Mathematical Modelling for Combinations of Immuno-Oncology and Anti-Cancer Therapies

- report of the QSP UK meeting -

Chappell M.[1], Chelliah V.[2], Cherkaoui M.[4,*], Derks G.[3], Dumortier T.[1], Evans N.[1], Ferrarini M.[5], Fornari C.[6,*], Ghazal P.[7], Guerriero M.L.[8], Kirkby N.[9], Lourdusamy A.[10], Munafo A.[11], Ward J.[12], Winstone R.[13], and Yates J.[8]

[1]University of Warwick — [2]EBI — [3]University of Surrey — [4]University of Nottingham — [5]University of Leeds — [6]University of Cambridge — [7]University of Edinburgh — [8]AstraZeneca — [9]University of Manchester — [10]University of Nottingham — [11]Merck — [12]University of Loughbourgh — [13]None
— [*]corrsponding author

Macclesfield, 14 - 17 September 2015

Contents

Acronyms **IO** - Immuno-Oncology; **ODEs** - Ordinary Differential Equations; **IR** Ionizing Radiation; **PD-L1** - Programmed Death-Ligand 1; **PKPD** - Pharmacokinetics/Pharmacodynamics

1 (Mathematical) Cancer Immunology

Cancer is a multi-faceted disease that is well characterised by the *Hallmarks Of Cancer* [1]. An important hallmark that has emerged is the ability of solid tumours to evade detection by the hosts immune system. This has resulted in the discovery and development of new anti-cancer treatments targeted to enable the immune system to attack the tumour based upon the cancer immunity cycle [2]. The approaches range from vaccines to targeting tumour cells ability to inhibit local T-cell response. This latter approach aims to reset the immune system whereby the immune system acquires and retains the ability to "see" the tumour and effectively kill tumour cells. Some of these approaches have seen encouraging results in the clinic: some patients respond to these agents, with few cases of relapse observed.

Despite the fact that much research into these tumour-immune interactions has been done, there are still many open questions in the field. Quantitative approaches, that combine mathematical modelling with experimental and clinical data, can significantly accelerate this discovery process. In fact, mathematical models of tumour-immune interactions offer an analytic framework that allows to answer some of the crucial questions concerning the dynamics between tumour and immune system [3, 4]. Cancer and systems immunology modellers have been working over recent decades in order to improve the understanding of how the immune system reacts to the growth of a tumour [5–8]. Several approaches have been used, such as differential equations, statistical physics and stochastic modelling [9–11], depending on the specific biological questions to be addressed. A quantitative understanding of the tumour-immune interactions is also crucial in designing treatment strategies, such as dosing and timing, and in predicting the response to a specific treatment [12].

The mathematical model presented in this report provides a high-level abstraction of the interaction between tumour and immune cells, and it explores the advantages of combining Immuno-Oncology (IO) agents with standard anti cancer treatments, such as chemo and radio threapies. As a case study, the model is applied to study the effects of a combination treatment with IO and Ionizing Radiation (IR), i.e. radiotherapy. Understanding the interplay between a growing tumour and the host's immune system is fundamental for optimizing current treatments - in terms of doses and schedules, proposing new ones, and designing rational therapeutic regimens combining chemo- and immunotherapeutic agents.

2 Taking Advantage of the Cancer-Immunity Cycle

2.1 Modelling the cancer-immunity cycle

The immune system recognises, protects and heals our bodies from harmful infective, damaged and abnormal cells such as (ideally) cancer cells. In fact, tumours develop mechanisms to protect themselves against the immune response, which becomes unable to *recognize* and attack them. There are two main arms of the immune system, the *innate* arm, comprising myeloid cells, and the *adaptive* arm, comprising lymphocyte cells, B- and T-cells. An important feature of the *adaptive* arm is that these cells are designed to generate memory against specific harmful agents (this is the reason why vaccines work). The two arms of the immune system communicate with each other to give a "Go/NoGo" signal for making an appropriate response, which serves as a central *check point* in controlling the immune response. *Check point* therapies are designed to break this control, such that tumour cells can be killed.

An antigen (usually an abnormal or different protein from a cancer cell) is first recognised by the *innate* immune cells, such as the macrophage and dendritic myeloid cells. Upon antigen recognition these cells become *activated* and migrate to the lymph nodes secreting signalling molecules, known as cytokines and chemokines which attract other immune cells, in particular CD4 T-cells. The CD4 T-cells, once in the vicinity of the activated myeloid cells form a synapse with them and directly communicate with each other to give positive or negative signals. When a positive signal is given, this allows other types of T-cells known as CD8 cytotoxic T-cells to function as killer cells to seek out and destroy the cancer. Conversely, if negative signals are provided, then these will block CD8 T-cells from becoming activated. However, these negative signals can be blocked, and with positive signals stimulate CD8 T-cells to seek out and destroy the cancer cell. This intricate process is known as the cancer-immunity cycle [2], and it is well summarized by Figure 1.

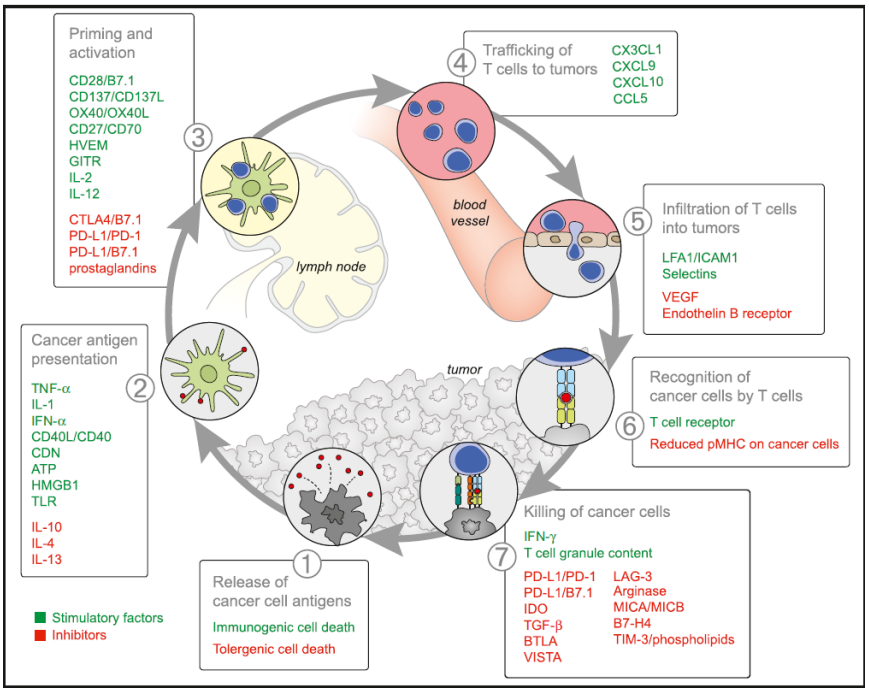


Figure 1: The cancer immune cycle. Extracted from [2].

