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# Cancer Immunotherapy: Planning Report

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# 1 Project Specification

The aim of the cancer immunotherapy project is to use computational models to improve our understanding of the immune mechanisms behind the CBD-IL-12 immunotherapy, ultimately to characterise the responder profile for the treatment.

**Objective 1:** validate the computational model of the immune response developed by previous researchers, and make modifications if necessary

**Objective 2:** identify key biological factors in mice that determine the outcome of the treatment, along with the corresponding threshold that separates CR from non-CR

**Objective 3:** associate each key factor to a corresponding biomarkers in mice

**Objective 4:** (*potential!*) understand how the boundary between CR and non-CR depends on the treatment characteristics (number and frequency of doses, combination with other treatments, etc.)

# 2 Ethical Analysis

The project's ethical foundation rests upon several crucial pillars. Scientific integrity is paramount, underlining the adherence to rigorous methodologies and transparent reporting of findings derived from animal tests on mice for cancer treatment. The project upholds scientific collegiality, fostering collaboration and open dialogue within the scientific community, enabling the exchange of insights and methodologies to advance cancer immunotherapy.

Regarding the subjects and specimens used, the project prioritizes the protection of human subjects by using animal models for initial testing, aiming to minimise potential risks to human health. This approach ensures stringent safety assessments and efficacy evaluations before any human trials. Animal welfare remains a cornerstone, demanding meticulous care and ethical considerations in handling and testing procedures, striving to minimise discomfort and stress for the animal subjects involved.

In the broader context of social responsibility, the implications of this work are profound. The potential to advance cancer immunotherapy represents a significant stride toward addressing a critical health concern, holding promise for enhancing treatment outcomes and potentially saving human lives. The long-term effects signify a potential shift in cancer treatment paradigms, ushering in more targeted and effective therapies.

The impact extends beyond the scientific realm, influencing colleagues, the College, society, and the environment. Collaborators and peers benefit from shared knowledge and advancements, fostering a culture of innovation and progress. The College gains recognition for its commitment to pioneering research with potential life-altering implications. Societal implications are vast, offering hope for improved cancer treatment and outcomes, positively affecting countless lives. Additionally, the project emphasizes environmental responsibility by ensuring ethical use of resources and minimising any potential ecological impact associated with research activities.

Ultimately, this project embodies a commitment to ethical, responsible, and impactful scientific research, rooted in principles of integrity, collegiality, human subject protection, animal welfare, social responsibility, and environmental mindfulness.

### 3 Background

Cancer is a large class of diseases that is the second leading cause of death in the United-States [1]. While the immune system has the potential to target and eliminate cancer cells, cancer often finds ways to evade these natural defenses. [2]. Traditional methods, such as chemotherapy or surgery, rely on using destructive external agents to kill the cancerous cells. However, introducing foreign agents in the body often results in heavy side effects [cite!]. This prompted the development of immunotherapies, a type of treatment aimed at countering cancer’s ability to escape immune detection, which thus has the potential to be less toxic. Several viable strategies exist for immunotherapy. The specific treatment of concern in this project is a combination of cytokine-based treatments and immune checkpoint inhibitors. We first review the general principles behind these strategies.

Cytokine-based therapies rely on the injection of specific cytokines (small proteins that act as signalling molecules during the immune response) to control tumour growth [5]. One of the most promising cytokine thus far is the interleukin-12 (IL-12), that was shown to have potent antitumour effects [6]. While it does not directly affect tumour cells, it mediates the production of other molecules or cells that have a more direct effect [8]. First, it activates the production of tumour-infiltrating cytotoxic cells, mainly  $CD8^+$  [7]. These are a type of T-lymphocytes whose main function is to carry out cytotoxic activity (i.e. killing the malignant cells) after detecting tumoural antigen [4]. Secondly, they induce production of another type of cytokine, called interferon- $\gamma$  ( $IFN\gamma$ ).  $IFN\gamma$  in turn affects the tumour microenvironment by stimulating production of cytotoxic cells [10], reducing angiogenesis [9] and upregulating antigen-presenting pathways within tumour cells [11]. Lastly, IL-12 facilitates T-cell proliferation (including  $CD8^+$ ) by reducing negative regulatory pathways that lead to immunosuppression [12]. It does so by inhibiting the effect of immune checkpoint PD1, following a similar strategy to checkpoint inhibitor (CPI) treatments (the more detailed mode of action is described in the following paragraph). While these three pathways indicate that IL-12 has a very robust antitumour effect, clinical studies demonstrated that systemic injection of IL-12 is exceedingly toxic as it triggers a large immune response throughout the whole body [cite]. These severe treatment-related adverse effects (trAEs) dampened research about IL-12 until a safer, more localised delivery method is found.

