A Hybrid Cellular Automata Model for the Role of Reactive Oxygen Species in Novel Tumour Treatments Strategies

by

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Cancer is a disease that entails uncontrolled proliferation and a disruption of tissue function that often results in a poor clinical outcome. Furthermore, it has continued to elude any universal treatment or cure in part because of the complexity seen across multiple spatial and temporal scales. *In silico* modelling techniques, such as hybrid cellular automata (HCA), provide an inexpensive and versatile means of incorporating experimental factors that are either impossible or infeasible to control and investigate when using *in vivo* or *in vivo* experimental settings. Reactive oxygen species (ROS) are critical molecules in cellular processes and have been demonstrated to induce effects on cellular processes ranging from proliferation to metabolic reprogramming and cell death. Dichloroacetate (DCA) and intravenous vitamin C (VC) are two proposed treatments that induce death via ROS and have begun to gain attention from the oncology community for their relative lack of toxicity and potential efficacy. Thus, investigating a HCA model to optimize their effects can be influential in the success of these drugs. The model employed uses discrete rules for the evolution of each cell, and continuous equations for diffusible elements so that multiple time scales may be incorporated effectively. The implementation of components such as necrotic cell removal and accurate pharmacokinetics in the model has a considerable effect on outcome of VC treatment individually and in combination. Furthermore, VC is most effective at removing tumour cells entirely when its plasma concentration is maximized, while DCA's cytotoxic action plateaus as its concentration increases and is only effective at removing cell types that exist within the interior of the tumour.

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List of Abbreviations

Adenosine Triphosphate – (ATP)

Basement Membrane – (BM)

Dichloroacetate – (DCA)

Epithelial-Mesenchymal Transition – (EMT)

Extracellular Matrix – (ECM)

Glucose – ($C_6H_{12}O_6$)

Hybrid Cellular Automata – (HCA)

Hydrogen Peroxide – (H_2O_2)

Hypoxia-inducible-factor (HIF-1)

Lactate – ($C_3H_5O_3^-$)

Pyruvate Dehydrogenase Kinase – (PDK)

Platelet-Derived Growth Factor – (PDGF)

Reactive Oxygen Species – (ROS)

Receptor Tyrosine Kinase – (RTK)

Vascular Endothelial Growth Factor – (VEGF)

Vitamin C / Ascorbate – (VC)

Chapter 1

Biological Background

1.1 Introduction

Despite improving the overall quality of health and life expectancy, modern medicine has been unable yet to counter the detrimental effects of cancer. Rather than elucidating a concise cause and mechanism of action in the progression of cancer, decades of research have revealed the complexity underlying the disease. Simply stated, cancer is the result of uncontrolled cell proliferation. Unfortunately, the manner in which it presents itself varies significantly among those afflicted. This is in part due to the vast array of genetic, epigenetic and environmental factors that can result in sustained cell division [Feinberg and Tycko 2004, Hanahan and Weinberg 2011]. Since these factors may accumulate over a timespan on the scale of years, focusing instead on the properties which enable the survival and success of various cancerous phenotypes is a promising approach to understanding the disease and implementing effective treatments. To illustrate, there are many possible genes that can promote proliferation (i.e., proto-oncogenes) or prevent the appropriate arrest of cell cycle

processes (i.e., tumour suppressor genes). However, mutations in these genes can have elaborate effects on the outcome of a cell through their role individually and also in conjunction with others. These effects are further diversified by the timing of when the normal function of these genes is disrupted. Since a single mutation is not sufficient for the development of malignant disease, the frequency of tumourigenic mutations, and not just the specificity, has been illustrated to be correlated with incidences of cancer [Croce 2008, Weinberg 2007].

Approaches in understanding the disease have revealed models in which the environment of a tumour plays a critical role in regulating the progression of cancer. Through a number of mechanisms explained later in this section, cells that make up a tumour are able to take advantage of the hostile environment that results from their formation. In particular, the environment varies in distribution of blood vessels, oxygen concentration and pH profile. It is the limited viability of this environment that is thought to provide the selective pressures driving the emergence of phenotypes of varying aggressiveness [Anderson et al. 2006, Molavian et al. 2009, Smallbone et al. 2007]. As a result, the growth dynamics of the tumour will be the result of its adaption to the environment. Despite the large and intricate nature of the signalling networks governing growth, mitosis, cell death, inflammation, and vascularization, the progression of tumour cells to an invasive phenotype has an intimate link with the composition of the microenvironment.

One method of cellular signalling that has received increasing attention in oncology research is via reactive oxygen species (ROS). While it has been well established that ROS are crucial in sustaining operation of the electron transport chain, recent evidence has

revealed a far more elaborate role. Aside from their toxic potential, ROS have been discovered to act as messenger molecules within transformed cells. While the range of effects vary from metabolic restructuring to division to programmed cell death, the actual network and interactions involved have not yet been fully explained [Behrend et al. 2003, Irani et al. 1997, Schimmel and Bauer 2002, Suh et al. 1999]. Nevertheless, the key proteins regulating the hypoxic response have been catalogued and shown to be stabilized by mitochondria-derived ROS [Chandel et al. 2000, Hamanaka and Chandel 2010]. Since cancer cells have often been observed to express increased concentrations of intracellular oxidants, the dynamics between ROS-induced metabolic reprogramming and cell death will be of particular interest herein [Trachootham et al. 2009].

As with all novel scientific discoveries in cancer research, potential treatment strategies have been proposed to disrupt the signals favouring growth for the cancer cells in the relationship. It is interesting to note that both anti-oxidant and pro-oxidant therapies have been investigated, but pro-oxidant theories will be investigated herein.

Two drugs of interest are ascorbate, or vitamin C (VC), and dichloroacetate (DCA) because of their intriguing effects on the microenvironment and phenotypic composition of tumours. Although VC has been described as being an anti-oxidant in physiological concentrations, blood concentrations achievable only by intravenous infusion have been demonstrated to induce the formation of the ROS, hydrogen peroxide (H_2O_2) [Chen et al. 2007]. Because of their toxic potential, intracellular ROS levels are maintained using anti-oxidant mechanisms. Since most tumour cells have consistently higher levels of ROS, it is reasonable to assume they are less apt than normal cells at surviving exogenous ROS stress,

as depicted in figure 1.1 [Trachootham et al. 2009, Wartenberg et al. 2003]. The reasoning is that antioxidant mechanisms are under more basal stress and cannot compensate for the increase. Thus, administering pro-oxidant drugs has been proposed as a possible treatment strategy that would cause little or no permanent damage to healthy tissues. Similar to VC, DCA has been used for decades for medicinal purposes but has only recently been recognized for its potential benefits in cancer [Michelakis et al. 2008]. Originally intended for the treatment of congenital lactic acidosis, DCA restores aerobic metabolism in cells with mitochondrial deficiencies [Stacpoole 1998]. Michelakis and his colleagues found that in addition to restoring metabolic structure of the mitochondria, a cascade of effects promoting cell death also occurred. For these reasons, DCA is currently in Phase II clinical testing for assessment in cancer treatment [Michelakis et al. 2008, Michelakis et al. 2010].

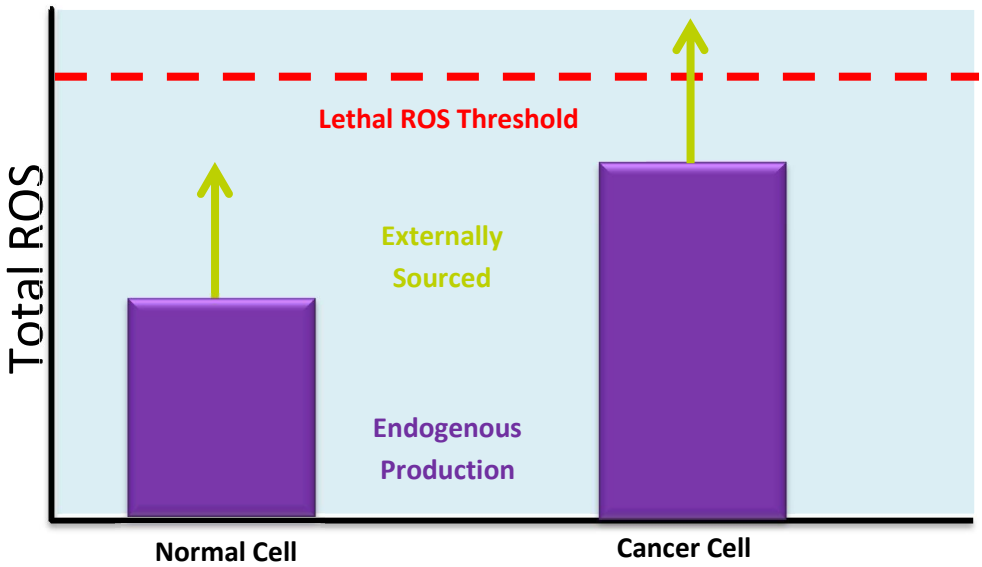

Figure 1.1: Rationalization for pro-oxidant treatment strategies. Adapted from [Trachootham et al. 2009].

However, these drugs have already been approved for use in medical settings not involving cancer and with little or no toxic side effects; thus, they have been administrated by

alternative health care specialists already [Padayatty et al. 2010]. As a result, there is a current need to maximize the response of the tumour to the treatment through detailing how to optimize schedules. In this thesis, previously explored models used to describe tumour metabolism are expanded to incorporate ROS-based treatments. Novel treatment strategies using realistic pharmacokinetics and combining both DCA and VC are investigated using tumours that are responsive to the effects ROS on metabolism and cell death. This reveals the underlying optimal strategies and tumour behaviour that results from each treatment. However, before attempting to create the model to do so, a thorough explanation of the relevant biological processes is necessary.

1.2 The Progression of Tumour Formation

1.2.1 Introduction to Cancer

One of the significant challenges presented in studying cancer is the large diversity of diseases through which it presents itself [Hanahan and Weinberg 2000]. For this reason, understanding what factors drive the progression from a normal cell to one with a high proliferation rate to one that is capable of invading other tissues is the focus of much research. Metastasis, the formation of tumours at secondary sites from invasive cells of the original tumour, are frequently observed and associated with poor prognoses. Similarly, acquired drug resistance is a trait observed to arise late in treatment. Thus, improving

clinical outcome necessitates a detailed understanding of the factors driving phenotypic evolution.

Cancer can be first initiated by a mutation in any of the genes responsible for regulating DNA repair or the cell cycle and death. However, it does not usually progress to a stage that can be diagnosed until there have been multiple mutations acquired. For instance, the number of genomic instability events observed in colorectal cancers was measured to be greater than 10^4 [Stoler et al. 1999]. Nevertheless, there is a set of characteristics that is observed almost universally in cells at some point or another in the progression from normalcy to malignancy; these have been dubbed the hallmarks of cancer [Hanahan and Weinberg 2000, Hanahan and Weinberg, 2011]. It is these defining characteristics that allow researchers to observe and discuss the intricate behaviour of tumours through a compartmentalized set of properties.

1.2.2 Promoting Growth While Evading Death

The most fundamental phenotypic change acquired by cells during tumour pathogenesis is the ability to promote proliferation through self-sustaining signals [Hanahan and Weinberg 2011]. During the normal formation of tissues and wound healing, a cell will begin and progress through the cell cycle only when growth-promoting signals are sustained. The signalling molecules are secreted by neighbouring tissues and are most often processed through the family of cell membrane-spanning proteins, receptor tyrosine kinases (RTK). Because of the difficulty in empirically measuring the paracrine-like signalling involved

between tissues *in vivo*, there is relatively little known on the pathways driving proliferative signalling in normal tissues. In contrast, much more is known about this process in

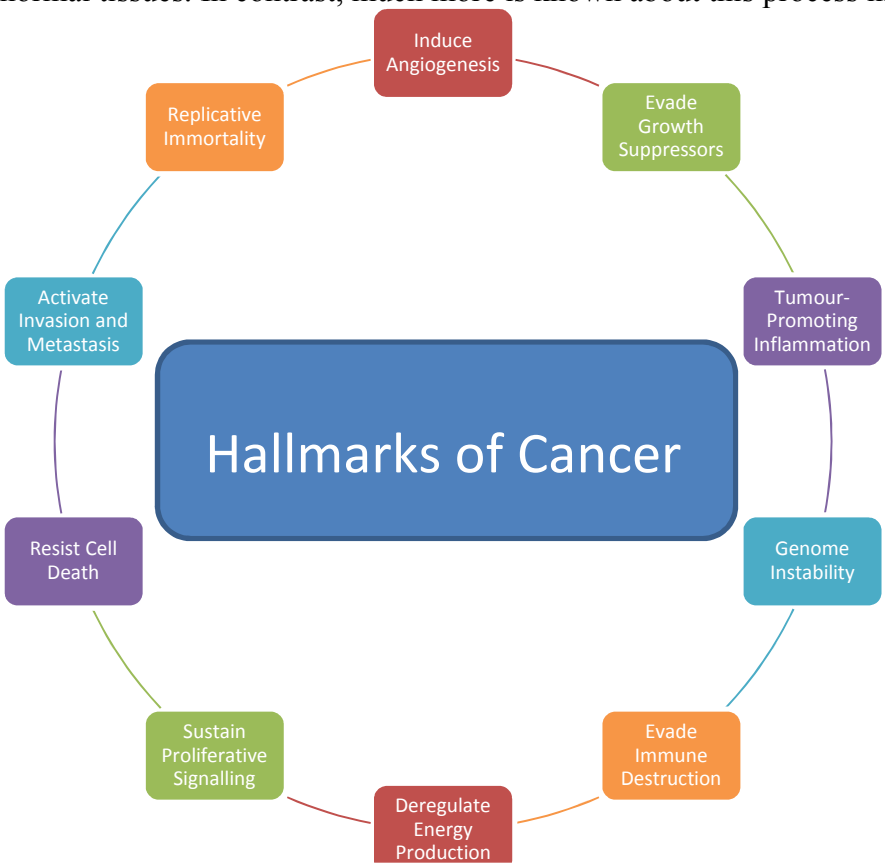

Figure 1.2: The hallmarks of cancer, including the emerging concepts and enabling characteristics described in [Hanahan and Weinberg 2011].

malignant tissues [Hanahan and Weinberg 2011]. For instance, there have been numerous mechanisms observed through which cancerous tissues are able maintain self-sufficient proliferative signalling. This can be accomplished through increased secretion or expression of either the ligand or the corresponding receptor by the cancer cell. Alternatively, they may recruit aid from normal cells in the surrounding tissues by initiating their release of growth stimulating factors [Cheng et al. 2008, Bhowmick et al. 2004]. To illustrate further, one of the most common mutations present in human cancers is in the signalling mediator, Ras. Cells may possess a mutant form of this receptor that is active without stimulation by growth

factors, or alternatively, a mutant form of any of the downstream proteins in the signalling pathway [Hanahan and Weinberg 2011]. However, these mechanisms alone are not sufficient to maintain rapid growth.

To prevent uncontrolled growth, normal tissues have a number of control mechanisms that operate on a variety of aspects of the cell cycle. When proliferative signalling pathways like those mentioned above are stimulated, negative feedback mechanisms are often in place to gradually dampen the effect of the signal. However, the proteins responsible for this homeostatic response are not always functioning in cancer. With respect to the Ras signalling pathway, it is the protein GTPase that is responsible for ensuring that proliferative signals are transitory [Weinberg 2007]. It is important to note that when the intrinsic signalling mechanisms are faulty and over-promote growth, it is not guaranteed that the cell will continue to divide indefinitely. Senescence and apoptosis have been observed following the excessive stimulation of signalling pathways through proteins such as Myc, Ras, and Raf. [Collado and Serrano 2010, Evan and d'Adda di Fagagna 2009, Lowe et al. 2004]. Thus, in order to establish neoplastic disease, cells must evade the regulatory mechanisms employed during cell cycle events.

The mechanisms utilized by healthy cells to ensure the proper progression of mitotic and cell cycle events are understandably thorough [Hanahan and Weinberg, 2011]. By having a controlled response to both the intracellular and extracellular factors affecting mitosis and survival, the collective fitness of the cells can be maximized. The canonical tumour suppressor proteins discussed in cancer research are Rb and p53. The Rb protein acts as a gatekeeper for cell-cycle progression. As such, division may only occur in the absence

of exogenous growth-inhibitory signals. In contrast, p53 is responsive to the intracellular factors that may result in a decreased survival probability. Specifically, this includes damage to the genome and low levels of nucleotide pools, glucose or oxygen. Rather than simply arresting the cell cycle and preventing division, p53 is able to induce programmed cell death that results in the attenuation of mutations [Weinberg and Hanahan 2011]. Interestingly, cell homeostasis is not immediately affected by a loss of normal function of either tumour suppressor or proto-oncogenes individually and neoplastic effects do not result until later in life [Lipinksi and Jacks 2010, Ghebranious and Donehower 1998]. This further supports the notion that malignant diseases are the result of many acquired mutations.

In addition to the explicit signalling pathways that regulate cell cycle progression, there are other mechanisms in place that ensure the proper formation and maintenance of tissues. One of the most essential traits acquired in a tumour's evolution to malignancy is the ability to evade contact inhibition and gain anchorage-independence [Smallbone et al. 2007]. In epithelial tissues, cells are joined together in single layers by a series of junctions that serve a variety of signalling, transport, and structural purposes [Lodish 2008]. Recent literature has revealed the role of two proteins, Merlin and LKB-1, in promoting epithelial integrity. Merlin, for example, accomplishes this task by coupling RTK's to the proteins that maintain the junction, effectively decreasing their ability to respond to signals in the environment [Hanahan and Weinberg 2011, Curto et al. 2007, Okada et al. 2005]. Conversely, although LKB-1 has been found to promote cell polarity and the formation of cell junctions, the exact protein interactions responsible for the response are yet unknown. Nonetheless, the presence of a mutated form of LKB-1 has been shown to promote a phenotype that is conducive to invasive growths [Partanen et al. 2012]. When these

mechanisms are not in place, and thus the cell is not anchored to its adjacent neighbours or the extracellular matrix (ECM), a programmed form of cell death, anoikis, usually results [Weinberg 2007]. Even when these properties are possessed by a cell, this does not confer immortality. This is due to the inevitable loss of genetic material at the ends of chromosomes after each division. This region of a chromosome is known as a telomere and consists of multiple tandem repeats that are often truncated after each division. Incidentally, a cell that is dividing rapidly will quickly lose the stability of its genome as chromosome ends begin to fuse [Hanahan and Weinberg 2011]. Clearly, this presents a hurdle that must be overcome in the progression of a malignant disease. Telomerase is a DNA-binding protein that specializes in extending the telomeric regions of DNA. In doing so, unlimited replicative potential is incurred to the cell [Blasco 2005]. Although increasing the expression of telomerase early in tumour formation is expected to accelerate the process, genetic damage consistent with telomere-shortening has been observed in a number of tumours prior to acquiring malignancy [Raynaud et al. 2010, Chin et al. 2004]. Thus, it has been proposed that the mutations acquired following telomere-shortening events may actually contribute to the tumour-promoting potential [Hanahan and Weinberg 2011]. In either scenario, the obstacles that need to be overcome by cells forming a neoplasm are complex and vary considerably in nature.

1.2.3 Metabolic Supply and Demand

Unsurprisingly, the regulation of metabolic pathways and biosynthesis also becomes dysfunctional in neoplastic disease. Although this trait has been known to be prominent

among many types of cancer for decades, the extent to which it has an effect on tumour growth dynamics and how it arises is not yet fully understood [Hanahan and Weinberg 2011]. In normal cells, the primary form of energy metabolism is aerobic respiration. This involves the catabolism of glucose ($C_6H_{12}O_6$) to pyruvate in the cytoplasm, followed by its reduction to CO_2 in the mitochondria through the citric acid cycle and electron transport chain. This process, known collectively as cellular respiration, can be represented by the following chemical equation:

$$\textbf{\textit{Respiration}}: \quad C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \sim 36ATP \qquad (1)$$

Clearly, oxygen is a critical reactant for the complete metabolism of glucose. Specifically, it is required for the mitochondrial branch of the metabolic network. However, *in vivo* there is an oxygen diffusive limit of roughly 100 μm [Hanahan and Weinberg 2000]. Therefore, as cells in an early tumour divide away from the basement membrane (BM), they will approach a threshold point where aerobic metabolism is no longer possible. This is compensated for by eliminating the dependence on the mitochondria for energy. As a result, the pyruvate produced from glycolysis is converted to lactate ($C_3H_5O_3^-$) and exported from the cell. This is in accordance with the following reaction:

$$\textbf{\textit{Glycolysis:}} \quad C_6H_{12}O_6 \rightarrow 2C_3H_5O_3^- + 2H^+ + 2ATP \qquad (2)$$

It becomes immediately apparent that the energetic yield is far less for a cell only using glycolysis, even when accounting for the oxidative ATP production actually being less than 36 in practice. Despite this inefficiency, many cancers have been observed to rely predominantly on glycolysis in what is known as the Warburg effect [Casciari et al. 1992,

Vander Heiden et al. 2009]. As with all perturbations to homeostatic cellular processes, an inability to return to the steady state can result in survival complications. Thus, cancer cells must adapt by multiple means in order to survive and thrive in collaboration with this metabolic shift.

1.2.4 The Microenvironment

A tumour's surroundings during the stages of development continuously change as a result of both intrinsic and extrinsic factors. That is, there exist effects on the microenvironment that are produced by the abnormal tissue directly and others which are produced indirectly in response to the abnormal tissue surrounding cells. Upon initiation of unrestricted growth and division, available nutrients within the microenvironment become limiting factors and an inflammatory response may be initiated. Specifically, glucose and oxygen are the most prevalent sources of energy for cells in healthy tissues. Once the environment progresses to a hypoxic state, the insufficient levels of oxygen result in a seemingly inefficient output of energy and dysfunction of the mitochondria. In response to this stress, the family of hypoxia-inducible factor (HIF-1) proteins are stabilized and affect metabolic pathways to decrease the dependence on oxygen, while also promoting the formation of new vasculature. During normoxia, the subunit HIF-1α is rapidly degraded in the cytoplasm; however during hypoxia, it is stabilized via ROS and combines with the constitutively active HIF-1β to be activated as a transcription factor. Genes that are activated as a result of this include enzymes in the glycolytic metabolic pathway, the membrane-bound glucose transport proteins GLUT-1 and GLUT-3, and also a variety of factors

promoting angiogenesis, proliferation and survival [Harris 2002]. Of notable mention is lactate dehydrogenase, a protein stabilized by HIF-1 that normally converts pyruvate to lactate in the cytosol. As a result, available pools of pyruvate are depleted and other mechanisms that prevent aerobic metabolism are amplified. Simultaneously, vascular endothelial growth factor (VEGF) is secreted to promote angiogenesis. This ligand activates endothelial cells to proliferate and create new vasculature while erythropoietin is expressed to activate the production of red blood cells, or erythrocytes, in the bone marrow. These effects are made more intricate by the influence of surrounding cells and structural factors. As a result, it has been proposed that a tumour should be considered as being comprised of both the malignant cells that originate from healthy tissues, as well as cells in the surrounding environment that contribute to the growth of a mass [Albini and Sporn 2007]. For this reason, an attractive area of research has been targetting the microenvironmental, or extrinsic, factors that contribute to the growth of the tumour.

