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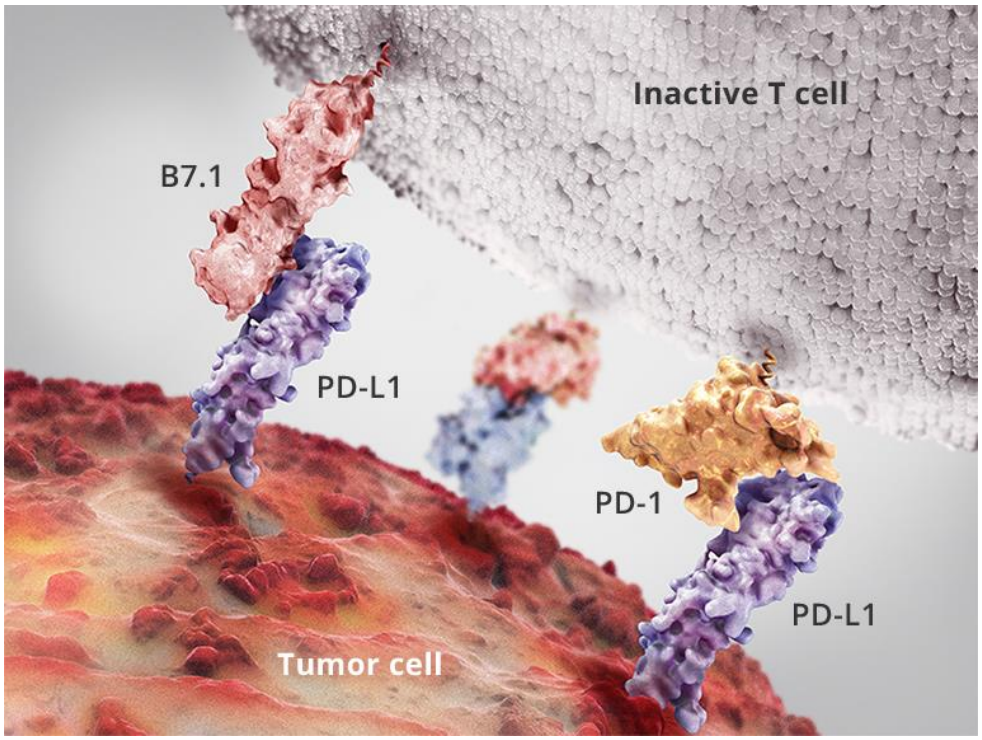
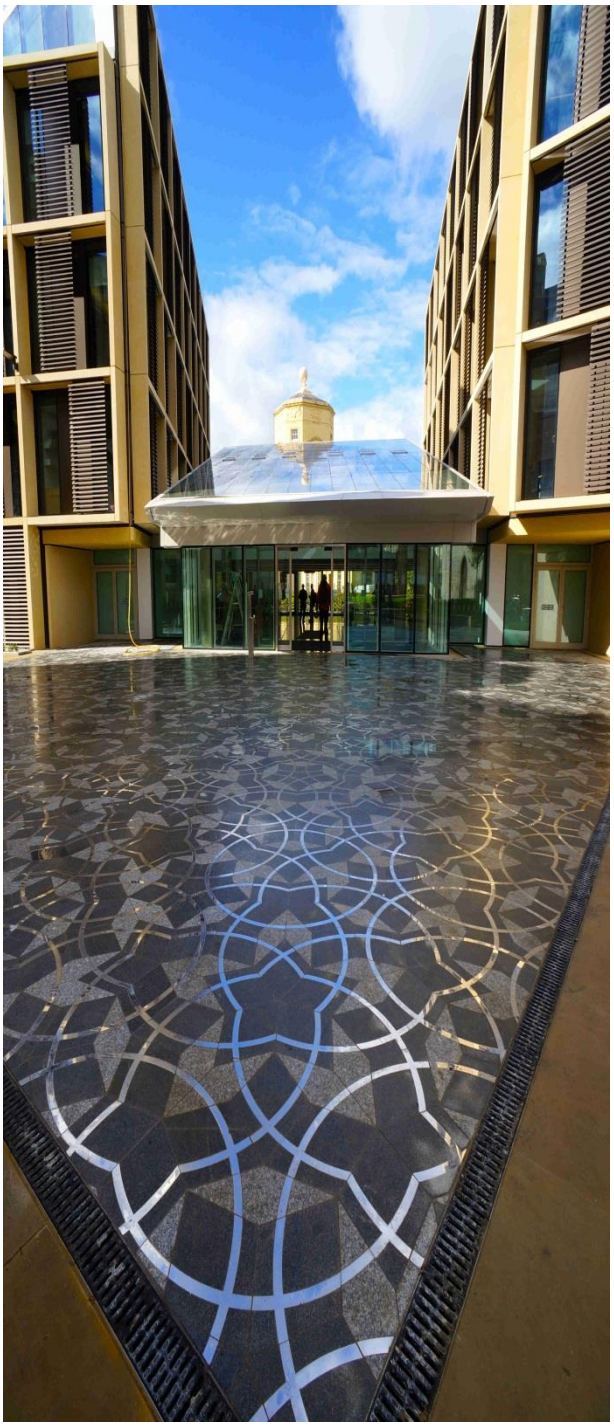


