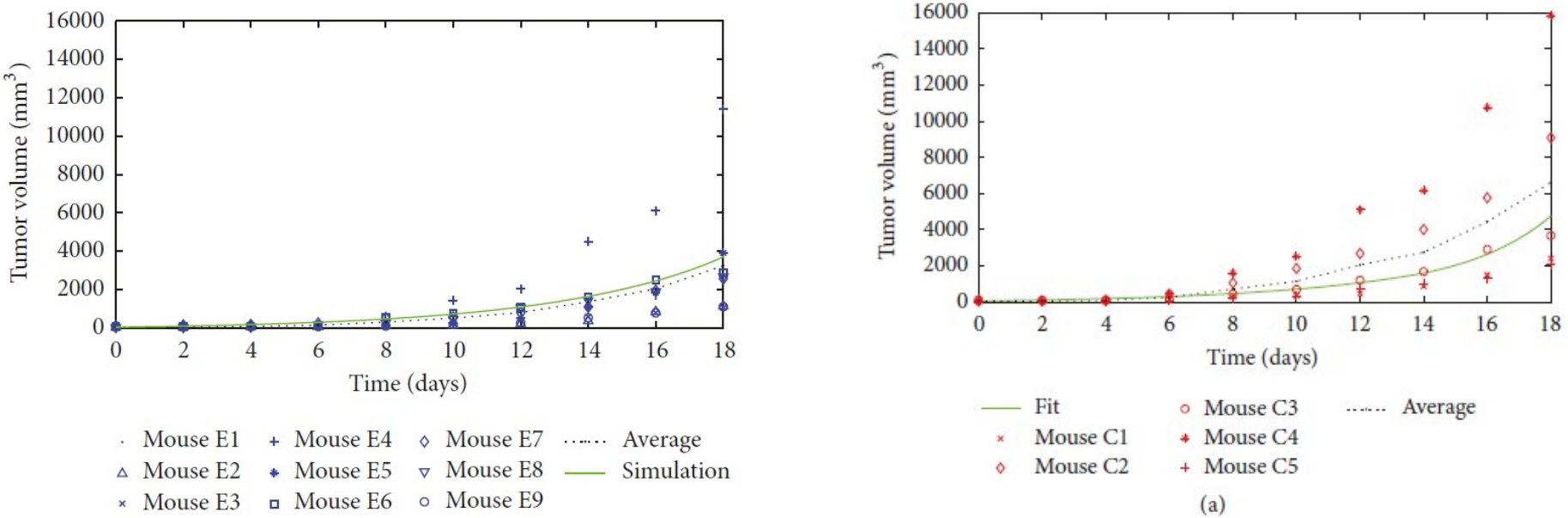


Figure 2: Experimental data: 1) Continuous dosage treatment of 1/18mg/Kg (9.5×10^{-4}) for 18 days. 2) Single dosage treatment of initial dosage 0.171mg/ml.

System Network

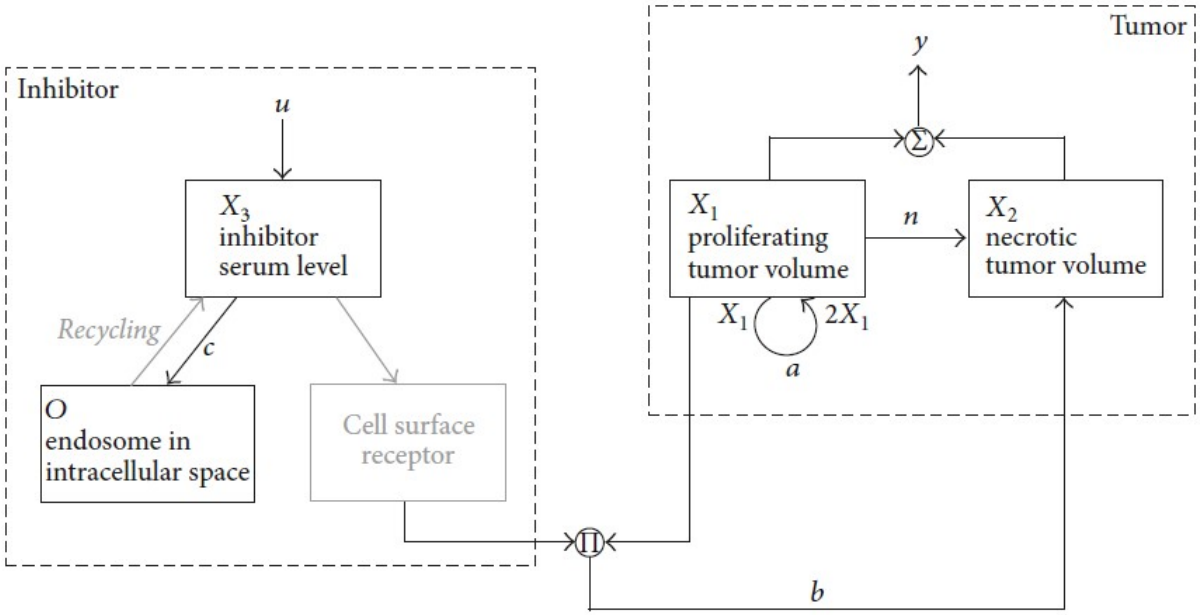


Figure 3: System network as shown in [7]. The items in grey represents extra details happening in the biological background which is not explicitly described in the model but is taken care of by the Michealis-Menten kinetics [7].

