Biological Networks - Simulation

Academic Year 2019-2020 Professors: Luca Marchetti and Federico Reali

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1st September 2020

1 Introduction

In our project we focused on a 2019 paper published on Mathematical Biosciences by Sigal Daniel et al. [1]. The paper aims to establish a mathematical model to study the interaction between different components of the immune system and different cancer cell types. In particular cancer cells are distinguished in cancer stem cells (CSCs) and all other cancer cells (nCSCs): the first ones have the maximum potential for self-renewal and differentiation, whereas nCSCs group all other cancer cells that can have varying degrees of self-renewal and differentiation depending on the specific case. On the other hand the cells of the immune system taken into account by the model are dendritic cells (DCs) and cytotoxic T-cells (CTCs), where both can be specific for either one of the two cancer cell types specified previously.
Other models are available to describe the phenomenon by modelling a homogeneous cancer population; this study, however, aims to take into account the difference between CSCs and nCSCs during the interaction with the immune system, hopefully providing more insights as to why certain therapies might fail in a given situation.
The model was used to investigate different therapeutic strategies and, eventually, to identify the best combination of therapies by optimizing tumor size reduction; in particular the strategies considered were dendritic cell vaccination, T-cell treatment and chemotherapy.

2 Model

In order to build the model 5 main assumptions were made:

1. CSCs self-renew and nCSCs have different degrees of self-renewal depending on the cancer itself. CSCs division may produce two CSCs (symmetric division), one CSC and one nCSC (asymmetric division) or two nCSCs (interconversion); at the same time nCSCs may also revert to CSC because of tumor plasticity.

2. Immature dendritic cells (iDCs) assume antigens from dead or live cancer cells. This process is specific to the type of cancer cell: if a DC consumes a CSC it will present antigens from the CSC, and if it consumes a nCSC it will present antigens from the nCSC. DCs are protected from death during the first interaction with CTCs but won't be protected anymore from further interactions.

3. Mature antigen-presenting dendritic cells (mDCs) present antigens to naive cytotoxic T-cells (nCTCs), inducing them to proliferate and differentiate into activated cytotoxic T-cells (aCTCs); in this state they can kill other cells presenting the same antigen that they have been activated with. They assume that if a CTC is presented with antigen from a CSC then it will only kill CSCs and DCs that present the same CSC antigen.

4. All cells (with the possible exception of CSCs in some cases) die by natural processes.

5. T-helper cells are omitted to reduce model complexity; in reality, however, they have an important role in CTCs activation.

Furthermore, when considering tumor size, 1 mm^3 of tumor was assumed to contain 10^5 cells (either CSCs or nCSCs). The main variables considered in the model are the following: S, P, Ts, Tp, Ds, and Dp, respectively the population of CSCs, nCSCs, activated CSC-specific CTCs, activated nCSC-specific CTCs, mature CSC-specific DCs, and mature nCSC-specific DCs. In addition, we let C to be the concentration of chemotherapeutic agent.
The dynamics of the system are reduced to 7 ODEs aimed at describing the variation overtime of the different cell types considered in the model:

$$
\begin{aligned}
\frac{dS}{dt} &= \alpha_S S + \rho_{PS} P - \rho_{SP} S - \beta_S S T_S - \delta_S S - \Gamma_S S C \\
\frac{dP}{dt} &= \alpha_P P + \alpha_{SP} S + 2\rho_{SP} S - \rho_{PS} P - \beta_P P T_P - \delta_P P - \Gamma_P P C \\
\frac{dT_S}{dt} &= \kappa_{T_S} T_S^n \frac{D_S}{s_{T_S} + D_S} - \delta_{T_S} T_S \\
\frac{dT_P}{dt} &= \kappa_{T_P} T_P^n \frac{D_P}{s_{T_P} + D_P} - \delta_{T_P} T_P \\
\frac{dD_S}{dt} &= \gamma_{D_S} D S - \beta_{D_S} D_S T_S - \delta_{D_S} D_S \\
\frac{dD_P}{dt} &= \gamma_{D_P} D P - \beta_{D_P} D_P T_P - \delta_{D_P} D_P \\
\frac{dC}{dt} &= - e_C C
\end{aligned}
$$

