Western Graduate&PostdoctoralStudies

Western University
Scholarship@Western

Electronic Thesis and Dissertation Repository

June 2015

Using Cellular Automata and Lattice Boltzmann Methods to Model Cancer Growth: Analysis of Combination Treatment Outcomes

Jenna L. Butler
The University of Western Ontario

Supervisor
Dr. Mark Daley
The University of Western Ontario

Graduate Program in Computer Science

A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

© Jenna L. Butler 2015

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Part of the Other Computer Sciences Commons

Recommended Citation

Butler, Jenna L., "Using Cellular Automata and Lattice Boltzmann Methods to Model Cancer Growth: Analysis of Combination Treatment Outcomes" (2015). *Electronic Thesis and Dissertation Repository*. 2871.
https://ir.lib.uwo.ca/etd/2871

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact tadam@uwo.ca.

المنارة للاستشارات

www.manaraa.com

USING CELLULAR AUTOMATA AND LATTICE BOLTZMANN METHODS TO MODEL CANCER GROWTH: ANALYSIS OF COMBINATION TREATMENT OUTCOMES

(Thesis format: Monograph)

by

Jenna Leah Butler

Graduate Program in Computer Science

A thesis submitted in partial fulfilment
of the requirements for the degree of
Doctorate of Philosophy

The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

© Jenna Leah Butler 2015

المنارة للاستشارات

www.manaraa.com

Abstract

In Canada it is estimated that 76,600 people will die of cancer in 2014. Cancer, a collection of over 200 diseases, has differences existing between globally, between individuals and overtime in one individual. Treatment options are similarly varied. These differences make selecting the best possible treatment for every type of cancer very challenging. In addition, with no single cure for cancer, treatments are often combined in different ways to form the best overall option.

In an attempt to synthesize the properties of these diseases into a collection of common cellular changes, Hanahan and Weinberg proposed "the hallmarks of cancer" – 10 differences between healthy cells and cancer cells, present in almost every cancer. There exists the potential for treatments that are broadly applicable if they reverse these general properties.

This work seeks to simulate early cancer growth, specifically looking at these hallmarks, and detect the best combinations of hallmarks to remove in order to stop cancer growth. This hybrid simulation combines a discrete model of cancer cells using cellular automata, with a continuous model of blood flow using lattice Boltzmann methods. Hallmarks relevant during the early growth stages of solid tumour development are simulated using rules in the cellular automata. Hallmarks were removed in pairs, triplets and quadruplets in order to model combination therapy, abstracting drugs that target these properties as the removal of the hallmark from the system. Overall growth of the tumours with "treatments" applied were compared to tumours where all hallmarks were present.

It was found that many combinations had no effect on tumour growth. In some cases combinations even increased growth, selecting for the most aggressive hallmarks since weaker hallmarks were unavailable. However, in general, as more treatments were applied, cancer growth decreased. This work is the first to simulate removing hallmarks in pairs, triplets and quadruplets from a model with biologically relevant oxygen flow. It provides a proof of concept that not all combinations are equally effective, even if the individual treatments are effective. This work suggests some combinations should be avoided while others could potentially be beneficial in a variety of diseases.

Keywords: Cellular automata, cancer hallmarks, lattice Boltzmann methods, combination treatment, oxygen, simulation

المنارة للاستشارات
www.manaraa.com

Co-Authorship Statement

In Chapter 3, Dr. Frances Mackay and Dr. Colin Denniston contributed research direction, editing and code review. Dr. Frances Mackay also contributed much of Section 3.3.

المنارة للاستشارات
www.manaraa.com

Dedication

This Doctoral thesis is dedicated to my mother, Linda Cameron, who in life taught me to explore, and in death gave me the topic to study. Most of all she taught me to love – God, my family and myself.

المنارة للاستشارات

www.manaraa.com

Acknowlegements

I would first like to thank my incredible supervisor, Dr. Mark Daley. Like a good parent, he has always supported me, let me fall (but not too far) and encouraged me to pursue my passion. He branched out into a different field to support my desire to do cancer research, and has always supported my desire to teach throughout my graduate career. Mark has been incredibly helpful throughout my graduate career, never micromanaging me, but allowing me to learn on my own with the knowledge that someone was watching out for me. I am thankful for his steering and direction throughout this process, but mostly for his support of my life throughout my thesis. He encouraged me to continue to live life outside graduate school, including having children, and his support of my family life have been invaluable. Thank you Mark.

I'd also like to thank the members of my laboratory, both past and present. Mike Burrell and Franziska Biegler welcomed me into the lab when I was a Masters student and have helped me along the way. Beth Locke has been a coauthor, office mate and friend whose intelligence and excitement for learning have always amazed me. Our newer additions, Mehrsa Golestaneh, Casey Wood, Celine Gravelines and Andrew Kope have been inspiring in their passion and dedication. Ethan Jackson and James Hughes, as well as Jason Morgenstern, William Callaghan and Heather Carlson, our newest recruits, have brought fresh excitement and much fun to our lab. Thank you all.

I would like to give a very special thank you to my editors, who tirelessly read this very long document, most of whom volunteered to boot! Thank you to Dr. Matthew McCann, physiologist; Catherine Bond-Mills, oncology pharmacist and pharmacy lead, Southwest Regional Cancer Program; and Bruce Cameron (I usually call him dad) who discovered I had misspelled "Philosophy" on the title page of this thesis.

Many other people have been incredibly supportive during my time here at Western. I'd like to thank Dr. Mike Katchabaw, Dr. Hanan Lutfiyya and Dr. Robert Mercer for giving me the opportunity to teach courses in our department while I pursued my PhD – an endeavour that made graduate school even more enjoyable and fulfilling. I'd also like to thank the many professors I have had throughout my 10 years at Western, especially our graduate chair Dr. Roberto Solis-Oba who is one of the most talented teachers I have had the pleasure of learning from. Thank you to my own personal cheerleader Laura Reid and "the office ladies", as I refer to them, Cheryl, Janice, Dianne and Angie, who always have an ear willing to listen. Thank you also to Bozena, Tom and Rob for keeping me in candy and comfort throughout my time in Middlesex College.

Thank you to my friends and church family for your support over the years. Frances and Dr. Ian Swentek have provided hours of free babysitting as I pursued my PhD and we thank you

للاستشارات المنارة
www.manaraa.com

for this. Thank you to the Tuesday Morning Prayer ladies for your continual prayer, support and listening ears.

To my very large family – thank you. Kelly, Cathy, Rick, Breanne, Reagan, Isaac, Barb, John and Noreen, you have all been such an encouragement. Your prayers, cheerful words, and love have been an encouragement to my heart throughout this long process. Thank you for being a part of my life.

My father, Bruce Cameron, is one of the most incredible people in my life. Thank you for working so hard to be able to pay for my education and give me these opportunities. You have never ceased to encourage me to reach higher and I know your personal tenacity and drive have formed much of my personality. You are caring, giving of your time and energy, and thoughtful, and have never ceased to support me. Thank you for being my dad, my friend, and even my mom when needed.

Now to the lights of my life – Caleb and Elora, and baby-to-be. Thank you! Ellie, your smile, pats on the arm, and encouragement to "keep working mama" have motivated me more than any award, scholarship or deadline ever could. You are joy personified and I thank God for you daily. Caleb, thank you for being my best friend. You have had the single biggest impact on the creation of this document. The every day support you have provided, from raising our child, to cooking our meals, to editing this document, have been invaluable. The especially hard work you have done over this last year has been transformational and I'm sure this document would not have come together the same way without it. You make it possible for me to give my best at work, knowing Ellie is taken care of, and returning home to you both each night is the best part of my day.

Lastly, to the Light of my life. I would like to thank the Lord for his guidance and love everyday of my life. He has always shown up and has made this document what it is. There were many times I did not think getting my PhD was possible, and was always reminded "I can do all things through Christ Jesus who gives me strength".

للاستشارات المنارة
www.manaraa.com

Contents

Abstract ii

Co-Authorship Statement iii

Dedication iv

Acknowledgements iv

List of Figures xi

List of Tables xii

List of Appendices xiii

1 Introduction and Literature Review 1

1.1 Introduction 2

1.2 Simulated brain tumour growth dynamics using a three-dimensional cellular automaton 5

1.2.1 Introduction 5

1.2.2 The model 5

The lattice 5

The proliferation algorithm 6

1.2.3 Results 8

1.2.4 Conclusions 9

1.3 An evolutionary hybrid cellular automaton model of solid tumour growth . . . 10

1.3.1 Introduction 10

1.3.2 The model 10

The cell 11

The network 11

Cell metabolism 12

Cell death 13

المنارة للاستشارات

www.manaraa.com

Cell movement 13
Proliferation 13
Mutations 13
Chemical fields 14
Cellular automaton 14
Parameters 15
1.3.3 Results and discussion 15
1.3.4 Conclusions 17
1.4 Simulating the hallmarks of cancer 19
1.4.1 Introduction 19
1.4.2 Methods 20
Overview 20
Hallmarks 20
The model 22
1.4.3 Results 24
1.4.4 Discussion 25
1.4.5 Conclusions 27
1.5 Study of cancer hallmarks relevance using a cellular automaton tumor growth model 28
1.5.1 Introduction 28
1.5.2 Methods 28
The hallmarks considered 29
Event model 29
1.5.3 Results 30
Hallmark accumulation for different parameter sets 31
1.5.4 Conclusions 32
1.6 Overall conclusions 35

2 Biological Background and Model Rationale **37**
2.1 Introduction 38
2.2 Cancer Hallmarks 41
2.2.1 Introduction to the hallmarks of cancer 41
2.2.2 Self-sufficiency in growth signals 42
2.2.3 Evading growth suppressors 44
2.2.4 Resisting cell death 45
2.2.5 Enabling replicative immortality 46

المنارة للاستشارات
www.manaraa.com

2.2.6 Inducing angiogenesis 48
2.2.7 Emerging hallmarks and enabling characteristics 50
Genetic instability 51
Reprogramming energy metabolism 52
Evading immune destruction 54
2.2.8 Features not simulated 56
Tumour-promoting inflammation 56
Invasion and metastasis 57
2.3 Cellular automaton modelling 59
2.3.1 The automaton states 60
Alive 60
Apoptotic 60
Necrotic 61
Quiescent 63
Glycolytic 64
Dead 65
2.4 Current treatment approaches and challenges 65

3 Impact of paired hallmarks on cancer growth 73
3.1 Introduction 74
3.2 Methods 75
3.2.1 Modelling the hallmarks 75
Sustained growth 76
Evading growth suppressors 76
Avoiding programmed cell death 76
Enabling replicative immortality 76
Inducing angiogenesis 77
Genetic instability 77
Evading immune destruction 77
3.2.2 A note on hallmarks not included 78
3.2.3 Event queue 78
3.2.4 Lifecycle pseudocode 78
3.3 Lattice-Boltzmann implementation details 80
3.3.1 Parameter values 83
3.3.2 Implementation details 84
3.4 Results 84

المنارة للاستشارات
www.manaraa.com

3.5 Discussion . . . 90
3.6 Conclusions . . . 91

4 Triplet and quadruplet knockouts **93**
4.1 Introduction . . . 94
4.2 Methods . . . 97
Life cycle pseudocode . . . 97
4.2.1 Oxygen modelling . . . 99
4.2.2 Knockouts . . . 99
4.2.3 Parameters . . . 99
4.3 Results . . . 101
4.4 Discussion . . . 108
4.5 Conclusions . . . 111

5 Conclusions **113**

Bibliography **121**

A Glossary **138**

B Abbreviation List **141**

C Raw p-values for all knockout data **143**
C.1 Doublet knockout data . . . 143
C.2 Triplet knockout data . . . 144
C.3 Quadruplet knockout data . . . 146

D Drugs that target hallmarks **149**

Curriculum Vitae **151**

المنارة للاستشارات
www.manaraa.com

List of Figures

2.1 Summary of current cancer statistics in Canada 66
2.2 Percent distribution of estimated new cancer cases in Canada 67
2.3 Cancer distribution by age in Canada 68
2.4 Cancer deaths and mortality rates in Canada 69

3.1 Simplified UML diagram of simulation 84
3.2 Cell counts and growth images for baseline tumour 85
3.3 End of simulation images for 4 knockout pairs 86
3.4 Total cancer cell count for all pairs over time 87
3.5 Top 10 phenotypes 88

4.1 A sample simulation of tumour growth for a baseline tumour 102
4.2 Final cancer growth stage for 9 hallmark removal sets 105
4.3 Comparisons of success of hallmark knockouts in triplets and quadruplets. . . . 106
4.4 Total cancer growth overtime for all triplet and quadruplet knockouts 107

5.1 Comparisons of hallmark knockout success for doublet, triplet and quadruplet knockouts 116
5.2 A symmetric heat map of co-occurrences of hallmarks in knockout treatments . 117

المنارة للاستشارات
www.manaraa.com

List of Tables

1.1 Summary of time-dependent functions and input parameters 8
1.2 Input and output nodes. 12

3.1 Parameters used in simulations. 83
3.2 Phenotype codes and the corresponding hallmarks present in the phenotype. . . 89

4.1 Parameters used in simulations. 100

C.1 Hallmark paired knockout data 144
C.2 Hallmark triplet knockout data 146
C.3 Hallmark quadruplet knockout data 148

للاستشارات

www.manaraa.com

List of Appendices

Appendix A Glossary 138
Appendix B Abbreviation List 141
Appendix C Raw p-values for all knockout data 143
Appendix D Drugs that target hallmarks 149

للاستشارات المنارة
www.manaraa.com

Chapter 1

Introduction and Literature Review

المنارة للاستشارات
www.manaraa.com

1.1 Introduction

Cancer has plagued humans for about as long as we have any knowledge of the disease. Evidence of cancer has been found as far back as 3000 BC. The Edwin Smith Papyrus is a copy of part of an ancient Egyptian textbook on trauma surgery, and describes tumours of the breast that were removed via cauterization. In the text, the writing says of the disease – “There is no treatment” [12].

Knowledge of cancer, the diagnosis, treatment and recovery from it have all come a long way since then. In 1761, Giovanni Morgagni began the common practice of performing autopsies which assisted in scientists relating illness seen while the patient was living with the mass of growth inside the body [12]. Around the same time, Scottish surgeon John Hunter suggested that if a tumour seems “moveable” and has not invaded adjacent tissue one should remove it – beginning the practice of surgical removal of cancer. In the 19th century, Rudolf Virchow began investigating cancer using the microscope, which gave a better understanding of the damage cancer had done, as well as allowing for more complete diagnoses and detection of whether a cancer had been completely removed by surgery [12]. Later, in 1915, cancer was induced in a lab animal for the first time at Tokyo University by Yamagiwa and Ichikawa, aiding in our knowledge of carcinogens (cancer causing agents). Throughout the 20th century cancer knowledge grew as scientists around the world discovered more carcinogens and viruses that caused cancer, the genetic code of life, the possibility and then eventuality of mutations to this code and familial cancer links [12]. Now we are at a place of great understanding where some cancers are almost completely curable (thyroid and prostate, for example, have survival rates above 97% [158]). However, progress in some cancer treatments has stalled, and as of 2009, cancer was the leading cause of death in Canada [154] and the second leading cause of death in the United States [39]. Clearly, much still needs to be done to combat this disease.

Kasia Rejniak and Alexander Anderson published a brief review titled “State of the art in computational modelling of cancer” in 2011 in the journal *Mathematical Medicine and Biology* [140]. They pointed out that while modelling of cancer has been occurring for over 60 years, it has only recently begun being seen as a real tool in the fight against cancer. In fact, they state:

> The reductionist approach that has traditionally guided biological research has taught us a great deal about the initial steps in cancer development and subsequent progression and still has great potential as a tool to aid the tailoring of patient-specific treatments. But given the diversity of favourite genes, proteins or pathways and their limited therapeutic success, many cancer biologists are beginning

المنارة للاستشارات
www.manaraa.com

> to accept that we need to integrate our knowledge (across scales) and start to look at cancer in a whole new way." [140]

Rejniak and Anderson, along with many others including Vito Quaranta, Philip Gerlee, David Basanta, Robert Gatenby, Heiko Enderling, Anuraag R. Kansal, José Santos and Ángel Monteagudo, have been doing this difficult and important work of integrating our knowledge. They have gathered knowledge gained over hundreds of years into models and simulations that allow clinicians and computational scientists to work together to battle cancer. Many cancer models currently exist, including: Anderson *et al.'s* multiscale mathematical model of 2-dimensional tumour growth [18]; Lloyd *et al.'s* computational framework for solid tumour growth, which comprised models at the tissue, cellular and subcellular levels [100]; and Ramis-Conde *et al.'s* hybrid discrete-continuum model which looked at tissue invasion by cancer cells [137]. Models focus on different aspects of tumour growth (including the use of the glycolytic phenotype [67], evolution of cell motility [68] and confined environments [69]) and employ different modelling approaches (mathematical, [80], [137], hybrid, [141], [66], agent-based [106], [157]).

Currently the state of the art in cancer modelling is spread across these different modelling techniques. A recent review paper looking at cancer invasion discusses the use of both hybrid discrete-continuous (HDC) models and immersed boundary models of a cell (IBCell). HDC allows for cells to be modelled discretely but microenvironmental variables such as nutrients and oxygen to be modelled using reaction-diffusion equations. The IBCell model is beneficial for capturing the morphology of a tumour cell as the cells in this model are deformable [88]. In addition to these two types of agent-based models, cellular automaton (CA) models are also used frequently. Gerlee and Anderson created an evolutionary hybrid cellular automaton model where the cancer cells are modelled using cellular automata to capture the behaviour of the tissue as a whole, while using an artificial neural network for cell decisions [66]. This type of hybrid cellular automaton model has recently been built on by Shrestha *et al.* who used a similar model to look at large-scale growth of tumours [148]. Recently, CA models have been used to look at the hallmarks of cancer [1], [25], [143] as proposed by Hanahana and Weinberg [74], [75].

Many reviews of the field of cancer modelling exist, and readers who wish a broader prospective are directed to the following review articles: [15], [17], [139]. Here I will outline four major works that have lead to the questions addressed in this thesis. These papers are representative of a path from some of the earliest accurate cellular automata models through to the current state of the art of CA models with respect to cancer "hallmarks". These four works

المنارة للاستشارات
www.manaraa.com

reflect the journey my research question took. The first paper, by Kensal *et al.*, showed that simple CA models are capable of achieving complex results – including accurately predicting time of diagnosis and death. The next works, by Gerlee and Anderson, provided the foundation of my model. They outlined the lifecycle for CA models of cancer and showed the importance of accurately modelling oxygen around the tumour. Next, the work of Abbott *et al.*, was the first to include the hallmarks of cancer in the rule set of the cellular automata. Lastly, Santos and Monteagudo provided a launching point for my research. They set out to find the relative importance of the hallmarks of cancer, and looked at the impact of removing each from a cancer simulation. This lead me to question how removing these hallmarks in pairs, triplets and quadruplets would impact the system. Each of these bodies of work were influential in bringing me from an interest in the field to a specific, attainable question, and I will describe each in detail in order to show both a representation of the field of cellular automata cancer modelling with respect to hallmarks as well as how my research question came about.

المنارة للاستشارات

www.manaraa.com

1.2 Simulated brain tumour growth dynamics using a three-dimensional cellular automaton

1.2.1 Introduction

When this paper [89], by A. R. Kansal, S. Torquato, G. R. Harsh IV, E. A. Chiocca and T. S. Deisboeck was published in January 2000, human glioblastoma multiforme (GBM) tumours left patients with a median survival time of only eight months. Due to the confined nature of the tumour (typically encased by the skull), GBM tumours were a good choice for mathematical modelling as they are fairly self contained, spherical tumours.

Another positive about modelling GBM tumours, is that they are able to be accurately modelled as a multicellular tumour spheroid (MTS) (an early stage tumour which is easier to model than a full grown GBM). Modelling a GBM as an oversized MTS is possible because a GBM, like a large MTS, is comprised of a large necrotic centre with a surrounding shell of proliferating cells. Kansal *et al.* developed a CA model that allows for GBMs to be modelled as MTSs and included several novel features at the time. The model treated cell division in a new way, used the Voronoi tessellation to study the dynamics of tumour growth in a cellular automaton and used a varying density of lattice sites (an adaptive grid lattice). The model is able to grow from roughly 1000 cells to a size of 10^{11} cells, the thickness of the proliferative rim and the non-proliferative shell are linked to the overall tumour radius by a 2/3 power relation which gives more biologically accurate results and the model included mutant phenotype "hot spots" in the tumour. This was a very advanced model and a seminal paper.

1.2.2 The model

The lattice

This model uses an underlying Delanuay triangulation lattice structure, which is the dual lattice of the Voronoi tesselation. To create the lattice a selected number of points are distributed randomly in space. The triangulation is created such that no point is inside the circumcircle of any triangle (this maximizes the minimum angle in the triangle, helping avoid skinny triangles). Connecting the centres of these circles creates the Voronoi tesselation.

The lattice used in this model was designed with a variable grid size. The density was allowed to vary with the radius from the centre of the tumour. Every lattice cell corresponded to

المنارة للاستشارات
www.manaraa.com

some number of biological cells. Where the density was high, there would be fewer biological cells per lattice cell and where it was low there would be more biological cells per lattice cell. Lattice cells near the center had significantly higher densities than those at the edge to allow for better resolution. Each cell in the centre of the tumour represented approximately 100 real cells, however the cells on the outside edge of the tumour each represented close to 10^6 real cells. The average distance between lattice sites is represented by ζ and is defined as:

$$\zeta = \frac{1}{6} r^{\frac{2}{3}}, \tag{1.1}$$

where r is the radial position where it is being measured. The $\frac{2}{3}$ in the exponent is intended to incorporate a surface-area-to-volume-like relation in order to better represent the diffusion of nutrients to the centre of the tumour.