The Model

- Let X_1 denotes the proliferating tumor volume(mm^3), X_2 denotes the necrotic tumor volume(mm^3) and X_3 denotes the inhibitor (Bevacizumab) serum level (mg/ml), O denotes the compartment outside the model. The following equations describe the dynamics of tumor and the serum [7]:
- (1) $X_1 \longrightarrow 2X_1$ this defines the growth of tumor cells with the rate a. Hence using mass action kinetics $\dot{x_1} = ax_1$.
- (2) $X_1 \longrightarrow X_2$ the proliferating tumor cells becoming necrotic tumor cells at the rate n. Hence by the mass action kinetics we obtain $\dot{x_1} = -nx_1$ and $\dot{x_2} = nx_1$.

- (3) $X_3 \xrightarrow{c} O$ represents the clearance rate of the drug or the outflow of the drug to compartment outside the model at the rate coefficient c. By using Michealis-Menten kinetics we get $\dot{x_3} = -c\frac{x_3}{K_B+x_3}$ where K_B is the Michealis-Menten constant for inhibitor.
- (4) $X_1 + X_3 \xrightarrow{b} X_2$ represents the inhibitor binding to the VEGF molecules whose concentration is proportional to the volume of tumor with the rate coefficient b. Hence by using the Michealis- Menten kinetics we get $\dot{x_1} = -bx_1\frac{x_3}{ED_{50}+x_3}$, $\dot{x_2} = bx_1\frac{x_3}{ED_{50}+x_3}$ and $\dot{x_3} = -b_kx_1\frac{x_3}{ED_{50}+x_3}$. Where $b_k = bk$ and k is a constant with dimension $mg/ml/mm^3.day$.
- ED_{50} is the median effective dosage which is found from [6] to be $5 \times 10^{-5} mg/ml$.

Continuous dosage model

The differential equations can be written as follows:

$$\dot{x_1} = (a - n)x_1 - bx_1 \frac{x_3}{ED_{50} + x_3}. \tag{1}$$

$$\dot{x_2} = nx_1 + b\frac{x_1 x_3}{ED_{50} + x_3}. \tag{2}$$

$$\dot{x_3} = -b_k \frac{x_1 x_3}{ED_{50} + x_3} - c\frac{x_3}{K_B + x_3} + u. \tag{3}$$

u is a function of the input dosage rate of the inhibitor. u will vary according to the type of treatment used.

Model for the single dosage treatment

$$\dot{x_1} = (a - n)x_1 - bx_1 \frac{x_3}{ED_{50} + x_3}. \tag{4}$$

$$\dot{x_2} = nx_1 + b\frac{x_1 x_3}{ED_{50} + x_3}. \tag{5}$$

$$\dot{x_3} = -b_k \frac{x_1 x_3}{ED_{50} + x_3} - c\frac{x_3}{K_B + x_3}. \tag{6}$$

with the condition that $x_3(0) = 0.171 mg/ml$(10mg/Kg dosage), which is the initial serum level of the inhibitor.

Mathematical Analysis

Theorem

The system (1)-(3) has a unique solution and $\{(x, y, z) : x \geq 0, y \geq 0, z \geq 0\}$ *is a positive invariant set for the system.*

Proof.

Note that $\dot{x_1}|_{x_1=0} \geq 0$, $\dot{x_2}|_{x_2=0} = nx_1 + b\frac{x_1 x_3}{Ed_{50}+x_3} \geq 0$ if x_1, $x_3 \geq 0$ and $\dot{x_3}|_{x_3=0} = u \geq 0$. Hence it follows that $\{(x, y, z) : x \geq 0, y \geq 0, z \geq 0\}$ is a positive invariant set for the system ([2], Proposition 2.1) .
The equations on the right hand side of (1)-(3) are C^1 functions in the domain $\{t : t \geq 0\} \times \mathbb{R}^3_+$. By the existence and uniqueness theorem the system (1)-(3) has a unique solution in $\{t : t \geq 0\} \times \mathbb{R}^3_+$. □

Theorem

The proliferating tumor volume is bounded by exponential functions as follows

$$x_1(0)e^{(a-n-b)t} \leq x_1(t) \leq x_1(0)e^{(a-n)t}. \tag{7}$$

If $(a-n) < 0$ *then eventually all the proliferating tumor will become necrotic tumors.*

Proof.

From (1) it follows that

$$(a-n-b)x_1 \leq \dot{x_1} \leq (a-n)x_1.$$

Hence it follows that the solution $x_1(t)$ satisfies the inequality

$$x_1(0)e^{(a-n-b)t} \leq x_1(t) \leq x_1(0)e^{(a-n)t}.$$

Note that from (1)-(3) it follows that the dynamics of proliferating tumor (x_1) and the inhibitor (x_3) is independent of the dynamics of necrotic tumor (x_2). Hence we focus on the system

$$\dot{x_1} = (a - n)x_1 - bx_1 \frac{x_3}{ED_{50} + x_3}. \tag{8}$$

$$\dot{x_3} = -b_k \frac{x_1 x_3}{ED_{50} + x_3} - c\frac{x_3}{K_B + x_3} + u. \tag{9}$$

For all the further analysis let us assume that u is a constant.

Theorem

The system (8)-(9) has a locally asymptotically stable equilibrium point $(0, u.\frac{K_B}{c-u})$ *if* $a - n \leq 0$.

Proof

Solving the equations

$$(a-n)x_1 - bx_1\frac{x_3}{ED_{50}+x_3} = 0.$$

$$-b_k\frac{x_1x_3}{ED_{50}+x_3} - c\frac{x_3}{K_B+x_3} + u = 0.$$

we get two equilibrium points

$$(\bar{x_1}, \bar{x_3}) = (0, u.\frac{K_B}{c-u})$$

and $(x_1^*, x_3^*) = (\frac{b}{b_k.(a-n)}.(u - c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}), \frac{(a-n)ED_{50}}{b-a+n})$.