One of the most prominent environmental cell types that are involved in tumourigenesis are pericytes. These cells can be anchored to the BM and have finger-like protrusions that span the endothelial cells and allow them to act as both structural promoters and growth and survival messengers. For example, they assist in anchoring of the smooth muscle cells to the endothelial cells, which provide structural support to counter the pressure variations resulting from pulsatile bloodflow. Simultaneously, they provide signalling factors like VEGF and platelet-derived growth factor (PDGF) to regulate quiescence or proliferation in endothelial cells. Angiogenesis requires the presence of pericytes and so investigating their significance has been related to developing novel treatment strategies [Raza et al. 2010,

Hanahan and Weinberg 2011]. Examples of other elements in the microenvironment that play a critical role are the macrophages, T and B lymphocytes, neutrophils and mast cells. They are significant for the inflammatory response that they induce to combat the volatile environments that arise during malignant evolution [Hanahan and Weinberg 2011]. During normal tissue damage or infection, these cells of the innate immune response are recruited to the site of injury and begin releasing critical growth factors, chemokines, and various other messenger proteins. The increased heat, redness and swelling at the injury site is indicative of fighting an infection, but it also indicates the regeneration of the damaged tissues. The immune cells that promote this process have been manipulated in tumourigenesis to favour growth, motility, vascularization, and metastasis. Although it may seem confounding how the cytotoxic lymphocytes are able to promote growth when their normal function is to destroy abnormal cells, it is now believed that they are responsible for the recruitment of other lymphocytes whose inflammatory response mechanism may be helpful to the tumour [Hanahan and Weinberg 2011]. Interestingly, the macrophages that assist in promoting tumour survival are phenotypically distinct from healthy macrophages and can respond appropriately to the environment presented [Lewis and Pollard 2006]. During the initial inflammatory response, they may first promote mutagenesis and tumour progression through increasing ROS. Subsequently, they assist hypoxic regions of the tumour through the promotion of vasculature [Qian and Pollard 2010]. While there are many more cells that may assist in tumour progression, the last ones discussed are cancer-associated fibroblasts. Their particular role is intriguing because of their involvement in maintaining the ECM, a collection of various fibrous proteins and carbohydrates that serve structural purposes like the basement membrane for epithelial cells , as well as a medium for the transduction of

cellular signals [Juliano and Haskill 1993]. Similarly to macrophages, there is a distinct cancer-promoting phenotype that is expressed by certain fibroblasts that results in increased likelihood of metastasis. In fact, the presence of myofibroblasts, which normally remodel the ECM and induce angiogenesis, in the tumour stroma has been associated with a poor prognosis for tumours. There exist many types of fibroblasts despite a lack of thorough knowledge surrounding their interactions in the tumour microenvironment [Franco, et al. 2010]. Nonetheless, the crucial role that the microenvironment plays during tumour formation has been recognized and has become a target in designing therapies.

1.2.5 Metastasis and Cancer Stem Cells

A characteristic of carcinomas frequently associated with a poor prognosis is the development of tumours at secondary locations through the process known as metastasis. After a series of morphological changes to both the cells and the environment, cells from the tumour acquire the characteristics of a motile cell and migrate to a secondary site where they may begin to divide and differentiate (figure 1.3). This first change that must take place for this to occur is collectively known as the epithelial-mesenchymal transition (EMT). During this process, cellular composition is remodelled to support the new purpose. For example, the filamentous protein vimentin becomes significantly upregulated. As opposed to utilizing the conventional structural proteins such as actin and specific collagens, mobile cells use vimentin to maintain cellular structure and organization. The reason for this change is believed to be vimentin's high tolerance for mechanical stress, which would be intensified

for cells attempting to migrate through the ECM. This cellular remodelling was first observed to be instrumental in the developmental stages of growth and also the adult response to damaged tissue when morphogenetic changes and differentiation of mesenchymal stem cells are frequent and necessary [Weinberg 2007]. Incidentally, this connection gave rise to the theory of cancer stem cells, which are a phenotypically distinct subset of tumour cells with important properties. Firstly, these cells must be capable of initiating the growth of a tumour when transplanted into immunodeficient mice. Furthermore, these cells must be able to reproduce the heterogeneity of cell types observed in the original tumour [Sampieri and Fodde 2012]. Cells possessing such qualities have now been observed to be present in a variety of solid cancers but accurately identifying and sorting them is a task complicated by the variety of membrane-bound proteins that may be used for their identification and deciphering their individual contribution to cancer progression.

However, the successful colonization of a secondary tumour is not guaranteed by initiating EMT or the CSC phenotype alone since there are many obstacles that determine the success of an invasive cell in propagating growth. Aside from the cytokines that are sufficient to induce dedifferentiation in epithelial cells, many environmental factors have been observed to be influential in determining the outcome of a migrating cell. In particular, the ECM and BM act as the first impediments by physically restricting the available space for cell motility. Although the incorporation of vimentin in the cytoskeleton increases a cell's elasticity and ability to deform, the lack of permeability in the ECM necessitates further action for migration. To address this issue, matrix metalloproteinases, among other

proteases, are secreted and begin disassembling the surrounding infrastructure. Interestingly, it has been proposed that certain tumour cells are released into the environment through passive mechanisms that result from poor vascular development and high interstitial pressure [Bockhorn et al. 2007]. Indeed, poor vascular development has already been associated with increased potential for metastasis. The pericytes that assist in developing and maintaining the vasculature of a tumour have been implicated in permitting intravasation because of their sparse distribution when compared with healthy tissues [Xian et al. 2006, Raza et al. 2010].

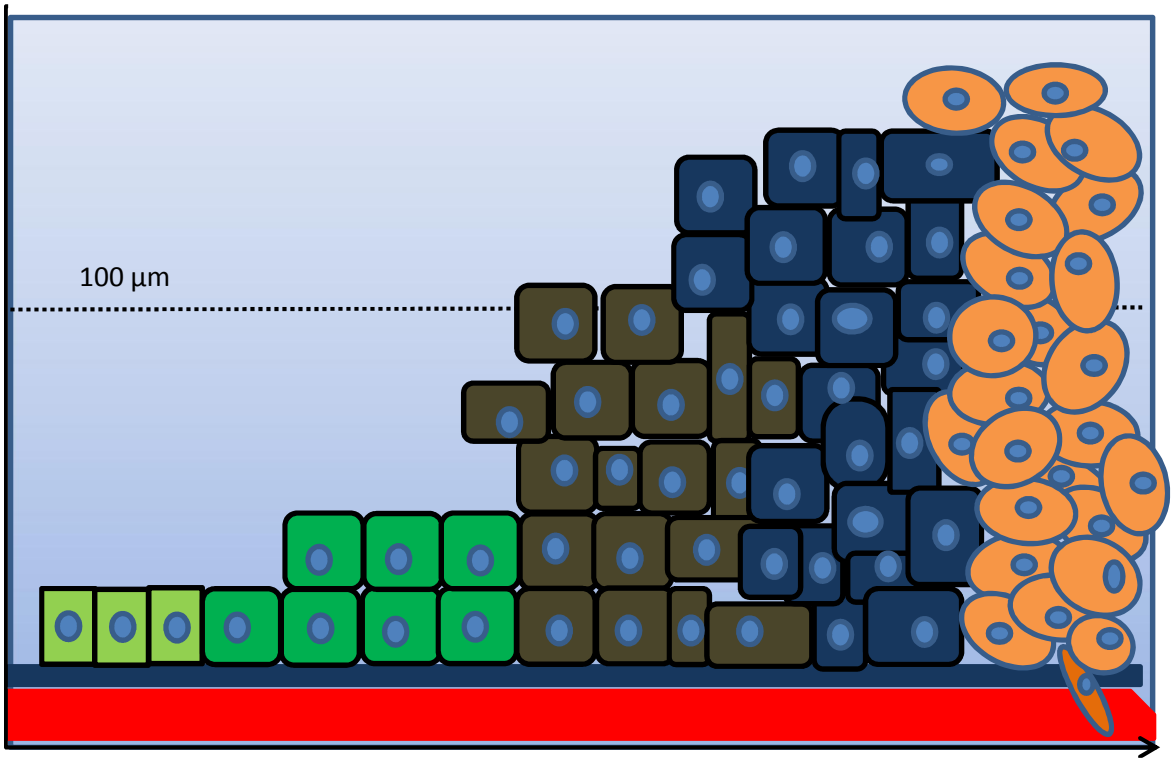

Figure 1.3: A simplification of the progression from normal epithelial cells to invasive cancerous ones. Initially cells form a monolayer adjacent to the basement membrane and blood vessel. As the cells progress in the increasingly limiting environment, traits that promote survival become more prominent until intravasation and metastasis through the BM may occur.

Similarly, macrophages have been observed to promote intravasation and prime secondary environments for the colonization of migrating tumour cells, while tumour-associated fibroblasts have been observed to promote EMT [Cirri and Chiarugi 2012, Qian

and Pollard 2010]. While the factors leading to extravasation are presumed to be rate-limiting *in vitro*, successful colonization events seem to be much less predictable *in vivo* [Chambers and Matrisian 1997, Bockhorn et al. 2007]. Alternatively, when the environment is not viable, dormancy is an option chosen by many cells.

1.3 Reactive Oxygen Species

Reactive oxygen species have garnered attention for their diverse roles in molecular biology that are a result of their high chemical activity in various settings. In particular, high levels of ROS act in a toxic manner on the composition of DNA and cell membranes, while moderate levels may act as signalling molecules to enhance a proliferative phenotype. Both of these effects are mediated by the reactivity induced by the presence of free radicals, which are unpaired valence electrons in molecules. By definition, ROS are any molecules possessing or capable of inducing the production of free radicals. Their use in metabolic processes result in the presence of ROS such as hydroxyl ($OH^{\cdot}$), superoxide ($O_2^{\cdot -}$), singlet oxygen (O_2^{*}) and H_2O_2 [Mates and Sanchez-Jimenez 2000, Wiseman and Halliwell 1996]. Due to the potential potency of their presence, anti-oxidant mechanisms are utilized by cells to mitigate their effect and ultimately determine the redox state of the cell. Much of the intracellular ROS produced by cells is in the highly reactive superoxide form; however, it is converted to H_2O_2 with assistance of superoxide dismutases (SOD), as summarized in the following chemical reaction:

$$2O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (3)$$

Once in the less toxic ROS form, radical removal mechanisms such as catalase, glutathione peroxidase and peroxiredoxin become effective [Halliwell 2006]. However, the reverse process is also possible with the presence of certain minerals like Fe^{2+} or Cu^{+}. Specifically, the Fenton reaction produces hydroxyl radicals from available H_2O_2 as follows:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-} \qquad (4)$$

Although these hydroxyl radicals have short half-life *in vivo*, they can be induced by elevated superoxide levels and are highly reactive with DNA [Mates and Sanchez-Jimenez 2000, Valko et al. 2007]. The aforementioned antioxidant mechanisms are able to remove excess ROS and maintain the redox state of the cell within a viable range. In general, catalase and peroxiredoxins both catalyze the formation of water from H_2O_2, albeit in distinct manners [Sun 1990, Valko et al. 2007, Rhee et al. 2001]. In addition to requiring an electron donation by thioredoxins to be in an active state, some peroxiredoxins are also involved in regulating H_2O_2 at the cell membrane, whereas catalase mainly operates in peroxisomes [Rhee et al. 2001, Woo et al. 2010, Veal et al. 2007]. Glutathione peroxidase varies from these two mechanisms in its ability to scavenge radicals from hydroxyl and superoxide molecules and not just H_2O_2 [Sun 1990, Valko et al. 2007]. If, however, the antioxidant mechanisms in place are unable to maintain a viable redox state within the cell, two possible situations may arise: cell death by apoptosis, or by necrosis. Depending on the extent of oxidant damage, a cell will either undergo a compartmentalized and timely deconstruction through rapid ATP depletion, or will expand and lyse [Chandra et al. 2000, Valko et al. 2007, Zong and Thompson 2006]. In the latter case, an inflammatory response

and potential damage to surrounding tissues may result. However, there is evidence that the inflammatory response may be beneficial to recruiting growth, survival, angiogenic, and metastastic factors in cancer [Wiseman and Halliwell 1996, Zong and Thompson 2006, Qian and Pollard 2010, Hanahan and Weinberg 2011].

Although counterintuitive at first, the notion that the harsh environment created by ROS in pre-cancerous and cancerous cells contributes to their progression to malignancy has gained considerable support. Firstly, there is an obvious effect on tumour progression that results from their toxicity. The sensitivity of DNA to damage by ROS incurs a greater possibility of mutatgenic events occurring. Thus, if the cell survives as mutations accumulate in proto-oncogenes and tumour suppressor genes, increasingly malignant properties may be acquired [Storz 2005, Mates and Sanchez-Jimenez 2000, Wartenberg et al. 2003, Wiseman and Halliwell 1996]. Notably, this instability has been purported to be a likely cause for the acquired drug resistance often observed in advanced tumours [Pelicano et al. 2004]. In addition, ROS play diverse roles in signalling pathways governing many aspects of growth, division and survival [Veal et al. 2007]. For example, consider the role of peroxiredoxin as a tumour suppressor. When it is downregulated or absent, H_2O_2 is not catalyzed and is able to accumulate at the cell membrane. As a result, RTK pathways such as PDGF and EGF are constitutively activated by the available H_2O_2, which acts as a signalling molecule by oxidizing cysteine residues on the proteins [Rhee et al. 2001, Woo et al. 2010, Choi et al. 2005]. Emerging evidence of low-level ROS as a messenger molecule has been observed in advancing cell cycle progression, as well as increasing the survivability of tumours through activating protein cascades and pathways [Boonstra and Post 2004, Veal et al. 2007, Storz

2005]. Additionally, transcription factors that promote cancer progression, such as NFκB and AP-1, are activated by ROS [Manna et al. 1998, Acharya et al. 2010]. Moreover, the hypoxic response, which entails a metabolic switch to glycolysis and the promotion of angiogenesis, and metastasis can be influenced by ROS signalling [Shi et al. 2009, Chandel et al. 2000, Goyal et al. 2004, Cannito et al. 2008]. While the stabilization of HIF-1 and degradation of aerobic metabolic factors by ROS moderate the hypoxic response, metastasis is promoted through stabilizing matrix degrading enzymes and inducing an EMT in the cell so that ultimately, the angiogenic growth will further promote successful extravasation [Li et al. 2013, Cannito et al. 2008, Wu 2006]. Given the potential opportunities that exist for tumour cells to use ROS in optimizing growth, it is not surprising that cancer cells have been observed to express higher levels of endogenous ROS when compared to normal cells *in vitro* and *in vivo*. However, it is important to consider that the redox state and profile of any tumour cell is dependent on the cell line, or clonal population, and maturity [Storz 2005, Wartenberg et al. 2003].

1.4 Vitamin C Therapy

Despite being known as a necessary nutrient in maintaining proper health for almost a century, the exact therapeutic potential of VC continues to be debated [Bessey and King 1933]. While its role as an antioxidant and its ability to prevent scurvy were relatively well-known, Cameron and Pauling investigated its effects as a supplement to cancer treatment that would inhibit tumour growth and published promising results [Cameron and Pauling 1976, Cameron and Pauling 1974]. Subsequently, conflicting results were published by researchers at the Mayo Clinic and the debate over the benefits of VC in cancer therapy

began [Creagan et al. 1979, Moertel et al. 1985]. It has since been elucidated that the method of drug delivery is likely the source of disparity between the results of the studies conducted. In particular, intravenous ascorbate was able to induce anti-tumourigenic effects, while oral administration did not. Clearly, the biochemistry of VC is critical to understanding how to optimize cytotoxic delivery for therapeutic purposes.

The biological significance of VC arises from its ability to donate electrons and the resulting behaviours have effects that cascade across many aspects of cell and tissue biology. Some of these effects include collagen production, assisting to destabilize HIF-1 during normoxia, and DNA modifications that regulate gene expression; all of which require active forms of iron to be replenished by VC for enzymatic reactions [Verrax and Calderon 2008, Du et al. 2012]. Most importantly, both pro-oxidant and antioxidant effects may be attained by supplmental VC [Du et al. 2012, Acharya et al. 2010, Wang and Yi 2008] . The realization of either redox role is determined by the concentration of VC, which itself is determined exogenously because of the inability of humans to produce it naturally. When consumed orally, ascorbate acts as an antioxidant to radical species like superoxide and hydroxyl and becomes the ascorbate radical [Padayatty et al. 2003]. This form is unreactive and so the radical can be converted back to ascorbate without causing damage [Du et al. 2012, Chan 1993]. However, the absorption of VC through the gut is tightly regulated and limits the plasma concentration to values below 80 μmol/L. In constrast, intravenously administered ascorbate can produce plasma and urine concentrations exceeding 30mmol/L [Padayatty et al. 2004, Monti et al. 2012].

Conventional therapies involving VC have accordingly been concerned with determining how to optimize their anti-tumourigenic effects [Du et al. 2012, Duconge et al. 2008, Gonzalez et al. 2012]. The theoretical strategy behind treatment methods that promote ROS damage have been well-studied but the actual mechanism through which VC exerts anti-carcinogenic effects has not yet been fully elucidated [Trachootham et al. 2009, Acharya et al. 2010, Wilson et al. 2014]. Specifically, the elevated internal levels of ROS

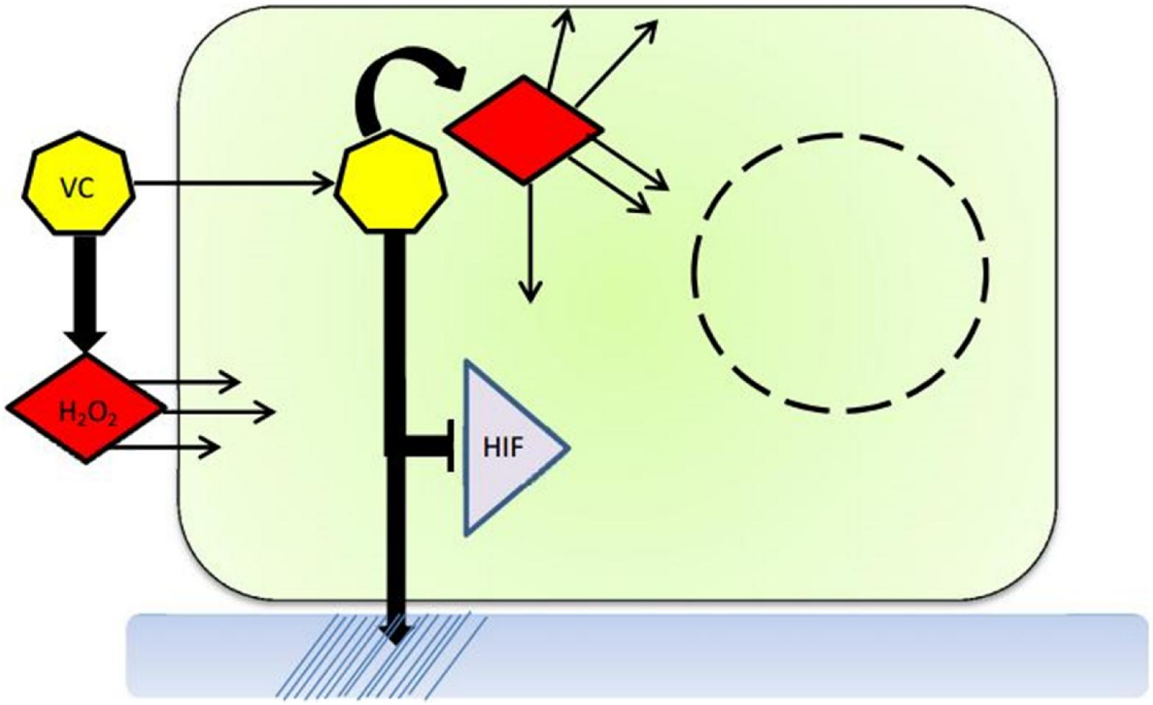

Figure 1.4: Mechanisms of action of ascorbate. ROS may be produced in the exterior or interior of the cell to produce damage. Alternatively, VC could indirectly inhibit cancer progression by fortifying the BM or inhibiting the hypoxic response.

render transformed cells more sensitive to further stress and damage by exogenous ROS, while the antioxidant machinery in normal cells functions properly to protect the cell [Trachootham et al. 2009]. Fortunately, Chen and his colleagues discovered that H_2O_2 is produced as a direct result of pharmacological (i.e., millimolar) plasma concentrations of

ascorbate and that this can be lethal to many cell lines when present in a concentration greater than 10 mmol/L [Chen, et al. 2007].

Since high concentrations of antioxidants exist in the blood, H_2O_2 production is limited to tissues where there are multiple proposed mechanisms of cytotoxicity. Firstly, the extracellular mechanism proposes that a catalyst such as Fe^{3+} induces the formation of ascorbate radicals, which then donate radicals to oxygen to produce superoxide [Chen et al. 2007]. Alternatively, intracellular mechanisms have been proposed to contribute to the demise of cancerous cells through uptake of VC via GLUT-1 or specific VC transporters [Du et al. 2012]. Subsequently, H_2O_2 may be generated from the VC, or it may stabilize factors that contribute to the production of superoxide [Chatterjee et al. 2008]. Chen demonstrated that the likely cause of the cell death is H_2O_2-mediated depletion of ATP, but there is potential for anti-tumourigenic effects by VC not directly producing ROS. Such effects include the inhibition of the hypoxic response and metastasis through reenforcement of the ECM and basement membrane [Wilson et al. 2014]. In any case, the potential health risks associated with mega-dose ascorbate therapy are relatively minor in those with properly functioning kidneys and without a glucose-6-phosphate-dehydrogenase deficiency [Cameron and Campbell 1974, Riordan et al. 2005].