In order to generate the points for the lattice, the random sequential addition (RSA) process was used. The RSA method checks each generated point to ensure it is not too close to all the other points. This was used to make sure there were no areas with extremely high density. They also tried to closely approach the "jamming limit" of the RSA in order to eliminate the chances of there being a low density spot. The minimum distance between points (R_s) could vary and was calculated as

$$R_s = 0.146 r^{\frac{2}{3}} \tag{1.2}$$

The proliferation algorithm

The algorithm is designed to grow the tumour from a few cells (approximately 1000 biological cells) to full macroscopic size. The idealized version of the tumour is a group of concentric spheres. The inner most sphere represents the necrotic region. This region has radius R_n which is a function of time, t, and distance from the proliferation rim, δ_n. The next sphere represents cells that are alive but in the G0 cell cycle state (a resting state of the cell cycle), and is termed the non-proliferative region and is also defined in terms of its distance from the edge of the tumour, δ_p. In order for the cells to be alive and not necrotic they must have access to nutrients from the blood vessels surrounding the tumour so the thickness is the maximum distance the nutrients can diffuse to. The outer, thinnest sphere, contains the actively dividing cells. Each cell can only divide if there is space available where it will still be able to access nutrients (again, δ_p).

R_t is the radius of the tumour. Since tumours are not perfectly spherical, the values of R_t and

المنارة للاستشارات
www.manaraa.com

R_n vary depending on the radii of cells on the edge of the model. The value of these parameters in the paper are the average of the values defined by:

$$R_t = \frac{\sum_{i=1}^{N_p} r_i}{N_p} \tag{1.3}$$

$$R_n = \frac{\sum_{i=1}^{N_n} r_i}{N_n} \tag{1.4}$$

where N_p is the number of cells on the edge of the proliferative region and N_n is the number of cells on the edge of the necrotic core.

Cells that do not lie on the very edge of the tumour are allowed to proliferate, but an algorithm was needed to ensure there was no discontinuous division. The algorithm allows for expansive growth via intracellular mechanical stress (IMS). Non-tumour cells are treated as empty cells and can be "filled" by a cancer cell dividing into their space. They are then pushed into the surrounding area and are not considered. Only if a cell can divide into a space where nutrients are still available can it divide.

The four main properties being monitored are R_t, δ_p, δ_n and p_d (the probability of division). In order to find these as functions of time the simulation utilizes four microscopic parameters: p_0, a, b and R_{max}. These parameters effect cell-doubling time, the nutritional needs of growth-arrested cells, the nutritional needs of dividing cells, and the effects of confinement pressure, respectively. The algorithm Kansal *et al.* used to calculate the quantities is as follows:

- Initial Setup: The cells at the center of the grid are set to proliferative, all others are non-tumour cells.
- At each time step:
  - Each cell is checked for type
  - Non-tumours cells and necrotic cells do nothing
  - Non-proliferative cells that are now more than δ_n from the tumour's edge become necrotic. The edge of the tumour is taken to be the nearest non-tumour cell as calculated by:

$$\delta_n = aR_t^{2/3}, \tag{1.5}$$

where a is the base necrotic thickness measured in units $(length)^{1/3}$.

المنارة للاستشارات
www.manaraa.com

Table 1.1: Summary of time-dependent functions and input parameters

	Functions within the model (time dependent)	
R_t	Average overall tumour radius	$R_t = \frac{\sum_{i=1}^{N_p} r_i}{N_p}$
δ_p	Proliferative rim thickness	$\delta_p = bR_t^{2/3}$
δ_n	Non-proliferative rim thickness	$\delta_n = aR_t^{2/3}$
p_d	Probability of division	$p_d = p_0(1 - \frac{r}{R_{max}})$
	Parameters (constant inputs to the model)	
p_0	Base probability of division	$p_0 = 0.192$
a	Base necrotic thickness	$a = 0.42mm^{1/3}$
b	Base proliferative thickness	$b = 0.11mm^{1/3}$
R_{max}	Maximum tumour extent	$R_{max} = 37.4mm$

  - Proliferative cells are checked for division. If they will divide is a random process, though the probability of division, p_d, is dependent on the location of the cell (r). The probability of division is calculated as:

$$p_d = p_0(1 - \frac{r}{R_{max}}) \tag{1.6}$$

    where R_{max} is the maximum extent of the tumour.

  - If a cell attempts to divide it will try to find an empty space within the proliferative range (where nutrients are available), which is calculated by:

$$\delta_p = bR_t^{2/3} \tag{1.7}$$

    where b is the base proliferative thickness again in units $(length)^{2/3}$.

  - If the cell cannot divide it becomes a non-proliferative cell.

- After a certain amount of the time, volume and radius are plotted as function of time.

Please see Table 1.1 for all of the time-dependent functions used by the algorithm, as well as the real values used in the simulations.

1.2.3 Results

The simulations were run according to the above algorithm and compared to experimental data for untreated GBMs on the basis of cell number, growth fraction, necrotic fraction and volumetric doubling time. The parameters a, b, R_{max} and P_0 were chosen to fit the test case.

المنارة للاستشارات
www.manaraa.com

The data from the simulation matched experimental data quite closely with a 25 mm sized tumour in both the simulation data and real data at time of death. The growth fraction data showed 9% and the simulation showed 11% and volume doubling time for the simulation was 100 days and 105 days for the real life data. The simulation was very accurate for tumours that were essentially spherical. This does not illustrate the more realistic and complex case of multiple distinct tumour clones. In order to simulate this case, second strain parameters were defined to be:

$$p_0 = 0.384, a = 0.42mm^{1/3}, b = 0.11mm^{1/3}, R_{max} = 37.55mm \quad (1.8)$$

This second strain has a faster doubling time, roughly 1.7 days as opposed to 4 days in the previous strain. A mutation in the tumour is chosen as a randomly selected cellular automaton cell (representing roughly 10^5 real cells) and is given the parameters above. This second genotype represents approximately 0.01% of all the cells. Gradually the second strain takes over the tumour.

Preliminary results show that this model does fit both experimental and clinical data for brain tumours. It also shows that for mutational strains to be dominate they need to have a large competitive advantage, such as the quick volume doubling time shown here.

1.2.4 Conclusions

This model incorporates four parameters and predicts the composition and dynamics of malignant brain tumours. The results from the simulation match experimental and clinical data for very idealized tumours. This model incorporates the Voronoi tessellation to create a cellular automaton model and was the first model to do so. The ability for cells that are not on the tumour border (other cells in the proliferative rim) to divide was new to modelling at the time and allowed this model to be much more biologically accurate. Also the inclusion of heterogeneity, by way of different parameters for different cells, was new to this model. The group modelled very few different parameters but had relatively close to life results. There is a balance needed between a high level of abstraction that still generates a relatively accurate model, and a complex model which could be less accurate and has severely increased computation time and space demands. This model convinced me that a simple model of cancer growth was capable of achieving complex results – even biologically relevant results. It gave me hope that it would be possible to create a cancer simulation during the course of a PhD that while simple, could still contribute to the field in a meaningful way, and provided me with the basic modelling approach to do that – using cellular automata.

المنارة للاستشارات
www.manaraa.com

1.3 An evolutionary hybrid cellular automaton model of solid tumour growth

1.3.1 Introduction

This paper by P. Gerlee and A. R. A. Anderson was published in February 2007 and describes a cellular automaton model for modelling the prevascular stage of a tumour [66]. The aim of the work is to model this early tumour growth where growth is limited by the diffusion of nutrients. The main goal of the model is for it to include the evolution of subclones within the tumour. In order to effectively do this, this novel cellular automaton model equips each cell with a "genotype" that can be mutated as well as passed on to the daughter cells at each generation. In the model, this genotype controls the behaviour of the cell. The authors believed they had created a model that is simple enough to be computed in a reasonable amount of time but that is also complex enough to provide information on the dynamics of clonal evolution.

Each cell is equipped with a feed-forward artificial neural network that helps it decide what to do at each stage in the model. This network takes environmental variables as input, processes it and produces an output telling the cell whether it should grow, divide, die or take part in other events. These networks are governed by connection weights and thresholds which can be mutated to allow new cells to have different genotypes.

The microenvironment has been shown to play a big role in tumour development, influencing both morphology and phenotype. Early selective pressure due to limited nutrients could impact the later development and invasiveness of a tumour. Tumour hypoxia (caused by lack of oxygen in the microenvironment) has been shown to be directly linked to tumour morphology as well as cancer cell aggressiveness. There is a known pathological link between tumour morphology and invasive potential. The aim of this model is to allow for clonal evolution in tumours and then to see the impact of hypoxia and the microenvironment on the morphology and genetic make up of cancer cells during avascular growth.

1.3.2 The model

This CA model represents a cross section of the tumour and is represented on a two-dimensional grid where each automaton cell is approximately the same size as a real cell. This is a vast improvement on Kansal *et al.* (2000) where each automata cell represented approximately 100 up to 10^6 real cells and shows how far computational power had come in seven years. Each

المنارة للاستشارات
www.manaraa.com

automaton cell can be empty or filled with a cancer cell. Non cancerous cells are not modelled in order to keep the model more simple and to focus on the effects of oxygen, however it has been shown that the interactions between cancer cells and host tissue are very important. This model also excludes direct interaction between the tumour and blood supply as it is to be modelling avascular growth. All oxygen is supplied by the surrounding tissue, which is more biologically accurate for avascular growth. In the model each cancer cell is an individual agent. Each cell decides what its phenotype will be based on the surroundings and its genotype, therefore each cell is not just characterized by if it is a cancer cell or not, but where it is located, the microenvironment and its genotype – again an improvement on other models.

The cell

Each cell is a computing unit that takes as input the microenvironmental factors, processes them (determined by the "genotype" of the cell), and then develops a phenotype as the output. Each cell has an artificial feed-forward network that takes the input, processes it and produces the output, and the actions of each cell are based on this network, the microenvironment and the interactions between the two. Each cell will have an individual response to stimuli in the environment. This way, the fitness of a cell is not defined by only what type of cell it is, but where it is located and the network it is equipped with. For example, cells with the same genotype (network) but in different areas of the tumour might have different fitness levels. At the same time, two cells beside each other with different genotypes can have very different fitness levels as one might have a mutation that allows it to survive better in its environment.

The network

The neural network each cell contains is designed to be an abstract representation of cellular behaviour and cellular pathways. There is an input layer that takes real numbers (measurements from the environment), a hidden layer and an output layer that determines the action of the cell. These layers are connected by matrices that can have different weights, representing the signalling strength of the "receptors" (the input nodes). The hidden layer represents regulatory genes, it processes the information then sends this to the output layer, which is thought of as the phenotype of the cell. This system, as a whole, represents a mapping from the genes to the behaviour of a cell, and as an extension, altering the weights can be thought of as changing the level of expression of one of these genes or the strength of a receptor.

There are four input nodes that take the value for the number of neighbours, the oxygen concentration, the glucose concentration and the hydrogen ion concentration. The input vec-

المنارة للاستشارات
www.manaraa.com

Table 1.2: Input and output nodes.

Node	Function	Variable
ζ_1	No. of neighbours	$n(\vec{x}, t)$
ζ_2	Oxygen concentration	$c(\vec{x}, t)$
ζ_3	Glucose concentration	$g(\vec{x}, t)$
ζ_4	Acidity $[H^+]$	$h(\vec{x}, t)$
O_1	Proliferation	P
O_2	Quiescence	Q
O_3	Apoptosis	A
O_4	Metabolism	M
O_5	Movement	Mov

tor is $\zeta = (n(\vec{x}, t), c(\vec{x}, t), g(\vec{x}, t), h(\vec{x}, t))$ where $n(\vec{x}, t)$ is the number of neighbours, $c(\vec{x}, t)$ is the oxygen concentration, $g(\vec{x}, t)$ is the glucose concentration and $h(\vec{x}, t)$ is the hydrogen ion concentration. There are also five output nodes. These are the phenotypes for the cell and are: proliferation, quiescence, apoptosis, metabolism and movement. The behaviour/phenotype for the cell with the strongest response is chosen. Please see Table 1.2 for an overview of the nodes, their function and the variable representing them.

Cell metabolism

The metabolic rate of cancer cells is often higher than normal cells, and frequently cancer cells rely on anaerobic metabolism instead of the more traditional aerobic metabolism [48]. The anaerobic pathway is 18 times less efficient at producing ATP, and since hydrogen ions are a by-product of ATP production, in order for an anaerobic cell to produce the same amount of ATP, it must also produce much more hydrogen [173], [175], [6]. This causes the cell's environment to be more acidic and this may be why cancer cells prefer anaerobic metabolism – the increased hydrogen ions can kill normal cells.

In order to model cells utilizing different pathways, the anaerobic cells are allowed to consume 18 times the glucose of normal cells and not consume any oxygen. The magnitude of the network output is then assumed to be proportional to the cell metabolism (higher output, higher metabolic rate). In order to model this there must be a response that corresponds to normal metabolism and a function relating this to the network. The authors have created a "target response" T_r and a modulation function $F = F(R)$, which depends on the response R of the network. To give the metabolism of each cell, F is multiplied by the base consumption/production rates, and the response R is set to the highest value for the lifecycle nodes. The

المنارة للاستشارات
www.manaraa.com

modulation function is:

$$F = max((k, R - T_r) + 1, 0.25), \tag{1.9}$$

where k determines the strength of the modulation and the use of $max(\cdot, 0.25)$ makes sure the cell has a minimal metabolism (no less than a quarter of the initial one).

Quiescent cells consume less oxygen than active cells, so their consumption is divided by some factor q which is not very well determined yet.

Cell death

Cells can die in two ways: 1) if a cell tries to consume more oxygen or glucose than is available, it dies of starvation; 2) if the apoptosis node in the network gets the strongest response, the cell will die.

Cell movement

There is a value a_i, which is the internal adhesion value and represents the number of neighbours a cell prefers to adhere to. If the number of actual neighbours is higher than this value ($n(\vec{x}, t) \geq a_i$) and the movement node gets the highest response, the cell can move.

Proliferation

If the proliferation node gets the strongest response out of the life cycle nodes, the cell can divide if two conditions are met. The first is that there must be an open space for the daughter cell to go to in one of the four adjacent CA spaces. If not the cell fails to divide and becomes quiescent. The second is that the cell must be of proliferation age. Each time a new cell is created it is randomly assigned a proliferation age between $(A_p, A_p/2)$, where A_p is the base proliferation age. At each time step, an internal counter X_p is incremented by F (see above) until X_p A_p. If both of these conditions are satisfied the cell can divide.

Mutations

The number of mutations that will occur is chosen from a Poisson distribution with parameter p and these are then equally distributed over the matrices and threshold vectors. The mutations alter values inside the matrix and threshold vectors, which alters connection strength between the nodes, simulating up or down regulation of receptors or genes in the cell.

المنارة للاستشارات
www.manaraa.com

Chemical fields

In this model the researchers have chosen to focus on glucose, oxygen and hydrogen ions for the chemical modelling. To make it simpler, they are not looking at the decay of these but rather are looking at the production of oxygen and glucose in the boundary conditions by applying Dirichlet boundary conditions with constant functions. This is to model the situation where there is a tumour surrounded by blood vessels that allow for diffusion of glucose and oxygen and take away the hydrogen ions. The simplified equations for the time evolution of oxygen, glucose and hydrogen ions are:

$$\frac{\partial c(\vec{x},t)}{\partial t} = D_c \Delta c(\vec{x},t) - f_c(\vec{x},t), \tag{1.10}$$

$$\frac{\partial g(\vec{x},t)}{\partial t} = D_g \Delta g(\vec{x},t) - f_g(\vec{x},t), \tag{1.11}$$

$$\frac{\partial h(\vec{x},t)}{\partial t} = D_h \Delta h(\vec{x},t) - f_h(\vec{x},t), \tag{1.12}$$

where D_i are the diffusion constants and the $f_i(\vec{x},t)$ give the individual cell consumption or production of the chemical $i = c, g, h$ for the cell at position $\vec{x}$ at time t. The concentrations are solved on a grid, and these concentrations are defined for each individual cell. The $f_i(\vec{x},t)$ are defined as:

$$f_i(\vec{x},t) = \begin{cases} 0 & \text{if there is no tumour cell at that point} \\ r_i F(\vec{x}) & \text{if there is a tumour cell at that point} \end{cases} \tag{1.13}$$

where r_i are the base consumption/production rates and $F(\vec{x})$ is the modulated energy consumption of the individual cell occupying the automaton element at $\vec{x}$ described earlier.

Cellular automaton

The grid for this model is an NxN grid with a grid constant d that represents the spacing between cells. The grid points are labelled by a coordinate, $\vec{x} = d(i, j), i, j = 0, 1, ..., N-1$. The partial differential equations mentioned above are discretized using standard five-points finite central difference formulas with space step d and time step δt. The chemical concentrations are solved at each time step and all the cells are updated.

At each time step for each cell (each is updated in a random order):

- the input vector is calculated from the environment

المنارة للاستشارات
www.manaraa.com

- response is calculated from the network
- the cell consumes nutrients as is required
- cell carries out end point decision (proliferation, apoptosis, quiescence)

If a cell dies via apoptosis, the grid space where the cell was becomes empty. However, if a cell becomes necrotic, the grid space remains filled by the cell.

Parameters

The parameters can be split up into three main areas in the model: cell behaviour, tissue structure and modelling parameters. One of the most important is the original cell structure because these make up the seed cells. The features the group incorporates into the cells to make them more closely resemble cancer cells are:

- cells perform apoptosis if the oxygen concentration falls below a certain threshold
- cells die if the glucose concentration falls below a certain threshold
- the cell will not divide if there is no space for the daughter
- cells perform apoptosis if the acidity is above a certain threshold
- cells switch to anaerobic metabolism if the oxygen concentration falls below a certain threshold
- cells are allowed to move if the number of neighbours exceeds the internal adhesion value

The real values for these thresholds can be found in the paper. After non-dimensionalising the equations and setting all the parameter values from various references, the group made the grid of size $N = 400$ which allows simulation of a tumour with radius 200 cells, which in three dimensions would be a tumour approximately $8x10^6$ cells.

1.3.3 Results and discussion

Due to the complexity of the model, the group decided to only investigate the dynamics of a subsystem of the model consisting of the effects of oxygen. Using this “minimal” system they hoped to see the effects of background oxygen concentration on the system.

المنارة للاستشارات
www.manaraa.com

For the lowest oxygen concentration they tested (normal levels divided by 10), cell death emerged early as diffusion could not get oxygen to the centre of the tumour. Only cells on the boundary were able to get oxygen leading to a fingering morphology. Cells had to compete with neighbours for oxygen which lead to this morphology where the proliferating cells were on the tips of the fingers and all other cells were necrotic or quiescent. This growth is like a race among the cells for oxygen.

With an increase in oxygen concentration (normal levels divided by 2.5), a slightly fingered morphology occurred. The tumour grew larger before the centre became hypoxic, after which point the growth was dominated by a proliferating rim on the outside of the tumour. For normal oxygen levels, hypoxia occurs late and the tumour grows as a mostly circular tumour with a small proliferating rim.

In all simulations the tumour begins as mostly quiescent cells and a thin rim of proliferating cells. This breaks down as oxygen cannot get to the centre of the tumour and cells begin to die. If there is enough oxygen, the cells leave a homogeneous wave of dead cells behind them as the proliferating edge grows. With less oxygen, this is not possible, as the proliferating cells begin to compete for resources. If a cell divides too slowly it may get trapped in the tumour where it will not have access to oxygen. Also, the tips of the fingers represent the surviving cells that have the most exposure to oxygen, but since their competition are the other living cells on other finger tips, these cells differentiate and the tumour is filled with many dominate subclones. When there is more oxygen, living cells are near each other and compete, which gives rise to only a few dominate subclones as they take over the surrounding cells. With the fingered morphology, there can be a different clone for each finger.

Cells in low oxygen environments will sacrifice normal consumption for a low proliferating age (the two are linked through the response modulation). Proliferation age is under stronger selective pressure, and although a high consumption increases the possibility of death by starvation, this appears to be less important than the advantage of a faster growing cell due to a younger proliferating age.

Overall, the results match those of other models and experiments which have showed that low oxygen concentrations created more fingered morphology and more aggressive populations [19], [18], [130]. The hypoxic environment creates selective pressure and the cells with slight growth advantages due to mutations tend to create dominate subclones. Low oxygen concentration also gives rise to phenotypes with a smaller apoptotic potential, in that on aver-

المنارة للاستشارات
www.manaraa.com

age cells in low oxygen concentrations tend to be less likely to die from apoptosis. Avoiding apoptosis is a hallmark of cancer cells [74].

1.3.4 Conclusions

This paper demonstrated that the base level of oxygen affects both the growth dynamics and the evolutionary dynamics of the tumour. When there is low oxygen levels a fingered morphology emerges, containing phenotypes that are very far evolved from the original tumour cells. These are also more aggressive compared to the tumour in normoxic conditions which grows with a round morphology. The main points are:

- lower oxygen concentrations cause fingered morphology
- fingered morphology tumours have more aggressive cells as they compete for oxygen at the tips of the fingers
- lower oxygen concentration also results in more diverse subpopulations because cell clones on different fingers cannot compete directly with each other
- there is higher heterogeneity in populations in harsh growth conditions
- harsh environments give rise to a lower variant in tumour size
- evolution in harsh environments is more directed towards aggressive phenotypes as opposed to just larger cells

From this, one can infer that cutting off the blood supply to a tumour could be very detrimental to the patient. While the growth of the tumour might be slowed, the cells that do remain are likely to be very powerful cells. They will be strong enough to survive in hypoxic conditions and possibly will be embedded in normal tissue if very thin fingers have grown.

Shortly after this paper was published, Gerlee and Anderson published a second paper, *A hybrid cellular automaton model of clonal evolution in cancer: The emergence of the glycolytic phenotype* [127]. This second paper shows a model that is designed to determine the microenvironmental conditions that lead to a glycolytic tumour. It is a continuation of the model presented in their earlier work, however it uses the full version of the model unlike the previous paper. It also includes information on the extracellular matrix which is new to the model.

المنارة للاستشارات
www.manaraa.com

Some cancer cells exhibit an anaerobic phenotype while others have a more typical aerobic phenotype. During anaerobic metabolism, oxygen is not used to convert glucose to ATP, but rather glucose is converted into less ATP with a by-product of lactic acid created. The anaerobic phenotype is much less efficient and the excess creation of acid can be detrimental to cells. However, certain cancer cells utilize this anaerobic metabolism and seem to thrive better because of it. This paper tries to determine under which conditions the glycolytic phenotype emerges and is successful. Using a model that is on the level of a single cell, a feed-forward neural network in each cell allows each individual cell to interact with the microenvironment and grow accordingly. This continuation from the paper above utilizes the full abilities of the previous model and is extended to include the interaction with the extracellular matrix (ECM). The model focuses on tissue oxygen concentration and extracellular matrix density, varying these two parameters to see what combination creates the glycolytic phenotype.