Since the tumour and immune system interactions are highly complex, a full model which includes all the significant cell types and signalling molecules would be difficult to build, unrealistic to parametrize, and not robust in its predictions. Therefore, in selecting the variables to consider in our model, a number of assumptions were made, as summarised in Figure 2.
Our mathematical model describes the interactions between a growing tumour and the immune system and it explores the effects of radio and immuno therapies used as single agents and in combinations. Notice that the model can be easily modified to reflect the effect of a systematic chemotherapy (instead of radiotherapy).

Among the several types of immune cells playing a role in fighting cancer, we have focussed only on T-cells, as they have been suggested as one of the principal methods that the body uses to combat a tumour. We have assumed that cancer cells grow following a logistic law [13] and constitute a homogeneous population such that radiation therapy acts in the same way on each cell, and no cell cycle specificity has to be considered. The T-cell population is modelled as *unlimited*, but with a *limited* power of action, i.e. no logistic growth is assumed, but their efficacy in killing tumour cells is limited. When antigens are released by cancer cells, T-cells are activated and programmed to kill cancer cells. After a certain number of interactions with cancer cells, T-cells are *de-activated* and/or they die. As the tumour grows, it provokes an immune response from the T-cells in the host. The strength of this response depends on the tumour's antigenicity, which describes how much antigen a tumour presents, and also how sensitive the immune system is to this antigen. At the same time, immunosuppressive factors secreted by the tumour and invoked by the host can act to limit the immune response. In fact, a key feature in our model is the inclusion of immunosuppression of T-cells by the cancer cells, which is one of the main obstacles that the immune system faces when fighting a tumour. Immunotherapy enhances the strength of the immune system and its ability to recognise and fight the tumour.

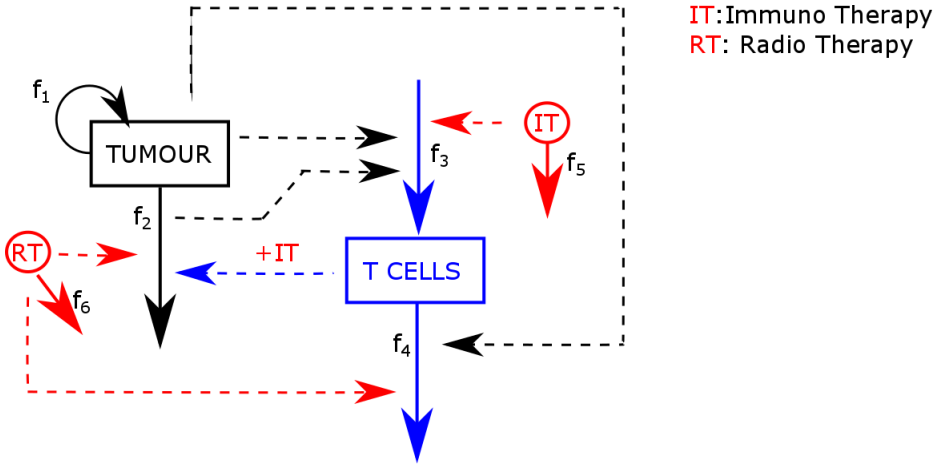


Figure 2: Schematic representation of the model, where tumour cells are coloured in black, T-cells in blue and therapies in red. Straight lines show direct interactions while dotted lines refer to indirect interactions, i.e. they connect elements with the ones that influence them.

Model equations We represented the cancer immune cycle showed in Figure 2 using the following system of Ordinary Differential Equations (ODEs):

$$\begin{aligned}
\tfrac{dS}{dt} &= f_1(S) - f_2(S,T,C) \\
\tfrac{dT}{dt} &= f_3(S,T,I,C) - f_4(S,T,C) \\
\tfrac{dI}{dt} &= -f_5(S,I) \\
\tfrac{dC}{dt} &= -f_6(S,C),
\end{aligned} \tag{1}$$

where system variables are:

S : size of the tumour (volume)
T : T-cells (density in tumour and microenvironment)
I : concentration of the immuno-agent (μM)
C : radioactivity administred (Gy)

Note that, for the sake of simplicity, we are considering only those T-cells that are active against the cancer.

Functions f_i are defined as:

$$
\begin{aligned}
f_1(S) &= rS\left(1 - \frac{S}{S_{max}}\right) \\
f_2(S,T,C) &= \frac{\delta_1 TS}{T_{50}+T} + \delta_2 CS + \frac{\delta_3 S}{1+k_1 T} \\
f_3(S,T,I,C) &= \frac{(\beta_1 + \beta_2 I)S + \phi_1 f_2 T}{1+k_2 CS} \\
f_4(S,T,C) &= \phi_2 \frac{\delta_1 TS}{T_{50}+T} + \delta_4 TC + \delta_5 T \\
f_5(S,I) &= \phi_3 \frac{\beta_2 IS}{1+k_2 CS} \\
f_6(S,C) &= \phi_4 \delta_2 CS
\end{aligned}
$$

Notice that we assumed no mass transfer resistance, i.e. equations were derived using the law of mass action. The meaning of these f_i functions can be explained by the following biological interpretation:

- f_1: tumour logistic growth;
- f_2: tumour death due to: (i) the immunotherapy via T-cell stimulus, (ii) the radiotherapy, and (iii) the NK-activity;
- f_3: T-cell *activation* due to: (i) interaction with the tumour, and (ii) activation by dead tumour cells;
- f_4: T-cell death and *inactivation* due to: (i) interaction with the tumour, (ii) chemotherapy, and (iii) T cell death;
- f_5: immunotherapy decrease;
- f_6: radiotherapy decrease.