HIF

1.5 DCA Therapy

Similarly to VC, the first medical discovery regarding the significance of DCA was not related to its role in cancer. Originally thought to be a hazardous waste produced from chlorination processes, DCA has since been used in the treatment of lactic acidosis in

children and adults [Stacpoole et al. 1998b]. For this reason, thorough investigations into the pharmacokinetics of DCA have been conducted and have revealed interesting trends. Firstly, the bioavailability, or the fraction of the administered drug that is absorbed into circulation unchanged, is nearly 100% [Stacpoole et al. 1998b]. Furthermore, the decay dynamics have been observed to differ from exposure history and among age groups [Stacpoole et al. 1998a]. While investigating these dynamics, the only toxic side effect observed was peripheral neuropathy, which was reversible with treatment arrest[Stacpoole et al. 1998]. However, Michelakis and colleagues identified similar mitochondrial dysfunction in cancers and discovered that DCA has potential benefits as a cancer treatment [Michelakis et al. 2008].

Specifically, it does so by eliminating some of the advantages gained by cells exhibiting the Warburg effect. In cancer and during the hypoxic response, mitochondrial function is often impaired and pyruvate that was destined for the mitochondria is converted to lactate in the cytosol. DCA has been identified for its ability to activate pyruvate dehydrogenase kinase (PDK), which in turn restores pyruvate transport and mitochondrial polarization [Michelakis et al. 2008]. Through mechanisms not yet fully understood, the restoration of mitochondrial function results in increased expression of ROS and p53, while also decreasing GLUT-1, HIF-1, and survival factors and altering cytokine signalling factors that normally promote survival and pH stress response [Kumar et al. 2012]. Cumulatively, these effects have been documented to inhibit the growth of many cancer types including breast, glioblastoma, Non-Hodgkins Lymphoma, pancreatic, and more [Sun et al. 2010, Michelakis et al. 2010, Strum et al. 2013, Monti et al. 2012, Wong et al. 2008].

Chapter 2

Hybrid Cellular Automata

2.1 Modelling Cancer

When designing biological experiments, *in vitro*, *in situ*, and *in vivo* models can be used to replicate the conditions present in nature in a controlled setting to varying extents. While *in vivo* models provide the most realistic experimental conditions, they tend to be more difficult to control and explore parametrically for technological and feasibility reasons [Kam et al. 2012]. Therefore, despite providing the most accurate model in which to study a disease, *in vivo* approaches do not always the most insight when studying the complexity of cancer. In contrast, *in vitro* methods provide the potential to investigate genetic and cellular events thoroughly and so they are generally more versatile for experimental investigations. With this and taking the increased availability of computing power into consideration, *in silico* methods provide a novel method by which results from *in vitro* experiments may be used to theoretically consider effects that may not be feasible under the prescribed experimental conditions used previously [Kam et al. 2012, Deisboeck et al. 2009]. However,

accurately incorporating more detailed aspects of tumour growth nevertheless requires ongoing efforts in developing new analytical and numerical approaches and techniques [Alarcon et al. 2004].

Modelling the biological processes governing cancer progression is a daunting task because of the complexity through which the disease presents itself on a variety of temporal and spatial scales, and also because of the variety of model types that may be appropriately applied [Ribba et al. 2004, Byrne 2010]. To illustrate, malignant cancer involves the disruption to processes at the molecular, cellular, tissue, and organismal levels [Preziosi 2003]. As a result, changes at the smallest scale, such as mutations or epigenetic changes to DNA, influence the overall behaviour of a cell through gene expression and protein production. If they are not fatal, such changes could induce proliferative or survival advantages in pre-malignant cells that ultimately transform the microenvironment. In turn, the microenvironment poses selective pressure on the developing tumour that may affect the gene expression profile of invasive cells [Anderson et al. 2007, Smallbone et al. 2007]. The resulting disruption to homeostasis and normal tissue function incurs health issues that pose a threat to the entire organism, thus creating a disease with interacting effects spanning a number of scales (figure 2.1).

Taking this into consideration, models that are able to encapsulate the multi-scale nature of cancer growth are ideal in revealing mechanistic behaviour during carcinogenesis. Early models describing tumour growth relied on continuous equations and a moving boundary problem to reproduce the growth behaviour of radially symmetrical tumours with basic intercellular forces [Greenspan 1972, Ribba et al. 2004]. Unfortunately, these models

are not very versatile and are computationally expensive to investigate. While tumour models have evolved to include a variety of progression factors and mathematical techniques, those which are capable of revealing the underlying behaviour of the biological processes driving cancer progression are most applicable in biology [Anderson and Quaranta 2008, Preziosi 2003].Therefore, a focus will be made herein to models that incorporate aspects of cancer progression at the subcellular, cellular, and extracellular (tissue) levels. Specifically, hybrid discrete continuum models permit a method of incorporating these multiple scales. Nevertheless, determining the advantages and disadvantages of techniques specific to such models is crucial to producing one that optimally encompasses the behaviour of the system being considered.

2.2 Hybrid Models

Through integrating traditional continuous methods with discrete elements, hybrid models of tumour growth can be constructed that are capable of handling growth on multiple temporal and spatial scales. These models are often ideal because they can describe complex biological behaviour that would otherwise produce a large system of equations requiring considerable computational power to solve. However, models such as this often cannot be extrapolated beyond the experimental settings used to derive parameter values and so they contribute little to revealing underlying mechanisms. Furthermore, the feasibility of producing such models operates under the assumption that sufficient experimental evidence

and insight has been collected to produce a realistic model [Ermentrout and Edelstein-Keshet 1993].

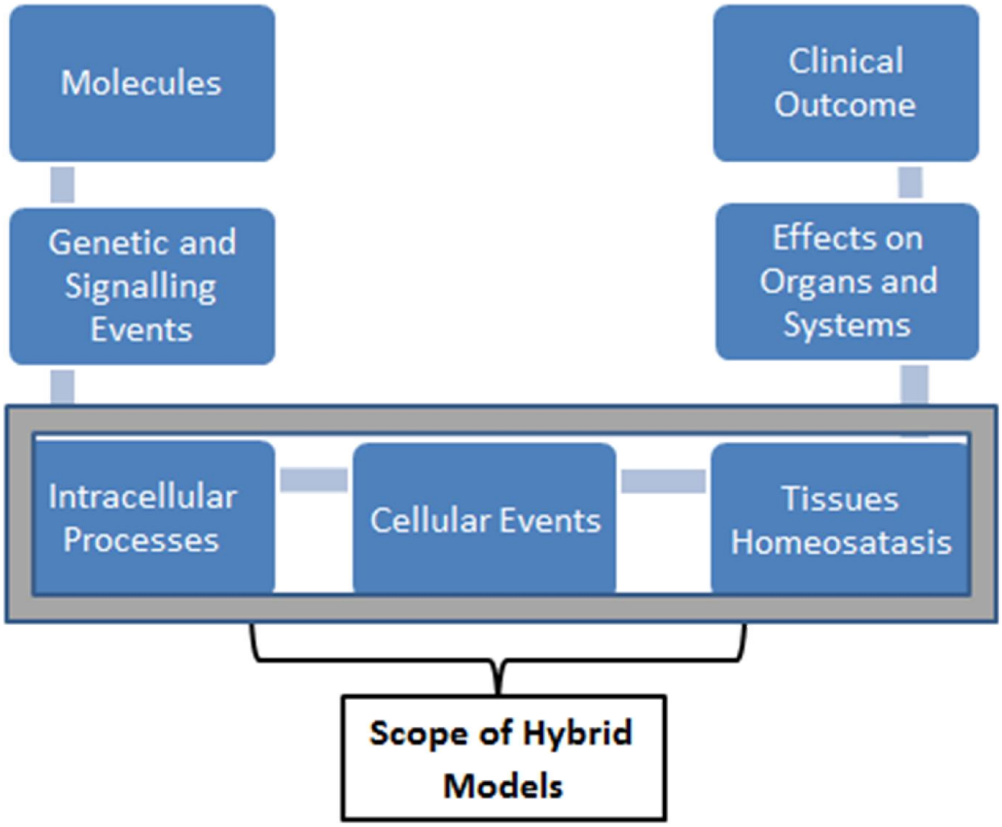

Figure 2.1: Implicated cellular and spatial scales involved in the modelling of cancer.

Explicitly speaking, discrete elements within hybrid models are manifested in the activity of and between cells, or possibly groups of cells, that proceed with each time step according to predetermined rules. Generally, these rules are simplifications of the actual dynamics governing phenotypic progression and metastasis but they can represent an accurate portrayal of tumour behaviour when coupled with stochastic elements. Simultaneously, continuous methods, such as ordinary or partial differential equations and

stochastic equations, can be implemented to capture aspects of tumour growth including, but not limited to, nutrient gradients and signalling factors.

Quantifying the rules driving the discrete progression of components in the model requires experimental evidence to ensure validity and to derive actual parameter values. In previous models, experimental results from multiple tumours grown in different environmental conditions have been compiled for this purpose; however, the combined properties may give rise to a model that may not actually correspond to any tumour observed yet. Additionally, gathering all of the parameter values necessary to describe a tumour may not be possible because of limitations imposed by current laboratory assay techniques [Kam et al. 2012]. For these reasons, collaboration between mathematicians and the biologists conducting research is critical to produce an accurate model capable of providing meaningful contributions to oncology. It should be noted that in addition to coordinating the design of experiments for preliminary parameter measurements, the refinement of models and hypotheses is a necessary step to ensure that the results obtained are realistic or verifiable [Byrne 2010]. This can entail comparing the model results to experimentally observed results, or comparing them to other models to determine underlying behaviours and the strengths and weaknesses associated with each type [Kam et al. 2012].

In the mathematical modelling of tumour progression, models may be broadly characterized as being descriptive or mechanistic in nature [Anderson and Quaranta 2008]. Furthermore, variations in hybrid models are presented in which aspects of carcinogenesis can be investigated in simulations. In general, models either tend to be able to manage large cell populations with simplified structures, or small populations of cells that are deformable

[Rejniak and Anderson 2011]. As a result, the former class of models allows for the investigation of the optimal response to treatments in tumours that are clinically-relevant in size, while the latter are capable of more thoroughly investigating the intercellular and environmental interactions that contribute to tumour growth and invasion. Consequently, designing a model that specializes in either application is dependent on how the cellular environment and interactions are implemented. Specifically, cells may either be fixed on a lattice, or categorized as off-lattice elements. What follows is an overview of some techniques that have been previously employed that reveal the benefits and disadvantages associated with implementing different hybrid schemes.

One of the most frequently used hybrid models is the square or cubed lattice HCA. As the name implies and as shown in figure 2.2, the tumour cells may occupy each grid site and are considered individually in the model. Avascular and vascular tumour growth, invasion, and a variety of environmental interactions stemming from available nurtrients, and stromal and tissue organization have been modelled with such methods [Dormann and Deutsch 2002, Owen et al. 2009, Smallbone et al. 2007, Gatenby et al. 2007, Anderson et al. 2006, Bankhead III et al. 2007]. Although the majority of models utilize a mutually exclusive organizational scheme for grid sites, certain types of models have been expanded to include lattices that allow multiple cells per site or multiple sites per cell in an attempt to capture different aspects of tumour progression. Allowing multiple cells to inhabit a lattice site has the advantage of producing larger and more relevant tumour sizes; however, the migration of cells becomes difficult to implement efficiently [Rejniak and Anderson 2011]. Conversely, representing cells with multiple grid sites provides the advantage of capturing

the heterogeneity of cell size through deformations and the resulting influence on cell division, invasive advantages, and vasculature formation [Jiang, et al. 2005, Rubenstein and Kaufman 2008, Poplawski et al. 2009, Shirinifard et al. 2009]. In particular, the overall cell size and shape are the result of stochastic forces that minimize the energy required for structural redesign in response to environmental and intracellular cues [Maree et al. 2007]. However, this approach is less applicable to more pronounced structural transformations, such as EMT. Hexagonal lattices have also been implemented in diversifying the structural entities used to construct the environment. Specifically, this allows one to investigate the effects of a having a unique local environment for cells (i.e. 6 possible neighbours) on the resulting tissue configuration. To this end, glioma cell migration has been reproduced and the effect of contact inhibition on the appearance of ductal tumours *in situ* has been investigated [Aubert et al. 2008, Shumate and El-Shenawee 2009].

An alternative to the aforementioned class of on-lattice models are the evolutionary HCA, which expand upon the simpler HCA by incorporating an artificial neural response network. Rather than using pre-determined thresholds or functions to induce cell changes such as proliferation, quiescence, or cell death, a network of nodes for each process are related to ranges of environmental conditions with varying affinities. Although the resulting behaviour of the cells as a tissue can be made to reproduce experimentally observed growth, their favourability as a model arises from less deterministic applications [Gerlee and Anderson 2007]. Notably, evolutionary HCA models are able to simulate events occurring at the genetic level and impose phenotypic changes that affect the overall behaviour of the system as a tissue. This is accomplished through adjusting the strength of the connection

between the environmental conditions tested and the node decided as outcome (i.e. division, quiescence, or death) after division to simulate a mutagenic event. While each mutagenic event in the model does not explicitly correspond to a single mutagenic event to DNA, the resulting phenotypic changes from these mutations emulate the physical morphology and clonal diversity observed to arise in cancers under varying environmental conditions [Gerlee and Anderson 2007].

In contrast to the on-lattice models presented thus far, off-lattice models are effective at capturing more realistic spatial configurations through disregarding the uniformly spaced grid that has been coupled with continous fields thus far [Rejniak and Anderson 2011]. To this end, cells have been implemented as spheres, elipses, tesselated structures, and most diversely as fully deformable entities with discretized boundaries capable of replicating phenotypic plasticity, polarity, and structural deformation [Ramis-Conde et al. 2008, Stolarska et al. 2009, Meineke et al. 2001, Anderson et al. 2007]. However, this versatility results in a necessity for more complex algorithms to handle the cellular environment and how it maps spatially to the continous fields that are implemented, which are normally calculated on a grid similar to those used in on-lattice models [Rejniak and Anderson 2011].

Despite all being capable of spanning multiple scales, each model is best equipped to investigate different aspects of tumour progression. For instance, standard HCA's excel at capturing events that effect the overall tissue dynamics, evolutionary HCAs model genetic changes and their effect on emerging phenotypes that influence tumour survival, and fully deformable cells are able to simulate cellular interactions in an environment that most accurately correlates to *in vivo* conditions [Anderson et al. 2007]. However, each model also

has inherent limitations in its ability to capture all dynamics relative to the progression of the disease. Thus, in conjunction with whichever aspects of the tumour environment are being investigated computationally, the use of multiple distinct models to investigate overlapping behaviour can be valuable in determining the underlying mechanistic behaviour that is significant for tumourigenesis [Kam et al. 2012]. Although verification of computational results by experimental evidence is ideal in determining model validity, cross-examining multiple models with unique assumptions can be useful when experimental verification is not feasible [Kam et al. 2012]. Due to its relevance in clinical outcomes, the appearance and dynamics of invasive phenotypes has been a target for research. For instance, a mutlicompartment model incorporating game theory employed by Basanta and colleagues demonstrates the cost-benefit relationship of introducing motile phenotypes in tumours with a heterogeneous environment [Basanta et al. 2008]. This work correlates with previous work by Smallbone that demonstrates heterogeneity in the environment influences the success of phenotypes that lead to motility and invasion; specifically, the effect that the distance from the blood supply has on nutrient availability, metabolic phenotype, and acidity [Smallbone et al. 2007]. Clearly, comparing and compiling evidence from multiple *in silico* approaches to determine the underlying behaviour is a promising approach to determing what patterns are significant in deciphering carcinogenesis.

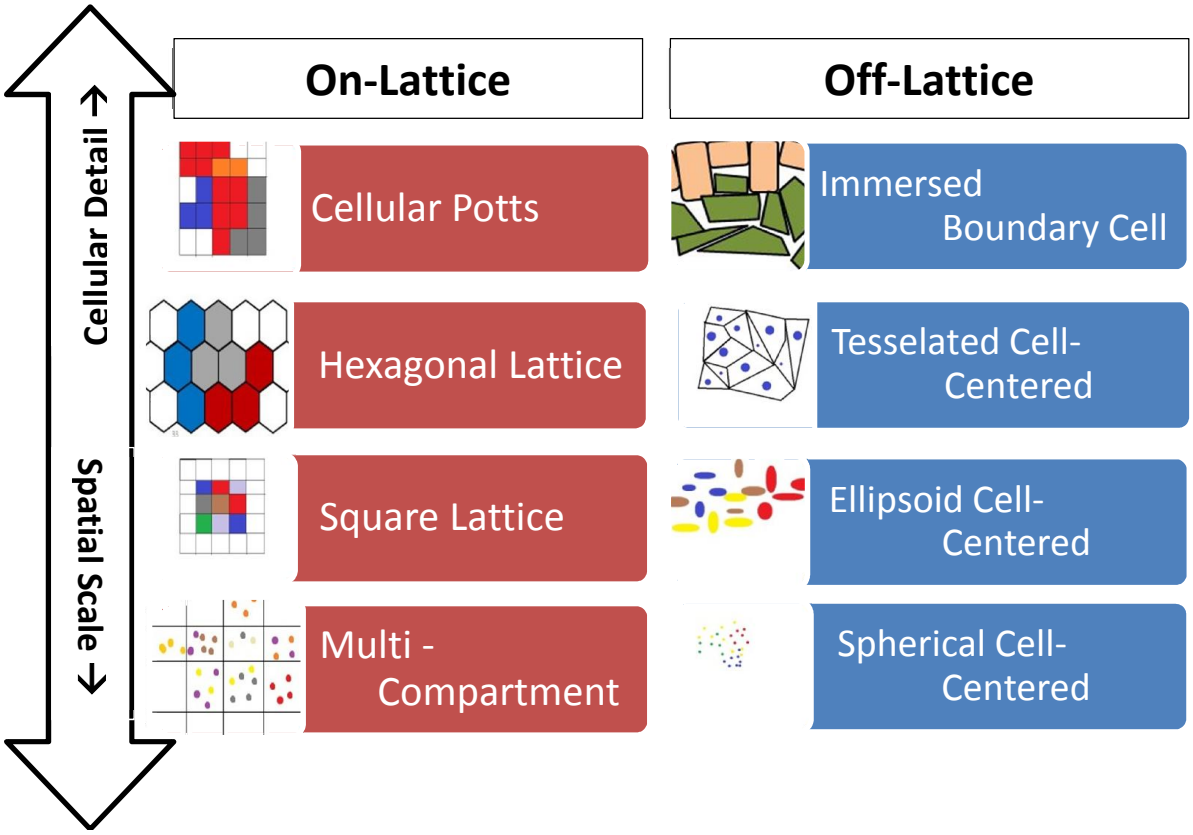

Figure 2.2: Select examples of the potential hybrid model types that can be useful in modelling cancer. Adapted from [Rejniak and Anderson 2011].

2.3 The Applied Model

With a particular interest in investigating the effects of ROS, metabolic phenotype, and microenvironmental factors on tumour response to treatment, a square-lattice HCA model is employed. As with most hybrid models, reaction-diffusion equations model the oxygen and glucose fields. In general, these equations produce the following system:

$$\frac{\partial C_G}{\partial t} + D_G \nabla^2 C_G = F_G(C_O, C_G) \qquad (5)$$

$$\frac{\partial C_O}{\partial t} + D_O \nabla^2 C_O = F_O(C_O, C_G) \qquad (6)$$

Here, D_i is the diffusion coefficient for element i, oxygen or glucose, and ∇ is the conventional Laplacian operator. Because each time step in the discrete progression of the system is an hour, a steady state approximation can be made that renders the time derivative zero and greatly reduces the complexity of solving the system. Similar equations are also used for the diffusion of VC and DCA, but they differ in the complexity of consumption dynamics. While many models have previously used a constant value for nutrient consumption, this fails to encapsulate the dynamic nature of the demand for metabolic precursors [Patel et al. 2001, Alarcon et al. 2003, Anderson et al. 2009]. Thus, the following functions are introduced for the consumption of oxygen and glucose:

$$F_G(C_O, C_G) = p_G \frac{C_G}{C_G + k_G} f_1(C_O) \qquad (7)$$

$$F_O(C_O, C_G) = r F_G \frac{C_O}{C_O + k_O} f_2(C_O)\,, \qquad (8)$$

where p_g is the normoxic consumption of glucose, k_G and k_O are the Michaelis parameters, and r is the ratio of glucose to oxygen consumption. When $r = 1$, the consumption reflects a cell with deficient oxidative machinery; whereas when $r = 6$, the cell would be using oxidative respiration efficiently and exclusively. The functions $f_i(C_O)$ are non-linear and were reverse-engineered from experimental data collected from *in vivo* tumours grown in the proximity of a single blood vessel [Molavian et al. 2009]. Overall, consumption obeying Michaelis-Menten dynamics was improved upon by including scaling that compensates for the effects of metabolic changes that occur in response environmental oxygen and glucose

cues ($f_i(C_O)$), as well as the inherent cellular preference (r). The functional form of f is given by:

$$f_i(C_O) = (1 - a_i C_O^{0.02} \exp(-100(C_O - 0.01)^4)) \qquad (9)$$

where $a_1 = \frac{2}{3}$, $a_2 = 1/2$, and units are all calculated in 10^{-2} mM. As shown in figure 2.3, oxygen below a concentration of 4.4 x 10^{-3} mM corresponds to a hypoxic response in metabolism; this presents itself as the glycolytic response and a shift towards independence from oxygen. Additionally, an oxygen value below 2 x 10^{-4} mM represents an anoxic environment where the effects of oxygen on metabolism are negligible.

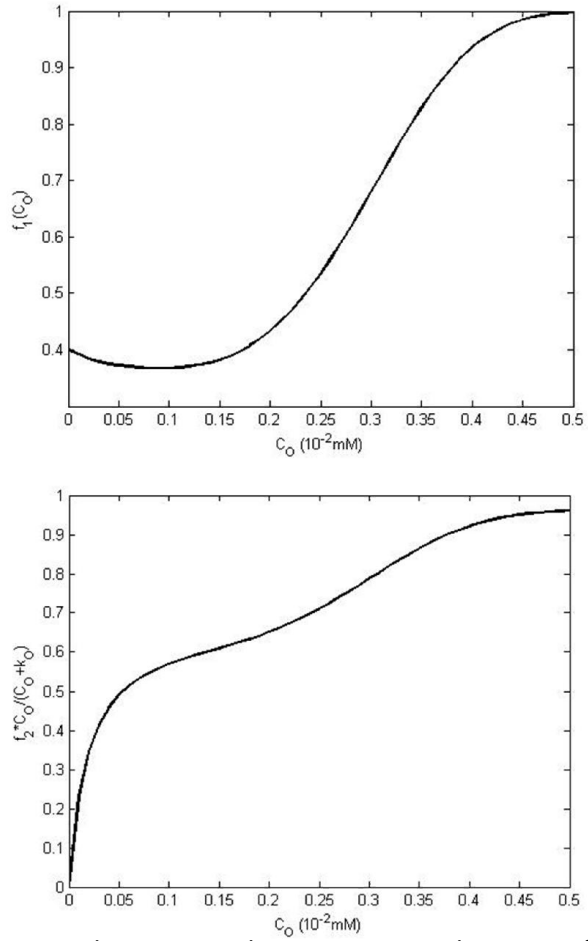

Figure 2.3: Functions determining the consumption dynamics of cancer cells in the model [Molavian et al. 2009].