It was found that low oxygen concentrations create tumours with a branched, fingered morphology. A dense matrix was found to create less fingered, more compact tumours. However, when both conditions were present in the environment (low oxygen concentration and dense matrix), the tumour was found to switch to the glycolytic phenotype. Therefore, the glycolytic phenotype emerges from a complex interaction between cancer cells, the microenvironment and the surrounding tissue.

These papers together provided me with a skeleton of how I could create my cellular automata model. The provided implementation details that were helpful in creating my own model, especially the lifecycle they provided. They also showed the importance of modelling oxygen in the system, which later led me to pair my cellular automata model with a lattice Boltzmann model of fluid flow. Lastly the authors provided a standard to shoot for, as they have continued to model in this area and regularly produce interesting papers that look at a variety of issues surrounding cancer growth.

المنارة للاستشارات

www.manaraa.com

1.4 Simulating the hallmarks of cancer

1.4.1 Introduction

This paper [1] is one of the earliest papers to use cellular automata to model cancer hallmarks, specifically coding the automata rules by using the concepts from the Hanahan and Weinberg paper, the Hallmarks of Cancer [74], [75]. It was written by Robert Abbott, Stephanie Forrest and Kenneth Pienta, published in the journal Artificial Life in 2006 and looks at the "dynamics and interactions" of the Hanahan and Weinberg hallmarks.

Abbott, Forrest and Pienta created a computer simulation of cancer growth titled CancerSim, which looks at the interactions of cell phenotypes. They were especially interested in finding the "pathways to cancer" - the order that hallmark mutations are sustained during the progression from healthy cell to cancerous cell. They found that some mutations have preconditions – other mutations that must occur before or concurrently – in order to be advantageous to the tumour. They also found that the order in which mutations occurred in their model was different than those pathways proposed by Hanahan and Weinberg as well as those predicted in an ordinary differential equation model of tumour growth.

The authors point out that progress on "the war on cancer" has been generally slow. They also note that research in cancer is often very specific, looking at individual cellular pathways or components, which means success in these research areas often only impacts a small, specific group or type of cancer. Hanahan and Weinberg on the other hand present a more general view of this group of diseases, distilling all cancers down to a set of six phenotypic changes they refer to as the hallmarks of cancer.

This general set of hallmarks allows for a more unified understanding of cancer progression, however the authors realize the need to not just understand individual hallmarks but also their dynamics in a cell or group of cells. They state that the effect of a mutation is often contextual in that it depends on nearby cells, the environment and the patient. This creates a disease with many different populations (different both genetically and phenotypically) competing to survive.

Knowing cancerous tumours have a heterogenous makeup of cells, the authors were interested in how this heterogeneity came to be and what pathways cells took to get there. Specifically, they were interested in:

المنارة للاستشارات
www.manaraa.com

- the relative frequency of different mutational pathways
- how long different pathways took to emerge
- the dependence of pathways on various parameters

They ultimately wanted to determine how much of cancer could be accounted for by the hallmarks of Hanahan and Weinberg. They hypothesized that if a model of individual cell behaviour, as described by the hallmarks, could not give rise to what is seen *in vivo* in a cancerous tumour than either the hallmarks are incomplete or the model is incorrectly implemented. If, however, the hallmarks and individual cell behavior could lead to whole tumour behavior consistent with reality then other phenomena arising in the model could generate hypotheses about cancer in a more general sense than often occurs in traditional cancer research.

1.4.2 Methods

Overview

CancerSim is an abstract model of cancer growth on the cellular level. The model is contained on a 3D Cartesian array which represents a tissue volume of 0.1 mm^3 growing over a time frame of approximately 60 years. A variety of parameters (many mentioned below) are changeable and cells differentiate over time by random mutation. Below, the methods used for modelling the hallmarks, implementing the cells and implementing the lifecycle are briefly described.

Hallmarks

Hanahan and Weinberg initially proposed six hallmarks: self-sufficiency in growth signals, ignorance of anti-growth signals, invasion and metastasis, replicative immortality, induction of angiogenesis and resistance of cell death [74]. Later, in 2011, they added two new “enabling characteristics”: genome instability and mutation, and tumour-promoting inflammation. They also added two emerging hallmarks: deregulating cellular energetics and avoiding immune destruction [75]. For a complete overview of all these hallmarks and characteristics, please see the exposition in Section 2.2. Here I will only explain those hallmarks modelled in CancerSim.

The first hallmark described is a cancer cell’s ability to grow regardless of growth signals – signals needed by healthy cells to initiate mitosis or cell division. To model this ability, healthy cells can only undergo mitosis while they are within a predefined spatial boundary. The authors think of this as the area where growth factor concentration is high enough to sustain growth.

للاستشارات المنارة
www.manaraa.com

Cells with the SUSTAINED GROWTH hallmarks are able to divide even when outside this boundary.

When cancer grows, the local blood supply is often not set up to handle the extra cells and once the early tumour reaches 1 - 2 mm in size it begins to die from lack of oxygen and nutrients [60]. Normal healthy cells cannot induce the creation of new vasculature (called angiogenesis), however this death process in a small tumour can activate angiogenesis and cause new vasculature to grow towards the tumour, infusing it with fresh oxygen and nutrients. This ability of cancer cells to induce angiogenesis is modelled similarly to the SELF GROWTH hallmark in CancerSim. Cells within a certain boundary are seen to have enough nutrients, while those outside of it do not. Those without enough but with the SUSTAINED ANGIOGENESIS hallmark activated can "induce angiogenesis" which just means (in the model) that those cells now do have enough nutrient even when outside the predefined nutrient boundary.

Along with needing signals to divide, healthy cells also receive signals to stop growing, allowing them to enter a mature, post-mitotic stage. One of the ways a cell receives this signal is through contact inhibition, which turns off growth for a cell when it is surrounded by many others. Cancer cells, in contrast, ignore this contact inhibition and will continue to grow, potentially causing overcrowding and overgrowth. In CancerSim, a cell on the grid has 26 neighbours (needing only to touch by at minimum a corner to be considered a neighbour). If all 26 neighbour slots are filled with a cell, the center cell will stop growing due to this "contact inhibition". Cells with the IGNORE GROWTH INHIBITION hallmark will attempt to grow anyways, entering into a battle for survival with one of its neighbours, winning it with some probability of success that is a tuneable parameter.

Healthy cells also have a mechanism for determining cell damage and if the damage is too great they can induce apoptosis (programming cell death to prevent the damage from being passed on to daughter cells). Cancer cells also manage to avoid this check, often dividing with many mutations. To simulate this, all cells about to undergo mitosis in CancerSim are checked for genetic damage. Damage is detected with a rate of n/e where n is the number of mutations the cell has sustained and e is a tunable parameter. Cells without the AVOID APOPTOSIS hallmark are killed if damage is detected, while cells with the hallmark completely avoid this mechanism. The n/e probability means that death by apoptosis is more common in cells with more genetic damage (mutations).

Yet another barrier to cancer is a cell's limited replicative potential. Even in the presence of growth factor, a cell cannot grow indefinitely. Cells have a limited number of replications

المنارة للاستشارات
www.manaraa.com

(approximately 50 - 70 in humans and differing in other organisms) that, once reached, stops the cell from replicating further. The mechanism controlling this is the telomere – a short bit of DNA on the ends of chromosomes that shortens with each division, eventually becoming too short to allow for division [74]. Cancer cells can extend their telomeres to effectively become immortal [74]. This is modelled by a cell variable that tracks telomere length / number of divisions. Each division decreases this number by one, and when it reaches zero the cell is no longer allowed to divide. If the cell has acquired a mutation activating the LIMITLESS REPLICATIVE potential hallmark, the simulator effectively ignores this greater than zero requirement.

While not identified as a hallmark, Hanahan and Weinberg have classified genetic instability as an "enabling characteristic" of cancer. The authors decided to include this attribute of cancer in their model. It is known that tumours are made up of a variety of cell phenotypes with all different mutations and it appears genetic instability allows for acquiring of changes that can be beneficial and confer a selective advantage. This means genetic instability can assist in generating this heterogeneous group, although the exact mechanisms of how are relatively unknown. In the model, cells that are "genetically unstable" are said to have a "mutator phenotype" and gain more mutations with increased likelihood.

The model

CancerSim is an agent based (a type of model which simulates the actions of autonomous agents), three-dimensional highly abstract model of very early cancer growth implemented in C++. All cubes in the 3D space can either be empty or filled with a cell. Along with the cancer cells, CancerSim also models vasculature, which can pass through any 3D cube, whether it has a cell or not. Both the vasculature and the cancer cells grow according to internal rules, in a roughly cellular automata fashion.

At the time of publication the authors were able to feasibly model roughly 1,000,000 cells, which corresponded to approximately 0.1 mm^3. While most tumours are not recognized by traditional methods until they are closer to 1 cm^3, it was not computationally feasible to model that many cells.

CancerSim starts with 1 single cell in the center of the simulation. At each cycle through the simulation the cell can either die via apoptosis, replicate or signal angiogenesis. The simulation ends when any one of three conditions are met:

- All cells die from old age

المنارة للاستشارات
www.manaraa.com

- 90% of the population is cancerous
- Some predefined number of steps has passed (the simulation could run forever if cells become immortal but healthy cells continue to grow as well, preventing cancer from completely taking over)

In order to model many cells in a reasonable amount of time, the authors chose to make each cell a highly simplified version of the biological cell, where the "genotype" is a boolean vector where each bit represents a phenotypic hallmark which is either on or off for that cell. Some state info, such as the length of the telomeres or the nutrient or oxygen around the cell, are stored in the cell along with this genotype vector. During the simulation, each cell goes through a rough version of the biological cell cycle. The cell enters G1 phase (a time of cell division when the cell grows and creates building blocks needed for DNA replication) if there is space around it for it to divide into. If the cell is successful in dividing, the daughter cell has all of the same hallmarks, unless a mutation occurs. Mutations occur with some parameter probability. At the G2 check phase (a point in the cell cycle when the cell makes sure it is ready to divide) apoptosis could occur, again based on some probability set as a parameter. Once division is over, the cells occupy the original space and one new neighbour space, and both are in the M phase of the cycle.

The authors included a highly simplified but quite elegant model of the vasculature surrounding tumour growth. Initially the simulation has 1 cell in the center, and that simulation cube also has a capillary. As cells grow, they get oxygen and nutrient from nearby vasculature and it diffuses towards the cell declining as it gets farther away according to the power law. When a cell has too little oxygen or nutrient it can signal for angiogenesis. This causes a new capillary to be added in a cell adjacent to the nearest existing capillary. This simple growth rule always creates a continuous vasculature with branches. While branches can occur anywhere, which is not the case *in vivo*, it creates fractal structures that look very similar to those seen in nature. Cells can only initiate this process when inside the predefined vasculature boundary, unless they have the angiogenesis hallmark activated.

To update the cells, instead of doing so with each time step (which would be computationally expensive when the vast majority of cells would not qualify for an update at every time step), CancerSim uses an event queue. Each cell has a future event scheduled during the current event for some time point in the future. At every iteration of the simulation the nearest event in time is processed.

المنارة للاستشارات
www.manaraa.com

A variety of parameters are required to run a model such as this. However, since the model presented here is so abstract, many parameters do not have a "real life" counterpart. This makes setting up the model challenging. All hallmarks had the same probability of getting mutated, however there were parameters associated with most hallmarks that could vary. With the wrong parameter a hallmark could basically become useless, but could also become extremely powerful, almost ruling out the other hallmarks. In order to find the best possible set of parameters, the authors did an 8-dimension parameter sweep 3 times with 1458 different parameter settings (a total of 4374 runs). This resulted in a set of parameters they published for future modelers.

Lastly, the authors did supply a rough visualization tool for the model. It represents the tumour in 2D but is moveable, showing each cell as a square. Since squares pack together completely one can only see the exterior surface of the tumour, which is useful for rendering speed. However, if desired, a user can select to see inside the tumour.

1.4.3 Results

Initially the authors ran 100 simulations with the set of parameters they discovered in their parameter sweep, only varying the random number generator seed. They were interested in what order the mutations appeared – the order often being referred to as the "pathway to cancer". Hanahan and Weinberg stated that "virtually all cancers must acquire the same six hallmark capabilities" which the Abbott *et al.* model does, however the pathway to this end state could be varied. In fact, even if every cell acquired all 6 mutations, there would still be 720 different possible pathways. The authors were interested in how the cancer arose and what combinations of hallmarks were dominant at the end of growth (a dominant pathway is one shared by 50% or more cells in the tissue at the end of simulation).

The authors noticed many interesting things about the pathways. By varying parameters they noticed dominant pathways would arise with different probabilities based on the initial base mutation rate. A low mutation rate allowed for more ecological dynamics. If the mutation rate was low, the initial, or close to initial, mutation would dominate as long as it was relevant. Once it was no longer as advantageous, it would die off and a new mutation that conferred more advantages at that particular growth phase would begin to take over. High mutations rates instead made it so a single mutation had less of an overall impact, and different mutations could arise more easily.

المنارة للاستشارات
www.manaraa.com

Since low (and more biologically likely) mutation rates seemed to create a tumour with a dominant pathway, the next question was whether a particular pathway repeatedly dominated. In one set of 100 runs, 96 of the runs finished with cancer and 90 of those had a dominant pathway. More interesting than that is the fact that all but one of those was formed from some permutation of the same 4 hallmarks, and only 7 different dominant pathways existed at all! In fact, one particular pathway dominated in 48% of the cancerous runs. Another interesting result that was sustained angiogenesis hallmark was not present in any of the dominant pathways. Many angiogenic cells were produced, however after they assisted in getting new vasculature set up they were not particularly advantageous and did not become the dominant group. Another hallmark not very common was genetic instability, which in CancerSim is only weakly selective. It does not convey any advantageous ability except the ability to potentially get more hallmarks, so it does not assist well early on.

Conversely, evade apoptosis was in every dominant pathway. It never occurred first but became advantageous early on when cells had accumulated damage and would be susceptible to apoptosis. Limitless replication usually occurred first. This surprised the authors as they thought an initial telomere length of 100 would mean cells would not be under much pressure to avoid becoming senescent. However the authors hypothesized that since cells with mutations have a high turnover as they are being targeted by apoptosis, then having the opportunity to replicate many times could be advantageous.

Lastly, the ignore growth inhibition mutation was near the end of the pathway, as it becomes advantageous once other cells have taken up most space.

In addition to the aggregate data from multiple runs, the authors also describe one particular run as an example of how mutations come and go early on before they are advantageous, and then how certain mutations eventually take over the growth. This is an enlightening walk through a possible progression of cancer and interested readers are pointed to the paper to read more [1].

1.4.4 Discussion

The authors point out that the pathways to cancer they discovered were different than those Hanahan and Weinberg proposed. Counter to CancerSim, Hanahan and Weinberg had insensitivity to anti-growth signals early on with limitless replication towards the end. The authors point out a few possible reasons for this discrepancy:

المنارة للاستشارات
www.manaraa.com

- The phenotypes may have been modelled incorrectly: the authors made many simplifications and assumptions that may have been incorrect

- The parameters could have been incorrect: the parameters have a large impact on how powerful each hallmark is, and if they were incorrectly chosen or not biologically relevant that could change the outcomes very much

- Hanahan and Weinberg's pathways could have been incorrect or insufficient: perhaps the six hallmarks are not enough to model cancer, or the pathways found were incorrect

The authors do point out that another model, an ODE model of cancer pathways [152], also found a different pathway and so perhaps modelling and implementation details are not the problem (as these were implemented very differently).

For future work, the authors suggested perhaps adding cancer stem cells to the model. It has been hypothesized that certain cancer cells have the ability to regenerate a whole tumour if it is almost completely destroyed, or if those cells migrate to a new location. If this is true, these cells would need to be destroyed to eradicate cancer. They also recommend breaking down the hallmarks into steps and modelling those individually, and adding a model of tissue invasion and metastasis.

The authors recognize that this is an early version of a highly abstract model, however they still found some interesting phenomena from studying it.

- Discrepancy between Hanahan and Weinberg's results and their own: There are many reasons this could be, pointed out above, however one possible reason seems to have been left out. Perhaps the differences are due to a relatively small number of runs. Only 100 runs were studied to find those pathways. Since there are more than 200 different kinds of cancer itself, all of which have different tumours with potentially different cell groups, it is conceivable certain cancers have certain dominate pathways, and others have different ones. It is possible both Hanahan and Weinberg and Abbott, Forrest and Pienta could have found a pathway to cancer. Perhaps factors like tissue location, age, environmental damage, etc, also impact the pathway, which could explain why different types of models and research result in different pathways.

- The role of LIMITLESS REPLICATION mutation: This appears early in their simulations as cells that have acquired mutations turn over quickly. They believe this signals a complication in cancer treatment. Obviously cancer cells need to be killed, but the act of killing

المنارة للاستشارات
www.manaraa.com

them might select for stronger cells. This has been seen *in vivo*. The childhood brain cancer, meduloblastoma, has an 80-90% survival rate when found early. It is treated with everything possible. If then the cancer ever recurs it has a nearly 100% fatality rate [23]. This is because any surviving cells that repopulate a tumour are most likely immune to the earlier forms of treatment, since they survived the first time.

1.4.5 Conclusions

This paper was an interesting first step in the area of modelling the hallmarks using an agent-based model. The large parameter sweep set the stage for future models (such as those by Santos *et al.*, described below [143]) and provided me with many needed parameters. The idea of looking at individual hallmarks and how they built up to the whole tumour using a model was quite inspiring to me, and I believe has great potential for learning more about how cancer operates in general. Since cancer is such a varied and complex disease, anything we can know that transcends location and type could be quite beneficial for large scale treatment, diagnosis or prevention. Abbott, Forrest and Pienta began a research trajectory that I hope to continue.

المنارة للاستشارات
www.manaraa.com

1.5 Study of cancer hallmarks relevance using a cellular automaton tumor growth model

1.5.1 Introduction

The last paper I will review in this introduction to the field is by José Santos and Ángel Monteagudo. It was published in 2012 in Lecture Notes in Computer Science, after it was presented in the conference Parallel Problem Solving in Nature [143]. The authors were motivated by both the previously mentioned paper by Abbott *et al.* [1] and the paper *Computational analysis of the influence of the microenvironment on carcinogenesis* by Basanta *et al.* [25]. The paper aims to identify the relative importance of each hallmark on overall cancer growth, and attempts to do so by simulating cancer growth with all hallmarks present, all but 1 hallmark present, and only 1 hallmark present at a time, with varying parameters. They use a 3D cubic lattice which is initially empty space, and begin with 1 healthy cell. Following closely the model shown by Abbott *et al.*, they allow for that single cell to begin the whole system, using an event queue to track mitotic events. The emergent behavior of the system is that of a cancerous tumour when the parameters are chosen in such a way to allow for cancer to grow. They use parameters similar to both Abbott and Basanta, and do a number of experiments on the growing system.

The authors remind us that Hanahan and Weinberg point out that while some hallmarks have helped identifiy potential therapeutic targets, they have not been entirely successful as cancer is adept at developing resistance to drugs which only target one property of cancer. They say that therefore, perhaps simultaneous targeting of more than one hallmark would be more beneficial [74], [75]. This lead Santos and Monteagudo to wish to identify which of the hallmarks are most critical to tumour growth. While the paper has no statistical proof of the significance of their results, I found the idea very interesting and this paper was the most influential on my ultimate choice of thesis question.

1.5.2 Methods

As mentioned above, the methods are closely related to those described previously from Abbott *et al.* [1]. Each cell in the model lives on a cubic lattice and has a "genome" that is a collection of hallmarks and cell specific parameters.

المنارة للاستشارات
www.manaraa.com

The hallmarks considered

Similar to Abbott *et al.*, Santos and Monteagudo did not model every one of the hallmarks introduced by Hanahan and Weinberg [74], nor each of the emerging hallmarks or enabling characteristics introduced in their updated paper, *The hallmarks of cancer: the next generation* [75]. They chose not to include hallmarks that are more relevant during later growth phases, such as mitosis and tissue invasion or tumour-induced angiogenesis. They also did not consider reprogramming of cell energetics, tumour promoting inflammation or evading immune destruction. The hallmarks incorporated into the cell's genome in this model include:

- Self Growth (SG): Cells with this hallmark are allowed to grow outside the predefined boundary of "growth factor", which is present in 85.7% of the total simulation growth space volume.
- Ignore Growth Inhibition (IGI): Typically, cell growth stops once the neighbouring space is full. Cells exert a pressure on neighbouring cells that causes them to stop growing, a way of signalling that the space is full and no more growth is needed. Cancer cells are often able to ignore this signalling and continue to grow, pushing other cells out of the way to make room.
- Evasion of Apoptosis (EA): Cells with this hallmark are able to completely avoid the cell mechanism of self death that is sometimes initiated in response to overwhelming cell damage.
- Effective Immortality (EI): The authors pose a limit on the number of times a healthy cell can divide in their model (ranging from 35 to 100). This represents some of the biological activity of telomeres, which shorten with every cell division and stop division once they reach a certain size. In this model, cells with the EI hallmark are able to continue to divide regardless of whether they have reached their "limit".
- Genetic Instability GI: Seen as a reason for the high number of mutations in cancer, genetic instability allows for accumulation of mutations (and possibly hallmarks) at an increased rate. Cells with this hallmark in the model have an increased base mutation rate, meaning they can accumulate hallmarks more quickly.