The usual partner of cytokine-based treatments are checkpoint inhibitors. To understand checkpoint inhibition, we must first review in more detail the negative regulatory pathways of  $CD8^+$  T-cells activity. The most potent pathway involves checkpoint molecules, either Cytotoxic T-lymphocyte antigen 4 (CTLA4) or programmed cell death 1 (PD1) [13]. Both molecules are membrane protein receptors that act with some delay to exhaust and deactivate T-cell functions after they are stimulated by antigen-presenting cells (APC). Both CTLA4 and PD1 function in similar ways, the main difference being the type of tissues they affect [15]. Although their original function was shown to be prevention of autoimmunity [14], they lead to immunosuppression in the presence of tumours. The idea of inhibiting these regulators to shift the tumour microenvironment away from immunosuppression hence seemed natural, and this is precisely the idea behind CPI treatments. Clinical trials demonstrated positive results in several types of cancers [cite], but performed poorly against immunologically cold tumours, i.e. tumours that do not normally elicit a strong immune response (they escape the immune system very effectively), such as melanoma [cite].

Recent endeavours in this field of immunotherapies led to the development by Mansurov, Ishihara et al. of a new molecule, CBD-IL-12, that demonstrated promising results to treat melanoma [16]. The CBD-IL-12 molecule consists of a collagen-binding protein (or collagen-binding domain, CBD) that is fused onto a IL-12 cytokine. The modified interleukin hence mainly accumulates in collagen-rich regions. As collagen is the main component of cancerous microenvironment [17], this effectively results in an enhanced

delivery method that can achieve high concentration of IL-12 specifically in cancerous microenvironment. In mice tumour-models, this novel molecule achieved a CR rate of up to 67% for melanoma, and 87% for breast cancer when combined with CPI drugs (a mix of both anti-PD1 and anti-CTLA4). While these results are very encouraging, the study showed that such high CR-rates could only be achieved in very specific settings (such as a tumour volume of 70mm<sup>3</sup> upon injection). Different settings (e.g. volume of 150mm<sup>3</sup>) elicited little to no response. This heterogeneity of treatment outcome could not be explained. The first step to improve efficacy of CBD-IL-12-based treatments would thus be to understand better what are the key parameters that control the treatment outcome.

To this end, T. Miyano (2019), under the supervision of R. Tanaka, proposed to use a computational modelling approach to the problem. He developed an initial mechanistic model based on Delay-Differential Equations (DDEs), parameterised by 21 parameters representing various relevant biological factors of a given mouse, such as the tumour growth rate or the degradation rate of IFN $\gamma$  [18]:

$$\begin{aligned}
\dot{g}(t) &= k_1 + k_2[d_{CBD}(t) + d_{12}(t)] - d_1g(t) \\
\dot{c}(t, t - t_d) &= k_3 + k_4g(t - t_d) - d_2c(t) \\
\dot{p}(t) &= k_5 - [d_3 + d_4g(t)]p(t) \\
\dot{v}_l(t) &= k_6 \left[ 1 - \frac{v(t)}{v_{max}} \right] v_l(t) - \left[ d_5 + \frac{\frac{d_6c(t)}{1+s_1p(t)(1-d_{CPI}(t))} + d_7g(t)}{1 + s_2v(t)} \right] v_l(t) \\
\dot{v}_d(t) &= \left[ d_5 + \frac{\frac{d_6c(t)}{1+s_1p(t)(1-d_{CPI}(t))} + d_7g(t)}{1 + s_2v(t)} \right] v_l(t) - d_8v_d(t)
\end{aligned}$$

The five state variables ( $g$ ,  $c$ ,  $p$ ,  $v_l$  and  $v_d$ ) are concentration of IFN $\gamma$ , of CD8+ and of PD1 along with volume of living and dead tumour, respectively. This was motivated by the fact that these are the key players in the immune response, as explained above. The meanings of each parameter are report in the Appendix. The model was investigated by C. Hines, who showed that the model could successfully reproduce experimental data by using a Genetic Algorithm for parameter fitting [19]. However, C. Hines also demonstrated in a subsequent analysis that the model was conflicting with findings from the biologists in two ways. A positive feedback loop, where IL-12-induced IFN $\gamma$  in turn produces IL-12, is missing from the model [20]. Additionally, C. Hines showed that the model outcome does not depend much on the initial tumour volume and treatment characteristics [19], which is opposite to results reported in [16].