The model prescribed here incorporates four vessels along the boundary of the lattice grid that provide nutrient-rich blood to the environment. However, the permeability of the vessel wall and resulting impact on flow across the vessel must be incorporated into the boundary conditions. As a result, the flux at the boundary is given by [Patel et al. 2001, Alarcon et al. 2003]:

$$D_i \vec{\boldsymbol{n}} \cdot \nabla C_i = \mu(C_k - C_i) \qquad (10)$$

where $\vec{\boldsymbol{n}}$ is the outward-facing unit vector, μ is the permeability of the vessel wall, C_i is the concentration of oxygen or glucose within the stroma, and C_v is the concentration of oxygen or glucose within the vessel.

In order to solve this system, the following finite difference scheme with step size Δ is applied to the interior of the domain for diffusible element *l* at site *(i,j)* :

$$\frac{D_l}{\Delta^2}\left(C_{i+1,j} + C_{i,j_{+1}} + C_{i-1,j} + C_{i,j-1} - 4C_{i,j}\right) - F_l\left(C_{i,j}^O, C_{i,j}^G\right) = 0 \qquad (11)$$

For any cells sharing a boundary with a vessel wall, which happens to be at location $C_{i-1,j}$ in this case, the following discretization of the boundary condition is imposed [Alarcon et al. 2003]:

$$-\frac{D_l}{\Delta}\left(C_{i,j} - C_{i-1,j}\right) = \mu\left(C_v - C_{i,j}\right) \qquad (12)$$

Upon isolating for $C_{i-1,j}$ and substituting into the discretization scheme, the following is derived in order to solve the system:

(13)

$$C_{i+1,j} + C_{i,j+1} + C_{i,j-1} - \left(3 + \frac{\Delta\mu + \Delta^2 F_l}{D_l}\right) + \frac{\Delta\mu}{D_l} C_v = 0$$

With the environment modelled, the values that are critical to determine the outcome of each cell can be calculated. Firstly, the age of each cell required for division can be found from Casciari's empirical modelling of the effects of the environment on the growth rate of cells. The ratio of the cell's doubling time, t_d, to the doubling time under optimal conditions (~13 hours) was fitted to the experimental data using the following expression [Casciara et al. 1992]:

$$f = G\left(\frac{C_O}{C_O + G_O}\right)\left(\frac{C_G}{C_G + G_G}\right) \qquad (14)$$

Other influential factors calculated in the model include the distribution of ECM and the diffusion of VC and DCA. The ECM concentration, or C_{ECM}, consists of randomly assigned values between 0 and 1 from a uniform distribution. A cell's ability to occupy a site is conditional on the ECM being sufficiently sparse. Alternatively, if the concentration of ECM proteins is within a predetermined threshold, there is a fixed probability of the cell being able to deform and occupy that site. In contrast, DCA and VC are calculated as diffusible entities according to equations similar to equations 5 and 6. In contrast, their consumption rate is presumed to be independent and constant. The diffusion coefficients for VC and DCA, as well as the permeability coefficients, are approximated and presumed to be similar to those for glucose and oxygen (Appendix). Furthermore, each treatment has a unique effect on ROS production and cell survival and metabolism. In untreated scenarios, the concentration of ROS, C_{ROS}, is a fixed proportion, α, of the oxygen consumed. When present, DCA causes a fixed increase in the proportion of oxygen converted to ROS during

metabolism, while VC is presumed to produce H_2O_2 independently in the extracellular space. The production of H_2O_2 is linearly related to the concentration of VC so it is easy to determine once the diffusion of VC has been calculated [Chen et al. 2007]. However, not all of the H_2O_2 produced will necessarily be internalized by the cell. Antunes and Cadenas measured the ratio of extracellular to intracellular H_2O_2 of cell membranes to be a constant value [Antunes and Cadenas 2000]. Thus, only a fixed proportion of the extracellular ROS contributes to influencing cellular dynamics. In any case, ROS damage is the primary source of toxicity for cells and is most often required for cell death by apoptosis or necrosis to occur. When not inducing death, ROS can induce a glycolytic phenotype.

Using a N x N array, each grid square has dimensions ΔxΔ, where $\Delta = 20\,\mu M$, to house individual cells. As the experiments used by Molavian et al. were conducted on a 2D plane, the model is most appropriately applied in two dimensions. The following rules are used to determine and dictate the progression of the cells, as summarize in figure 2.5:

1. Empty grid sites may become occupied if an adjacent cell undergoes division to that site. The only exceptions are the grid sites on the boundary that are occupied by vascular cells.
2. Oxygen and glucose are calculated with equations 5 and 6, with empty grid sites having a consumption rate of zero, and occupied sites consuming oxygen and glucose at a rate of F_O and F_G, respectively.
3. Cells occupying a grid site are selected at random to be updated at each time step. If a cell is older than the division age calculated from equation 14, it may divide into an adjacent cell. Each unoccupied site has an equal probability of housing

the new cell provided that it does not have too dense of an ECM. If,$C_{ECM} < \Psi_{def}$, the deformation threshold, a cell may divide freely. Alternatively, if$C_{ECM} \in [\Psi_{\text{def}}, \Psi_{\text{max}}]$, the cell has a fixed chance of deforming, P_{Def}. If, however, $C_{ECM} > \Psi_{\text{max}}$, the cell cannot divide into that site.

4. Living cells can be defined as quiescent, glycolytic, or oxidative. Any cell with a maturity age greater than 150 hours is considered to be quiescent. The glycolytic and oxidative phenotypes are determined by calculating $r_0 = F_O/F_G$. If $r_0 \geq 4$, the/ cell is considered oxidative. Otherwise, it is considered to be primarily glycolytic.
5. The amount of ATP produced can be calculated from $\phi_{ATP} = \frac{36F_O}{6} + 2(F_G - \frac{F_O}{6})$, where the second term representing energy harnessed from glycolysis.
6. The intracellular concentration of ROS, C_{ROS}, has a direct effect on both the metabolism and survival outcome of the cell. If $C_{ROS} > C_{ROS}^{gly}$, the cell will switch to a glycolytic metabolism [Shi et al. 2009]. Alternatively, if $C_{ROS} > C_{ROS}^{Apop}$, the cell will undergo necrosis or apoptosis depending on ϕ_{ATP}. However if $C_{ROS} > C_{ROS}^{Nec}$, necrotic cell death will be chosen independently of ATP levels [Leilli Jr. et al. 1998, Chandra et al. 2000, Zong and Thompson 2006].
7. If the concentration of oxygen falls below a value, C_O^{Nec}, the cell dies by necrosis. Alternatively, if the ATP that is generated is less than ϕ_{ATP}^{Nec}, necrosis also occurs. Necrotic cells at the core increase in age at each time step, but are not updated otherwise. Necrotic cells at the periphery of the tumour are removed randomly after a fixed time [Brouckaert et al. 2004]. Alternatively, if $\phi_{ATP}^{Nec} \leq \phi_{ATP} \leq$

ϕ_{ATP}^{Apop}, cells will die by apoptosis and be removed after the next time step [Leist, et al. 1997].

8. ROS are generated from oxygen that is consumed for respiration in the mitochondria. Approximately 1-2% of the oxygen consumed becomes a radical-inducing or possessing species, and so $C_{ROS} = \alpha * C_O$ [Orrenius 2007]. Additionally, hypoxia increases the proportion of oxygen committed to becoming ROS (i.e. $\alpha_{hyp} > \alpha$) [Goyal et al. 2004].
9. Ascorbate is administered according to the pharmacokinetic model described below. Vitamin C is degraded at a constant rate, k_{Asc} and $[C_{Asc}]_{in}$: $[C_{Asc}]_{out} = 7$ [Antunes and Cadenas 2000].
10. DCA concentrations decay from the time of dose administration in a first-order manner. When $C_{DCA} > C_{DCA}^{min}$, the metabolic pathway used by the cell will move preferentially towards respiration. Specifically, $F_G = 6F_O$ and so $r = 6$. In addition to restoring oxidative phosphorylation, cells are also more sensitive to ROS-induced death ($C_{ROS}^{Apop-DCA} < C_{ROS}^{Apop}$). For cells that are not under oxidative stress or DCA influence, $r = 5$ [Molavian et al. 2009].

These rules are programmed to determine the evolution of the system at each time step, but the pharmacokinetics of the treatment regimens must be considered carefully so that any results obtained replicate *in vivo* dynamics as closely as possible.

Riordan's extensive work in high-dose ascorbate therapy includes a two-compartment model describing tissue and plasma concentrations in response to intravenous administration [Riordan et al. 2000]. It can be described using the following diagram:

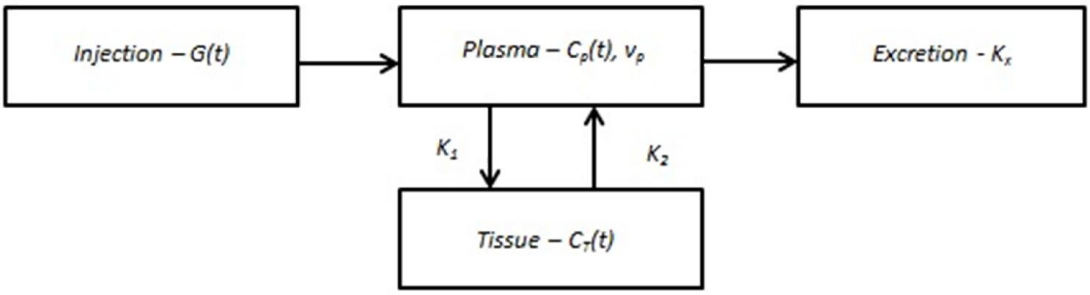

Figure 2.4: Schematic representation of the two-compartment model used for vitamin C removal

This yields the system of ordinary differential equations:

$$\frac{dC_p}{dt} = G(t) + k_2 C_T - (k_x + k_1) v_p C_p(t) \qquad (15)$$

$$\frac{dC_T}{dt} = k_1 v_p C_p(t) - k_2 C_T(t) \qquad (16)$$

where C_T is the accumulated concentration of VC in all tissues, C_p is the effective plasma concentration, v_p is the plasma volume (assumed constant), $G(t)$ is the administration schedule, k_x is the excretion rate, and k_1 and k_2 are the rates of blood-plasma and plasma-blood diffusion, respectively [Riordan et al. 2000].

In contrast with mega-dose VC therapy, the pharmacokinetics of DCA have been studied in greater detail among larger demographics over longer periods of time [Stacpoole et al. 1998]. While this research has revealed considerable insight into the toxic effects of DCA, the optimal dosing in cancer and its effects in adjuvant therapy have yet to be studied in detail. An attempt is made herein to explore the effects of both treatments on malignant growth so that the potential therapeutic benefits could be taken advantage of in clinical situations.

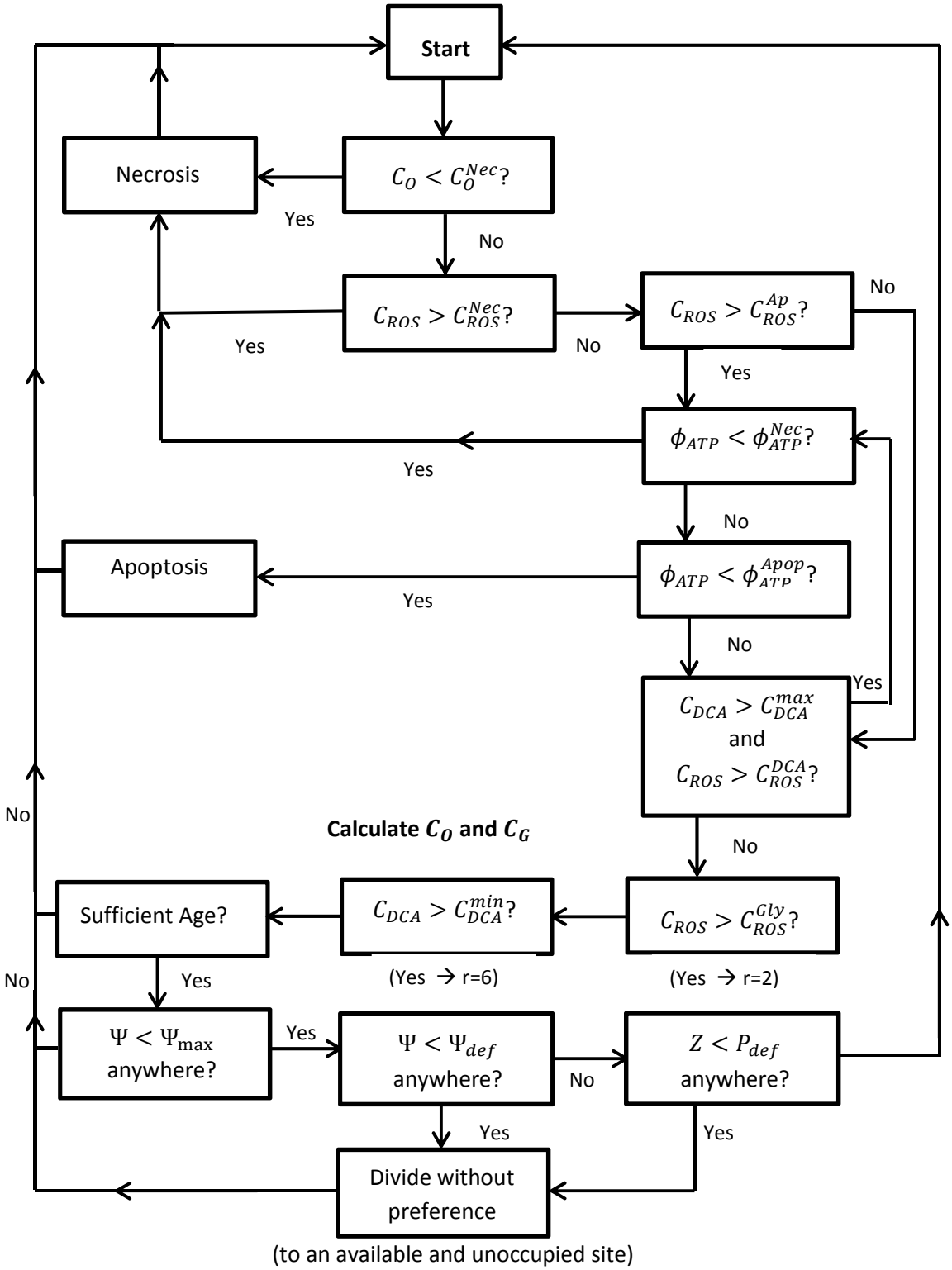

Figure 2.5: Flow chart for the square-lattice model applied in simulations

Chapter 3

Simulation Results

3.1 The Role of the Microenvironment

3.1.1. ECM Density and Cell Deformability

One of the most obvious factors that may influence the growth of a tumour in this model is the extracellular matrix. Specifically, the ability of each cell to divide into vacant sites in its neighbourhood is determined by the tolerance and ability to deform. In practice, this is a highly variable event that depends on intercellular junctions, surface proteins that regulate such processes, and matrix metalloproteinases contributing to a cell's anchorage and resulting mobility in an environment with a dense ECM. To investigate such variables, the effects of Ψ_{max} and Ψ_{def} are varied. In all simulations that follow, cell using glycolytic metabolism are labelled 1 (blue), oxidative cells are labelled 2 (light blue), necrotic cells labelled as 3 (yellow), apoptotic as 4 (orange), and quiescent cells are labelled 5 (red).

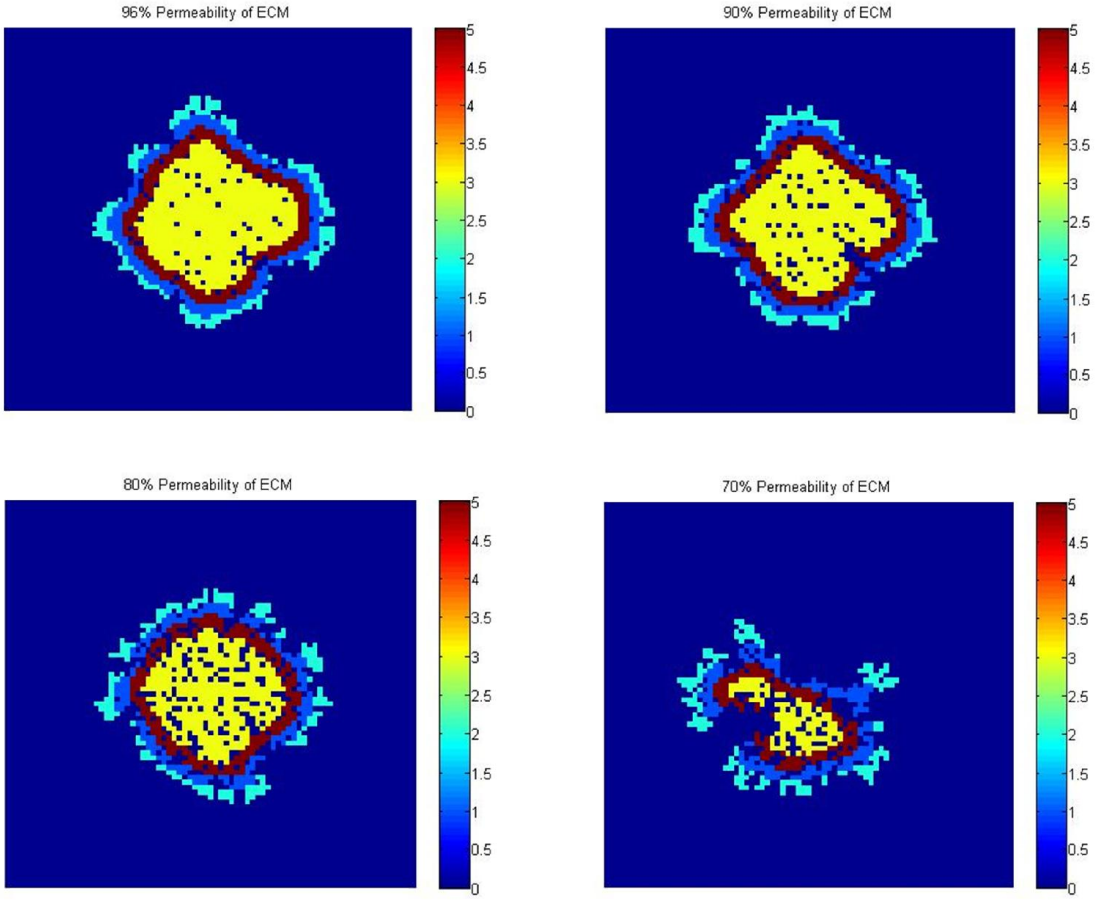

Figure 3.1: Effects of ECM permeability (Ψ_{max}) on tumour morphology after 600 hrs. *Top-left*: ψ_{max}=0.96. *top-right:* ψ_{max}=0.9, *bottom-left*: ψ_{max}=0.8, *bottom-right:* ψ_{max}=0.7

As evidenced above, the morphology of the tumour is greatly affected by the permeability of the ECM. The features of the less permissible ECM resemble the fingering morphology observed to be a result of the harsh environmental conditions modelled by Anderson and colleagues [Anderson et al. 2009]. Unsurprisingly, this also has an immediate effect on the size of the tumour. This can be seen in the plot of total number of living cells for each case in figure 3.2, where decreased permeability results in a smaller tumour.

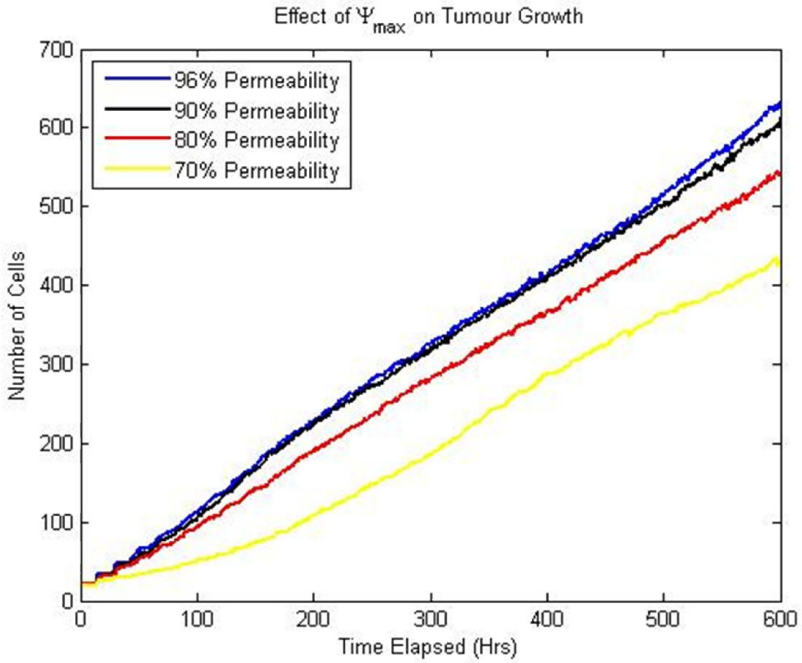

Figure 3.2: Number of living cells existing after 600 hrs as a result of varying Ψ_{max}

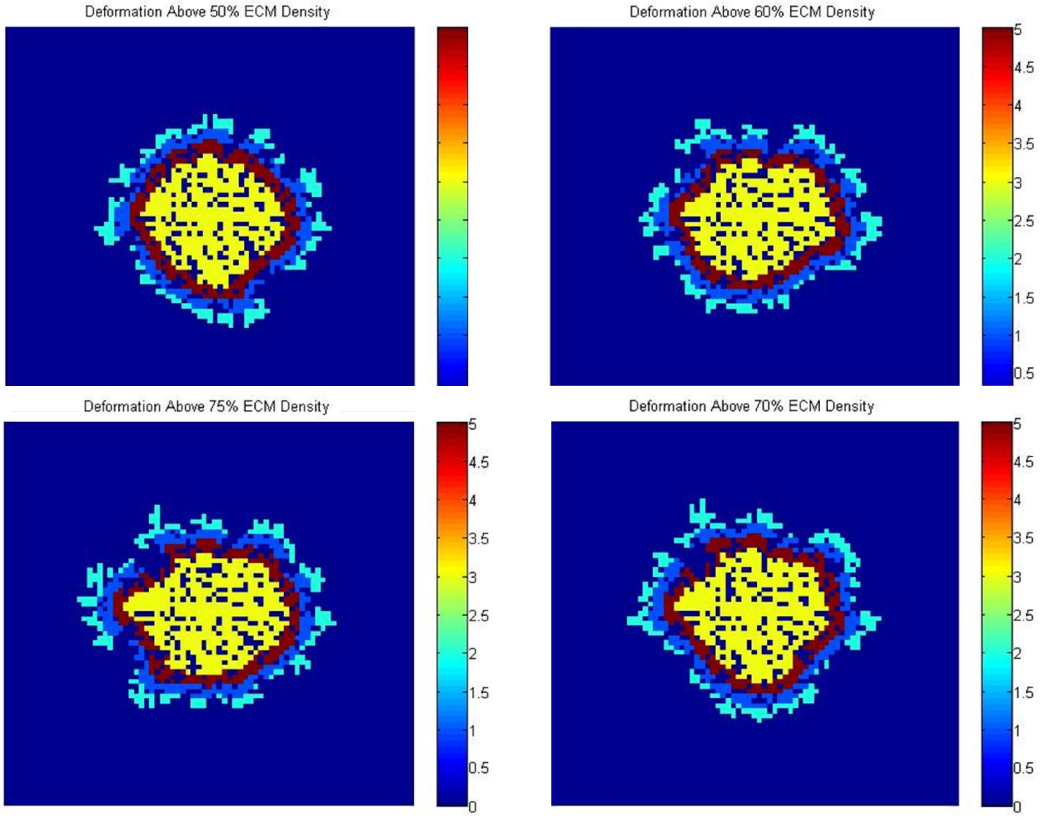

Figure 3.3: Effects of ECM deformable potential (Ψ_x) on tumour morphology after 600 hrs. *Top-left:* ψ_x=0.5, *top-right:* ψ_x=0.6, *bottom-left:* ψ_x=0.75, *bottom-right:* ψ_x=0.7

In contrast with the effects of decreasing Ψ_{max}, increasing the deformation potential of cells, Ψ_x, results in a recovery of the radially symmetric morphology observed in cells in an environment conducive for growth, as shown in figure 3.3. Unsurprisingly, this also increases the potential size of the tumour by increasing the number of available lattice sites into which a cell may divide provided deformation is possible. As seen below, this effect seems to be more modest than when varying Ψ_{max}.