Parameter	Description	Units
α_i	reproduction rate of cell type i	day^{-1}
α_{ij}	production of cell type j through the asymmetrical division of cell type i	$\text{nCSC/CSC} \cdot \text{day}^{-1}$
ρ_{ij}	conversion rate of cell type j to type i	day^{-1}
β_i	death rate of cell type i due to CTCs	$\text{aCTC cells}^{-1} \cdot \text{day}^{-1}$
δ_i	death rate of cell type i due to natural processes	day^{-1}
T_i^n	population of naive CTCs specific to cell type i (constant)	mDCs
κ_i	saturated activation rate of CTCs due to activation by mDCs	$(\text{aCTCs/nCTCs}) \cdot \text{day}^{-1}$
s_i	mDC EC_{50} for CTC activation rate	mDCs
γ_i	maturation rate of DCs due to consumption of cancer cells	$(\text{mDCs/iDCs}) \cdot \text{day}^{-1} \cdot \text{cancer cell}^{-1}$
D	population of iDCs (constant)	iDCs
Γ_i	rate of killing of cell type i by the chemotherapeutic agent	$\text{day}^{-1} \cdot (\mu\text{g/mL})^{-1}$
e_C	elimination rate of the chemotherapeutic agent	day^{-1}

Parameters Γs, Γp, and e_C depend on the particular chemotherapeutic agent, the other parameters are host-specific ones, depending on the cancer type and the tumor micro-environment.
For simplicity the following parameters were considered in all simulations:

$$r_S \equiv \alpha_S - \delta_S, \quad r_P \equiv \alpha_P - \delta_P, \quad \tilde{\kappa}_{T_S} \equiv \kappa_{T_S} T_S^n, \; \tilde{\kappa}_{T_P} \equiv \kappa_{T_P} T_P^n, \; \tilde{\gamma}_{D_S} \equiv \gamma_{D_S} D, \text{ and } \tilde{\gamma}_{D_P} \equiv \gamma_{D_P} D.$$

3 Simulations

We started by replicating the model proposed in the article in order to verify its behaviour. At first we replicated the simplest case proposed, aiming to model the tumor growth in absence of any kind of therapy. In the simulation we adjusted parameters for tumoral cells growth at day 12; this was done in order to reflect the experimental time needed for cancer cells to take root in mice. The experiment was performed firstly inoculating only 50,000 CSCs and in the second case 50,000 nCSCs instead. All the other populations have been set to zero in order to simulate the absence of immune response in nude mice.

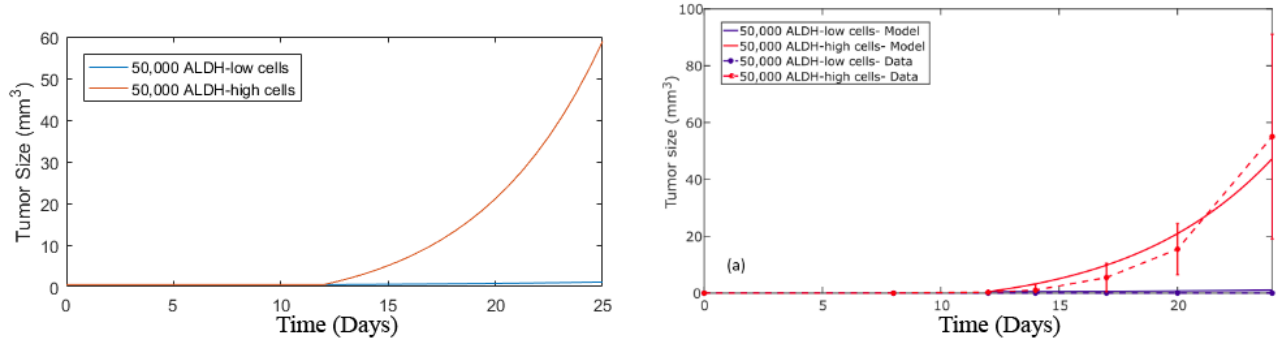


Figure 1: On the left our model, on the right the model presented in the article. Parameters set: $r_s = 0.3$, $r_p = 0.05$, $\rho_{sp} = 0.15$, $\alpha_{sp} = 1.8$, $\rho_{ps} = 5.3 * 10^{-4}$, $\delta_{Di} = 0.2$

