Mathematical modelling of tumour growth and interaction with host tissue and the immune system

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tumour, immune, differential equation, cellular automata, optimal control, forward-backward sweep, Runge-Kutta, dendritic cell, natural killer cell, cytotoxic T cell, lymphocyte, helper T cell, CD4\(^+\), CD8\(^+\)
Abstract

There is strong evidence in the literature for the hypothesis that tumour growth is directly influenced by the cellular immune system of the human host. For example, NK cells and CD8\(^+\) T cells are components of the immune system that are well known to be able to kill tumour cells. Dendritic cells (DCs) as antigen-presenting cells (APCs), play an important role in stimulating, recruiting and activating the immune system. In recent years, it has been reported that DCs can also directly lyse tumour cells. Some tumours present DCs and the presence of such cells has a potential role in tumour control. Currently, mechanisms involved in immune system interactions with growing tumours are not fully understood.

There are still many unanswered questions related to how the interaction between a growing tumour and immune system and regarding which components of the immune system play important roles in this mechanism. The mathematical models to be developed in this project will provide theoretical descriptions of biological systems that can be used to arrive at quantitative and qualitative understandings regarding answers to such questions as well as providing the ability to simulate experiments *in silico* using computers. These *in silico* experiments will help to extend current understanding and opinion related to tumour-immune system interactions and allow for the development of more refined hypotheses that can be tested via laboratory experiments.

In this thesis, three new mathematical models describing the growth of solid tumours incorporating the host tissue and immune system response are developed and investigated. We describe various biological aspects of tumour growth and immune system interactions using mathematical models based on findings extracted from the biological literature.

In the first investigation, two submodels are constructed to provide a description of the interaction between a growing tumour and cells of the innate and specific immune system. In these models, we assume that NK and DCs, (the innate immune system), and CD8\(^+\) T cells (the specific immune system) can kill tumour cells. To describe this interaction, the models are comprised of four ordinary differential
equations. We analyse the stability of the model as well as the simple bifurcation behaviour. Numerical solutions of the models are also presented and interpreted. A preliminary model is constructed to serve as an introductory description of the biological system, before a more complex mathematical model is provided which builds on the preliminary model with greater biological realism. Both of the models demonstrate the compound effects of elements of the immune system on the dynamics of tumour growth through their effects on other immune system components. From numerical solutions, it is found that increasing the source term of DCs is more useful than increasing the source term of NK cells.

In the second investigation, we study an optimal control treatment strategy for a model of the interactions between a growing tumour and the host immune system. To obtain the optimal control model, we extend the model of the first investigation (described above) by introducing a time-varying dendritic cell-based treatment strategy and also describing an objective functional that we seek to minimise in this modelling study. Further, a discussion of a necessary condition for an optimal strategy is presented to set the background of the model. The existence of the optimal control is then proved. This then allows for a presentation of the optimality system that will be solved numerically. The numerical scheme itself, a forward-backward sweep method, is then applied to the model and a number of important numerical solutions of the optimal control model are presented. From simulations, increasing the strength of the DCV reduces the tumour burden and the required time duration of the treatment. This model suggests that the best way to fight the tumour is to give the first high DCV concentration at the beginning of the treatment and reduce the treatment over the remaining treatment period.

In the final part of the thesis, we present a mathematical model of a growing tumour and the interaction between the tumour cells and the host immune system using a hybrid cellular automata (HCA) model. This model can describe the system in much more detail, including cell-cell interactions of every single cell in the system. To include the effect of a chemokine in this model, we recognise the significantly smaller size of such molecules compared with biological cells and introduce a partial differential equation to describe the concentration of chemokine secreted by the tumour. We combine the numerical solution of the partial differential equation with a number of biologically motivated automata rules to govern the evolution of various cell populations to form the HCA model. From numerical simulations, the tumour evolution shows the characteristic exponential and linear growth phases of solid, avascular tumours (see for example, Folkman and Hochberg), as well as a slower growing population of necrotic cells. The distribution of the growing tumour is demonstrated with results qualitatively matching those of existing work due to Mallet and de Pillis.
We also use the HCA model to investigate the growth of a tumour in a number of computational “cancer patients”. We present the results of these simulations using a simulated Kaplan-Meier survival curve. From simulation, the model indicates that increasing the number of immature DC in the domain results in significantly longer “survival” of simulated patients.
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Statement of Original Authorship

The work contained in this thesis has not been previously submitted for a degree or diploma at any other higher educational institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signed: __________________________

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Chapter 1

Introduction

1.1 Overview

Cancer is one of the leading causes of death worldwide, with 7.6 million people dying as a result of cancer in 2008 alone. This is projected to rise to over 13.1 million by 2030 [131]. A similar report [4] states that in Australia in 2007, cancer was the second most common cause of death and that 108,368 new cases of cancer were diagnosed. For those diagnosed with cancer between 1998 and 2004, the 5-year relative survival for all cancers combined was 61%. Clearly, cancer is a major concern for public health officials around the world and a greater understanding of cancer has potential to save many lives.

There is evidence that the immune system is capable of recognising and eliminating tumour cells [16, 31, 106, 122]. Therefore, some researchers intensively continue to develop and investigate theoretical and experimental approaches regarding the interactions between growing tumours and the immune system. However, it is difficult to observe and to control experimentally all of the interacting elements of a growing tumour due to the sophistication of the biological system which depends on many factors involved in the process [92]. Mathematical models play an important role in the development of knowledge in this field of research, since we can use models to understand the general behaviour of a phenomenon in different situations, to perform in silico experiments or simulations, to carry out new experiments, and to test theoretical assumptions and suggest modifications of theories [85]. The development of mathematical models of tumour growth and immune responses requires knowledge in two different areas, in particular: the understanding of biological phenomena involved in the growth and response processes, and also in using a variety of mathematical tools to obtain both qualitative and quantitative predictions [108].

There are still many unanswered questions related to how the interaction be-
tween a growing tumour and immune system and regarding which components of
the immune system play important roles in this mechanism [39]. The mathemati-
cal models to be developed in this project will provide theoretical descriptions of
biological systems that can be used to arrive at quantitative and qualitative un-
derstandings regarding answers to such questions as well as providing the ability
to simulate experiments \textit{in silico} using computers. These \textit{in silico} experiments
will help to extend current understanding and opinion related to tumour-immune
system interactions and allow for the development of more refined hypotheses that
can be tested via laboratory experiments. It is important to study or develop such
models, because these models can provide valuable input to the development and
refinement of tumour treatment strategies.

In this thesis, three new mathematical models describing the growth of solid
tumours incorporating the host tissue and immune system response will be de-
veloped and investigated. We attempt to describe the various biological aspects of
tumour growth and immune system interactions using mathematical models based
on findings extracted from the biological literature. First, a differential equation
model will be constructed to investigate the relationship between tumour growth,
host tissue and immune system components. Then we will expand the model to
explore immunotherapeutic treatment in the form of dendritic cell application, and
the effects of such treatment on tumour growth. This will be explored using an
optimal control framework. We also investigate a hybrid cellular automata model
of tumour-immune system interactions to undertake an investigation that not only
allows for the investigation of spatial variation in tumour growth and immune
response, but also for variation across computational “cancer patients”.

There are three aims that will be carried out in this thesis. They are:

Aim 1: To develop a differential equation-based mathematical model of a growing
tumour, host tissue and immune system, the associated interactions and
resulting outcomes. Based on de Pillis and Radunskaya model [31] and
Castiglione and Piccoli [26], we construct new mathematical models that
explain more detail the role of DCs incorporating with NK cells, CD8+ T cells and tumour cells. These models can provide a tool to describe
qualitative relationships based on particular laboratory findings.

Aim 2: To develop a mathematical model of DC-based immunotherapeutic treat-
ment of a growing tumour, that can provide a basic level of understand-
ing and feedback to experimentalists to assist in designing more effective
and/or efficient laboratory experiments.

Aim 3: To develop a cellular automata model of tumour-immune system inter-
actions that explicitly accounts for cell-cell interactions, incorporates a
multi-dimensional spatial viewpoint and allows for stochastic variation to simulate virtual patients. Based on Mallet and de Pillis model \cite{89}, we build a new cellular automata model that describes the immune system in more detail. The effect of chemokines in a growing tumour and the simulation of virtual patients using similar Kaplan-Meier curve also provide a new contribution to the literature. This model then provides a deeper level of understanding of the immune system interactions with a growing tumour.

To this end, the research requires a working understanding of biological and mathematical literature and methods. The mathematical models developed incorporate differential equations, partial differential equations, cellular automata and optimal control theory and require the application of numerical schemes such as the finite difference method, forward-backward sweep with Runge-Kutta differential equation solvers, and purpose-written algorithms for cellular automata.

The remainder of this chapter is organised as follows. First a review of biological literature of relevance to the problem of interest is presented. This includes a discussion of the immune system, the growth of tumours and the interaction of the immune system with a growing tumour. Then a brief review of some relevant mathematical models is presented. This review includes models which simply look at tumour growth alone, as well as modelling that incorporates elements of the host immune response. Technical aspects are also reviewed and in particular, a discussion is presented of the use of optimal control theory and of cellular automata in modelling tumour growth. Finally, the structure of the thesis as a whole, as well as its main findings are summarised.

### 1.2 A review of relevant biological literature

#### 1.2.1 Tumours

A general discussion regarding what a tumour is can be found in many references. For example, the Australian Institute of Health and Welfare discussion forms an appropriate basis for this work and is summarised as follows \cite{5}. Normally, cells grow and replicate in an orderly way and the generation of tissue and organs occurs to satisfy a particular function in the body. Uncommonly, however, after being affected by a carcinogen, or after developing a random genetic mutation, cells replicate in an uncontrolled way and form a mass which is called a tumour or neoplasm.

Tumours can be classified into two groups, namely benign tumours that do not spread to other tissue and malignant tumours that spread or invade to other tissue.
Benign tumours are often thought to be less dangerous than their invasive malignant counterparts, however it should be noted that the growth of a benign tumour can be dangerous due to the resulting obstruction of natural bodily functions. The main characteristic of a malignant tumour is its ability to grow in an uncontrolled way and to invade or spread to other tissues in the body. Tumour cells spread to other parts of the body when the bloodstream or the lymphatic system carries some cancer cells and lodges them some distance away. They can then start to form a new tumour and continue to invade again. Some tumours can stay in the body for years without showing any symptoms. Others can grow, invade or spread quickly, and cause death in a short period of time.

Cell migration is an essential factor in tumour cell invasion. Migration can be promoted by numerous factors including chemical substances, pressure gradients and components of the extracellular matrix (ECM). Normally, cells in tissue are attached to the ECM and also to one another in specific ways related to the purpose of those cells. Interruption of this adhesion leads to increased motility of cells and possible invasiveness of cells through the ECM. Cell surface receptors that mediate cell adhesion are called integrins. The interactions between cells, via such receptors, are also crucial in the normal regulation of proliferation and differentiation of cells [117]. These interactions are mediated by the cell adhesion molecules (CAMs) which is a family of molecules expressed at the surface of the cell [112] [117].

1.2.2 The immune system

An immune system is a collection of biological mechanisms and processes inside an organism with the purpose of protecting the organism against diseases and infections by identifying and killing non-self (foreign) matter such as viral particles, parasites, and which is of importance here, tumour cells. There is a number of ways to provide a classification for the human immune system and one involves splitting the system into two components, namely the innate and adaptive systems. The innate immune system can recognise foreign matter without the requirement for previous priming by specific non-self antigens. The adaptive immune system can recognise specific targets, after antigens have been processed and presented in combination with a self-receptor or major histocompatibility complex (MHC) molecule on the surface of a cell, resulting in the production of memory immune cells [117].

Innate immunity can involve phagocyte cells such as neutrophils and macrophages, natural killer (NK) cells, dendritic cells (DCs), cytokines, and the complement system. NK cells, DCs and macrophages are important in tumour recognition. Numerous observations have indicated that activated macrophages also play a sig-
significant role in the immune response to tumours [73]. In preventing the development of clinical tumours, NK cells also play a key role by destroying abnormal cells before they replicate and grow [39]. NK cells have the ability to recognise certain tumour antigens and to kill the tumour cells [117]. In recent years, DCs have been identified as an important component in controlling the growth of tumours. Also DCs have a potential role in directly killing the tumour cells [81].

Dendritic cells are known as antigen-presenting cells (APCs), which have an important role in activating the immune system. DCs uptake and process the antigen in peripheral tissue and then migrate to lymphoid tissue where antigen presentation to the immune system occurs [11, 123]. They present antigens to CD8+ T cells through MHC class I molecules and they are very effective in activating CD4+ helper T cells through MHC class II molecules. Once activated, CD4+ helper T cells then secrete chemokines that can enhance immunoglobulin production. Dendritic cells also play a vital role as the major regulator in cytotoxic T lymphocytes (CTLs) and NK cell activation [30, 51, 81].

Adaptive immunity can involve antigen presenting cells, T and B lymphocytes, cytokines and the MHC system. T cells and B cells are derived from the process of hematopoiesis in the bone marrow. T cells are involved in the cell-mediated immune response while B cells are involved in the humoral immune response (that is, antibody-mediated). These two arms of the immune system are illustrated in Figure 1.1. Furthermore, T cells can be split into two groups: the T helper cells and the cytotoxic T cells. T Helper cells regulate to activate both the innate and adaptive immune responses and can only recognise antigen that is presented together with class II MHC on the cell surface aided by a coreceptor on the T cell, called CD4+, whereas cytotoxic T cells generally can recognise antigen combined with the MHC Class I aided by the CD8+ coreceptor [73]. On the other hand, the B cell antigen-specific receptor is an antibody molecule on the B cell surface and is presented with the MHC class II [117].

Tumour immunology involves the study of the host immune response to antigens on tumour cells [73]. There are two types of tumour antigens that have been identified on tumour cells: tumour-specific transplantation antigens (TSTAs) and tumour-associated transplantation antigens (TATAs). Tumour-specific antigens which are unique to tumour cells do not occur in normal cells. They may result from a mutation in the tumour cells that can alter cellular proteins. They are then presented with class I MHC molecules, invoking a cell-mediated response by tumour-specific CTLs. However, tumour-associated antigens are not unique to tumour cells which may be proteins. They are expressed on normal cells during fetal development when the immune system is immature and unable to respond.

As a result of destruction of tumour cells, tumour antigens are produced that
influence both the humoral and cell-mediated immune responses \[73\]. In general, the cell-mediated response appears to play the major role in a response to a growing tumour. A number of tumours have been shown to induce tumour-specific CTLs that recognise tumour antigens presented by class I MHC on the tumour cells. Research has shown that costimulatory signal required for activation of CTL precursors can enhance tumour immunity. A variety of experimental and clinical approaches have been developed to use recombinant cytokines to augment the immune response against cancer either singly or in combination with each other.

**Treatment**

There are three traditional therapy procedures practised for treatment of tumours: surgery, radiation therapy, and chemotherapy \[98\]. Surgery attempts to directly remove tumours and hence reduces tumour burden. Radiation therapy destroys and kills tumour cells by the direct application of radiation to the affected area. Chemotherapy attempts to destroy tumour cells with drugs. However, all these procedures are identified by a relatively low efficacy and high toxicity for the patient. The prospect of treatment through immunotherapy is a relatively new regarding tumour treatment and offers promise due to the fact that it mimics the natural response to non-self entities.

Immunotherapy usually involves the use of cytokines together with adoptive cellular immunotherapy (ACI). Cytokines are protein hormones that mediate both natural and specific immunity \[77 \[98\]. Among the cytokines that have been evaluated in cancer immunotherapy are interferon-\(\alpha\) (IFN-\(\alpha\)), IFN-\(\beta\), and IFN-
The cytokines are produced mainly by activated T cells (lymphocytes) during the cell-mediated immune response. Interleukin-2 produced by CD4$^+$ T cells is the main cytokine responsible for lymphocyte activation, growth and differentiation. ACI refers to the injection of cultured immune cells with anti-tumour reactivity, into a tumour bearing host [77]. This type of treatment consists of two approaches:

- **LAK (lymphocyte-activated killer cell) therapy:** These cells are obtained from the high concentration of IL-2 in peripheral blood leukocytes taken from patients, via *in vitro* culturing. The LAKs are then injected back at the tumour site.

- **TIL (tumour infiltrating lymphocyte) therapy:** These cells are obtained from *in vitro* incubating with a high concentration of IL-2 lymphocytes recovered from the tumour itself and are comprised of activated NK cells and CTL cells. They are then injected back at the tumour site.

### 1.2.3 Summary

In this section we have presented a brief review of relevant biological literature. In particular, tumours themselves have been discussed in terms of classification, composition and means of affecting the host. The immune system has also been discussed and in particular we have described different types of immune response as well as different arms of the immune system and the associated cell types. Finally, a short overview of different types of tumours treatment, including the relatively new approach of immunotherapy, has been presented. This background information is important as it is referred to, built upon and relied on in the construction of the mathematical models presented later in this thesis. It is this information, along with further detail presented in the subsequent chapters, which will inform the biological relevance of the models.

### 1.3 The mathematical approach

In this thesis, two fundamentally different mathematical methodologies are employed in the modelling of tumour growth and subsequent immune system interactions. In particular, first we use ordinary differential equations to form a description of growth and interactions, that is then coupled to an optimal control strategy to investigate tumour treatment. Then we employ a hybrid cellular automata model to undertake an expanded investigation of the system.
The ordinary differential equations (ODEs) modelling approach are extremely well tested in this context (see the review presented in the coming sections), but while it has several strong points as a modelling strategy it is not without its disadvantages. Ordinary differential equations, particularly those traditionally used to model tumour growth (which are not terribly nonlinear) are fairly easily solved in the numerical sense as well as in many cases being amenable to analytical investigations such as via phase plane, stability and bifurcation analysis. ODEs allow the researcher to look at changes in the dynamics of the system in a sense that is similar to how experimental researchers conduct their investigations – that is, the natural output of a system of ODEs is the time course of each variable of interest, just as experimentalists record observations of a system over time. However, restricting a study to the use of ODEs imposes an assumption that the system is spatially well-mixed. This is of course problematic when spatial variation is important in a system. Furthermore, ODEs of the type used in most tumour modelling work only allow for consideration of population-level interactions. When individual interactions between cells and/or between cells and other matter are important, then the usefulness of ODE models is decreased.

More recently, discrete approaches such as cellular automata (CA) and hybrid cellular automata (HCA) models have been used for modelling tumour systems. While simple conceptually, such models do not facilitate analytical investigations except in the most basic (completely unrealistic) cases. However, CA models are straightforward to implement computationally and the lack of a means of general analysis is compensated by the ease with which the models can be simulated \textit{in silico}. Simple simulation also means that where system parameters are unknown, it is possible to computationally investigate the possible parameter space. A further advantage of CA models is that they do allow for individual level interactions to be captured. In particular, each individual cell can be investigated for interactions with other cells, other matter in the region, forces, chemical gradients and even internal variation, at any point in the progression of the model solution.

A review of some of the relevant existing mathematical models of tumour systems is now presented in the remainder of this section. In this review, some of the ideas discussed above are elaborated upon in the context of the studies that have already taken place. The review also demonstrates that the methodologies applied in the current research are well-placed in terms of existing work in the field. Presented first is a review of some of the earliest models of tumour growth based on nutrient diffusion, as well as an inspection of some cell migration models. Then models that incorporate the immune system and use an ODE approach are covered, including ODE models of tumour growth and the immune system that are coupled with optimal control analysis. Finally, we briefly discuss CA-based models of tumour-
1.3.1 Tumour growth models

The dynamics of tumour growth and the interactions of growing tumours with the host immune system have been a significant focus for mathematical modelling over the past four decades [89]. Araujo and McElwain [10] recently presented an excellent review of the mathematical modelling of tumour growth. Starting from early chemical diffusion and differential equation models of Burton [22] and Greenspan [63, 64], tumour modelling has expanded to include ordinary differential equations (ODE) models, partial differential equation (PDE) models [2, 14, 62, 89, 92, 107, 109, 128] and cellular automata (CA) models [89]. Increasingly, models are being explicitly tied to experimental results and data, for example, in [74], a mathematical model based on the diffusion of nutrient especially for oxygen and glucose is developed and is validated using in vitro tumour growth data.

Multicell spheroids have been studied for some time now as laboratory models for in vivo tumours and over the past few decades, they have become the focus of modelling efforts of applied mathematicians. Multicellular spheroids can consist of three distinct regions: a central necrotic core, an inner shell of quiescent cells and an outer shell of proliferating or viable cells. Among the earliest models of tumour spheroids was the work of Greenspan [63] who developed a simple model of growth depending on nutrient diffusion. Since then, many others have modelled various aspects of tumour spheroid growth including nutrient limited growth, immune system interactions, effects of stresses and cell migration (see for example, Pettet et al. [110]). Here we present a summary of the pioneering work of Greenspan to set the scene for the modelling to be considered in subsequent sections.

Greenspan (1972)

Greenspan proposed a simple mathematical model of tumour growth by diffusion in order to investigate the evolution of solid carcinoma [63]. The growth of a solid tumour in the earliest stage is regulated by the direct diffusion of nutrient and wastes, to and from surrounding tissue. By simple diffusion, each cell receives adequate nourishment when the size of the tumour is very small and the resulting growth rate of the population is exponential. There is a critical tumour size where the growth rate of the tumour then diminishes markedly. Greenspan describes the tumour shape as a sphere that consists of three layers: a central necrotic core, a layer of viable non-proliferating cells and the outer shell where all mitosis occurs. To model this tumour growth, the following assumptions are made.

1. The shape of a solid tumour is a sphere and complete spherical symmetry
prevails at all times.

2. When the concentration of a crucial nutrient falls below a critical level, tumour cells die.

3. The vital nutrient is consumed by living cells only.

To describe the characteristics of a growing tumour and the dormant steady state, there are some new hypotheses and approximations that must be added, such as adhesion, disintegration of necrotic cellular debris, and the production of a chemical that inhibits the mitosis of cancer cells without causing their death. Finally, it is assumed that the carcinoma is in a steady state of diffusive equilibrium at all times. To construct the model of tumour growth, a conservation of mass principle is applied such that

\[ A = B + C - D - E, \]  

(1.1)

where \( A \) represents the total volume of living cells at any time \( t \), \( B \) is the initial volume of living cells, \( C \) denotes the total volume of cells produced in \( t > 0 \), \( D \) is the total volume of necrotic debris at time \( t \) and \( E \) represents the total volume lost in the necrotic core in \( t > 0 \).

These terms can be written mathematically as

\[
A = \frac{4\pi}{3} (R_0^3(t) - R_i^3(t)), \\
B = \frac{4\pi}{3} (R_0^3(0)), \\
C = 4\pi \int_0^t dt \int_{\max(R_i(t), R_g(t))}^{R_0(t)} S(\sigma, \beta)r^2 dr, \\
D = \frac{4\pi}{3} R_i^3(t), \\
E = \frac{4\pi}{3} \int_0^t 3\lambda R_i^3(t) dt,
\]

where \( R_0(t) \) is the outer radius of the tumour at any time \( t \), \( R_i(t) \) is the radius of necrotic core, \( R_g(t) \) is the radius at which cell proliferation ceases, \( S(\sigma, \beta) \) is the proliferation rate of cells, \( \sigma \) and \( \beta \) represent the concentrations of nutrient and inhibitor respectively, and \( 3\lambda \) is the proportionality constant for the rate at which the necrotic core loses cell volume.

Substituting each of these expressions into equation (1.1) and differentiating the resulting equation with respect to \( t \) gives the integro-differential form of the outer boundary condition

\[
\frac{dR_0}{dt} = \int_{\max(R_1, R_g)}^{R_0} S(\sigma, \beta)r^2 dr - \lambda R_i^3,
\]
which relates the tumour radius to the volume production due to mitosis and volume loss due to necrosis.

In this paper, a model of growth retardation due to necrosis and wastes from living cells is also derived. For the growth retardation due to necrosis model, during the first phase, it is found that the tumour grows at an exponential rate until the first cell at the centre of the sphere dies due to lack of sufficient nutrient. In the second phase, the growth either tends to a final steady state or it terminates at some point. Finally, in the third phase, a period of retarded tumour growth occurs because of the death of cells and because chemical inhibition of mitosis is achieved. There are also three phases of development in tumours which exhibit growth retardation due to wastes from living cells. In the first phase, the tumour grows at an exponential rate until growth retardation occurs at the centre of the sphere. The growth rate changes from the initial exponential rate to one that is approximately linear in time in the second phase. In the last phase, this model indicates that the rate of volume loss per unit volume in the necrotic core controls the tumour growth resulting in a steady state tumour volume.

**Greenspan (1976)**

In a subsequent paper, Greenspan explained the distribution of nutrients related to the growth and movement of certain cell cultures and solid tumours [64]. He investigated the unstable development of tumours when the internal pressure forces overcome surface tension and adhesion. The processes and mechanisms of cell culture growth are very complex and in order to reproduce the main qualitative features of shape and structure, Greenspan made a number of simplifying assumptions. Some of these have been discussed by Greenspan in [63] (see above). Others include:

1. The culture and surrounding medium is essentially in diffusive equilibrium at all times. It is assumed that the tumour has two-layers: an outer shell and a larger core of necrotic debris.

2. When the available concentration of a vital nutrient, denoted by \( \sigma(x, y, z, t) \), falls below a critical level \( \sigma_1 \), cells begin to die. If \( h \) is the local thickness of the thin layer of living cells and this depth depends only on \( \sigma_1 \) and the value of \( \sigma \) at the outer surface of tumour, this relationship can be written as follows

\[
h = \begin{cases} 
\nu \sqrt{\sigma - \sigma_1}, & \sigma > \sigma_1 \\
0, & \sigma < \sigma_1.
\end{cases}
\]

3. The mitotic index is a constant.
4. Necrotic debris breaks down continually into simpler compounds.

5. The force of surface-tension $\Gamma$ is proportional to the mean deflection $\kappa$ of the boundary.

6. Internal pressure differentials which cause the motion of cellular material are produced by the birth or death of cells and it is assumed that

$$\mathbf{q} = -\nabla p,$$

where $\mathbf{q}(x, y, z, t)$ is the particle velocity and $p(x, y, z, t)$ is proportional to the internal pressure.

Using these assumptions, the mathematical model of cell cultures and solid tumours is formulated as

$$\nabla^2 p = S_i \text{ inside } \Gamma = 0,$$

$$\nabla^2 \sigma = 0 \text{ outside } \Gamma = 0,$$

where $S_i$ is the rate of volume loss per unit volume, and on $\Gamma(x, y, z, t) = 0$ we have

$$p = \frac{\alpha}{2}(\kappa_1 + \kappa_2) = \lambda \kappa,$$

$$\mathbf{q}_+ \cdot \mathbf{n} = -\mathbf{n} \cdot \nabla p + \lambda \sqrt{\sigma - \sigma_1},$$

$$\mathbf{q}_+ \times \mathbf{n} = -\nabla p \times \mathbf{n},$$

$$\mathbf{n} \nabla \sigma = \mu \sqrt{\sigma - \sigma_1}.$$

The bounding surface is defined by

$$\frac{d\mathbf{r}}{dt} = \mathbf{q}_+,$$

and the initial parametric prescription

$$\mathbf{r} = a(\xi, \zeta) \text{ at } t = 0.$$

This model describes the relationship between the nutrient concentration, the pressure on the surface and the surface-tension force. Greenspan states that if the tumour reaches a critical size beyond which surface tension is overcome by pressure forces, the tumour becomes unstable. It is shown that in the necrotic core, the propensity of the colony to distort by either growth or the elimination of material can reverse the effect of stability on the surface tension and on the other hand, by controlling the distribution of nutrient, a steady state equilibrium can be reached.