Event model

The driving force of the model is cell mitosis, which for each cell is kept track in one large event queue. The initial starting cell has a mitotic event scheduled for 5-10 time steps in the

www.manaraa.com

future (representing the different cell division lengths, which Santos and Monteagudo say can range from 15 - 24hrs) and after that each cell has an event scheduled and the model goes so long as events are currently scheduled. The cellular automata on the model can exist in 2 states: alive or dead. When they are alive, they can also be going through mitosis, but that is for a short period of time taken up during 1 event, not a state the cell remains in for any more than 1 moment. When an event is popped from the queue, there are a set of tests that take place:

- Random Cell Death Test: It is possible that any cell could die randomly, based on a given probability (provided in the paper and varied for different runs of the simulation)
- Genetic Damage Test: If the cell has sustained any damage, there is a chance for random death. This chance increases with the number of mutations. Cells with the EA hallmark will not be killed regardless of the outcome of this test
- Mitosis Tests: A collection of tests which all must be passed for a cell to divide
  - Growth Factor Test: The cell must either be within range of growth factor or have the SG hallmark activated
  - Ignore Growth Inhibition Test: The cell must either have empty space, or have the IGI hallmark activated
  - Replicative Potential Test: The cell must either have some “telomere” left (variable keeping track of number of remaining divisions must not be at zero) or have the EI hallmark activated
- If mitosis occurs: Mutations can possibly be sustained by the parent or daughter cells and the two cells both have mitotic events scheduled for the future and added to the queue
- If no mitosis occurs: The original cell has another mitotic event scheduled and added to the queue

It is important to note that Santos and Monteagudo have followed the rule of Abbott [1] and stipulated in their model that once a hallmark has been activated, no other mutations can inactivate it.

1.5.3 Results

The authors tracked total healthy cell and cancerous cells numbers, as well as the number of cells with each hallmark activated, over three different experiments: growth under different parameter values including high and low rates of mutation and the parameter set presented by

www.manaraa.com

Abbott *et al.* chosen to "facilitate the appearance of cancer cells" [1]; growth numbers when one hallmark is missing from the simulation; and growth numbers when only 1 hallmark is available for activation in the simulation. The numbers presented were all the average of 5 runs. The execution and results of each are described in more detail below.

Hallmark accumulation for different parameter sets

The authors used default parameters (provided in the paper) with a mutation rate of both 100 and 1000 (during any mitotic event, there was a $1/m$ chance of developing a mutation) and tracked the total number of healthy cells, cancerous cells, and cells with each type of hallmark activated. They found that with $m = 1000$, the cancerous cells did not "take off" and healthy cells dominated the growth. With m=100, cancerous cells quickly outnumbered healthy cells, and the evades apoptosis (EA) hallmark was most dominate in the group. The second most common hallmark was ignores growth inhibition (IGI). The authors believe with high mutation rates cells need the EA hallmark in order for any cancerous cells to survive, since they quickly accumulate enough hallmarks to greatly increase their chances of dying from apoptosis. Also, the authors think IGI becomes important as it allows growth to continue once the space is almost full. With the lower mutation rate, Self Growth (SG) was more dominant than either EA or IGI. This makes sense since with lower mutation rates, cells would be less likely to be killed by apoptosis and therefore not need to avoid it as much, and cells might be less aggressive and so happy to grow slower so long as they can continue to expand outward (an ability conferred by SG).

When the authors used the parameter set provided by Abbott *et al.* in [1], they found other hallmarks to dominate. The parameter set lowered the telomere length from 100, to 35, and changed m to be 100,000. This time in the simulation, cancer cells never dominated (up to time step 5000) however they did steadily increase in number. The most dominant hallmark was effective immortality (EI). Other hallmarks present in large number at time 5000 were EA and SG. The other hallmarks were present in such small amounts that they are not detectable on the graph presented. Due to the drastic decrease in max telomere length (100 to 35), it is not surprising that EI would dominate to such a large degree.

Next, the authors wanted to investigate the relative importance of each hallmark, and so performed the same growth experiment (growth to 1000 time steps for m=100) with each hallmark missing. They looked at the total number of cancer cells that managed to grow with the hallmark missing and infered importance based on the decrease in cancer cells from the baseline, that is, all hallmarks being available for activation. For m=100 (a high mutation rate),

المنارة للاستشارات
www.manaraa.com

they found the most critical hallmark to be EA. This again is not surprising since with a high mutation rate cells would be highly susceptible to apoptosis, so avoiding it would be highly advantageous. The next most important was IGI. The authors suggest that once time hits roughly 200 time steps space is mostly full, and so without IGI cells cannot continue to grow (except on the very outer limits, and only there until they run out of growth factor). A close third in relevance at this mutation level was genetic instability (GI). Without it cells would have fewer mutations over all and therefore less hallmarks. Since a cell needs at least 1 hallmark activated to be considered cancerous, it is not surprising that without GI there are less overall cancer cells.

Using the same set of parameters they investigated what happened when only 1 hallmark was available for activation. In this case they found that again EA and IGI were most relevant to the simulation. Now however GI is completely insignificant, as the only real benefit it confers is the ability to accumulate hallmarks faster and in this experiment only 1 hallmark could exist in the system at a time.

Lastly, they repeated the one-hallmark-missing and one-hallmark-present experiments with the parameter set given in Abbott's model [1]. Now that the mutation rate was much slower and the telomere length much shorter, the only hallmark that causes cancer growth when only one is included is EI. This effect is even more pronounced than when only one hallmark was present in the m=100 parameter set. This parameter set is chosen to help facilitate cancer, and yet the simulation has removed almost everything that defines cancer, so it is not surprising that the simulation fails to produce cancer in almost every instance when all but one hallmark is removed. With these parameters however the impact of removing any one hallmark seems to be fairly uniform. Aside from removing EI (which basically obliterates any cancer growth whatsoever), removing any hallmark has as small impact on the total number of cancer cells compared to any other. This again shows the huge power imbalance in the hallmarks due to the choice of parameters.

1.5.4 Conclusions

The authors set out to determine the relative importance of each hallmark included on overall cancer growth. It was shown that different parameter sets change the relative importance. They concluded by saying that "the simulations can help to analyze what are the most relevant hallmarks which can be targeted in each multicellular system simulation". While this slightly over states the contribution of the paper, a side effect of their parameter choice emphasizes an even

المنارة للاستشارات
www.manaraa.com

bigger contribution.

While the paper lacked any statistics (so one can't say if one hallmark is really more important statistically), it did show plainly that the choice of parameters had a huge impact on relative importance. It was not at all surprising that the only hallmark that made any impact on the system when telomere initial lengths were shortened to 35 was EI, since with telomeres that short, cells without it would quickly be stunted. A lot of the results in this paper could be artifacts of parameter selection. Aside from the over-dependence on EI, IGI also played a large role. This may have been for two reasons. One, the simulation was almost completely full of growth factor (85.7% of the space had growth factor), and so the relative roll of SELF GROWTH (SG) may have been artifically down played, making IGI look more important as the real fight for growth space was within the tumour. Secondly, in real systems oxygen availability has a big impact on growth. Often the centre of a tumour dies from a lack of oxygen and actively proliferating cells are only on the very rim where oxygen is available. This would greatly decrease the importance of IGI as cells on the boundary would automatically have space. It might also increase the importance of SG as cells might reach the boundary faster.

However, the fact that many of the results could be artifacts of parameters is a great result in and of itself! Since the authors are trying to model a generalize tumour, using the hallmarks of cancer which are common across almost all cancers, it is extremely challenging to pick specific parameters. They cannot use ones from any specific cancer without impacting the generalization of the model. This has a direct application in medicine. Different cancers from different patients, in different parts of the body and different tissues, may all have different "parameter sets". For example, the cells may have different division rates, mutation rates, division maximums, etc. Because of this their treatment may need to be altered, as we can see that different parameters expose a different "Achilles heel" in cancer growth.

While this paper had some shortcomings, it was an introduction to this idea and the authors have since expanded it to look at other properties of cancer growth including stem cell abilities [114], [115]. Of all the papers, it excited me most as I have always found the hallmarks of cancer to be fascinating and have wanted to create a broadly applicable model of cancer. I also noticed that Santos and Monteagudo said they wanted to find the relevance of hallmarks because Hanahan and Weinberg had noted cancer's ability to develop resistance to a particular treatment, and therefore multiple treatments should be used. I found it odd that the authors stopped at knocking out just one hallmark, and thought investigations were needed to see the impact of knocking out multiple hallmarks together. Using the hallmarks as part of the cellular

المنارة للاستشارات
www.manaraa.com

automata rules seemed brilliant, and excited me to create my own model based on the work of both Abbott *et al.* and Santos et al. which had more hallmarks, relevant oxygen, and an investigation of targeted combination knockouts.

المنارة للاستشارات
www.manaraa.com

1.6 Overall conclusions

These four works offer a brief but fairly representative look at the history of cancer modelling, from the perspective of the modelling approach I will be employing in this thesis. The initial paper, *Simulating brain tumour growth dynamics using a three-dimensional cellular automaton* by Kansal *et al.* shows the early promise of cancer modelling. Their model used only four varying parameters (cell-doubling time, nutritional needs of growth-arrested cells, nutritional needs of dividing cells, and the effects of confinement pressure) and was able to model a simple real life case, matching tumour doubling time and size of the tumour at death almost exactly. They were the first model to allow cells not on the boundary to divide which gave the model another element of realism previously missing. In addition to this, they allowed cells to have different parameters, modelling a heterogeneous tumour type, yet another unique feature of the Kansal *et al.* model. This model was basic in its chosen parameters but well thought out and the best for the time in its abstraction. It set the stage for many future models and solidified the theory that a simple, well abstracted model could get biologically relevant results.

Gerlee *et al.'s* work initially sparked my interest in this area, actually putting me on the path for this thesis. They set out to model early tumour growth, at the prevascular growth stage, incorporating fairly advanced "genotypes" for each cell that could be passed on to daughter cells with each division. This model was simple enough that it was computationally feasible at the time (2007) but complex enough to actually provide some insights into the interesting concept of clonal evolution. To study this clonal evolution, Gerlee and Anderson specifically looked at the impact of nutrient diffusion, finding that lower oxygen concentrations caused fingered morphology and more aggressive cells. It sparked thoughts on the interesting idea of tumour-induced angiogenesis (the development of new blood vessels caused by tumour growth). It was known that angiogenesis gave a tumour fresh oxygen and nutrients as well as a network with which to travel through the body. One would assume this could only be detrimental. However this model revealed that low oxygen to a tumour could possibly select for a more aggressive tumour, which could be even more dangerous than an oxygenated one. Many more models were put forth after this investigating the impact of oxygen, vascularization, etc, and encouraged me to study in more detail the impact of oxygen availability on cancer growth.

Building on this model, Gerlee and Anderson published another paper looking at the impact of nutrient availability, specifically looking at what kind of microenvironmental conditions would lead to a glycolytic tumour (one in which many cells are getting energy via anaerobic glycolysis). They introduced elements of the extracellular matrix on top of what was already

المنارة للاستشارات
www.manaraa.com

included in the previous model. The results of this model gave a rough framework for what kinds of microenvironmental conditions (meaning properties like oxygen availability, acid and glucose levels, and density of the extracellular matrix) lead to what properties in the tumour (such as glycolytic or not, how quickly a necrotic core appeared, how soon a quiescent rim appeared, etc). This model encouraged me more to study the impact of oxygen, and the glycolytic phenotype related well to the emerging hallmark of cancer REPROGRAMMED METABOLISM, which I have incorporated into my model.

The last two papers, *Simulating the hallmarks of cancer* by Abbott, Forrest and Pienta [1] and *Study of cancer hallmarks relevance using a cellular automaton tumour growth model* by Santos and Monteagudo [143] introduced me to the idea of modelling Hanahan and Weinberg's hallmarks of cancer [74], [75] in order to learn about the growth dynamics of early tumour growth. Using the hallmarks to model the tumour allows one to model the most basic principles that are shared by almost all tumours, hopefully giving unified results that are applicable to a variety of cancer types.

Abbott, Forrest and Pienta published their model in 2006 and looked at "pathways to cancer" - the order that hallmarks were acquired during progression from healthy cell to cancerous cell. They came up with many simple abstractions that allowed them to model 5 of the 6 hallmarks of cancer, as well as genetic instability, using cellular automata. They also included a simple but elegant model of tumour-induced vasculature that is similar to what I used in this model. They chose and validated a set of parameters for modelling hallmarks that Santos and Monteagudo [143] have used and that I have also used as a jumping off point in the following models.

Overall, these works present a historical and informative look at the progression of cancer models in the last 15 years. All have influenced the model presented throughout this thesis in some way and were unique papers in the field. These papers greatly excited me and gave me the question I posed in this thesis: how do cancer hallmarks impact overall growth in a simulation with accurate oxygen modelling? I appreciate their contribution and hope to have even a small amount of the impact they had in the future.

المنارة للاستشارات
www.manaraa.com

Chapter 2

Biological Background and Model Rationale

المنارة للاستشارات
www.manaraa.com

2.1 Introduction

I have created a highly abstract cellular automaton model of early cancer growth with a dynamic binary fluid model of oxygen flow in blood that investigates the impact of knocking out pairs of “cancer hallmarks”. As of 2009, cancer was the leading cause of death in Canada [154] and the second leading cause of death in the United States [39]. While much time, money and research are dedicated to cancer, the statistics are still grim, with little to no progress in some cancers – for example, there has been no significant improvement in 5 year survival rates of pancreatic cancer since 1971 in England and Wales, with similar numbers in North America [38].

While the traditional reductionist approach to studying cancer has been successful in targeting some forms of the disease, new approaches are needed that can study cancer across scales [140]. *In silico* modelling of cancer is a nascent approach available to attack this problem. Specifically, multiscale *in silicio* modelling is a powerful tool for cancer simulation as it allows modelling at the cellular level as well as at the fluid level in order to accurately model oxygen flow. The availability of oxygen is one of the most critical factors in whether a tumour remains in the relatively safe avascular growth phase or switches to the more deadly vascular growth phase. A small, avascular tumour (approximately 1-2mm) cannot support its own growth and will begin to die unless new vasculature is developed to deliver oxygen and other nutrients [60]. If angiogenesis (the development of new vasculature) does occur, the tumour will have a direct supply of oxygen and nutrients, allowing almost exponential growth, and providing a direct route through which to spread in the body. Since oxygen availability is one of the key factors in whether the tumour becomes vascularized, modelling both scales (cellular level and fluid level) provides a more realistic model. Many cancer models currently exist, including: Anderson *et al.'s* multiscale mathematical model of 2-dimensional tumour growth showing harsh microenvironments can produce fingering outlines and aggressive subclones (often seen *in vivo*) [18]; Lloyd *et al.'s* computational framework for solid tumour growth, which comprised models at the tissue, cellular and subcellular levels [100]; and Ramis-Conde *et al.'s* hybrid-discrete model which looked at tissue invasion by cancer cells, modelling the cells discretely and the spatio-temporal interactions of variables using PDEs [137] but many do not have a strong focus on oxygen modelling beyond simple diffusion [18], [137], [106].

Models focus on different aspects of tumour growth (including the use of the glycolytic phenotype [67], evolution of cell motility [68] and confined environments [69]) and employ different modelling approaches (mathematical [101], [80], [137], hybrid [54], [141], [66],

المنارة للاستشارات
www.manaraa.com

agent-based [157], [172], [106]). Readers who wish to know more are directed to the following review articles: for a review of three hybrid models and how they can be used to bridge scales and relate to one another, see [15]; for more on the field of integrative mathematical oncology, see [17]; lastly, for a review of hybrid models, see [139]. One type of agent-based model that has gained popularity uses cellular automata to model the states, and state transitions, of individual cells [135], [150], [89], [133]. These models have been used to look at the hallmarks of cancer [1], [25], [143] as proposed by Hanahan and Weinberg [74], [75].

Today, survival rates for cancers vary widely, as different cancers are approached in different ways. For example, the 5 year survival rate as recorded from 2003-2009 in the United States for prostate cancer, breast cancer, colon cancer, lung cancer and pancreatic cancer were 99.2%, 89.2%, 64.9%, 16.6% and 6% respectively. While different cancers are approached differently (surgery, radiation, chemotherapy, brachytherapy, etc) and have different survival rates, Hanahan and Weinberg proposed that almost all cancers actually share six common phenotypic changes: self sufficiency in growth signals, insensitivity to anti-growth signals; avoidance of programmed cell death; limitless reproductive potential; sustained angiogenesis; and tissue invasion and metastasis [74]. More recently, in 2011 they updated this list to include two new hallmarks: deregulated metabolism and evading the immune system [75]. They also proposed two "enabling characteristics" of cancer: inflammation and genetic instability. These general hallmarks and characteristics of cancer have been investigated as potential treatment targets for cancer since a drug targeting these issues might be successful with multiple types of cancer [75].

Both Abbott *et al.* and Santos *et al.* have developed models looking at these hallmarks [1], [143]. Abbott *et al.* (as discussed extensively in Section 1.4) primarily focused on looking at the order in which hallmarks were acquired in the growing tumour. Abbott's results differed from the pathway to cancer proposed by Hanahan and Weinberg, as did the results of an ordinary differential equation model looking at the pathway to cancer [152]. Abbott's model was an agent based model that simulated the progression of cancer from a single healthy cell to a tumour with at least 90% cancer cells. They found that hallmarks that confer an advantage to all cells (such as SUSTAINED ANGIOGENESIS which creates blood vessels carrying oxygen into the tumour which all nearby cells can benefit from), do not dominate a cancer clone, whereas hallmarks such as LIMITLESS REPLICATION appear early and dominate as they turn over very quickly.

Santos *et al.* (also discussed thoroughly in Section 1.5) built on the work of Abbott by using a similar modelling approach, but instead of looking at pathways to cancer focused on

المنارة للاستشارات
www.manaraa.com

the impact of removing different hallmarks on tumour growth. They investigated how critical to growth each hallmark was by removing it from the system and comparing the total number of cancerous and healthy cells present without the hallmark present, to growth totals with all hallmarks present. They used a cellular automaton model which determined cell division and apoptosis based on internal rules and acquired hallmarks. They found that with high mutation rates, the most critical hallmark is AVOIDS APOPTOSIS, while in tumours with little room to grow the IGNORE GROWTH INHIBITION hallmark proved most impactful on overall growth. In addition they found when cells had reached their proliferation potential the impact of LIMITLESS REPRODUCTIVE POTENTIAL (called EFFECTIVE IMMORTALITY in their paper) became great.

I have used similar model parameters and methods as those outlined in Abbott *et al.'s* work to build upon Santos *et al.'s* hallmark relevance study. I have created a high level abstract model of early tumour growth. This model uses cellular automata to model individual cancer cells, and uses various rules to model the hallmarks as outlined by Hanahan and Weinberg. In this model I have implemented five of the six original hallmarks as well as three of the four newly introduced hallmarks and enabling characteristics. This thesis specifically focuses on those relevant during initial tumour growth, not metastasis, as this early tumour growth is critical to patient survival. In general, finding and treating cancer during the early phase (before it metastasizes) increases the chances of patient survival [10], [11], [36], [37].

Using this model I have investigated the impact of knocking out hallmarks (in pairs, triplets and quadruplets) on overall tumour growth. These investigations have lead to the conclusion that attacking multiple avenues of tumour growth is often the best way to treat the tumour. Moreover, the results show that only certain combinations of "hallmarks" are more advantageous than their singular counterparts. This research shows that some combinations are not additively better, and in fact are worse, than individual treatments. It also shows that *in silico* modelling is a useful tool in determining what hallmark combinations are most useful for halting cancer growth in a high level scenario.

Henderson and Samaha stated that "in the most general sense, combinations of therapies, whether drugs and/or other modalities, will always play an important role in the management of diseases for which there exists no single specific and totally effective treatment" [78]. Combination treatment involves pairing multiple drugs with the hope that two in combination will not just be an additive advantage but a multiplicative one. Targeted therapy involves identifying key pathways or molecules involved in cancer progression and creating drugs to target these entities. This model simulates targeted combination therapy as we remove key cancer

المنارة للاستشارات
www.manaraa.com

properties in pairs, triplets and quadruplets and compare cancer growth rates to tumours with all hallmarks active. We hypothesize that knocking out pairs of hallmarks will not necessarily have just a slightly greater effect than knocking the hallmarks out separately but rather will sometimes have an even greater, potentially multiplicative, combined impact.

2.2 Cancer Hallmarks

2.2.1 Introduction to the hallmarks of cancer

Hanahan and Weinberg noticed over the years that cancer research had created such a large body of knowledge that it was almost beyond measure [26]. They recognized the benefit of being able to distil this knowledge into a small number of underlying principles describing this broad disease category, and attempted to begin that endeavour by putting forth what they called *The hallmarks of cancer* [74]. They stated that these hallmarks were “rules that govern the transformation of normal human cells into malignant cancers” and they believed they applied to all, or at least almost all, cancers [74].

Cell biology teaches that mammalian cells share similar machinery for common cell processes such as proliferation and death, so the idea of a common transformation step (or set of steps) to cancer seems plausible. Cancer often appears in stages; a series of intermediate steps transforms normal tissue into an invasive cancer [170]. Cancers are found to contain multiple aberrations, likely because of these progressive changes.

This stepwise process has been speculated to be similar to Darwinian evolution [74]. A cell sustains a small mutation and if it confers strength to the cell, it gets passed down to daughter cells. It can get added to by more advantageous mutations until a select group of changes is so powerful as to allow for an overpowering of normal cells by this stronger population.

A major barrier to the study and treatment of cancer is that cancer itself is actually a collection of over 160 different diseases. The question remains: what are their similarities, if any, and how can we use them to treat more cancers more efficiently?

Hanahan and Weinberg proposed that the large array of cancer types and genotypes is actually the impact of six essential changes to the cell [74]:

- Self sufficiency in growth signals

المنارة للاستشارات
www.manaraa.com

- Insensitivity to anti-growth signals
- Evasion of programmed cell death (apoptosis)
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis

Cells have built in defenses to all of these and cancer is the successful breakdown of these defense mechanisms. The number of defences that need to be broken down could be why cancer is somewhat rare, usually only appearing once per life time and later in life. Clearly the idea of a set of six common cancer abilities is appealing to researchers. Many drugs attempt to stop one or more of these "hallmarks" and over time it has become apparent that the idea of Hanahan and Weinberg has strong merit [56], [21]. Since so much treatment and research is based on these six abilities, I have chosen to use them as my basis for modelling cancer. They are arguably what defines cancer, and therefore they form how cancer is programmed in my model. Below I will outline each hallmark and describe a bit about the biology of it as well as how it is represented in my model simulation.

2.2.2 Self-sufficiency in growth signals

Normal cells are generally in a quiescent state where they are functioning but not actively proliferating. In order for a cell to become mitotically active (divide), it needs mitogenic growth signals, which stimulate it to move from the quiescent state to a dividing one. It is believed in fact that normal cells cannot divide in the absence of growth signals.