Afterwards we proceeded by implementing a more complex model still present in the original article. In this case the effect of different therapies was evaluated. Firstly a control experiment was run with the inoculation of 95,000 nCSCs and 5,000 CSCs in mice, letting the cell populations evolve over time without any kind of treatment being performed. Secondly, 10^6 ALDH-low and ALDH-high specific T-cell were introduced in the previous system in separated simulations on days 20 and 27. Then 6,000 μg/mL of cytotoxic agent was added to the system daily on days 20–24 and on days 27–31, lastly the system was left to evolve over time. Finally the combined experiment was performed simply adding the aforementioned amount of cell and chemotherapy at the same time steps.
In our case, instead of providing all 6,000 μg/mL of cytotoxic agent at the same time each day, we designed our simulation to add a total of 6,000 μg/mL of this agent distributed across the whole day. In this way, the drug introduced in the whole system, will be adsorbed and reach its target gradually over time. We feel like this makes more sense rather than adding the whole dose at the same time as originally proposed by the authors of the paper.

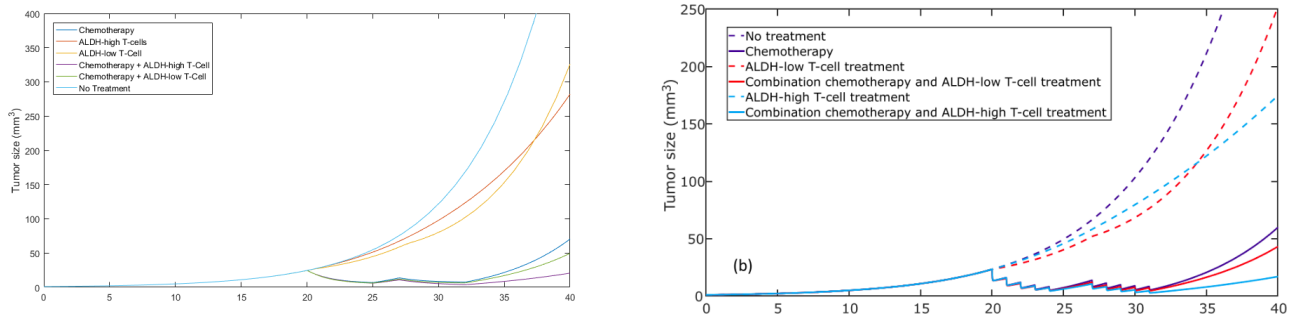


Figure 2: On the left our model, on the right the model presented in the article. $r_s = 0.3$, $r_p = 0.05$, $\rho_{sp} = 0.15$, $\alpha_{sp} = 1.8$, $\rho_{ps} = 5.3 * 10^{-4}$, $\delta_{Di} = 0.2$, $\Gamma_s = 1.4 * 10^{-3}$, $\Gamma_s = 5 * 10{-}3$, $e_C = 50$

We then translated the first deterministic simulation, present in Figure 1 in a stochastic one, keeping the same parameters and amount of cells inoculated. In this way we were able to assess the impact that stochasticity had on the system.

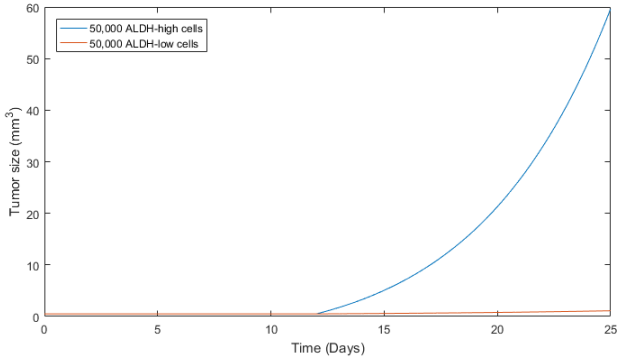


Figure 3: Stochastic simulation with direct method. Parameters set: Parameters set: $r_s = 0.3$, $r_p = 0.05$, $\rho_{sp} = 0.15$, $\alpha_{sp} = 1.8$, $\rho_{ps} = 5.3 * 10^{-4}$, $\delta_{Di} = 0.2$