Model parameters are described in Table 1, which also reports those values used for the numerical experiments.

Parameters	**Biological meanings**	**Units**	**Values**
r	tumour growth rate	d^{-1}	0.2
S_{max}	tumour carrying capacity	mm^3	3000
δ_1	maximum tumour death rate by T-cells	d^{-1}	15
δ_2	tumour death rate by radiation threapy	$\mathrm{d}^{-1}\,\mathrm{Gy}^{-1}$	0.07
δ_3	maximum tumour death rate by NK-activity	d^{-1}	0
δ_4	T-cell death rate by radiotherapy	$\mathrm{d}^{-1}\,\mathrm{Gy}^{-1}$	0.05
δ_5	natural T-cell death rate	d^{-1}	0.05
β_1	maximum T-cell activation rate by tumour	$10^9\,\mathrm{cells}/(\mathrm{L\,d\,mm}^3)$	0.1
β_2	maximum T-cell activation rate by T-cells & tumour interaction	$10^9\mathrm{cells}/(\mathrm{L\,d\,mm}^3\,\mu\mathrm{M})$	0.3
ϕ_1	T-cell activation coefficient by tumour death	mm^{-3}	12.13
ϕ_2	T-cell death coefficient by T-cells & tumour interaction	$10^9\mathrm{cells}/(\mathrm{L\,mm}^3)$	30
ϕ_3	immunotherapy decreasing coefficient by T-cells & tumour interaction	$\mu\mathrm{M}/(10^9\mathrm{cells/L})$	0.0001
ϕ_4	radiotherapy decreasing coefficient by radiotherapy & tumour interaction	$\mathrm{Gy}/\mathrm{mm}^3$	0.0001
k_1	limitation of the NK-cell activity by T-cells	$\mathrm{L}/10^9\mathrm{cells}$	0
k_2	limitation of the T-cell activity by radiotherapy	$\mathrm{mm}^{-3}\mathrm{Gy}^{-1}$	0.001
T_{50}	half maximum effective concentration of T-cells for tumour death by T-cells	$10^9\mathrm{cells/L}$	100

Table 1: Model parameters - biological meanings and values used in the simulations.

2.2 Mathematical analysis of the model

2.2.1 Model equilibria

The first steady state of system 1 is given by E_0:

$$\hat{S} = \hat{T} = 0,$$

with arbitrary values for I and C. Notice that this equilibrium corresponds to the *hypothetical* situation in which there is no tumour and T-cells are inactive.

The second steady state is given by E_1:

$$\hat{S} = 0, \quad \text{i.e. there is no tumour;}$$
$$\hat{C} = -\delta_5/\delta_4,$$

with arbitrary values for T and I. Assuming that all of the parameters are positive numbers, then E_1 is not biologically possible.

A third *family* of steady states relates to the situation in which:

$$\hat{I} = \hat{C} = 0,$$
$$\hat{S} = \frac{S_{\max}\left(k_1\hat{T}^2\left(r-\delta_1\right) + \hat{T}\left(r\left(T_{50}k_1+1\right) - \delta_1 - \delta_3\right) + T_{50}(r-\delta_3)\right)}{r\left(k_1\hat{T}^2 + \hat{T}\left(T_{50}k_1+1\right) + T_{50}\right)} \tag{2}$$

where $\hat{T}$ is a root of the following equation:

$$\begin{aligned}
&k_1^2\hat{T}^5\left(S_{\max}\delta_1\phi_1(r-\delta_1) - r\delta_5\right) + \hat{T}^4\big(k_1S_{\max}(k_1(r\delta_1(T_{50}\phi_1-\phi_2) + \delta_1^2\phi_2 + \beta_1(r-\delta_1)) \\
&\quad + \phi_1(r(2\delta_1+\delta_3) - 2\delta_1(\delta_1+\delta_3))) - 2r\delta_5k_1(k_1T_{50}+1)\big) + \hat{T}^3\big(S_{\max}(k_1^2T_{50}(r(2\beta_1 - \delta_1\phi_2) - \beta_1\delta_1) \\
&+ 2k_1(T_{50}\phi_1(r(\delta_1+\delta_3) - \delta_1\delta_3) + \delta_1\phi_2(\delta_1 - r) + 2\beta_1(r-\delta_1)) + \delta_1(\delta_3k_1\phi_2 + r\phi_1) + \delta_3(r\phi_1 - \beta_1k_1) \\
&\quad - \phi_1\delta_1(\delta_1+2\delta_3) - \delta_3^2\phi_1) - r\delta_5(T_{50}^2k_1^2 + 4T_{50}k_1 + 1)\big) + \hat{T}^2\big(S_{\max}(T_{50}(rT_{50}k_1(\beta_1k_1 + \delta_3\phi_1) \\
&+ \delta_1k_1\phi_2(\delta_3 - 2r) + 4r\beta_1k_1 + r\phi_1(\delta_1+2\delta_3) - 2(\beta_1k_1 + \delta_3\phi_1)(\delta_1+\delta_3) + \delta_1(\delta_1\phi_2 - r\phi_2 + \delta_3\phi_2) \\
&\quad + \beta_1(r - \delta_1 - \delta_3)) - 2rT_{50}\delta_5(T_{50}k_1+1)\big) + \hat{T}\big(S_{\max}T_{50}(T_{50}(\beta_1k_1(2r-\delta_3) + \delta_3\phi_1(r-\delta_3)) \\
&\quad + \delta_1\phi_2(\delta_3 - r) + \beta_1(2r - \delta_1 - 2\delta_3)) - rT_{50}^2\delta_5\big) + S_{\max}T_{50}^2\beta_1(r-\delta_3) = 0
\end{aligned} \tag{3}$$

With the parameter values used in the MATLAB simulations (see Table 1) the steady states collapse into the trivial one (E_0) together with two impossible ones ($\hat{S} < 0$) and one physically meaningful $E^* = [\hat{S} = 2949.5; \quad \hat{T} = 0.0224; \quad \hat{I} = \hat{C} = 0]$.