Normal, healthy tissues regulate the creation and release of growth signals very carefully, balancing the number of actively dividing cells so no area is overburdened with resource requests. Cancer cells de-regulate these signals. In fact, self-sufficiency in growth signals is said to be the most fundamental trait of cancer cells [75].

The signals involved with cell proliferation are often bound by cell-surface receptors, which in turn are often involved in even more pathways, including cell-survival and energy metabolism. Altered regulation of the proliferation pathway, particularly upstream enzyme modulators, can initiate a cascade of detrimental changes that may present themselves later in the cell's life cycle. The full set of events governing the creation and release of these signals is poorly understood due to a variety of issues making experimentation difficult. These issues include temporal

المنارة للاستشارات
www.manaraa.com

and spatial regulation of the signals, the complexity of the networks involved and the highly localized and specialized mechanisms involved.

Interestingly, the deregulation of these signals in cancer is much better understood [176], [131]. Cancer cells can sustain their growth through a variety of mechanisms including:

- sending growth signals to surrounding cells which reciprocate by releasing even more growth factors.
- increasing their own cell-surface receptors for growth signals so that basal levels of signal can have an enhanced effect.
- activation of growth pathways downstream of signal reception, which enhances proliferation but also reduces their dependency on external stimuli.
- interference with negative feedback loops which normally lessen signals and therefore assist in maintaining homoeostasis. Hanahan and Weinberg have predicted that "compromised negative-feedback loops" will be found to be "wide spread among human cancer cells" [75]. They also believe cancer cells may use this ability to disturb feedback loops to gain resistance to drugs which target cell cycle signalling.

Interestingly, very high levels of some oncoproteins (proteins created from an oncogene, a gene with the potential to cause cancer that is often mutated in cancers) such as RAS (a family of proteins which are involved in cell growth) and MYC (a regulator gene that is involved in cell cycle progression and apoptosis) are implicated in cell senescence, a cell state that is viable but non-proliferative [75]. This is counter-intuitive to the thought that high expression of signalling proteins would lead to increased growth. It is hypothesized by Hanahan and Weinberg [75] that cells may have built in safety mechanisms to avoid excessive growth and therefore induce senescence in cells with high levels of oncoprotein. Furthermore they postulate that cancer may be involved in a delicate trade-off between increased signalling for fast growth and not growing so fast as to activate these safety mechanisms. It is also possible that cancer cells could somehow be deactivating these safety mechanisms.

Many cancer hallmarks have an impact on more than just the cancer cell itself, and self growth is no different. Indeed, cancer's ability to "co-opt" neighbouring normal cells by stimulating them as well, helps create a rich tumour microenvironment where cancer cells can thrive and be supported by normal cells. Signal changes, including in growth factors, can conscript normal cells as active agents in a tumour's growth.

المنارة للاستشارات
www.manaraa.com

In order to model this hallmark, I have set up healthy cells to only be able to divide within a certain area of the total growth environment. Healthy cells can only divide up to some predefined boundary (which is a gradual boundary), which is akin to an area with growth factor present. In certain areas (outside the boundary) there is not enough growth factor to signal proliferation, and therefore healthy cells become senescent in this area. Cancer cells however can obtain the SUSTAINED GROWTH (SG) mutation, allowing them to actively divide outside of the predefined boundary, modelling the ability to initiate growth themselves via a variety of mechanisms. My model is a high level model, modelling a general solid-mass tumour, and so no specific pathways or proteins are involved. Rather the use of the boundary models self-sufficient growth via any means the cancer cell may be using.

2.2.3 Evading growth suppressors

Coupled with a tissue's tight regulation over growth inducing mechanisms, tissues also have strong negative regulation over cell growth, actively suppressing it as opposed to passively not taking part due to the absence of growth promoting factors. The genes which do this are often referred to as tumour suppressor genes (genes that when damaged and lose function often allow cancer to grow) and many have been identified via gain- or loss-of-function experiments in mice [75]. These genes can actively limit cell growth and proliferation through a variety of mechanisms including stopping the cell cycle and cell-cell contact inhibition.

One tumour suppressor gene often discussed and referred to as "the guardian of the genome" is TP53 (tumour protein p53, often abbreviated p53). Cells have built-in stress and abnormality sensors that p53 can utilize to detect unfavourable conditions or cell damage. If p53 detects such things, it can pause the cell cycle and stop division until the damage has been fixed and/or the conditions have changed for the better. If dire circumstances are detected, or extreme cell damage is found, p53 can actually initiate apoptosis (the process of programmed cell death), preventing such damage from being passed on to daughter cells [98].

Similar to the internal guardian p53, the RB gene product (pRb) processes both internal and external signals to ensure the extracellular and intracellular environment is sufficient for growth and division. It can repress the cell cycle if conditions are not as they should be [79]. Both p53 and pRb are part of complex networks with much built in redundancy [75].

Damage to either of these pathways, including but not limited to pRb and p53 themselves, can allow a cell to circumvent the anti-proliferative cell mechanisms. In fact, the TP53 gene is

المنارة للاستشارات
www.manaraa.com

found to be mutated in 50% of all cancers.

In addition to the functioning of cell cycle regulators, cell growth is also negatively controlled by the pressure exerted on cells by other cells. When cells grow together the cell-to-cell contact has an inhibitory effect on growth. This mechanism, called contact inhibition, is often turned off or perhaps ignored in many cancer cells. An example of a gene involved in this pathway is the NF2 gene, the loss of which triggers human neurofibromatosis, a disease which deposits tumours throughout a patient's body. The gene product of NF2, Merlin, strengths cell-cell adhesion and also sequesters growth factors, limiting cell growth.

In order to model cancer's ability to stop responding to anti-growth signals, I have implemented a space requirement for growth. This models one of cancer's anti-growth avenues – avoiding contact inhibition. In my model, healthy cells stop actively growing once there is no more space available adjacently or diagonally on the lattice. Cancer cells in this system can have the IGNORE GROWTH INHIBITION (IGI) hallmark activated which allows cells to grow even without space (thereby modelling the impact of mutations which cause a cell to ignore contact inhibition). These cells have a "competition" factor (c). If a cell with the IGI hallmark attempts to grow and is out of space, they compete with cells around them and can potentially take over the space another cell is occupying in order to grow. Each time a cell competes (done once every attempt to divide without space), the cell has a $1/c$ probability of successfully gaining the space, where c is the competition factor provided in Table 3.1.

2.2.4 Resisting cell death

Apoptosis, or programmed cell death, is a normal process in animal tissue. Apoptosis assists in the balance of healthy alive cells with dead or dying cells, keeping cell populations in check and dealing with damaged or old cells. Apoptosis involves controlled death of the cell, including breaking up the cell whose individual pieces/components subsequently get removed from the area. In addition to helping maintain balance, it is also a safety mechanism which prevents damaged cells from passing on damage to future generations of cells [103], [58].

Apoptosis has been found to be triggered by different events, many of which are common during the progression from normal, healthy cell to cancerous cell, such as high levels of oncogene expression and DNA damage. Although it is known that DNA damage and oncogene overexpression can lead to cancer which should trigger apoptosis, it has also been found that cancer cells sometimes manage to avoid apoptosis [75].

المنارة للاستشارات
www.manaraa.com

The machinery involved with apoptosis includes upstream regulators and downstream effectors [4]. The regulators both listen for extracellular death-inducing signals and intracellular signals of problems. When apoptosis is induced, normally latent proteases (an enzyme that breaks down proteins) are activated and begin a cascade of proteolysis (protein breakdown) which disassembles the cell. The cell lysate (fluid made up of the content of the broken down cell) is then consumed by neighbouring cells or phagocytic cells (cells which consume and kill other cells, often involved with the immune system) [75].

Various cell conditions leading to apoptosis have been identified, with one of the most notable being DNA damage [87]. Major DNA breaks or chromosomal abnormalities are sensed by tumour-suppressor TP53 which can in turn activate apoptosis if the damage cannot be fixed. While cancer cells evolve many mechanisms to avoid apoptosis, one of the most common is loss of TP53 function (found to be gone in 50% of all cancers). This allows a cell to build up DNA damage unchecked which can lead to additional mutations and passing down of damaged DNA.

I have chosen to model the apoptotic pathway primarily as a sensor for DNA damage. Once a healthy cell has sustained any mutations (in this model meaning its rules have been altered from the healthy cell version), it is possible for it to enter the apoptotic state and be killed via apoptosis. Since apoptosis can be activated due to genetic damage, the chances of a cancer cell dying via apoptosis increases with each subsequent mutation (in this model). A cell has an m/a probability of being killed by this mechanism, where m is the number of mutations already sustained, and a is an apoptosis chance variable contained in each cell (see Table 3.1 for exact parameters). Cells with a mutation in this mechanism cannot die by apoptosis, regardless of the number of mutations acquired. This AVOIDS APOPTOSIS mutation is referred to as AA throughout this thesis.

2.2.5 Enabling replicative immortality

Most normal healthy cells have a limit to how many times they can divide before they enter a viable but non-proliferative state called senescence. Healthy cells that manage to avoid senescence often instead enter a crisis state, ending with cell death. In contrast, cancer cells seem to require unlimited replication to grow to a tumour of microscopic size. In culture when cells are propagated, leading to senescence, and then for some to crisis, many of the cells die. At this point it occasionally happens that a cell line comes up displaying this unlimited replication,

المنارة للاستشارات
www.manaraa.com

effectively becoming immortal, continuing to grow without hitting senescence or crisis.

It is believed cells have a feature which only allows them a certain number of replications – telomeres. These are segments of DNA made up of multiple repeating 6-nucleotide segments capping the ends of chromosomes. Chromosome replication is not a perfect process and always results in the loss of some material at the ends of chromosomes. Telomeres protect the "necessary" DNA by themselves being shortened at each replication. Eventually however they become too small to effectively protect the DNA and at this point it is believed senescence can be triggered.

Telomerase is a DNA polymerase that builds these telomeres. Normally it is not active in healthy cells, however it is found to be turned on in approximately 90% of suddenly immortal cells [75]. Telomerase then can continuously extend the ends of DNA making it so they never reach a size small enough to trigger senescence or crisis. Accordingly, the presence of telomerase is correlated with resistance to both of these fates [75]. It is believed both of these events are natural barriers to cancer. Rogue cells may develop mutations, be growing out of control of the body's signalling, and begin rapid division. These cells can be abruptly stopped when their full replicative potential is reached, causing them to not be able to divide further and not make it to a macroscopic tumour. Cells which manage to activate telomerase however keep their telomeres long enough to avoid senescence and crisis and therefore forever pass on their mutations. As such telomere shortening is thought to be one of the barriers cancer cells must defeat to progress into a dangerous tumour [75].

One particular example supporting this hypothesis is the work of Artandi and DePinho who found that mice genetically predisposed to certain cancers had weakened tumorigenesis when born without telomerase [20]. The early shortening of telomeres may have pushed the cells into senescence more quickly, and the lack of telomerase would prevent immortalization of the cells.

The exact roll of telomerase and telomeres in cancer is complicated by evidence that a lack of telomerase may sometimes encourage neoplastic progression (abnormal growth, a characteristic of cancer) [92], [76]. As chromosomes shorten, they risk being involved in "breakage-fusion-bridge" cycles, whereby chromosome ends lacking telomeres fuse together. During cell division these fused chromatids form a bridge and then are ripped apart, but often not at what would be the correct place, where they fused. The resulting chromosomes are uneven and the two daughter cells have improper chromosomes. This often leads to large scale rearrangement,

المنارة للاستشارات
www.manaraa.com

such as deletions and amplifications of DNA, potentially activating oncogenes or silencing tumour suppressors. In this case the lack of telomerase has enhanced a cancer cell's mutability and possibly fitness. Over time these cells may activate telomerase thereby immortalizing these potentially dangerous mutations. In fact, pre malignant legions in the human breast have been found to have normal levels of telomeres as well as chromosomal aberrations while malignant lesions had telomerase activity and fixed aberrant karyotypes presumably from earlier in the cancer's progression [138], [42].

We have included in our model the ability for a cell to become immortal. Every cell is equipped with a "telomere" variable that decreases by one with every cell division. This variable limits healthy cells to 60 cell divisions. Normal healthy cells go through roughly 25 to 70 divisions [74] and other models have chosen a number to lie in this range [153]. Some simulations chose a higher initial telomere length (100 in both [143] and [1]) however it was found that in models higher initial lengths cause rapid cancer development, while a number closer to 55 causes later cancer onset [153], suggesting the number of cell divisions allowed in reality has been optimized (perhaps via evolution) to reduce cancer occurrences. We chose a value that would not lead to rapid cancer development but would be more similar to reality. Cells can acquire the IGNORE TELOMERE hallmark which allows them to effectively ignore this limitation and not be bound by their telomeres. This replicates the biological activity of telomerase which continues increasing telomeres after they are shortened, preventing their length from impeding growth. Cells with the IGNORE TELOMERES (IT) hallmark activated can divide forever, regardless of telomere length, so long as all other required conditions are met (oxygen, nutrients, space, etc).

2.2.6 Inducing angiogenesis

Vasculature, the system of blood vessels in the body, serves two major purposes to cell groups: delivering nutrients and oxygen, and removing waste products and carbon dioxide. Both healthy and cancerous cells depend on and need this system. Typically, vasculature is quite stable. It is originally developed during embryogenesis, when the processes of vasculogenesis (the birth of new endothelial cells and their development into tubes) and angiogenesis (sprouting) occur. After embryogenesis is complete, angiogenesis is only turned on transiently during wound healing and as part of the female reproductive cycle. A key early development in tumour growth is the activation of this normally quiescent angiogenic process, causing new vasculature to sprout towards and even into tumours [74], [75]. This is extremely dangerous as it not only provides the tumour with fresh oxygen, nutrients, and waste removal, but also gives it a system

المنارة للاستشارات
www.manaraa.com

to use to travel through the body.

It is believed that the process of angiogenesis is regulated by counteracting factors that induce or oppose angiogenesis. Vascular endothelial cells have surface receptors which can bind inhibitory or stimulating cell surface receptors – one of the most well known of which is the angiogenesis inducer vascular endothelial growth factor A, or VEGF-A [75].

The VEGF-A products are involved in the process of new blood vessel sprouting in at least three different situations: embryonic and post natal development, homoeostatic survival of endothelial cells and disease situations. The VEGF pathway is complex and involved in a variety of situations, and it can be upregulated by both hypoxia (lack of oxygen, common in tumour growth) and oncogeneic signalling. Even more situations can impact this pathway, such as sequestration (biological accumulation of a compound) and subsequent activation and release of inactive VEGF ligands by matrix degrading proteases and upregulated pro-angiogenic factors such as fibroblast growth factor (FGF) [75].

When angiogenesis is induced in tumours it often results in poorly set up vasculature with issues such as leakiness, erratic blood flow and excessive and convoluted branching. While this process was previously thought to occur later in tumour development, such as once the tumour was rapidly growing and macroscopic in size, research in the past two decades has found it can begin as early as the pre-malignant microscopic stage of growth [136].

Tumours exhibit widely varied tumour-induced vasculature, even within the same organ. For example, adenocarcinoma of the pancreatic ducts is hypovascularized [124], while pancreatic neuroendocrine carcinomas can be densely vascularized [53]. The variety of tumour induced angiogenesis seen suggests that angiogenesis is initially switched on, but complexly regulated and impacted throughout tumour growth. While the mechanism of angiogenesis switch activation can vary, the net result is a common inductive signal (e.g. VEGF). In some cases oncogenes activate angiogenesis (such as RAS and MYC). These also can stimulate proliferation which means other unique hallmarks (such as sustained growth) can possibly be activated by the same rogue players.

Since it is believed angiogenesis is at least initially switched on in tumour growth, and this model aims to simulate early tumour growth, we have included this hallmark. In the simulation, angiogenesis is modelled at a very basic level. Cells that have the INDUCE ANGIOGENESIS (A) hallmark activated are thought to be on the path of new vasculature. In the simulation, every

المنارة للاستشارات
www.manaraa.com

lattice location has an associated oxygen value that changes over time based on consumption and supply, and is modelled using lattice Boltzmann methods (LBM) (for a full explanation of this, please see Section 3.3). At each division a cell checks if it has enough oxygen to survive by checking with the lattice Boltzmann simulation, for how much oxygen is present where it is located. The lattice Boltzmann model simulates the flow of oxygen in the blood around the tumour and how it diffuses towards cells. If a cell does not have enough oxygen it either becomes quiescent or dies (depending on how much oxygen is present). However, if it is on the path of vasculature because it is inducing angiogenesis, it is getting oxygen directly from the vasculature so it does not require enough oxygen in its lattice cell as calculated by the LBM. Neighbours of cells on the vasculature also benefit from this via diffusion from the new vasculature and are able to survive in places there would not otherwise be enough oxygen.

In this model angiogenic cells also have a higher chance of being killed by the immune system, as immune-associated cells travel via the vasculature. Angiogenic cells and their neighbours have a $(ai) * (1/i)$ chance of being killed by the immune system at each replication step, where ai is the angiogenesis immunity parameters and i is the immune death likelihood parameter (Table 3.1).

2.2.7 Emerging hallmarks and enabling characteristics

Hanahan and Weinberg describe the hallmarks of cancer as "acquired functional capabilities that allow cancer cells to survive, proliferate, and disseminate" [75]. They are acquired over time by a variety of distinct mechanisms in many different tumour types. In order to acquire these hallmarks, Hanahan and Weinberg have identified two "enabling characteristics": genomic instability and tumour promoting inflammation. The most prominent of the two, genetic instability, is included in this model and described below.

Other research has found cancer cell attributes that seem similar to hallmarks, in that they are perhaps attributes allowing cancer cells to survive, proliferate and disseminate [45], [121]. Of the attributes noted, two seem particularly well suited to potentially be hallmarks. The first is the fact that many cancer cells are found to have reprogrammed metabolism, increasing their use of glycolysis, a less efficient form of energy generation. Secondly, tumour cells have been observed to somehow avoid destruction by the immune system. These two attributes have been deemed "emerging hallmarks" by Hanahan and Weinberg and are also discussed in greater detail below [75].

المنارة للاستشارات
www.manaraa.com

Genetic instability

Cancer cells acquire the above mentioned hallmarks in large part because of successive changes to the genome of neoplastic cells [75]. Some mutational changes will confer an advantage to the cell, allowing it to grow and dominate in an environment. Therefore, the many steps from normal cell to cancerous cell, and the subsequent stages of tumour growth, can be viewed as the successive accumulation of favourable chance mutations each creating a new cell clone group. However, not all clonal expansions need be caused by mutations, as research has shown that epigenetic changes (genetic influences that are not related to the actual sequence of DNA) can also impact gene expression [29], [86].

Many innate cell systems are able to detect and repair DNA damage; consequently, the number of spontaneous mutations in a cell generation tends to be low. Cancer cells however often have higher than normal mutation rates, which can be achieved in a variety of ways. There can be an increased sensitivity to mutagenic agents or breakdown in any of the genetic maintenance machinery or pathways. Also, a disturbance in the cell machinery that detects and fixes mutations (for example, TP53, mentioned earlier) can lead to an increased mutation rate. The process of detecting and fixing DNA damage is complex, and so has a lot of potential places breakdowns can happen, such as:

- Machinery that detects damage and activates repair pathways
- Machinery that repairs DNA
- Machinery that eliminates a cell if too much damage is acquired
- The pathways involved with the inactivation and interception of mutagenic molecules

Other cell components can be included in the list of "caretakers" that watch over the genome. As mentioned above, the loss of telomeres leads to increased chromosomal mutations and instability, another avenue for rapid mutation accumulation. Korkola and Gray found that there may even be certain areas of the genome where aberrations are likely to lead to neoplastic growth, an interesting observation made after seeing recurrence of specific amplifications and deletions at certain sites in breast cancer [95].

In the future, the current development of more efficient and affordable sequencing technology should allow full sequencing of tumours, which may show even more the impact of genetic instability. Already early research has shown that there are distinctive patterns of DNA mutations across different tumours [75]. More knowledge in this area should help explain the

المنارة للاستشارات
www.manaraa.com

prevalence of the apparently random mutations across cancer cells and may reveal some logic and causation behind some of these changes.

Currently it is known that the genetic changes in cancer vary across tumours, tissues and types, however one thing that is agreed upon is the vast array of cell maintenance and repair mechanisms that can be damaged as well as the prevalence of copy number changes to genes in cancers. These wide spread changes point to genome instability as an enabling characteristic of cancer in general, and possibly one that is causing the acquisition of cancer hallmarks [75].

Working with this belief, that genome instability could cause cells to acquire some hallmarks, we have added it as an enabling characteristic in this cancer simulation (for simplicity, all hallmarks, enabling characteristics and emerging hallmarks and simply referred to as "hallmarks" throughout this thesis). While there are many different pathways and mechanisms that can be impacted in cancer growth and can cause genetic instability, the end result is a genetically unstable cell which has a higher likelihood of mutation. We model this characteristic with the GENETICALLY UNSTABLE hallmark (GU) that when active increases the probability of mutation in each mitotic event by a factor of (1/gif) where gif is the genetic instability factor parameter. Modelling it in this way allows for hallmarks to still be acquired spontaneously due to any number of factors, but also allows cells to accumulate mutations more quickly if they are "genetically unstable".

Reprogramming energy metabolism

Cells need to be able to generate energy to sustain themselves and proliferate. Typically, healthy cells perform aerobic metabolism, where they take glucose in the presence of oxygen and convert it into adenosine triphosphate (ATP - a molecular unit for energy in the cell) and carbon dioxide in the mitochondria. If cells are in an anaerobic environment (one with little oxygen), they preferentially upregulate glycolysis, a form of energy production which uses very little oxygen and creates much less ATP. Glycolysis is considered to be less effective then aerobic metabolism (as approximately 18x less ATP are generated by glycolysis relative to aerobic metabolism). However, cancer cells often seem to reprogram their glucose metabolism to rely largely on this mechanism. This was first observed by Otto Warburg, and has since been termed "the Warburg effect" [173], [175].

This effect has been observed repeatedly in the years since, although it seems counter-intuitive since glycolysis produces so much less ATP. Cancer cells seem to have ways to compensate for this. One such method is the increased intake of glucose into the cell, and increased

المنارة للاستشارات
www.manaraa.com

utilization of it. This has been found by positron emission tomography and reported by various studies and reviews [109], [50], [82].