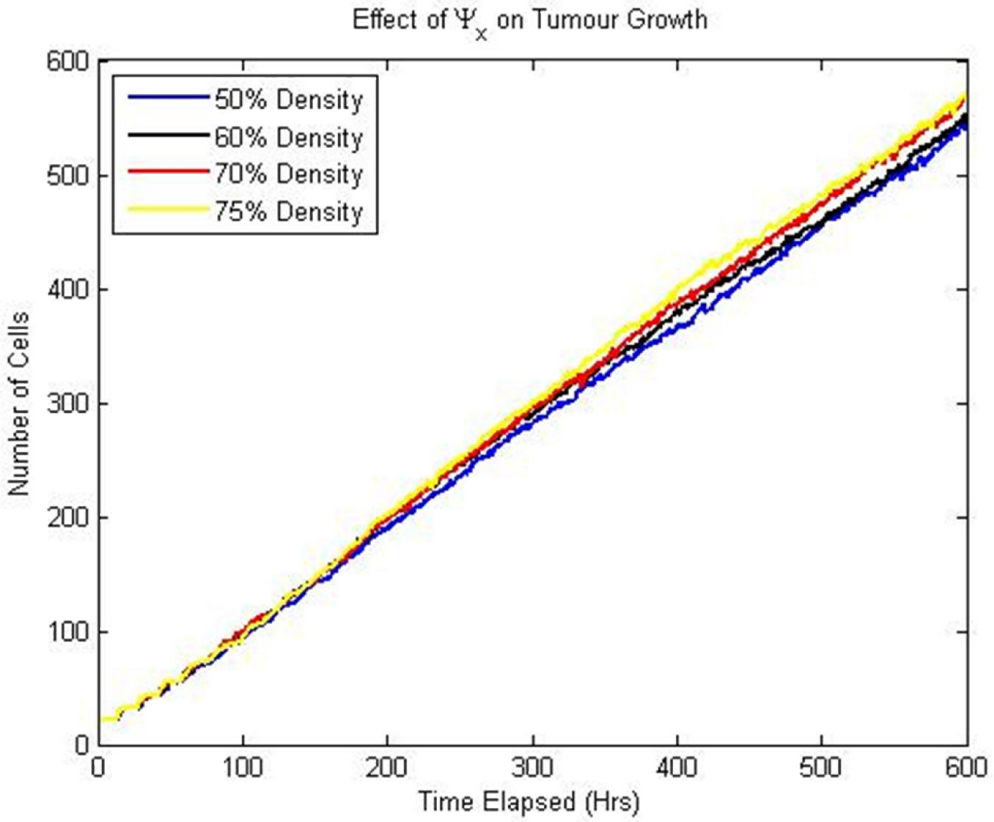

Figure 3.4: Number of living cells existing after 600 hrs as a result of varying Ψ_x

3.1.2. Calculating ROS Production

In addition to the effects of the ECM, the production of ROS has a considerable effect on facets of tumour growth like metabolism and cell death. Naturally, the method in which ROS are calculated in the model and the resulting effect on tumour growth is of interest. To investigate these possibilities, the proportion of and method in which oxygen is converted to ROS are varied according to different schemes. Firstly, a fixed increase in α, the constant proportion of oxygen converted to ROS, is implemented. In particular, $\alpha^* = \frac{3}{2}\alpha$.

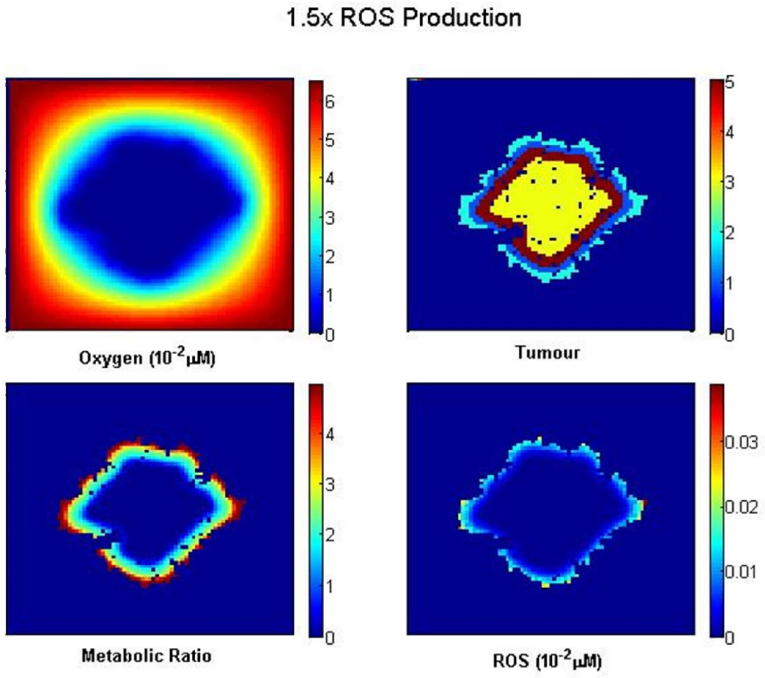

Figure 3.5: Effects of using $\alpha^* = \frac{3}{2}\alpha$ to calculate endogenous ROS productions. *Top-left:* oxygen distribution, *top-right:* tumour structure, *bottom-left:* metabolic ratio of cells (*r*), *bottom-right:* ROS concentration

Similarly, an increase in ROS that is directly proportional to the age of the cell is implemented. In this case, $\alpha^* \epsilon [0.01,0.03]$, where the value of α^* is increased by 25% of α for each 25hrs that the cell has been alive. In either case, the resulting dynamics of the system are not significantly influenced.

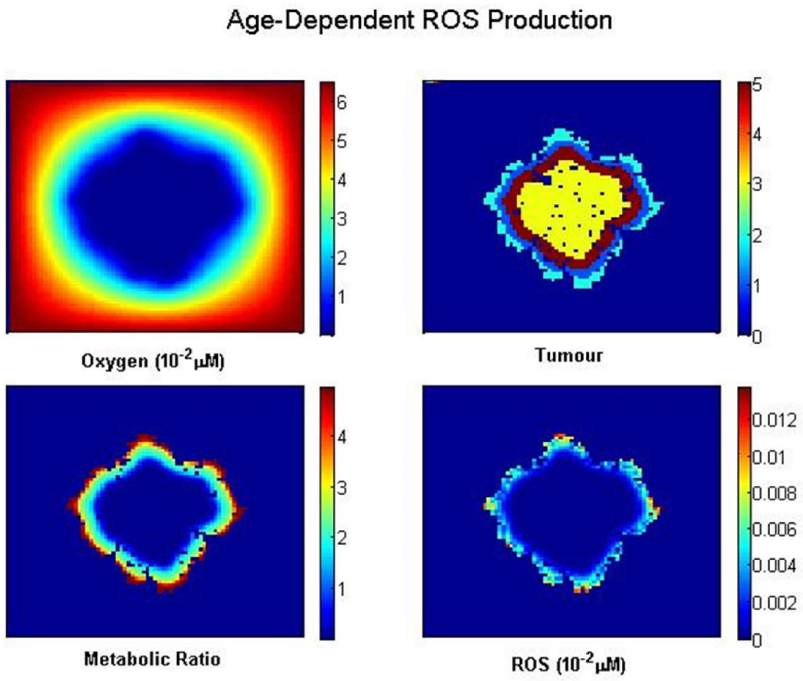

Figure 3.6: Effects of using an age-dependent scheme for α to calculate endogenous ROS production

In contrast, more complicated methods of determining each cell's ROS-producing potential can be implemented. In particular, a linear mutation scheme is imposed for ROS production in which α is increased after each division in which a stochastically determined mutation occurs. This mutation scheme results in a range of ROS production values ranging from 1% to 3.5% of the environmental oxygen calculated. Consequently, cells on the periphery of the tumour are likely to have an increased relative ROS production; they

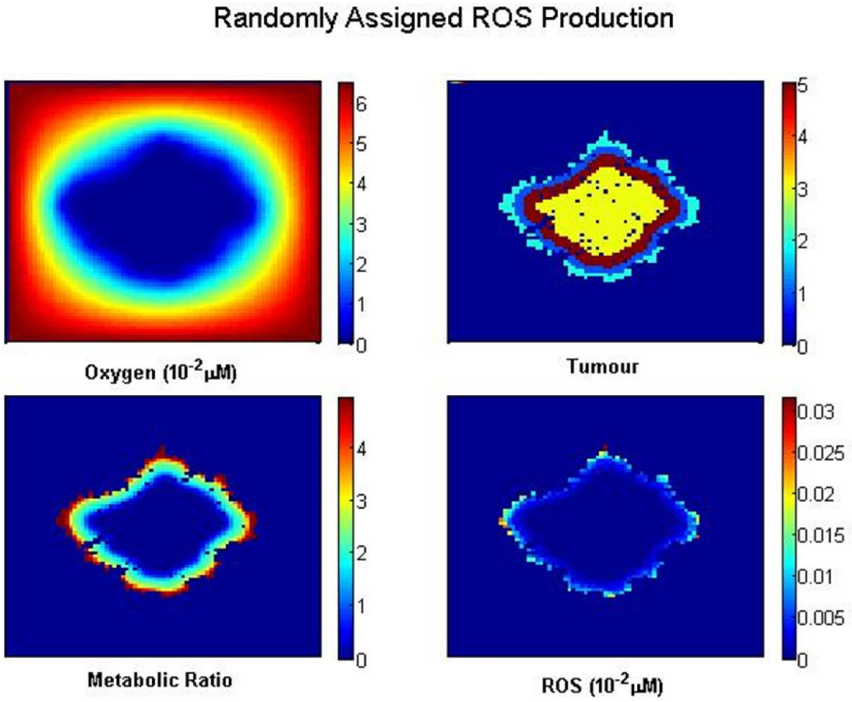

Figure 3.7: Effects of using a randomly assigned values for α to calculate endogenous ROS production

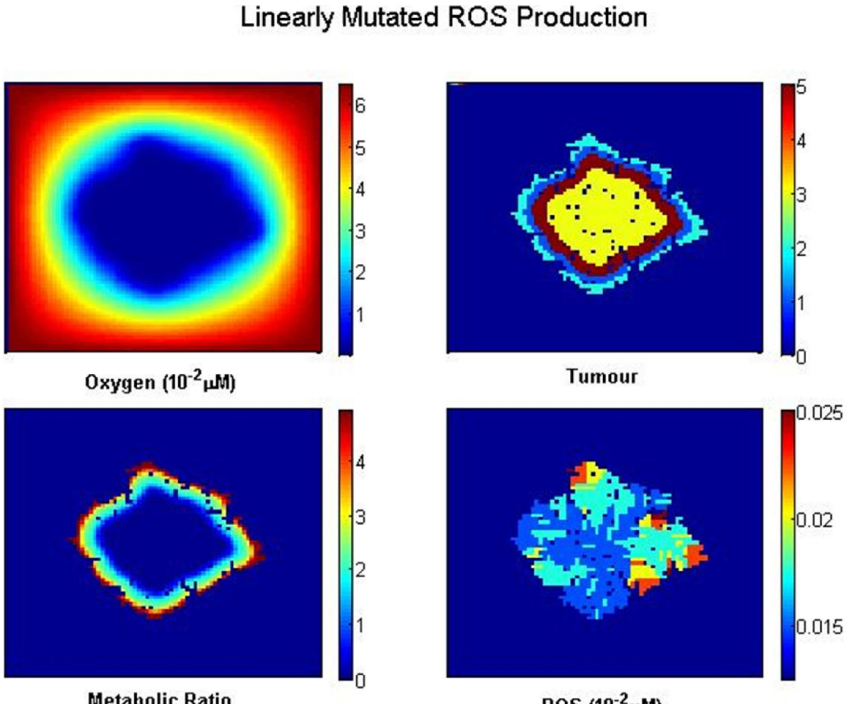

Figure 3.8: Effects of using a linear mutation scheme to determine α for calculating endogenous ROS production

represent cell lines that have collectively had the most opportunities to undergo a mutagenic event. Lastly, ROS are calculated through assigning random values of α to cells. Similarly to the previous cases, the range of α varies between 1% and 3%. However, the morphological and environmental properties remain relatively unchanged in each case.

As observed experimentally, the effects of ROS can have varying results. In particular, an increased ROS concentration that is not sufficiently high so as to induce toxic effects on cell growth may instead contribute to the proliferative potential of cancerous cells. Clearly, the fixed increase of ROS production has the greatest impact on the resulting size of the tumour. As ROS increases, the method of ATP production shifts away from respiration and towards glycolysis. As a result, a decrease in the amount of glucose available could increase the amount of oxygen available while glucose remains readily available. While the *in vivo* justifications for increased proliferative potential involve activation of signalling pathways, the change observed is likely due to a decrease in the maturity age of the cell required for division (*Eq. 14*).

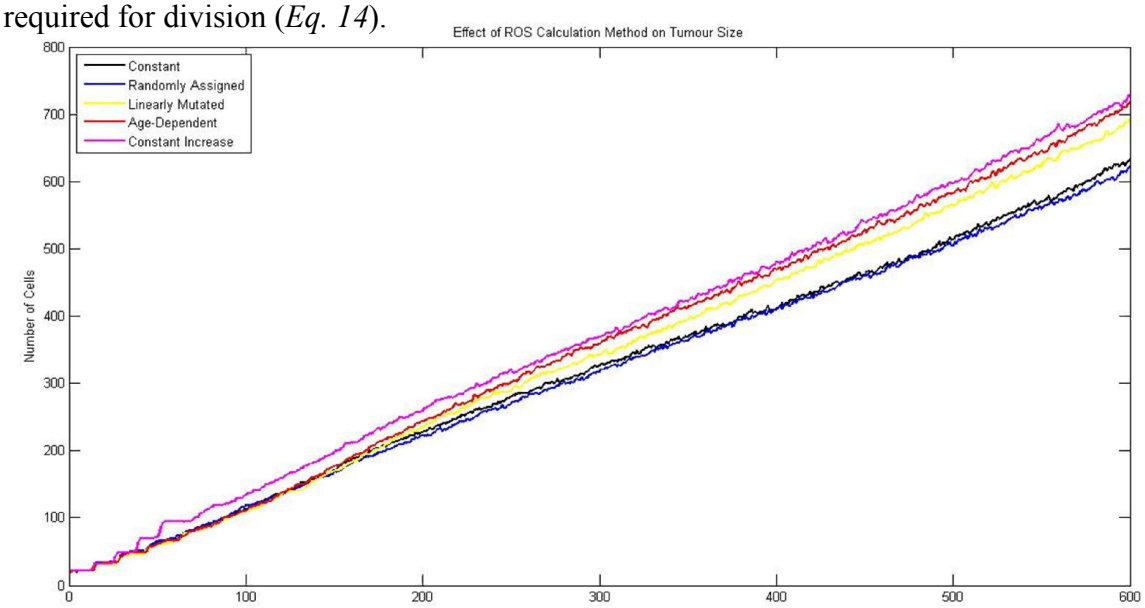

Figure 3.9: Number of living cells at each time as a result of using each scheme for α when calculating endogenous ROS production

3.2 Mega-Dose VC Therapy

Previous Phase I studies have investigated the toxicity of VC in doses around 2g/kg of body weight, or around 100g on average, thrice weekly to achieve plasma concentrations upwards of 30 mM [Monti et al. 2012]. These studies have been undertaken with the convention that a plasma concentration of 10mM is sufficient to induce H_2O_2 production and cell death.

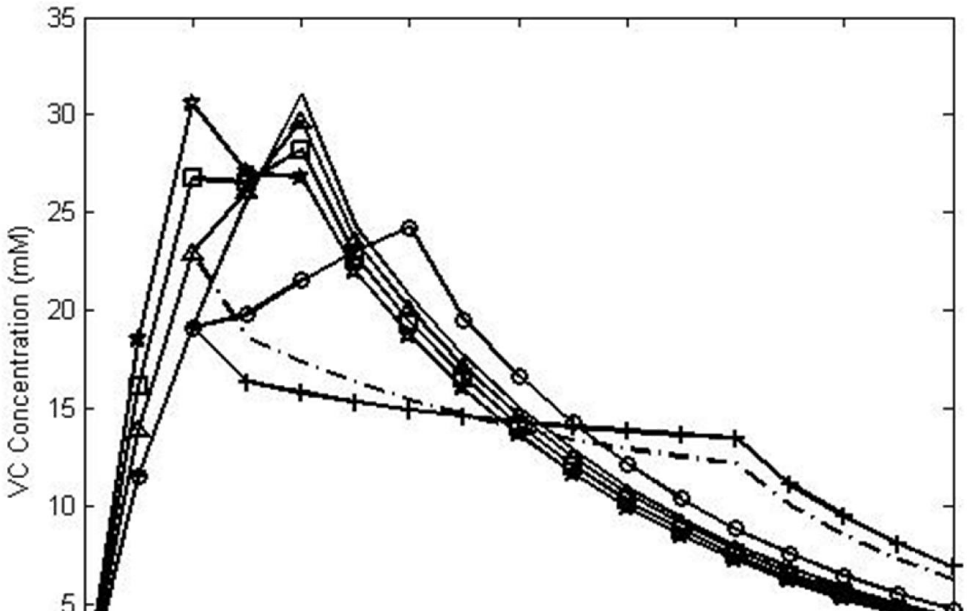

Figure 3.10: Calculated plasma concentration of VC in the hours following each administration schedule. **(–)** Schedule 1: 50g/hr x 2 hrs. **(o)** Schedule 2: 50g/hr x 1hr,then 25g/hr x 2 hrs. **(+)** Schedule 3: 50g/hr x1h then 10g/hr x 5hrs. **(– - –)** Schedule 4: 60g/hr x 1hr, then 8g/hr x 5hrs. **(Δ)** Schedule 5: 60g/hr x 1hr, then 40g/hr x 1hr. **(■)** Schedule 6: 70g/hr x 1hr then 30g/hr x 1hr. **(*)** Schedule 7: 80g/hr x 1hr then 20g/hr x 1hr.

Given the multitude of possibilities regarding schedules with a constant dose that maintain concentrations above 10 mM, *in silico* techniques are particularly attractive to investigate such options.

3.2.1 Simplified Administration Schedules and Dynamics

It has already been posited that the administration schedule of VC may be optimized by adjusting the rate of drug administration so that the time interval during which VC concentrations are above 10 mM is maximized rather than the peak VC concentration [Gonzalez et al. 2012]. To investigate this claim in the model, a dose of 100g was applied at various rates.

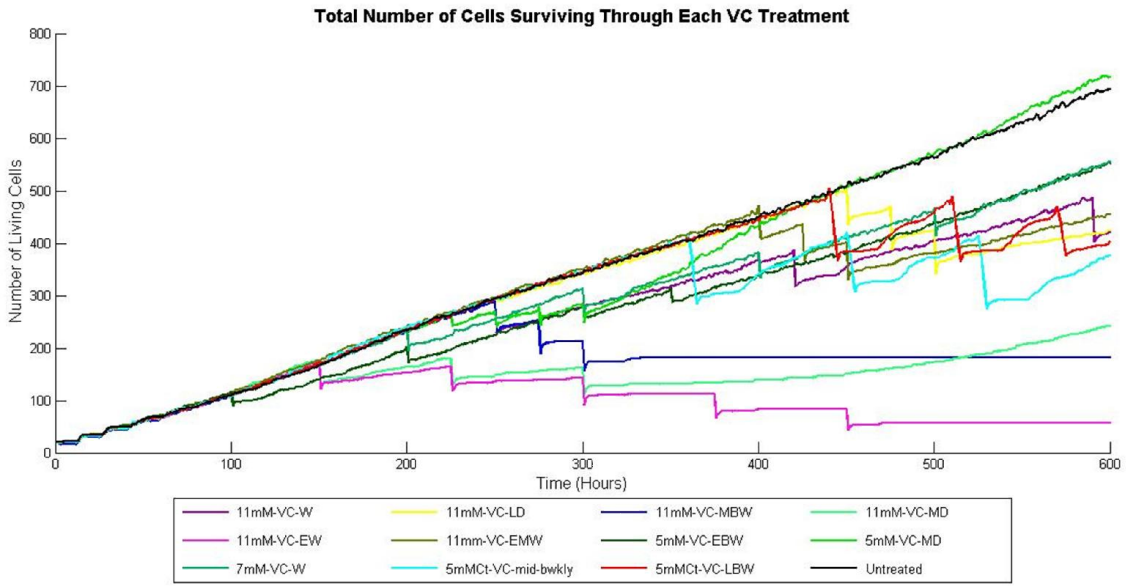

Figure 3.11: Number of living cells at each time as a result of using simplified growth dynamics and numerous administration schedules.