Proof Cont.

Evaluating the Jacobian of the system (8)-(9) we get

$$J|_{(\bar{x_1},\bar{x_3})} = \begin{bmatrix} a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} & 0 \\ \frac{-b_k.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} & \frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2} \end{bmatrix} \tag{10}$$

$$J|_{(x_1^*,x_3^*)} = \begin{bmatrix} 0 & \frac{-b.x_1^*.ED_{50}}{(ED_{50}+x_3^*)^2} \\ \frac{-b_k.x_3^*}{(ED_{50}+x_3^*)} & \frac{-b_k.x_1^*.ED_{50}}{(ED_{50}+x_3^*)^2} - \frac{c.K_B}{(K_B+x_3^*)^2} \end{bmatrix} \tag{11}$$

Proof Cont.

For the equilibrium point $(\bar{x_1}, \bar{x_3})$, it follows that $c \geq u$ (equilibrium point should be a positive value). From (10) it follows that the eigenvalues are $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})}$ and $\frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2}$. If $a - n \leq 0$ then the eigenvalues are negative. Hence the equilibrium point $(\bar{x_1}, \bar{x_3})$ is locally asymptotically stable. This equilibrium is a 'favourable' equilibrium since the proliferating tumor $\bar{x_1} = 0$ and from (2) it follows that necrotic tumor is stable (i.e $\dot{x_2}|_{(\bar{x_1},\bar{x_3})} = 0$).

Proof Cont.

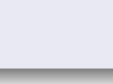
We can neglect the equilibrium point (x_1^*, x_3^*) since it is biologically unreasonable. This is because $\dot{x_2}|_{(x_1^*, x_3^*)} = x_1^*(n + b.\frac{x_3^*}{ED_{50}+x_3^*}) = a.x_1^*$, if $x_1^* \neq 0$ then the necrotic tumor x_2 increases in mass for all future time, which is absurd because the proliferating tumor is at equilibrium (with the value x_1^*) and the serum inhibitor level is a constant (with the value x_3^*). Hence we are left with the case when $x_1^* = 0$ which is possible if and only if the dosage rate is a fixed value of $u = c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$. At this dosage rate both the equilibrium points (x_1^*, x_3^*) and $(\bar{x_1}, \bar{x_3})$ are equal.

Theorem

If $a - n > 0$ *and* $K_B.\frac{b-a+n}{a-n} + ED_{50} > 0$ *then the dosage rate* $u = c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ *is the bifurcation value for the equilibrium point* $(0, u.\frac{K_B}{(c-u)})$ *of the system (8)-(9) .*

Proof.

From (10) it follows that the eigenvalues are $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})}$ and $\frac{-c.K_B}{(K_B+\frac{u.K_B}{c-u})^2}$. It follows that $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} < 0$ if and only if $u > c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ and $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} > 0$ if and only if $u < c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$. Hence if $u > c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ then the equilibrium point is locally asymptotically stable (eigenvalues are negative). If $u < c.\frac{ED_{50}}{(K_B.\frac{b-a+n}{a-n}+ED_{50})}$ then the equilibrium point is a saddle (eigenvalues are of opposite sign).



Theorem

There is no periodic orbit for the system (8)-(9) in the region $\{(x_1, x_3) : x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}\}$.

Proof.

Let $f = (a-n)x_1 - bx_1\frac{x_3}{ED_{50}+x_3}$ and $g = -b_k\frac{x_1x_3}{ED_{50}+x_3} - c\frac{x_3}{K_B+x_3} + u$. Since $\frac{\partial f}{\partial x_1} + \frac{\partial g}{\partial x_3} = (a-n) - \frac{b.x_3}{(ED_{50}+x_3)} - \frac{b_k.x_1.ED_{50}}{(ED_{50}+x_3)^2} - \frac{c.K_B}{(K_B+x_3)^2} \leq 0$ when $(a-n) - \frac{b.x_3}{(ED_{50}+x_3)} \leq 0$ or equivalently when $x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}$. Hence by the Dulac's criteria the system (8)-(9) has no periodic solution in the region $\{(x_1, x_3) : x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}\}$.

Theorem

The system (1)-(3) has no periodic orbit in the region $\{(x_1, x_2, x_3) : x_1 \geq 0, x_2 \geq 0, x_3 \geq 0\}$.

Proof.

Equation (2) implies that x_2 is an increasing function. Hence it is impossible to have a periodic orbit in the positive region. □

Parameter estimation and data fitting

- The value of ED_{50} was obtained from [6] to be 5×10^{-5} mg/ml.
- The value of the clearance rate c of Bevacizumab dosage of $5 - 10$ mg/Kg was obtained from [4] to be 0.18 L/day. Which is equivalent to the clearance of 0.0855-0.171 mg/ml of drug in the serum per day.
- The growth rate a of the tumor volume was estimated to be $0.2 - 0.7$ per day.
- The value of n is taken to be between 0 and 1. If $n \geq 1$ then tumor volume is decreases according to eq(1) which is not an interesting case.
- The value of b is estimated to be between 0 and 1 from [3].
- The value of K_B was obtained as 0.4409 from [3].

- The parameters a, n, b, b_k, c, $x_1(0)$ were identified by fitting to the data using the LSQCURVEFIT function in MATLAB(R2020b).
- This was done by minimizing the distance of the simulated total tumor volume and the experimentally observed total tumor volume.
- A set of parameters were identified such that the model eq(1)-eq(3) fit the experimental data for continuous dosage (1/18mg/Kg per day) (table(24)(a)).
- Similarly another set of parameters were identified such that the model eq(4)-(6) fit the experimental data for single dosage (single initial dosage of 10mg/Kg)(table(24)(b)).