The work of Greenspan is important here as it provides the basis for much of the tumour modelling that followed. Ordinary and partial differential equation-based mathematical models of tumour growth, nutrient supply, contaminant removal and so on, provide a starting point for all of the mathematical models developed in this thesis.
1.3.2 Modelling of cell migration

The cell migration-dependent invasion of tumour cell colonies is examined by a number of authors (see for example [88, 107, 108, 109]). Mallet [88] for example, explained the role of haptotaxis in tumour growth. Perumpanani [107] constructed a mathematical model of tumour growth that considers the way in which chemotaxis and haptotaxis cooperate to regulate invasion, whereas in [109], Perumpanani and coauthors developed and analysed a model for malignant invasion that combined both proteolysis and haptotaxis. Integrins, as described in [90], also play an important role in cell migration and recently integrins have become the topic of some experimental investigations [113]. Below we discuss the background, construction and analysis of some of these models.

Perumpanani et al. (1998)

During the process of migration, cells use a combination of changes in adhesion, proteolysis and motility (directed and random). Haptotactic gradients, which cells use to move in a directed fashion, are produced by proteolysis of the ECM. The ability of the migratory cells to secrete enzymes and digest ECM as they move through it, is a critical feature of cell migration. Invasive cells in vivo bind to surrounding ECM molecules via receptors such as integrins. Perumpanani contrasts the terms haptotaxis and chemotaxis, noting that chemotaxis is used to describe cell motility caused by responses to gradients in soluble attractants, while haptotaxis describes motility towards insoluble, substratum-bound attractants such as laminin and fibronectin.

Perumpanani et al. [107] developed a mathematical model for the invasive process in one space dimension. They considered the way in which chemotaxis and haptotaxis cooperate to regulate invasion. The model is comprised of the partial differential equations

\[
\begin{align*}
\frac{\partial u}{\partial t} &= \text{cell division} - \text{chemotaxis} + \text{haptotaxis}, \\
\frac{\partial c}{\partial t} &= \text{proteolysis} - \text{diffusion}, \\
\frac{\partial s}{\partial t} &= \text{proteolysis} + \text{diffusion}, \\
\frac{\partial p}{\partial t} &= \text{MMP-2 production} - \text{MMP-2 degradation}.
\end{align*}
\]
where $u(x,t), c(x,t), p(x,t)$ and $s(x,t)$ represent cells, intact fibronectin, matrix metaloprotease-2 and the matrix metaloprotease-2 digested soluble fibronectin respectively. The functions $\psi(s)$ and $\chi(c)$ are the coefficients of chemotaxis and haptotaxis. The proteolysis of fibronectin is proportional to the interaction between protease $p$ and fibronectin $c$. The term $h(p,s)$ is used to represent the action of proteases.

Based on numerical simulation of this model, the authors obtained that by increasing chemotactic sensitivity the invasiveness of cells will decrease. There is dependence of the invasion speed on MMP-2 production with and without $h(p,s)$. In all cases, the authors concluded that the solution is a traveling wave moving in the positive $x$-direction and the model predicts that, counterintuitively, if the chemotactic coefficient $k_3$ is increased then the invasiveness will decrease. This implies that

“ECM chemotaxis actually inhibits invasion, contrary to its conception as a pro-invasive factor; this is because the gradient in degraded soluble ECM $s$ is in opposite direction to invasion. The predictions of the model depend crucially on the function of $h(p,s)$.” (p. 2349 in [107]).

If the $h(p,s)$ term is excluded from the model, by increasing the rate of protease production, $k_5$, then the invasiveness of the cell population will increase continuously. However, if $h(p,s)$ is included, the model estimates a decrease in invasiveness at high rates of protease production. The model of Perumpanani et al. predicts that invasion occurs when a cell is between two regions, namely, a haptotactic gradient of insoluble ECM and a chemotactic gradient of soluble ECM.

In this paper, the competing gradients of digested and undigested fibronectin are also described. The mathematical model predicts that digested fibronectin retards cell migration, that is, the cells tend preferentially to the undigested fibronectin if both the digested and undigested fibronectin are at low concentrations, but as the concentrations of both these forms of fibronecting are increased, the number of migrating cells decreases drastically and the migration levels will reduce. The results of this study show that combining anti-protease therapy with haptotactic blockade can effectively prevent the unintended augmentation of invasion caused by antiproteases.

**Perumpanani et al. (1999)**

Perumpanani et al. in [109] developed and analysed a model for malignant invasion that combined both proteolysis and haptotaxis. The process of malignant tumour migration into surrounding tissue can be divided into three main parts: adhesion,
proteolysis and migration. The model presented in this paper can be thought of as a reduced or simplified version of that in [107].

In this paper, the effects on malignant cells of haptotaxis and protease production are studied. The authors derived a model for invasion by a combination of haptotaxis and proteolysis based on a continuum approach in which \( u(x,t), c(x,t) \) and \( p(x,t) \) represent the concentration of the invasive cells, ECM and protease, respectively.

In developing the model of malignant invasion, Perumpanani et al. consider the movement of the cells spatially, as well as the proliferation of the malignant cells. They model spatial movement through a description of directed cell migration up an ECM gradient, represented by \( \frac{\partial c}{\partial x} \). This leads to a haptotactic cell movement term proportional to

\[
\frac{\partial}{\partial x} \left( u \frac{\partial c}{\partial x} \right).
\]

The increased proliferation of malignant cells relative to normal cells is assumed to obey a logistic-type growth. The motility of the ECM is negligible, since the movement of ECM elements occurs over a much longer timescale than that of cell migration and protease movement. Hence the authors model the dynamics of connective tissue by the activity of the tissue protease which is represented by the term \(-g(c,p)\). Perumpanani et al. assume that the protease decays linearly, with half-life \( \kappa \), and that protease diffusion is negligible. The authors introduce the function \( h(u,c) \) to represent the dependence of protease production on local concentration of tumour cells and ECM. Combining the above explanation, they present the model with the partial differential equations

\[
\begin{align*}
\frac{\partial u}{\partial t} &= f(u) - \frac{k_3}{\partial x} \left[ u \frac{\partial c}{\partial x} \right], \\
\frac{\partial c}{\partial t} &= -g(c,p), \\
\frac{\partial p}{\partial t} &= h(u,c) - \kappa p, \\
\end{align*}
\]

(1.2)

where \( f, g \) and \( h \) are increasing functions of \( u, c \) and \( p \).

The authors present a number of simple cases for the functions \( f, g \) and \( h \), then nondimensionalise and simplify the model in the limit of fast protease dynamics to present a parameter-free, two equation system to model tumour cell invasion. The model is given by the equations

\[
\begin{align*}
\frac{\partial u}{\partial t} &= u(1 - u) - \frac{\partial}{\partial x} \left[ u \frac{\partial c}{\partial x} \right], \\
\frac{\partial c}{\partial t} &= -uc^2. \\
\end{align*}
\]

(1.3)
Figure 1.2: The dimensionless tumour cell density resulting from the numerical solution of the Perumpanani et al. model given by equations (1.3) using MATLAB PDE solver pdepe for $t = 0, \ldots, 30$, $x = 0, \ldots, 20$, $n = 100$ spatial points (fine mesh, short domain) and $\delta t = 0.001$.

The stability and the numerical solution of this simplified model are analysed, including a traveling wave analysis. Numerical simulations suggest that the addition to the model of a small amount of cell diffusion results in no significant change in either the form or speed of the traveling wave solution corresponding to invasion, except that the discontinuity in the derivative of $u$ is lost. Numerical simulations are however easier to calculate with the addition of diffusion.

We present some numerical results for equations (1.3) using MATLAB’s in-built PDE solver pdepe. Figure 1.2 describes the migration of tumour cells at various times over the domain $x \in [0, 20]$ with 100 spatial discretisation points. It shows that there are some oscillations in the solution around the left end of the domain. These oscillations remain throughout the integration and are not dampened as $t$ increases, however these oscillations will decrease if we use a longer domain spatial domain (see Figure 1.3).

In Figure 1.3 we demonstrate the numerical result taken from pdepe using a
Figure 1.3: The dimensionless tumour cell density resulting from the numerical solution of the Perumpanani et al. model given by equations (1.3) using MATLAB PDE solver pdepe for $t = 0, \ldots, 30, x = 0, \ldots, 40, n = 40$ spatial points (coarse mesh, long domain) and $\delta t = 0.001$.

Figure 1.4 gives even better results. In this simulation we use the same domain and time length as in Figure 1.3, however here the mesh is refined to include $n = 100$ spatial points. Comparing the depth of migration at $t = 30$ for the simulations with $n = 40$ and $n = 100$, the front of tumour cells nearly reaches a point $x = 40$ in the first case, while for the second, the front has reached only as far as $x \approx 20$. This reflects the restriction that the mesh must be quite fine in order to obtain appropriate numerical solutions for models of this type using the solver pdepe.

While the solver pdepe can be used to obtain approximate solutions in some parameter ranges, it is not entirely suited to this hyperbolic PDE model. More appropriate results for PDE models such as these should be calculated using numerical solvers written by hand.
Figure 1.4: The dimensionless tumour cell density resulting from the numerical solution of the Perumpanani et al. model given by equations (1.3) using MATLAB PDE solver pdepe for $t = 0, \ldots, 30$, $x = 0, \ldots, 40$, $n = 100$ spatial points (fine mesh, long domain) and $\delta t = 0.001$. 
In this paper, a mathematical model of haptotaxis which includes the adhesion receptors known as integrins was developed. The model consisted of five species contributing to the temporal and spatial behaviour of the model system. In the model, they described both active and inactive integrins as continua in space and time. The non-dimensionalised model is given by

\[
\begin{align*}
\frac{\partial A}{\partial t} &= aIE - bA, \\
\frac{\partial I}{\partial t} &= \frac{\partial}{\partial x} \left( d_1 I \frac{\partial I}{\partial x} + d_{cN} I \frac{\partial N}{\partial x} - d_{c\eta} I \frac{\partial A}{\partial x} \right) - aIE + c(N + dA - I), \\
\frac{\partial N}{\partial t} &= \frac{\partial}{\partial x} \left( d_N N \frac{\partial N}{\partial x} - \bar{\eta} N \frac{\partial A}{\partial x} \right) + N(1 - N), \\
\frac{\partial E}{\partial t} &= fN(1 - N) - gEP, \\
\frac{\partial P}{\partial t} &= d_P \frac{\partial^2 P}{\partial x^2} + \mu NE - \nu P,
\end{align*}
\]

where \(N(x,t), E(x,t), P(x,t), A(x,t)\) and \(I(x,t)\) denoted the density of cellular material, ECM, protease, and functionally active and functionally inactive integrins respectively.

They found that there exists a traveling wave solution for the model equations where the minimum wave speed depends on the ratio of integrin binding to integrin unbinding. The numerical solutions showed a biphasic relationship between the depth of cell migration and the magnitude of haptotactic response, where the migration of cells was slowed by small haptotactic coefficients. They also developed a reduced three-equation model of haptotactic cell migration which gives a good approximation to the full model. Finally, a travelling wave analysis was investigated for a further simplified version of the cell migration model.

Eikenberry et al. (2009)

Eikenberry et al. in [49] presented a mathematical model of melanoma invasion incorporating healthy cells and the immune response. They develop a spatially explicit model using partial differential equations to explain the dynamics of melanoma invasion in the skin. Then, they extend this model to explain the effect of the immune system and different levels of immune response. To construct this model, they use the following assumptions.

- Cell motility depends on contact with other cells and oxygen concentration and it can be modelled using Fick’s Law.
- Oxygen is a growth limiting nutrient and diffuses into the skin from the skin surface.
• The amount of space available and the concentration of oxygen mediate the proliferation of healthy and cancerous cells.

• Cancer cells that die become necrotic debris.

• Cancer cells produce angiogenic factors.

• Endothelial cells migrate into the system in response to angiogenic factors and form the tumour vasculature.

• A basement membrane separates the epidermis from the dermis and restricts cell migration.

The basic model consists of seven variables, namely $c$, the tumour cell density, $h$, healthy cell density, $v$, the tumour angiogenic factor (TAF) density, $b$, the blood vessel endothelial cell density, $d$, the necrotic debris density, $r$, the partial pressure of oxygen, and $l$ the basement membrane (basal lamina) density. Using the assumptions and variables above, the mathematical model is constructed as a rather detailed seven equation reaction-diffusion model.

To see the effect of the cellular immune response, they extend the basic model by introducing two new variables, namely $m$, the cytotoxic immune cell density and $a$, the “immune attracting factor” (IAF) density. In simulations using the basic model, it can be shown that the tumour spreads throughout the dermis and forms a significant necrotic core. When an immune response is considered, it usually inhibits tumour growth, often destroying the invasive tumour or holding the tumour to a steady state for many years. Immune activation plays the dominant role in the migration of cells near the primary tumour.

This model provides a segue from the discussion of cell migration models to a much more closely relevant discussion of mathematical models of the immune response to growing tumours. This discussion is presented in the following section.

1.3.3 Modelling tumours and the immune system

As has already been noted, it is a fundamental driver for the work of this thesis that the role of the immune system in responding to growing tumours is still not fully understood. An understanding of the effects of the immune system on growing tumours may be aided by constructing and analysing a model of tumour immune interaction and calculating the parameters of the model using empirical data.

In recent years several researchers have developed mathematical models of various aspects of the immune response associated with tumour growth [2, 12, 39, 77, 78, 85, 89, 130]. A review of non-spatial mathematical models of tumour and immune system interactions can be found in Eftimie et al. [48]. Specific aspects
include lymphocyte diffusion, proliferation and migration in solid tumours [92].

Tumour growth coupled with immunotherapy has also been investigated using
ODE models [9, 19, 20, 27, 28, 37, 44, 45, 41, 70, 76, 77, 80, 99]. Furthermore,
umerical and bifurcation analyses of the interaction between the growing tumour
and oncolytic virus models have been demonstrated by Novozhilov et al. [103].

Mukhopadhyay and Battacharyya combine this model with the immune system
and analyse results based on a deterministic model [98]. Eikenberry et al. (dis-
cussed above) presented a mathematical model of melanoma invasion into healthy
cells and the subsequent immune response [49]. They developed a spatially explicit
model using partial differential equations to explain the dynamics of melanoma in-
vasion in the skin. They then extended this model to explain the effect of the
immune system in different levels of immune response. Mathematical models of
growing tumours coupled with macrophage response can be found in [17, 97, 104].

de Pillis et al. have developed mathematical models of tumour-immune system
interactions that concentrate on the role of certain effectors in anticancer responses
[37, 39]. A mathematical model describing multi-layered cell growth of a solid tu-
mour under control of an immune response such as that due to tumour-infiltrating
cytotoxic lymphocytes is investigated in [92]. This model also considers the spatio-
temporal dynamics of a solid tumour. de Pillis et al. in [39] focus on the role of
NK and CD8+ T cells in a mathematical model describing the interaction between
a growing tumour and immune system. Furthermore, a model of the interaction
of tumour growth with the immune system using NK cells as the innate immune
system and cytotoxic T lymphocytes as a specific immune system is developed in
[89].

In a study of tumour therapy, Kirkby et al. [75] proposed a mathematical
model of tumour cell response to radiotherapy. Immunotherapy using the cy-
tokine interleukin-2 (IL-2) has had the most impact for cancer treatment [23, 77].
Isaeva and Osipov [67] studied a dynamical model for the tumour-immune response
based on the interactions between interleukin-2 (IL-2) and intercellular cytokine-
mediated responses. They found that, for example, in the case of medium level
antigen presentation, the growth of a tumour depends on the initial tumour size
and the condition of the immune system. Currently, DCs are seen as a potential
mechanism for cancer therapy [25, 81, 118].

Wu et al. proposed a mathematical model that describes the dynamics of in-
teractions of CD8+ T cells and DCs in lymph node, however this model did not
include the growth of a tumour [132]. Most of the work of de Pillis and coauthors
[33, 34, 35, 36, 37, 39] describes a growing tumour interacting with an immune sys-
tem without DCs whereas in [25, 81] DCs are shown to play an important role in
tumour immunotherapy. Castiglione and Picolli in [25] constructed a mathemati-
cal model to investigate the effect of tumour immunotherapy, especially dendritic cell vaccine (DCV), for a generic solid avascular tumour. They applied the theory of optimal control to find the optimal approach to applying DCV. The model that they build consists of an immune system including CD4$^+$ T helper and CD8$^+$ T cells, but this model does not include NK cells.

Below, a more detailed summary is presented for a selection of models of the interaction between tumours and the immune system. These specific models are discussed as they form a framework for the ODE-based models to be developed and analysed in this thesis.

**Kirschner and Panetta (1998)**

Some research suggests that immunotherapy with the cytokine interleukin-2 may boost the immune system to fight tumour growth and in this paper due to Kirschner and Panetta, a mathematical model describing the dynamic interaction between tumour cells, immune effector cells and IL-2 is illustrated [77]. Also examined are the effects of adoptive cellular immunotherapy and the circumstances under which the tumour can be eliminated are described.

To construct the model, Kirschner and Panetta define three populations, namely the activated immune-system cells (called effector cells), $E(t)$, such as cytotoxic T-cells, macrophages, and NK cells that are cytotoxic to the tumour cells; the tumour cells themselves, $T(t)$; and the concentration of IL-2, $I_L(t)$. Kirschner and Panetta describe the interaction between the effector cells, tumour cells, and the cytokine IL-2 using the model

\[
\frac{dE}{dt} = cT - \mu_2 E + \frac{p_1 EI_L}{g_1 + I_L} + s_1,
\]

\[
\frac{dT}{dt} = r_2(T)T - \frac{aET}{g_2 + T},
\]

\[
\frac{dI_L}{dt} = \frac{p_2 ET}{g_3 + T} - \mu_3 I_L + s_2.
\]

The first equation describes the dynamics for the effector cell population. The Michaelis-Menten form in this equation indicates the saturation effects of the immune response. The average of the lifespan of the effector is $1/\mu_2$. The external source of effector cells such as LAK and TIL cells is represented by the constant influx term, $s_1$. The second equation explains changes in the tumour cell population as a result of growth proportional to $T$ and a Michaelis-Menten term reflecting the limited immune response to the tumour. The final equation gives the dynamics for the concentration of the IL-2. Again, Michaelis-Menten kinetics are used to describe the limiting production of IL-2, IL-2 is depleted at a constant rate $\mu_3$, and $s_2$ represents an external input of IL-2 into the system.
Kirschner and Panetta analyse the bifurcation behaviour of the model. It is found that in the absence of $s_1$ the trivial cancer-free steady state, $E_0$, is always unstable. In the presence of $s_1$, there is a more realistic tumour-free state, $(E_0, 0, 0)$. This implies that effector cells can clear the tumour if the equilibrium is stable. If the treatment is increased to very high levels then the tumour can be completely cleared. When $s_2$ is small, the dynamics are found to be similar to those of the untreated case. If $s_2$ is large, then the effector cells will grow uncontrolled and the only stable state is $(\infty, 0, s_2/\mu_3)$. Finally, they combine both adoptive cellular immunotherapy and IL-2, finding that the treatment will succeed for tumours of high antigenicity.

**de Pillis et al. (2001)**

De Pillis and Radunskaya [34] proposed a competition model of tumour growth that includes both an immune system and drug therapy, and which uses ordinary differential equations to describe the system dynamics. The model describing the interaction between immune cells, tumour cells and host cells and is represented by the system of differential equations

\[
\frac{dI}{dt} = s + \frac{\rho IT}{\alpha + T} - c_1 IT - d_1 I, \quad (1.4) \\
\frac{dT}{dt} = r_1 T(1 - b_1 T) - c_2 IT - c_3 TN, \quad (1.5) \\
\frac{dN}{dt} = r_2 N(1 - b_2 N) - c_4 TN, \quad (1.6)
\]

where $I(t)$ represents the number of immune cells at time $t$, $T(t)$ the number of tumour cells at time $t$, and $N(t)$ the number of normal, or host, cells at time $t$.

The source of the immune cells is considered to be outside of the system, so a constant influx rate, $s$, is assumed. Next, in the absence of any tumour, the cells will die off at per capita rate $d_1$. The presence of tumour cells stimulates the immune response, denoted by the positive nonlinear growth term for the immune cells,

\[
\frac{\rho IT}{\alpha + T},
\]

where $\rho$ and $\alpha$ are positive constants. Furthermore, the interaction of immune cells and tumour cells is reflected in both the death of tumour cells and the inactivation of immune cells, modelled using the terms $-c_1 IT$ and $-c_2 IT$. Proliferation of both the tumour cells and the normal cells is modelled by a logistic growth law, with parameters $r_i$ and $b_i$ denoting the per capita growth rates and reciprocal carrying capacities of the two types of cells.

The system of equations (1.4)-(1.6) models only the growth of cells and interactions between the immune system, host and tumour. To add the effect of the
drug therapy on the system, the function $u(t)$ is used to denote the amount of drug at the tumour site at time $t$. The authors assume that the drug response is represented by an exponential function of the form

$$F(u) = a(1 - e^{-ku}),$$

where $F(u)$ is the fraction cell kill for a given amount of drug, $u$, at the tumour site. Here, $a$ represents the kill rate coefficient for a particular cell type. de Pillis et al. take $k = 1$ and then the system with drug interaction is given by

$$\frac{dI}{dt} = s + \frac{\rho IT}{\rho + T} - c_1 IT - d_1 I - a_1 (1 - e^{-u}) I,$$

$$\frac{dT}{dt} = r_1 T (1 - b_1 T) - c_2 IT - c_3 TN - a_2 (1 - e^{-u}) T,$$

$$\frac{dN}{dt} = r_2 N (1 - b_2 N) - c_4 TN - a_3 (1 - e^{-u}) N,$$

$$\frac{du}{dt} = v(t) - d_2 u.$$

where the function $v(t)$ represents the rate at which the drug is delivered to the tumour site and $d_2$ is the decay rate for the drug.

From numerical simulations, it is found that the tumour-free equilibrium without providing drugs is locally stable if

$$r_1 < \frac{c_2 s}{d_1} + c_3.$$

This represents a comparison between the growth rate of the tumour cells, $r_1$, to the “resistance coefficient”, $c_2 s / d_1$, which describes how effectively the immune system competes with the tumour cells. Also, the authors found that the behaviour of the coexisting equilibrium is very sensitive to the value of $\rho$, the tumour response rate, and to $s_1$, the source rate of the immune cells.

In the optimal control model developed as part of this thesis, we will adopt this kind of modelling strategy and build upon this particular work to incorporate the different species considered in the preliminary models of this research.

de Pillis et al. (2005)

A mathematical model that describes tumour-immune system interactions, with a focus on the role of NK and CD8$^+$ T cells is presented in [39]. In their model, de Pillis et al. note a clear distinction between the dynamics of NK and CD8$^+$ T cells. The authors investigated the parameter estimation and model validation using data from published mouse and human experimental studies. They also conducted a sensitivity analysis for the model parameters. To construct this model, they consider three cell populations, namely tumour population at time $t$, denoted
$T(t)$, total level of NK cell effectiveness at time $t$, $N(t)$, and $L(t)$ representing the total level of tumour-specific CD8$^+$ T cell effectiveness at time $t$. The model is given by

\[
\frac{dT}{dt} = aT(1 - bT) - cNT - D, \\
\frac{dN}{dt} = \sigma - fN + \frac{gT^2}{h + T^2}N - pNT, \\
\frac{dL}{dt} = -mL + \frac{jD^2}{k + D^2}L - qLT + rNT,
\]

where

\[D = \frac{(L/T)^\lambda}{s + (L/T)^\lambda}T.\]

Here tumour cells proliferate logistically with growth rate $a$ and reciprocal carrying capacity $b$, are lysed at a rate given by $D$ (explained further below) and are destroyed by NK cells at a rate $c$. Natural killer cells are supplied constantly at a rate $\sigma$, die exponentially with rate constant $f$, and are destroyed in interactions with tumour cells at rate $p$ and are recruited to the site of the tumour at a rate proportional to $gT^2/(h + T^2)$. Finally, CD8$^+$ T cells die exponentially with rate constant $m$, are destroyed in interactions with tumour cells with rate constant $q$, and recruited by NK cells with rate constant $r$ and are also self-recruited at a rate proportional to $jD^2/(k + D^2)$.

The somewhat unusual $D$ term is used to represent the effect of enhancing ligand expression on tumour cells – this is of importance as a framework for constructing individual cell level interactions for the cellular automata modelling undertaken as part of the current research. de Pillis et al. found that traditional power-law representations did not fit well to available experimental results, while on the other hand, their newly introduced rational law, $D$, can predict cell lysis quite accurately. From sensitivity analyses, the authors found that the most significant parameters in the model were the tumour cell growth rate and $\lambda$, the power to which the ratio of CD8$^+$ T cells to tumour cells was raised in the term $D$.

Novozhilov et al. (2006)

Novozhilov et al. present a mathematical model of tumour therapy using oncolytic viruses that specifically target tumour cells – a promising anti-cancer therapeutic agent [103]. Oncolytic viruses are viruses that specifically infect and kill cancer cells but not normal cells. Such viruses have been shown to be relatively effective for reducing or eliminating tumours in clinical trials. To describe the spread of viral infection of the tumour, the authors introduce a functional response, which is related to the ratio of uninfected to infected tumour cells. The model of Novozhilov
\[ \begin{align*}
\frac{dX}{dt} &= r_1X \left( 1 - \frac{X + Y}{K} \right) - \frac{bXY}{X + Y}, \\
\frac{dY}{dt} &= r_2Y \left( 1 - \frac{X + Y}{K} \right) + \frac{bXY}{X + Y} - aY,
\end{align*} \]

where \( X \) is the size of the uninfected cell population, \( Y \) is the size of the infected cell population, \( r_1 \) and \( r_2 \) are the maximum per capita growth rates of uninfected and infected cells correspondingly, \( K \) is the carrying capacity for all cells, \( b \) is the transmission rate of the virus, and \( a \) is the rate at which infected cells are killed by the virus. Rescaling with

\[ x(\tau) = \frac{X(t)}{K}, \quad \gamma(\tau) = \frac{Y(t)}{K}, \quad \tau = r_1 t, \]

leads to the system

\[ \begin{align*}
\frac{dx}{d\tau} &= x(1 - (x + y)) - \frac{\beta xy}{x + y}, \\
\frac{dy}{d\tau} &= \gamma y(1 - (x + y)) + \frac{\beta xy}{x + y} - \delta y,
\end{align*} \] (1.7) (1.8)

where \( \beta = b/r_1, \gamma = r_2/r_1, \) and \( \delta = a/r_1. \)

The authors conduct a detailed bifurcation and phase plane analysis and it is found that, in a certain region of the parameter space, both infected and uninfected tumour cells can theoretically be eliminated in finite time (see Figure 1.5) – that is, clearance of the tumour as a result of the introduction of the oncolytic virus is possible.

de Pillis et al. (2009)

de Pillis et al. note that understanding the immune system is essential to understanding the growth of a tumour and if immunotherapy is to be used in a clinical setting, its dynamic interaction with chemotherapy and the tumour itself must be understood. In this paper, the authors update the model of de Pillis et al. to allow for endogenous IL-2 production, IL-2-stimulated NK cell proliferation and IL-2-dependent CD8\(^+\) T cell self-regulation.