4 Materials and Methods

4.1 Deterministic Model

To implement our version of the simulation algorithm we began by creating a function that generates the ODEs taking as input a vector of species ("populations") and a vector for the specific constants ("parameters"). Parameters values were directly taken from the paper, in which they were estimated starting from experimental data.
We grouped some parameters according to the simplifications made in the paper, in order to simplify and group terms for specific simulations.
To solve numerically our system we used the Matlab implementation of Runge Kutta 45 algorithm (*ode45* function).

By adjusting the population and parameters vectors, we were able to replicate some of the different conditions tested in the paper.

4.2 Stochastic Model

A simpler model was implemented and simulated through a discretized version of the direct method; this enabled us to perform the least amount of approximations and compare the results with the ones from the deterministic model, giving us further information about the viability of approximations and assumptions made in the deterministic case. In particular the stochastic approach was only applied to tumor growth in absence of any therapies, leading to a much simpler model overall: the only species described by the model are S (CSCs) and P (nCSCs); moreover, all terms correlated with dendritic cells (D_i), cytotoxic T-cells (T_i) and the chemotherapeutic agent (C) were disregarded.
ODEs implemented for the deterministic model were converted into a set of 5 "reactions", each characterized by a stochastic rate c equal in value to one of the parameters proposed in the original model:

1. $S \rightarrow 2S$ $\quad c = r_S$
2. $P \rightarrow S$ $\quad c = \rho_{PS}$
3. $S \rightarrow 2P$ $\quad c = \rho_{SP}$
4. $P \rightarrow 2P$ $\quad c = r_P$
5. $S \rightarrow S + P$ $\quad c = \alpha_{SP}$

When necessary during computations, a volume $V = N_A^{-1}$ was considered to obtain values comparable with the deterministic counterpart.
The simulation itself was performed from day 12 onwards to properly depict the time it took the inoculated cells to take root and start to grow.

4.3 Parameter Estimation

The model proposed by the authors employed many different parameters (20 independent parameters were needed even without considering chemotherapy). This resulted in a high probability of over fitting. In order to avoid this issue they tried to estimate each parameter independently whenever possible.
It wasn't possible to estimate an exact value for all parameters since some of them are host specific or drug specific. Collecting information from literature they were able to set boundaries useful for the actual experiment-specific parameter estimation process.

Host-specific parameter	Value
α_S	$0.14 - 0.76\ \text{day}^{-1}$
α_{SP}	$0.4 - 6\ \text{day}^{-1}$
α_P	$0 - 0.8\ \text{day}^{-1}$
ρ_{PS}	fit as needed to keep %CSCs within 1–10% (see A.3)
ρ_{SP}	$0 - 0.76\ \text{day}^{-1}$
δ_S	$0 - 0.25\ \text{day}^{-1}$
δ_P	$0 - 0.39\ \text{day}^{-1}$
δ_{D_S}, δ_{D_P}	$0.2 - 0.8\ \text{day}^{-1}$
Non-host-specific parameter	Value
β_S, β_P	$6.2 \times 10^{-8}\ \frac{1}{\text{aCTCs-day}}$
$\kappa_{T_S} T_S^n$, $\kappa_{T_P} T_P^n$	$4.5 \times 10^4\ \frac{(\text{aCTCs}/\mu\text{L})}{\text{day}}$
s_{T_S}, s_{T_P}	$2.5 \times 10^4\ \text{mDCs}/\mu\text{L}$
δ_{T_S}, δ_{T_P}	$0.02\ \text{day}^{-1}$
$\gamma_{D_S} D$, $\gamma_{D_P} D$	$0.0063\ \frac{\text{mDCs}/\mu\text{L}}{\text{day-cancer cell}/\mu\text{L}}$
β_{D_S}, β_{D_P}	$6.2 \times 10^{-8}\ \frac{1}{\text{aCTC cells}/\mu\text{L-day}}$
Drug-specific parameter	Value
Γ_S	$7.8 - 14 \times 10^{-4}\ \text{day}^{-1}\cdot(\mu\text{g/mL})^{-1}$
Γ_P	$5.2 - 7.0 \times 10^{-3}\ \text{day}^{-1}\cdot(\mu\text{g/mL})^{-1}$
e_C	$49 - 124\ \text{day}^{-1}$