Parameter	Dimension	Parameter name	1.Continuous dosage	2.Single dosage
a	$\frac{1}{day}$	Tumor growth rate	0.5696	0.6443
n	$\frac{1}{day}$	Necrosis rate	0.2564	0.2337
b	$\frac{1}{day}$	Inhibition rate	0.2556	0.2326
b_k	$\frac{mg}{ml}/mm^3/day$	Modified inhibition rate	9.498×10^{-6}	9.5×10^{-6}
c	$\frac{mg}{ml}$/day	Clearance rate	0.1502	0.1039
K_B	$\frac{mg}{ml}$	Michealis-Menten constant for inhibitor	0.4409	0.4409
$x_1(0)$	mm^3	Initial tumor volume	67.3819	47.7666
ED_{50}	mg/ml	Median effective dosage	5×10^{-5}	5×10^{-5}

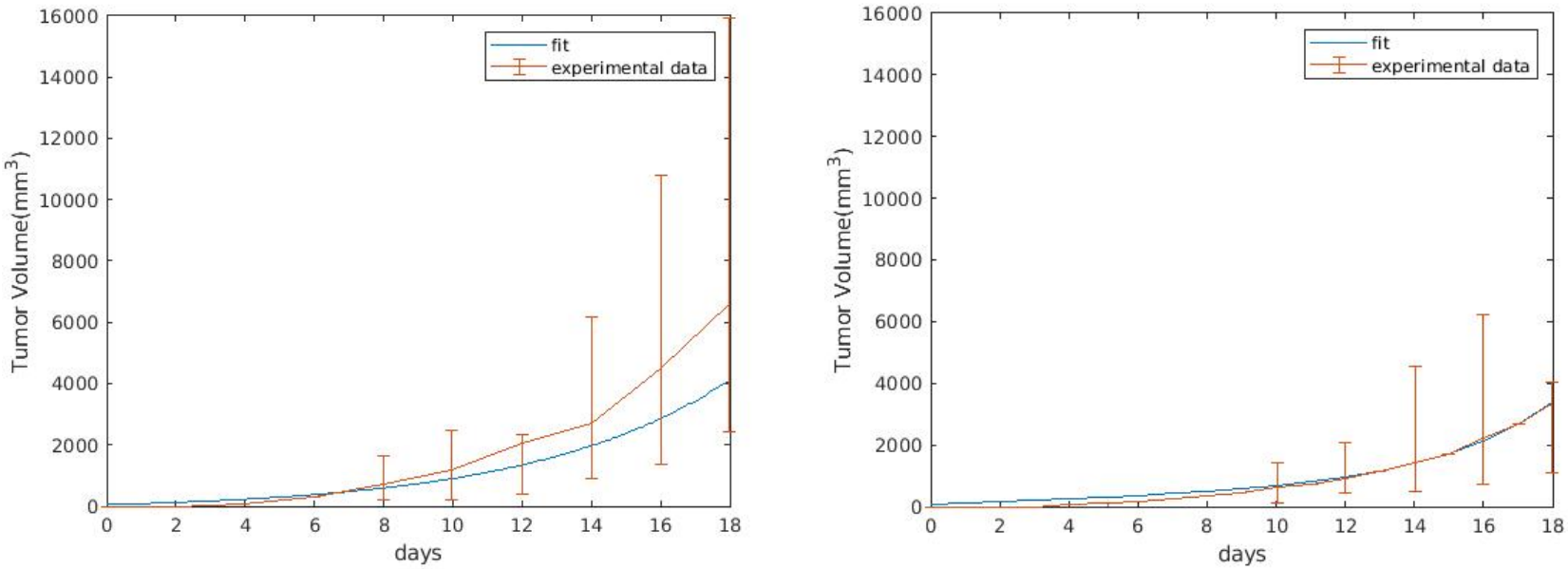
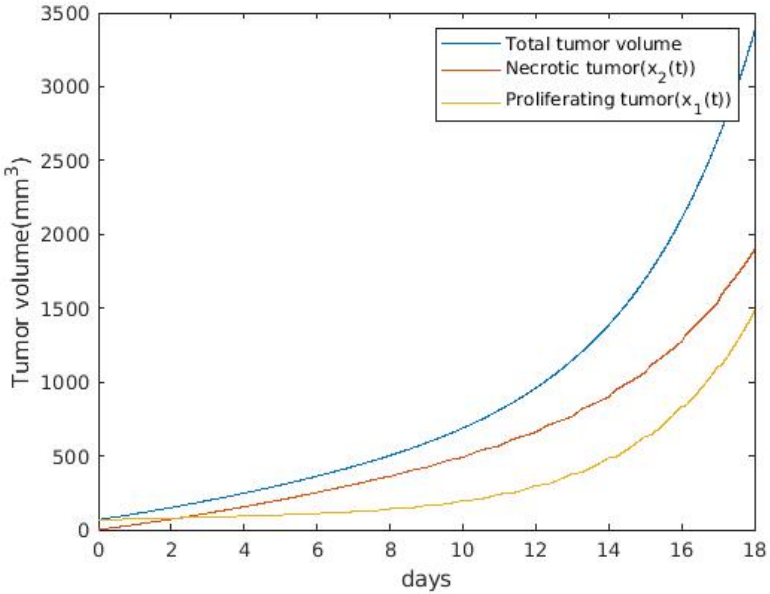


Figure 4: The figure shows the result of fitting the models eq(1)-(3) and eq(4)-(6) to the experimental data obtained from the continuous dosage and single dosage treatment respectively. (a) The experimentally observed average tumor volume (necrotic + proliferating tumor) in five mice (C1-C5) with treatment1 (single initial dosage of 10 mg/Kg) and the simulated total tumor volume. (b) The experimentally observed average tumor volume (necrotic + proliferating) in nine mice (E1-E9) with treatment2 (continuous dosage of 1/18 mg/Kg per day) and the simulated tumor volume.