It has been shown that glycolysis in cells is associated with both activated oncogenes and mutant tumour suppressors. In addition, tumours often exist in a hypoxic environment (one with low oxygen) and hypoxia is an activator of the glycolytic pathway. The cells response to a hypoxic environment can both up-regulate glucose transporters to increase glucose uptake, and increase creation of enzymes involved in the glycolytic pathway.

Although there is evidence for hypoxia, oncoproteins and tumour suppressors activating this pathway, the reason why it would be advantageous to the cell is still somewhat unknown. One hypothesis by Potter [134] which was recently reviewed by Vander Heiden *et al.* [168] suggests that by switching to this pathway, cells use some of the intermediate molecules created during glycolysis for other important biosynthetic pathways - such as creating molecules and organelles needed for new cells. Since cancer divides so rapidly, it would be advantageous to have more available building blocks for the process. Supporting this hypothesis is evidence of Warburg-like metabolism in rapidly-dividing embryonic tissue.

An interesting symbiosis within cancer tumours has also been observed with respect to energy metabolism. Some tumours have been found to contain two subpopulations - one which relies on glycolysis for energy and secretes lactate as a waste product, and a second which relies on lactate as their main energy source. This behaviour of coordinating metabolism between lactate-secreting and lactate-utilizing cells is not unique to cancer cells and indeed is used in operating muscle cells [75]. In addition, the oxygenation of a cancer cell varies across the tumour and over time, most likely as a result of poorly orchestrated tumour vasculature, and so having cells that operate efficiently in both normoxic and hypoxic environments could be advantageous.

Hanahan and Weinberg were unsure whether altered energy metabolism was independent and necessary enough to be listed with the other six hallmarks when they published the next generation [75]. On the one hand, it has been observed to be as wide spread as many other cancer traits, but on the other, it appears it may be programmed by proteins that already are involved in programming hallmarks. Due to the ubiquity of reprogrammed metabolism in cancer tumours, but the lack of clarity in whether it is independent from other hallmarks, Hanahan and Weinberg have given it the same designation as evading immune destruction – that of an "emerging hallmark" [75].

المنارة للاستشارات
www.manaraa.com

While the independence of this emerging hallmark is unknown, its prevalence in cancer is not, and as such I have chosen to include it in this high level model of cancer. In this model, cancer cells can get a mutation allowing them to switch to the "glycolytic phenotype". These cells do not require oxygen present in their environment, and so can survive in poorly oxygenated environments. This model does not look at the impact of energy requirements or metabolism by-products but is specifically interested in the role oxygen plays in early tumour growth, and as such the modelling of the glycolytic phenotype only confers the ability for cells to survive in low oxygenated environments.

Evading immune destruction

Listed as an "emerging hallmark" in the updated cancer hallmarks paper, the ability for cancer to seemingly evade destruction by the immune system is an unresolved issue that appears to play a big role in cancer growth [75]. It has long been believed that the immune system is like a constant surveillance system, watching the body for signs of foreign cells or incipient cancer cells, eradicating them if they are found. By this logic, cells that manage to grow into full macroscopic tumours must have somehow avoided detection or destruction by that same system.

One piece of evidence in the argument that the immune system is involved in the early detection and eradication of cancer cells is the fact that individuals who are immunocompromised have a much higher incidence of certain cancers [165]. However many of these are cancers caused by viruses, and so it would seem that perhaps the role of the immune system in cancer prevention is just minimizing the viral load on a body. Recently however, some studies have shown that even in non-virus-induced cancer, the immune system still plays a significant role as a barrier to cancer progression [75].

Studies with genetically engineered mice back up this claim. Mice that had been engineered to lack various pieces of the immune system had an increased incidence of tumour formation. Mice that were engineered to be deficient in multiple pieces of the immune system had even higher rates of cancer [162], [94].

Other interesting work on this emerging hallmark has been done involving transplantation of cancer cells between immunocompromised and immunocompetent mice [162], [94]. It has been shown that cancer cells that originated in immunodeficient mice often cannot initiate tumour growth when transplanted into immunocompetent hosts; conversely, cancer cells

المنارة للاستشارات
www.manaraa.com

that initially grow in an immunocompetent host are just as able to develop cancer in either immunocompetent or immunocompromised hosts. One theory is that the immune system routinely edits cell growth, deleting any immune-susceptible early cancer cells, and so if cancer develops it is a collection of cells able to avoid the immune system. If these are put in any host the same outcome will occur. However cancer cells which survive in an immunocompromised mouse have not yet had to come up against a competent immune system and so are often eliminated when that occurs, upon transplantation into a immunocompetent host [151].

Interestingly, some human organ transplant recipients have been found to develop donor-derived cancers while the donor did not present with cancer. It is believed the healthy donor was able to keep early cancer growth in check via their immune system, however the transplant recipient is not able to do so [155]. Other work in this area has found that patients with ovarian and colon cancers have a better prognosis when large amounts of natural killer and cytotoxic T lymphocyte cells are found in their tumour [128].

It is not a given that immunosuppressed patients will get cancer, however it should be noted that many immunocompromised people are only lacking in certain immune system functions, and still have others, for example, natural killer cells. Perhaps the reason for a lack of a strong increase in cancer among immunocompromised patients is that they still contain certain components of the surveillance system, perhaps a more critical component than that which is compromised.

Hanahan and Weinberg believe there is some evidence of anti-tumour immunity but that it has yet to be proven ubiquitously enough to be considered a core hallmark and as such have set it as an "emerging hallmark", along with reprogramming energy metabolism. We have chosen to include both of these hallmarks in this study, as we are interested in a high-level, abstract model. We could assume this general model is of a type of solid mass tumour that is affected by both the immune system and the reprogramming of energy metabolism. This simulation has a very simple, basic model of immune system surveillance. In the model, cells with mutations have a probability of being killed by the immune system at every life cycle (exact numbers can be found in Table 4.1). We then model cancers ability to possibly avoid this with the AVOIDS IMMUNE SYSTEM hallmark (AI) which when activated lowers the chances of a cancer cell being killed by the immune system by a factor of aip, the avoids immune system parameter.

المنارة للاستشارات
www.manaraa.com

2.2.8 Features not simulated

This model specifically models 5 of the original 6 hallmarks of cancer, both of the emerging hallmarks, and one of the two enabling characteristics. The choice of what to model was a complex one, and was largely impacted by the scope and type of study, the biological relevance at the early growth phase, and the computational and modelling feasibility. The hallmark and enabling characteristic that were not included in this model are described in more detail below for completeness. A rational for not including these hallmarks follows each description.

Tumour-promoting inflammation

Immune cells are present in virtually every neoplastic legion in some amount. Some lesions have very small infiltrations that can only be detected using cell type-specific antibodies, however others have such large densities of immune cells they can be seen using standard histochemical staining [128]. As mentioned above, there is an immune response that attempts to stop tumour growth, however in the last two decades there have been hints that the tumour-associated inflammatory response might paradoxically also be promoting tumour growth [52], [181], [72]. In fact, research in the last decade has found multiple ways that the innate immune system actually contributes to neoplastic progression and hallmark acquisition, largely by supplying bioactive molecules to the microenvironment of the tumour such as:

- Growth factors that maintain signalling for proliferation [52], [72], [181]
- Survival factors that assist in avoiding cell death [72], [181]
- Proangiogenic factors that facilitate in the development of tumour vasculature [49], [47], [123]
- Extracellular matrix-modifying enzymes that can assist in angiogenesis, invasion and metastasis [46], [179]
- Inductive signals that lead to hallmark-facilitating programs [52]

Hanahan and Weinberg point out that inflammation is sometimes present at very early stages of neoplastic progression, and has been found to be clearly capable of assisting in the progression from incipient neoplasias to fully functioning cancer tumours [75]. Also, some inflammatory cells release reactive oxygen species which are mutagenic to the surrounding cells, possibly assisting these cells in gaining advantageous mutations. As such, Hanahan and Weinberg have classified inflammation as an “enabling characteristic” of cancer, however they stated that of the two enabling characteristics, the most prominent is genomic instability. I

المنارة للاستشارات
www.manaraa.com

have chosen not to include tumour promoting inflammation in our simulation. The role of inflammation is still mostly unknown. Many of the cell types that lead to inflammation, such as cells of the innate immune system, play a dual role of assisting in cancer development and trying to stop it. Inflammation appears to be involved in a host of developments, specific to what cells are present, the microenvironment, etc. As such, there is no obvious single impact on early tumour growth that can be abstracted to a parameter that could be modelled in the type of high level, broadly applicable model we are interested in studying. I have chosen to model the tumour growing in an empty environment, only looking at the development from 1 cell onward, focusing on the impact of oxygen in the environment and no other microenvironmental conditions. As such, I believe it is sufficient to model the 8 other hallmarks and characteristics mentioned above. In the future adding in a more realistic microenvironment would be beneficial and would allow the addition of inflammatory modelling.

Invasion and metastasis

When Hanahan and Weinberg originally proposed the hallmarks of cancer, the process of tissue invasion and metastasis was largely unknown [74], [75]. It had been noticed however that cancer cells often lose E-cadherin, a cell-to-cell adhesion molecule. Epithelial cells are joined together by E-cadherin forming sheets and helping maintain quiescence. Cancer cells lose this adhesion and are then more able to migrate and not remain in a quiescent state. It has been found that increased expression of E-cadherin slowed invasion and metastasis, and that a reduction in it would increase the likelihood of invasion and metastasis [75].

Interestingly, other cell-to-cell and cell-to-ECM (extracellular matrix) adhesion molecules have been found to be undeniably altered in some highly aggressive cancers [75]. In addition to this, some molecules that are active during normal cell migration, such as during embryogenesis, are found to upregulated in some cancers [75].

Since the initial publication of the hallmarks, more information on cancers ability to migrate has come out, however the entire process is still somewhat elusive. Certain mechanisms have been implicated, such as the epithelial-mesenchymal transition (EMT) whereby cells lose their epithelial traits, such as staying in sheets and being quiescent, and gain more traits of mesenchymal cells including migratory and invasive behaviors [75]. Also, many transcriptional factors have been identified in this process – factors which contribute to things like expression of matrix-degrading enzymes, increased motility and increased resistance to apoptosis [75]. There has even been implication that the transition from stationary cancer cells to invasive or metastatic cancer does not require any additional mutations on top of those which occur when

المنارة للاستشارات
www.manaraa.com

the initial tumour is being formed.

Complicating matters is the existence of multiple forms of invasions. There is “collective invasion” where whole groups of cells will migrate into adjacent tissue, and “amoeboid invasion” where cells slither to new regions without clearing a path (which is done in EMT migration). There is also some thought into whether a patient’s own inflammatory response is involved in invasion and metastasis.

Beyond the initial problem of migrating, a second piece of the metastasis puzzle is foreign tissue colonization. Cells may manage to be invasive and migrate away from the primary tumour, but the ability to seed a new macroscopic tumour in a new location is not a given. In fact it is possible that colonization may be even more challenging than the initial issue of migrating. A variety of interesting situations has been noticed in the problem of cell dissemination and colonization. For example, cells that migrate may be prevented from colonization by antigrowth forces exhibited by the primary tumour. In some cases after primary tumour resection many distant colonies have suddenly arose [51]. It has also been observed that limited nutrient availability can cause distant micrometastasis to shrink and become dormant, only to be reawoken when environmental conditions change [93], [104]. Distant site tumour cells may also lay dormant because of tumour surpressors in the environment or normal antigrowth signals in the extracellular matrix [24], [162], [5].

Other factors complicating the situation include the microenvironment of the new cells. The cancer cells may have been perfectly adapted to the primary location, however when they migrate they may not have the mutations necessary to thrive in the new environment. The cells may require many changes to be able to proliferate in the new environment.

There are many interesting and open research problems in this area, such as:

- When do cancer cells acquire the ability to spread?
- When do cancer cells acquire the ability to colonize?
- What sets of genes (or “metastatic signatures”) are responsible for the ability to spread and colonize?
- What kind of microenvironment is needed to facilitate tumour development at distant sites?

المنارة للاستشارات
www.manaraa.com

These questions are quite complex and beyond the scope of this research, however included for completeness. This particular cancer simulation is focused on the early, pre-metastatic growth phase of cancer. I am interested in early growth, the appearance of the hallmarks, the time when tumours reach visible size and what hallmark-stopping efforts are most useful at this time. Patients whose tumours are found before they spread have a much better chance of survival and we are interested in this early phase [13], [9], [14], [8]. For completeness, I have included a small summary of this hallmark. Those interested in learning more are directed to the revised Hallmark paper, *Hallmarks of cancer: The next generation* [75] and the papers referenced therein. This simulation looks specifically at early tumour growth and the development of the primary tumour, and as such does not include this hallmark in the simulation.

2.3 Cellular automaton modelling

A cellular automaton is a discrete unit that can exist in a finite number of states based on how an internal rule set processes given information. Automata are commonly placed on a grid structure and are updated at discrete time intervals to reflect their new states based on changes in the environment. In this model they are autonomous units that are independent from the states of those cells around them, and their next state is selected at each time point solely based on internal rules and input from the environment.

Cellular automata are a natural tool for modelling cancer cells; much like cancer cells, they operate somewhat autonomously and choose their next state based on the input they receive from their environment. This mimics cancer's loss of collective behaviour – cancer ignores contact inhibition signals from surrounding cells, stops listening to growth or anti-growth signals, and operates in a manner resembling "survival of the fittest" focusing on itself. Cancer cells receive information from their environment, such as the amount of oxygen or nutrients present, and then this data is transmitted through various biochemical pathways that eventually lead the cell to make a decision such as divide, move, die, or do nothing. The state-transition model implicit in the evolution of cellular automata capture this property. The logic and usefulness of using cellular automata to model cancer has been proven time and again, and the reader is directed to Chapter 1, the literature review, as well as Section 2.1 to learn more about previous models of cancer based on cellular automata.

The cellular automata used in the model described herein can exist in one of six states: Alive (healthy or cancerous), Apoptotic, Necrotic, Quiescent, Glycolytic and Dead. At time

www.manaraa.com

point zero in the simulation, there is a single healthy Alive state cell surrounded by empty grid sites. At every time step in the simulation, each currently alive or quiescent cancer cell is updated based on current environmental variables and the cellular automaton's internal rule set. These "rules" represent the phenotype of an individual cancer cell, and can change due to mutations. Each automaton is given its own phenotype (a collection of parameters and rules), and at each replication it is possible for the phenotype to be altered based on sustained mutations.

In this introduction I have outlined the biological relevance of each state with high level information as to how the state is represented in the model. More specific implementation details as to how the phenotype of each cell is defined, what transition rules are, how parameters can impact transition rules, what parameters were used and how overall growth occurs in the simulation can be found in Sections 3.2 and 4.2.

2.3.1 The automaton states

Alive

The initial cell in the simulation starts in the alive state. This original cell has no mutations and has therefore acquired no hallmarks. Throughout the simulation, cells that are not dead (by any means), quiescent or glycolytic are alive, regardless of if they have mutations or not. Whether a cell in the Alive state is cancerous or not is determined by its genotype and phenotype. These consist of a variety of parameters that impact the rules of the cellular automaton. Cells in this state are bound by things like growth factor, local vasculature, cell-cell adhesion and telomere length – all of which can be impacted by mutations that change cell rules.

Apoptotic

Apoptosis is the process of programmed cell death. The cell is deconstructed and packaged up for elimination by both the neighbouring cells and autophages, and this occurs in a coordinated, programmed manner. Apoptosis is a critical part of the cell lifecycle, as just as cells need to grow and divide, they also need to die in order to maintain homeostasis. In fact, misregulation of apoptosis causes a variety of diseases. AIDS and various neurodegenerative diseases are the result of too much apoptosis with a lack of proper cell replenishment, while cancer and many auto-immune diseases are riddled with a lack of apoptosis [180]. Apoptosis can be triggered by both intrinsic pathways, where signals inside the cell activate the process, or extrinsic pathways, where death ligands (molecules that bind to others) bind to death receptors on the cell surface. Regardless of which pathway initiates the process, caspases are activated and begin a chain reaction which destroys the cell [180]. The cell is then disassembeled and consumed by

المنارة للاستشارات
www.manaraa.com

both neighbouring cells and phagocytic cells [75].

Apoptosis can be triggered by a variety of mechanisms, not all of which are completely clear at this point. A key distinguisher of the apoptotic form of cell death is that while outside forces and trauma often initiate it, the end state of the cell is self-determined [182]. One well known initiating force of apoptosis is DNA damage, the most notable player in this pathway being tumour suppressor protein 53 (often referred to as p53 or TP53) [75]. p53 induces apoptosis after sensing large amounts of irreversible DNA damage by upregulating certain proteins that begin the caspase cascade [75].

Since the most well known inducer of apoptosis is DNA damage, we have chosen to model apoptosis as a state a cell can enter once a mutation has occurred. Once the cell is in the apoptotic state, it is also dead. The cell will have no further events scheduled for it and will not take part in any cell activities including consumption of oxygen or mitosis. The space where the cell resided is made available again in the system, to model the apoptotic cell being broken up and consumed, no longer taking up space. In this model, the apoptotic state can be entered only once a cell has sustained at least one mutation. During each event progression, the cell is checked for entering into the apoptotic state. Every time a mutated cell goes through the event process, there is a $(numberOfMutations)/evadeApoptosisFactor$ probability the cell will enter the apoptotic state. Cells with fewer mutations have a smaller probability of entering the apoptotic state since their damage is presumably less and may not be large enough to trigger apoptosis. As the cell sustains more damage the probability of apoptosis increases, both in cell biology and our model. The base $evadeApoptosisFactor$ can be found in Table 3.1.

Necrotic

In addition to apoptosis, cells can also die via necrosis. This process has long been thought to be the result of extreme damage to the cell – a process which is beyond the cells control. The term necrosis comes from the Greek "nekros" for corpse, and has been used to describe pathologic cell death [182]. New research is showing that this process may be under genetic control in some circumstances, similar to apoptosis, however the actual process of how the cell dies is different than in apoptosis [182]. In contrast to apoptosis, necrosis does not involve the organized dismantling of a cell but rather is characterized by cell bloating, loss of cell energetics and membrane rupture [182]. In fact, necrotic cells often remain and cause issues for the host. Gangreen is a potentially serious condition caused by a lack of blood supply in which cells die via necrosis and remain in the body, forming a dead mass of tissue.

المنارة للاستشارات
www.manaraa.com

While it was thought that this was a processes not controlled by the cell, various pieces of evidence are showing that may be incorrect, including the observations that necrotic death can be induced by certain ligands, necrotic death can be upregulated by various factors and that when apoptotic mechanisms are not available cells die via necrosis [64]. If a cell's ability to create ATP is damaged, these same events can occur, suggesting that loss of ATP creation can lead to necrosis over apoptosis, as ATP is needed for the process of self-degredation [182]. The relationship between apoptosis and necrosis is complex, however it is believed that the two lie on a continuum, with necrosis being the result of extreme insults to the cell, and apoptosis being the response to less extreme insults [182].

If ATP creation is lessened in the cell, it can lead to necrosis. The cytoplasmic membrane of the cell has ATP dependent ion pumps that maintain electrical balance in the cell. If their functioning is reduced, it can open the "death channel" in the membrane making it selectively permeable to anions. This leads to entry of cations and cytoplasmic membrane swelling and eventual rupture [182]. Lack of oxygen can cause this ATP depletion, as oxygen is needed to power the most efficient pathway for ATP creation. Necrosis is often observed in the center of tumours where nutrient and oxygen supplies are limited [182].

In our model, the necrotic state is entered whenever a cell severely lacks oxygen. Sometimes cells can survive in low oxygen environments by entering a quiescent state (described below), however when oxygen levels severely drop (such as at the center of a tumour) the cell cannot survive and, in our simulation, dies via necrosis. This is supported by both the observation that tumours have a central necrotic core [182], [61], [159] and that lack of ATP can lead to necrosis (ATP being created in a process that is oxygen dependent). If a cell enters the necrotic state, it no longer partakes in any new events (no growth and no oxygen consumption) however it remains in the grid. This is in contrast to the apoptotic state. If a cell dies via apoptosis, that space is reclaimed and new cells can enter that location. This models the biologic process where the cell is neatly packaged up and consumed. However, when a cell dies via necrosis, it bloats and the membrane becomes damaged, but the cell material often builds up, forming a necrotic core. Because of this, cells in the necrotic state remain physically present but inactive in the simulation, taking up space just as in cancer biology. A cell enters the necrotic state when there is not enough oxygen to survive actively or in a quiescent state, and this oxygen threshold varies based on how aggressive the cancer cell is and what form of metabolism it is dependent on. Baseline oxygen thresholds can be found in Table 3.1.

المنارة للاستشارات
www.manaraa.com

Quiescent

Cell division is tightly regulated within healthy tissue. Cells take part in a lifecycle, which ensures that DNA is completely replicated and that the cell splits into two equal daughter cells at each division [147]. Various pathways control the transitions between different cell states including G0 (a resting state where cells are neither dividing nor preparing to divide), G1 (a gap phase where cells grow in size and prepare for division), S (where DNA replication occurs and all chromosomes are duplicated), G2 (a second gap phase where the cell continues to grow in size and prepare to divide) and M phase (where the cell stops growing and divides in two). Cancer is often thought of as a disease of this cycle, since uncontrolled growth is one of the main characteristics of cancer ([108]). In fact, cancer cells often sustain mutations in pathways critical to regulating this cycle, throwing off the delicate homestasis and trapping cells in a state of constant replication. Cancer cells often have mutations in the RB (retinoblastoma) pathway or the p53 pathway, both of which can cause them to replicate indefinitely ([147]).

Quiescent cells are cells that are neither preparing for division nor actively dividing [160]. They can be in the G0 state of the cell cycle, in which case they can reenter the cycle if conditions favour it, or they can be post-mitotic where they are very unlikely to ever divide again. Studies of tumour microspheroids have shown that as the tumour grows it develops a necrotic core, followed by a quiescent ring and then a small proliferating rim of actively dividing cells [160]. Often this necrotic core and quiescent rim is the result of a lack of oxygen and/or nutrients getting to these cells. In fact, quiescent cells survive while consuming half as much oxygen as proliferating cells, and quiescent cells also have roughly half the rate of respiration [62], [63].