To construct this model, they denote by \( T(t) \) the total tumour cell population, by \( N(t) \) the concentration of NK cells per litre of blood, by \( L(t) \) the concentration of CD8\(^+\) T cells per litre of blood, by \( C(t) \) the concentration of lymphocytes per litre of blood, not including NK cells and CD8\(^+\) T cells, by \( M(t) \) the concentration of chemotherapeutic drug per litre of blood, by \( I(t) \) the concentration of IL-2 per litre of blood, by \( v_L(t) \) the number of tumour-activated CD8\(^+\) T cells injected per day per litre of blood volume, by \( v_M(t) \) the amount of doxorubicin injected per
Figure 1.5: Phase portrait of the Novozhilov et al. model given by equations (1.7)–(1.8) using parameter values $\beta = 1.5$, $\gamma = 0.7$, and $\delta = 1$. A number of sample trajectories in the uninfected tumour cell–infected tumour cell phase space are presented and we observe for this parameter set the stability of the trivial steady state, reflecting clearance of the tumour.
day per litre of blood volume, and \( v_I(t) \) the amount of IL-2 injected per day per litre of blood volume.

The model is given by the relatively comprehensive system of ordinary differential equations

\[
\frac{dT}{dt} = aT(1 - bT) - cNT - DT - K_T(1 - e^{-\delta T M})T, \\
\frac{dN}{dt} = f \left( \frac{e^f}{f} C - N \right) - pNT + \frac{p_N NI}{g_N + I} - K_N(1 - e^{-\delta N M})N, \\
\frac{dL}{dt} = \frac{\theta_m L}{\theta + I} + \frac{T}{k + T} - qLT + (r_1 N + r_2 C)T - \frac{uL^2CI}{\kappa + I} \\
- K_L(1 - e^{-\delta L M})L + \frac{p_I LI}{g_I + I} + \nu_L(t), \\
\frac{dC}{dt} = \beta \left( \frac{\alpha}{\beta} - C \right) - K_C(1 - e^{-\delta C M})C, \\
\frac{dM}{dt} = -\gamma M + \nu_M(t), \\
\frac{dI}{dt} = -\mu_I I + \phi C + \frac{\omega LI}{\zeta + I} + v_I(t),
\]

where again the authors employ the ratio-form for the CD8\(^+\) T cell related killing of tumour cells, given by

\[
D = \frac{d(L/T)\lambda}{s + (L/T)\lambda}.
\]

Compared with the de Pillis et al. model presented in [37], the authors here remove the term, \( gT^2N/(h+T^2) \), from the model because of its previously observed insignificance within the context of the model system and due to the additional complexity it introduces to the form of the model. New terms were added however such as an IL-2 induced NK cell proliferation term, \( \frac{p_N NI}{g_N + I} \).

From the model simulation with no therapy, for large initial tumour size, the immune system is not able to destroy the tumour and the tumour cell populations grow to the high tumour equilibrium. However, when the chemotherapy is initiated, the tumour cell population is rapidly eliminated. For combined therapy, the tumour cells can also be destroyed, activated CD8\(^+\) T cells and NK cells drop slightly but still in agreement with Jurisitc et al. [71]. In this paper, they also present the sensitivity analysis for model parameters and found that the model is very sensitive to some of its parameters such as \( a, K_T, \delta_T, u, \gamma, d, \lambda \) and \( s \).

The authors suggest that if individual data for CD8\(^+\) T cell effectiveness in killing the tumour can be obtained, it will be possible to determine the feasibility of using immunotherapy to combat the growing tumour. Their model also indicates that if the CD8\(^+\) T cells kill tumour cells more effectively than immunotherapy, it may be more useful to give immunotherapy in conjunction with chemotherapy.
Whereas, immunotherapy might not be effective in eliminating the tumour cells if the patient has low immune efficacy.

Wilson and Levy (2012)

In a recent study, Wilson and Levy propose a mathematical model related to immunotherapy using transforming growth factor β (TGF-β) \[^{[129]}\]. They analysed the effect of anti-TGF-β treatment when combined with a vaccine as treatments for tumour growth. The model is constructed using the five ordinary differential equations

\[
\begin{align*}
\frac{dT}{dt} &= a_0T(1 - c_0T) - \delta_0 \frac{ET}{1 + c_1B} - \delta_0TV, \\
\frac{dB}{dt} &= a_1\frac{T^2}{c_2 + T^2} - dB, \\
\frac{dE}{dt} &= \frac{fET}{1 + c_3TB} - rE - \delta_0RE - \delta_1E, \\
\frac{dR}{dt} &= rE - \delta_1R, \\
\frac{dV}{dt} &= g(t) - \delta_1V,
\end{align*}
\]

where \(T(t)\) denotes the tumour size, \(B(t)\) represents TGF-β concentration, \(E(t)\) denotes activated cytotoxic effector cells, \(R(t)\) is regulatory T cells and \(V(t)\) is vaccine-induced cytotoxic effector cells.

The authors numerically solve four cases, namely, no treatment, vaccine treatment, anti-TGF-β treatment and combined treatment. The vaccine treatment and the anti-TGF-β treatment alone can not eliminate the tumour mass although it can reduce the tumour size. However, the solution of the model suggests that a combined anti-TGF-β and vaccine treatment can eliminate the tumour.

From a sensitivity analysis of the model, in the case of combined treatment, Wilson and Levy found that the system was sensitive to parameters such as the maximal production rate of TGF-β, \(a_1\); the size at which a tumour starts to produce TGF-β, \(c_2\); and the rate of antigenicity, \(f\).

1.3.4 Optimal control applied to tumour-immune system model

Over approximately the last decade, theoretical researchers have began investigating tumour treatment strategies. In particular, the ordinary differential equation models discussed already, have been coupled with chemotherapy and/or immunotherapy models. Mathematically, this type of investigation falls neatly into
the class of problems analysable via optimal control theory. Optimal control theory allows the researcher to investigate a dynamical system (such as a growing tumour) being affected by some controlling influence (such as a treatment), and then to optimise the application of that control such that some quantity is optimised (such as minimising tumour size at some end-time). In this section, we review a selection of such models that influence the model development in this thesis.

**de Pillis and Radunskaya (2003)**

de Pillis and Radunskaya [36] constructed a mathematical model of tumour growth and immune system interactions. This model consists of three populations of cells, namely, $I(t)$ denoting the number of immune cells at time $t$, $T(t)$ representing the number of tumour cells at time $t$, and $N(t)$ denoting the number of normal or healthy host cells at time $t$. While this in itself provides a fairly simple three ODE model of tumour-host interaction, the authors went on to add the effect of drug therapy to the system where the strategy of application of the drug was to be solved for. They then applied optimal control theory to solve the problem for a specified optimal situation. The aim of the optimal control problem is to determine the chemotherapy administration schedule that minimises the tumour cell population, while also minimising the destruction of normal cells.

To this end, they attempt to find the control, or drug administration schedule, $v(t)$, and free final time $t_f$ that minimises some objective functional

$$J(v, t_f) = \phi(x(t_f, t_f)),$$

subject to the state equations

$$\frac{dI}{dt} = s + \frac{\rho IT}{\rho + T} - c_1 IT - d_1 I - a_1(1 - e^{-u})I,$$

$$\frac{dT}{dt} = r_1 T(1 - b_1 T) - c_2 IT - c_3 TN - a_2(1 - e^{-u})T,$$

$$\frac{dN}{dt} = r_2 N(1 - b_2 N) - c_4 TN - a_3(1 - e^{-u})N,$$

$$\frac{du}{dt} = v(t) - d_2 u,$$

and a state constraint

$$N(t) \geq 0.75, \quad 0 \leq t \leq t_f,$$

which implies that normal cells must remain above some threshold level required for a minimal level of patient health. Here $u(t)$ represents the amount of drug in the system which is administered at rate $v(t)$ and decays naturally with rate constant $d_2$. The state equations represent the influx, recruitment and production of the various cells, the interactions between cells leading to tumour death.
and neutralisation or removal of healthy and immune cells, and the effects of the chemotherapeutic drug on each of the cell types.

The authors used a direct collocation method to solve the optimal control problem over a simulated time period of 150 days. From numerical simulations, they found that for a weaker immune system, traditional therapy fails to bring the system to the zero-tumour burden state. However, by applying the drug therapy using the optimal control solution it is shown that the tumour cell population is driven to zero, while keeping the normal cell population above the constraint level.

Burden et al. (2004)

Based on the Kirschner and Panetta model [77], Burden et al. [20] developed a model to determine, using optimal control, under what circumstances a tumour can be eliminated by an interleukin-based treatment. As described in section 1.3.3, the model includes the activated immune system cells, denoted \( x(t) \), the tumour cells, denoted \( y(t) \), and \( z(t) \) to denote the concentration of IL-2. The model has the form

\[
\begin{align*}
\frac{dx}{dt} &= cy - \mu_2 x + \frac{p_1 x z}{g_1 + z} + u(t)s_1, \\
\frac{dy}{dt} &= r_2(y)y - \frac{axy}{g_2 + y}, \\
\frac{dz}{dt} &= \frac{p_2 xy}{g_3 + y} - \mu_3 z + s_2,
\end{align*}
\]

where \( c \) represents the antigenicity of the tumour, \( \mu_2 \) is the natural death rate of the effector cells, \( p_1 \) and \( g_1 \) affect the shape and rate at which interleukin/effectector cell interactions recruit more immune cells, \( s_1 \) represents the strength of the treatment in activating immune cells and \( u(t) \) represents the control. The tumour cells proliferate at a rate given by \( r_2(y) \) and are killed by immune cells with rate constant \( a \) and at a rate shaped by \( g_2 \). The interleukin is constantly sourced from outside the system, decays naturally with rate constant \( \mu_3 \) and is produced by tumour/immune cell interactions with rate constant \( p_2 \) and shaping parameter \( g_3 \).

The objective functional for the problem is defined to be

\[
J(u) = \int_0^T \left[ x(t) - y(t) + z(t) - \frac{1}{2}B(u(t))^2 \right] dt,
\]

which reflects the intention to maximise the amount of effector cells and interleukin-2 as well as to minimise the number of tumour cells and the cost of the control. \( B \) represents a cost weighting factor.

From numerical solutions, run over a 350 day simulation period, it is found that the cancer cells can be controlled with the tumour reaching a peak between days 200 and 250 (using \( s_1 = 500, B = 5 \) and \( c = 0.25 \)). The authors also show the
delicate nature of the dynamics with respect to the system parameters, noting that
the cancer cells also can be controlled nearly all of the time, except approximately
at days 320, the tumour starts to grow again because of the unstable dynamics in
the system (using $s_1 = 500$, $B = 5$ and $c = 0.25$). Changing only the strength
coefficient for the treatment, namely $s_1 = 550$, the maximum drug level occurs
from days 5 to 100 and is able to keep the cancer within acceptable levels, with
a peak between days 200 to 250. From day 250 until at the end of the simulated
time interval, the tumour cells can be controlled.

**Castiglione and Piccoli (2006)**

A mathematical model that includes DCs was presented by Castiglione and Pic-
ccoli [26]. They investigated the effect of tumour immunotherapy using a DCV.
The model consists of five variables representing tumour cells and elements of the
immune system. The model can be written as

$$\begin{align*}
\frac{dH}{dt} &= a_0 - b_0 H + c_0 D \left( d_0 H \left( 1 - \frac{H}{f_0} \right) \right), \\
\frac{dC}{dt} &= a_1 - b_1 C + c_1 I (M + D) \left( d_1 C \left( 1 - \frac{C}{f_1} \right) \right), \\
\frac{dM}{dt} &= \left[ d_2 M \left( 1 - \frac{M}{f_2} \right) \right] - e_2 MC, \\
\frac{dD}{dt} &= -e_3 DC, \\
\frac{dI}{dt} &= a_4 HD - c_4 CI - e_4 I, \\
\end{align*}$$

(1.15)

where $H$ denotes the tumour-specific CD4$^+$ T helper cells, $C$ represents the tumour-
specific CD8$^+$ T cells or cytotoxic T lymphocytes, $M$ represents the cancer cells
which expose the tumour-associated antigen, $D$ denotes mature DCs which are
loaded with the antigen and $I$ represents interleukin-2 secreted by CD4$^+$ T cells
and responsible for tumour cell growth. The control variable is added to the fourth
of equations (1.15), and the optimal control problem requires finding $u$ such that

$$\varphi(x, (T, u)),$$

with $x(0) = x_0$, is minimised. Here $\varphi$ represents the final cost, $T$ is the terminal
time, $x$ denotes the cell population and $x_0$ is the initial condition. To solve this
problem, they use typical tools of optimal control to find the effect of the DC vac-
cine. They compute the gradient of the cost function with respect to the schedule
and use the steepest descent method for the optimization algorithm. The authors’
numerical results indicate that the treatment is always able to reduce the tumour
burden, however the extent of reduction varies.
de Pillis et al. (2007)

de Pillis et al. [40], also investigating tumour-immune system interactions, used quadratic and linear optimal control problems applied to find strategies for optimally administering treatment to a growing tumour. Their model consists of four populations including drug concentration, similar to their earlier work. The model describes the growth, death, and interactions of these populations with a chemotherapy treatment, and is given by

\begin{align*}
\frac{dT}{dt} & = aT(1 - bT) - c_1 NT - K_T MT, \\
\frac{dN}{dt} & = \alpha_1 - f N + g \frac{T}{h + T} N - pNT - K_N MN, \\
\frac{dC}{dt} & = \alpha_2 - \beta C - K_C MC, \\
\frac{dM}{dt} & = -\gamma M + V_M(t),
\end{align*}

where \( T(t) \) denotes the tumour cell population, \( N(t) \) represents the effector-immune cell population, \( C(t) \) is the circulating lymphocyte population and \( M(t) \) is the chemotherapeutic drug concentration.

The authors conduct a stability analysis showing that the \( T = 0 \) equilibrium point is locally asymptotically stable if

\[ a - \frac{c_1 \alpha_1 \gamma}{\gamma f + K_N V_M} - \frac{K_T V_M}{\gamma} < 0. \]

For other equilibrium points, the equilibrium locations and their stability were calculated numerically using Maple.

The objective functional employed by de Pillis et al. in this work is given by

\[ J(V_M) = \int_0^{t_f} \left( T(t) + \frac{\epsilon}{2} V_M^2(t) \right) dt, \]

and the aim of the optimal control problem, as modelled via this functional, is to minimise the tumour burden over the time interval while also minimising the total drug administered. The authors prove existence of an optimal control and also compare the results using a number of software packages, including Miser3, RIOTS and DIRCOL. The numerical solutions presented cover a predetermined (rather than free) time interval of 365 days. From the solution of the optimal control problem, it is shown that the initial level of drug administration is high for a short period of time, after which it falls rapidly. In contrast, the two immune cell populations, that is NK cells and circulating lymphocytes, continue to increase over time. The tumour population regrows if the low-dose of medicine is shut off. Clinically, this implies that a patient is required to take low-dose medication regularly for the rest of his or her life. Both of the models, linear and quadratic control, demonstrated
that the best way to fight the tumour is to provide a burst of treatment at the beginning of the treatment period.

**Castiglione and Piccoli (2007)**

Another mathematical model describing the interaction between cancer and the immune system was constructed by Castiglione and Piccoli [27]. They applied optimal control theory to answer the question of how to decide when and how much of an anticancer drug to inject so as to induce a prolonged and effective immune response to the cancer. In this model, the authors use a DCV as an immunotherapeutic treatment for cancer. The model is similar to that described in equations (1.15) as developed by the authors in [26]. Castiglione and Piccoli considered the optimal control problem using three methods, namely continuous-in-time controls with no jumps, impulsive controls and hybrid controls. From their numerical solutions, they summarised the results as follows. To reduce the tumour burden, a high dose of vaccination should be administered early in the treatment regime, then smaller doses of vaccination should be distributed over the remainder of the treatment. These findings are seen to be similar to those reported by de Pillis et al. above.

**Cappucio et al. (2007)**

Cappuccio et al. [24] also described a mathematical model related to cancer immunotherapy. They produced a model that used an optimal control approach to attempt to minimise the tumour growth and the toxicity of the drug to the patient. Unlike the previous model due to Castiglione and Piccoli [27], which optimised only the dosage of the vaccine to be administered to the site of the tumour, Cappucio et al. also optimise the timing of each drug administration. The mathematical model of the tumour-immune system to which they couple an optimal control problem, is based on the Kirschner and Panetta model [77]. They investigated three types of immunotherapy, namely, CTL therapy, IL-2 therapy and combined therapy. From the numerical analysis of their model, the authors found that the most successful method to control the tumour growth, keeping the tumour size below a stated threshold, is the combined CTL/IL-2 therapy. For monotherapy, IL-2 treatment is found to be less effective than CTL treatment.

**Ghaffari and Naserifar (2010)**

In 2010, Ghaffari and Naserifar [61] applied optimal control to a mathematical model of tumour-immune system interactions, again based on the Kirschner and Panetta model [77]. In their optimal control problem, Ghaffari and Naserifar
minimised the number of tumour cells during and at the end of the treatment. Such a strategy involves the definition of an objective functional of the form

\[ J(u) = -y(t_f) + \int_0^{t_f} \left[ x(t) - y(t) + z(t) - \frac{1}{2} B(u(t))^2 \right] \, dt. \]

where compared with the objective functional used by Burden et al. For example, Ghaffari and Naserifar added the term

\[ -y(t_f), \]

which introduces the possibility to minimise the tumour cell population at the end time in addition to the burden over time.

Numerical solutions indicate that the addition of this term to the objective functional allows a treatment strategy to be determined that causes the tumour cells to be eliminated in a significantly shorter time than in the work of Burden et al. with identical parameter values. Furthermore, the peak of tumour cells during therapy is smaller than that seen in [20].

### 1.3.5 Modelling tumours and the immune system using cellular automata

One of the earliest hybrid cellular automata models of tumour growth was constructed by Ferreira et al. Ferreira et al. produced a mathematical model for the growth of an avascular tumour, including cell proliferation, motility, and death, each of which is incorporated via a discrete, individual cell-based cellular automata. The hybrid nature of the model is due to the inclusion of continuous reaction-diffusion equations, used to model the concentration of nutrients that are considered essential and nonessential for cell proliferation. These nutrient concentration equations have the dimensionless forms

\[
\frac{\partial N}{\partial t} = \nabla^2 N - \alpha^2 N \sigma_n - \lambda_N \alpha^2 N \sigma_c,
\]

\[
\frac{\partial M}{\partial t} = \nabla^2 M - \alpha^2 M \sigma_n - \lambda_M \alpha^2 M \sigma_c,
\]

where \(N\) and \(M\) represent the concentrations of the two nutrients which diffuse in a Fickian manner, are consumed by tumour cells and also by healthy host cells. The equations are coupled with boundary conditions representing a constant source of nutrients via a blood vessel.

In the discrete cellular automata component, each tumour cell can undergo division, migration or death. The probability for cancer cell division is given by

\[
P_{\text{div}}(x) = 1 - \exp \left[ -\left( \frac{N}{\sigma_c \theta_{\text{div}}} \right)^2 \right],
\]
where $\theta_{\text{div}}$ controls the shape of the probability curve. The probability of cancer cell migration takes the form

$$P_{\text{mov}}(x) = 1 - \exp \left[ -\sigma_c \left( \frac{M}{\theta_{\text{mov}}} \right)^2 \right],$$

and the probability for cancer cell death is

$$P_{\text{div}}(x) = \exp \left[ - \left( \frac{M}{\sigma_c \theta_{\text{del}}} \right)^2 \right].$$

From numerical simulations, it is found that three patterns of tumour growth can be produced by the model, namely, compact tumours, ramified morphologies and disconnected patterns.

Based on this model, Mallet and de Pillis [89] presented a mathematical model of tumour immune-system interactions using a hybrid cellular automata model. In this model, the authors also employed reaction-diffusion partial differential equations to describe the distributions of nutrient species which are involved in the interaction between the immune system and the growing tumour. However they added to the Ferreira work by incorporating an immune system. In the absence of the immune system, the authors produced similar results as in the work of Ferreira et al.. That is, for lower nutrient consumption rates more compact tumours were simulated, whereas for higher consumption rates ‘branchier’ morphologies were produced. Also, they found that at the beginning of the growth period the tumour cells grow exponentially, then linearly until a steady-state of existence is reached. The numerical simulations demonstrated that there is oscillatory behaviour for the tumour and immune system cell populations which depend on the strength of the immune system parameters. Under certain conditions, the tumour can be eliminated, slightly oscillatory or grow unstable.

1.3.6 Summary

This brief literature review indicates that there is a significant body of research in the area of tumour growth incorporating interactions with host tissue and indeed more recently with the immune system. This review also suggests that the mathematical modelling of tumour-immune system interactions with specific focus on cells of the innate (NK cell and DCs) and specific immune system has not yet been fully studied. The new models developed as part of this research will expand upon existing models to provide a more complete picture of the interaction between growing tumours and the host immune system, especially with regard to individual level cell interactions and the potential for immunotherapeutic treatments.
1.4 Thesis structure

The remainder of the thesis comprises three chapters where the primary contribution to the field of research are presented. In particular, three new mathematical models of tumour growth and immune system interactions are presented.

Chapter 2 describes a mathematical model of the interaction between a growing tumour and cells of the innate (NK cells and DCs) and specific (CD8+ T cells) immune system. To describe this interaction, the model is comprised of four ordinary differential equations (ODEs). We analyse the stability of the model as well as the simple bifurcation behaviour. Numerical solution of the model is also carried out and allows for an investigation of the effect of DCs and other components of the immune system on the tumour cell population. Two mathematical models are presented here to give a different overview of the model. Before a more complex mathematical model is provided, first the preliminary model is constructed to serve as an introductory example to build upon. This model is extended for the optimal control model that will be presented in Chapter 3.

In Chapter 3, a DCV is added to the mathematical model that is built in the previous chapter. The aim of this model is to find the optimal DCV to be administered to the patient that minimises the tumour burden. To solve this problem, we apply optimal control theory to a modified version of the model given in Chapter 2. The proof of the existence of an optimal control is presented in this chapter. We solve the optimal control model using the forward-backward sweep method, which is also explained in the text. The effect of varying the amount of DCs to be administered to the tumour site is demonstrated.

Furthermore, a hybrid cellular automata model is presented in Chapter 4 to describe the interactions between tumour cells and the immune system in much more detail. This model allows us to investigate the complexity of the biological system, including cell-cell interactions of every single cell in the system. We include the effect of chemokines in the model by introducing two partial differential equations to describe their concentrations and changes due to interactions with the cellular species in the model. Then, stochastic rules based on descriptions of the various cell types comprising the host-tumour environment are introduced to the model via a cellular automata modelling strategy. We use the CA model to simulate the growth of a tumour in a number of computational “cancer patients” and present outcomes in a similar manner to Kaplan-Meier survival curves reported in clinical studies. We define “death” of a patient as the situation where the cells of the tumour reach the boundary of our model domain which effectively represents tumour metastasis.

Finally, Chapter 5, summarises the results of the thesis presented in Chapter 2-4. Before we conclude with a discussion for future research, contributions of this
research are presented in this chapter.
Chapter 2

ODE models of specific immune cell interactions
with a population of tumour cells

2.1 Introduction

In this chapter, a new mathematical model is constructed to provide a description of the interaction between a growing tumour and cells of the innate and specific immune system. Commonly, NK cells and CD8$^+$ T cells are included in mathematical models, because both of these cells can lyse tumour cells [15, 59]. However, in recent years, it has been reported that DCs can also lyse tumour cells [81]. Some tumours present DCs and the presence of such cells has a potential role in tumour control. In this model, we assume that NK and DCs, (the innate immune system), and CD8$^+$ T cells (the specific immune system) can kill tumour cells. To describe this interaction, the model is comprised of four ordinary differential equations. We analyse the stability of the model as well as the simple bifurcation behaviour. The numerical solution of the model is also considered. Before a more complex mathematical model is provided, a preliminary model is first constructed to serve as an introductory example to build upon. This model also forms the basis for the optimal control model that will be presented in the following chapter of this thesis.

As has already been noted in the introductory chapter, there is evidence that the immune system is capable of recognising and eliminating tumour cells [16, 31, 106, 122]. Because of this, much research has been done regarding the activation of the anti-tumour response and stimulation of the immune system with vaccines or by direct injection of T cells or cytokines.

Currently, the mechanisms involved in immune system interactions with tumour cells are still not fully understood. Mathematical models can provide important tools and information to support the early stages of development of biological tumour therapy. In particular, the model developed here is focused on the attack of
tumour cells by immune cells and how the behaviour of the tumour cells is changed because of the presence of these immune cells. We present a simple mathematical model of a growing tumour cell population incorporating an immune system comprised of NK cells, DCs and CD8\textsuperscript{+} T cells.

In recent years, DCs have been identified as an important component in controlling tumour growth. Also DCs have a potential role in directly killing cancer cells, besides their primary role in the regulation of the adaptive antitumour immune response [81]. This mechanism can be described as in Figure 2.1. Dendritic cells can stimulate resting NK cells, which in turn, after activation, may induce dendritic maturation. However, NK cells also can kill immature DCs in peripheral tissues [96].

This research presented in this chapter proposes a mathematical model to describe the dynamics of interaction of the growing tumour and the immune system. We focus on an immune system comprised of NK cells, DCs and CD8\textsuperscript{+} T cells. Wu et al. propose a mathematical model that describes the dynamics of interactions of CD8\textsuperscript{+} T cells and DCs in the lymph node, however this model does not include the
growth of the tumour. Most of the de Pillis papers such as [33, 34, 35, 36, 37, 39] present mathematical models of a growing tumour interacting with the immune system without DCs whereas experimental studies (see for example [51]) indicate that DCs play an important role in the modelled tumour immunotherapy. Castiglione and Picolli in [25] construct a mathematical model to investigate the effect of tumour immunotherapy, especially the dendritic cell vaccine (DCV), for a generic solid avascular tumour. They apply the theory of optimal control to find the optimal DCV. The model that they build consists of an immune system comprised of CD4+ T helper and CD8+ T cells, but this model does not include NK cells.

The approach adopted in this thesis is to build a mathematical model describing the dynamics of interaction between a growing tumour and the immune system. This model consists of four populations: tumour cells and three components of the immune system, namely DCs, NK cells and CD8+ T cells. In the subsequent work, we investigate the effect of DCs and hence, the immune system more generally, on the growing tumour in more detail than has been described in the literature above. Beside their function as an antigen presenting cells, DCs can also kill tumour cells directly. DCs can also be killed by NK cells and CD8+ T cells. Such a mathematical model of tumour-immune system interactions which specifically considers cells of the innate (NK cells and DCs) and specific immune systems (CD8+ T cells) has not yet been fully studied.