While the above mechanistic model mentioned plays a defining role in the computational modelling of the CBD-IL-12 immunotherapy, it is not sufficient by itself. We also require additional tools to analyse it and extract useful data that help us improve the therapy. Regarding analysis of population-level data heterogeneity, a study by Rosenbaum et al. (2020) shows that hierarchical Bayesian inference is of particular relevance [21]. They studied the dynamics of predator-prey systems (as defined by Fussmann et al. [22]), which display two types of behaviour depending on the value of some parameters (either exponential decay or orbits), analogous to the treatment outcome of CBD-IL-12, where a patient can either go into CR or non-CR. By fitting data about a collection of predator-prey system dynamics to a Bayesian model, they were not only able to extract a specific set of parameters for each population; but they could also determine the patterns in parameter value that led to radically different types of behaviour. While this approach looks promising, it suffers from one weakness: very sensitive to the ‘‘Curse of dimensionality’’, according to which the immune-response model is likely to be too large to

be analysed this way. A solution proposed by Vasquez-Cruz et al. [23] to reduce dimensionality of computational models is to use sensitivity analysis. This analysis tool is used to classify parameters by the level of impact they have on the model. By using both Sobol’ method and extended Fourier amplitude sensitivity test (eFAST) in conjunction with parameter fitting, they were able to parameterise a reduced model that could still accurately reproduce experimental data.

## 4 Implementation Plan

In light of the knowledge gathered through the literature review presented above, the proposed plan to fulfill the 4 (3?) objectives is as follows.

### **Task 1.1 – Evaluation of the initial model (already completed)**

This includes numerical stability/bifurcation analysis and sensitivity analysis to find the boundary between CR and non-CR in parameter space. This step is necessary to understand to general design principles that guide the development of a mechanistic model, and can potentially reveal some of the model weaknesses that need to be addressed. The sensitivity analysis is also key to reduce the dimensionality of the problem, since the model is otherwise too large and would result in intractable computations.

### **Task 1.2 – Non-hierarchical model validation (already completed)**

The second step is to verify that the model can be used to perform Bayesian inference, as this will be the core of the analysis. Following the the procedure highlighted by Gelman et al, 2020, in their *Bayesian Workflow* paper [25], the validation consists of three main steps: prior predictive check, fake data check and posterior predictive check. We first validate two versions of the model, with complete- and no-pooling, since they are the basic, simpler blocks that we will use in task 2.2 to construct the full hierarchical model.

The above validation procedure might fail in two ways : either the model cannot produce results (for example, the MCMC chains cannot explore the posterior distribution well), or it produces wrong results. For each outcome, we present below the steps we plan to take to overcome these.

#### **Task 1.2a (fall-back 1) – Validate using Approximate Bayesian Computation (1-2 month)**

In case the MCMC chains do not converge, it would be due to the high complexity of the likelihood function (the mechanistic model of the immune response), a set of five Delayed Differential Equations that cannot be solved analytically. One way to reduce this complexity would be to use Approximate Bayesian Computation (ABC), which is a likelihood-free framework [26]. This approach is a relatively easy to implement method that prevents convergence problems, at the cost of slightly less exact results.

#### **Task 1.2b (fall-back 2) – Validate using simplified likelihood function (2-3 months)**

As suggested in the *Bayesian Workflow* paper, another method to solve convergence issues would be to simplify the likelihood function, for example by using Ordinary Differential Equations instead of DDEs. This is an alternative to ABC, and would require more time to implement as it needs to completely review the tumour model. However, contrary to ABC, it is still a traditional Bayesian inference and hence does not results in approximation errors that can have a non-negligible impact, as highlighted by Robert et al, 2011 [27]

#### **Task 1.2c (fall-back 3) – Modify the model (3 months) [need refo.]**

In case the inference can be run but produces wrong results, the most sensible approach would be to modify the Bayesian model. Using literature about cancer immunology will be crucial to ensure that all the key interactions active during the immune response are correctly translated into the mechanistic model. The first missing pathway that we plan to implement is the positive feedback loop mentioned in Section 3. During this process, it is likely that we will need to use a Genetic Algorithm for parameter fitting. This would be useful to test that the mechanistic model can reproduce the data, without having to resort to a full Bayesian inference, where many additional elements interact together and can make precise diagnostic harder.