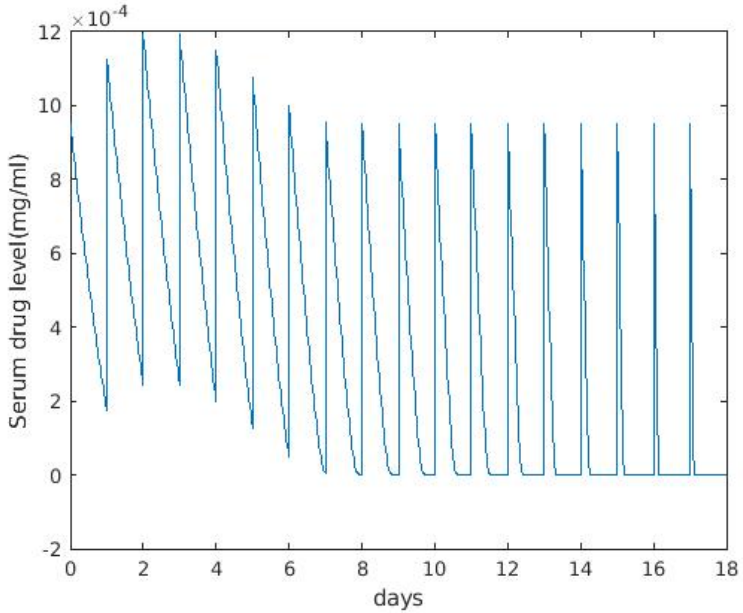


Figure 5: (1) The figure shows the simulation result of the necrotic tumor volume, proliferating tumor volume and the total tumor volume for the continuous treatment. (2) The figure shows the simulation result of the serum drug level in the continuous dosage treatment.

Simulation Results

- Using the identified parameters for the continuous dosage treatment (table(24)(b)) the eigenvalue $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} = 0.1191 > 0$ for $u = \frac{9.5\times10^{-4}}{18}$ and hence by eq(10) it follows that the equilibrium point $(0, \frac{u.K_B}{c-u})$ for the system eq(8)-eq(9) is a saddle node.
- From the calculations it also follows that $ED_{50} + \frac{b-a+n}{a-n}K_B = -0.0825 < 0$.
- It can be shown that given $a - n > 0$ then $a - n - \frac{b.u.K_B}{(c-u).(ED_{50}+\frac{u.K_B}{c-u})} \leq 0$ iff $c.ED_{50} \leq u.(K_B(\frac{b-a+n}{a-n}) + ED_{50})$.
- Since $c.ED_{50}$ is a positive value and $ED_{50} + \frac{b-a+n}{a-n}K_B = -0.0825$, the last inequality doesn't hold for any value of dosage rate u. Hence there is no bifurcation with the given set of parameter values as there is no change in the sign of the eigenvalues of the system eq(8)-eq(9).

- By the theorem(5) there is no limit cycle in the region $\{(x_1, x_3) : x_3 \geq \frac{(a-n)ED_{50}}{(b-a+n)}\}$. Since $\frac{a-n}{b-a+n} < 0$ for the given set of parameters, hence in particular there is no limit cycle in the region $\{(x_1, x_3) : x_3 \geq 0\}$ i.e there is no limit cycle in the positive region.