This chapter is organised as follows. In Section 2.2, we construct a simple mathematical model describing the dynamics of interaction between a population of tumour cells and three components of the host immune system. Then, steady state and stability analysis of the model is presented and discussed. In Section 2.3, a more complex model, that includes saturation effects regarding immune system recruitment, is presented and analysed in a similar manner to the introductory model. Finally, we discuss the results of both models in Section 2.4.

2.2 Initial mathematical model

The model consists of four populations, namely tumour cells, NK cells, DCs and CD8+ T cells. The model is described using a series of coupled ordinary differential equations where the populations of each cell type at time $t$ are denoted by:

- $T(t)$, tumour cells,
- $N(t)$, NK cells,
- $D(t)$, DCs,
• $L(t)$, CD8$^+$ T cells.

To construct our initial mathematical model describing a growing tumour interacting with the immune system, we use assumptions based on knowledge of the immune system and some assumptions stated in [35]. These assumptions can be summarised as follows.

• The growth of the tumour cell populations is assumed to be logistic in the absence of an immune response [35].

• NK cells, CD8$^+$ T cells and DCs can kill the tumour cells [43, 72, 81].

• As part of the innate immune response, NK cells and DCs are normally present in the body, even when no tumour cells are present [37].

• After some number of interactions with tumour cells, NK cells and CD8$^+$ T cells become inactive [3].

• Dendritic cells can prime and increase the activity of NK cells and CD8$^+$ T cells [30, 51, 58, 81, 96].

• NK cells can kill immature DCs [96].

• Mature CD8$^+$ T cells can clear out the DCs [25, 132].

Using these assumptions, the model takes the form

\[
\frac{dT}{dt} = aT(1 - bT) - (c_1N + jD + kL)T, \tag{2.1}
\]

\[
\frac{dN}{dt} = s_1 - c_2NT + d_1DN - eN, \tag{2.2}
\]

\[
\frac{dD}{dt} = s_2 - f_1LD - d_2DN - gD, \tag{2.3}
\]

\[
\frac{dL}{dt} = f_2DT - hLT - iL, \tag{2.4}
\]

where $a$ represents the per capita growth rate of tumour cells, $b^{-1}$ denotes the carrying capacity of tumour cells, $c_1$ and $c_2$ are the competitive coefficients between tumour cells and NK cells, $d_1$ and $d_2$ are the competitive coefficients between NK cells and DCs, $f_1$ represents the rate DCs are killed by CD8$^+$ T cells, $f_2$ represents the rate of CD8$^+$ T cell expansion due to the interaction between DCs and tumour cells, $s_1$ and $s_2$ are the constant sources of NK cells and DCs respectively. Parameters $e$, $g$ and $i$ are the per capita death rates of the NK cells, DCs and CD8$^+$ T cells respectively and $j$ is the coefficient of competition between tumour and DCs. Finally, $k$ denotes the coefficient of competition between tumour and CD8$^+$ T cells.
Proliferation of the tumour cells is modelled by a logistic growth law, with parameters $a$ and $b$ denoting the per capita growth rates and reciprocal carrying capacities of the tumour cells. The competition of NK cells and tumour cells can result in either the death of tumour cells or inactivation of the NK cells, resulting in two competition terms: $-c_1NT$ and $-c_2NT$. This form of competition also occurs between CD8$^+$ T cells and tumour cells resulting in the terms: $-kLT$ and $-hLT$. The competition of NK cells and DCs can result in activation of the NK cells, represented by $d_1ND$, also NK cells can kill DCs, $d_2ND$. The mature CD8$^+$ T cells can clear out the DCs, and the interaction between DCs and tumour cells can activate the CD8$^+$ T cells, resulting in terms $-f_1DL$ and $f_2DT$.

The general initial conditions of equations (2.1)–(2.4) are

$$T(0) = T_0, \quad N(0) = N_0, \quad D(0) = D_0, \quad L(0) = L_0,$$

where each of these initial values is non-negative and specific values are introduced later when numerical solutions are presented.

We have developed a new ODEs model describing the interaction between a growing tumour and the immune system. This model is not the same as that models described in Chapter 1. Kirschner and Panetta model \[77\] and Wilson and Levy model \[129\] proposed a mathematical model describing a growing tumour and the immune system, whereas these models do not include DCs. Most of de Pillis papers such as \[33, 34, 35, 36, 37, 39\] present mathematical models of a growing tumour interacting with the immune system without DCs. Experimental studies (see for example \[81\]) indicate that DCs play an important role in the modelled tumour immunotherapy. The model constructed by Castiglione and Picolli in \[25\] described a mathematical model of specific tumour cells interaction with immune system include the DCs, however our model describe more detail in DCs population. The model that we construct in this section also is not the same as other models in the literature.

### 2.2.1 Non-dimensionalisation

To facilitate model analysis, we non-dimensionalise the system as follows. Let the non-dimensionalised state variables be

$$\hat{T} = bT, \quad \hat{N} = \frac{d_2}{g}N, \quad \hat{D} = \frac{d_1}{g}D, \quad \hat{L} = \frac{f_1}{g}L, \quad \hat{t} = gt,$$

and the corresponding parameters be

$$a^* = \frac{1}{g}a, \quad c_1^* = \frac{c_1}{d_2}c_1, \quad c_2^* = \frac{1}{bg}c_2, \quad e^* = \frac{1}{g}e, \quad i^* = \frac{1}{g}i,$$

$$f_2^* = \frac{f_1}{bd_1g}f_2, \quad h^* = \frac{1}{bg}h, \quad j^* = \frac{s_2}{g^2j}, \quad s_1^* = \frac{d_2}{g}s_1, \quad s_2^* = \frac{d_1}{g}s_1.$$
Leaving the other parameters unchanged, and dropping the hats and stars for notational clarity, the non-dimensionalised system is given by

\[ \frac{dT}{dt} = aT(1 - T) - (c_1N + jD + kL)T, \tag{2.6} \]

\[ \frac{dN}{dt} = s_1 - c_2NT + DN - eN, \tag{2.7} \]

\[ \frac{dD}{dt} = s_2 - LD - DN - D, \tag{2.8} \]

\[ \frac{dL}{dt} = f_2DT - hLT - iL. \tag{2.9} \]

The general initial conditions of equations (2.6)–(2.9) are

\[ T(0) = T_0, \quad N(0) = N_0, \quad D(0) = D_0, \quad L(0) = L_0, \]

where each of these initial values is non-negative.

### 2.2.2 Steady state and stability analysis

To understand the behaviour of this model, first we analyse the dynamics of the system. Let \( E(T^*, N^*, D^*, L^*) \) be an equilibrium point of the system described by the equations (2.6)–(2.9). At an equilibrium point, we have

\[ \frac{dT}{dt} = \frac{dN}{dt} = \frac{dD}{dt} = \frac{dL}{dt} = 0. \]

As long as \( s_1 \neq 0 \) and \( s_2 \neq 0 \), there is no trivial equilibrium. That is

\[ E(T^*, N^*, D^*, L^*) \neq (0, 0, 0). \]

However, it is found that there are two tumour-free equilibrium points, namely, \( E_1 = (0, N^*, D^*_1, 0) \) and \( E_2 = (0, N^*, D^*_2, 0) \) where

\[ N^* = \frac{s_1}{e - D^*}, \]

\[ D^*_{1,2} = \frac{(s_2 + s_1 + e) \pm \sqrt{(s_2 + s_1 + e)^2 - 4(es_2)}}{2}. \]

These tumour-free equilibrium points have biological meaning or exist if,

1. \( (e - D^*) > 0 \), and
2. \( (s_2 + s_1 + e) \geq 2\sqrt{es_2} \).

This means that if the death rate of NK cells, \( e \), is greater than the value

\[ e_{\text{critical}} = D^*, \]
and if the source term of NK cells, $s_1$, is greater than the value

$$s_1\text{critical} = 2\sqrt{es_2} - (s_2 + e),$$

then all of the tumour-free equilibrium points have positive values, otherwise they do not have biological meaning, that is, the values are negative.

The Jacobian matrix for the linearisation of (2.6)–(2.9) about an equilibrium point, is given by

$$\begin{bmatrix}
1 - 2aT^* - c_1N^* - jD^* - kL^* & -c_1T^* & -jT^* & -T^* \\
-c_2N^* & -c_2T^* + D^* - e & N^* & 0 \\
0 & -D^* & -(L^* + N^* + 1) & -D^* \\
f_2D^* - hL^* & 0 & f_2T^* & -hT^* - i
\end{bmatrix}.$$  

Evaluating the Jacobian matrix at the general tumour-free equilibrium point reduces it to

$$J_1 = \begin{bmatrix}
a - c_1N^* - jD^* & 0 & 0 & 0 \\
-c_2N^* & D^* - e & N^* & 0 \\
0 & -D^* & -(N^* + 1) & -D^* \\
f_2D^* & 0 & 0 & -i
\end{bmatrix}.$$  

Henceforth we let submatrix $A_{11}$ be defined by

$$A_{11} = \begin{bmatrix}
D^* - e & N^* \\
-D^* & -(N^* + 1)
\end{bmatrix}.$$  

The eigenvalues of $J_1$ are given by

$$\lambda_1 = a - c_1N^* - jD^*, \quad \lambda_2 = -i,$$

and the eigenvalues of $A_{11}$ which are given by

$$\sigma(A_{11}) = \{\lambda_q| \det(A_{11} - \lambda I) = 0, \quad q = 3, 4\},$$

$$= \{\lambda_q|\lambda^2 - \text{tr}(A_{11})\lambda + \det(A_{11}) = 0, \quad q = 3, 4\}.$$  

Using the Routh-Hurwitz conditions, the eigenvalues of $A_{11}$ have negative real parts, that is, $\text{Re}\{\sigma(A_{11})\} < 0$, if $\text{tr}(A_{11}) < 0$ and $\det(A_{11}) > 0$. If all the eigenvalues of the Jacobian matrix $J_1$ are negative then the tumour-free equilibrium point is stable.

Since, from the existence conditions, the value of $D^* - e < 0$, the trace of matrix $A_{11}$, that is, $(D^* - e) - (N^* + 1)$ is always negative, and the determinant of matrix $A_{11}$ is always positive. This means that the eigenvalues of matrix $A_{11}$ are always negative. Furthermore, the tumour-free equilibrium points are stable only if

$$a - c_1N^* - jD^* < 0.$$
This means that if the rate of tumour growth, $a$, is greater than

$$a_{\text{critical}} = c_1 N^* - j D^*,$$

then the tumour cell population grows to the high equilibrium points, otherwise it can be eliminated.

We now analyse the steady-states when there are no source terms, that is both $s_1$ and $s_2$ are zero. There is one trivial equilibrium point where all the populations are zero, namely $E(T^*, N^*, D^*, L^*) = (0, 0, 0, 0)$. The Jacobian matrix here is given by

$$
\begin{bmatrix}
a & 0 & 0 & 0 \\
0 & -e & 0 & 0 \\
0 & 0 & -1 & 0 \\
0 & 0 & 0 & -i
\end{bmatrix}.
$$

Then the eigenvalues of the Jacobian matrix are $a$, $-e$, $-1$, and $-i$. Therefore, the steady-state (0,0,0,0) is always a locally unstable saddle point.

The equilibrium points representing a tumour coexisting with the immune system, $E_{\text{coexist}} = (T^*, N^*, D^*, L^*)$, can be found by solving for the solutions of the nonlinear simultaneous equations (2.6)–(2.9) with left sides set to zero. In this case, the values of the equilibrium must be solved for numerically. Similarly, the stability of the coexisting equilibrium points can be found by numerically calculating the eigenvalues of the Jacobian matrix.

From the above analysis, it can be concluded that

- If the source terms for both NK cells and DCs are nonzero, then the tumour cells can be eliminated if the tumour growth rate is less than the critical tumour growth rate, where $a_{\text{critical}} = c_1 N^* - j D^*$.

- If there is no constant source term, that is both of the source terms for NK cells and DCs are zero, then the tumour-free equilibrium is always unstable and the tumour grows without bound.

### 2.2.3 Numerical results

The solutions presented in this section are calculated using the `ode45` function in MATLAB to solve equations (2.1)–(2.4) along with initial conditions (2.14). Parameter values used in this simulation are given in Table 2.1. Maple is used to calculate the equilibrium points which must be solved for numerically. In the simulations shown we use initial values $T_0 = 100$, $N_0 = D_0 = L_0 = 1$ to investigate the effects on the growing tumour of initial values corresponding with a weak immune system.
<table>
<thead>
<tr>
<th>Param.</th>
<th>Description</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>Tumour growth rate</td>
<td>$4.31 \times 10^{-1}$</td>
<td>[37]</td>
</tr>
<tr>
<td>$b$</td>
<td>$b^{-1}$ tumour carrying capacity</td>
<td>$2.17 \times 10^{-8}$</td>
<td>[37]</td>
</tr>
<tr>
<td>$c_1$</td>
<td>NK cell tumour cell kill rate</td>
<td>$3.5 \times 10^{-6}$</td>
<td>[39]</td>
</tr>
<tr>
<td>$c_2$</td>
<td>NK cell inactivation rate by tumour cells</td>
<td>$1.0 \times 10^{-7}$</td>
<td>[37]</td>
</tr>
<tr>
<td>$d_1$</td>
<td>Rate of dendritic cell priming NK cells</td>
<td>$1.0 \times 10^{-6}$</td>
<td>Estimate</td>
</tr>
<tr>
<td>$d_2$</td>
<td>NK cell dendritic cell kill rate</td>
<td>$4.0 \times 10^{-6}$</td>
<td>Estimate</td>
</tr>
<tr>
<td>$e$</td>
<td>Death rate of NK cell</td>
<td>$4.12 \times 10^{-2}$</td>
<td>[79]</td>
</tr>
<tr>
<td>$f_1$</td>
<td>CD8$^+$T cell dendritic cell kill rate</td>
<td>$1.0 \times 10^{-8}$</td>
<td>Estimate</td>
</tr>
<tr>
<td>$f_2$</td>
<td>Rate of dendritic cell priming CD8$^+$T cell</td>
<td>0.01</td>
<td>Estimate</td>
</tr>
<tr>
<td>$g$</td>
<td>Death rate of dendritic cell</td>
<td>$2.4 \times 10^{-2}$</td>
<td>[132]</td>
</tr>
<tr>
<td>$h$</td>
<td>CD8$^+$T inactivation rate by tumour cells</td>
<td>$3.42 \times 10^{-10}$</td>
<td>[37]</td>
</tr>
<tr>
<td>$i$</td>
<td>Death rate of CD8$^+$T cells</td>
<td>$2.0 \times 10^{-2}$</td>
<td>[37]</td>
</tr>
<tr>
<td>$j$</td>
<td>Dendritic cell tumour cell kill rate</td>
<td>$1.0 \times 10^{-7}$</td>
<td>Estimate</td>
</tr>
<tr>
<td>$k$</td>
<td>NK cell tumour cell kill rate</td>
<td>$1.0 \times 10^{-7}$</td>
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<tr>
<td>$s_1$</td>
<td>Source of NK cells</td>
<td>$1.3 \times 10^4$</td>
<td>[79]</td>
</tr>
<tr>
<td>$s_2$</td>
<td>Source of dendritic cell</td>
<td>$4.8 \times 10^2$</td>
<td>[132]</td>
</tr>
</tbody>
</table>

Table 2.1: Parameter values and their associated sources used in numerical solutions in the present research.

Most of the parameters here have been taken from [37] which have been fitted to the experimental data of Diefenbach [43]. Most of these parameters also have been used for numerical simulation of the modelling of immunotherapy and chemotherapy of tumours in [37].

From the earlier analysis, it is found that with parameter values as reported in Table 2.1, the tumour-free equilibrium is

$$E_0 = (0, 3.1839 \times 10^5, 3.6992 \times 10^2, 0),$$

which is a stable equilibrium point. Using parameter values from Table 2.1, it is found that there are no equilibrium points with coexisting tumour and immune cell populations that have biological meaning.

Figure 2.2 shows the evolution of the tumour cells, NK cells, DCs and CD8$^+$T cells, over time. The tumour cells grow to the maximum value, then can be eliminated after a couple of days, see Figure 2.2 (top left). This means that in this case a small immune system is able to clear the tumour cells. NK cells gradually increase to the maximum value and remain constant to the steady state. Initially,
Figure 2.2: The evolution of tumour cells, NK cells, DCs and CD8$^+$ T cells using parameter values from Table 2.1 with the model given by equations (2.1)–(2.4), showing a tumour cell population which grows initially to a peak prior to full immune clearance.

DCs quickly increase to a high value then as soon as the population of CD8$^+$ T cells increase, the DCs drastically decrease because immature DCs are killed by CD8$^+$ T cells. This decreases the tumour population followed by decreasing CD8$^+$ T cells and as a result DCs tend to a steady-state indicating that CD8$^+$ T cells can affect the growth of DCs.

Using parameter values from Table 2.1 bifurcation occurs at $c_1 \approx 1.3536 \times 10^{-6}$. After bifurcation, tumour cells go to the high tumour equilibrium, even if we start with one tumour cell. However, before bifurcation the tumour cell population can be eliminated, as shown in Figure 2.2 (top left).

The evolution of the tumour cells, NK cells, DCs and CD8$^+$ T cells, over time, with $c_1 = 3.5 \times 10^{-7}$ (lower than the critical value for clearance of the tumour cells) can be seen in Figure 2.3. In this case, the tumour cells cannot be destroyed. There is oscillation at the beginning of the growth period, then the tumour cell population remains constant after approximately 500 days.
Figure 2.3: The evolution of tumour cells, NK cells, DCs and CD8+ T cells using parameter values from Table 2.1 except for $c_1 = 3.5 \times 10^{-7}$, with the model given by equations (2.1)–(2.4), showing a tumour cell population which grows initially to a peak prior to an oscillatory response to the immune system and reaching a final, non-zero equilibrium.
DCs play an important role in the activation of the immune system since DCs can activate NK cells and CD8$^+$ T cells. Figure 2.4 and 2.5 show the effect of varying the source term for DCs on the resulting population of NK cells and CD8$^+$ T cells. Increasing the source term of DCs causes increases to the NK cells and CD8$^+$ T cells populations. Furthermore, these can affect the growth of tumour cells since both NK and CD8$^+$ T cells can lyse tumour cells. Figure 2.5 shows the quite significant role played by DCs in recruiting CD8$^+$ T cells, especially early in the tumour growth period. Finally, increasing the source term for DCs will decrease the growth of tumour cells as shown in Figure 2.6. This indicates that an external source of DCs might be used as treatment if the patient lacks sufficient DCs in their body. From these simulations, it is shown that DCs alone are not fully effective for tumour treatment. It is also necessary that the NK cell population is high enough for an effective anti-tumour response. Finally, we note that while the peak of the tumour cell population is decreased, the time to clearance is actually not altered significantly.

From these numerical results we have found that, if the rate, $f_1$, at which CD8$^+$ T cells kill DCs is big enough, the DC population decreases in very short time. It can reduce the activation of NK cells and CD8$^+$ T cells that cause the tumour cells to grow to the higher value. This result can explain that CD8$^+$ T cells can inhibit the function of DCs as an antigen presenting cell (see Figure 2.7).

While we have obtained quite useful results with this simple, initial model, it does have its shortcomings. An important drawback is that if the rate at which NK cells kill DCs is very small or the constant source term of DCs is large enough, the population of NK cells will grow in an uncontrolled manner (see Figure 2.8). This problem can be avoided by introducing to the rate of NK cell activation, a saturation effect of DCs (rather than a simple linear interaction). We revise our model in the next section to overcome these inconsistencies.

### 2.3 A model incorporating Michaelis-Menten dynamics

In this section, we build on the findings of the previous section to construct a more complex mathematical model that incorporates saturation effects in a number of dynamic interactions. In particular, the kinetic terms used to model the activation of NK cells, the recruitment of DCs and the activation of CD8$^+$ T cells will all be modified from the linear interaction terms of the previous section and instead be described using Michaelis-Menten saturating kinetics.

As described in section 2.2.3 in certain parameter regimes, the NK cell population can grow in an uncontrolled manner. To overcome this problem, here we
Figure 2.4: The evolution of NK cells showing the effect of varying the source term of DCs in numerical solutions of the model given by equations (2.1)–(2.4). Increasing the source term of DCs increases the peak NK cell population due to the NK cell recruitment role played by DCs and leads to more effective tumour cell clearance. Other parameters for these simulation taken from Table 2.1 with values of $s_2$ as indicated on the graph.
Figure 2.5: The evolution of CD8$^+$ T cells in numerical solutions of the model given by equations (2.1)-(2.4), showing that increasing the source term of DCs increases the peak CD8$^+$ T cell population again due to the role played by DC-CD8$^+$ T cell interactions in activating the T cells. The effect here is more pronounced than it was for NK cells. Other parameters for these simulation taken from Table 2.1 with values of $s_2$ as indicated on the graph.
Figure 2.6: The evolution of tumour cells in numerical solutions of the model given by equations (2.1)–(2.4), showing the effect of varying the source term of DCs. Increasing the source term of DCs decreases the peak tumour cell population not only due to an increased DC population but also the increases in NK and CD8+ T cells observed in Figures 2.4 and 2.5. Other parameters for these simulations taken from Table 2.1 with values of $s_2$ as indicated on the graph.
Figure 2.7: The evolution of tumour cells in numerical solutions of the model given by equations (2.1)–(2.4), showing the effect of varying the rate at which CD8$^+$ T cell kill DCs, $f_1$, with $s_1 = 5.000$. Other parameters for these simulation taken from Table 2.1 with values of $f_1$ as indicated on the graph.
Figure 2.8: The evolution of NK cells of the model given by equations (2.1)–(2.4) show the population of NK cell growing in an uncontrolled manner.
include saturation of the effect due to DCs in equation (2.11) such that NK cells are activated by DCs through a term of the form

$$\frac{DN}{m_1 + D}.$$

By adjusting the value of $m_1$, the rate of NK cell population growth can be controlled. This form is a modification of the familiar Michaelis-Menten term (see for example [77, 79, 37]).

Similarly, the recruitment of DCs is affected by the number of tumour cells and this will now be reflected by a Michaelis-Menten term included in equation (2.12), namely

$$\frac{DT}{m_2 + T}.$$

CD8$^+$ T cells are also activated by DCs that have come in contact with tumour cells and this is incorporated in the model through a further Michaelis-Menten term,

$$\frac{DT}{m_3 + T},$$

to provide saturation in the effect of the tumour cells (see equation (2.13)). As a result of these changes, the revised model can be written as

$$\frac{dN}{dt} = s_1 - c_2 NT + d_{11} \frac{DN}{m_1 + D} - eN,$$

$$\frac{dD}{dt} = s_2 + l \frac{DT}{m_2 + T} - f_1 LD - d_2 DN - gD,$$

$$\frac{dL}{dt} = f_{22} \frac{DT}{m_3 + T} - hLT - iL,$$

where $d_{11}/m_1$ represents the maximum rate of NK cell activation, $l/m_2$ represents the maximum dendritic cell recruitment rate by tumour cells, $f_{22}/m_3$ denotes the maximum rate of CD8$^+$ T cell activation. Parameters $m_1$, $m_2$ and $m_3$ control the steepness of the NK cell activation curve, the tumour cell recruitment curve and the CD8$^+$ T cell activation curve, respectively. The remaining parameters are the same as in Section 2.2.3.

The general initial conditions of equations (2.10)–(2.13) are, as in the previous section,

$$T(0) = T_0, \quad N(0) = N_0, \quad D(0) = D_0, \quad L(0) = L_0,$$

where each of these initial values is non-negative and where specific values will be introduced in the numerical solutions.
2.3.1 Steady state and stability analysis

To understand the behaviour of this model, first we analyse the dynamics of the system. Let $E(T^*, N^*, D^*, L^*)$ be an equilibrium point of the system described by the equations (2.10)–(2.13). At an equilibrium point, we have

$$\frac{dT}{dt} = \frac{dN}{dt} = \frac{dD}{dt} = \frac{dL}{dt} = 0.$$ 

Provided $s_1 \neq 0$ and $s_2 \neq 0$, again we see that there is no trivial equilibrium. That is

$$E(T^*, N^*, D^*, L^*) \neq (0, 0, 0, 0).$$

However, for this revised model, it is found that there is only one tumour-free equilibrium point, namely, $E_{01} = (0, N^*, D^*, 0)$ where

$$D^* = \frac{s_2}{d_2 N^* + 1},$$

$$N^* = \frac{(d_2 s_1 m_1 + d_11 s_2 - e m_1 - e s_2) + \sqrt{A}}{2d_2 e m_1},$$

and

$$A = (d_2 s_1 m_1 + d_11 s_2 - e m_1 + e s_2)^2 + 4 (e d_2 e m_1) (s_1 m_1 + s_1 s_2).$$

The Jacobian matrix of the linearisation of system (2.10)–(2.13) about an equilibrium point is given by

$$J = \begin{bmatrix}
B & -T^* & -jT^* & -T^* \\
-c_2 N^* & -c_2 T^* + \frac{d_11 D^*}{m_1 + D^*} - e & m_1 d_11 N^* & 0 \\
\frac{m_2 D^*}{(m_2 + T^*)^2} & -d_2 D^* & C & -f_1 D^* \\
\frac{m_3 f_22 D^*}{(m_3 + T^*)^2} - hL^* & 0 & \frac{f_22 T^*}{m_3 + T^*} & -hT^* - i
\end{bmatrix},$$

where

$$B = (a - 2a T^* - c_1 N^* - jD^* - L^*),$$

and

$$C = \left( \frac{IT^*}{m_2 + T^*} - f_1 L^* - d_2 N^* - 1 \right).$$

The Jacobian matrix about the tumour-free equilibrium point, $E_{01} = (0, N^*, D^*, 0)$, is given by

$$J_2 = \begin{bmatrix}
a - c_1 N^* - j D^* & 0 & 0 & 0 \\
-c_2 N^* & \frac{d_11 D^*}{m_1 + D^*} - e & \frac{m_1 d_11 N^*}{(m_1 + D^*)^2} & 0 \\
\frac{I D^*}{m_2} & -d_2 D^* & -d_2 N^* - 1 & -f_1 D^* \\
\frac{f_22 D^*}{m_3} & 0 & 0 & -i
\end{bmatrix}.$$
Henceforth we let $A_{22}$ define the submatrix

$$A_{22} = \begin{bmatrix} \frac{d_{11}D^*}{m_1 + D^*} - e & \frac{m_1 d_{11}N^*}{(m_1 + D^*)^2} \\
-m_1D^* & -d_2D^* - d_2N^* - 1 \end{bmatrix}. $$

The eigenvalues of $J_2$ are given by

$$\lambda_1 = a - c_1N^* - jD^*, \quad \lambda_2 = -i,$$

and the eigenvalues of $A_{22}$ are given by

$$\sigma(A_{22}) = \{\lambda_q | \det(A_{22} - \lambda I) = 0, \quad q = 3, 4\},$$

$$= \{\lambda_q | \lambda^2 - \text{tr}(A_{22})\lambda + \det(A_{22}) = 0, \quad q = 3, 4\}.$$

Using the Routh-Hurwitz conditions, the eigenvalues of $A_{22}$ have negative real parts, that is, $\text{Re}\{\sigma(A_{22})\} < 0$, if $\text{tr}(A_{22}) < 0$ and $\det(A_{22}) > 0$. If all the eigenvalues of the Jacobian matrix $J_2$ are negative then the equilibrium point is stable.