### **Task 1.3 – Validation of the hierarchical model (??)**

Once two component of the hierarchical model are validated (the no-pooling and complete-polling models), it is necessary to validate the hierarchical model itself. We will follow the same procedure (include the fall-backs) as for the non-hierarchical models. [anything else to mention here?]

### **Task 2.1 – Extensive numerical analysis of the computational model**

This is the first set of analysis we plan to do on the validated model. First we perform a sensitivity analysis, using the eFAST method as justified in Section 3. This is necessary to know if a given parameter should be set as a free or fixed parameter. This makes practical implementation of the subsequent analysis possible, while minimising the error we introduce. Then, we plan to perform a bifurcation analysis to evaluate the boundary surface in parameter space that separates CR from non-CR. We expect a grid-search bifurcation to be sufficient, since only trans-critical bifurcation can happen (Hopf would not make biological sense... ?).

#### **Task 2.1a (fall-back 1) – ?**

What happens if the model does not capture the bifurcation behaviour? Does it mean that the model is wrong?

### **Task 2.2 – Full Bayesian Inference**

I do not really know what to say here.

### **Task 3 – Biomarker Identification**

Having identified the key parameters that encode the outcome heterogeneity along with their bifurcation point, we will link each of them to a corresponding mouse biomarker. We suspect that the exact methodology will depend on which parameters are selected for this step, but we propose to follow to general methodology for biomarkers identification presented in Section 3, relying on genomic data and statistical tools. We will also make use of the already-existing literature about cancer biomarkers that measure potential response to therapy.

#### **Task 3.a (fall-back 1) – Collect genomic data**

If genomic data is not available, means that we will have to collect it first. It is likely that the project will thus not be finishable, so will only be able to design computational/theoretical framework for biomarker identification without practical implementation.

## **5 Risk Register**

The main risks are :

Risk	Likelihood	Impact	Mitigation Strategy
not finishing the project	dedes	3	4

Table 1: Table of the different risks associated with the project’s objectives

## 6 Evaluation

Below we present a list of the key components of the cancer immunotherapy project, along with a way to verify that they function correctly. The Bayesian encompasses the mechanistic model, as the likelihood function.

### *Mechanistic Model*

The mechanistic model is the core element of the project. Its ‘quality’ can be assessed by two criteria: it should be able to reproduce the data obtained in the lab by Dr. Ishihara (see Appendix), and it should make “biological sense”. To assess the former, we propose to use the standard method of parameter fitting through a Genetic Algorithm (GA), which has already been used in the past for this purpose [20]. This enables us to find a parameterisation of the model that leads to the best simulation, along with the Mean-Squared Error to the true data. This metric can be used to validate that the model can produce data close enough to the experimental time series. To assess the second criteria, we will use a sensitivity analysis (namely, the eFAST method [should justify?]). It enables us to check that the model is sensitive to the same quantity as shown in the lab [wording is terrible here, need to rephrase], for example to the treatment specification or to the initial tumour volume, which is not the case for the current one. [need to describe experiments more?]

### *Bayesian Model*

To validate the Bayesian model, which is an extension of the mechanistic model, we will follow the Bayesian Workflow procedure, as explained on Section 4. Each validation test is evaluated differently:

- **Prior Predictive Check:** we sample 1,000 sets of parameter from the priors and simulate tumour growth for each of them. The 95% credible interval of the resulting collection of time series should contain our expected range of curves we can expect. Evaluation of this step is mostly qualitative.
- **Fake Data Check:** we first need to generate a artificial dataset using known values of parameters, and then fit the Bayesian model to these fake datasets. By comparing the results of the model (i.e. the estimated parameter value in the form of the posterior distribution) to the true values, we can conclude whether the model can successfully perform parameter estimation or not. A possible quantitative approach detailed in [21] involves subtracting the true parameter value from the posterior. This results in a “error” distribution that should theoretically be zero-mean.
- **Posterior Predictive Check:** this is analogous to the Prior Predictive Check, except that parameter are drawn from the posterior distributions instead of the prior distributions. This results in a collection of simulated time series. The median curve can be compared with the true curve obtained in the lab through a difference metric (MSE is the typical error metric for time series).