Characterising the behaviour of systems with parameter distribution

Ana Osojnik

UNIVERSITY OF OXFORD

AstraZeneca



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1. Introduction

Motivation

Cancer is a deadly disease that 1 in 2 people in the UK will get in their lifetime. It starts when gene changes make one cell or a few cells begin to grow and multiply in an uncontrolled way. These cells are called tumour cells, and have the potential to invade or spread to other parts of the body, forming secondary tumours called *metastases*. Despite being toxic and carcinogenic, radiation and chemotherapy are widely accepted as two of three standard treatments of care for cancer, with the third being invasive surgery. The metastatic nature of cancer makes it difficult for the disease to be controlled by these treatments since, for example, tumour recurrence remains the leading cause of mortality in patients receiving radiotherapy. Therefore, there is an urgent need for new and more effective treatments.

The heterogeneity of tumour responses to immunotherapy treatments emphasizes that better understanding of the mechanisms governing tumour-immune interactions is needed

New promising tools are immunotherapy treatments, now starting to be approved for clinical use in the UK. As a pharmaceutical company, AstraZeneca is committed to delivering such therapies. The difficulty they are facing is the heterogeneity of responses to treatment, as tumours shrink in some subjects and not in others. This emphasises the importance of gaining an increased understanding of the mechanisms forming the basis of these treatments. Due to the complexity of interactions in the tumour micro-environment, obtaining sufficient understanding has proven difficult, and our aim is to make use of mathematical modelling to gain more insight into the dynamics of tumour growth.

The cancer-immunity cycle

In the last 20 years, evidence has accumulated that our immune systems can recognize and eliminate malignant tumours. The cancer-immunity cycle describes the mechanisms by which this occurs. Mutated tumour cells are coated with substances called tumour-specific *antigens*, which make them distinguishable from normal cells. Such antigens are loaded onto the surface of cells called antigen-presenting cells, which travel through blood from the tumour to lymph nodes. There they interact further with immune cells to activate them and orchestrate a powerful immune response. For example, T cells become activated if they recognise these antigens through their receptors, and receive an additional stimulatory signal through their co-receptors. Activated T cells then divide further to build up in numbers, and travel to the tumour site, where they infiltrate the tumour and scan surrounding tumour cells until they find their target tumour cells to kill. The same antigen which activated the T cell must be present on the surface of the tumour cell, and again a co-stimulatory signal must be transmitted between the interacting cells for the T-cell-programmed death of the tumour cell to occur.

Immune checkpoint therapy

The three stages of immunoediting set foundations for understanding the dual host-protective and tumour-promoting actions of immunity

In cancer patients, the cancer-immunity cycle does not perform optimally, enabling tumour cells to avoid attack. This process can be described via the three stages of immunoediting:

- **Elimination**: Tumour cells are destroyed by a competent immune system.
- **Equilibrium**: A tumour population which manages to survive immune attack may undergo division and editing, but remain in a state of immune-mediated dormancy.
- **Escape**: Immune-resistant tumour cells begin to grow progressively, become clinically apparent, and establish an immunosuppressive tumour micro-environment.

Escape from immune surveillance often occurs due to negative co-stimulatory signals transmitted through co-receptors during cell interactions in the lymph nodes or in the tumour bed. In a healthy organism, the step in the cancer-immunity cycle where this occurs (a checkpoint) plays the role of preventing over-inflammation but, in the case of cancer, they can promote its progress. These findings form the basis of types of immunotherapy called immune checkpoint therapies, which attempt to block the binding of immunosuppressive co-receptors, such as PD-1/PD-L1 at the tumour site or CTLA-4 in

the lymph nodes (see Figure 1). By targeting the immunosuppressive step, it is hoped that these therapies can reinitiate a self-sustaining cycle of cancer immunity, enabling it to amplify and propagate, but not so much as to generate unrestrained autoimmune inflammatory responses.