If we take $d_{11} < e$ then the we have

$$\frac{d_{11}D^*}{m_1 + D^*} - e < 0.$$

Furthermore the trace of matrix $A_{22}$ is negative, that is

$$\left(\frac{d_{11}D^*}{m_1 + D^*} - e\right) - (d_2N^* + 1) < 0,$$

and also the determinant of matrix $A_{22}$ is always positive. This means that the eigenvalues of matrix $A_{22}$ are always negative. Finally, we have then that the tumour-free equilibrium points are stable if

1. $d_{11} < e$

2. $a - c_1N^* - jD^* < 0.$

This means that if the rate of tumour growth, $a$, is less than

$$a_{\text{critical}} = c_1N^* - jD^*,$$

and the rate of NK cell activation is less than the death rate of NK cells, then the tumour cell can be eliminated, otherwise it grows to the nonzero equilibrium value.

The equilibrium point corresponding with coexistence of a tumour cell population and the immune system, $E_{\text{coexist}} = (T^*, N^*, D^*, L^*)$, can be found by solving for the solutions of the nonlinear simultaneous equations (2.10)–(2.13) with left sides set to zero. In this case, the values of the equilibrium must be solved for numerically. Similarly, the stability of the coexisting equilibrium points can be found by numerically calculating the eigenvalues of the Jacobian matrix.
### Table 2.2: Parameter values used in numerical solution of equations (2.10)–(2.13) in addition to those in Table 2.1.

<table>
<thead>
<tr>
<th>Param.</th>
<th>Description</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{11}$</td>
<td>Rate of NK cell activation</td>
<td>0.05</td>
<td>Estimate</td>
</tr>
<tr>
<td>$f_{22}$</td>
<td>Rate of CD$^+$T cell activation</td>
<td>0.01</td>
<td>Estimate</td>
</tr>
<tr>
<td>$l$</td>
<td>DC recruitment rate by tumour cells</td>
<td>0.01</td>
<td>Estimate</td>
</tr>
<tr>
<td>$m_1$</td>
<td>NK cell activation rate steepness coeff.</td>
<td>$1.0 \times 10^4$</td>
<td>Estimate</td>
</tr>
<tr>
<td>$m_2$</td>
<td>Tumour cell recruitment rate steepness coeff.</td>
<td>$1.0 \times 10^4$</td>
<td>Estimate</td>
</tr>
<tr>
<td>$m_3$</td>
<td>CD$^+$T cell activation rate steepness coeff.</td>
<td>$3.0 \times 10^3$</td>
<td>Estimate</td>
</tr>
</tbody>
</table>

#### 2.3.2 Numerical results

Similarly to Section 2.2.3, the solutions presented in this section are calculated using the `ode45` function in MATLAB to solve equations (2.10)–(2.13). MAPLE is again used to calculate the equilibrium points which must be solved for numerically. Parameter values used in this section are given in Table 2.1 and Table 2.2.

From the above analysis, it is found that the tumour-free equilibrium for the tabulated parameters is

$$E_{01} = (0, 3.2934 \times 10^5, 3.5784 \times 10^2, 0),$$

and this, as demonstrated in the previous section, is a stable equilibrium point. Using parameter values from Table 2.1 and 2.2, it is found that there are two equilibrium points with coexisting tumour and immune cell populations that have biological meaning, specifically

$$E_u = (7.0431 \times 10^5, 1.2122 \times 10^5, 9.6188 \times 10^2, 4.7320 \times 10^2),$$

which is unstable, and

$$E_s = (4.4741 \times 10^7, 2.9000 \times 10^3, 1.8710 \times 10^4, 5.2996 \times 10^3),$$

which is a stable equilibrium point.

Figure 2.9 shows the evolution of the tumour cells, NK cells, DCs and CD$^+$T cells, over time, using parameters from Table 2.1 and 2.2. The tumour cells grow to their maximum value and then are eliminated after approximately 40 days, as shown in Figure 2.9 (top left). NK cells gradually increase to their maximum value, however, CD$^+$T cells move away from the tumour location (decrease) as soon as the tumour cells are reduced in number. Dendritic cells increase rapidly in very
Figure 2.9: The evolution of tumour cells, NK cells, DCs and CD8$^+$ T cells found by numerically solving the model given by equations (2.10)–(2.13) using parameter values from Tables 2.1 and 2.2, showing a tumour cell population which grows initially to a peak prior to full clearance by the combined immune system.

short time and after reaching a peak, decrease again to a steady-state. When the tumour cells are eliminated, NK cells remain at a maximum level, however DCs and T cells approach a lower equilibrium value.

Using parameter values from Table 2.1 and 2.2, bifurcation occurs at $c_1 = 1.62509 \times 10^{-6}$.

Below the bifurcation values, the tumour cell population reaches the nonzero tumour equilibrium, even if the system starts with one tumour cell (see Figure 2.10). However, above the critical $c_1$ value, the population of tumour cells that grows from the initial single cell can be eliminated, as shown in Figure 2.11.

The evolution of the tumour cells, NK cells, DCs and CD8$^+$ T cells, with $c_1 = 3.5 \times 10^{-7}$ (a value below the bifurcation value) and $l = 0.1$ is shown in Figure 2.12. In this case, the tumour cells cannot be destroyed. There is oscillation
Figure 2.10: In the numerical solution of the model given by equations (2.10)–(2.13), using parameters from Table 2.1 and 2.2 except for \( c_1 \), bifurcation occurs at \( c_1 = 1.62509 \times 10^{-6} \). Shown here is that below the bifurcation value, for example \( c_1 = 1.6250 \times 10^{-6} \), one tumour cell, \( T(0) = 1 \), grows to the nonzero tumour equilibrium.
Figure 2.11: In the numerical solution of the model given by equations \((2.10)-(2.13)\), using parameters from Table 2.1 and 2.2 except for \(c_1\), bifurcation occurs at \(c_1 = 1.62509 \times 10^{-6}\). In contrast to Figure 2.10 shown here is that above the bifurcation value, for example \(c_1 = 1.6251 \times 10^{-6}\), an initial population \(T(0) = 1\) grows but is then controlled by the immune system, returning to a stable zero tumour equilibrium.
Figure 2.12: Numerical solution of equation (2.10)–(2.13) using data from Table 2.1 and 2.2 except for $c_1 = 3.5 \times 10^{-7}$ and $l = 0.1$. Here the tumour grows to a high peak population initially before the immune system is primed. The immune system is only able to reduce the tumour population rather than clear it completely.

in the four populations of cells near the beginning of solution time period and following initial growth of the tumour population to a high level. This is followed by a steadying of the cell populations which all remain at a constant equilibrium level after approximately 400 days. Clearly, this numerical solution represents an example of the case where the immune system is not able to eliminate tumour cells.

A number of numerical solutions using parameter values from Tables 2.1 and 2.2 but with different initial values for the tumour cell population are presented in Figure 2.13. The solutions show that the growth of a tumour can be completely controlled for some initial values. This means that it is possible that even a weak immune system is able to clear the tumour cells for certain initial populations. However, with a larger initial population of tumour cells, the immune system cannot eradicate the tumour cells. In this case, the tumour cells grow to a nonzero
Figure 2.13: The evolution of tumour cells given by solutions of equations (2.10)–(2.13) using parameters from Table 2.1 and 2.2 with different initial values (indicated on the plot). This graph indicates that there is a critical initial value of the tumour cell population, where all numerical solutions of the model with initial value below the critical value grow to the stable zero tumour equilibrium. However, all solutions with initial value above the critical value grow to the nonzero equilibrium point.

DCs play an important role in the activation of the immune system since DCs can activate NK cells and CD8\(^+\) T cells. Figure 2.14 (b) and (c) show the effect of varying the strength of the DC source on the NK cells and CD8\(^+\) T cells. Increasing the DC source term causes increases to the NK cell and CD8\(^+\) T cell populations. Furthermore, such change can affect the population of tumour cells since both of NK and CD8\(^+\) T cells can lyse tumour cells. Finally, increasing the source term of DCs will decrease the growth of tumour cells as shown in Figure 2.14 (a). This suggests that an external source of DCs can be used as treatment if the patient lacks sufficient DCs in their body. These numerical solutions show however that DCs alone are not fully effective for tumour treatment. It is also necessary that...
the NK cell population is high enough for an effective anti-tumour response.

Again from the numerical solutions, if the rate at which CD8$^+$ T cells kill DCs, $f_1$, is above a critical level, the DC population decreases in very short time. This reduces the activation of NK cells and CD8$^+$ T cells and causes excessive growth of tumour cells. This result indicates that CD8$^+$ T cells can also inhibit the function of DCs as antigen presenting cells. Increasing the parameter, $l$, the dendritic cell recruitment rate, will cause decreases to the number of tumour cells.

Figure 2.15 (a) shows that increasing the source of NK cells causes decreases to the number of tumour cells. However, increasing NK cells can result in decreasing of CD8$^+$ T cells and DCs as shown in Figure 2.15 (b) and (c). Because of this effect on the other parts of the immune system, NK cells are considered to be a less appropriate choice for use as a direct cell treatment. This leaves DCs as a better choice for immunotherapeutic treatment of tumour cells – a concept investigated in the following chapter.

2.4 Discussion

In this chapter, two mathematical models of the interaction between a growing tumour and the immune system have been presented. The models describe how DCs and NK cells, as the innate immune system, and CD8$^+$ T cells, as the specific immune system, affect the growth of the tumour cell population. While the models of de Pillis et al. [34, 35, 36, 37, 39, 33] represent the interaction between a growing tumour and the immune system without DCs, our model includes more detail through incorporation of three immune system components. This allows for the investigation of, for example, the effect on tumour growth of the antigen presenting cell role of DCs.

The first model developed in this chapter was a relatively simple ordinary differential equation-based model describing the dynamics of populations of tumour cells, DCs, NK cells and CD8$^+$ T cells. The first model used only constant, linear and linear interaction terms to represent system dynamics. Equilibrium, stability and numerical analysis of this model, which was similar in structure to many of the existing mathematical models discussed in the introduction and earlier literature review chapter, allowed an understanding of the dynamics to be developed and uncovered some shortcomings in a model of this level of abstraction. In the second model, saturation of effects (such as activation of immune cells by other cells) were introduced to deal with some of the downfalls of the initial model.

Using the models developed in this chapter, it was possible to investigate the growth of the tumour cells and the effect of three components of the immune system (NK, dendritic and CD8$^+$ T cells) on the growth of the tumour cell popu-
Figure 2.14: The evolution of (a) tumour cells, (b) CD8$^+$ T cells and (c) NK cells showing the effect of varying the DC source term. Other parameters for these simulations are taken from Table 2.1 and Table 2.2 with $s_1 = 7.000$ and values of $s_2$ as indicated on the graph.
Figure 2.15: The evolution of (a) tumour cells, (b) CD8$^+$ T cells and (c) DCs showing the effect of varying the NKs source term. Other parameters for these simulations are taken from Table 2.1 and 2.2 with $s_2 = 7.000$ and values of $s_1$ as indicated on the graph.
lation. A stability analysis of the equilibria of the mathematical models revealed that under certain conditions, tumour cells can be completely eliminated by the immune system. This type of behaviour, for both the initial model and the improved model, is shown Figure 2.2 and Figure 2.9. On the other hand, when the relevant conditions found via the stability analysis, are not satisfied, the tumour cell population reaches a nonzero equilibrium, indicating coexistence of the tumour and immune cell populations, as shown in Figure 2.10.

Both of the models demonstrate the compound effects of elements of the immune system on the dynamics of tumour growth through their effects on other immune system components. In particular, the role of DCs as an antigen presenting cell is shown to play an important role in the effector cell activation, enhancing the immune system activity (in this case, NK cells and CD8+ T cells).

For small sources of DCs and NK cells, the immune system is not able to eliminate the tumour cell population, however for large sources of DCs and NK cells, the tumour cells can be eliminated. Furthermore, in the second model, increasing the rate of dendritic cell recruitment, $l$, will decrease the number of tumour cells.

As described in Mazzolini et al. [93], some patients showed increased NK cell activity after injection of up to $50 \times 10^6$ DCs. This is represented qualitatively by our results indicating that an increased DCs source term can cause increases in the NK cell population which implies increased NK cell activity. Moreover, our result can show that this also can increase the magnitude of the CD8+ T cell population.

The models analysed in this chapter provide support for investigating the use of DCs as an immunotherapeutic treatment. In the following chapter, the model given here will be extended to include a dendritic cell treatment as a tumour therapy. This problem will be analysed using optimal control theory, using the model developed in this chapter, to determine an optimal method for administering DCs as a treatment with a view to minimising tumour burden.
Chapter 3

An optimal control model of dendritic cell treatment of a growing tumour

3.1 Introduction

In this section, we study an optimal control treatment strategy for a model of the interactions between a growing tumour and the host immune system. To obtain the optimal control model we expand the model developed in Chapter 2, consisting of four ODEs, by adding the control function, \( u \), which represents a possibly time-varying application of a dendritic cell treatment as well as an optimal control statement reflecting an aim to minimise some functional of \( u \). A forward-backward sweep method, which is based on Runge-Kutta scheme, is then used to solve the optimal control problem.

The aim of this chapter is to construct an optimal control model of the interaction between a growing tumour and the immune system subjected to an externally supplied dendritic cell treatment. The model again consists of four variables representing the populations of tumour cells and three immune cell populations: NK cells, DCs and cytotoxic T cells. By applying the theory of optimal control, we find the optimal control strategy for the application of the DC treatment to be administered while minimising the tumour burden.

Some research related to the mathematical model of tumour immunotherapy has been undertaken already, including for example \[19, 20, 23, 24, 25, 37, 50, 60, 61, 67, 77\]. Kirschner and Panetta \[77\] illustrated the effect of adoptive cellular immunotherapy through a mathematical model which consists of tumour cells, immune-effector cells and interleukin-2. This model describes under what conditions the tumour can be destroyed as a result of the therapy. de Pillis et al. \[37\] developed a mathematical model describing the growing tumour with combination immune, vaccine and chemotherapy treatments. In addition, Cappuccio et al. \[23\]
introduced a mathematical model of tumour immune system interactions focusing on NK and T cell immunity, combined with interleukin-21 as an immunotherapy. However, there are few models which study the effect of DC vaccines on a growing tumour, for example [25], but these treat the immune system in a different manner to the present research.

Furthermore, a number of studies (see for example, [20, 27, 29, 40, 50, 55, 56, 60, 61, 68, 91, 94, 126]) have applied an optimal control strategy to determine a treatment method to obtain the minimum of some cost (such as monetary costs and harm to the patient) while minimising the tumour cell population. Where most of these papers described drug and chemotherapy for the tumour treatment, only a few of them investigated immune cells as a treatment. For example, based on the Kirschner-Panetta model [77], Burden [20] and Ghaifari and Naserifar [61] developed an optimal control strategy to minimise tumour burden. Both of their models attempt to maximise the amount of effector and interleukin-2 cells and minimise the number of tumour cells and the cost of the control. A review of optimal control theory in cancer chemotherapy can be found in Swan [125].

Castiglione and Piccoli constructed a mathematical model to describe the interaction between immune system cells and tumour cells. Then, they applied the optimal control methods to find the optimal quantity of DCs to be administered to the tumour site. In this research, we focus on immunotherapy-based treatment, specifically a dendritic cell-based vaccine, since DCs are safe and have minimal side effects to the human patients. This research is different from Castiglione and Piccoli model, since in this model, we include NK cells and also describe in more detail the role of DCs in tumour control.

This chapter is organised as follows. In Section 3.2 an extension of the ODE model of Chapter 2 is introduced that incorporates a time-varying dendritic cell-based treatment strategy and also an objective functional that we seek to minimise in this modelling study. Further, a discussion of a necessary condition for an optimal strategy is presented to set the background for the remainder of the chapter. The existence of the optimal control is then proved for the model presented in this chapter. This allows for a presentation of the optimality system that will be solved numerically. The numerical scheme itself, a forward-backward sweep method, is then outlined in Section 3.3 along with a number of important numerical solutions of the optimal control model. Finally, we discuss the results of the model in the context of the tumour growth problem.
3.2 Optimal control problem

Recall from Chapter 2 the revised ODE model (incorporating Michaelis-Menten dynamics) given by equations (2.10)–(2.13) describing tumour growth and interaction with the NK, dendritic and CD8$^+$ T cells of the host immune system. In particular, with $T(t)$ denoting the tumour cell population, $N(t)$ denoting the NK cell population, $D(t)$ denoting the dendritic cell population and $L(t)$ representing the population of CD8$^+$ T cells, the tumour system was described by the equations

\[
\frac{dT}{dt} = aT(1 - bT) - (c_1N + jD + kL)T, \quad (3.1)
\]
\[
\frac{dN}{dt} = s_1 - c_2NT + \frac{DN}{m_1 + D} - eN, \quad (3.2)
\]
\[
\frac{dD}{dt} = s_2 + \frac{l}{m_2 + T} \frac{DT}{T} - f_1LD - d_2DN - gD, \quad (3.3)
\]
\[
\frac{dL}{dt} = f_22 \frac{DT}{m_3 + T} - hLT - iL, \quad (3.4)
\]

along with appropriate initial conditions, governing the time evolution of the four species of interest. For a description of the individual parameters and terms in the equations, see Chapter 2.

In this section, we extend this ODE model further to incorporate a representation of a dendritic cell immunotherapeutic strategy for treatment of the growing tumour. We consider the problem of controlling the immunotherapy in the most advantageous way by casting the model as an optimal control problem.

In view of the equations above, we add a dendritic cell treatment to aid in decreasing the tumour burden by altering equation (3.3). Restating the full model for later reference, we have

\[
\frac{dT}{dt} = aT(1 - bT) - (c_1N + jD + kL)T, \quad (3.5)
\]
\[
\frac{dN}{dt} = s_1 - c_2NT + \frac{DN}{m_1 + D} - eN, \quad (3.6)
\]
\[
\frac{dD}{dt} = s_2 + \frac{l}{m_2 + T} \frac{DT}{T} - f_1LD - d_2DN - gD + w(u(t)), \quad (3.7)
\]
\[
\frac{dL}{dt} = f_22 \frac{DT}{m_3 + T} - hLT - iL, \quad (3.8)
\]

where $w$ is the strength of the treatment and $u(t)$ represents the time varying rate of application of the dendritic cell treatment, satisfying $a \leq u(t) \leq b$, with $a$ and $b$ some minimum and maximum rates of cell treatment.

Through the application of the dendritic cell treatment, we seek to minimise the tumour burden over time while simultaneously minimising the amount of treatment required. As such, we consider the treatment rate to be a control and cast the model
along with these aims as an optimal control problem. The objective functional, to be minimised here, is then given by

$$J(u) = \int_0^{t_f} \left( T(t) + \frac{B}{2} u^2(t) \right) \, dt,$$

where \(t_f\) is the specified final time. The first term represents the tumour cell burden, to be minimised during the treatment, and the second term reflects a cost of the dendritic cell treatment (such as monetary costs, harm to patient, etc) and is also to be minimised. \(B\) is a weighting factor that represents the effect of treatment for patient. The aim is to minimise the number of tumour cells as well as the cost of vaccine to be administered over all acceptable control functions \(u(t)\). Note that this is not the only acceptable functional, for example the final tumour cell population could be included to give \(J_{\text{alt}}(u) = T(t_f) + J(u)\), however the objective functional as given in equation (3.9) is consistent with others used in the literature. This objective functional is the same as objective functional employed by de Pillis et al. [40].

Hence, we attempt to find an optimal control function \(u(t) = u^*\), in other words the optimal treatment, such that

$$J(u^*) = \min_{u \in \mathcal{U}} \{ J(u) \},$$

where \(\mathcal{U} = \{ u(t) | a \leq u(t) \leq b, \ t \in [0, t_f] \}\) is the control set.

With the optimal control problem defined, we now move to proving the existence of an optimal control that minimises the given objective functional.

### 3.2.1 Derivation of necessary condition

In this section, we derive a necessary condition for the optimal control of a system of the form investigated here. More detail can be found in [82], which forms the basis for the derivation presented here.

Consider an optimal control problem comprised of ordinary differential equations

$$x'(t) = g(t, x(t), u(t)),$$

where \(x(t)\) represents the state variable and \(u(t)\) is the control variable. The basic optimal control problem consists of finding a piecewise continuous control function \(u(t)\) and the associated state variable \(x(t)\) to maximise (minimise, with reversed directions on inequalities) the given objective functional. That is, finding \(x(t)\) and \(u(t)\) to satisfy

$$\max_u \int_{t_0}^{t_f} f(t, x(t), u(t)) \, dt,$$
subject to
\[ x'(t) = g(t, x(t), u(t)), \]
where \( x(t_0) = x_0 \) is given and \( x(t_f) \) is free.

Suppose \( u^* \) is an optimal control and \( x^* \) the corresponding state. So we have \( J(u) \leq J(u^*) < \infty \) for all controls \( u \). Now, let \( h(t) \) be a piecewise continuous variation function and \( \epsilon \in \mathbb{R} \). Then \( u' = u^*(t) + \epsilon h(t) \) will be another valid piecewise continuous control function. Let \( x'(t) \) be the state corresponding to \( u^*(t) + \epsilon h(t) \). Then we have
\[
\frac{d(x^*(t))}{dt} = g(t, x^*(t), u^*(t)),
\]
and the objective functional at \( u' \) is
\[
J(u') = \int_{t_0}^{t_f} f(t, x^*(t), u^*(t)) \, dt. \tag{3.10}
\]

Now, let \( \lambda(t) \) be a piecewise differentiable function to be determined. By the Fundamental Theorem of Calculus we have
\[
\int_{t_0}^{t_f} \frac{d}{dt} [\lambda(t)x^*(t)] \, dt = \lambda(t_f)x^*(t_f) - \lambda(t_0)x^*(t_0),
\]
which implies
\[
\int_{t_0}^{t_f} \frac{d}{dt} [\lambda(t)x^*(t)] \, dt + \lambda(t_0)x_0 - \lambda(t_f)x^*(t_f) = 0.
\]
Adding this equation to equation (3.10) gives
\[
J(u') = \int_{t_0}^{t_f} \left( f(t, x^*(t), u^*(t)) + \frac{d}{dt} (\lambda(t)x^*(t)) \right) \, dt + \lambda(t_0)x_0 - \lambda(t_f)x^*(t_f)
= \int_{t_0}^{t_1} (f(t, x^*(t), u^*(t)) + \lambda'(t)x^*(t) + \lambda(t)g(t, x^*(t), u^*(t))) \, dt + \\
\lambda(t_0)x_0 - \lambda(t_f)x^*(t_f), \tag{3.11}
\]

Since, the maximum of \( J \) with respect to \( u \) occurs at \( u^* \), then the derivative of \( J(u') \) with respect to \( \epsilon \) is zero, namely,
\[
0 = \frac{d}{d\epsilon} J(u^*) \bigg|_{\epsilon=0} = \lim_{\epsilon \to 0} \frac{J(u^\epsilon) - J(u^*)}{\epsilon}.
\]
Differentiating equation (3.11) and using the above result gives
\[
0 = \frac{d}{d\epsilon} J(u^*) \bigg|_{\epsilon=0}
= \int_{t_0}^{t_f} \frac{\partial}{\partial \epsilon} \left( f(t, x^*(t), u^*(t)) + \lambda'(t)x^*(t) + \lambda(t)g(t, x^*(t), u^*(t)) \right) \, dt \bigg|_{\epsilon=0}
- \frac{\partial}{\partial \epsilon} \lambda(t_1)x^*(t_1) \bigg|_{\epsilon=0}.
\]
Applying the chain rule to $f$ and $g$, it follows that

$$0 = \int_{t_0}^{t_f} \left( f_x \frac{\partial x^\epsilon}{\partial \epsilon} + f_u \frac{\partial u^\epsilon}{\partial \epsilon} + \lambda'(t) \frac{\partial x^\epsilon}{\partial \epsilon} + \lambda(t) \left( g_x \frac{\partial x^\epsilon}{\partial \epsilon} + g_u \frac{\partial u^\epsilon}{\partial \epsilon} \right) \right) \bigg|_{\epsilon=0} \, dt$$

$$- \lambda(t_f) \frac{\partial x^\epsilon}{\partial \epsilon}(t_f) \bigg|_{\epsilon=0}.$$ 

$$= \int_{t_0}^{t_f} \left( f_x + \lambda(t) g_x + \lambda'(t) \frac{\partial x^\epsilon}{\partial t} \bigg|_{\epsilon=0} + (f_u + \lambda(t) g_u) h(t) \, dt \right)$$

$$- \lambda(t_f) \frac{\partial x^\epsilon}{\partial \epsilon}(t_f) \bigg|_{\epsilon=0}.$$ 

(3.12)

Now, we want to choose the adjoint function, $\lambda(t)$, to simplify equation (3.12) by forcing to zero the coefficients of

$$\frac{\partial x^\epsilon}{\partial \epsilon}(t_f) \bigg|_{\epsilon=0},$$

both in the integrand and in the boundary condition term. Thus, we choose the function $\lambda(t)$ to satisfy

$$\lambda'(t) = -f_x(t, x^*(t), u^*(t)) - \lambda(t) g_x(t, x^*(t), u^*(t)),$$ 

(3.13)

as well as the boundary condition

$$\lambda(t_f) = 0.$$ 

(3.14)

Here, equation (3.13) is referred to as the adjoint equation and equation (3.14) is called the transversality condition.

Now if we take

$$h(t) = f_u(t, x^*(t), u^*(t)) + \lambda(t) g_u(t, x^*(t), u^*(t)),$$ 

(3.15)

then substitute equation (3.13), equation (3.14) and equation (3.15) into equation (3.12), we have

$$0 = \int_{t_0}^{t_f} (f_u(t, x^*(t), u^*(t)) + \lambda(t) g_u(t, x^*(t), u^*(t)))^2 \, dt,$$

which implies the optimality condition

$$f_u(t, x^*(t), u^*(t)) + \lambda(t) g_u(t, x^*(t), u^*(t)) = 0,$$ 

(3.16)

for all $t_0 \leq t \leq t_f$. Equations (3.13), (3.14) and (3.16) form a set of necessary conditions that an optimal control and state must satisfy. In practice, the above equations need not be re-derived for each application. Furthermore, the above necessary conditions can be generated via the Hamiltonian of the problem, denoted $H$, which is defined as follows

$$H(t, x, u, \lambda) = f(t, x, u) + \lambda g(t, x, u).$$
That is, the Hamiltonian is equal to the integrand of the objective functional added to the product of the adjoint function and the right hand side of the differential equation system.

So in terms of the Hamiltonian the above necessary conditions can be written as

Optimality condition: \( \frac{\partial H}{\partial u} = 0 \) at \( u^* \Rightarrow f_u + \lambda g_u = 0 \),

Adjoint equation: \( \lambda' = -\frac{\partial H}{\partial x} \Rightarrow \lambda' = -(f_x + \lambda g_x) \),

Transversality condition: \( \lambda(t_f) = 0 \),

and have the state equation

\[ x' = g(t, x, u) = \frac{\partial H}{\partial \lambda}, \quad x(t_0) = x_0. \]

### 3.2.2 Existence of an optimal control

To prove the existence of an optimal control for the system of equations presented in Section 3.2, we use the fact that supersolutions \( T, \overline{N}, \overline{D}, \overline{L} \) of

\[
\frac{dT}{dt} = aT, \\
\frac{d\overline{N}}{dt} = s_1 + d_{11} \overline{N}, \\
\frac{d\overline{D}}{dt} = s_2 + l \overline{D} + u, \\
\frac{d\overline{L}}{dt} = f_{22} \overline{D},
\]

are bounded on a finite time interval.