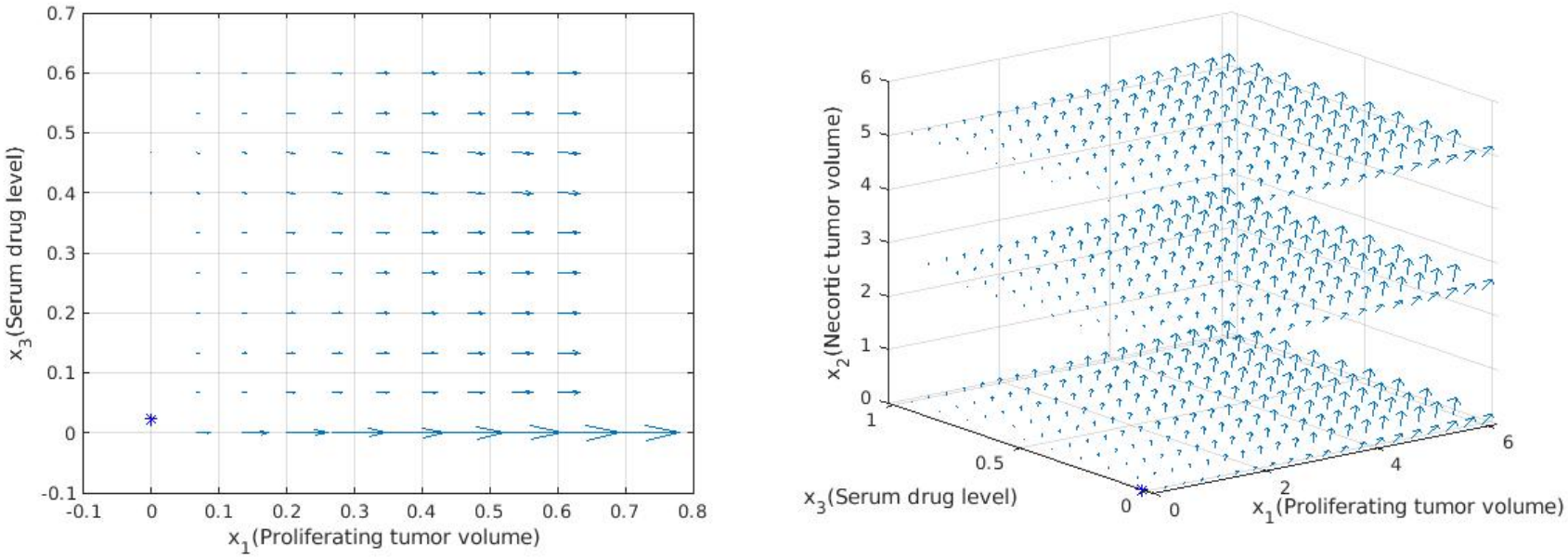


Figure 6: (a) The local vector field for the system eq(8)-eq(9). (b) The local vector field for the system eq(1)-eq(3). The star mark in the plots represent the equilibrium points $(0, u.\frac{K_B}{(c-u)})$ and $(0, 0, u.\frac{K_B}{(c-u)})$ respectively. The parameters used for plotting the vector fields are given in the table(24)(a). Here $u = \frac{9.5\times10^{-4}}{18}$mg/ml representing the average dosage rate for the continuous treatment.

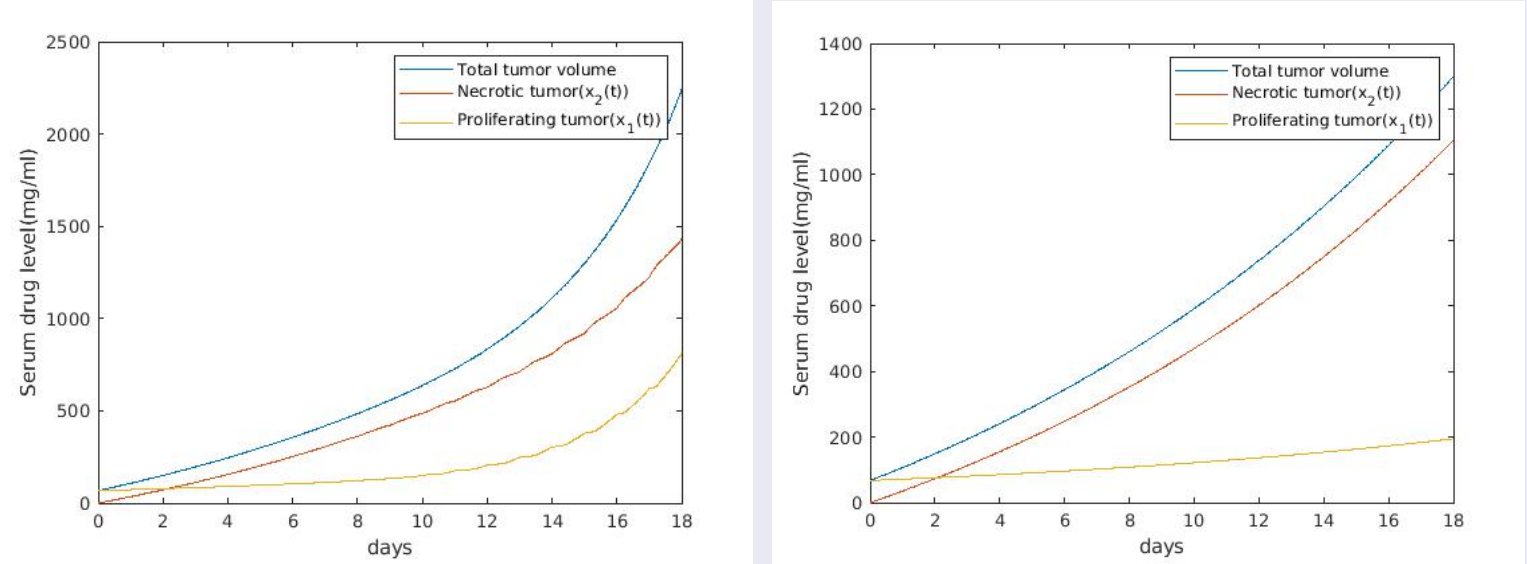


Figure 7: The figure shows the simulation result of the tumor volume for 18 days for the treatment of (a) $1.11 \times 10^{-3} mg/ml$ dosage of drug per day (b) $0.005 mg/ml$ dosage of drug per day.

- The figure shows the results for treatment of (a) $1.11 \times 10^{-3} mg/ml$ dosage of drug per day (b) $0.005 mg/ml$ dosage of drug per day.
- The final tumor volume at the end of 18 days for the two treatments are $2239.39 mm^3$ and $1299.09 mm^3$ respectively.
- Increasing the dosage rate decreases the final tumor volume.

- Comparing with the final tumor volume for the single initial dosage treatment fig(4), it follows that the continuous dosage treatment is much more effective in reducing the tumor volume.
- The total amount of drug used for 18 days in the case of continuous treatment with $1.11 \times 10^{-3} mg/ml/day$ is $0.02 mg/ml$, which is lower than the single dosage treatment of $0.171 mg/ml$.
- The fig(8) shows the simulation results of the tumor volume for 18 days when the continuous dosage of 9.5×10^{-4}mg/ml was stopped at the tth day for $t = 1, 6, 12, 18$.