Immune checkpoint therapy attempts to reinitiate a self-sustaining cycle of cancer immunity by blocking immunosuppressive mechanisms, which have evolved to promote cancer

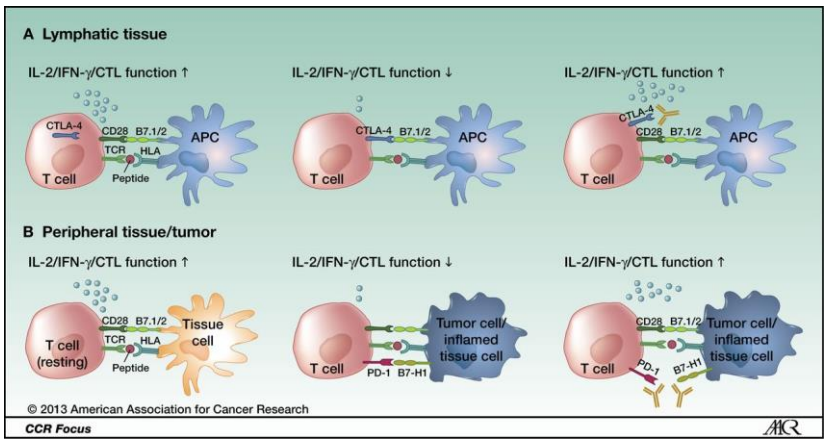


Figure 1: Immune checkpoint therapy via blockade of receptors CTLA-4, or PD-L1/PD-1

Glossary of terms

- Metastasis: spread of cancer to a different part of the body from where it started
- Antigen: a molecule capable of producing an immune response in a host organism
- T cell: a type of white blood cell that plays a central role in cell-mediated immunity
- Immunoediting: a dynamic process that consists of immune eradication of a tumour and its progression
- Bifurcation: sudden appearances of qualitatively different model solutions when some model parameter is slightly varied
- Steady state: state of a dynamical system in which variables do not vary with time
- Phase portrait: a graphical representation of the trajectories of a dynamical system in a coordinate system with axes being the values of system variables
- Posterior distribution: a description of a set of possible values in terms of their probabilities after taking into account the relevant data

Experimentally driven models of tumour response to immune checkpoint therapy in mouse experiments do not capture the full range of responses well

In mouse experiments conducted by AstraZeneca, immune checkpoint therapies resulted in a dichotomous response from the tumour as shown in Figure 2; sometimes the tumour is seen to shrink (or even get eradicated), while there are also situations where the tumour grows. AstraZeneca fit an experimentally driven mathematical model for tumour volume to this dataset. This involves formulating an equation for tumour volume that depends on time and some physical parameters. Values of parameters are chosen so that the difference between the time-dependent solution to the equation and the data is as small as possible. New tumour volume data are also simulated using the model, with parameters slightly perturbed from their fitted values to account for subject variability. The resulting range of data fails to capture the separation of response (shrinkage) vs no response (escape) to treatment, which suggests a better model is needed.

Our aim is to develop a more mechanism-based model of tumour-immune interactions. The desired key characteristic of the model is allowing the switch between the three typical behaviours observed during immunoediting, i.e. elimination, equilibrium, and escape. We hypothesize that the propagation through these stages may be represented by *bifurcations* in a model of immunoediting; in other words, if we represent immunotherapy and variability between subjects through changes in parameter values, then the model should allow a large qualitative change in behaviour when these are varied across a threshold representing, for example, the transitions between tumour escape and eradication that are seen in the data.