The existence of the optimal control can be obtained by using the following result due to Fleming and Rishel [54]. Before we present the existence theorem of the optimal control, the existence theorem of initial value problem is introduced. The proof of the theorem can be found in, for example, [87].

**Theorem 3.1.** Given the initial value problem

\[
\frac{dx(t)}{dt} = f(t, x(t)), \quad x|_{t=\tau} = \xi.
\]

This problem has a solution if for some

\[ R_{a,b} = (t, x) : |t - \tau| \leq a, |x - \xi| \leq b, \]
centred about \((\tau, \xi)\) the restriction of \(f\) to \(R_{a,b}\) is continuous in \(x\) for fixed \(t\), measurable in \(t\) for fixed \(x\), and satisfies

\[
|f(t, x)| \leq m(t), \quad (t, x) \in R_{a,b},
\]

for some \(m\) integrable over the interval \([\tau - a, \tau + a]\).

Generally speaking, this theorem tells us that the solution to the initial value problem exists if the function \(f\) in equation (3.17) is bounded.

**Theorem 3.2.** Consider the control problem with system equations (3.5)–(3.8). There exists an optimal control, \(u^* \in U\) such that

\[
\min_{u \in U} J(u) = J(u^*),
\]

if the following conditions are satisfied.

(i) The set of controls and corresponding state variables is not empty.

(ii) The control set \(U\) is convex and closed.

(iii) Each right hand side of the state system (3.5)–(3.8) is bounded above by a linear function in the state and control variable.

(iv) The integrand of the objective functional is convex on \(U\) and is bounded below by \(-c_2 + c_1 u^2\) with \(c_1, c_2 > 0\).

**Proof.** For the given model, we prove each condition in turn.

(i) Using Theorem 3.1 we can prove the existence of solutions of (3.5)–(3.8) with bounded coefficients.

(ii) The control set is convex and closed by definition.

(iii) The right hand side of the state system is linear in the state and control variable. We let \(\delta(t, X)\) be the right hand side of system (3.5)–(3.8) without the control function \(u(t)\). That is,

\[
f(t, X, u) = \delta(t, X) + \begin{bmatrix} 0 \\ s_1 \\ s_2 + u \\ 0 \end{bmatrix}, \quad \text{with} \quad X = \begin{bmatrix} T \\ N \\ D \\ L \end{bmatrix}.
\]
Using the boundedness of the solutions, we see that
\[
|f(t, X, u)| \leq \begin{bmatrix} a & 0 & 0 & 0 \\ 0 & d_{11} & 0 & 0 \\ 0 & 0 & l & 0 \\ 0 & 0 & f_{22} & 0 \end{bmatrix} \begin{bmatrix} T \\ N \\ D \\ L \end{bmatrix} + \begin{bmatrix} 0 \\ s_1 \\ s_2 + \omega u \end{bmatrix}
\]
\[
\leq \begin{bmatrix} 0 \\ s_1 \\ s_2 \\ 0 \end{bmatrix} + \begin{bmatrix} a & 0 & 0 & 0 \\ 0 & d_{11} & 0 & 0 \\ 0 & 0 & l & 0 \\ 0 & 0 & f_{22} & 0 \end{bmatrix} \begin{bmatrix} T \\ N \\ D \\ L \end{bmatrix} + \begin{bmatrix} 0 \\ \omega u \end{bmatrix}
\]
\[
\leq C_1 (1 + |X| + |u|),
\]
where \( C_1 \) depends on the coefficients of the system. Hence, the right hand side of the state equation (3.5)–(3.8) is bounded above by a sum of the state and control variable.

(iv) To prove that the integrand of the objective functional is convex, we recall that a real valued function \( f \) on an interval is said to be convex if, for any two points \( x \) and \( y \) in the interval and for any \( p \in [0, 1] \),
\[
f(px + (1 - p)y) \leq pf(x) + (1 - p)f(y).
\]
Hence, to prove the integrand of the objective functional is convex, we need to show that
\[
J(t, T, pu + (1 - p)v) \leq pJ(t, T, u) + (1 - p)J(t, T, v),
\]
where \( u, v \in U, 0 < p < 1 \). We see the difference of the left and right sides of the inequality can be written as
\[
J(t, T, pu + (1 - p)v) - (pJ(t, T, u) + (1 - p)J(t, T, v))
\]
\[
= T(t) + \frac{B}{2}(v^2 - 2pu^2 + p^2v^2 + p^2u^2 - 2p^2vu + 2pvu)
\]
\[
- \left( T(t) + \frac{B}{2}pu^2 + \frac{B}{2}v^2 - \frac{B}{2}v^2p \right)
\]
\[
= \frac{B}{2}(p^2 - p)(v - u)^2.
\]
Since, \( p \in (0, 1) \), we have \((p^2 - p) < 0 \) and obviously \((v - u)^2 > 0 \), so it follows that the expression \( B(p^2 - p)(v - u)^2/2 \) is negative. Hence, the condition
\[
J(t, T, pu + (1 - p)v) \leq pJ(t, T, u) + (1 - p)J(t, T, v),
\]
is satisfied. Furthermore, from the objective functional we have
\[
T + \frac{B}{2}u^2 \geq \frac{B}{2}u^2 \geq -c_2 + \frac{B}{2}u^2,
\]
which gives \(-c_2 + c_1u^2\) as the lower bound, where \( c_1, c_2 > 0 \).
The conditions are satisfied and hence, we conclude that there exists an optimal control.

Theorem 3.2 and similar proof can be found in, for example, [20, 40].

### 3.2.3 Optimality system

Having proved the existence of an optimal control for minimising the functional (3.9) subject to the equations (3.5)–(3.8), we now derive the necessary conditions for this particular optimal control using Pontryagin’s maximum principle [111].

Using the functional given in equation (3.9), the Hamiltonian of the system is given by

\[ H = \left[ T + \frac{B}{2} u^2 \right] + \sum_{i=1}^{4} \lambda_i g_i, \]  

(3.18)

where the functions \( g_i \) are the right hand sides of the state equations (3.5)–(3.8). Then the Lagrangian is defined as

\[ L_g = H - \omega_1 (t) (u - a) - \omega_2 (t) (b - u), \]

where \( H \) is the Hamiltonian as defined in (3.18) and \( \omega_1 (t), \omega_2 (t) \geq 0 \) are penalty multipliers satisfying

\[ \omega_1 (t) (u^* - a) = 0, \]

\[ \omega_2 (t) (b - u^*) = 0. \]

Hence,

\[ L_g = \left[ T + \frac{B}{2} u^2 \right] \]

\[ + \lambda_1 \left( aT(1 - bT) - c_1 NT - jDT - kLT \right) \]

\[ + \lambda_2 \left( s_1 - c_2 NT + d_{11} \frac{DN}{m_1 + D} - eN \right) \]

\[ + \lambda_3 \left( s_2 + l \frac{DT}{m_2 + T} - f_1 LD - d_2 DN - gD + wu (t) \right) \]

\[ + \lambda_4 \left( f_{22} \frac{DT}{m_3 + T} - hLT - iL \right) \]

\[ - \omega_1 (t) (u - a) - \omega_2 (t) (b - u), \]

Note that at \( u^* \), \( H = L_g \).

**Theorem 3.3.** Given optimal control \( u^* \) and solutions \( T^*, N^*, D^* \) and \( L^* \) of the corresponding state system (3.5)–(3.8), there exist adjoint functions \( \lambda_1, \lambda_2, \lambda_3 \) and
\( \lambda_4 \) satisfying
\[
\lambda_1' = -1 + \lambda_1 (2abT - a + c_1 N + jD + kL) + \lambda_2 c_2 N - \lambda_3 l \left( \frac{m_2 D}{m_2 + T} \right) \\
+ \lambda_4 \left( -f_{22} \frac{m_3 D}{(m_3 + T)^2} + hL \right),
\]
\[
\lambda_2' = \lambda_1 c_1 T + \lambda_2 \left( c_2 T - d_{11} \frac{D}{m_1 + D} + e \right) + \lambda_3 d_2 D,
\]
\[
\lambda_3' = \lambda_1 jT - \lambda_2 d_{11} \frac{m_1 N}{(m_1 + D)^2} + \lambda_3 \left( f_1 L + d_2 N + g - t \frac{T}{m_2 + T} \right) - \lambda_4 f_{22} \frac{T}{m_3 + T},
\]
\[
\lambda_4' = \lambda_1 kT + \lambda_3 f_1 D + \lambda_4 (hT + i).
\]

and \( \lambda_1(t_f) = \lambda_2(t_f) = \lambda_3(t_f) = \lambda_4(t_f) = 0 \) (the transversality conditions). Furthermore
\[
u^*(t) = \min \left\{ \max \left\{ a, \frac{-\lambda_3 w}{B} \right\}, b \right\}.
\]

**Proof.** The form of the adjoint equations and transversality conditions are standard results from Pontryagin's Maximum Principle [111]. We differentiate the Lagrangian with respect to the states, \( T, N, D \) and \( L \) respectively, and then the adjoint system can be written as
\[
\lambda_1' = -\frac{\partial L_g}{\partial T} = -1 + \lambda_1 (2abT - a + c_1 N + jD + kL) + \lambda_2 c_2 N - \lambda_3 l \left( \frac{m_2 D}{m_2 + T} \right) \\
+ \lambda_4 \left( -f_{22} \frac{m_3 D}{(m_3 + T)^2} + hL \right),
\]
\[
\lambda_2' = -\frac{\partial L_g}{\partial N} = \lambda_1 c_1 T + \lambda_2 \left( c_2 T - d_{11} \frac{D}{m_1 + D} + e \right) + \lambda_3 d_2 D,
\]
\[
\lambda_3' = -\frac{\partial L_g}{\partial D} = \lambda_1 jT - \lambda_2 d_{11} \frac{m_1 N}{(m_1 + D)^2} + \lambda_3 \left( f_1 L + d_2 N + g - t \frac{T}{m_2 + T} \right) \\
- \lambda_4 f_{22} \frac{T}{m_3 + T},
\]
\[
\lambda_4' = -\frac{\partial L_g}{\partial L} = \lambda_1 kT + \lambda_3 f_1 D + \lambda_4 (hT + i).
\]

The optimality equation is
\[
\frac{\partial L_g}{\partial u} = 0 \quad \text{at} \quad u^*.
\]

Thus,
\[
\frac{\partial L_g}{\partial u} \bigg|_{u^*} = Bu + \lambda_3 w - \omega_1 + \omega_2 = 0
\]
\[
\Rightarrow Bu^* = -\lambda_3 w + \omega_1 - \omega_2.
\]

Hence we have
\[
u^*(t) = \frac{-\lambda_3 w + \omega_1 - \omega_2}{B}.
\]  (3.19)
Now, we consider all possible values of optimal control $u^*$, of which there are three cases.

1. For $\{ t : a < u(t) < b \}$.
   
   In this case, since $\omega_1 = \omega_2 = 0$, then from equation (3.19), the optimal control is
   
   $$ u^* (t) = -\frac{\lambda_3 w}{B}. $$

2. For $\{ t : u(t) = b \}$.
   
   Then, from equation (3.19), we have
   
   $$ b = u^* (t) = -\frac{\lambda_3 w + \omega_1 - \omega_2}{B}. $$

   In this case, $\omega_1 = 0$, consequently
   
   $$ b = u^* (t) = -\frac{\lambda_3 w - \omega_2}{B}. $$

   or
   
   $$ b + \frac{\omega_2}{B} = -\frac{\lambda_3 w}{B}. $$

   Since $\omega_2 \geq 0$, then
   
   $$ b + \frac{\omega_2}{B} \geq b, $$

   Thus,
   
   $$ b = u^* \leq -\frac{\lambda_3 w}{B}. $$

   So, in this case, we have
   
   $$ u^* (t) = \min \left\{ -\frac{\lambda_3 w}{B}, b \right\}. $$

3. For $\{ t : u(t) = a \}$.
   
   Then, from equation (3.19), we have
   
   $$ a = u^* (t) = -\frac{\lambda_3 w + \omega_1 - \omega_2}{B}. $$

   In this case, $\omega_2 = 0$, consequently
   
   $$ a = u^* (t) = -\frac{\lambda_3 w + \omega_1}{B}, $$

   or
   
   $$ a - \frac{\omega_1}{B} = -\frac{\lambda_3 w}{B}. $$

   Since $\omega_1 \geq 0$, then
   
   $$ a - \frac{\omega_1}{B} \leq a. $$
Thus,

\[ a = u^* \geq -\frac{\lambda_3 w}{B}. \]

So, in this case, we have

\[ u^*(t) = \max \left\{ a, -\frac{\lambda_3 w}{B} \right\}. \]

Combining these three cases, we conclude

\[ u^*(t) = \begin{cases} 
-\frac{\lambda_3 w}{B}, & a < -\frac{\lambda_3 w}{B} < b \\
 a, & -\frac{\lambda_3 w}{B} \leq a \\
b, & -\frac{\lambda_3 w}{B} \geq b.
\end{cases} \]

In compact notation,

\[ u^*(t) = \min \left\{ \max \left\{ a, -\frac{\lambda_3 w}{B} \right\}, b \right\}. \]

\[ \Box \]

### 3.3 Numerical solutions

In this section we provide a description of the numerical scheme employed to solve the optimal control problem developed in Section 3.2. We then present a number of numerical solutions of the model to demonstrate its implementation and possible outcomes.

#### 3.3.1 Forward-backward sweep method

In this thesis, the numerical solution of the optimal control problem given by equations (3.5)–(3.8) along with the objective functional equation (3.9), is obtained using the iterative scheme referred to as the forward-backward sweep method. In short, the method involves first solving the state system and its associated initial condition, forward in time. Then, the adjoint system with its initial condition obtained from the current iteration of the state system is solved backward in time to meet the transversality condition. An initial guess for the control, \( u \), is updated by using a convex combination from the optimality equation. The iteration is repeated until the difference between values of state, adjoint and control variable of the current iteration and previous iteration are within some acceptable tolerance level.

In this numerical solutions, the ODE is solved using Runge-Kutta method. The `ode45` function in MATLAB can not used any more since the forward-backward sweep method requires a uniform step size. The parameters used in the numerical
solutions in this chapter are taken from Table 2.1 and Table 2.2 in Chapter 2, and we now expand upon this informal description of the scheme.

To solve our optimal control problem

$$\min \int_{t_0}^{t_f} f(t, x(t), u(t)) \, dt,$$

subject to

$$x'(t) = g(t, x(t), u(t)),$$

with the initial condition $x(t_0) = x_0$, we introduce the Hamiltonian function

$$H(t, x, u, \lambda) = f(t, x, u) + \lambda^T g(t, x, u).$$

The problem then becomes

$$x'(t) = g(t, x(t), u(t)), \quad x(t_0) = x_0,$$

$$\lambda' = -\frac{\partial H}{\partial x} = -(f_x + \lambda g_x), \quad \lambda(t_f) = 0,$$

$$0 = \frac{\partial H}{\partial u} = f_u + \lambda g_u, \text{ at } u^*.$$
where
\[ k_1 = f(t, x(t)) \]
\[ k_2 = f(t + \frac{h}{2}, x(t) + \frac{h}{2}k_1) \]  
(3.20)
\[ k_3 = f(t + \frac{h}{2}, x(t) + \frac{h}{2}k_2) \]
\[ k_4 = f(t + h, x(t) + hk_3) \]

4. Using the transversality condition \( \lambda_{N+1} = \lambda(t_f) = 0 \) and the values for \( u_i \)
and \( x \), solve \( \lambda' = -(f_x + \lambda g_x) \) backward in time.

5. Update \( u_i \) by entering the new \( x_i \) and \( \lambda_i \) values into the optimal control. In
equations (3.20) and (3.21), we calculate \( k_2 \) and \( k_3 \) at time \( t + \frac{h}{2} \). Since, \( u \) is
a function of time, then we approximate \( u_{i+h/2} \) with the average
\[ \frac{u_i + u_{i+1}}{2}. \]

6. Check convergence where we aim for a relative error to be negligibly small.
That is,
\[ \frac{\|u_i - u_i^{old}\|}{\|u_i\|} \leq \eta, \]
where \( \eta \) is the acceptable tolerance, \( u_i \) represents the current approximation,
\( u_i^{old} \) denotes the previous approximation and \( \|\cdot\| \) refers to the sum of the
absolute value of the terms. To avoid the problem of zero controls, multiply
both sides by \( \|u_i\| \), to give
\[ \eta \|u_i\| - \|u_i - u_i^{old}\| \geq 0, \]
or
\[ \eta \sum_{i=1}^{N+1} |u_i| - \sum_{i=1}^{N+1} |u_i - u_i^{old}| \geq 0. \]
Similarly, we apply this convergence criteria to the state and adjoint variables. So for the state variables, we have
\[ \eta \sum_{i=1}^{N+1} |x_i| - \sum_{i=1}^{N+1} |x_i - x_i^{old}| \geq 0, \]
and for the adjoint variables, we have
\[ \eta \sum_{i=1}^{N+1} |\lambda_i| - \sum_{i=1}^{N+1} |\lambda_i - \lambda_i^{old}| \geq 0. \]
If the values of the state, adjoint and optimal control variables in this iteration and the last iteration are negligibly close, output the current values as solutions. Otherwise return to Step 3, using the new \( u_i \) values.
3.3.2 Simulation and results

Figure 3.1 illustrates the evolution over time of the tumour cell population calculated via the numerical solution of system (3.5)–(3.8) using different strengths for the DCV. The parameter values presented in Tables 2.1 and 2.2 in Chapter 2 and the initial value $T(0) = 100, N(0) = 1, D(0) = 1$ and $L(0) = 1$ are again used in this simulation. With the initial value $T_0 = 100$ cells, without vaccine, tumour cells grow to a maximum value of approximately 1000 cells within just 12 days. The remaining numerical solutions show, not surprisingly, that increasing the strength of the DCV to be administered to the tumour site decreases the number of tumour cells and also shortens the time at which the tumour cell population reaches its peak level. By adding a significantly strong vaccine, for example $5 \times 10^6$, the...
Figure 3.2: The evolution of two tumour cell populations using a large initial tumour cell population, showing the impact of the vaccine-based control strategy. The dashed line shows the tumour population growth resulting from no control, while the solid line (more easily seen inset) shows the tumour cell population eradication resulting from the optimal control vaccine strategy.

Figure 3.2 presents a plot of the tumour cell population using a large initial value of tumour cells, demonstrating the impact of the vaccine-based control strategy. The dashed line shows the tumour population growth resulting from no control, while the solid line (more easily seen inset) shows the tumour cell population eradication resulting from the optimal control vaccine strategy. With initial value $T_0 = 42310$ cells, without vaccine, tumour cells grow to the nonzero tumour equilibrium after around 120 days. However this can be eliminated in just under 40 days using the optimal control strategy. The inset plot shows that the evolution of tumour cells in 40 days where without control, the number of tumour cells remains
Figure 3.3: The optimal control, $u$, used for treatment of the tumour cell population shown in Figure 3.2.
Figure 3.4: The evolution of three NK cell populations showing the impact of the DC vaccine-based control strategy for three different maximum vaccine levels (as shown in the legend). This plot indicates that increasing the maximum vaccine level increases the peak NK level and decreases the time to reach that peak.

Increasing the strength of the DC vaccine not only impacts the tumour cell population, it also causes increases in the numbers of NK cells (see Figure 3.4) and CD8$^+$ T cells (see Figure 3.5). These effects in turn also reduce the growing of tumour cell population as shown in Figure 3.6. The effect is very significant in increasing the number of CD8$^+$ T cells, consequently reducing the tumour burden. This particular numerical solution shows that DCs alter the population of CD8$^+$ T cells more so than NK cells.

Figures 3.7a, 3.7b and 3.8 display the strength of the DC vaccine to be applied
**FIGURE 3.5**: The evolution of three CD8$^+$ T cell populations showing the impact of the DC vaccine-based control strategy for three different maximum vaccine levels (as shown in the legend). This plot indicates that increasing the maximum vaccine level increases the peak CD8$^+$ T cell level and slightly decreases the time to reach that peak.
Figure 3.6: The evolution of three tumour cell populations showing the impact of the DC vaccine-based control strategy for three different maximum vaccine levels (as shown in the legend). This plot indicates that increasing the maximum vaccine level decreases the peak tumour cell level and slightly decreases the time to reach that peak.
Figure 3.7: The control, $u$, used for treatment with initial value of tumour cells $T_0 = 42310$ and where the maximum vaccine to be administered is (a) 2000 units and (b) 20000 units.
Figure 3.8: The control, \( u \), used for treatment with initial value of tumour cells \( T_0 = 42310 \) and where the maximum vaccine to be administered is 200000 units.
over time given a maximum DC vaccine strength of 2000 units, 20000 units and 200000 units, respectively. Increasing the strength of the DC vaccine also reduces the required time duration of the treatment.

Similarly, Figure 3.9 represents the evolution of the number of tumour cells using parameter value taken from Tables 2.1 and 2.2 except for $c_1 = 1.5 \times 10^{-6}$ and parameter $B = 0.9 \times 10^{-11}$. The dashed line shows the evolution without control/vaccine and the solid line represents the tumour cell population change with optimal control application of the vaccine. With initial value $T_0 = 100$ cells, without vaccine, the tumour cell population grows to a nonzero tumour equilibrium after around 80 days. However this can be eliminated in 100 days using the optimal control vaccine strategy. The inset plot shows the evolution of the tumour cell populations on a compressed vertical scale. We see more clearly that while the controlled tumour is eradicated in around 100 days, the tumour cell population...
Figure 3.10: The control, $u$, used for treatment with an initial value of tumour cells $T_0 = 100$ and where the maximum vaccine to be administered is (a) 2000 units and (b) 20000 units. Parameter values are taken from Tables 2.1 and 2.2 except for $c_1 = 1.5 \times 10^{-6}$. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.10a.png}
\caption{(a)}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.10b.png}
\caption{(b)}
\end{figure}
without vaccine remains at a high level at this time. The DC vaccine to be administered during this treatment can be seen in Figure 3.10a. To eliminate the tumour cells using a maximum strength of 2000 units, the vaccine should be administered during the overall treatment, whereas for a much higher maximum strength the time of treatment is reduced significantly (see Figure 3.10b). From this simulation, it is found that increasing the value of parameter $B$ causes an decreasing in the number of DC vaccine to be administered.

Figure 3.11: The control, $u$, used for treatment with an initial population of tumour cells $T_0 = 100$ and where the maximum vaccine to be administered is 200000 units. Parameter values are taken from Tables 2.1 and 2.2 except for $c_1 = 1.5 \times 10^{-6}$.

Again, increasing the strength of the DC vaccine causes an increase in the number of NK cells and CD$8^+$ T cells (data not shown), however this reduces the growing of the tumour (see Figure 3.12). The effect is very significant in increasing the number of CD$8^+$ T cells, consequently reducing the tumour burden. This simulation shows that DCs impact on the CD$8^+$ T cell population more so than NK cells. Furthermore, Figure (3.10)a, (3.10)b and (3.11) represent the form of the vaccine strategy to be used with a maximum DC vaccine level of 2000 units, 20000
Figure 3.12: The evolution of three tumour cell populations using optimal control with varying maximum maximum vaccine levels to be administered (as shown on the figure legend). Parameter values are taken from Table 2.1 and 2.2, except for $c_1 = 1.5 \times 10^{-6}$. Intuitively, the plot indicates that increasing the maximum value for the vaccine decreases both the peak of tumour cell population and the length of time to eliminate the tumour cells.
units and 200000 units respectively. Increasing the strength of the DC vaccine also reduces the required time duration of the treatment.

### 3.4 Discussion

In this chapter, optimal control theory has been to determine appropriate dendritic cell treatments to apply to a growing tumour. Using two conditions which indicate that the tumour cells grow to the nonzero tumour equilibrium, we have successfully implemented an optimal control model and been able to find optimal treatment strategies that result in eradicating tumour cell in optimal time.

The numerical solutions indicate that if the optimal DC treatment is administered to the growing tumour, then it eliminates the tumour quickly. However, it should be kept in mind that there is a limitation of DC treatment when it is applied in the form of an injection to the patient. As mentioned in Mazzolini et al. [93], treatment using DCs in colorectal cancer was safe and well tolerated, with injection of up to $5 \times 10^7$ DCs.

This model suggests that the best way to control the tumour cell population is to give a high DC vaccine concentration at the beginning of the treatment and then reduce the treatment after a specific period of time. This optimal strategy coincides with the previous work by Castiglione and Piccoli [27]. They found that to reduce the tumour cells, the high dose of the first vaccination should be given at the beginning of treatment period, then the smaller doses of vaccination should be administered later the treatment. This result also supports findings of de Pillis et al. [40], they found that the best way to fight the growing tumour was to apply the bulk of chemotherapy at the beginning of the treatment.

In practice however, the scheduling of tumour immunotherapy is periodical. For example, in a study due to Burgdorf et al., DCs were administered biweekly with a total of 10 vaccinations per patient [21]. It is however hypothesised that in the future, DC vaccines can be administered to the patient in what mathematically would be represented as a continuous function of time, using portable pumps [68].

In future work, we intend to consider a particular case of multitherapy, for which different factors (DCs and CTL cells or drug treatment) are delivered at the same time to the patient. As described in de Pillis et al. [37] combined immunotherapy and chemotherapy are more effective than immunotherapy or chemotherapy alone. Further developments of the current study include the extension to consider specific kinds of tumour and also investigation of spatial variations in the development of the tumour and the effect of the immune system.
4.1 Introduction

Presented in Chapter 2 was an ODE-based mathematical model of the interactions between tumour cells and components of the host immune system. Based on this model, a mathematical model for tumour treatment using an optimally controlled vaccine application also has been discussed in Chapter 3. In this section, we present a mathematical model of a growing tumour and the interaction between the tumour cells and the host immune system using a cellular automata (CA) model. This model can describe the system in much more detail, including spatial variations and cell-cell interactions of every single cell in the system. Based on Mallet and de Pillis [89] model, we construct a 2D CA model instead of 3D model, since this model can provide a preliminary basis for the project in the future.

As has already been discussed, there is strong evidence in the literature for the hypothesis that tumour growth is directly influenced by the cellular immune system of the human host. We stress this point further and note some specific interactions that, with the cellular automata modelling strategy, we are now able to incorporate directly into our mathematical model. For example, Hart [65] states that DCs, found in many types of tumours, are the dominant antigen presenting cells for initiating and maintaining the host immune response. They are critical in activating, stimulating and recruiting T lymphocytes: cells with the ability to lyse tumour cells. Also, Sandel et al. [119] discuss the influence of DCs in controlling prostate cancer. Furthermore, tumour infiltrating DCs are a key factor at the interface between the innate and adaptive immune responses in malignant...
diseases. Beside their primary role in the induction and regulation of the adaptive antitumoural immune response, more recent studies have shown that DCs have a capacity to directly kill cancer cells \[81\]. Natural killer cells and cytotoxic T lymphocyte cells also play important roles in the response of the immune system against the tumour as described in Kindt et al. \[73\].