Since tumours are heterogeneous and contain not only actively dividing and dead cells, I have incorporated a state of “quiescence” into the cellular automaton model. Since oxygen availability is one of the factors in a cell becoming quiescent, and since this work focuses on modelling early cancer growth with an emphasis on biologically relevant oxygen modelling, I have used oxygen availability as the regulator of cells entering the quiescent state. Cells that do not have some minimum amount of oxygen available for consumption (specific values can be found in Tables 3.1 and 4.1), but who do have a minimum amount needed to survive, enter into a quiescent state. Cells that are quiescent cannot divide, even if they otherwise meet requirements for division (available space, telomere length, etc). As the oxygen concentration in the model is fluid, the cell could eventually exit the quiescent state if it again has access to enough oxygen. At this point, it becomes a normal proliferating cell again. Cells can also be forced into quiescence if they do not have any space to divide. Once a cell is completely

المنارة للاستشارات
www.manaraa.com

surrounded, if it has not attained the IGNORE GROWTH INHIBITION mutation, it has no way of dividing. These cells also become quiescent as there is no need for them to be consuming high levels of oxygen and nutrients or preparing to divide. Cells in the quiescent state do not have a mitotic event scheduled, but are still checked regularly for having enough oxygen to survive, still consume oxygen, and can transition to the Alive state (where they can proliferate) at any time if space and oxygen are available in the correct amounts.

Glycolytic

As mentioned in 2.2.7, cancer cells often transition to a glycolytic phenotype – one in which the cell preferentially upregulates glycolysis for metabolism. Glycolysis involves breaking down glucose to yield energy and acid, however it is negatively regulated by the presence of oxygen [65]. The presence of oxygen usually makes a cell perform aerobic metabolism which is 18x more efficient at producing energy molecules for the cell (ATP). In cancer there is an increase in glycolysis, which was first reported by Warburg in the 1920s (cancer cells upregulating glycolysis is now termed "the Warburg effect") [173]. This change has a few repercussions on the growing community. Firstly, glycolysis has a by-product of acid, making the environment the tumour is growing in highly acidic. Secondly, the cell is less dependent on oxygen since glycolysis requires significantly less oxygen then aerobic respiration, the process used by most healthy cells for ATP creation. It has been observed that cancer cells often use glycolysis even in the presence of oxygen, although it is less efficient, and the reason for this is still somewhat unknown. It was proposed however that cancer cells use glycolysis even in the presence of oxygen as an adaptation to intermittent hypoxia in early growth, as often oxygen levels will drop below that required for aerobic metabolism throughout growth [65]. Also, cancer cells that adapt to survive in this kind of an environment, one with high acid and low oxygen, have a selective advantage over nearby healthy cells allowing for faster growth and invasion [65].

Reprogramming of energy metabolism was identified as an "emerging hallmark" in the updated hallmarks of cancer [75]. I have included it in this model as a hallmark that can be acquired by cancer cells. This allows the cells to live in low oxygen environments, effectively eliminating their need for oxygen. This particular hallmark is actually a state the cellular automata cells can enter. When cells acquire this hallmark they automatically enter the "glycolytic" state and no longer require oxygen for growth. Cells that acquire hallmarks never lose them in this simulation, so cells in the glyolytic state remain in that state until they are forced to transition to another state. Of the other states, these cells can either become quiescent (if they run out of space to grow), apoptotic, or dead. They cannot become necrotic (in this simulation, death via lack of oxygen) as they no longer have an oxygen requirement [127].

المنارة للاستشارات
www.manaraa.com

Dead

The last state a cell can enter is the dead state. While other states mean a cell is dead (such as apoptotic or necrotic), the dead state is for cells that have been killed via random cell death or the immune system. Cells in this state cannot divide nor consume oxygen, but do remain in their grid location. For the sake of analysis, which mechanism kills the cell (random death or the immune system) is recorded, but has no impact on the cells behaviour and so these are not represented as separate states in the cellular automata.

2.4 Current treatment approaches and challenges

Currently, cancer treatment in Canada has a 63% five-year survival rate, in that the likelihood of surviving for at least 5 years after a cancer diagnosis is 63%. This is a broad figure, encompassing survival rates for all cancers, which actually vary widely (for example, thyroid is 98% while pancreatic is 8%) [33]. In Canada it is estimated that 22 people are diagnosed with cancer every hour, and males have a 45% lifetime chance of getting cancer with females close behind with a 41% chance [33]. Even with the seemingly high survival rate, there will still be an estimated 76,600 deaths from cancer in Canada alone in 2014.

Some cancers are more prevalent than others. For example, in men, prostate, colorectal and lung make up approximately 49% of all new cancer cases, while in women the top three (breast, lung and colorectal) make up 51% of all cases. That still leaves another approximately 50% of 191,300 new cancer cases this year, which are split between over 150 different diseases. Figure 2.2 shows just the top 20 different cancers that will impact Canadians this year. Not only is cancer varied across the population, it is extremely varied over the course of a person's lifetime. Different age populations have very different cancer rates, with childhood cancer diagnoses being largely made up of leukemia and cancers of the central nervous system, and elderly diagnoses largely lung and colorectal (see Figure 2.3 for a more complete breakdown). The vast diversity seen with cancer diagnoses shows that a treatment regime capable of treating multiple types at once is highly desirable. This research aims to give support to targeted combination therapy by simulating cancer in general, hopefully allowing the results to be broadly applied to many different cancers.

Much research is done every year to improve these statistics. This includes trying to better understand causes so that its incidence can be reduced and/or prevented, learning how to improve patient outcomes once a cancer diagnosis has been made, and how to improve quality

المنارة للاستشارات
www.manaraa.com

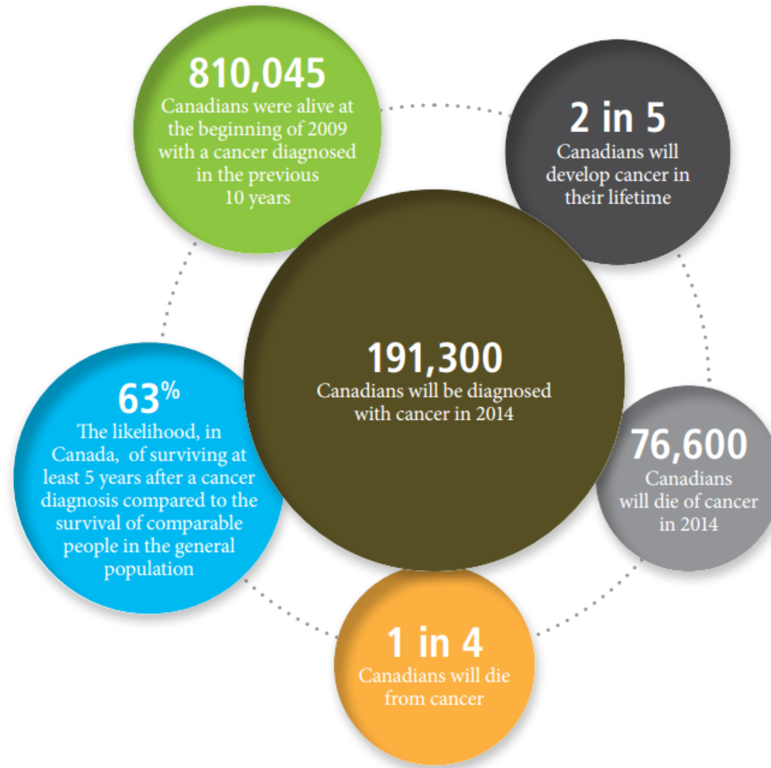

Figure 2.1: A summary of current cancer statistics in Canada. Reproduced with permission from [33].

of life and progression-free survival time. A large amount of money is funnelled towards this endeavour. The Canadian Cancer Society, which is the largest supporter of cancer research in Canada, donated $44,989,000 to cancer research in Canada last year. In the United States, the National Cancer Institute, which is that country's primary agency for cancer research, spends half of its annual 4.9 billion dollar budget on basic science and cancer specific research [119]. Research into cancer is helping, and mortality rates of most cancers are declining. Figure 2.4 shows the overall decreases in cancer mortality that have been occurring over the last 20 years.

These decreases are due to a variety of reasons, including more information on causes, better preventive care and better treatment options. Treatment options are given with a variety of intents, including to cure (curative), to relieve symptoms when a cure is not possible (palliative) and as preparation for other treatments. Currently treatment options are varied and include:

- **Surgery:** For most cancers the most effective treatment option, surgery involves physically removing the cancer from the body. Surgery can be used in a variety of ways including prevention (removing tissues with high risk of developing cancer), diagnosis, primary treatment (as the main treatment – for most cancers if possible this is the best

المنارة للاستشارات
www.manaraa.com

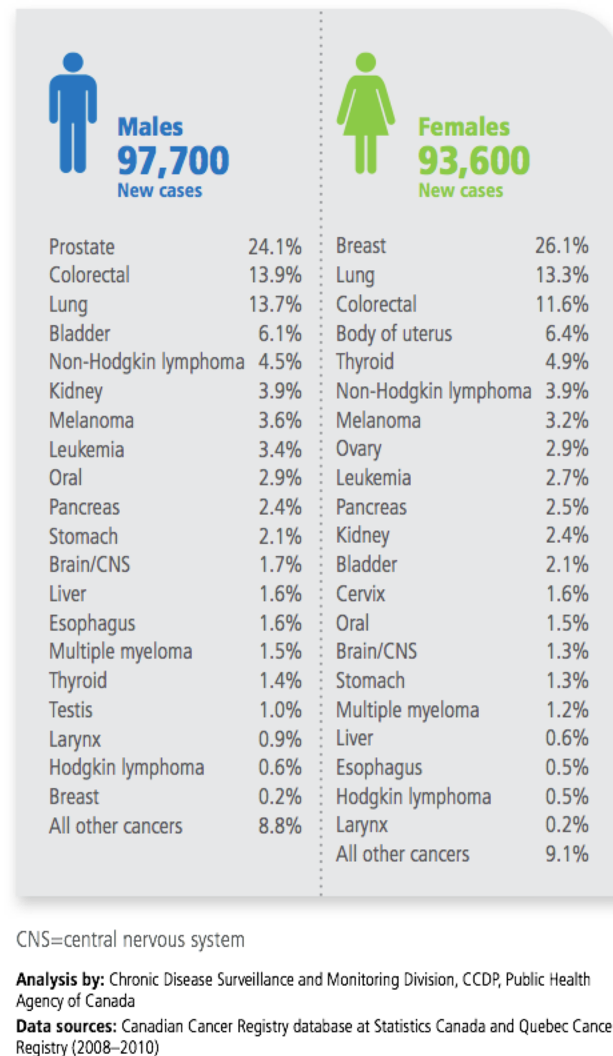

Figure 2.2: Percent distribution of estimated new cancer cases, by sex, in Canada in 2014. Reproduced with permission from [33].

chance for a cure), debulking (to stop it from severely hurting an organ) and relieving symptoms [110].

- **Chemotherapy:** The use of drugs to kill cancer cells. Often, chemotherapy drugs are given in combination with other drugs or treatment regimes, such as radiation therapy or biological therapy. Chemotherapy can be given in an attempt to cure cancer (often successful in some leukaemias [118]), prolong life or reduce symptoms (e.g. gemcitabine for pancreatic cancer). Traditional chemotherapy is cytotoxic in that it targets and kills cells that rapidly divide (one of the hallmarks of cancer). Since some healthy cells rapidly divide, such as bone marrow, blood cells, and hair follicles, chemotherapy often kills these cells as well, resulting in unpleasant side effects. These side effects are not only

المنارة للاستشارات
www.manaraa.com

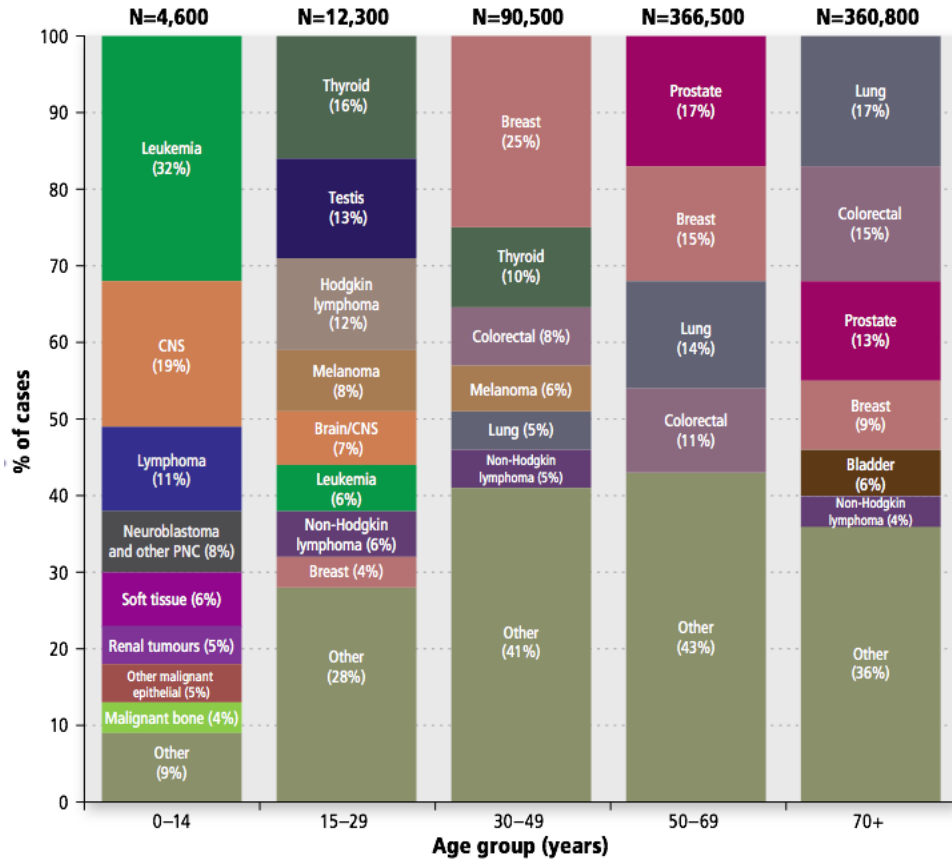

Figure 2.3: Cancer distribution for certain cancers in Canada by age. N is the total number of cases over 5 years (2006-2010) for each age group; CNS = central nervous system; PNC = peripheral nervous cell tumours. Reproduced with permission from [33].

unpleasant, but can be life threatening. Bone marrow suppression, for example, can lead to fatal sepsis as a result of a dramatic reduction of circulating neutrophils in the blood. Chemotherapy has other limitations including that many chemotherapy agents cannot easily pass through the blood-brain barrier and reach brain tumours. Also since tumour vasculature is often poorly developed, actually delivering the drugs into tumours can be challenging.

- **Radiation therapy:** Also called radiotherapy, radiation therapy uses ionizing radiation to kill cancer cells. Often radiation is used with chemotherapy, either before, during or after, and is also used after surgery to prevent tumour recurrence. Since ionizing radiation damages DNA and causes cell death of both cancerous and healthy cells, shaped beams are used from several different angles all pointed at the tumour so the intersection gives the tumour the highest dose, while sparing the surrounding healthy tissue as much as

المنارة للاستشارات
www.manaraa.com

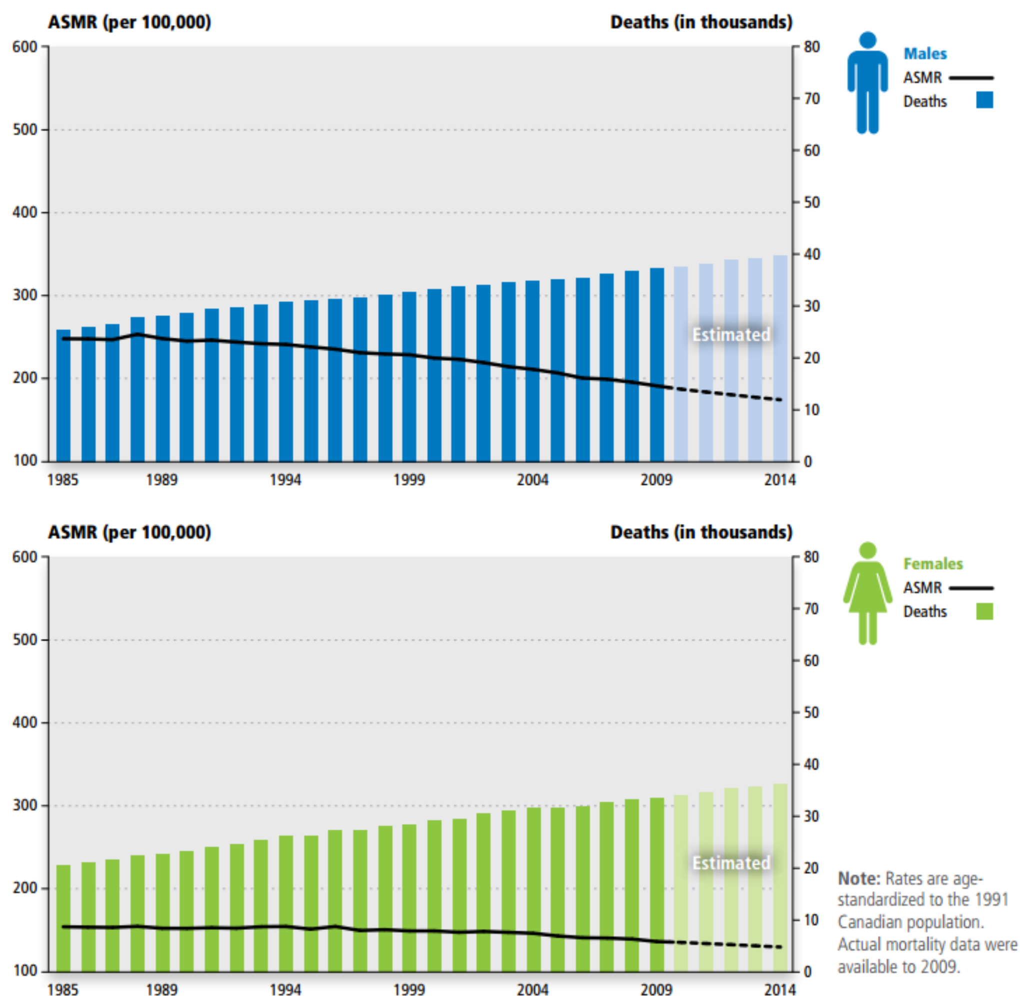

Figure 2.4: Deaths and age-standardized mortality rates (ASMR) for all cancers in Canada between 1985 and 2014. Reproduced with permission from [33].

possible. Some tumours respond very well to radiation, including germ cell tumours and lymphomas, while others are largely radioresistant, such as renal cell cancer and melanoma. In general early stage cancers are better targets for radiation therapy since they are still contained. Once cancer has spread it is usually incurable with radiation alone as it is not safe to radiate the whole body. One specific type of radiation therapy is brachytherapy, which is often used to treat prostate and breast cancer. In brachytherapy, radioactive seeds are placed inside the area to be irradiated and radiate outwardly to radiate the tumour without hurting surrounding tissue.

- **Hormone therapy**: Hormones are a type of chemical messenger produced by glands and organs in the body. They regulate a wide variety of processes including growth and development, sexual function and reproduction. Some organs are more responsive to

المنارة للاستشارات
www.manaraa.com

hormones than others – such as the breast, prostate and ovaries. When these organs have cancer, the disease is often hormone-driven, as hormones have a strong impact on gene expression. Cancers can be tested for their hormone sensitivity, and those that are sensitive can often be treated by either preventing the hormones driving growth from being created or preventing their impact on the tumour [35]. One of the most common uses of hormone therapy is for breast cancer. Hormone-receptor positive breast cancer has receptors to bind the hormone oestrogen, which when bound, stimulates growth. Tamoxifen also can bind into this receptor, blocking oestrogen from reaching the receptor, and stopping the growth stimulating impact of oestrogen. [35]. Therefore, tamoxifen can be thought of as stopping (or at least, slowing down) the "self growth" hallmark of cancer.

- **Immunotherapy** As discussed in Section 2.2.7, the immune system appears to be constantly on surveillance for early cancer growth, finding it and eradicating it. However, obviously this does not always work as cancer still grows and is diagnosed in 1 out of 3 people [33]. Immunotherapy involves activating or strengthening the immune system to fight the cancer itself. It was named "Breakthrough of the Year" in 2013 by the journal Science. Immune therapy can work via a variety of mechanisms including immune checkpoint modulation, immune cell therapy, vaccines and immune modifying agents [35]. The immune system is designed to only attack foreign cells and as such needs a way to recognize these cells. Proteins and other chemical signals are present on cells and can either attract or elude the immune system. In cancer it is possible the "checkpoint proteins" may be abnormal and assist cancer in avoiding the immune system. Blocking these checkpoints can let the immune system know that the cells are not meant to be in the body. This class of drugs was started in 2011 when ipilimumab was approved for the treatment of metastatic melanoma [117]. Another form of immune therapy, immune cell therapy, involves taking cancer-fighting T-cells out of the body, harvesting them in the lab, and then reinjecting them into the patient. Currently vaccines are also under development and involved in trials for the treatment of certain cancers, including the already-approved Gardasil™for prevention of cervical cancer, and sipuleucel-T for the treatment of metastatic prostate cancer [90]. Lastly, immune modifying agents such as antibodies, can be used to enhance a persons immune response to cancer. One such agent, bevacizumab, actually targets one of the cancer hallmarks – angiogenesis – and is approved for use in a variety of cancers [144].

- **Targeted therapy** Lastly, targeted therapy involves drugs that target certain molecules involved with cancer growth. Targeted therapy is part of the new medical field of "per-

المنارة للاستشارات
www.manaraa.com

sonalized medicine" where knowledge of an individual's genetics influences the course of treatment. Hormone therapy and immunotherapy are two examples of targeted therapy. Another common target for cancer therapy are proteins which are over-expressed in tumours. Vemurafenib is an example of such a treatment approved for use in metastatic melanoma [120].