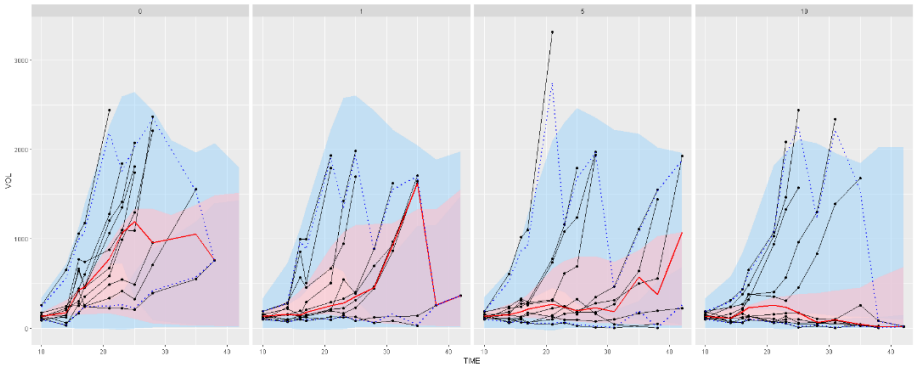


Figure 2: Graph showing tumour volume of different mice (black) vs time at increasing treatment dosages (grey), the range of medians of simulated data sets of tumour volume (pink region), and their 95% confidence interval (blue region).

2. Mathematical model

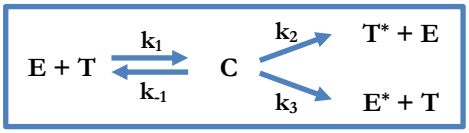
There are a large variety of models of tumour-immune interactions available. We choose one of the simplest models (Kuznetsov et al. [1]) that admits immunoediting behaviour. The model describes the interactions in a fixed region representing the tumour site, of five different cell species, which are:

A conjugate is a binding of a tumour and effector cell, when they are exchanging signals; these determine the outcome of their interaction, such as tumour cell kill, effector cell inactivation or no damage

- **effector cells** (E): activated immune cells, free to bind to tumour cells and kill them,
- **tumour cells** (T): free to bind to effector cells,
- **E-T conjugates** (C): interacting tumour and effector cell,
- **inactivated effector cells** (E^*): inactivated through interaction with tumour cells,
- **lethally hit tumour cells** (T^*): programmed for death by effector cells.

Kinetic scheme

The differential equation model is based on the following kinetic scheme, which describes the competition between tumour and effector cells as observed in vitro.



The model assumptions are that when tumour and effector cells interact through their receptors, they temporarily form structures called conjugates. As a result of the interaction within a conjugate, the tumour cell can either be programmed for death with the effector cell staying intact, or the effector cell can become inactivated while the tumour cell remains undamaged. The rates of change of species due to such kinetics are modelled as proportional to concentrations of respective "reactant" species.

Cell growth, death and migration

The model incorporates a simple kinetic scheme and additional cell growth, death and migration terms

In addition to kinetics, the model includes cell proliferation, migration, and death. The growth of tumour cells is assumed to be logistic, so that growth is exponential for small tumour populations and, as the number of cells gets larger, it slowly approaches a maximum size. Tumour cells are assumed not to migrate out of the region. The main source of effector cells is assumed to be their migration from the lymph nodes, which is modelled as a baseline constant influx rate and a tumour-stimulated influx rate due to an increased immune response. The latter is assumed proportional to the number of conjugates (interacting effector cells), and inversely proportional a linear term in T, which implies an influx rate saturation as the number of tumour cells in the region gets large. This assumption accounts for limitations in the rate of transport through blood vessels to the

tumour site. Lastly, per capita death rates of effector cells, their inactivated counterparts, and lethally hit tumour cells are all assumed constant.

3. Model analysis

Quasi-steady-state assumption

A *quasi-steady-state assumption* (QSSA) is normally applied to systems where one component reacts much quicker than another. The disparity in the timescales means that we can treat this component as if it were in a steady state; this means it varies parametrically over the longer timescale on which the other components vary significantly. In [1] the model is simplified through the quasi-steady-state assumption, since the time scale of formation and dissociation of conjugates (minutes to hours) is shorter than multiplication and migration of effector cells into the tumour region (tens of hours).

Assumptions about parameter sizes enable mathematical verification of the quasi-steady-state assumption

We challenge this assumption by revisiting the underpinning biological assumptions. It is known that effector cells are constantly scanning and interacting with tumour cells in order to locate their target cells, but a very small proportion of these interactions result in tumour cell death or the inactivation of effector cells. This translates into the assumption that per capita rates k_{-1} and k_1E_0, where E_0 is the typical number of effector cells, are much larger than k_2 and k_3. We can also translate the assumption of growth, death or migration occurring on a much slower time scale than formation/detachment of conjugates to supposing per capita rates associated with these effects are of similar size as k_2.