In the tumour micro-environment, the tumour produces chemokines that can attract immune system components including DCs, NK cells and T cells to sites of tumour growth. These chemokines also function to activate DCs which, in turn, can stimulate and activate the immune system \[124\]. Chemokines are a family of small cytokines, or proteins secreted by many different cell types, including tumour cells. They can affect cell-cell interactions and play a fundamental role in the recruiting or attracting of cells of the immune system to sites of infection or, of interest in the present research, tumour growth.

As discussed in Chapter 1, the dynamics of tumour growth and the interactions of growing tumours with the host immune system have been studied intensively using mathematical models over the past four decades (see also Araujo and McElwain for a review to the early 2000s). Most of these models are presented using ordinary differential equations (ODEs) or partial differential equations (PDEs) that impose restrictions on the modelled system’s time-scales, as described in Ribba et al. \[115\]. However, a CA model can describe more complex mechanisms in the biological system without such restrictions by detailing phenomena at the individual cell or particle level. The classic definition of a CA model imposes that they involve only local rules that depend on the configuration of the spatial neighbourhood of each CA element. Hybrid cellular automata (HCA), on the other hand, extend the CA to incorporate non-local effects, often via coupling the CA with PDEs, and it is this modelling strategy that we employ in this chapter.

The interactions of a tumour and the host immune system using the CA framework have been modelled previously by, for example, Mallet and de Pillis \[89\] and de Pillis et al. \[38\], where they presented the first multidimensional, hybrid cellular automata model of the process that incorporated important signalling molecules. However, these models and others neglected to describe DCs and chemokines and their roles in tumour growth and control. This present research attempts to improve on their work by explicitly describing more of the host immune system.

The purpose of the model developed in the present research is to investigate the growth of a small solid tumour when that growth is affected by the immune system. To this end, in this study, we present a hybrid cellular automata model of the interaction between a growing tumour and cells of the innate and specific immune system, including the role of DCs and signalling molecules known as chemokines.

To include the effect of a chemokine in this model, we recognise the significantly
smaller size of such molecules compared with biological cells and introduce a partial differential equation to describe the concentration of chemokine secreted by the tumour. We combine the numerical solution of the partial differential equation model with a number of biologically motivated automata rules to govern the evolution of various cell populations from the HCA model. We use the hybrid cellular automata model to simulate the growth of a tumour in a number of computational “cancer patients”. Each computational patient is distinguished from others by way of patient-specific characteristics reflected through particular parameter choices. We define the “death” of a patient as the situation where the cells of the tumour reach the boundary of our model domain; effectively this represents tumour metastasis.

In the sections to follow, we present a discussion of the role of DCs and chemokines related to cell-cell interactions in tumour growth. Furthermore, the development of the HCA model is considered before analysing numerical simulations. We conclude with a discussion of the results.

4.2 The role of DCs and chemokines

Before starting to construct the model using the HCA framework, in this section, we present a more detailed discussion of the role of immune system (especially DCs) and chemokines as related to cell-cell interactions in tumour growth. This discussion provides us the basis for making rules in HCA model.

The immune system plays an important role in defending the body against pathogens by identifying and killing non-self (foreign) matter such as viral particles, parasites and importantly here, tumour cells [117]. The immune system may be considered to consist of two components, namely the innate and adaptive systems. The innate immune system (for example: DCs, NK cells, macrophages) can recognise antigen without the requirement for previous priming by specific non-self antigens [117]. However, the adaptive immune system (for example: cytotoxic T cells, helper T cells and B cells) need the antigen to be processed and presented in a histocompatibility complex through antigen presenting cells (APC) [11 117]. DCs are known to be the most efficient APC and express high levels of MHC class I and II molecules [59].

Current evidence is that there is a large number of functional states for DCs and the immunogenic capacity depends on the microenvironment [84]. The tumour microenvironment is a complex system that consists of extracellular matrix [114] and stromal cells including fibroblasts, endothelial cells, lymphocytes, macrophages, DCs, and neutrophils, which supports and regulate tumour growth [114 124].

In the tumour microenvironment, DCs play a crucial role in activating, stimu-
uating and recruiting the immune system. DCs can be activated by chemokines secreted by tumour cells or after direct interactions with the tumour itself \[114\] \[124\]. These activated DCs can stimulate and regulate components of the immune system including CTL cells \[121\] and NK cells, which after activation can directly kill tumour cells \[15\] \[59\]. Furthermore, helper T cells that are activated by DCs \[121\] can produce chemokines which, in turn, lead to CTL cell stimulation \[124\].

Dendritic cells found in various types of solid tumours, are antigen presenting cells that initiate and regulate immunity as well as shape the host response to tumours \[42\] \[65\]. They play an important role in activating, stimulating, recruiting and developing the immune response. Therefore, it can be concluded that tumour-infiltrating DCs play a key role in a cellular antitumour immune response by infiltrating, capturing, and processing tumour antigens, recruiting and activating the immune system \[42\] \[119\].

Dendritic cells play a vital role as the major regulator in CTL cell and NK cell activation \[30\] \[51\] \[81\], they also can control the activation of B cells \[11\]. As well, DCs have a unique function depending on their stage of maturation. Immature DCs (iDC) are very efficient in antigen uptake and are capable of presenting captured antigens through their surface receptors. After antigen uptake, DCs migrate from peripheral tissues to the lymph nodes, where antigen presentation to the immune system occurs \[11\] \[123\]. In the secondary lymphoid tissues, DCs are mature and able to attract, interact and activate the immune system including T lymphocytes and helper T cells to initiate a primary immune response \[1\].

Cytotoxic T cells are activated by DCs through antigens presented to MHC class I molecules. Also, DCs activate CD4 helper T cells which then secrete chemokines that can enhance immunoglobulin production. CD4 helper T cells are activated by binding via their T cell receptor (TCR) to MHC class II molecules. Then they can be recognized by specific CD4 helper T cells in the cell membrane, where MHC peptide complex is presented to the CD4 helper T cells by DCs. CD4 cells can be categorised according to the type of signalling that they receive, Th1 CD4 helper T cells and Th2 CD4 helper T cells. Th1 CD4 helper T cells secrete chemokines such as interleukin-2, thereby stimulating cell-mediated immunity by activating CTL cells. Th2 CD4 helper T cells mediate an antibody response by releasing chemokines such as IL-4 and IL-10 \[59\].

On the other hand, active CTL cells can kill mature DCs \[26\], as a result of presenting antigen on their surface. In a recent study, cytokine-induced killer T cells (CIK), expanded T cells from ex vivo have been shown to selectively eliminate iDC by direct cytotoxicity \[69\]. Furthermore, Moretta \[96\] notes that NK cells can down regulate the function of DCs by killing iDCs in peripheral tissues, and also states that NK cells might have a role in killing mature DCs.
Dendritic cells are considered as the most potent component of the immune system because they facilitate transport of antigen-presenting cells to the lymphoid tissue and provide efficient stimulation of T cells \[86\]. From experimental studies in mice, DCs have been shown to be very efficient in stimulating CTL cells \[86\]. Because of their unique role in initiating and regulating the immune response, currently DCs are exploited in the hope of becoming a novel tool for cancer therapy. It has been proven that DCs are feasible, safe \[21, 93, 120\], and efficient for treatment of some “cancer patients”, especially if DCs are matured and activated \[1\]. The first study related to DC vaccination was carried out by Hsu, et al. in 1996 \[66\]. They investigated the ability of DCs pulsed \textit{ex vivo} to stimulate host anti-tumour immunity in patients with B cell lymphoma. Other research, for example, Schuler et al. \[120\], Fong et al. \[58\] and Burgdorf et al. \[21\], also discussed the use of DCs in cancer immunotherapy.

Chemokines and chemokine receptors, important in immune homeostasis and surveillance, also play an important role in the tumour environment. In the tumour microenvironment, chemokines are secreted both by stromal cells (fibroblasts, endothelial cells and infiltrating leukocytes) and by the tumour itself \[7, 124\]. Tumours, such as glioblastoma, melanoma, and neuroblastoma produce high levels of chemokines. These chemokines can promote tumour growth and induce stromal cells to produce cytokines or chemokines which, in turn, can regulate angiogenesis, tumour growth, and metastasis in the tumour microenvironment \[124\]. Tumour cells can activate DCs, lymphocytes, fibroblasts, macrophages and neutrophils, through cell-cell interactions or cytokines or chemokines produced by tumour cells \[124\].

Chemokines secreted by tumour or stromal cells can also attract a large number of leukocytes such as DCs, NK cells, and T cells (helper T and cytotoxic T lymphocytes) to the tumour site which may result in tumour regression and elimination \[124\]. Chemokines secreted by tumour cells can stimulate or inhibit tumour growth, modulation of chemokine activity in a selective manner at the site of the tumour can lead to tumour cell apoptosis. These kinds of chemokines can be chosen for their potential to attract immune cells to the tumour site that may result in successful tumour treatment. From experiments involving mouse tumours, chemokines such as CCL5 and CCL21 are promising avenues for cancer therapy investigations \[102, 127\].

### 4.3 Mathematical model

In this model, we consider the early growth of a solid tumour and its interaction with the immune system and a tumour-secreted chemokine. The model is
Figure 4.1: Schematic showing the partitioning of the problem domain into cellular automata elements (squares) and mesh-points for numerical solution of the partial differential equation.

Comprised of a system of partial differential equations to describe the chemokines secreted by the tumour and CD4$^+$ T cells, coupled with a discrete, stochastic cellular automata that describes the various cell types comprising the host-tumour environment.

Following Ferreira et al. [52, 53] and de Pillis and coworkers [38, 89] the tumour environment is modelled as a square shaped computational domain of side length $L$ (see Figure 4.1). Each square element in the grid represents a location that may contain a healthy cell, tumour cell or immune cell. The domain is partitioned into a regular square grid with each element of the grid representing a space approximately corresponding with the size of a tumour cell (around 10-20$\mu$m; [6, 83]). These elements are the discrete locations considered in the cellular automata component of the model, while the midpoint of each element will be used as a mesh-point in the numerical scheme used to solve the partial differential equation component.

Initially, non-cancerous healthy cells cover the whole of the model domain, then the tumour mass is allowed to grow from one cancer cell placed at the centre cell of the grid. Cells of the host immune system are initially spread randomly over the domain throughout the other healthy cells. Four separate immune cell populations are considered here – the NK cells and DCs of the innate immune system and cells of the specific immune response, represented by the CTL cells and helper T cells. Each of these may exist in either an active or inactive state.

Computationally, the CA grid is stored as a two dimensional data structure
<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>healthy cell</td>
</tr>
<tr>
<td>1</td>
<td>tumour cell</td>
</tr>
<tr>
<td>2</td>
<td>necrotic debris</td>
</tr>
<tr>
<td>3</td>
<td>inactive CD8$^+$ cytotoxic T cell</td>
</tr>
<tr>
<td>4</td>
<td>active CD8$^+$ cytotoxic T cell</td>
</tr>
<tr>
<td>5</td>
<td>inactive dendritic cell</td>
</tr>
<tr>
<td>6</td>
<td>active dendritic cell</td>
</tr>
<tr>
<td>7</td>
<td>inactive CD4$^+$ helper T cell</td>
</tr>
<tr>
<td>8</td>
<td>active CD4$^+$ helper T cell</td>
</tr>
<tr>
<td>9</td>
<td>active NK cell</td>
</tr>
<tr>
<td>10</td>
<td>inactive NK cell</td>
</tr>
</tbody>
</table>

**Table 4.1**: The different cell species tracked in the cellular automata and the numerical value given to each in the computational implementation.

(matrix) with correspondence between CA elements and matrix elements. The number stored in the matrix corresponds with the type of cell occupying that element in the domain, according to the definitions given in Table 4.1.

The model solutions are progressed via discrete time steps, at which each spatial location is investigated to determine its contents and whether or not certain actions will occur. This is summarised in Algorithm 1.

**Algorithm 1** Brief pseudocode for the full model algorithm.

1. Draw set parameters for current computational patient
2. Initialise CA domain contents
3. Solve PDEs
4. **for** each time step **do**
   1. **for** each CA element **do**
      1. Determine cell type in element
      2. Characterise neighbourhood of element
      3. Update PDEs solution (chemokine concentration)
      4. Test whether event will occur and update state
   2. **end for**
5. **end for**
6. Export data

The rules for the cellular automata component as well as the form of the diffusion
equation for chemokines are presented below.

4.3.1 Diffusion equation for chemokine concentration

Chemokines are small (8-14 kDa, in size [114]), cell-secreted protein molecules that can effect cell-cell interactions. In this model we consider two different chemokine molecules such as interleukins. Given that such molecules are very small compared with the size of tumour and host cells, we treat them essentially as a continuum and use a partial differential equation to model changes in their concentration in space and time. Denoting the concentration of the chemokines as $C_1(x, y, t)$ and $C_2(x, y, t)$ we have,

$$
\frac{\partial C_1}{\partial t} = D_{C_1} \left( \frac{\partial^2 C_1}{\partial x^2} + \frac{\partial^2 C_1}{\partial y^2} \right) + \sigma T, \tag{4.1}
$$

$$
\frac{\partial C_2}{\partial t} = D_{C_2} \left( \frac{\partial^2 C_2}{\partial x^2} + \frac{\partial^2 C_2}{\partial y^2} \right) - \lambda C_2 + \gamma + \alpha D^A H - \beta C_2 I, \tag{4.2}
$$

where $D_{C_1}$ and $D_{C_2}$ are the coefficients of random motility of the chemokines. The parameter $\sigma$ is the rate of secretion of chemokine by tumour cells. The rate of chemokine secretion by CD4$^+$ helper T cells is represented by $\lambda$ as a natural degradation rate and $\gamma$ as a natural production rate. The constant $\alpha$ represents the rate of secretion of chemokine resulting from interactions between activated DC cells and helper T cells, while $\beta$ represents the rate at which chemokine is used up in activating CD8$^+$ cytotoxic T cells. The description of model variables can be seen in Table 4.2.

Initially and on the boundaries, we assume that there are no chemokines. However these partial differential equations must be solved at each time step of the HCA model. Given that chemokines are secreted by the tumour cells and when CD4$^+$ T helper cells and DC cells come in contact (in the cellular automata component of the model), at later times the initial condition used in a HCA time step becomes nonzero and is in fact provided by the outcomes of the cell level interactions.

4.3.2 Cellular automata rules

In this model, we consider a number of biological cell types including normal healthy cells, tumour cells (necrotic, dividing and migrating), DCs, NK cells, CTL cells and helper T cells. To build the CA model, we define ‘rules’ that draw upon the biological literature to describe cell-cell interactions, cell effects on the environment, and effects of the environment on cells.

The evolution of the cell species involved in the tumour-host interactions considered here is governed by a set of discrete, stochastic rules which are presented
## Variable Description

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_1(x, y, t)$</td>
<td>concentration of chemokine secreted by tumour cells</td>
</tr>
<tr>
<td>$C_2(x, y, t)$</td>
<td>concentration of chemokine secreted by CD4$^+$ T cells</td>
</tr>
<tr>
<td>$N_{i,j}$</td>
<td>healthy host cell</td>
</tr>
<tr>
<td>$T_{i,j}$</td>
<td>tumour cell</td>
</tr>
<tr>
<td>$D_{i,j}^I$</td>
<td>inactive dendritic cell</td>
</tr>
<tr>
<td>$D_{i,j}^A$</td>
<td>active dendritic cell</td>
</tr>
<tr>
<td>$I_{i,j}^I$</td>
<td>inactive CD8$^+$ cytotoxic T cell</td>
</tr>
<tr>
<td>$I_{i,j}^A$</td>
<td>active CD8$^+$ cytotoxic T cell</td>
</tr>
<tr>
<td>$H_{i,j}^I$</td>
<td>inactive CD4$^+$ helper T cell</td>
</tr>
<tr>
<td>$H_{i,j}^A$</td>
<td>active CD4$^+$ helper T cell</td>
</tr>
<tr>
<td>$K_{i,j}^I$</td>
<td>inactive NK cell</td>
</tr>
<tr>
<td>$K_{i,j}^A$</td>
<td>active NK cell</td>
</tr>
</tbody>
</table>

### Table 4.2

The variables used in the hybrid cellular automata model. Here $x$ and $y$ are the spatial variables for the PDE component, $t$ denotes the time variable, and $i$ and $j$ represent spatial locations in the cellular automata component.
below. Each particular cell-level action or interaction has associated with it, a probability of success. Generally speaking, we calculate the number

\[ P_{\text{cell}}^{\text{event}} = f(\cdot), \]

where \( f \) depends on relevant cell types and conditions in the neighbourhood. We compare \( P_{\text{cell}}^{\text{event}} \) with a pseudo-random number, \( r \), drawn from the uniform distribution on the interval \([0,1]\). If \( r < P_{\text{cell}}^{\text{event}} \) then the event is carried out, otherwise it is deemed to have failed to occur. To describe the evolution of the cell population, we introduce the general algorithm of cellular automata rules as presented in Algorithm 2.

**Algorithm 2** Pseudocode for testing occurrence of individual events.

\[
\text{Draw } r \sim U[0,1] \\
\text{Calculate } P_{\text{cell}}^{\text{event}} \text{ using current state of CA} \\
\text{if } r < P_{\text{cell}}^{\text{event}} \text{ then} \\
\quad \text{update state (the event occurs)} \\
\text{end if}
\]

We now consider each cell type in turn and introduce the specific forms of the CA rules utilised in the model.

**Host cells**

Following from the work of Ferreira *et al.* [52] and of Mallet and de Pillis [89], we assume that the healthy host cells are effectively passive bystanders in the interaction. They do not hinder the growth of the tumour cells or the movement of any cell type.

**Tumour cells**

In this model, we consider tumour growth to be influenced by the immune system via NK cells, CTL cells and DCs. The tumour cells undergo the processes of division, migration and lysis resulting from interactions with components of the immune system such as NK cells, CD8\(^+\) cytotoxic T cells, and DCs. We assume lysis is dependent upon the local strength of the immune system and model division to be influenced by crowding due to the presence of other tumour cells, respectively. At each time step, the neighbourhood of each tumour cell is surveyed to determine whether the cells of the active immune system are present or not. If immune cells are present, the tumour cell is marked for potential lysis whereas if there are no active immune system cells in the neighbourhood then the tumour cell is
marked for potential division or migration. A stochastic rule is then implemented to determine whether or not the action (division, migration or lysis) will be carried out. While the time-scales of these processes can vary (between or within cell types), for generality we consider equal time-scales and tie to this the time step of the numerical solution method. With this in mind, we impose the following cellular automata rules for tumour cells.

**Cell division:** For cell division, we adopt a probability of division of the similar form as that used by Mallet and de Pillis [89]. When a tumour cell is marked for division, that action is carried out with a probability that depends upon the density of tumour cells in the neighbourhood of the dividing cell. In particular, we have

\[ P^T_{\text{div}} = \exp \left( - \left( \theta_{\text{div}} \sum_{i,j \in \eta} T_{i,j} \right)^2 \right), \]

where \( \theta_{\text{div}} \) controls the shape of the curve allowing it to capture qualitative understanding of the biology and \( \sum_{i,j \in \eta} T_{i,j} \) is the number of tumour cells in a one cell radius of the cell of interest.

When division occurs, the resulting daughter cell is placed in an element of the neighbourhood of the dividing cell in the following order: filling an empty element, replacing (killing and consuming/removing) a healthy host cell, adding to the tumour burden of the least filled neighbouring element. From Figure 4.2(a), it can be seen that tumour cell division is more likely when there is space in the neighbourhood for the resulting daughter cell.

**Lysis by active immune cells:** Again for tumour lysis, we adopt a probability of lysis of the similar form as that used by Mallet and de Pillis [89]. Active immune cells such as CD8\(^+\) T cells, NK cells and DCs are able to directly lyse tumour cells when they share a local neighbourhood. We assume that the intensity of the immune system effect is proportional to the number of active CD8\(^+\) T cells, NK cells and DCs in the neighbourhood of the tumour cell. The probability of tumour lysis depends on the strength of the active immune system in the neighbourhood of the tumour cell (see Figure 4.2(b)), and is given by

\[ P^T_{\text{lysis}} = 1 - \exp \left( - \left( \theta_{\text{lysis}} \sum_{i,j \in \eta} (I^A_{i,j} + K^A_{i,j} + D^A_{i,j}) \right)^2 \right), \]

where again \( \theta_{\text{lysis}} \) controls the shape of the curve allowing it to capture qualitative understanding of the biology and

\[ \sum_{i,j \in \eta} (I^A_{i,j} + K^A_{i,j} + D^A_{i,j}) \]

is the number of active immune cells in a one cell radius of the tumour cell of interest.
Cell migration: At each time step the tumour cells marked for migration do so with a constant probability, $k_1$ as given below

$$P^{\text{T}}_{\text{mig}} = k_1.$$ 

These rules are presented in pseudocode form in Algorithm 3.

**Algorithm 3** Pseudocode for tumour cell related events.

```pseudocode
if (location holds tumour cell) then
    Calculate the number of tumour cells in the neighbourhood
    Calculate the number of immune cells in the neighbourhood
    Find new location at random in the neighbourhood
    Draw $r \sim U[0, 1]$  
    Calculate $P^{\text{T}}_{\text{div}}$ using current state of CA
    Calculate $P^{\text{T}}_{\text{lysis}}$ using current state of CA
    if $r < P^{\text{T}}_{\text{div}}$ and (new location holds healthy cell) then
        filling new location by tumour cell
    else if $r < P^{\text{T}}_{\text{lysis}}$ then
        current surface become necrotic debris
    else if $r < P^{\text{T}}_{\text{mig}}$ then
        cell move
    end if
end if
```

**CD4$^+$ helper T cells**

Inactive CD4$^+$ helper T cells are subject to change as a result of natural replenishment and activation due to direct interaction between dendritic cells and existing inactive CD4$^+$ helper T cells. At each time step, the neighbourhood of each inactive CD4$^+$ helper T cell is surveyed to determine whether DCs are present. If any DCs are present, then the CD4$^+$ helper T cell is marked for potential activation, otherwise the CD4$^+$ helper T cell is marked for random migration. A stochastic rule is then implemented to determine whether or not the action (migration or activation) will be carried out. A normal background level of inactive CD4$^+$ helper T cells is also maintained at each time step. To this end, we impose the following cellular automata rules.

**Activation following DC and inactive CD4$^+$ helper T cell contact:** When inactive helper T cells come in contact with DCs they have probability

$$P^{\text{CD4}}_{\text{act}} = 1 - \exp \left( - \sum_{i,j \in \eta} (D^4_{i,j})^2 \right),$$
Figure 4.2: The form of the curves used to determine the probability of (a) tumour cell division and (b) tumour cell lysis, given different neighbourhood conditions.
of becoming active CD4+ T cells which are then able to secrete chemokines to activate cytotoxic T cells (see equation (4.2)). Here

\[ \sum_{i,j \in \eta} (D^A_{ij}) , \]

is the number of active DC cells in a one cell radius of the cell of interest. 

**CD4+ T cell migration:** If any DCs are present, then the CD4+ helper T cell is marked for potential activation, otherwise the CD4+ helper T cell is marked for random migration with probability of migration given by

\[ P_{\text{CD4 mig}} = k_2. \]

**Natural replenishment to background level:** A near-constant minimum background level of inactive and active helper T cells is ensured at each time step by replacing some healthy cells on the boundary with inactive helper T cells. This mimics replenishment of the CD4+ population from external sources (such as the lymph nodes). At each time step we determine the proportion of all locations in the domain occupied by inactive and active helper T cells. Whenever this is less than the minimum background level, \( H_0 \), each healthy cell on the boundary of the domain has probability

\[ P_{\text{CD4 rep}} = H_0 - \frac{1}{n^2} \sum_{i,j \in \eta} (H^I_{ij} + H^A_{ij}) , \]

of being replaced with an inactive CD4+ T cell from outside of the problem domain, where \( H_0 \) is the ‘normal’ density of inactive CD4+T helper cells and \( n^2 \) is the total number of CA elements.

These rules are presented in pseudocode form in Algorithm 4.

**CD8+ cytotoxic T cells**

Inactive CD8+ cytotoxic T cells are subject to change as a result of natural replenishment and activation as a result of direct interaction between existing inactive CD8+ cytotoxic T cells and either active DCs, tumour cells or cytokines produced by active CD4+ helper T cells. At each time step, the neighbourhood of each inactive CD8+ cytotoxic T cell is surveyed to determine whether active DCs or tumour cells are present. If any DCs or tumour cells or cytokines are present, then the inactive CD8+ cytotoxic T cell is marked for potential activation, otherwise the CD8+ cytotoxic T cell is marked for random migration or movement towards regions of higher chemokine concentration. If the chemokine level secreted by active CD4+ T cells is greater than some threshold concentration, \( C_{20} \), then the inactive CD8+ cytotoxic T cell is marked for activation. A stochastic rule is then implemented to
Algorithm 4 Pseudocode for CD4⁺ helper T cell related events.

\[
\text{if (location holds inactive CD4⁺ helper T cell ) then}
\]

\[
\begin{align*}
\text{Calculate the number of tumour cells in the neighbourhood} \\
\text{Find active DCs in the neighbourhood} \\
\text{Calculate the number of active DCs in the neighbourhood} \\
\text{Draw } r \sim U[0, 1] \\
\text{Calculate } P_{\text{act}}^{\text{CD4}} \text{ using current state of CA} \\
\text{if The number of tumour cells in the neighbourhood } \geq 1 \text{ then} \\
\text{current surface replace with healthy cell} \\
\text{else if } r < P_{\text{act}}^{\text{CD4}} \text{ then} \\
\text{current surface replace with active CD4⁺ helper T} \\
\text{else if } r < \text{constant} \text{ then} \\
\text{inactive CD4⁺ helper T cell moves towards the higher chemokine concentration} \\
\end{align*}
\]

\[
\text{end if}
\]

\[
\text{end if}
\]

determine whether or not the action (migration or activation) will be carried out.

A normal background level of inactive CD8⁺ T cells is also maintained at each time step. As a result, we impose the following cellular automata rules.

**Activation following DC and/or tumour cell and inactive CD8⁺ T cell contact:** When inactive cytotoxic T cells come in contact with DCs and/or tumour cells they have probability

\[
P_{\text{act}}^{\text{CD8}} = 1 - \exp \left( - \sum_{i,j \in \eta} D_{i,j}^A \right)^2,
\]

of becoming active CD8⁺ T cells, which are then able to lyse tumour cells. Here

\[
\sum_{i,j \in \eta} (D_{i,j}^A),
\]

is the number of active DC cells in a one cell radius of the cell of interest. We assume that active CD8⁺ cytotoxic T cells can lyse tumour cells more than once. CD8⁺ cytotoxic T cells are neutralised if there are no more tumour cells in the neighbourhood. CD8⁺ cytotoxic T cells can also kill active DCs. At each time step, the neighbourhood of each active CD8⁺ cytotoxic T cell is surveyed to determine whether active DCs are present. If any DCs are present, then the CD8⁺ cytotoxic T cells lyse the DCs.