2.2.2 Stability analysis

The Jacobian matrix of system 1, evaluated in $\hat{E} = [\hat{S}; \quad \hat{T}; \hat{I} = \hat{C} = 0]$ is given by:

$$J_m = \begin{pmatrix}
\dfrac{-a_1}{S_{\max}(T_{50}+\hat{T})(\hat{T}k_1+1)} & \dfrac{-a_2}{(T_{50}+\hat{T})^2(\hat{T}k_1+1)^2} & 0 & -\delta_2\hat{S} \\
\dfrac{a_3}{(T_{50}+\hat{T})(\hat{T}k_1+1)} & \dfrac{a_4}{(T_{50}+\hat{T})^2(\hat{T}k_1+1)^2} & \beta_2\hat{S} & \dfrac{-a_5}{(T_{50}+\hat{T})(\hat{T}k_1+1)} \\
0 & 0 & -\phi_3\beta_2 & 0 \\
0 & 0 & 0 & -\phi_4\delta_2\hat{S}
\end{pmatrix} \tag{4}$$

where

$$\begin{aligned}
a_1 &= S_{\max}\left(\hat{T}\left(\hat{T}k_1(\delta_1 - r) - r(T_{50}k_1+1) + \delta_1 + \delta_3\right) + T_{50}(\delta_3 - r)\right) \\
&\qquad + 2\hat{S}r\left(\hat{T}\left(k_1(\hat{T}+T_{50}) + 1\right) + T_{50}\right) \\
a_2 &= \hat{S}\left(\hat{T}\left(\hat{T}k_1\left(T_{50}\delta_1k_1 - \delta_3\right) + 2T_{50}k_1(\delta_1 - \delta_3)\right) + T_{50}(\delta_1 - T_{50}\delta_3k_1)\right) \\
a_3 &= \hat{T}^3\delta_1k_1\phi_1 + \hat{T}^2\left(k_1(\beta_1 - \delta_1\phi_2) + \phi_1(\delta_1+\delta_3)\right) + \hat{T}\left(T_{50}(\beta_1k_1 + \delta_3\phi_1) - \delta_1\phi_2 + \beta_1\right) + T_{50}\beta_1 \\
a_4 &= \hat{S}\big(\hat{T}^4\delta_1k_1^2\phi_1 + 2\hat{T}^3\delta_1k_1\phi_1\left(T_{50}k_1+1\right) + \hat{T}^2\left(T_{50}\delta_1k_1(4\phi_1 - k_1\phi_2) + \phi_1(\delta_1+\delta_3)\right) \\
&\quad + 2\hat{T}T_{50}\left(\delta_1(\phi_1 - k_1\phi_2) + \delta_3\phi_1\right) + T_{50}\left(T_{50}\delta_3\phi_1 - \delta_1\phi_2\right)\big) - \delta_5\big(\hat{T}^4k_1^2 + 2\hat{T}^3k_1\left(T_{50}k_1+1\right) \\
&\quad + \hat{T}^2\left(T_{50}^2k_1^2 + 4T_{50}k_1 + 1\right) + 2\hat{T}T_{50}\left(T_{50}k_1+1\right) + T_{50}^2\big) \\
a_5 &= \hat{S}^2k_2\left(\hat{T}^3\delta_1k_1\phi_1 + \hat{T}^2\left(\beta_1k_1 + \phi_1(\delta_1+\delta_3)\right) + \hat{T}\left(T_{50}(\beta_1k_1 + \delta_3\phi_1) + \beta_1\right) + T_{50}\beta_1\right) \\
&\quad - \hat{S}\phi_1\left(\hat{T}^3\delta_2k_1 + \hat{T}^2\left(T_{50}\delta_2k_1 + \delta_2\right) + \hat{T}T_{50}\delta_2\right) + \hat{T}^3\delta_4k_1 + \hat{T}^2\left(T_{50}\delta_4k_1 + \delta_4\right) + \hat{T}T_{50}\delta_4
\end{aligned}$$

Notice that the eigenvalues of the Jacobian matrix evaluated at the steady state $\hat{E}$ are all real and negative ($\lambda = [-0.1966, -13030, -0.0885, -0.0206]$) indicative if a steady state locally asymptotically stable.

2.2.3 Nullclines

Under the condition that $I = C = 0$ we can consider the following nullcines from the system equations (with parameters substituted):

$$S + \frac{6000(37T - 50)}{100 + T} = 0$$
$$S - \frac{T(100 + T)}{3639T^2 - 8998T + 200} = 0$$

These are shown in Figure 3. The first equation (i.e. the nullcline for $\dot{S}$) represents the effect of the immune system on the tumour size. Stronger is the immune response, smaller is the tumour. The second equation expresses the responses of the immune system to the tumour size: bigger is the tumour size, stronger is the response. The second equation suggests that the response of the immune system is sensitive to the tumour size only initially and then reaches an equilibrium. The second of these equations (the nullcline for $\dot{T}$) rises towards a vertical asymptote near the steady state value.

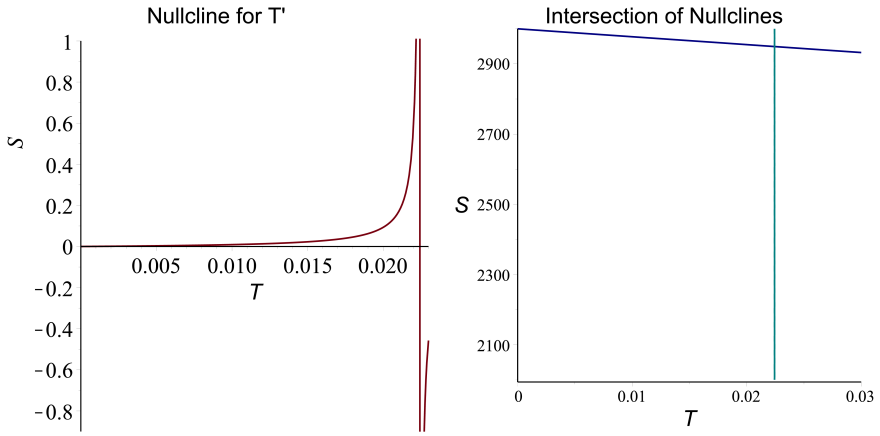


Figure 3: Nullcline for $\dot{T}$ equation (left) and intersection of $\dot{S}$ and $\dot{T}$ nullclines (right)

2.3 Synergistic effects of immuno and radio therapy

According to the cancer immune cycle, and to clinical and pre-clinical results, it can be postulated that tumours evolve through three distinct steps of interaction with the immune system: (i) malignant cells are recognized and eradicated by immune effector cells (elimination phase); (ii) small tumours are still held in check by less proficient immune responses (equilibrium phase); and (iii) neoplastic cells lose their antigenic properties or establish potent immunosuppressive networks, thus avoiding any control by the immune system (escape phase); see Figure 4.