We are then able to choose a small parameter ε as the ratio of k_2 and $k_{-1}+k_2+k_3$ that appears in a scaled version of the model. By exploiting the smallness of this parameter, we able to make mathematical approximations, and reduce our system of model equations in a systematic way. The resulting system does indeed reach quasi-steady behaviour, but this is not a valid assumption during the pre-steady-state period, when system dynamics are predominantly governed by fast kinetics of conjugate binding.

Long-term model behaviour

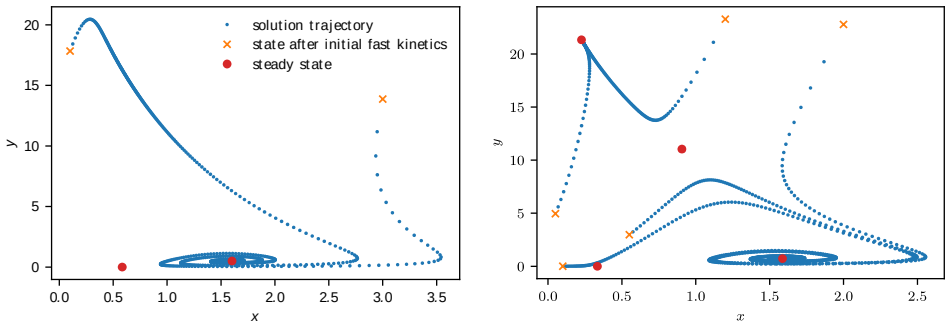


Figure 3: Graphs showing time-dependent paths of system solutions for different initial states in phase portraits of scaled numbers of tumour cells (y) vs effector cells (x) in different parameter regimes.

When parameters are varied, model solutions are descriptive of immunoediting in long-term

Next we investigate how the system behaves in the long term. The types of behaviours we observe are attraction with possible oscillations towards a steady state without a tumour (as seen in Figure 3a) or with a small tumour that is controlled by the immune system. In addition, some parameter regimes with certain initial conditions allow tumour escape, which is characterised by a large-tumour steady state (as seen in Figure 3b). We can thus say that our system describes the stages of immunoediting, as desired. The rich behaviour is a result of bifurcations that occur when varying the parameters, such as for instance the constant per capita rate of influx of effector cells (σ), effector cell per capita death rate (δ), or the ratio of tumour killing versus effector cell inactivation (μ).

In simulations, we observe that by carefully changing μ for example, we can change system behaviour in a way that tumour escape becomes impossible. We can thus model immunotherapy treatment as a change in therapy-relevant parameters. Small parameter

changes due to heterogeneity of tumours can also result in a large behavioural change if close enough to the bifurcation region of the parameter space, which could explain large differences in tumour responses to treatment observed in the experiments.

Parameter estimation

A crucial step in evaluating how well the mechanistic model describes the experimental data is the resulting estimation of the model parameters. We test a method called Approximate Bayesian Computation (ABC), which enables estimation of the *posterior distribution* of the parameters given incomplete and scarce data. From the posterior, we can then infer the most probable parameter value. We use synthetic data of the number of tumour cells only, obtained by solving the model at a set of consecutive times. We first estimate σ, μ, and δ while fixing other parameters, and afterwards perform estimation on the full parameter set.

Parameters for tumour growth can be much more readily estimated from synthetic tumour data than those that determine the dynamics of effector cells

We find that parameter estimation is a much easier problem for a smaller data set, where, despite using only tumour data, parameters defining the behaviour of effector cells can be well estimated; σ and μ in particular. On the full parameter set we find that the parameters with the smallest uncertainties are the per capita tumour growth rate (α) and the inverse of the carrying capacity (β). Larger uncertainties in other parameters suggest difficulties in estimation of the full parameter set for this model.

The uncertainty level associated with a certain parameter gives some indication of how effective a possible immunotherapy that changes this parameter would be as predicted by the model, or how influential variability in this parameter over the tumour population would be on the predicted tumour response. This means that posteriors indicate how prone the system is to qualitatively changing behaviour when the parameter is perturbed. The smaller the interval of posterior values, the larger the effect of perturbation. The key is identifying parameters with the largest effect, such as μ, α and β with smaller uncertainties.