In this example, the effect of necrotic cell death is particularly evident. Once cells on the periphery of the tumour are exposed to high-concentration VC, they become necrotic. Since these cells are not removed in the simulation, they present a physical barrier that inhibits the space available for cell division. As a result, the tumours exposed to necrosis-inducing VC concentrations shrink and are unable to expand further (figure 3.11).

Furthermore, each treatment with VC is either implemented to induce a constant concentration for 1 hour, or 5 hours before being removed immediately; thus, this is a general approximation of the pharmacokinetics modelled by Riordan. It is important to note that *in vivo* tumour growth would most likely allow for the pushing of cells from the interior outwards, thus negating the effect of the peripheral necrotic ring. These effects on tumour growth are investigated in more detail below.

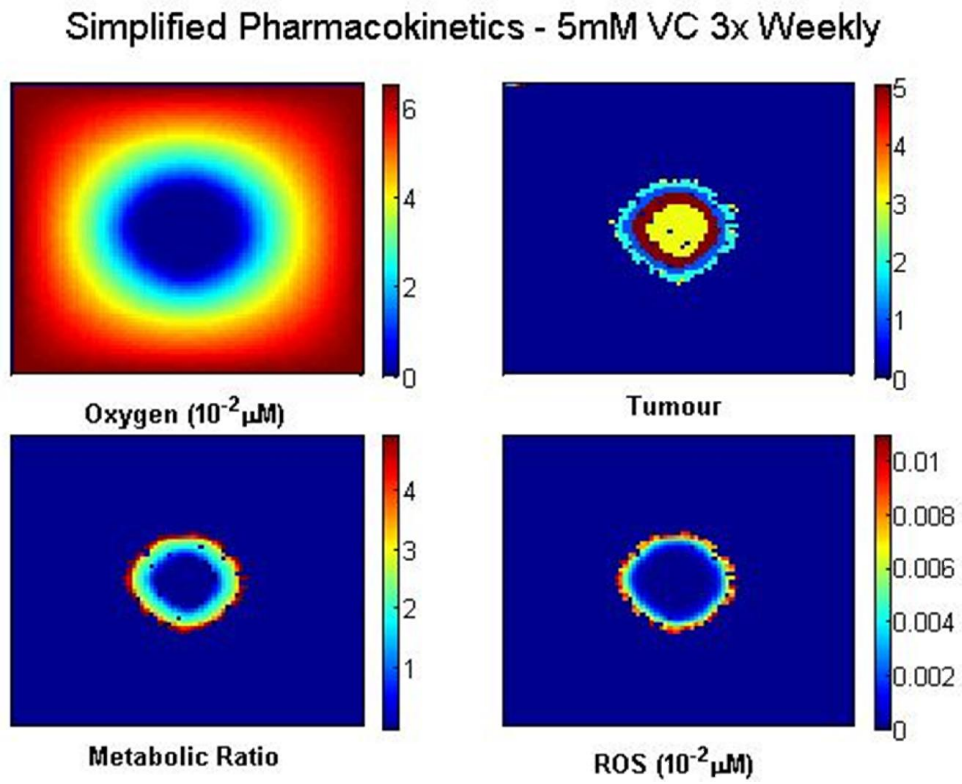

Figure 3.12: Effects of the simplified, low concentration VC treatment on tumour dynamics.

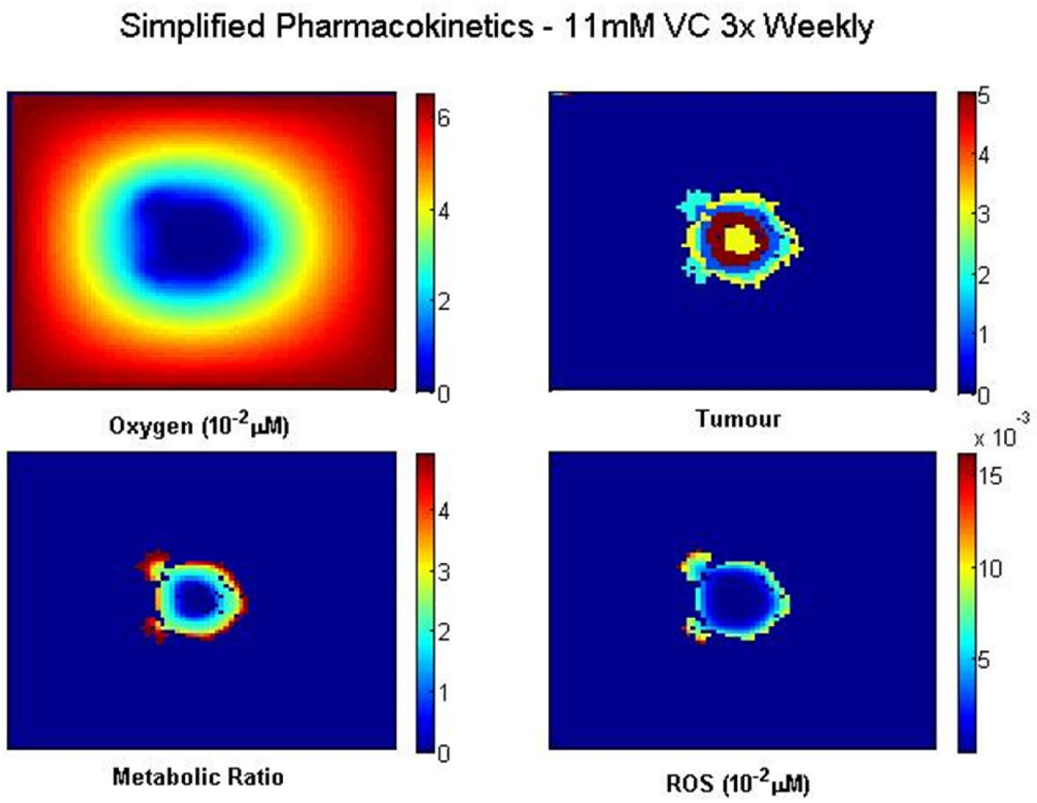

Figure 3.13: Effects of the simplified, high concentration VC treatment on tumour dynamics.

As demonstrated in these two examples of the various simplified treatment protocols presented, the increased proportion of necrotic cells imposes a limit on the potential tumour size by encapsulating most of the cells. Whereas growth is predominantly inhibited by physical restrictions with higher concentrations of VC, lower concentrations could inhibit growth to a similar extent when applied over a longer period of time. However, the effects of the treatment using 5mM VC are not long-lasting; the rate of growth immediately resumes at pace similar to growth before treatment (figure 3.14). In contrast, 11mM VC treatment results in a much slower rate of growth upon recovery. At any rate, the necrotic cells would normally be removed and degraded over time with the assistance of cells of the immune system [Brouckaert et al. 2004]. Thus, it is more appropriate in the context of biological

applications to consider these effects in detail so that the merits and disadvantages of each treatment strategy can be distinguished.

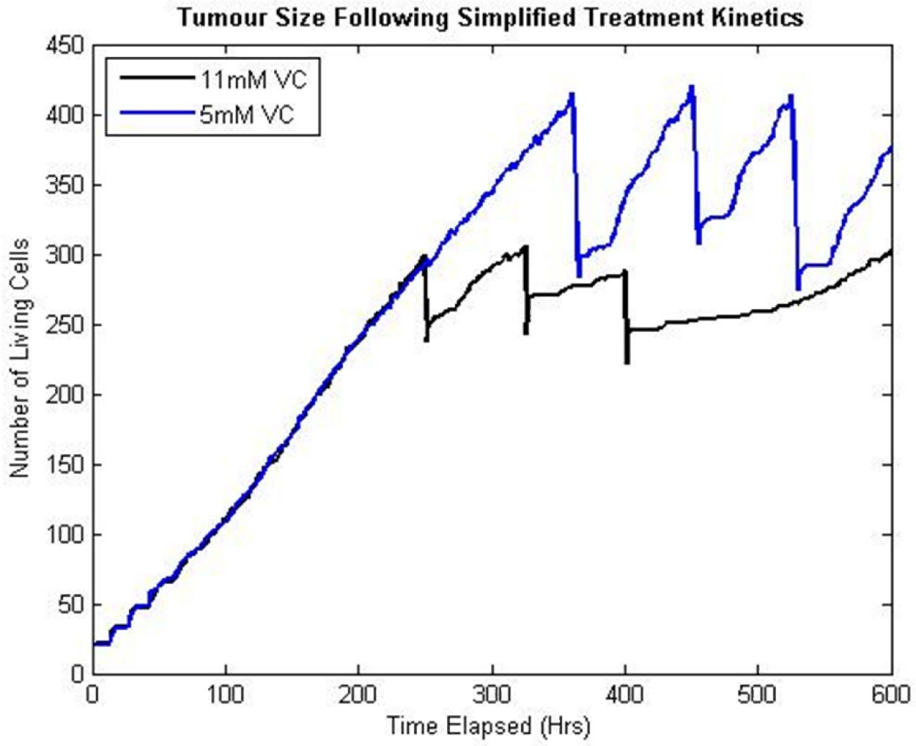

Figure 3.14 : Number of living cells resulting from the simplified VC treatments at each time.

3.2.2 Increased Complexity of Treatment Dynamics and Response

Incorporating the pharmacokinetic models derived by Riordan and colleagues with the removal of cells dying by necrosis at the tumour periphery reveals insights into the results obtained and their implications. To do so, a constant total dose of 100g of VC is administered in each schedule at varying rates. This results in the different concentration curves in figure 3.10. The schedules used herein were derived as follows:

1. 50g/hr administered over 2 hrs
2. 50g/hr administered for 1hr, followed by 25g/hr for 2 hrs
3. 50g/hr administered for 1hr, followed by 10g/hr for 5hrs
4. 60g/hr administered over 1hr, followed by 8g/hr for 5hrs
5. 60g/hr administered over 1hr, followed by 40g/hr for 1hr
6. 70g/hr administered over 1hr, followed by 30g/hr for 1hr
7. 80g/hr administered over 1hr, followed by 20g/hr for 1hr.

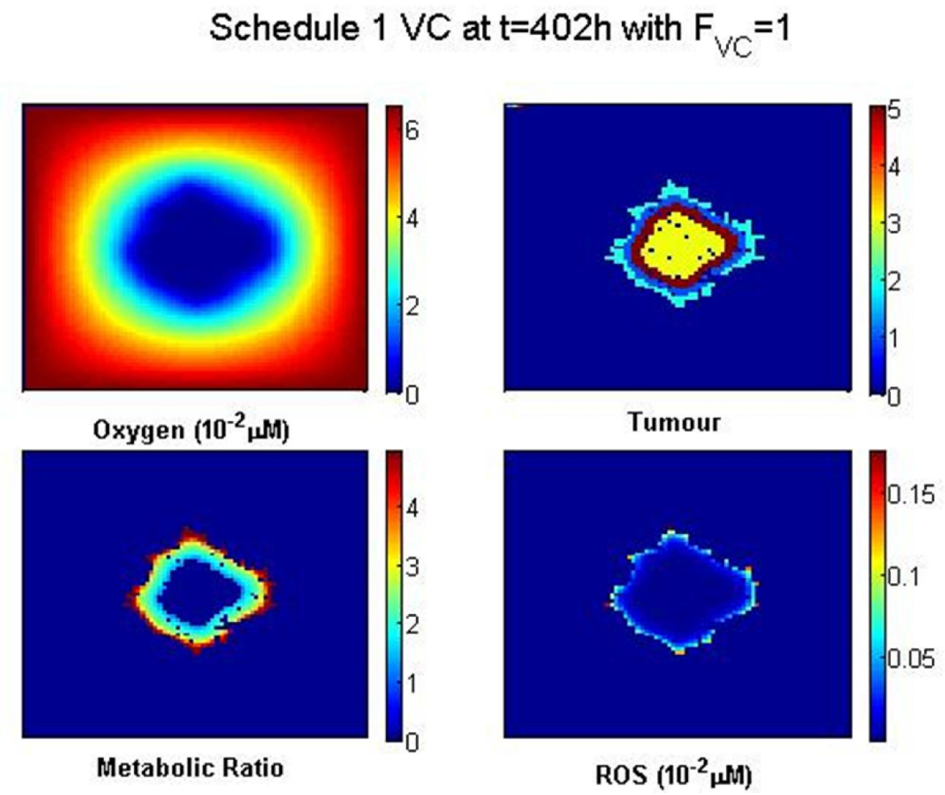

Figure 3.15: Effect of Schedule 1 VC with relatively high VC consumption.

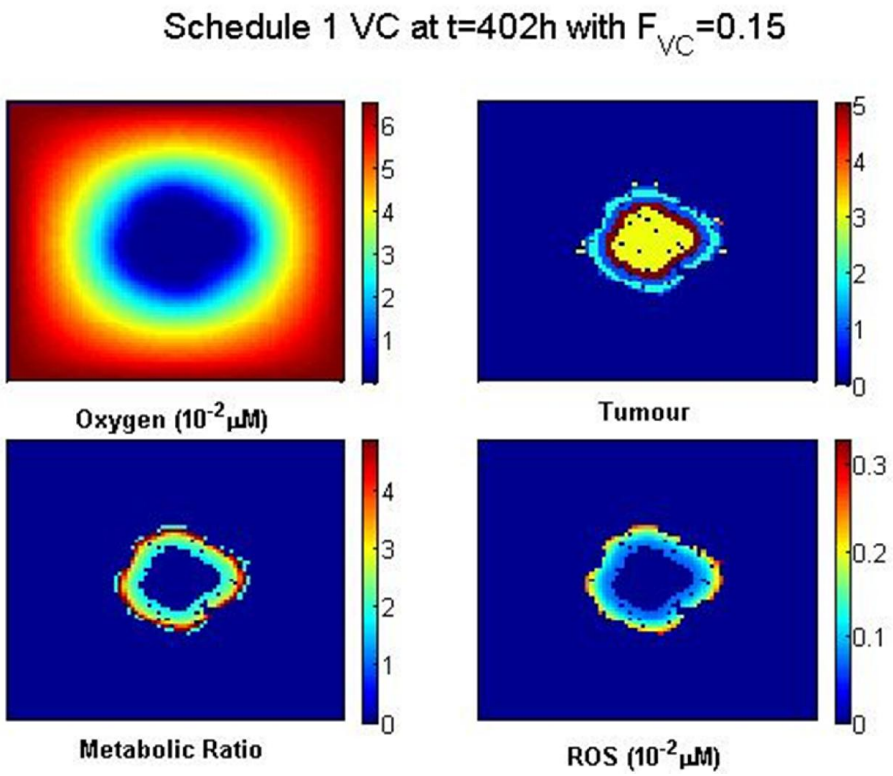

Figure 3.16: Effect of Schedule 1 VC with moderate VC consumption.

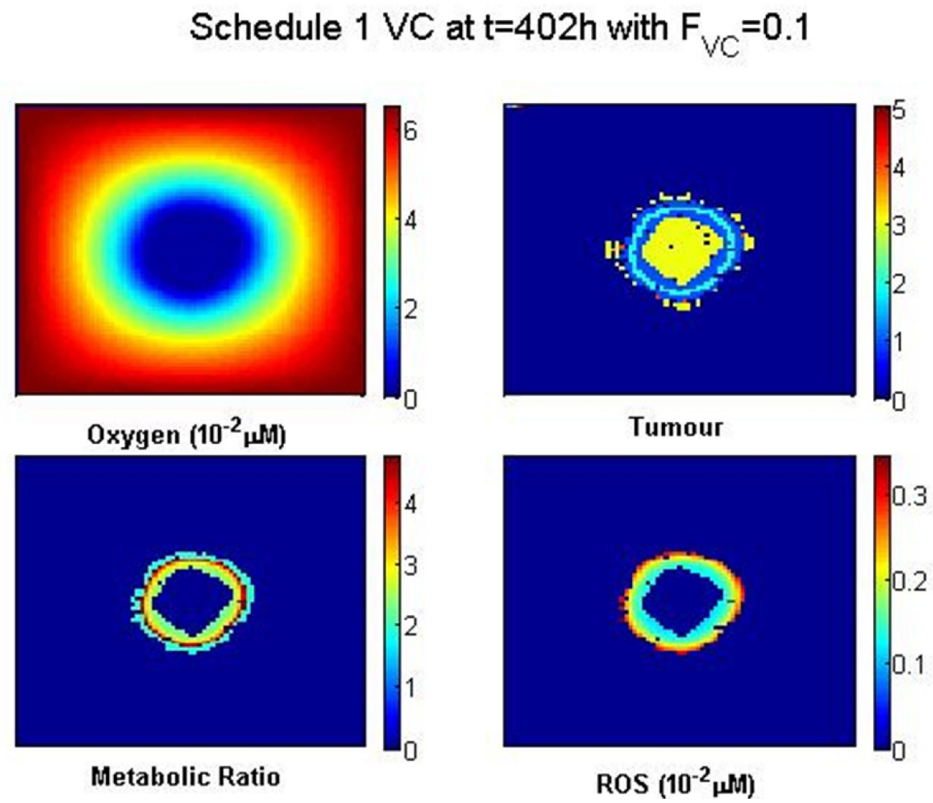

Figure 3.17: Effect of Schedule 1 VC with moderate VC consumption.

Firstly, the effects of achieving a maximal VC concentration are investigated using a constant infusion of 50g/hr. As one might expect, the rate of consumption of VC, F_{VC}, will have a direct impact on the amount of cell death that occurs. In particular, VC that is metabolized or sequestered in its role of promoting collagen formation or the hypoxic response is unable to act as a pro-oxidant and will not be effective in anti-tumourigenic behaviour. Thus, a greater consumption of VC is likely to have a decreasing effect on cell death.

As demonstrated in figure 3.15, the survival of a tumour in response to VC treatment appears dependent on the rate at which VC can be metabolized. Specifically, values of F_{VC} greater than 0.1 result in an insufficient production of ROS to promote cell death. Thus, tumours that consist of cells capable of metabolizing elevated levels of VC in the microenvironment will have a resistance to treatment-induced cell death. Interestingly, comparing the metabolic ratio in figures 3.15-3.17, increased VC metabolism is associated with a more oxidative phenotype overall. The peaks observed in figure 3.18 for F_{VC}=0.15 reveal an interesting behaviour. Particularly, there may be a proliferative advantage acquired when VC is not cytotoxic. The efficiency at inducing cell death in each simulation is further demonstrated by considering the total number of necrotic, apoptotic, and living cells presented in figure 3.21.

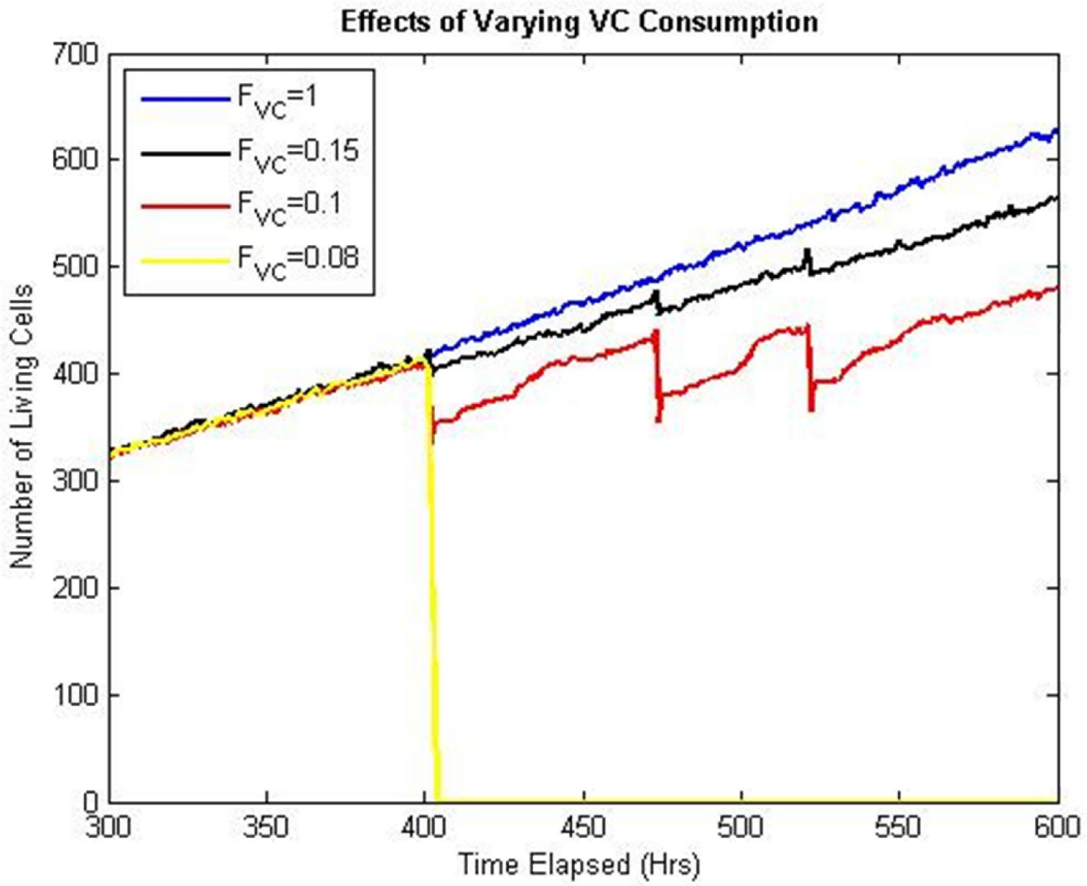

Figure 3.18: Number of living cells at each time resulting from the simplified Schedule 1 treatments with varying values of F_{VC}

Similarly, the size of the tumour at the time of the treatment appears to affect the efficacy of the treatment in removing cancerous cells entirely. While this scenario may not always be predictable in clinical situations, the behaviour is nonetheless interesting in determining the frequency of doses that is optimal. However, it is important to note that F_{VC} for the tumour undergoing Schedule 3 treatment in figure 3.19 is less than that of tumour undergoing Schedule 1 treatment. This is to ensure that we compare tumours that are both responsive to treatment.