This relationship between cancer and immune system supports the ideas of treating tumours via therapies that induce immunosurveillance, and to combine these treatments with conventional chemotherapies and radiotherapies. Moreover, chemo and radio therapies appear to stimulate the antigenicity of malignant cells due to their potential to induce a cell death-independent stress response that may mimic that elicited by viral infection [14, 15]. A review of two promising classes of antibodies, antiCTLA-4 and antiProgrammed Death-Ligand 1 (antiPD-L1), used as monotherapy and in combination with cancer therapies can be found in [16].

As a case study to test our model, we have focussed on the work of Deng *et al.* [15] that shows how irradiation and immunotherapy synergistically promote anti-tumour immunity in mice.
Localized IR has been shown to mediate tumour regression in a T-cell dependent fashion. Therapeutic blockade of the T-cell negative regulator PD-L1 can enhance T-cell effector function when PD-L1 is expressed in chronically inflamed tissues and tumours, as it happens in tumour microenvironment after IR. PD-L1 expression in the tumour microenvironment has been associated with poor outcomes following radiotherapy in cancer patients. Therefore, PD-L1 expression in the tumour microenvironment provides an opportunity for therapeutic intervention using regulators such as antiPD-L1 and antiPD-1. Deng

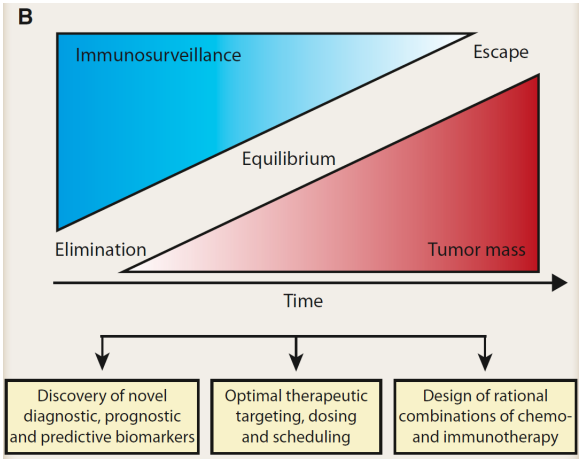


Figure 4: Tumour growth and immunosurvelliance. Adapted from [14]

et al. showed that the administration of antiPD-L1 enhanced the efficacy of IR through a cytotoxic T-cell dependent mechanism. Concomitant with IR-mediated tumour regression, they observed that IR and antiPD-L1 synergistically operate in reducing tumour growth; see Figure 5.

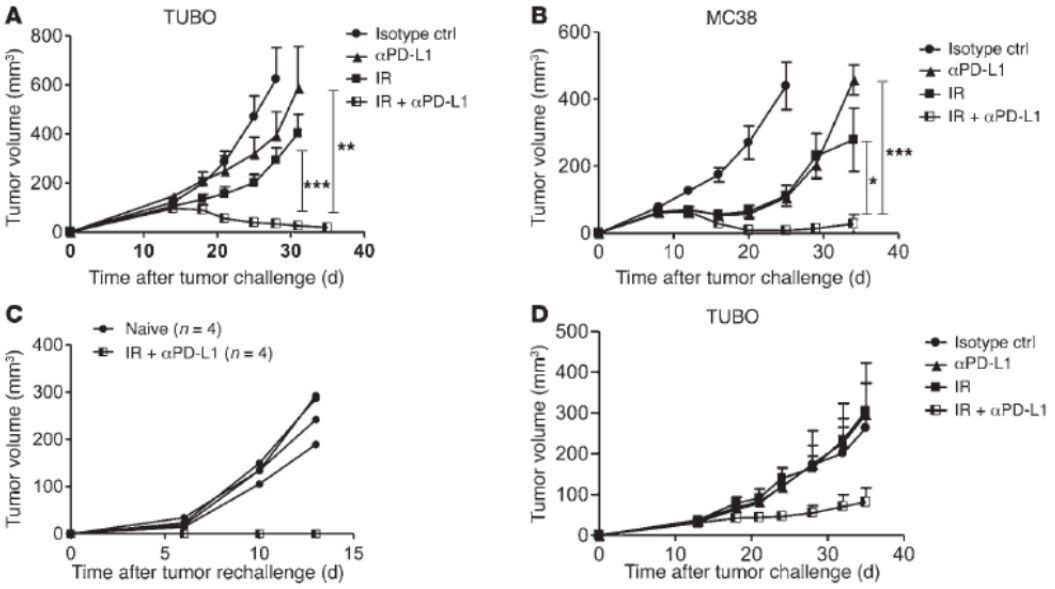


Figure 5: Combination of antiPD-L1 and irradiation significantly enhanced the inhibition of tumour growth in mice. Extracted from [15]