Simulated treatment

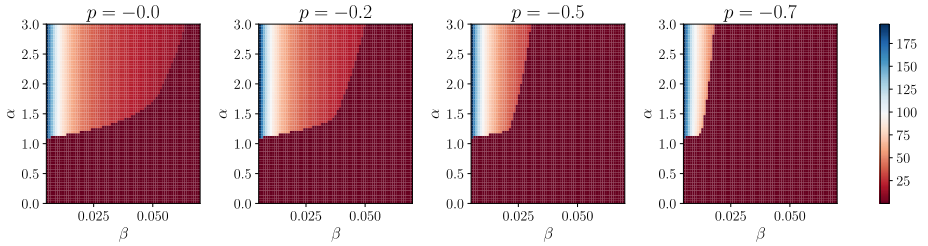


Figure 4: Graphs showing the scaled number of tumour cells at the steady state, when μ is changed at time t=10 to (1+p)μ, as α and β are varied.

A simple model simulation of treatment predicts variability in tumour responses, and shows that treatment modelled via a certain parameter can influence long-term outcomes

Using results from our parameter estimation, we perform a simple simulation of treatment. We solve the model until time t=10, when we change the μ parameter by some percentage, which represents immunotherapy treatment. We then continue the simulation using the perturbed parameter from this point on, until a steady state is reached. We record the final tumour size, and repeat this for different values of α and β which represent tumour population variability. The results in Figure 4 show variability in the resulting tumour response, where the region of the α-β parameter space with tumour escape (blue to bright red) is sharply separated from the region of tumour suppression (dark red). By decreasing μ or increasing the strength of treatment, there is a considerable decrease in the parameter region of tumour escape.

4. Discussion, Conclusions & Recommendations

We have analysed a simple mechanistic model of tumour-immune interactions from [1]. The model incorporates fast effects of formation/dissociation of conjugates of interacting tumour and effector cells, and slow effects of immunologically initiated killing of tumour cells, inactivation of effector cells, and their migration to the tumour site.

By revisiting the model assumptions and sizes of parameters, we showed that, after the initial period of fast relaxation from initial conditions, a quasi-steady state is reached and the model can be simplified.

Nevertheless, looking at the long-term behaviour of the quasi-steady simplified model, we showed that, by varying parameters, the bifurcating system is descriptive of immunoediting. The sudden switch between the qualitatively different solutions caused by a small parameter change suggests that tumour-immune dynamics can play an important role in immunotherapy effectiveness. Our results suggest the large differences in tumour responses may be explained by a distribution of parameters in the tumour population.

Parameter inference using only tumour data performed well for parameters of tumour growth, but we found difficulties with parameters that determine the dynamics of effector cells. The parameters with smaller uncertainties were thought to be the influential ones, which we tested in simulations. Clear separation between tumour escape and tumour control in responses is a promising result, as it resembles outcomes of AstraZeneca's immune checkpoint therapy trials.

Our first attempts at assessing whether experimental data from immunotherapy trials can be explained better with a bifurcating mechanistic model gave encouraging results. However, further testing and assessment of the current model, and parameter estimation using experimental data, will be needed to confirm our hypothesis.

Extending the model

The key extensions for future work are:

Future model extensions should balance mechanistic complexity and characteristics of data from experiments

- Incorporation of effects due activation and proliferation of effector cells that occur in the lymph nodes, and not at the tumour site;
- Incorporation of effects of inactivated effector cells, which can stay at the tumour site and compete for tumour binding spots, as well as become reactivated by immune checkpoint therapy;
- Transformation of the model into a stochastic system by adding random effects.

However, we must bear in mind that the aim of our model is to reflect a balance between its mechanistic complexity and the characteristics of AstraZeneca's experimental data.

5. Potential Impact

Further progress on aligning a simple but mechanistic model with data from immune checkpoint therapy trials will provide a proof of concept for modelling immune-oncology treatments for AstraZeneca. A more general model describing combinations of cancer treatments should enable AstraZeneca to interpret existing datasets, and inform the design of future experiments to increase understanding of these treatments. Modelling of immunotherapy treatments could in long-term contribute to optimisation of treatment regimen, and understanding of what immune characteristics predict a likely response to it.

James Yates, Principal Scientist at AstraZeneca, commented "*Understanding the sources of variability that contribute to response vs non-response to immune checkpoint inhibitors is clearly challenging. However, identifying these sources of variability are key to the best use of these treatments in patients, especially as part of combination of treatments that targets tumour cells as well as mobilises the immune system. The results of this mini-project give progress in this area by demonstrating the feasibility of developing a model that can describe the reasons for variability.*"

References

1. VA Kuznetsov, IA Makalkin, MA Taylor, AS Perelson (1994) *Nonlinear dynamics of immunogenic tumors: Parameter estimation and global bifurcation analysis*. Bull. Math. Biol. **56.2**, 295-321.