Figure 4: Boundaries set by the authors for each parameter, exact values were estimated only when there is no case of host specificity or drug specificity

4.3.1 α_S, α_{SP}, α_P, ρ_{PS}, ρ_{SP}, δ_S and δ_P

These parameters are particularly difficult to obtain independently; hence they were all obtained as a group by using a model with as few parameters as possible to fit experimental data. It was not specified which algorithms were employed for fitting parameters, hence it would be hard for us to propose different techniques that could perform better in this setting.

4.3.2 β_S and β_P

β_S was estimated by fitting experimental data to the exponential $f_D(\tilde{T_S}, t) = 1 - e^{-\beta_S * \tilde{T_S} * t}$; $\tilde{T_S}$ represents the concentration of aCTC (activated cytotoxic T-cells) and $f_D(\tilde{T_S}, t)$ is the ratio of dead target cells to total cells at time t. The same process was applied to β_P. Eventually a Welch's t-test found that the two parameters are not significantly different, meaning that we could actually merge the two parameters into one to simplify the model.

4.3.3 K_Ts, K_Tp, S_Ts and S_Tp

K_{Ts} and S_{Ts} were estimated together by fitting experimental data to the relation $f(\tilde{D_S}) = K_{TS}\frac{\tilde{D_S}}{\tilde{S_{TS}} + \tilde{D_S}}$; in particular $\tilde{D_S} = D_S/V_{DS}$ and $\tilde{S_{TS}} = S_{TS}/V_{DS}$ where V_{DS} is the volume considered to be inhabited by DC cells. Given the scarce amount of experimental data for CD8+ T-cells available, data from CD4+ T-helper cells were used instead. Parameters K_{Tp} and S_{Tp} were assumed to equal K_{Ts} and S_{Ts} during numerical simulations.

4.3.4 δ_{TS} and δ_{TP}

These parameters were directly derived from the half-life of activated cytotoxic T-lymphocytes using the relation $\delta_{TS} = \delta_{TP} = \frac{ln(2)}{half-life}$.

4.3.5 γ_{DS} and γ_{DP}

γ_{DS} was obtained by fitting the data to the expression $\frac{\tilde{D_S}}{\tilde{D}} = \gamma_{\tilde{D}S} * \tilde{S} * t$. To obtain the correct value of γ_{DS} it will then be necessary to divide $\gamma_{\tilde{D}S}$ by the volume V_S, considered as the volume inhabited by CSCs. The value of γ_{DP} was considered to be the same as γ_{DS} during numerical simulations.

4.3.6 β_{DS} and β_{DP}

The lack of literature concerning the killing of DCs by CTCs forced the authors to approximate their values to the ones obtained for other parameters. In particular β_{DS} and β_{DP} were assigned the same values as β_S and β_P; doing this we are basically assuming that CTCs kill with similar rates tumor cells and DCs.

4.3.7 δ_{DS} and δ_{DP}

These parameters were estimated by fitting experimental measuraments to the following expression $\frac{D_i(t)}{D_i(0)} = e^{-\delta_{Di} * t}$ where D_i can identify either CSC-specific (D_S) or nCSC-specific (D_P) DCs.

4.3.8 $\Gamma_s$and Γ_p

$\Gamma_s$and Γ_p depend on the drug being used, in this case oxaliplatin and 5-FU. They are expressed with the following formula
$S(t) = S_0 * e^{-\Gamma_s SC}$.
Fitting it with experimental data it is possible to obtain parameter values.