2.4 Model validation and numerical results

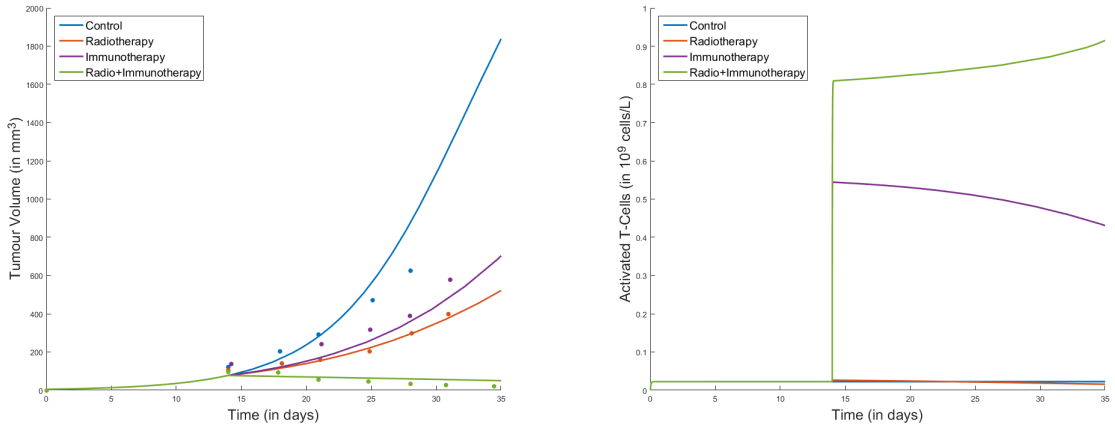
As mentioned in the previous section, we have used some data by Deng *et al.* [15] as a case study to test our model. Specifically, values of model parameters were *adjusted* to reproduce the tumour growth observed in mice - with and without treatments - reported in Figure 5 **A**. Proper parameter estimation and sensitivity analysis will be performed to explore the parameter space and find a robust set of estimated parameter values.
Parameter values are shown in Table 1, while Table 2 reports the initial conditions used in each numerical simulation.

In our numerical experiments mice were treated after 14 days, i.e. when tumour mass is around 100 mm^3; see Figure 6(a). As a first approximation we modelled treatments as constant over time. However,

Variables	No treatments	Immunotherapy	Radiotherapy	Immuno & Radio-therapy
S(0)	5	5	5	5
T(0)	0	0	0	0
I(0)	0	6	0	6
C(0)	0	0	1.3	1.3

Table 2: Initial conditions.

model 1 could be expandend to include PK/PD studies, and specific kinetics for the immuno and the radio therapies.



(a) Tumour Volume

(b) T-Cells

Figure 6: In silico tumour and T-cell growth reproducing in vivo data.

As can be appreciated from Figure 6(a), our model reproduces the *in-vivo* observations by Dang *et al.* [15]. Notice that, both the radio and the immuno therapy - when used as single agents - are not able to reduce significantly the tumour mass; see red and violet lines in Figure 6(a). However, tumour mass is reduced when IR and IO are used in combination; see green line in Figure 6(a). This dual agent treatment is able to efficaciously stimulate the immune system against the tumour cells. In fact, the number of *activated* T-cells is higher when IR and IO are combined than when they are used as single agents; see Figure 6(b). From these results it can be suggested that the immune system requires a *certain* stimulus before it decides to act, as if it worked in a threshold like manner. For combinations of IO with radiation (or other cancer treatments), this might be dependent upon a number of factors, importantly what the radiation "does" to cancer cells and the immune system, and hence it is crucial to define proper timing and sequence.

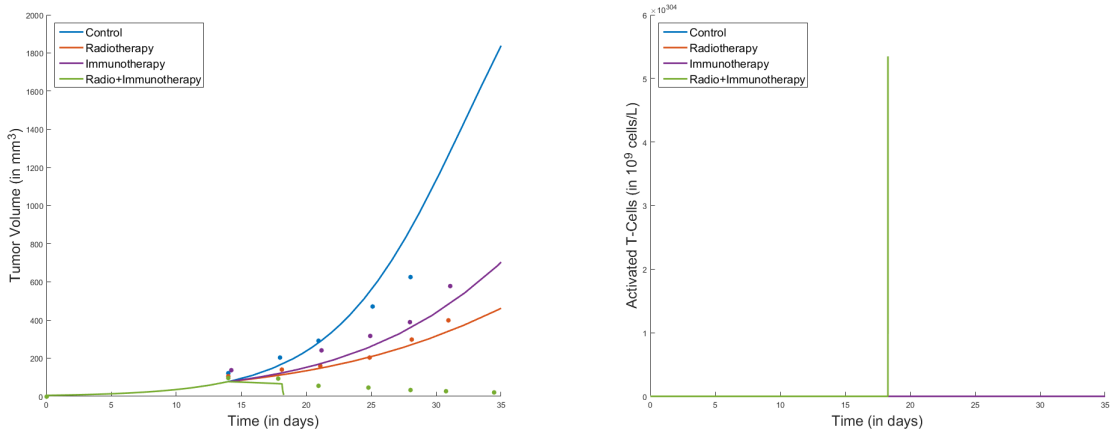
3 Future work

This report summarizes the work that our group did during the Quantitative Systems Pharmacology meeting in Macclesfield, from 14 to 17 September 2015.
In those days we designed the model showed in this report, we performed some analyses, and we ran numerical simulations too. However, it is worth to investigate the following aspects too.

Sensitivity Analysis A sensitivity analysis is necessary to identify those parameters which impact the most on the model and to identify correlations among parameters.
An example of *why* it is important to analyze model parameters is provided by Figure 7. The Figure shows model simulations started from initial conditions slightly different from those used in Figure 7; this set of initial conditions is reported in Table 3.

Variables	No treatments	Immunotherapy	Radiotherapy	Immuno & Radio-therapy
S(0)	5	5	5	5
T(0)	0	0	0	0
I(0)	0	6	0	6
C(0)	0	0	1.4	1.4

Table 3: *New* initial conditions.



(a) Tumour Volume

(b) T-cells

Figure 7: Model simulations with initial conditions showed in Table 3.

Notice that in the combination treatment the number of *activated* T-cells increases exponentially and reaches an *unrealistic* threshold; see green lines in Figure 7(b). This behaviour suggests the need to constrain T cell growth in the model or either by changing its definition in system 1, or by properly identifying the parameter space.

Data Fitting Parameter values of Table 1 were defined trying to reproduce data of Figure 5 **A**. Once more data of tumour size and T-cell number for various dosages will be available, a more adequate parameter estimation will be performed. This process is fundamental to validate the model design, i.e. to assess whether the model was designed considering the right assumptions. Moreover, model design should take into account also the source of the data. For instance, T-cell concentration is often measured in the blood, whereas the *effective* T cells are those within the tumour and its microenvironment. This should be taken into account in model designing and variable definition.