### Responder Profile

Once the responder profile has been characterised, its accuracy can be evaluated by comparing the predicted treatment outcome against the true outcome on a new batch of cancerous mice. The procedure to inoculate skin cancer, inject the immunotherapy and measure tumour volume should be the same as defined in [16] and [18]. To be robust against all cases, only half of the batch of cancerous mice should be predicted as complete responder according to the responder profile. This ensures that we can collect data about both the rate of true positives and of true negatives.

## 7 Preliminary Results

### 7.1 Sensitivity Analysis

In order to restrict the parameter space for subsequent analysis, and also to understand the main mechanisms behind the immune response, we performed a eFAST sensitivity analysis, which is a variance decomposition method. As it can only decompose variance of a scalar metric, it does not natively support time-series. Hence we chose to apply it to the integral of the tumour growth curve simulated by Miyano’s model. Results are shown in Fig. 1. The total height of the bar represent the fraction of the variance that is imputable to the corresponding parameter. The first observation we can make is that model is mostly sensitive to  $k_6$ ,  $d_1$ ,  $d_7$  and  $s_2$ . However, seen, we can see that the main effect indices (in blue) are almost always negligible compared to the interaction indices (orange). According to a study by Vazquez-Cruz et al. (2012), this is a typical sign of non-identifiability [23] that will significantly hinder Bayesian inference. Additionally, results indicate that the treatment characteristics (labelled  $\mathbf{tem}$ ,  $\mathbf{td}$  and  $\mathbf{ti}$ ) have almost no impact on the treatment outcome, which is conflicting with the results experimentally obtained in the CBD-IL-12 study [16].

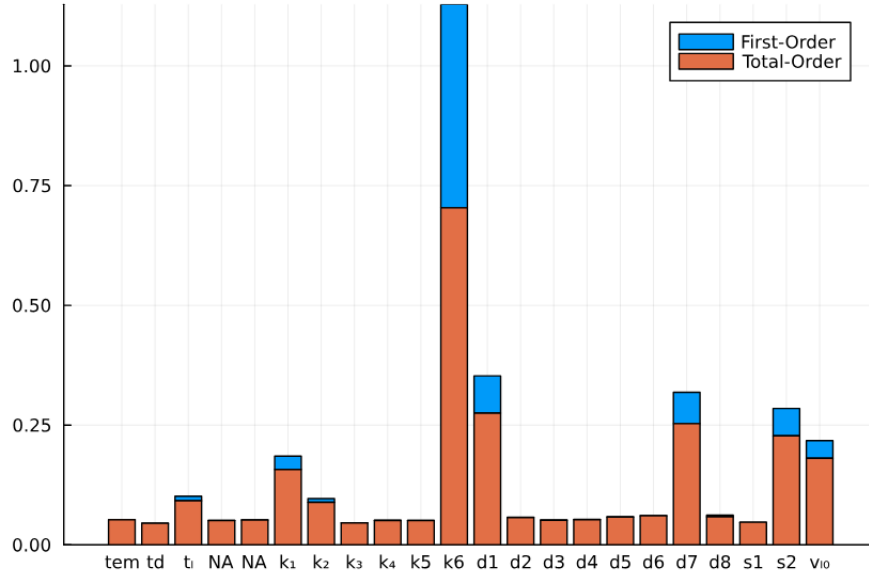


Figure 1: Results of the eFAST sensitivity analysis on the initial model

## 7.2 Numerical Stability Analysis

The very first aspect of Miyano’s model that we wanted to verify was its ability to capture two specific treatment outcome: CR vs non-CR. As these behaviours can essentially be characterised by the fixed-points of the model (if the steady-state behaviour of the model converges to high values of tumour volume, it is a non-CR behaviour, and vice-versa). We opted for a grid-search stability analysis, meaning that we sample regularly-spaced points in parameter space and classify them as either CR

## 7.3 Bayesian Model Validation

In this section we show how the Bayesian model was validated, following the procedure highlighted in Sections X and Y. We focus on a reduced model with only three free parameters,  $k_6$ ,  $d_1$ ,  $s_2$ , which were identified by C. Hines are the most impactful ones [19]. All other parameters of the model were fixed to arbitrary, —“realistic” values.