**CD8⁺ T cell migration:** At each time step the inactive CD8⁺ T cells marked for migration do so with a constant probability given by,

\[
P_{\text{mig}}^{\text{CD8}} = k_3.
\]
Natural replenishment to background level: Similarly to the helper T cells, a near-constant minimum background level of inactive and active cytotoxic T cells is maintained at each time step by replacing some healthy cells on the boundary with inactive cytotoxic T cells. At each time step we determine the proportion of all locations in the domain occupied by inactive and active cytotoxic T cells. Whenever this is less than the minimum background level, $I_0$, each healthy cell on the boundary of the domain has probability

$$P_{\text{rep}}^{\text{CD8}} = I_0 - \frac{1}{n^2} \sum_{i,j \in \eta} (I_{i,j}^I + I_{i,j}^A),$$

of being replaced with an inactive CD8$^+$ T cell from outside of the problem domain, where $I_0$ is the ‘normal’ density of inactive CD8$^+$ T cells and $n^2$ is the total number of CA elements.

These rules are presented in pseudocode form in Algorithm 5.

**Algorithm 5** Pseudocode for inactive CD8$^+$ T cell related events.

```plaintext
if (location holds CD8$^+$ T cell) then
    Calculate the number of tumour cells in the neighbourhood
    Find active DCs in the neighbourhood
    Calculate chemokine concentration in the neighbourhood
    Find new location at random in the neighbourhood
    Draw $r \sim U[0, 1]$,
    Calculate $P_{\text{act}}^{\text{CD8}}$ using current state of CA
    if the number of tumour cells in the neighbourhood $\geq 1$ then
        current surface replace with active CD8$^+$ T cell
    else if $r < P_{\text{act}}^{\text{CD8}}$ then
        current surface replace with active CD8$^+$ T cell
    else if chemokine concentration $> \text{threshold chemokine concentration}$ then
        current surface replace with active CD8$^+$ T cell
    else if $r < k_3$ then
        inactive CD8$^+$ T cells move to the new location
    end if
end if
```

Dendritic cells

Inactive DCs are activated when they come in contact with either chemo-kines secreted by tumour cells or with the tumour itself. DCs process the tumour associated antigens and present the antigen on their cell surface. Active DCs play an important role in the activation of T cells and can also be lysed by activated CD8$^+$
cytotoxic T cells as a result of presenting antigen on their surface. At each time step, the neighbourhood of each inactive DC is surveyed to determine whether tumour cells or chemokines are present nearby. If either are present, the DCs are marked for potential activation. The neighbourhood of each active dendritic cell is surveyed for the presence of active CD8$^+$ T cells. If the chemokine concentration level secreted by tumour cells is greater than some threshold concentration, $C_{10}$, then the inactive DC is marked for activation. When active cytotoxic T cells reside nearby, the DC is marked for potential lysis. A stochastic rule is then implemented to determine whether or not the action (migration, lysis or activation) will be carried out. A normal background level of inactive DCs is also maintained at each time step. As a result, we impose the following cellular automata rules.

**Activation by interaction with chemokines and/or tumour cells**: Inactive DCs process and present tumour associated antigen upon interaction with tumour cells. That is, active DCs are activated with a probability given by

$$P_{DC_{act}} = 1 - \exp \left( - \theta_{act} \sum_{i,j \in \eta} T_{i,j} \right)^2,$$

where $\theta_{act}$ controls the shape of the curve allowing it to capture qualitative understanding of the biology and $\sum_{i,j \in \eta} T_{i,j}$ is the number of tumour cells in a one cell radius of the cell of interest.

**Lysis by active CD8$^+$ T cells**: CD8$^+$ T cells kill DCs presenting antigen with probability

$$P_{DC_{lysis}} = k_4.$$

**Dendritic cell migration**: At each time step the inactive DCs marked for migration do so with a constant probability

$$P_{DC_{mig}} = k_5.$$

**Natural replenishment to background level**: Similarly, a near-constant minimum background level of inactive and active DCs is maintained at each time step by replacing some healthy cells on the boundary with inactive DCs. At each time step we determine the proportion of all locations in the domain occupied by inactive and active DCs. Whenever this is less than the minimum background level, $D_0$, each healthy cell on the boundary of the domain has probability

$$P_{rep}^{inactDC} = D_0 - \frac{1}{n^2} \sum_{i,j \in \eta} \left( D^I_{i,j} + D^A_{i,j} \right),$$

of being replaced with an inactive DC from outside of the problem domain, where $D_0$ is the ‘normal’ density of inactive DCs and $n^2$ is the total number of CA elements.

These rules are presented in pseudocode form in Algorithm 6.
Algorithm 6 Pseudocode for DCs related events.

if (location holds inactive DCs) then
    Calculate the number of tumour cells in the neighbourhood
    Calculate chemokines concentration in the neighbourhood
    Find new location at random in the neighbourhood
    Draw \( r \sim U[0, 1] \)
    Calculate \( P_{act}^{DC} \) using current state of CA
    if number of tumour cells in the neighbourhood \( \geq 1 \) then
        if \( r < P_{act}^{DC} \) then
            current surface replace with active DCs
        end if
    else if chemokine concentration > threshold chemokine concentration then
        current surface replace with active DCs
    else if \( r < k_5 \) then
        inactive DCs move to the new location
    end if
end if

Natural killer cells

Inactive NK cells are subject to change as a result of natural replenishment and activation as a result of direct interaction between existing inactive NK cells and either active DCs, tumour cells or cytokines produced by tumour cells. At each time step, the neighbourhood of each inactive NK cell is surveyed to determine whether active DCs or tumour cells are present. If any DCs or tumour cells or cytokines are present, then the inactive NK cell is marked for potential activation, otherwise the NK cell is marked for random migration. If the chemokine level secreted by tumour cells is greater than some threshold concentration, \( C_{10} \), then the inactive NK cell is marked for activation. Active NK cells will survey their neighbourhood to determine whether tumour cells are present. If any tumour cells are present, then the active NK cell is marked as having potential to lyse tumour cells. A stochastic rule is then implemented to determine whether or not the action will be carried out. A normal background level of inactive NK cells is also maintained at each time step. To this end, we impose the following cellular automata rules.

Activation by interaction with DCs: When inactive NK cells come in contact with DCs, they have probability

\[
P_{act}^{NK} = 1 - \exp \left( - \left( \sum_{i,j \in \Omega} D_{i,j}^{A} \right)^2 \right),
\]
of becoming active NK cells, which are then able to lyse tumour cells. Here

$$\sum_{i,j \in \eta} D_{i,j}^A,$$

is the number of active DCs in a one cell radius of the cell of interest.

*NK cells migration:* At each time step the inactive NK cells marked for migration do so with a constant probability given by,

$$P_{\text{NK mig}} = k_6,$$

where $k_6$ is a constant.

*Natural replenishment to background level:* Similarly, a near-constant minimum background level of inactive and active NK cells are maintained at each time step by replacing some healthy cells on the boundary with inactive NK cells. At each time step we determine the proportion of all locations in the domain occupied by inactive and active NK cells. Whenever this is less than the minimum background level, $K_0$, each healthy cell on the boundary of the domain has probability

$$P_{\text{rep inact NK}} = K_0 - \frac{1}{n^2} \sum_{i,j \in \eta} \left( K_{i,j}^I + K_{i,j}^A \right),$$

of being replaced with an inactive NK cell from outside of the problem domain, where $K_0$ is the ‘normal’ density of inactive NK cells and $n^2$ is the total number of CA elements.

These rules are presented in pseudocode form in Algorithm 7.

### 4.4 Simulation and Results

The numerical simulation of the model involves two main steps. Spatial changes for the chemokine species are determined by solving the partial differential equation using an explicit forward in time, central in space (FTCS) method [18]. Then the cell-level phenomena (such as cell-cell interactions, cell death, division and migration) are carried out, dependent on the updated chemokine levels, by updating the cellular automata component of the model. We tie the time step of this iteration process to approximate period of tumour cell division (approximately 0.5-10 days; see for example, [77, 116]).

We combine the solution of the PDE with the CA as described in Section 4.3.2 to simulate the evolution of the growing tumour. Here, a two-dimensional regular $100 \times 100$ square domain is used with 100 cell cycles and a Moore neighbourhood is considered for the cellular automata rules. In this simulation, we solve the PDE model using the finite difference method and an estimated value of diffusion
Figure 4.3: The growing tumour and host immune system. After 25 cell cycles (a). After 50 cell cycles (b). After 75 cell cycles (c). After 100 cell cycles (d).
Algorithm 7 Pseudocode of NK cell related events.

\begin{algorithm}
\textbf{if} (location holds inactive NK cell) \textbf{then}
\begin{algorithmic}
\State Calculate the number of tumour cells in the neighbourhood
\State Find active DCs in the neighbourhood
\State Calculate chemokine concentration in the neighbourhood
\State Find new location at random in the neighbourhood
\State Draw $r \sim U[0,1]$
\State Calculate $P_{NK}^{\text{act}}$ using current state of CA
\State \textbf{if} the number of tumour cells in the neighbourhood $\geq 1$ \textbf{then}
\State \hspace{1em} current surface replace with active NK cell
\State \textbf{else if} $r < P_{NK}^{\text{act}}$ \textbf{then}
\State \hspace{1em} current surface replace with active NK cell
\State \textbf{else if} chemokine concentration $> \text{threshold chemokine concentration}$ \textbf{then}
\State \hspace{1em} current surface replace with active NK cell
\State \textbf{else if} $r < k_6$ \textbf{then}
\State \hspace{1em} inactive NK cells move to the new location
\end{algorithmic}
\textbf{end if}
\end{algorithm}

The distribution of the growing tumour after 25, 50, 75 and 100 cell cycles is shown in Figure 4.3, with results qualitatively matching those of Mallet and de Pillis [89] and commonly found in tumour modelling literature.

Figure 4.4 shows the evolution of the tumour cell and necrotic cell densities over 100 cell cycles. This plot shows the characteristic exponential and linear growth phases of solid, avascular tumours (see for example, Folkman and Hochberg [57]), as well as a slower growing population of necrotic cells. Figure 4.5 shows the number of tumour cells for 100 simulations (thin lines) and the median simulation (thick line) of the CA model over 100 cell cycles. In addition, increasing the number of immature DCs in the domain causes a decrease the number of tumour cells (this result is run by 100 simulations), see Figure 4.6. This result strongly supports the hypothesis that DCs can be used as a cancer treatment.

In Figure 4.7(a) we see that initially, the number of mature DCs is zero until immature DCs come in contact with tumour cells, at which point the matured DCs commence killing the tumour cells. Also, immature DCs are activated by chemokines secreted by the tumour cells. As expected, due to the nature of equation (4.3), the populations of immature and mature DC cells remain approximately steady over the extent of the tumour growth. Similarly, the same behaviour occurs in the populations of CTL cells and helper T cells, see Figure 4.7 and Figure 4.8.
**Figure 4.4**: Total cell counts of tumour and necrotic cells after 100 cell cycles. This plot shows a slower growing population of necrotic cells and an exponential growth of tumour cells.
Figure 4.5: Total cell counts of tumour cells for 100 simulations (thin) and the median simulation (thick) of CA model over 100 cell cycles.
Figure 4.6: Total cell counts of tumour cells for 100 simulations showing the effect of varying the DCs as indicated on the graph over 100 cell cycles. This plot shows that increasing the population of DCs decreases the number of tumour cells.
Figure 4.7: Total cell counts of CD8\(^+\) T cells (a) and DCs (b), after 100 cell cycles.
Figure 4.8: Total cell counts of CD4⁺ helper T cells (a) and NK cells (b), after 100 cell cycles.
Figure 4.9: The evolution of CD8$^+$ T cells, Dendritic cells, T Helper cells, NK cells, healthy cells, tumour cells and necrotic cells for 5 simulations over 300 cell cycles. Parameters used are: $I_0 = 0.005, D_0 = 0.002, H_0 = 0.002, K_0 = 0.001, P_{div} = 0.5, P_{mig} = 0.2$
Figure 4.10: The evolution of CD8$^+$ T cells, Dendritic cells, T Helper cells, NK cells, healthy cells, tumour cells and necrotic cells for 5 simulations over 300 cell cycles. Parameters used are: $I_0 = 0.009$, $D_0 = 0.002$, $H_0 = 0.002$, $K_0 = 0.001$, $P_{div} = 0.9$, $P_{mig} = 0.2$. 

(a) CTL cells

(b) Dendritic cells

(c) T helper cells

(d) Natural killer cells

(e) Healthy, tumour and necrotic cells
Figure 4.9 represents the evolution of cytotoxic T cells, DCs, T Helper cells, NK cells, healthy cells, tumour cells and necrotic cells for 5 simulations over 300 cell cycles. Parameters used are: \( I_0 = 0.005, D_0 = 0.002, H_0 = 0.002, K_0 = 0.001, P_{\text{div}} = 0.5, P_{\text{mig}} = 0.2 \). The initial number of CD8\(^+\) T cells is approximately 50 cells or 0.005 % of the total cells in the domain (see Figure 4.9 (a)). In this case, the tumour cells reach the boundary after approximately 140 cell cycles (see Figure 4.9 (e)). However, increasing the probability of tumour cell division, \( P_{\text{div}} = 0.9 \), the tumour cells reach the boundary only after approximately 100 cell cycles (see Figure 4.10 (e)). In this case, we also increase the initial value of CD8\(^+\) T cells. It means that the probability of tumour cell division is more dominant to affect the growth of the tumour than increasing immune cells.

We also use the hybrid cellular automata model to investigate the growth of a tumour in a number of computational “cancer patients”. Each computational patient is distinguished from others by altering model parameters. We define “death” of a patient as occurring when the tumour is able to metastasise. Effectively, this is when the cells of the tumour reach the boundary of our model domain. We define Kaplan-Meier “survival” estimates as

\[
S(t) = \frac{\text{number of individuals “surviving” longer than } t}{\text{total number of individuals studied}},
\]

where \( t \) is a time from initial diagnosis to “death”. We present the results of these simulations using a simulated Kaplan-Meier survival curve, shown in Figure 4.11 and Figure 4.12.

Figure 4.11 describes that metastasis sets in for the first patients after approximately 80 cell cycles. In addition, at 300 cell cycles after one tumour cell is allowed to grow, the metastasis of the simulated tumours occurred in approximately 30% of patients with lowest iDC in the domain, but approximately 50% of the patients with the highest iDC in the domain. This means that increasing the number of immature DC in the domain result in significantly longer “survival”. These results qualitatively agree with experimental data, see for example, Becker et al., Daud et al. and Nagorsen et al. [13, 32, 100].

Similarly, as shown in Figure 4.12, the patients showed better “survival” as the number of inactive CTLs within the domain increases. These results also agree with experimental data as explained in [101].

### 4.5 Discussion

In this chapter, we have developed a hybrid cellular automata model to describe the interaction between a growing tumour and the immune system including chemokines. The model is able to describe the effect of the immune system and chemokines on a
Figure 4.11: Simulated Kaplan-Meier curve with different initial values of immature DCs as indicated on the graph. This plot shows that increasing the population percentage of DCs in patients will increase “survival” rate.
Figure 4.12: Simulated Kaplan-Meier curve with different initial values of immature CTL as indicated on the graph. This plot shows that increasing the population percentage of CTL cells in patients will increase “survival” rate.
growing tumour. Increasing the number of immature DCs in the domain causes a
decrease in the number of tumour cells. This result strongly supports the hypoth-
thesis that DCs can be used as a cancer treatment. Furthermore, we also use the
hybrid cellular automata model to investigate the growth of a tumour in a number
of computational “cancer patients”. Using these virtual patients, the model can
explain that increasing the number of DCs in the domain causes longer “survival”. Not surprisingly, the model also reflects the fact that the parameter related to
tumour division rate plays an important role in tumour metastasis.

In previous work, Duchting and Vogelsaenger [47] pioneered the use of discrete
cellular automata for modelling cancer, in an investigation of the effects of radio-
therapy. Ferreira et al. [52] modelled avascular cancer growth with a CA model based on the fundamental biological processes of proliferation, motility, and death, including competition for diffusing nutrients among normal and cancer cells. Mal-
let and de Pillis [89] constructed a hybrid cellular automata cancer model that
built on the work of Ferreira et al. to include NK cells as the innate immune sys-
tem and CTL cells as the specific immune system. The Mallet and de Pillis model
was lacking in its detail of the immune system and in this present research we have
improved on their work by explicitly describing more of the host immune system. While direct comparison of the models is difficult, the results as presented in this
chapter qualitatively reflect the findings of Mallet and de Pillis and of Ferreira et
al. while extending them to incorporate greater realism in the description of the
immune system.

While models based on differential equations allow for analytical investigations
such as stability and parameter sensitivity analyses, and ease of fitting the model
to experimental data, these types of models cannot capture the detailed cellular
and sub-cellular level complexity of the biological system. On the other hand,
HCA models can describe in far greater detail, the intricacies of the biological
process such as the interaction between individual cells. In current work comple-
mentary to the present research of this chapter, we have included greater realism in
the modelling of tumour-secreted chemokines by allowing secretion due to cell-cell
interaction. Currently, chemokines and their receptors in the tumour microenvi-
ronment are being extensively investigated to produce therapeutic interventions
to combat cancer, (see for example, Allavena et al. [7] and Murooka et al. [95]).
Future developments based upon this model will allow for simulation-based and
theoretical investigations of such interventions.

In this chapter, we have developed a useful model that can be employed as a
preliminary investigative tool for experimentalists who conduct expensive in vitro
and in vivo experiments to test and refine hypotheses prior to entering the lab. With further cross disciplinary collaboration, this type of model can be refined to
provide a more accurate description of the underlying cancer biology and hence yield more relevant predictions and tests of hypotheses.
Chapter 5

Conclusions and Discussion

5.1 Summary of thesis aims

This section summarises the achievement of all aims given in Chapter 1, which are restated here as follows.

1. To develop a differential equation-based mathematical model of a growing tumour, host tissue and immune system, the associated interactions and resulting outcomes. Based on de Pillis and Radunskaya model [34] and Castiglione and Piccoli [26], we construct new mathematical models that explain more detail the role of DCs incorporating with NK cells, CD8+ T cells and tumour cells. These models can provide a tool to describe qualitative relationships based on particular laboratory findings.

2. To develop a mathematical model of DC-based immunotherapeutic treatment of a growing tumour, that can provide a basic level of understanding and feedback to experimentalists to assist in designing more effective and/or efficient laboratory experiments.

3. To develop a cellular automata model of tumour-immune system interactions that explicitly accounts for cell-cell interactions, incorporates a multi-dimensional spatial viewpoint and allows for stochastic variation to simulate virtual patients. Based on Mallet and de Pillis model [89], we build a new cellular automata model that describe more detailing in immune system. The effect of chemokines in a growing tumour and the simulation of virtual patients using similar Kaplan-Meier curve also provide a new contribution of the literature. This model then provide a deeper level of understanding of the immune system interactions with a growing tumour.
Achievement of aim 1

We have developed two new ODE models describing the interaction between a growing tumour and cells of the innate and specific immune system as presented in Chapter 2. These models consist of four populations, namely, tumour cells and three components of the immune system: NK cells, DCs and CD8\(^+\) T cells. Under certain conditions, the first model has a drawback, that is, the evolution NK cells will grow uncontrolled. To overcome this problem, the model is revised to produce a new more detailed model by including a saturation effect for a number of the dynamic processes modelled via Michaelis-Menten kinetics. Also, the recruitment of DCs which is affected by the number of tumour cells is added in the second model.

Furthermore, we analysed the stability of the models as well as the simple bifurcation behaviour. Both of these models exhibit similar stability behaviour around their system equilibria. As long as the source terms for NK cells and DCs are nonzero, there is no trivial equilibrium. If there is no constant source term, that is both of the source terms for NK cells and DCs are zero, there is a trivial equilibrium which is always unstable. There is a critical initial value for tumour cells, where below this critical initial value, the tumour cells can be eliminated, whereas above this critical initial value, the tumour cells always grow to the nonzero tumour equilibrium. We also analysed the bifurcation behaviour. Under certain conditions, tumour cells can be eliminated as given in Figure 2.2 and 2.9 on the other hand, when these conditions are not satisfied the nonzero tumour equilibrium is reached. This analysis illustrates the important parameters in achieving a tumour-free equilibrium, such as the growth rate of tumour cells, the rate at which tumour cells are killed by NK cells and the rate at which DCs lyse tumour cells.

Finally, numerical solutions of the model demonstrated that the role of DCs as antigen presenting cells was important in enhancing the immune system. By increasing the source term for DCs, the population of NK cells and CD8\(^+\) T cells was increased which in turn decreases the number of tumour cells. These results suggested that an external source of DCs might be used as a treatment for a patient who lacks sufficient DCs in their body. From these simulations, it is shown that DCs alone are not fully effective for tumour treatment. It is also necessary that the NK cell population is high enough for an effective antitumour response. On the other hand, increasing the source term of NK cells causes decreases to the number of DCs and CD8\(^+\) T cells, suggesting that according to this model NK cells are not useful as a treatment strategy when compared with DCs. Based on these models, we undertook further investigation by extending the model to include a DCV as a tumour therapy strategy.
Achievement of aim 2

Presented in Chapter 3 is a mathematical model to describe the dynamics of the interaction of a growing tumour and the host immune system including a DCV as a tumour treatment. The second model in Chapter 2 is extended to include the DCV. Optimal control theory is then applied to this model to find the optimal choice for the timecourse of administration of DCs to the tumour site, with a view to minimising the tumour burden. In this problem, we are interested in the solution of the model in Chapter 2 where the growing tumour tends to the nonzero tumour equilibrium state.

To provide an overview to the reader, before solving the problem, we derive the necessary condition for the existence of an optimal control for such a problem. Based on these conditions, we prove the existence of the optimal control for this particular problem and then use the Hamiltonian function to solve the objective functional which has the aim to minimise the number of tumour cells as well as the cost of the DCV to be administered.

The forward-backward sweep method, which is based on an order 4 Runge-Kutta scheme, is used to numerically solve the optimal control problem. First, a forward method is used to solve the state system with its associated initial condition, then a backward method is applied to solve the adjoint system with an initial condition obtained from the current iteration of the state system, to meet the transversality condition. Numerical solution of the model provided the optimal strategy by which DCs should be administered to the tumour site while minimising the tumour burden. While the solutions obtained here are of a different form from strategies commonly used in clinical practice, we note that it has been hypothesised that portable pumps (not yet developed for clinical use) could be used to administer a DCV to the patient as a continuous function of the form found in Chapter 4.

Achievement of aim 3

Chapter 4 of the thesis included the development of a mathematical model of tumour-immune system interactions that explicitly accounts for cell-cell interactions and spatial variations. The model developed is a two dimensional hybrid cellular automata for tumour-immune system interactions. Besides providing more detail of the host immune system than either previous HCA models or the ODE models developed in this thesis, this model also include chemokines which function to activate DCs. To include the effect of chemokines in the model, we introduce a partial differential equation to describe the concentration of chemokine, and couple this with the discrete cellular automata used to model individual cells.
The cellular automata is built as a square shaped computational domain. Each square element in the grid represents a location that may contain a healthy cell, tumour cell or immune cell. Initially, one cancer cell is placed at the centre cell of the grid and non-cancerous healthy cells cover the remainder of the model domain. A stochastic rule is then applied to each cell to possibly change its current state due to the state of its neighbours. At each time step, a normal background level of inactive immune cells is also maintained.

The model described the evolution of tumour cells both in space and time. We also use the hybrid cellular automata model to investigate the growth of a tumour in a number of computational “cancer patients”. Each computational patient is distinguished from others by altering model parameters. We define “death” of a patient as occurring when the tumour is able to metastasise. Effectively, this is when the cells of the tumour reach the boundary of our model domain. From simulation, the model indicated that increasing the number of immature DCs in the domain result in significantly longer “survival”. This model is able to describe the complex mechanisms of the interactions between a growing tumour, immune system and chemokines in individual cells. In future work this model could be extended to incorporate a discrete analogue of the optimal control theory used to analyse vaccine treatment in the ODE models developed in this thesis.

The ODE models describe tumours of about $10^7$ cells whereas the CA model only describes tumours of at most $10^4$ cells. Due to this, care should be taken in making any comparisons between the work in Chapter 2 and 3 and that of Chapter 4. In order to increase the size of the tumour modelled using the CA method would require vast increases to the size of the CA grid and hence also to the computational power and programming complexity required. Such work was beyond the scope of this project.

5.2 Contribution of this thesis

The interactions between a growing tumour and the immune system with or without immunotherapy are still somewhat poorly understood from a biological and immunological point of view. Therefore, the models developed in this research will make a significant contribution by providing a level of theoretical understanding of these interactions. The significant impacts of this research include

- providing a tool to describe qualitative relationships based on particular laboratory experiments/findings,

- providing a basic level of theoretical understanding to experimentalists, with an aim to assisting in designing more effective and efficient laboratory exper-
iments related to tumour growth and resulting interactions with the immune system and immunotherapy,

- developing a deeper level of understanding of the immune system interactions with a growing tumour.

The ODE models given in this thesis contribute to the theoretical modelling literature especially for the interaction between a growing tumour and immune system. The models can provide information of use in designing laboratory experiments, regarding which parameters play an important role in eliminating the tumour cells. With further data from subsequent experiments, the models can easily be fit to the data, then further analysis could be conducted. The models are also able to explain how DCs can alter the growing tumour and other elements of the immune system. While there is a significant body of literature that describes a growing tumour and the host immune system, few of these describe the growing tumour and its interaction with DCs as well as a tumour treatment, and none in the way shown in this thesis.

In addition, by using optimal control theory the ODE models developed in Chapter 2 have been extended to determine optimal strategies for administering DCV to the patient in order to minimise the cost of the vaccine as well as the tumour burden. This model suggests that the optimal treatment strategy, namely to first administer a high level DCV at the beginning of the treatment schedule with a subsequent reduced treatment level, actually quite different from the standard methods used in clinical practice.

Finally, we present a new hybrid cellular automata model of far greater complexity than those in existing literature incorporating far more detail of the host immune system. This model is able to describe the complex mechanisms of the immune system and impact of chemokines on the tumour system. The model allows for a description of each individual cell and its interactions with other cells and the surrounding chemical fields. The effect of chemokines in a growing tumour and the simulation of virtual patients using similar Kaplan-Meier curve also provide a new contribution of the literature.

In summary, in this thesis a number of useful models have been developed that can be employed as preliminary investigative tools for experimentalists who conduct expensive in vitro and in vivo experiments to test and refine hypotheses prior to entering the lab. With further cross disciplinary collaboration, these type of models can be refined to provide a more accurate description of the underlying cancer biology and hence yield more relevant predictions and tests of hypotheses.
5.3 Future research

These mathematical models using ordinary differential equations, optimal control theory and hybrid cellular automata models provide new insights into the effects of the immune system on a growing tumour. Further development of the ODE models developed here will include the extension to consider the chemokine secretion involving CD4$^+$ T helper cells. Furthermore, development of PDEs models to include spatial effects from ODE models will be observed in future research. The consideration of combined immunotherapy-chemotherapy treatment strategies may also be analysed using optimal control theory.

Future developments based upon the CA model will involve considering specific types of cancer. Also, the effect of chemokines on the cell-cell interactions will be more deeply investigated. More complex partial differential equations related to chemokines secretion resulting from cell-cell interactions including the tumour treatment may also be introduced in future work. Finally, 3D CA model also will be considered in future work.