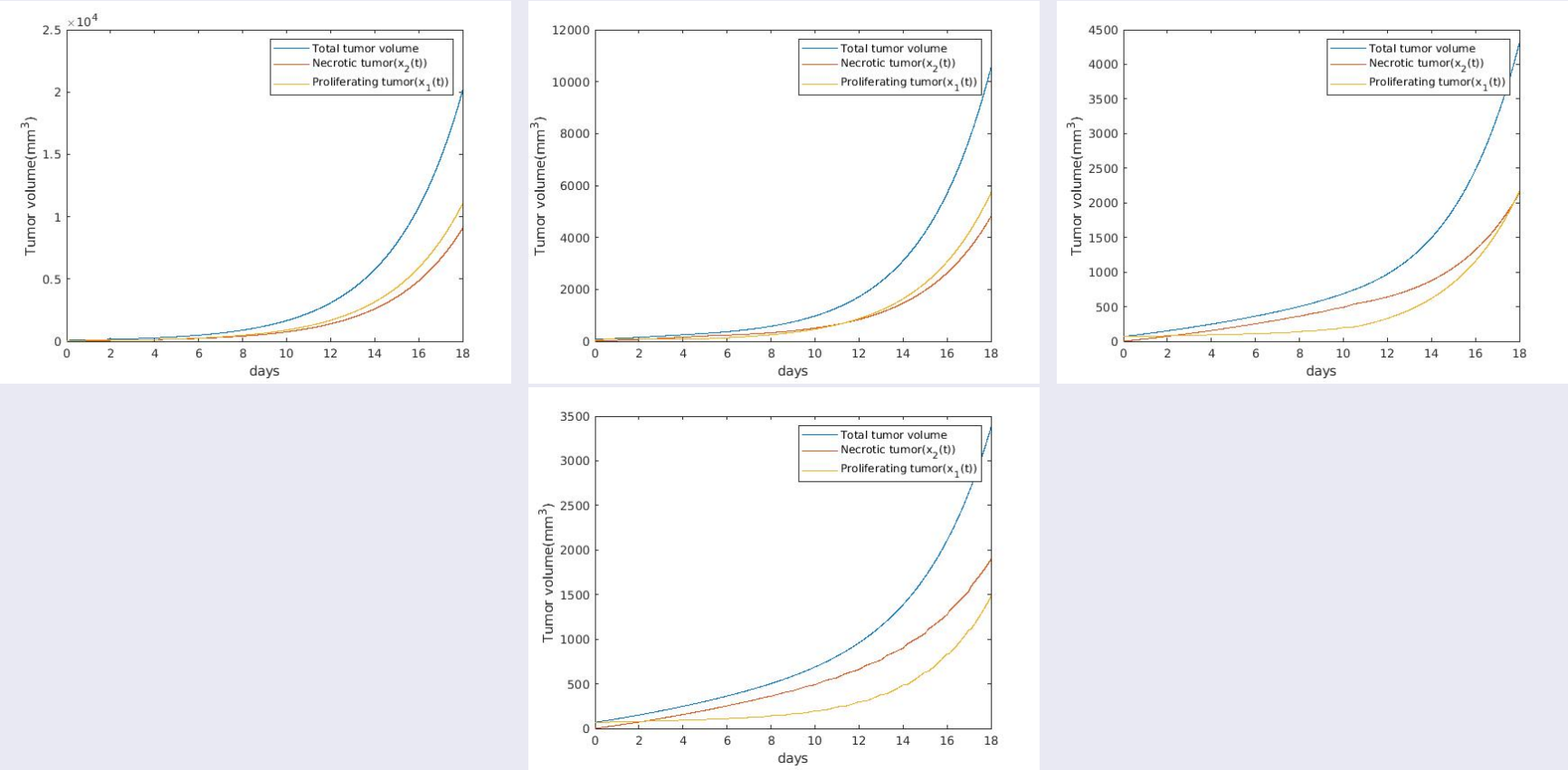


Figure 8: The figure shows the simulation result of the tumor volume for 18 days when the treatment of $1/18 mg/Kg$ dosage of drug per day was given till the (a) second day (b) First 6 days (c) First 12 days and (d) First 18 days.

Simulation Prediction

- By the fig(4) it is clear that the model for single dosage treatment is unable to fit the growth of the total tumor volume as experimentally observed.
- A possible reason for this can be that there is some other mechanism which is effecting the Bevacizumab drug which is hindering it's action with the VEGF molecules, hence a continuous dosage is necessary to maintain it's action.
- Hence the continuous dosage model is able to explain the experimental results in contrast to the single dosage model. Further experiments has to be done on the drug to find this unknown mechanism.

Limitations of the model and Future work

- Literature search for possible mechasims which can hinder Bevacizumab action.
- Incorporate this effect into modeling the tumor growth.
- Check whether the model is able to fit the experimental result for the single dosage treatment.

Coding: Continuous treatment model

disontinuous.m

```
function S= discontinuous(B, d)
tend=[];
Sv1=[];
Sv2=[];
Sv3=[];
T=[B(6)];
x0 = [B(6);B(6);9.5.*10^-4];
for index = 2:numel(d)
t = d(index-1:index);
[t, Sv] = ode45(@DifEq, t, x0);
T=cat(1,T,Sv(end,1));
tend= cat(1, tend, t);
Sv1 = cat(1, Sv1, Sv(:, 1));
Sv2 = cat(1, Sv2, Sv(:, 2));
Sv3 = cat(1, Sv3, Sv(:, 3));
```

```
x0 = Sv(end,:);
if index < 20
x0(3) = Sv(end,3)+ 9.5.*10^-4;
end
end
Sv(end,:);
S=T;

function dS = DifEq(~, x)
ED= 5.* 10^-5;
xdot = zeros(3,1);
xdot(1)=B(1).*x(2);
xdot(2)= (B(1)-B(2)).*x(2)-(B(3).*(x(2).*x(3))./(ED + x(3)));
xdot(3)= -(B(4).*x(2).*x(3))./(ED + x(3)) -(B(5).*x(3))./(0.449 +
x(3));
dS = xdot;
end
end
```