### 7.3.1 Prior Predictive Check

In this case, we do not have much data on the typical values of the parameters since it is impossible to measure it (we only know that it is a positive number whose typical value is between 0 and 1, as evidenced by [19]), so we aim to design an uninformative prior. Fig. 2 shows a plot of 1,000 tumour growth time-series. Each of them was simulated using a set of parameters drawn from the following prior distribution:

$$\begin{aligned}\ln(k_6) &\sim \text{Cauchy}^-(0, 1) \\ \ln(d_1) &\sim \text{Cauchy}^+(0, 1) \\ \ln(s_2) &\sim \text{Cauchy}^-(0, 1)\end{aligned}$$

As we exponentiate the Cauchy distribution, it means that  $0 < k_6 < 1$ . The blue shade represents the 95% credible interval, and the dark green line is the median growth curve. As we can see, the 95% credible interval is very wide and can virtually contain our expected range of curves, since it ranges from 0 (minimum volume) to 600 (maximum possible volume according to the equation), meaning that they are relatively uninformative priors. The median curve has the shape of the typical growth curve, as observed in the labs. Hence, we can say that the prior distribution is satisfying, as it could explain any potential growth curve while restricting the values of the parameters to a smaller subset of  $\mathbb{R}$ .

## 7.4 Fake Data Check

Each fake growth curve was generated by sampling a value of  $\theta$  from the prior distribution, and then simulating the tumour growth in the same way as for the Prior Predictive Check. However, as biological data is always noisy, we also added some noise to make the fake dataset closer to what we would actually expect from the labs. This was done in two different ways, resulting in two distinct datasets. For dataset A, we simply added a white standard Gaussian noise to the simulation. For dataset B, we use added white noise to the log of the simulated curve. The generation process is summarized in Table 2, where  $x_*$  denotes a noiseless data point. The reason for using two different noise generation is that we observed, in the experimental data from the labs, that data points are usually more dispersed when they have a high value, suggesting an exponential relationship.

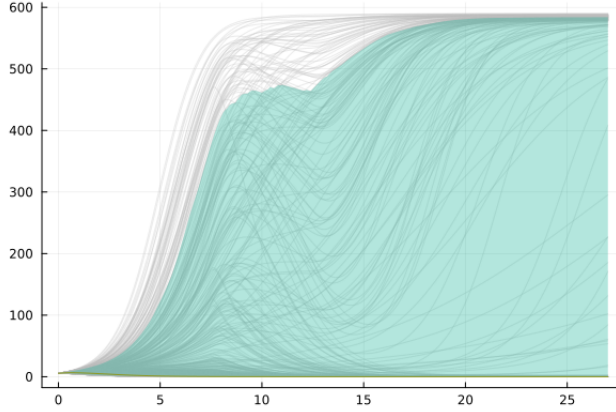
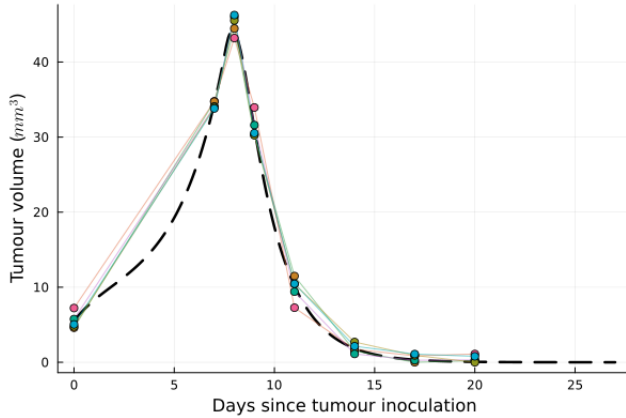


Figure 2: ODE solution for 1,000 parameter values sampled from the prior ( $\theta \in \mathbb{R}^1$ )