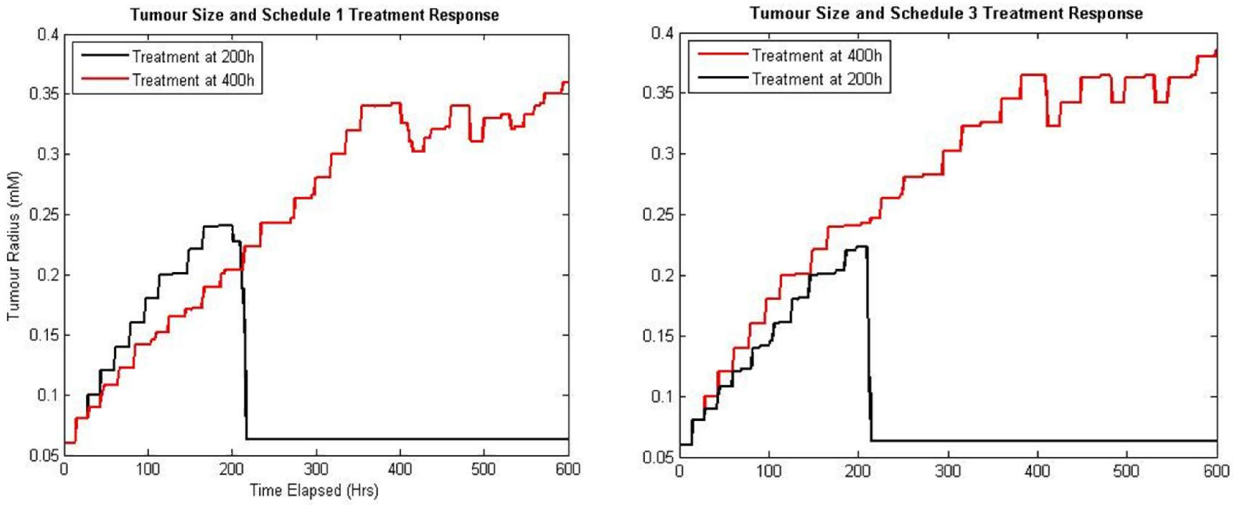

Figure 3.19: Tumour radius at each time for Schedule 1 (left) and Schedule 2 (right) treatments. Schedule 1 is treating a tumour with $F_{VC} = 0.1$ and Schedule 2 with $F_{VC} = 0.05$

When considering tumours with an identical rate of consumption of VC, the effects of tumour size are not as noticeable (figure 3.20). In other words, treating a tumour with Schedule 3 when it is smaller in size may not necessarily compensate for the resistive characteristics acquired from increased VC consumption.

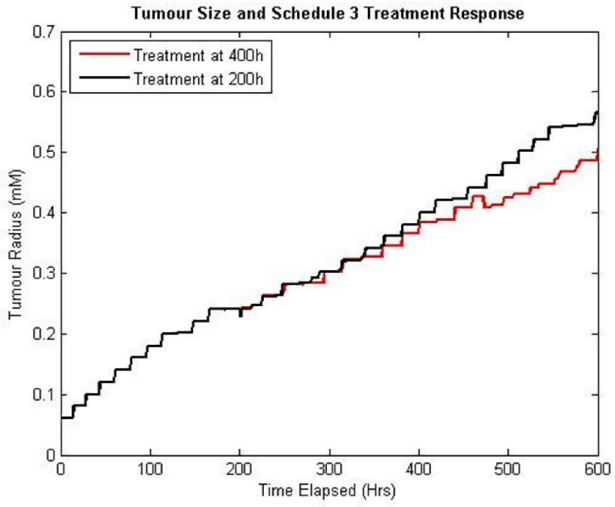

Figure 3.20: Tumour radius at each time for Schedule 3 treatment; $F_{VC} = 0.1$

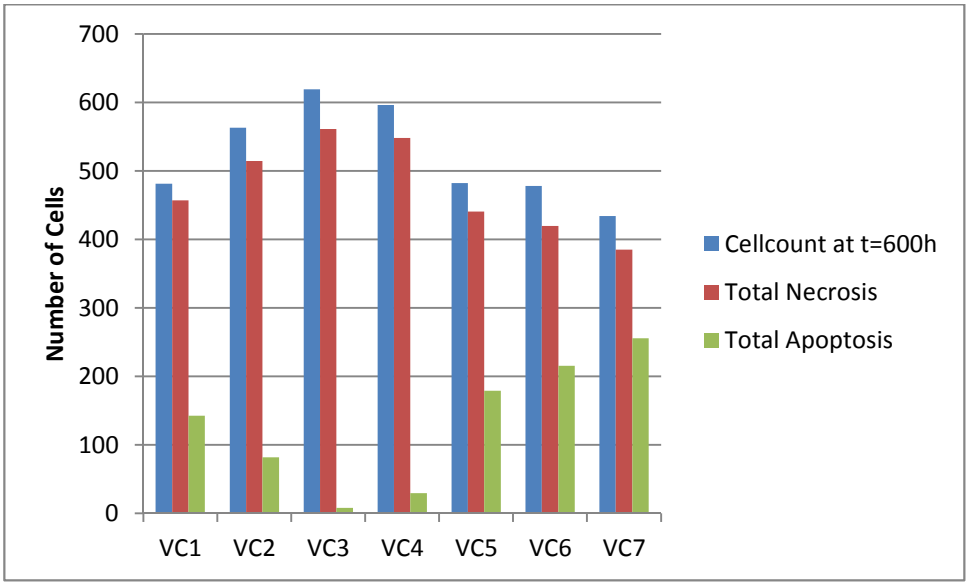

Figure 3.21: Total cell death by necrosis and apoptosis and final number of living cells after 600 hrs for each VC schedule.

3.2.3 Treatment Resistance

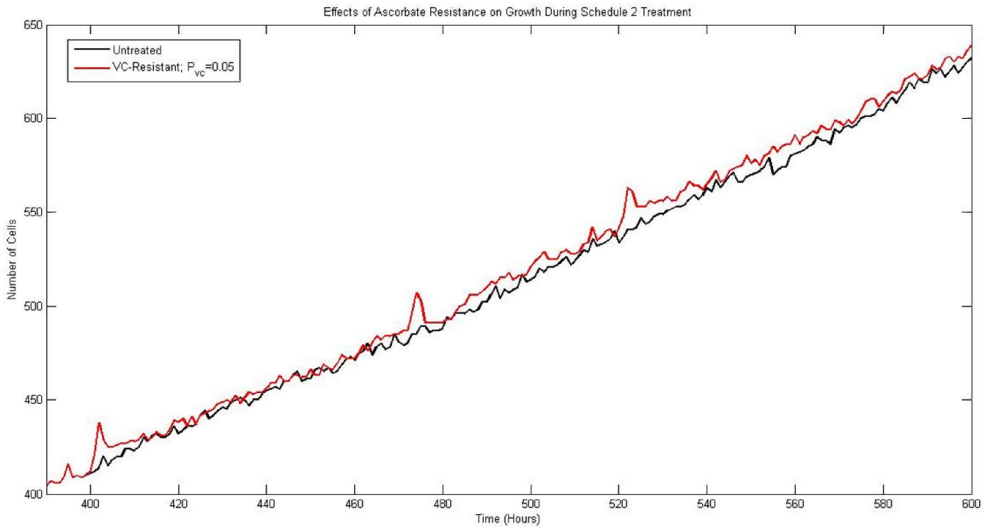

Figure 3.22: Number of living cells at each time for a VC-resistant cell undergoing Schedule 2 treatment.

Often in clinical situations, a resistance to treatments arises in progressive cancers that is indicative of a poor prognosis. With regards to VC, catalase is the antioxidant enzyme purported to infer treatment resistance in cells [Klingelhoeffer et al. 2012]. When the cells within the model are capable of surviving high-dose VC, the exogenous ROS can have a proliferative effect on the tumour cells.

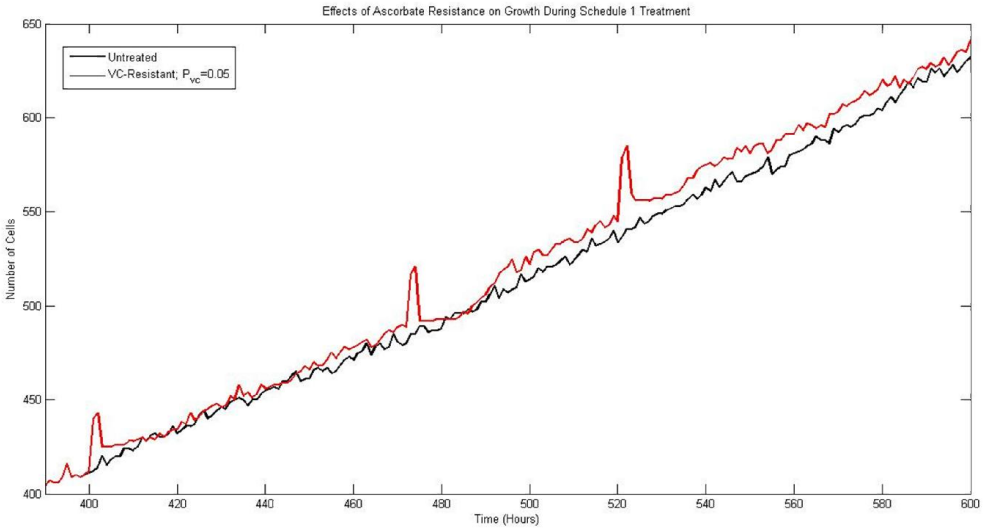

Figure 3.23: Number of living cells at each time for a VC-resistant cell undergoing Schedule 1 treatment.

This is accomplished in the model by increasing the thresholds at which ROS induces cell death: C_{ROS}^{Nec} and C_{ROs}^{Apop}. Within the context of the model, this is likely attributable to an increased availability of oxygen from the glycolytic switch, which in turn lowers the maturity age necessary for division. However, this rapid increase in tumour size results in an equally rapid increase in the size of necrotic core. Nevertheless, tumour growth within the model is hastened by VC treatment when resistance has been acquired by the cells, as demonstrated in figures 3.22-3.23.

3.3 DCA Therapy

Since the absorption, metabolism, and excretion of DCA vary among different demographics, the pharmacokinetics of healthy adults (i.e., not suffering from lactic acidosis) are applied. In particular, DCA is removed from the system much more quickly following the initial dose ($t_{1/2} = 1.03$hrs) than subsequent doses ($t_{1/2} = 6.23$hrs) where trough concentrations of 0.5 mM are maintained between doses. However, detailed treatment schedules for how trough concentrations arise would complicate analysis significantly with minimal gains, if any. Thus, unless otherwise specified, all doses are presumed to be administered to a system that has previously been exposed to DCA.

Despite the fact that the exact mechanisms that result in cell death are unknown, the effects of DCA on cell death in clinical and laboratory settings has been well-observed [Sun et al. 2010,Michelakis et al. 2010, Sun et al. 2011]. While an increase in the metabolic ratio is to be expected (i.e. an increase in cell respiration), events involving cell death are less predictable. Regardless, a treatment regime similar to those utilized in clinical settings is applied in our simulations. This correlates with doses between 6.5mg/Kg and 50mg/Kg that are able to induce plasma concentrations of upwards of 5mM daily [Michelakis et al. 2010, Flavin 2010]. A notable result from the simulations is the precise tumour region that is most affected by DCA treatment. Specifically, the hypoxic regions tend to be most affected by the cytotoxic effects of DCA (figure 3.25), as has been suspected but not yet verified in tumours [Papandreou et al. 2011].

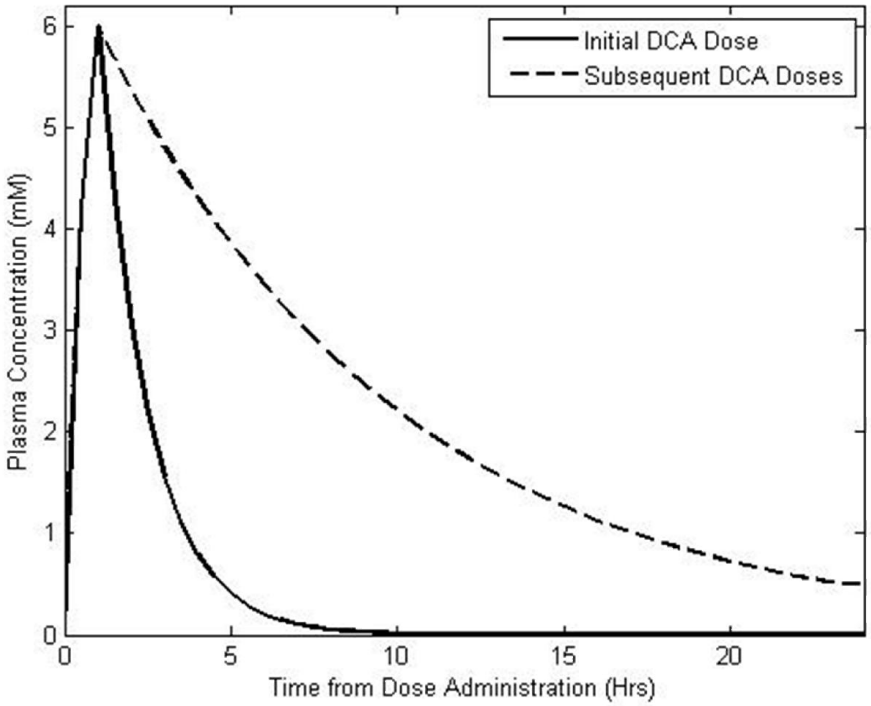

Figure 3.24: DCA dose curves for individuals with and without previous exposure to DCA

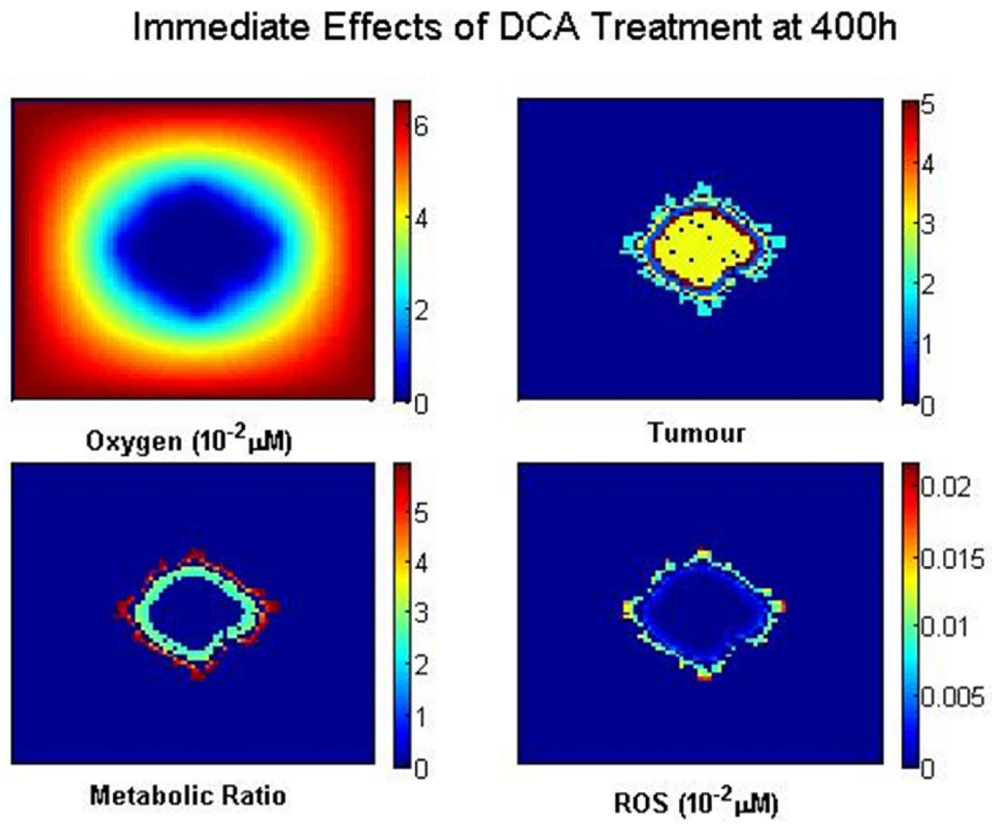

Figure 3.25: Environmental and morphogenic results immediately following a DCA treatment at 400h

Bearing in mind that the proportion of hypoxic cells will increase with the tumour's size, and hence with the time elapsed before the treatment, the effects of administering the dose at multiple times points was investigated. In particular, it is interesting to note that the greatest decrease in the number of cells occurs when treatment is administered after 400 hours (figure 3.26). Regardless, the rate of growth accelerates following each treatment once cells have been removed and resources become more available.

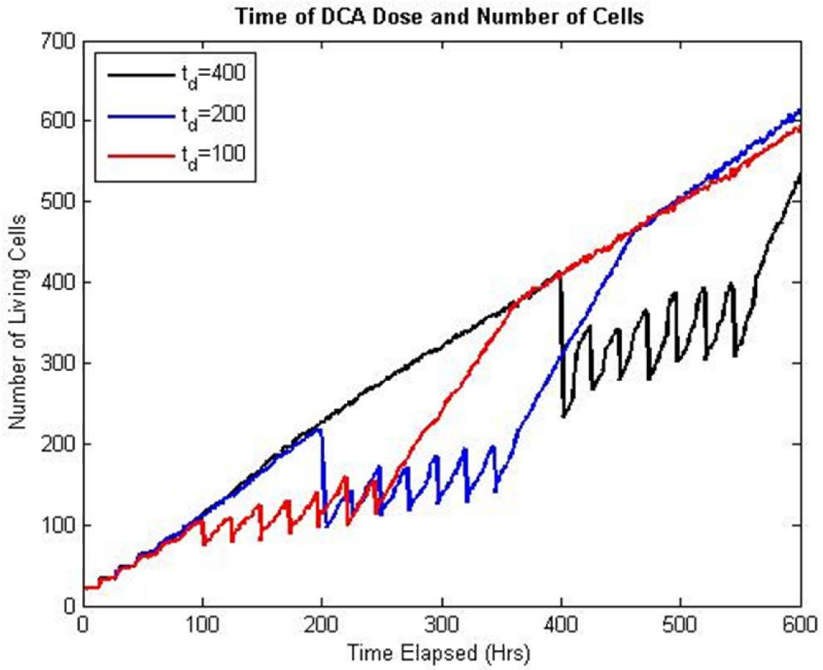

Figure 3.26: Effect of time of dose administration of DCA on number of living cells

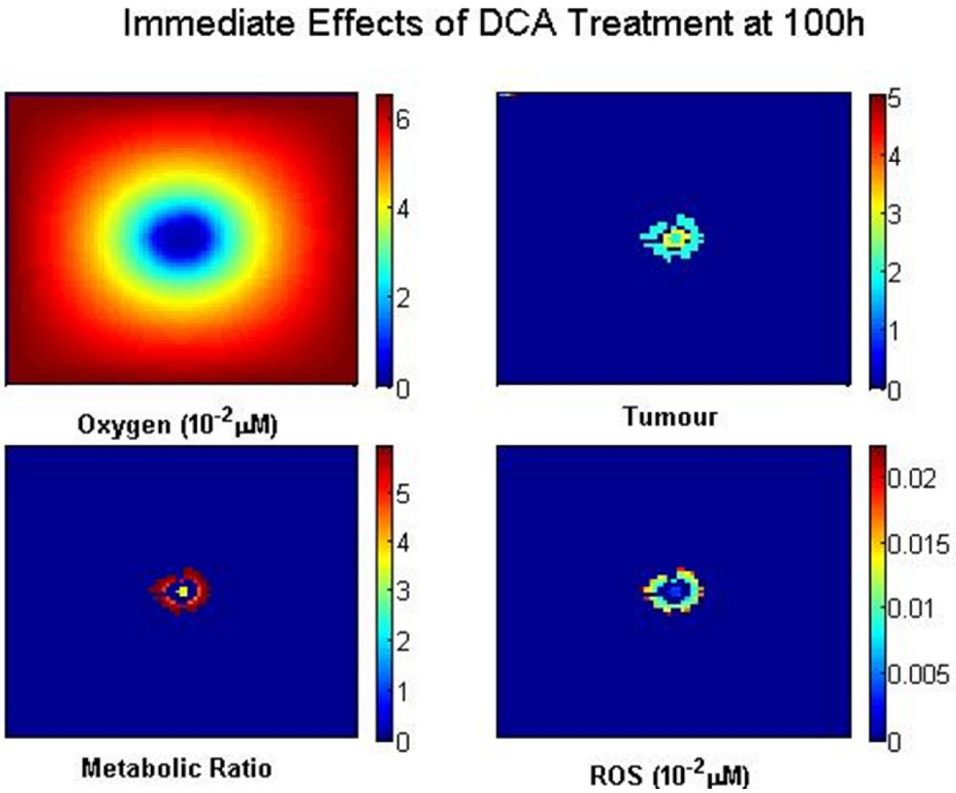

Figure 3.27: Environmental and morphogenic results immediately following a DCA treatment at 100h

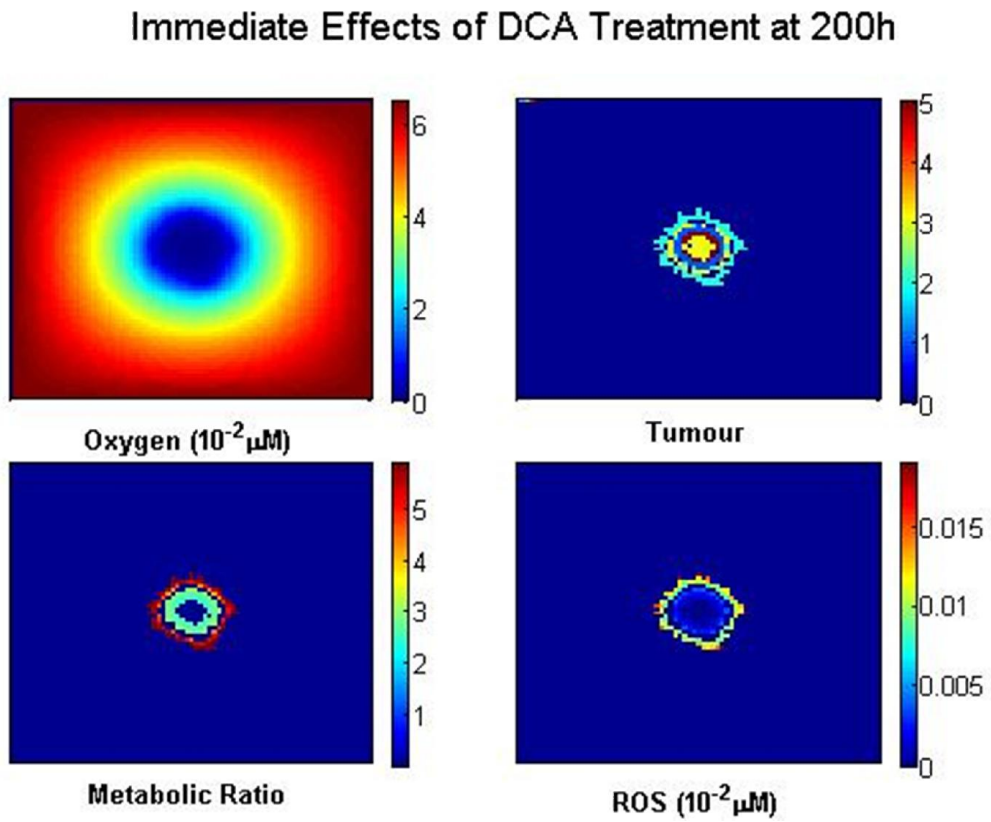

Figure 3.28: Environmental and morphogenic results immediately following a DCA treatment at 200h

Since it has been observed that certain cell lines are more responsive to DCA treatment than others, one must be cautious in relating the results obtained from simulations to realistic treatment behaviour [Michelakis et al. 2008, Wong et al. 2008]. With these considerations, the simulations indicate that the effect of DCA is not significantly moderated by the dose applied. In particular, the maximum effect is achieved with a peak concentration of 8mM (figure 3.29). This may be attributed to the fact that DCA allows for regulated cell death to be reinstated; thus, there is a theoretical saturation point after which all cells are consuming oxygen readily and responding to the hypoxic and oxidant death queues appropriately.