Further in-silico experiments Once model robustness is proved, several numerical experiments can be performed. Specifically, expanding model 1 with specific kinetics for the immuno and radio therapies and PK/PD studies, it can be used to predict the effects of different treatment schedules for both single and dual agent therapies.

References

[1] D. Hanahan and R. Weinberg. Hallmarks of cancer: The next generation. *Cell*, 144(5):646–674, 2011.

[2] D. S. Chen and I. Mellman. Oncology meets immunology: the cancer-immunity cycle. *Immunity*, 39(1):1–10, 2013.

[3] NL Komarova and W Wodarz. Computational biology of cancer. *Lecture Notes and Mathematical Modelling. Singapore: World Scientific*, 2005.

[4] RA Ramos, Jair Zapata, CA Condat, and Thomas S Deisboeck. Modeling cancer immunotherapy: Assessing the effects of lymphocytes on cancer cell growth and motility. *Physica A: Statistical Mechanics and its Applications*, 392(10):2415–2425, 2013.

[5] Daniel G Mallet and Lisette G De Pillis. A cellular automata model of tumor–immune system interactions. *Journal of Theoretical Biology*, 239(3):334–350, 2006.

[6] Lisette G de Pillis, Daniel G Mallet, and Ami E Radunskaya. Spatial tumor-immune modeling. *Computational and Mathematical Methods in medicine*, 7(2-3):159–176, 2006.

[7] SA Menchón, RA Ramos, and CA Condat. Modeling subspecies and the tumor-immune system interaction: steps toward understanding therapy. *Physica A: Statistical Mechanics and its Applications*, 386(2):713–719, 2007.

[8] RJ De Boer, Pauline Hogeweg, HF Dullens, Roel A De Weger, and Willem Den Otter. Macrophage t lymphocyte interactions in the anti-tumor immune response: a mathematical model. *The journal of immunology*, 134(4):2748–2758, 1985.

[9] John Adam and Nicola Bellomo. *A survey of models for tumor-immune system dynamics*. Springer Science & Business Media, 2012.

[10] Nicola Bellomo and M Delitala. From the mathematical kinetic, and stochastic game theory to modelling mutations, onset, progression and immune competition of cancer cells. *Physics of Life Reviews*, 5(4):183–206, 2008.

[11] A d'Onofrio. Tumor-immune system interaction: modeling the tumor-stimulated proliferation of effectors and immunotherapy. *Mathematical Models and Methods in Applied Sciences*, 16(08):1375–1401, 2006.

[12] L. G. dePillis, A. Eladdadi, and A. E. Radunskaya. Modeling cancer-immune responses to therapy. *Journal of Pharmacokinetics and Pharmacodynamics*, 41(5):461–478, 2014.

[13] S. Benzekry, C. Lamont, A. Beheshti, A. Tracz, J. M. L. Ebos, L. Hlatky, and P. Hahnfeldt. Classical mathematical models for description and prediction of experimental tumor growth. *Immunity*, 10(8), 2014.

[14] L. Zitvogel, L. Galluzzi, M. J. Smyth, and G. Kroemer. Mechanism of action of conventional and targeted anticancer therapies: Reinstating immunosurveillance. *Immunity*, 39(1):74–88, 2013.

[15] L. Deng, H. Liang, B. Burnette, M. Beckett, T. Darga, R. R. Weichselbaum, and Y. Fu. Irradiation and anti pd-l1 treatment synergistically promote antitumor immunity in mice. *The journal of clinical investigation*, 124(2):687–695, 2014.

[16] Lucy Lee, Manish Gupta, and Srikumar Sahasranaman. Immune checkpoint inhibitors: An introduction to the next-generation cancer immunotherapy. *The Journal of Clinical Pharmacology*, 2015.

Acknowledgements

The authors acknowledge Neil Evans, Marco Ferrarini (funding from the European Union Seventh Framework programme FP7/2007-20013 under grant agreement 317040; ITN QuanTI), Peter Ghazal, Maria Luisa Guerriero, Alain Munafo, and James Yates for their contributions to writing this report.
The authors also acknowledge Michael Chappell, Vijayalakshmi Chelliah, Gianne Derks, Thomas Dumortier, Norman Kirkby, Anbarasu Lourdusamy, John Ward and Richard Winstone for the interesting discussions that leaded to the model design during the meeting.

A Matlab Code

A.1 The ODE system

The `ODEs_QSP.m` function represents the ODE system 1, previously described.

```
function dydt =ODEs_QSP(t,y,par,PK_I,PK_C)

%If the PK is ignored and a constant concentration is assumed
if nargin == 3,
    PK_I=@(t,I)(0);
    PK_C=@(t,I)(0);
end

%PARAMETER MAPPING:
    %f1:
r = par(1);          %tumour growth rate.
Smax = par(2);       %tumour carrying capacity.
    %f2
d1 = par(3);         %maximum tumour death rate by T-cells.
T50 = par(4);        %half maximum effective concentration of T-cells for tumour death by T-cells.
d2 = par(5);         %tumour death rate by chemo/readio-therapy.
d3 = par(6);         %maximum tumour death rate by NK-activity.
k1 = par(7);         %imitation of the NK-cell activity by T-cells.
    %f3
b1 = par(8);         %maximum T-cell activation rate by tumour.
b2 = par(9);         %maximum T-cell activation rate by T-cells & tumour interaction.
phi1 = par(10);       %T-cell activation coefficient by tumour death.
k2 = par(11);        %limitation of the T-cell activity by radiotherapy.
    %f4
phi2 = par(12);      %T-cell death coefficient by T-cells & tumour interaction.
d4 = par(13);        %T-cell death rate by radiotherapy.
d5 = par(14);        %natural T-cell death rate.
    %f5
phi3 = par(15);      %immunotherapy decreasing coefficient by T-cells & tumour interaction.
    %f6
phi4 = par(16);      %radiotherapy decreasing coefficient by radiotherapy & tumour interaction

%PLACE MAPPING:
S = y(1);            %Tumour
T = y(2);            %T-cells
I = y(3);            %Immunotherapy
C = y(4);            %Chemo/Radio-therapy

%FUNCTION f1, f2, f3 & f4:

    %Tumour logistic growth:
f1 = r*S*(1-S/Smax);
    %Tumour decreasing rate due to:
f2 = d1*T*S/(T50+T)+...          % interaction between tumour and T-cells.
     d2*C*S+...                  % interaction between tumour and chemo/readio-therapy.
     d3*S/(1+k1*T);              % NK-activity.
    %T-cell increasing rate:
f3 = ((b1+b2*I)*S+phi1*f2*T)/(1+k2*C*S);
    %Tcell decreasing rate:
f4 = phi2*d1*T*S/(T50+T)+d4*T*C+d5*T;

    %Immunoth decreasing rate:
f5 = phi3*b2*S*I/(1+k2*C*S);

    %Chemo decreasing rate:
f6 = phi4*d2*C*S;


%ODE SYSTEM:
dS = f1 - f2;
dT = f3 - f4;
dI = PK_I(t,I)-f5;
dC = PK_C(t,C)-f6;
%....
dydt = [dS;dT;dI;dC];
```

```
end
```