4.3.9 e_c

Represents the drug decay rate and depends on the drug used. For these simulations this parameter was associated to oxaliplatin and 5-FU half life.

4.3.10 Our idea to optimize model parameters

In order to optimize the parameter of our model we propose the following approach. Assuming that we have the corresponding data that are shown in Figure 1 we can use the approach of a multi start non linear least square optimization. We could start by using a ODE solver that works well even in cases of stiff problems like ode15s, already implemented by Matlab. We could use as lower and upper bound of our optimisation the upper and lower bound of the ranges that the authors extracted from the experimental data (Figure 4), and starting by 50 runs the optimisation process, correct accordingly the number of iterations. The main idea is to try to keep the number of iterations limited in order to contain the problem of overfitting. At the same time we want our model to accurately describe our experimental data, hence the number of iterations can't be too low either. A process of trial and error will be obviously needed to adjust the number of iterations to the ideal value; since we were not able to retrieve appropriate experimental data we could not explore this concept any further.
We could use the Matlab implementation for the multi start process (MultiStart). In this way we can generate starting points using the latin hypercube approach to ensure a better spread of points when compared to the standard randomizers. In particular we propose to use the already implemented function lsqnonlin to solve the optimization problem each iteration. Since we didn't have any experimental data available to perform optimization, we simulated them starting from the model itself; afterwards we introduced noise following a standard distribution to better represent "real" biological data. At this point we performed optimization as described previously (Figure 5). Of course this is only the first step of the process, and should be validated in a simulation with a larger number of parameters like the one present in Figure 2.

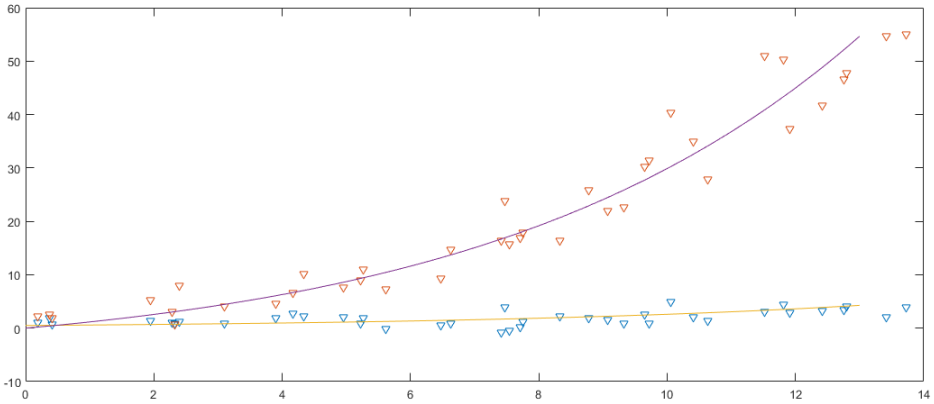
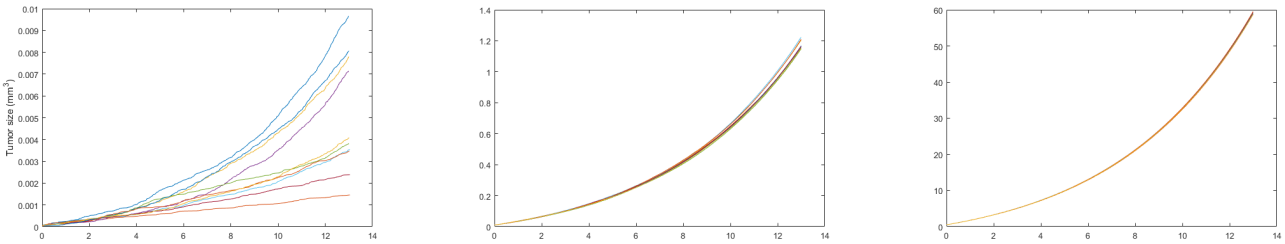