disconfit.m

$Time2 =$

[0; 1; 2; 3; 3.9860417516293 6; 5.11409752306497; 6.01652828969137;
7.04423702860133;7.9967374325748 9;8.97430728152725;
10.0269469601951;11.0295862541263;12.0070175978578;
12.9843796889788;14.0368116098154;14.9890350033472;
15.9910510237844;17.0181364892004;17.9698058618487];
d = [0;1;2;3;4;5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 16; 17; 18];
Sdata2= [0;0;0;0;63.6508437276353;113.389607491590;
162.020329488526;255.465185173787;348.540693603364;
441.739317784831;623.704399079705;717.026139013063;
898.621873052264;1124.41616202032;1438.97690810178;
1708.84663624681;2199.95537053994;2691.18722058495;
3314.64538816087];

```
options = optimset('disp',' iter',' LargeScale',' off',' TolFun', .00001,
'MaxIter',1000000,'MaxFunEvals',1000000);
B0=rand(6,1) * 100;
B= lsqcurvefit(@discontinuous,B0,Time2,Sdata2,[0.2; 0; 0; 0; 0; 0]
,[0.7; 1; 1; 9.5.*10^-6; 0.2; 100], options);
Mfit = discontinuous(B,Time2);
errneg=[0;0;0;0;0;0;0;0;0;0;485;0;475.1;0;968.2;0;1496.5;0;2200.3];
errpos=[0;0;0;0;0;0;0;0;0;0;807.496;0;1166.4;0;3087.5;0;4029.8;0;741.2];
plot(Time2, Mfit)
hold on
errorbar(Time2, Sdata2,errneg,errpos)
hold off
xlabel('days')
ylabel('Tumor Volume(mm^3)')
legend( 'fit','experimental data','Location','Northeast')
```

Coding: Single dosage treatment

singledosage.m

```
functionS = singledosage(B, t)
x0 = [B(6);B(6);0.171];
opt = odeset('MaxStep',0.5);
[T, Sv] = ode45(@DifEq, t, x0);
function dS = DifEq(~, x)
ED= 5.* 10.^-5;
xdot = zeros(3,1);
xdot(1)=B(1).*x(2);
xdot(2)= (B(1)-B(2)).*x(2)-(B(3).*(x(2).*x(3))./(ED + x(3)));
xdot(3)= -(B(4).*x(2).*x(3))./(ED + x(3)) -(B(5) .*x(3))./(0.4409 +
x(3));
dS = xdot;
end
S = Sv(:,1);
end
```

singledosagefit.m

$Time = [0; 2; 3.95000000000000; 5.97500000000000; 8.00000000000000;$
9.97500000000000;12.0000000000000;14;16.0000000000000;
17.9750000000000];
Sdata=[0; 0; 53.6883991429438;323.109886746250;
724.762779308233;1170.37037037037;2056.87174778084;
2722.92623201714;4490.90909090909;6611.44781144781];
$options =$
$optimset('disp',' iter',' LargeScale',' off',' TolFun', .00001,' TolX', 0.0001,$
'MaxIter',100000,'MaxFunEvals',100000);
B0=rand(6,1)*100;
$[B] =$lsqcurvefit(@singledosage,B0,Time,Sdata,[0.2;0;0;0;0;0],
$[0.7;1;1;9.5.*10.^{-6}; 0.2; 100], options);$

```
t = linspace(0, 18, 250);
errneg=[0;0;0;0;508.73;944.29;1644.31;1813.24;3130.5;4180.3];
errpos=[0;0;0;0;905.64;1309.5;295.2;3445.2;6318.1;9300.55];
Mfit = singledosage(B,t);
plot(t, Mfit)
hold on
errorbar(Time, Sdata, errneg, errpos)
hold off
xlabel('days')
 ylabel('Tumor Volume(mm^3)')
legend( 'fit','experimental data','Location','Northeast')
```

References I

[1] Filis Kazazi-Hyseni, Jos H. Beijnen and Jan H.M.Schellens.
Bevacizumab.
The Oncologist, 15:819–825, 2010.

[2] Wassim M.Haddad, Vijaysekhar Chellaboina and Qing Hui.
Nonnegative and Compartmental Dynamical Systems.
Second Edition. Princeton University Press, 2010.

[3] David Csercsik and Levente Kovacs.
Dynamical modeling of the angiogenic switch and it's inhibition by bevacizumab.
Hindawi Complexity, 2019:18, 2019.

[4] Erini Panoilia , Emilie Schindler et al.
A pharmacokinetic binding model for bevacizumab and vegf in colorectal cancer patients.
Cancer Chemotherapy and Pharmacology, 75:791–803, 2015.

References II

[5] Monika Bzowska et al.
Antibody based antiangiogenic and antilymphagiogenic therapies to prevent tumor growth and progression.
Acta biochimica Polonica, 60:263–275, 2013.

[6] Hans-Peter Gerber and Napoleone Ferrera.
Pharmcology and pharmacodynamics of bevacizumab as monotherapy or in combination with cytotoxic therapy in preclinical studies.
American Association for Cancer Research, 65, 2005.

[7] Daniel Andras Drexler, Johanna Sapi and Levente Kovacs.
Modeling of tumor growth incorporating the effects of necrosis and the effect of bevacizumab.
Hindawi Complexity, 2017:10, 1973.