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, utlimately 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 treaments, 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-State [1]. While the immune system has the potential to target and eliminate cancer cells, cancer often finds ways to evade hese 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 stratgeies.

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-γ (IFNγ). IFNg 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.

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. tumour 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 collegen is the main component of cancerous microenvironment [17], this effectively results in an enhanced delivery method that can achive 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^3 upon injection). Different settings (e.g. volume of 150mm^3) 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 treaments 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 developped 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 IFNγ [18]:

$$
\begin{aligned}
\dot{g}(t) &= k_1 + k_2[d_{CBD}(t) + d_{12}(t)] - d_1 g(t) \\
\dot{c}(t, t - t_d) &= k_3 + k_4 g(t - t_d) - d_2 c(t) \\
\dot{p}(t) &= k_5 - [d_3 + d_4 g(t)]p(t) \\
\dot{v}_l(t) &= k_6 \left[1 - \frac{v(t)}{v_{max}}\right] v_l(t) - \left[d_5 + \frac{\frac{d_6 c(t)}{1+s_1 p(t)(1-d_{CPI}(t))} + d_7 g(t)}{1 + s_2 v(t)}\right] v_l(t) \\
\dot{v}_d(t) &= \left[d_5 + \frac{\frac{d_6 c(t)}{1+s_1 p(t)(1-d_{CPI}(t))} + d_7 g(t)}{1 + s_2 v(t)}\right] v_l(t) - d_8 v_d(t)
\end{aligned}
$$

The five state variables (g, c, p, v_l and v_d) are concentration of IFNg, 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 successfuly 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 IFNg 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 is very sensitive to the "Curse of dimensionality", and 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 model is to use sensitivity analysis. These 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 conjuction with

parameter fitting, they were able to parameterise a reduced model that could still accurately reproduce experimental data.

A paragraph for litt review about cancer biomarker identification and already existing ones.

4 Implementation Plan

In light of the knowledge gathered through the litterature 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)
First we familiarise with the initial mechanistic model of the immune response developped by Takuya. This includes numerical stability/bifurcation analysis, 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 at 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. For each step, the paper also provides a list of potential methods to improve the model if it fails the corresponding test. The Bayesian analysis relies on using a hierarchical model, however we propose to first validate simpler, non-hierarchical model twice: with complete- and no-pooling. [Explain why! maybe cite as well]

Task 1.2a (fall-back 1) – Validate using Approximate Bayesian Computation (1-2 month)
The above validation procedure might fail in two ways : either the model produces wrong results, or it cannot even produce results (for example, the MCMC chains cannot explore the posterior distribution well). In case the latter happen, it would be due to the high complexity of the likelihood function (the mechanistic model of the immune response), a set 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 would enable us to prevent the inference from running into 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 approxmation 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.]
The two fall-backs described above are mostly usefull to address convergence issues, but may not help

much if the model produces wrong results. In this case, the most sensible approach would be to modify the Bayesian model (either the priors or the likelihood, depending on which test was not passed). Using litterature about cancer immunology will be key, as is especially suggests that a key feedback loop is missing in the current model [cite!!]. During this process, it is likely that we will need to use a Genetic Algorithm for parameter fitting. This is especially 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.

Task 1.3 – Validation of the hierarchical model (??)
Once two component of the hierarchical model are validated (the no-pooling and complete-pollingn 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 numerial analysis of the compuational 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 litterature 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 asessed 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. Describe here with more detail, but nothing else to be explained.

Responder Profile

lorem ipsum

7 Preliminary Results

7.1 Sensitivity Analysis

In order to restrict the parameter space for subsequent analysis, and also to understand to 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 nateivly 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 `tem`, `td` and `ti`) have almost no impact on the treatment outcome, which is conflicting with the results experimentally obtained in the CBD-IL-12 study [16].

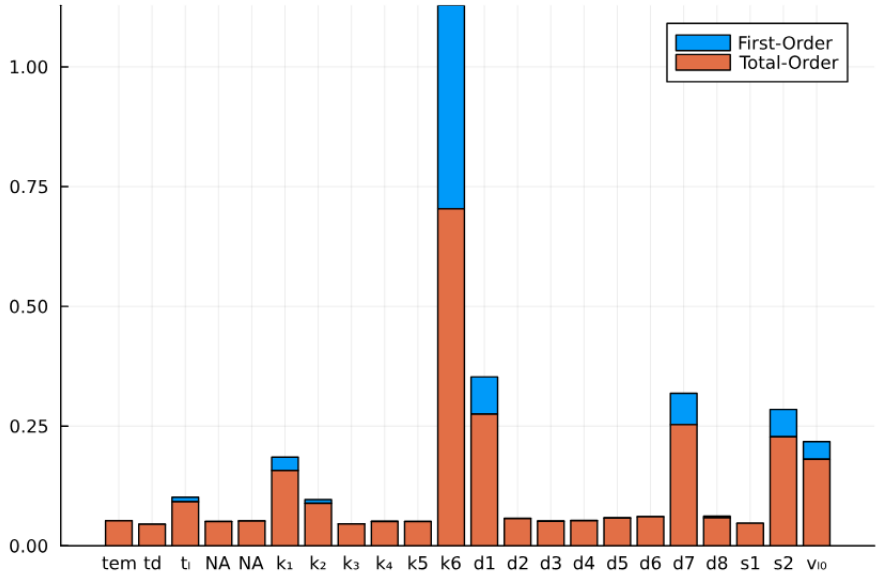

Figure 1: Results of the eFAST sensitivity analysis on the initial model

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 reguarly-spaced points in parameter space and classify them as either CR

7.3 Bayesian Model Validation

7.4 Switchng to Approximate Bayesian Computation

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