Figure 5: Model simulating tumor size relative to CSCs and nCSCs using parameters obtained through optimization. Experimental setting was the same shown in Figure 1. Triangles represent the points generated to simulate experimental data using the same parameters highlighted in Figure 1.
Optimized parameters are: $r_S = 0.4747$, $\rho_{PS} = 10^{-4}$, $\rho_{SP} = 0.76$, $r_P = 0.0279$, $\alpha_{SP} = 1.493$. These parameters resulted in $SSE = 73.861$

5 Results and Discussion

At first we reproduced in Matlab part of the simulations presented in the paper in a deterministic way (Figure 1 and Figure 2). Overall we obtained very similar results, some of the discrepancies from our work compared to the original one could be derived from the different simulation algorithm employed. In particular our simulations led to higher values of tumor size across the board that, actually, seem to better reflect experimental data. However, since the authors didn't specify what algorithm they relied on, we can't make further speculations.
Thereafter we translated the original system of ODEs in a stochastic setting following the rule of mass action kinetics. In this case we reproduced only the first experiment (Figure 3), due to the complexity that translating saturation terms in ODEs 3 and 4 entailed. In this case the stochastic simulation provided very similar results compared to the deterministic one and results didn't change that much repeating many times the simulation. That's probably due to the high number of cells involved in each time-step leading to a negligible effect of stochasticity. This hypothesis was tested by performing 10 independent simulations of the stochastic model using very different numbers of CSCs as initial condition (see Figure 6). As we increase the number of starting cells it is clear how simulations become more stable and lead to almost identical results; on the other hand only using 5 CSCs as starting point determines differences that can also be very significant.



(a) (b) (c)

Figure 6: Stochastic DM repeated with a starting amount of CSC 5 (a), 1000 (b), 50000 (c) respectively.

Summing up, the deterministic method is a viable approach to simulate our model and basically leads to the same results obtained by the stochastic one, with the advantage of being incredibly more efficient computation-wise. In fact the same simulation takes about 0.08 seconds in a deterministic setting when compared to the 67 seconds the stochastic model takes on average.
Quoting the authors, a stochastic model could be very useful to study the development of the first phases of the tumor when the number of cells is very low. In fact from Figure 6(a) the difference in routes the cell growth can

take is remarkable setting as 5 the initial amount of CSCs.
Additionally, the paper also considered the impact that the different treatments could have on CSC percentage. The results clearly showed that chemotherapy actually leads to an increase in CSC content in the tumor when compared to CSC-specific immunotherapy. This means that, while chemotherapy is the most efficient method to decrease tumor size in the short term, it is also associated to higher tumorigenicity and a higher risk of recurring tumors.
Overall the combination of CSC-specific immunotherapy and chemotherapy seems to be the ideal choice; this combination leads to a significant decrease in tumor size and, at the same time, tries to keep low the amount of CSCs.

5.1 Comments

The model developed in the paper is obviously still in an embryonic stage and must be evaluated on more samples data. As illustrated in the article we could collect patient-specific data by monitoring periodically the tumor size. Starting from this data we suggest to estimate host-specific parameters through optimization (as described previously). Parameters describing treatment response could then be estimated performing an average over documented cases in the literature with similar features to the patient of interest (gender, age, type of tumor). At this point the best treatment to apply could be inferred by the model itself without having to go through a long period of trial and error.
It is also important to consider that the model performs well in describing already developed tumors. As we proved previously stochasticity plays an important role in the initial phases of tumor growth, when the number of cells is limited, rendering this model inaccurate.
However identification of a tumor only occurs in later stages, characterized by a significant cancer biomass. This means that the lack of stochasticity doesn't really affect model applicability. The only exception to this would be describing early-stages growth of metastatic cancers.
Furthermore, we have to point out that the authors applied a deterministic approach to non-continuous values like cell number; this doesn't really affect model performance, probably due to high cell numbers considered. However it is important to point out the fact that this is a further approximation performed by the authors without properly explaining their choice. Moreover, they used an empirical method to estimate parameters ranges to be applied afterwards in fitting experimental data. It is not specified how they choose those parameters to perform their simulations and here we propose a possible solution.

References

[1] Sigal, Daniel, et al. "Mathematical modelling of cancer stem cell-targeted immunotherapy." Mathematical Biosciences 318 (2019): 108269.