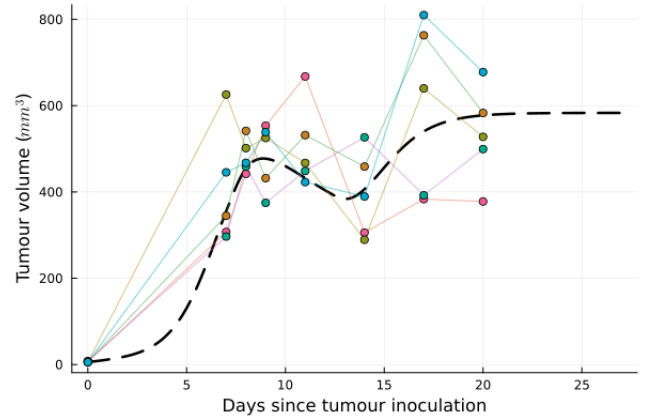
Additionally, each dataset contains 10 time series. Fig. 3 plots the fake data points against the original curve. For clarity, only 5 time series, selected at random, were shown. *Results*

Table 2: Summary of the generation process for the two datasets A and B

Dataset	Generation Process
A	$x_A = x_* + \mathcal{N}(0, 1)$
B	$x_B = x_* \times e^{\mathcal{N}(0, 0.3)}$



(a) Dataset A



(b) Dataset B

Figure 3: Plot of the fake data points (colored lines and scatter plot) along with the original growth curve (dashed line)

Before checking if the estimated values match the true ones, we first assess convergence of the MCMC chains. The R-hat values are reported in Table. 3 as the average R-hat value across the 3 parameters. It must be noted that in this case, for a given inference, some chains get trapped while some are well-mixed. In that case, the R-hat calculation excludes the trapped chains. This is indicated in the Tables

by reporting the number of chains used to calculate R-hat (out of the 5 chains). If none of the chain converged, we simply report N/A. Looking at Table 3, we can hence conclude that the chains did not

Table 3: Assesment of convergence for the MCMC chains for uninformative priors (3 free parameters)

Data set	Pooling Type	R-hat diagnostic	Number of Chains	Convergence
C	None	116.08	N/A	No
	Complete	298.67	N/A	No
D	None	1.092	3/5	Close to
	Complete	3.11	N/A	No

converged, meaning that the model cannot make inference with  $\theta \in \mathbb{R}^3$  and uninformative priors. To further diagnose the model, we performed another set of inferences, except that the priors where highly informative:

$$\begin{aligned}\ln(k_6) &\sim \text{Cauchy}^-(\theta_{k_6}, 1) \\ \ln(d_1) &\sim \text{Cauchy}^+(\theta_{d_1}, 1) \\ \ln(s_2) &\sim \text{Cauchy}^-(\theta_{s_2}, 1)\end{aligned}$$

where  $\theta_x$  represent the true value of parameter  $x$ . Convergence of this new set of inferences is shown in Table 4. As we can see, convergence of the MCMC chains are still very poor, even with highly informative priors centered on the true parameter values.

Table 4: Assesment of convergence for the MCMC chains for uninformative priors (3 free parameters)

Data set	Pooling Type	R-hat diagnostic	Number of Chains	Convergence
C	None	18.15	N/A	No
	Complete	45.96	N/A	No
D	None	1.505	3/5	Close to
	Complete	1.014	2/5	Yes

It might be objected that Cauchy distributions are by definition not too informative since a non-negligible portion of their mass stretches well beyond their standard deviation, contrary to normal distributions. This hence motivated us to perform one last fake data check, using the normal priors shown below to be even more informative:

$$\begin{aligned}\ln(k_6) &\sim \mathcal{N}^-(\theta_{k_6}, 0.3) \\ \ln(d_1) &\sim \mathcal{N}^+(\theta_{d_1}, 0.3) \\ \ln(s_2) &\sim \mathcal{N}^-(\theta_{s_2}, 0.3)\end{aligned}$$

Convergence results are shown in Table. 5. The main result is that chains converged or were close to convergence only for dataset D, showing that a log-normal transformation is key to make exploration of the posterior easier to perform. Whilst the overall convergence rate is still very low given the informative normal priors, this series of fake data checks for the case of three free parameters highlighted the key role of the log-transformation.

Table 5: Assesement of convergence for the MCMC chains for uninformative priors (3 free parameters)

Data set	Pooling Type	R-hat diagnostic	Number of Chains	Convergence
C	None	7.387	N/A	No
	Complete	39.17	N/A	No
D	None	1.066	3/5	Yes
	Complete	1.111	5/5	Close to

**7.5 Switchng to Approximate Bayesian Computation**

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