A.2 Solver

The `SolveImmunoChemo.m` function solve the ODE system of `ODEs_QSP`, using the MATLAB solver `ode15s`.

```
function [t,y] = SolveImmunoChemo(y0,Par,PK_I,PK_C,t_int,t_end)

%TIME SPAN OF THE SIMULATION
tspan = [t_int t_end];

%SOLUTION
[t,y] = ode15s(@(t,y)ODEs_QSP(t,y,Par,PK_I,PK_C),tspan,y0);

end
```

A.3 Therapy Simulation

The file `TherapySimulation.m` contains the script to simulate the therapies:

```
clear all
close all

%% Extract data from Paper:
[t_paper_cont,Y_paper_cont] =importPKCurveData('Controle.csv');
[t_paper_IR,Y_paper_IR] =importPKCurveData('IR.csv');
[t_paper_immuno,Y_paper_immuno] =importPKCurveData('Immuno.csv');
[t_paper_both,Y_paper_both] =importPKCurveData('Immuno_IR.csv');

%% Initial Conditions, Parameters Values and PK:

%Initial Conditions:
S0 = 5;
T0 = 0;

%Parameter values:
r = 0.2;                %tumour growth rate
Smax = 3000;            %tumour carrying capacity
d1 = 15;                %maximum tumour death rate by T-cells
T50 = 100;
d2 = 0.07;              %tumour death rate by chemo/radio-therapy
d3 = 0;                 %tumour decr rate by NK-activity
k1 = 0;
b1 = 0.1;               %T-cells incr rate by tumour
b2 = 0.3;               %T-cells incr rate by immunoth
phi1 = 12.13;           %T-cells incr rate by chemo/radio-therapy
k2 = 0.001;
phi2 = 30;              %T-cells death rate by tumour
d4 = 0.05;              %T-cells death rate by chemo/radio-therapy
d5 = 0.05;
phi3 = 0.0001;          %Immunoth decr rate by tumour-immunoth interact
phi4 = 0.0001;          %Chemo/radio-therapy decr rate by tumour-chemo interact

Par = [r,Smax,d1,T50,d2,d3,k1,b1,b2,phi1,k2,phi2,d4,d5,phi3,phi4];

%% Simulation Time:
t_end=500;

%% PK of I and C:
    %Start of the treatment
t_TreatStart=14;
    %Clearance
CL_I=0;
CL_C=0;
    %With an bolus injection at t=t_TreatStart
PK_I=@(t,I)(-CL_I*I);
PK_C=@(t,C)(-CL_C*C);
    %With an bolus injection at t=t_TreatStart (Second way to express the
    %PK)
```

```
% Sigma=0.001;
% D_I=0;
% D_C=0;
% PK_I=@(t,I)(D_I/Sigma*(heaviside(t-t_TreatStart)-heaviside(t-t_TreatStart-Sigma))-CL_I*I);
% PK_C=@(t,C)(D_C/Sigma*(heaviside(t-t_TreatStart)-heaviside(t-t_TreatStart-Sigma))*dirac(t-t_TreatStart)-CL_C

%% Initial Condition: Control

I0 = 0;
C0 = 0;
y0=[S0, T0, I0, C0 ];

%Excute main program:
    %Simulation until the injection of the strat of the treatment:
[t_0,y_0] = SolveImmunoChemo(y0,Par,PK_I,PK_C,0,t_TreatStart);
    %Simulation after the beginning of the tretment (In here no treatment):
[t_Cont,y_Cont] = SolveImmunoChemo(y0,Par,PK_I,PK_C,0,t_end);


%% Initial Condition: Chemo alone

I0 = 0;
C0 = 1.3;
y0=[y_0(end,1), y_0(end,2), I0, C0 ];      %IC at the begging of the treatment ->See T_0 & y_0 simulation

%Excute main program:
[t_C,y_C] = SolveImmunoChemo(y0,Par,PK_I,PK_C,t_TreatStart,t_end);
t_C=cat(1,t_0,t_C(2:end));
y_C=cat(1,y_0,y_C(2:end,:));

%% Initial Conditions: Immuno alone

I0 = 6;
C0 = 0;
y0=[y_0(end,1), y_0(end,2), I0, C0 ];      %IC at the begging of the treatment ->See T_0 & y_0 simulation

%Excute main program:
[t_I,y_I] = SolveImmunoChemo(y0,Par,PK_I,PK_C,t_TreatStart,t_end);
t_I=cat(1,t_0,t_I(2:end));
y_I=cat(1,y_0,y_I(2:end,:));

%% Initial Conditions: Chemo and Immuno alone

I0 = 6;
C0 = 1.3;
y0=[y_0(end,1), y_0(end,2), I0, C0 ];      %IC at the begging of the treatment ->See T_0 & y_0 simulation

%Excute main program:
[t_CI,y_CI] = SolveImmunoChemo(y0,Par,PK_I,PK_C,t_TreatStart,t_end);
t_CI=cat(1,t_0,t_CI(2:end));
y_CI=cat(1,y_0,y_CI(2:end,:));
```