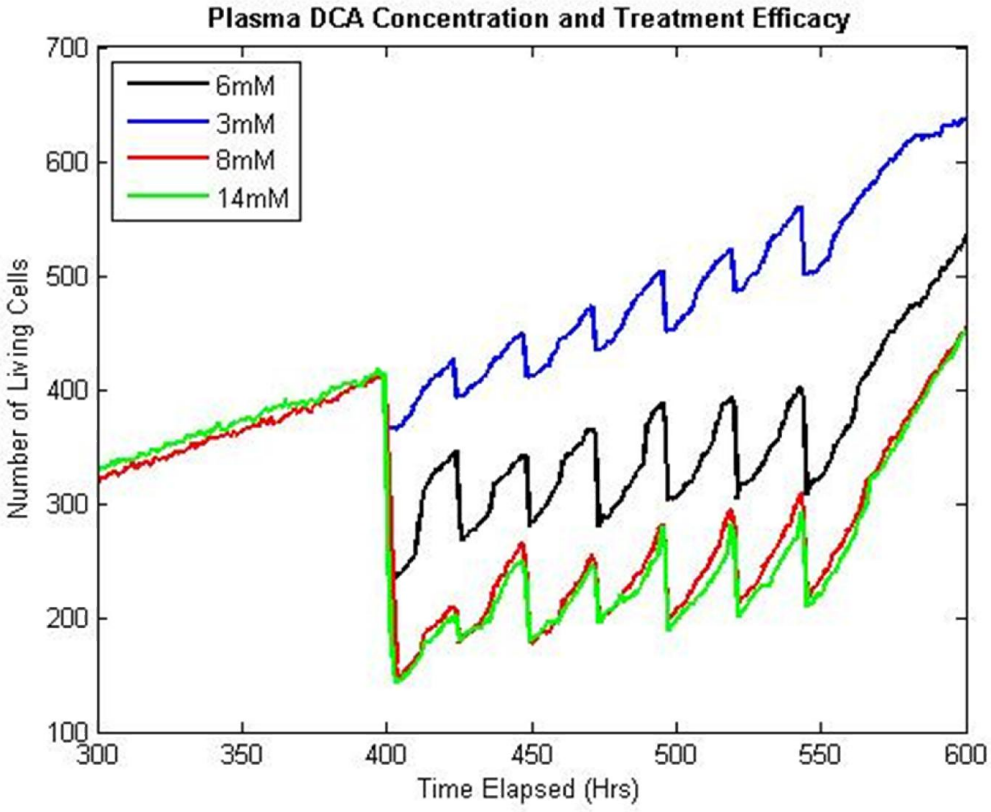

Figure 3.29: Effect of plasma concentration of DCA on number of living cells

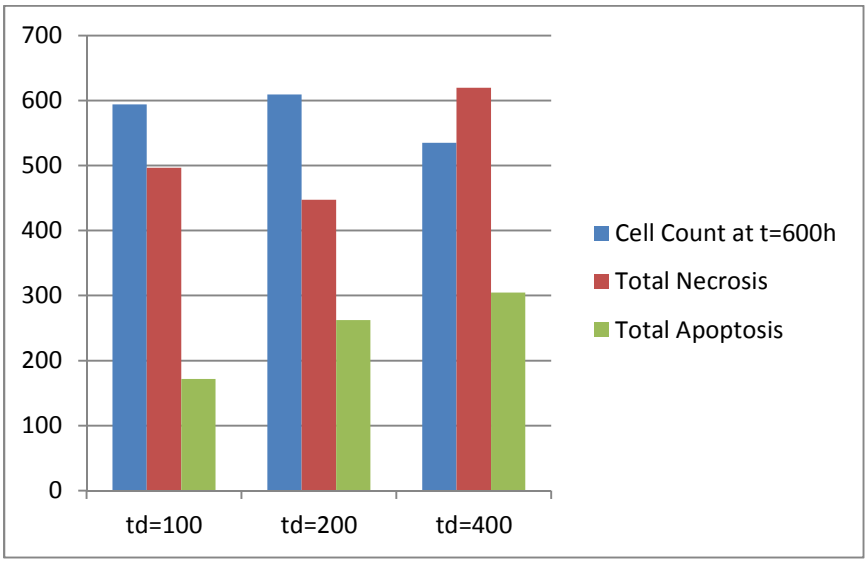

Figure 3.30 Total cell death by necrosis and apoptosis and final number of living cells after 8mM DCA administered at different times

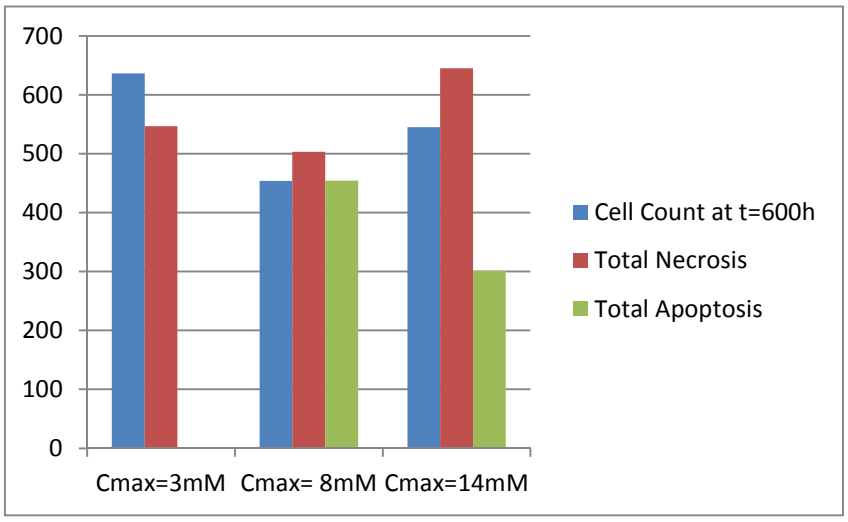

Figure 3.31: Total cell death by necrosis and apoptosis and final number of living cells for different C_{max} when administered at td=400.

3.4 Strategies for Combining Treatments

Often the studies conducted on VC and DCA have considered the effects of each as an adjuvant therapy; VC has been proposed to be more effective for palliative purposes during treatments and DCA for increasing the cytotoxicity of other treatments [Monti et al. 2012, Mastrangelo et al. 2013, Ayyanathan et al. 2012, Gonzalez and Miranda-Massari 2014]. Regardless, the effects of these treatments together have yet to be studied clinically.

Common techniques for applying multiple treatments involve concurrent, sequential, and adjuvant administration. Whereas concurrent involves simultaneous dosing, adjuvant therapy utilizes a staggered approach where a delay is imposed between the first and second administrations of the drugs. Sequential dosing involves administering the second dose once the first drug has been cleared from the system, or is no longer in significant concentrations. When applied together, many of these techniques actually succeed in killing all living cells

in the simulated tumour. For instance, concurrent administration yields the results shown in figure 3.32. Schedule 3 VC administration does not appear to have a significant effect on eradicating tumour cells, but more interesting is the fact that there is not a significant difference in tumour outcome when it is combined with DCA. Similarly, the effects of adjuvant administration are most pronounced with Schedules 1 and 7 (figure 3.33). This supports the notion that higher concentrations of VC are more effective than prolonged lower concentrations, even when the effects of DCA are contributing.

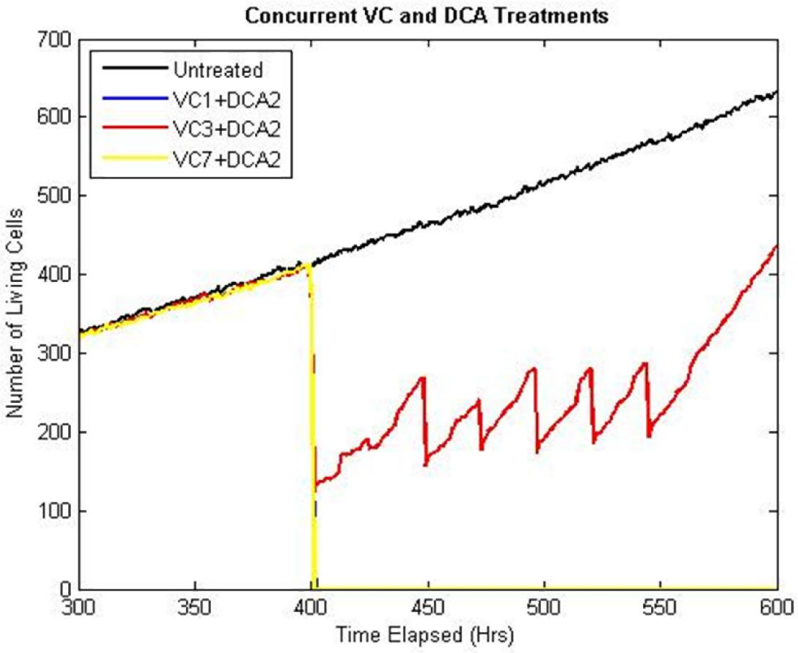

Figure 3.32: Concurrent administration of DCA and VC after 400 hrs results in tumour eradication, except when schedule 3 is used.

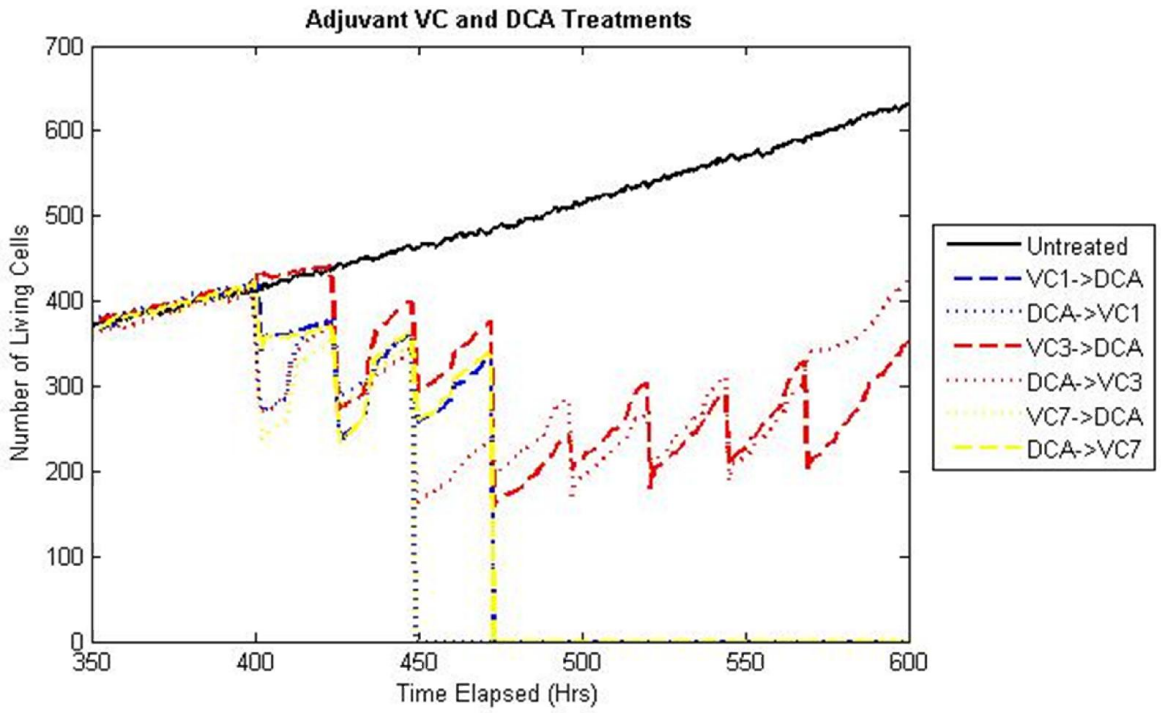

Figure 3.33: Adjuvant administration of DCA and VC after 400 hrs results in tumour eradication, except when schedule 3 is used.

The last class of combination therapy that is investigated is sequential administration. The ability to inhibit tumour growth seems to mostly affected by the timing of the administration of VC. For the schedules where DCA is administered first, it is after 1 week of daily DCA infusions that VC will be introduced; this corresponds to 468 steps (hours) into the simulation. Alternatively, VC being administered first corresponds to DCA being introduced at approximately 432 steps, or hours, into the simulation.

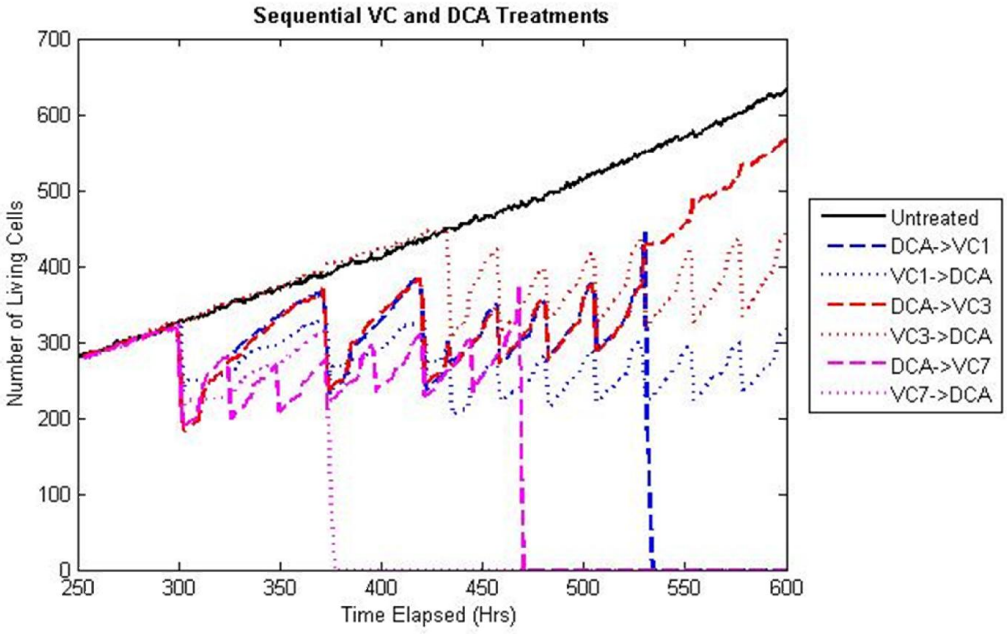

Figure 3.34: Sequential administration of DCA and VC after 300 hrs

3.5 Concluding Remarks and Future Directions

The HCA model applied herein reflects the sensitivity of the growth of a tumour to the environmental considerations. In particular, the ECM and means of calculating ROS impact the overall growth of the tumour despite being simple approximations. While more detailed ECM interactions have been studied elsewhere, the simple distribution utilized in this model nevertheless produces interesting and dynamic behaviour in the form of tumour size and morphology [Anderson et al. 2009, Zaman et al. 2005]. Furthermore, various methods of calculating the endogenous ROS production from the amount of oxygen consumed by cells produce similar morphological patterns, yet different rates of growth (figure 3.9). Thus, an improvement on the accuracy of the caculated ROS could yield

interesting and unforeseen results. Specifically, this would entail the use of an integrated differential equation model for the antioxidant mechanisms employed by cancer cells; i.e., catalase, glutathione peroxidase, and peroxiredoxin regulation.

As demonstrated by the implications of utilizing realistic pharmacokinetic models and necrotic cell removal, the model considerations have a signifiant impact on the results obtained. In particular, the cytotoxic effects and implications of a necrotic ring were exaggerated prior to the model improvements. While over-complicating the model may be counter-productive to the meaningfulness of the obtained results, including the effects of environmental cells not of epithelial origin could improve the dynamics. In particular, including additional phenotypes for macrophages that remove necrotic cells and induce inflammation (and hence provide an exogenous source of ROS production), could have an impact on the quantitative behaviour of the model. In addition to the more intricate antioxidant mechanisms, this can be manifested in the form of a decreased sensitivity to cell death and wider range of acceptable ROS levels.

The model itself demonstrates that schedules that maximize the plasma concentration of VC obtained are necessary to induce death. That is, there is no conclusive evidence that moderate concentrations of VC over longer periods of time are more effective in treating cancer. However, in cell lines that are resistant to H_2O_2-mediated cell death, VC could contribute to the growth of a tumour through reprogramming metabolic machinery. As intravenous VC use continues to be investigated in phase I and II clinical trials, the unpromising results that have been seen in advanced and recurring cancers using this treatment strategy prompt a more thorough investigation into the pharmacokinetics of

vitamin C in various cancer cell lines and in conjunction with other cytotoxic therapies [Hoffer et al. 2008, Gonzalez and Miranda-Massari 2014]. Previously observed effects of VC appear to show conflicting results for malignant growth, with some studies showing cytotoxic effects and others showing enhanced malignancy [Gonzalez and Miranda-Massari 2014]. Tumours that have responded to treatment with ascorbic acid have indeed shown to be completely eradicated, as the results from this model have indicated. However, this is not always clinically relevant. To account for the conflicting results observed, the systemic saturation hypothesis in addition to catalase over-expression may explain why excessive concentrations of plasma VC do not induce a proportional effect on inhibiting tumour growth. Specifically, it has been proposed that a chemical mechanism may exist in which larger VC concentrations result in an increase of the proportion that is in the oxidatively inactive form of dehydroascorbic acid [Gonzalez et al. 2012]. Nevertheless, controlled and randomized clinical trials investigating the efficacy of VC with other cancer therapies have yet to be published [Gonzalez and Miranda-Massari 2014]. As a result, improving computer simulations could prove to be a promising approach in contributing to the investigation of multiple treatment scenarios in chemotherapy and radiotherapy [Deisboeck et al. 2009].

DCA has been observed to exert differing cytotoxic effects to cell lines in *in vitro* and *in vivo* experiments. In the *in silico* model presented, insights into the experimentally observed tumour reduction could prove useful in explaining the persistence of cells capable of forming metastatic masses [Sun et al. 2010]. In particular, the sensitivity of cells in the hypoxic region of the model allow for the survival of cells in the oxidative region, ultimately resulting in its failure as an independent therapy.In other words, the inability of DCA to

eliminate tumours in this model is supported by the observed shrinkage and subsequent persistance of tumours treated with DCA alone [Michelakis et al. 2010, Sun et al. 2011]. This further indicates that particular attention should be made in designing clinical trials that incorporate alternative therapies. However, hypoxic and quiescent cells may be more sensitive because of decreased overall ATP production. Thus, the incorporation of other metabolic pathways, like glutaminolysis, may reveal why certain tumours are completely unresponsive to DCA treatment.

Appendix of Parameter Values

Parameter	Value	Reasoning
D_G	$1.1x10^{-6}$ cm^2/s	[Molavian et al. 2009]
D_O	$1.46x10^{-5}$ cm^2/s	[Molavian et al. 2009]
D_{DCA}	$1.1x10^{-6}$ cm^2/s	Empirical studies have yet to be published so this value is approximated
D_{Asc}	$1.1x10^{-6}$ cm^2/s	Given the similar chemical structure to glucose, the diffusion constant is chosen to be the same
μ_O	$3.0x10^{-5}$ cm/s	[Molavian et al. 2009]
μ_G	$3.0x10^{-5}$ cm/s	[Molavian et al. 2009]
μ_{Asc}	$3.0x10^{-5}$ cm/s	Given the similar chemical structure to glucose, permeability is chosen to be the same
μ_{DCA}	$1.2x10^{-4}$ cm/s	Its bioavailability of 100% of the administered dose indicates an increased potential for absorption across biological barriers [Stacpoole 1998b]
F_{VC}	$1x10^{-3}$ mM/s	Multiple values are explored, but this one produces realistic growth results
k_{DCA}	$5x10^{-4}$ mM/s	Empirical studies have yet to be published so this value is approximated
p_g	$1.9x10^{-3}$ mM/s	[Casciari et al. 1992]
α	$1.5x10^{-3}$	[Orrenius 2007]
α_{hyp}	$2.25x10^{-3}$	[Goyal et al. 2004]
Ψ_{def}	0.7	Multiple values explored
Ψ_{max}	0.96	Multiple values explored
C_O^{Nec}	$2x10^{-7}$ mM	Indicative of an anoxic and uninhabitable environment
C_{ROS}^{Gly}	$2.1x10^{-3}$ mM	Calculated ROS concentrations that would result from an environment with only ¼ of the ideal, as determined by [Casciari et al. 1992] oxygen levels available
C_{ROS}^{Apop}	$3.0x10^{-3}$ mM	Calculated ROS concentrations that would result from an environment with only $^1/_6$ of the

		ideal, as determined by [Casciari et al. 1992], oxygen levels available
ϕ_{ATP}^{Apop}	$1.8x10^{-2}$ mM	~30% of the ATP that would be produced in a nutrient-saturated environment (consumption of p_g and p_o) [Leist et al. 1997, Casciari et al. 1992]
ϕ_{ATP}^{Nec}	$1.29x10^{-2}$ mM	~20% of the ATP that would be produced in a nutrient-saturated environment (consumption of p_g and p_g) [Leist et al. 1997, Casciari et al. 1992]
C_{ROS}^{Nec}	$3.5x10^{-3-}$ mM	Calculated ROS concentration that would result from an environment with only $^1/_8$ of the ideal, as determined by [Casciari et al. 1992], oxygen levels available
$C_{ROS}^{Apop-DCA}$	$4.1x10^{-5}$ mM	Chosen to be a proportion of the normal apoptotic ROS level so that it reflects scenarios in which DCA is lethal to cells with ample nutrients

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