The sheer size of cancer treatment options available is overwhelming. Often treatment options are very limited for any given cancer and are given according to algorithms that have evolved over time, however selecting possible treatment combinations to research can be much more complicated. The advances in our understanding of cancer have been helpful (most cancers have shown an increase in 5-year survival over the last 30 years, with initial diagnoses made in 1975-2003 [83]), and new techniques such as machine learning and data mining are making it easier to see connections between symptoms, genetics, treatments, outcomes and more. However, cancer research at the bench is still a slow and meticulous process. Drugs, or even combinations, need to be approved over the course of many years. The process of getting an idea to an approved, in-use drug can take anywhere from 10-15 years, and involves many stages including preclinical testing, new drug applications and three phases of clinical (human) trials [164]. In addition to a large number of treatments, there are over 200 different types of cancer [35]. With treatments being tested in combination, and combinations requiring testing for each different type of cancer, there is a staggering amount of possibilities.

With such a large number of possibilities and potentially years to determine their efficacy, it would be highly beneficial to reduce the number of options. This research seeks to assist with this problem in the following ways:

- pair more accurate oxygen modelling with traditional modelling techniques to give more biologically relevant results that show the impact of oxygen availability on tumour growth
- show that combination treatments will not always work in an additive manner, but rather multiplicatively beneficial or potentially detrimental
- be a proof of concept that some treatment pairs need not be tested for certain cancers, environments, patients, etc. as they can be ruled out by accurate modelling

These aims were achieved using the cellular automata model of cancer growth and lattice Boltzmann model of oxygen distribution in the blood presented in the rest of this thesis. This model simulates early solid-mass tumour growth with sizes and time lines that match those seen *in vivo*. Once the model was capable of growing an "average" tumour, the model was used to

المنارة للاستشارات
www.manaraa.com

simulate pairs of "treatments" via hallmark knockouts that represent a high level abstraction of combination treatments. Hallmarks were removed from the system in pairs to simulate the impact of a combination cancer treatment that is targeted at stopping that particular behaviour of the cell (e.g. knocking out the hallmark SELF GROWTH simulates a drug that stops the overgrowth of cancer, paired with knocking out ANGIOGENESIS to model stopping the development of new blood vessels). For an example of actual cancer treatments aimed at each hallmark, please see Appendix D. This initial work supported my research aims and was extended to include triplet and quadruplet knockouts, eventually simulating over 150 different "treatment" combinations. The results are outlined throughout Chapters 3 and 4 and conclusions and future directions are outlined in Chapter 5.

المنارة للاستشارات
www.manaraa.com

Chapter 3

Impact of paired hallmarks on cancer growth

Sections of this chapter have been reproduced from:
Butler, J., Mackay, F., Denniston, C., Daley, M. Simulating cancer growth using cellular automata to detect combination drug targets. *Unconventional Computation and Natural Computation*. Springer International Publishing, 67-79, 2014.

المنارة للاستشارات

www.manaraa.com

3.1 Introduction

As of 2004, cancer was the leading cause of death in the developed world and the second leading cause of death in the developing world [177], with about 12.7 million cases of cancer in 2008 alone [59]. While much time, money and research are dedicated to cancer the statistics are grim, with little to no progress in some cancers - for example, there has been no significant improvement in survival rates of pancreatic cancer in two decades [145]. We have created a highly abstract cellular automaton model of early cancer growth and a lattice Boltzmann model of oxygen flow in blood that investigates the impact of knocking out pairs of "cancer hallmarks".

As described previously, both Abbott *et al.* and Santos *et al.* have developed models looking at the hallmarks of cancer [1],[143],[113],[114],[115]. Santos *et al.* built on the work of Abbott by using a similar modelling approach, but focused on the impact of removing different hallmarks on tumour growth.

We have used model parameters and methods similar to those outlined in Abbott *et al.'s* work to build upon Santos *et al.'s* hallmark relevance study. We have implemented five of the six original hallmarks as well as two of the newly introduced hallmarks and enabling characteristics (focusing on those relevant during initial tumour growth), and knocked them out in pairs to see which have the greatest combined effect.

Henderson stated that "in the most general sense, combinations of therapies, whether drugs and/or other modalities, will always play an important role in the management of diseases for which there exists no single specific and totally effective treatment" [78]. Combination treatment involves pairing multiple treatments with the hope that two in combination will not just be an additive advantage but a multiplicative one. Targeted therapy involves identifying key pathways involved in cancer progression and creating drugs to target these pathways. This model simulates targeted combination therapy as we remove key cancer properties (hallmarks) in pairs and compare cancer growth rates to tumours with all hallmarks active. We hypothesize that knocking out pairs of hallmarks will not necessarily have the additive effect of knocking the hallmarks out separately but rather will sometimes have an even greater, potentially multiplicative, combined impact.

المنارة للاستشارات
www.manaraa.com

3.2 Methods

We have chosen to model two dimensional cancer growth where the biological cells are represented by cellular automata and the oxygen in the environment is modelled as a two-phase fluid using the lattice Boltzman method. Most models in the literature currently restrict themselves to two dimensions as it is more computationally feasible and since cancer does not grow in a sphere but rather an oblate spheroid. Our 2D simulation is easily compared with both existing models and 2D biopsy slices. Here we will present a high level outline of the method, and each section will be covered in more detail below. Pseudocode describing the simulation is provided in Section 3.2.4. The simulation begins with a single healthy cell at the center of a 2-dimensional grid. An event queue keeps track of cellular events, and initially a single mitotic event is placed on the queue for the healthy cell. Each event dequeued from the queue is another loop in the model and puts that cell through a life cycle. The cell is checked for whether it still has enough oxygen to survive, is in a location with growth factor, has access to blood, has space to grow, and has sufficiently long telomeres. If all of these checks are successful, or if mutations confer these abilities, the cell enters a mitotic event. This creates a daughter cell and potentially introduces mutations into the daughter or parent. Both cells have events scheduled for some point in the future and are added to the event queue, then the next event is popped. Oxygen is consumed by cells when they divide or every 25 time steps of the main simulation if they are not actively dividing.

3.2.1 Modelling the hallmarks

We have simulated 5 of the 6 original hallmarks and two of the recently added characteristics and hallmarks that were described in [75]. This model is specifically interested in premetastatic growth, when a patient has the greatest chances of survival. Therefore, we have not modelled the sixth hallmark, tissue invasion and metastasis. To keep our results credibly comparable with previous work in this field, we have not included inflammation or energy metabolism in our model. Our model is inspired by work in artificial life where agent based and mathematical models have been used to simulate cancer growth and angiogenesis [107], [28]. In general, each hallmark is modelled as a change to the rules of the life cycle or automata. For example, if a cell needs to be within growth factor to grow, at each potential growth time point it is checked if there is growth factor present at the cell's location, and it can only grow if there is. However, if the hallmark SUSTAINED GROWTH has been activated in that cell, the rule no longer applies and the cell can grow regardless of the availability of growth factor. Here we will briefly describe each hallmark put forth by Hanahan and Weinberg that we are including in this model, as well as their implementation. At the end of this section pseudo code describing

www.manaraa.com

the cell lifecycle is included.

Sustained growth

Normal tissue function is regulated by growth signals which instruct cells when to grow. These signals are involved in a signalling cascade that eventually instructs the cell to divide. Mutations in genes that act early in this pathway have been thought to be cancer causing [32]. To model this process, healthy cells can only grow within a predefined boundary of growth factor. Outside of this, cells can not actively divide unless they have the SUSTAINED GROWTH (SG) mutation.

Evading growth suppressors

Similarly, healthy cells are instructed when to stop growing, and eventually reach a state of replicative maturity (senescence). One way cells receive this instruction is via contact inhibition - pressure from surrounding cells signals that the cell no longer needs to grow. Contact inhibition is not present in cancer cells in cultures. Contact inhibition is modelled in our system by a space requirement. Healthy cells stop actively growing once there is no more space adjacently or diagonally available on the lattice. Cancer cells in our system can have the IGNORE GROWTH INHIBITION (IGI) hallmark activated which allows cells to grow even without space. If a cell with the IGI hallmark attempts to grow and is out of space, it competes for space with its neighbour. The cancer cell has a $1/c$ likelihood of success, success being that it divides and takes over its neighbour's space.

Avoiding programmed cell death

When a healthy cell sustains too many mutations it can undergo programmed cell death (apoptosis) often initiated by TP53 which is mutated in approximately 50% of all cancers, allowing cells to continue living and dividing even after substantial genetic changes. In our simulation, apoptosis can occur to any cell that has a single mutation. Since apoptosis is initiated when aberrant activity is detected, the chances of a cancer cell dying via apoptosis increases with each subsequent mutation (m/a likelihood of death). Cells with a mutation in this mechanism (referred to as AA throughout) cannot die by apoptosis.

Enabling replicative immortality

One unique property of cancer cells is their ability to grow seemingly forever. Usually cells are limited to a certain number of replication cycles, which research suggests is controlled

المنارة للاستشارات
www.manaraa.com

by telomeres, DNA caps, that shorten and prevent replication once at a certain length [31], [146]. We have modelled telomeres as an integer in each cell that decreases by one after every division, and must be greater than zero for division to occur. Cells with the IGNORE TELOMERE (IG) hallmark turned on will replicate regardless of telomere length.

Inducing angiogenesis

Typically in humans the vasculature is quiescent, in a sleeping state, and not actively building new blood vessels except during certain circumstances (such as wound healing). Conversely in cancer there is an "angiogenic switch" that becomes and stays active sometime during cancer growth and develops new vasculature for the tumour, delivering nutrients, removing waste, potentially delivering drugs and immune cells, and giving a pathway to spread [73]. Cells with the ANGIOGENESIS (A) mutation in our model are thought to be on the path of new vasculature, and any cells neighbouring an angiogenic cell have access to oxygen regardless of existing vasculature or surrounding oxygen levels. These cells also have a higher chance of being killed by the immune system, as immune cells travel through the blood system. Anigogenic cells and their neighbours have a $(ai) * (1/i)$ (ai being the angiogenesis immunity parameter and i being the immunity death parameter, both described in Table 3.1) probability of being killed by the immune system.

Genetic instability

The body has a remarkable set of machinery that detects and resolves changes in the DNA which often prevents large amounts of mutations being propagated from one cell to the next. Cancer cells however display an increase in the rate of mutation, often as a result of the breakdown of this protective machinery [91]. We model this behaviour with the GENETICALLY UNSTABLE hallmark (GU) which, when active, increases the chances of mutation in each mitotic event by a factor of (1/gif) where gif is the genetic instability factor parameter.

Evading immune destruction

There is a theory that the immune system is always watching for the development of cancer cells, and is able to eradicate them quickly before they become solid tumours. Therefore it is believed that when a tumour does form, its cells must have somehow avoided or limited the impact of the immune system. We model this characteristic of cancer cells with the AVOIDS IMMUNE SYSTEM hallmark (AI) which when activated lowers the chances of a cancer cell being killed by the immune system by a factor of aip, the avoids immune system parameter.

المنارة للاستشارات
www.manaraa.com

3.2.2 A note on hallmarks not included

We have not modelled the emerging hallmark of "deregulated cellular energetics" in this version of the model (however it was included in the next iteration of the software, described in Chapter 4). This potential hallmark involves a cancer cell's ability to change their energy metabolism pathway from the high efficiency aerobic respiration, to the lower efficiency anaerobic respiration. As this is an emerging hallmark, Hanahan and Weinberg have not decided that it is common enough to be a full hallmark. Also, this high level model is only looking at one aspect of the tumour microenvironment - the impact of oxygen availability on the hallmarks. We are not specifically modelling ATP creation or glucose, and as such we have not included this emerging hallmark at this time.

3.2.3 Event queue

Mitotic events are the driving force in this model. An event queue keeps track of all events scheduled for the simulation. Initially, a starter cell has a mitotic event scheduled for 5-11 time steps in the future. When the event is dequeued, the time is checked. If the time for the event is beyond the current time in the simulator the current time is updated to reflect the new time.

When a mitotic event is scheduled a time is calculated for that event. If the cell is to grow in a North, East, South or West direction, the time is scheduled 5-11 time points in the future (chosen by random number). If the cell is growing on a diagonal on the grid then the event is scheduled for 7-14 time points in the future, to account for the increase in spatial distance.

Since the cells in the simulation (the biologic cells, not necessarily "cells" in the classical cellular automaton sense) are impacted by rules that use probability, this model deviates slightly from standard cellular automata. Also, at every time step only a handful of cells are actually updated (those that were scheduled for a mitotic event). As pointed out by Abbott *et al.*, the cells could instead have a counter that is updated each time as it progresses through the life cycle, but since real biological cells are not updating that often it makes more sense to schedule their events for some time in the future and save the computation time [1].

3.2.4 Lifecycle pseudocode

1: $firstCell \leftarrow$ Cell
2: $time \leftarrow 0$
3: $firstCell.scheduleMitoticEvent()$

المنارة للاستشارات
www.manaraa.com

```
4:  eventQueue.push(firstCell)
5:  S ← 0
6:  while eventQueue not empty do
7:      currentCell ← eventQueue.pop()
8:      if currentCell.isAlive then
9:          time ← currentCell.time()
10:         dead ← currentCell.died {check for random cell death}
11:         if currentCell.isMutated then
12:             apop ← currentCell.apoptosis {check for death via apoptosis if cell is mutated
                (avoided if apoptosis hallmark is on)}
13:         end if
14:         cangrow ← false
15:         if selfGrowthHall or withinGrowthRange then
16:             cangrow ← true
17:         end if
18:         if spaceToGrow then
19:             space ← true {if ignore growth inhibition is on, it can compete for space if no
                space available}
20:         end if
21:         telo ← false
22:         if currentCell.getTelomere > 0 or currentCell.ignoresTelomereHallark then
23:             telo ← true
24:         end if
25:         if currentCell.withinBlood or currentCell.isOrWithinAngiogeneic then
26:             blood ← true
27:         end if
28:         if currentCell.killedByImmune != true or currentCell.avoidsImmune then
29:             stillAlive ← true
30:         end if
31:         if currentCell.enoughOxygen then
32:             oxygen ← true
33:         end if
34:         if cangrow and space and telo and blood and stillAlive and oxygen then
35:             daugtherCell ← currentCell.mitosis {daughter cell may be mutated during mitosis
                event}
36:             currentCell.mitosisOccured() {opportunity for mutation during a mitosis event}
```

المنارة للاستشارات
www.manaraa.com

```
37:         currentCell.scheduleMitoticEvent()
38:         daughterCell.scheduleMitoticEvent()
39:         eventQueue.push(daughterCell, currentCell)
40:       end if
41:     end if
42: end while
```

3.3 Lattice-Boltzmann implementation details

Lattice-Boltzmann algorithms [41], [156], have become increasingly popular as methods used to model coarse-grained fluid dynamics. These methods use a discretization of time, space, as well as velocity in order to solve for the motion of a set of partial distribution functions, $f_i(\mathbf{x}, t)$, each corresponding to a discrete velocity vector, $\mathbf{e}_i$, which evolve according to a discretized version of the linearized Boltzmann equation. Macroscopic fluid quantities are then determined via moments of these distribution functions. Lattice Boltzmann algorithms have even recently been paired with a cellular automata cancer model in the 2012 paper by Alemani *et al.* wherein they coupled a 2-state cellular automata model of cancer with a lattice-Botlzmann fluid model of nutrient diffusion [7].

In order to model the transport of oxygen dissolved in blood, we use a two component lattice-Boltzmann algorithm. Here, the quantities of interest are the total density $\rho = \rho_B + \rho_O$ which should satisfy both the continuity and Navier-Stokes equations, and the density difference between the two components $\phi = \rho_O - \rho_B$, which will evolve according to a convection-diffusion equation. In these expressions, ρ_O corresponds to the density of oxygen in the blood, and ρ_B is the density of the remaining blood constituents. In order to model these two quantities we follow the binary fluid approach of [126], [161], and introduce two sets of distribution functions, f_i and g_i, whose moments correspond to the physical variables,

$$\rho = \sum_i f_i$$

$$\rho u_\alpha = \sum_i f_i e_{i\alpha} \tag{3.1}$$

$$\phi = \sum_i g_i, \tag{3.2}$$

where $\mathbf{u}$ is the local fluid velocity. The time evolution of these distribution functions is governed

المنارة للاستشارات
www.manaraa.com

by the following discretized Boltzmann equations,

$$\begin{aligned} f_i(\mathbf{x}+\mathbf{e}_i\Delta t, t+\Delta t) - f_i(\mathbf{x},t) &= -\frac{\Delta t}{\tau_\rho}\left(f_i(\mathbf{x},t) - f_i^{eq}(\mathbf{x},t)\right) \\ g_i(\mathbf{x}+\mathbf{e}_i\Delta t, t+\Delta t) - g_i(\mathbf{x},t) &= -\frac{\Delta t}{\tau_\phi}\left(g_i(\mathbf{x},t) - g_i^{eq}(\mathbf{x},t)\right) + h_i(\mathbf{x},t)\Delta t. \end{aligned} \tag{3.3}$$

The first term on the right hand side describes a single time relaxation towards the equilibrium distribution functions, f_i^{eq} and g_i^{eq} [30], while h_i is a forcing term we have introduced in order to remove oxygen locally from the system when it is consumed by the cancer cells. To model these equations we use a nine velocity, 2D algorithm with velocity vectors,

$$\mathbf{e}_i = [(0,0),(\pm v_c,0),(0,\pm v_c),(\pm v_c,\pm v_c)]\,, \tag{3.4}$$

where $v_c = \Delta x/\Delta t$, with Δx and Δt corresponding to the lattice spacing, and timestep respectively.

In order to satisfy conservation of mass and momentum we choose the equilibrium distribution functions according to,

$$\begin{aligned} \sum_i f_i^{eq} &= \rho \\ \sum_i f_i^{eq} e_{i\alpha} &= \rho u_\alpha \\ \sum_i g_i^{eq} &= \phi \qquad (3.5) \\ \sum_i g_i^{eq} e_{i\alpha} &= \phi u_\alpha, \qquad (3.6) \end{aligned}$$

and define the higher moments and forcing term by the following equations,

$$\begin{aligned} \sum_i f_i^{eq} e_{i\alpha} e_{i\beta} &= P_{\alpha\beta} + \rho u_\alpha u_\beta \\ \sum_i g_i^{eq} e_{i\alpha} e_{i\beta} &= \Gamma\mu\delta_{\alpha\beta} + \phi u_\alpha u_\beta \\ \sum_i h_i &= F \\ \sum_i h_i e_{i\alpha} &= 0. \end{aligned} \tag{3.7}$$

Here $P_{\alpha\beta}$ is the pressure tensor, Γ is the mobility, which is related to the diffusion constant,

المنارة للاستشارات
www.manaraa.com

μ is the chemical potential difference between the fluid components, and F is an oxygen sink term describing the amount by which ϕ changes at a given timestep. With these choices, a Chapman-Enskog expansion of our Boltzmann equations (equations (3.3)) can be shown to reproduce the continuity and Navier-Stokes equations,

$$\begin{aligned} \partial_t \rho + \partial_\alpha \rho u_\alpha &= 0 \\ \rho \partial_t u_\alpha + \rho u_\beta \partial_\beta u_\alpha &= -\partial_\beta P_{\alpha\beta} + \eta \nabla^2 u_\alpha, \end{aligned} \tag{3.8}$$

as well as a convection-diffusion equation with oxygen sink term, F,

$$\partial_t \phi + \partial_\alpha (\phi u_\alpha) = (\tau_\phi - \Delta t/2) \left[\Gamma \nabla^2 \mu - \partial_\alpha \left(\frac{\phi}{\rho} \partial_\beta P_{\alpha\beta} \right) \right] + F. \tag{3.9}$$

to second order in the derivatives. Here, the viscosity, η, is defined according to $\rho(\tau_\rho - \Delta t/2)v_c^2/3$. For the pressure, $P_{\alpha\beta}$, and chemical potential difference, μ, we use the equations given in [126], [161], which were derived based on a free energy description of the fluid mixture,

$$\begin{aligned} \mu &= -\frac{\lambda}{2}\frac{\phi}{\rho} + \frac{\xi}{2} ln \left(\frac{\rho + \phi}{\rho - \phi} \right) \\ P_{\alpha\beta} &= [\rho\xi + \phi\mu]\, \delta_{\alpha\beta}. \end{aligned} \tag{3.10}$$

Here λ and ξ are parameters determining the state of the system; for $\xi < \lambda/2$ phase separation of the two components occurs. We therefore always work in a regime where $\xi > \lambda/2$ and the oxygen remains mixed in the blood. In this framework, the diffusion constant for the model, D, is given by,

$$D = \frac{(\tau_\phi - \Delta t/2)\Gamma\lambda}{2\rho}. \tag{3.11}$$

For the lattice-Boltzmann algorithm, we use a density of $\rho_B = 300 mol/m^3$ [57] throughout the simulation domain, and set the density of oxygen to $\rho_O = 9 mol/m^3$ at the boundary, representing a continual supply of oxygen to the system. We choose a diffusion constant, $D = 0.134 mm^2/s$ [166], corresponding to the thermal diffusivity of colon cancer. The oxygen grid is updated every 25 time steps (time in the cellular automata model) and each cell at that point consumes oxygen if it has not consumed already during a mitotic event. The consumption level for normal cancerous cells is 0.019. The lattice Boltzmann grid is finer than the CA grid, and so at each time step of the cancer cells, each cancer cell calculates the amount of oxygen present in all lattice Boltzmann cells mapped to it and gets a total oxygen value. These are dimensionless and are parameters chosen by fixing the consumption and diffusion rates, and

المنارة للاستشارات
www.manaraa.com

Table 3.1: Parameters used in simulations.

Description	Symbol	Value	Ref
Initial telomere length	t	100	[1]
Evade apoptosis factor	ev	10	[1]
Mutation rate	m	500	Chosen to lay between two used in [143]
Random death rate	d	10000	Simulation
Competition likelihood	c	10	[1]
Angiogenesis immunity	ai	10	Simulation
Avoid immunity	aip	10	Simulation
Immunity death	i	1000	Simulation (equal to random cell death in [143])
Genetic instability factor	gif	10	Simulation
Blood density	ρ_B	300 mol/m^3	[57]
Boundary oxygen density	ρ_O	9 mol/m^3	[111]
Thermal diffusivity	D	0.134 $mm^2 * s^{-1}$	[166]

iteratively determining what requirements gave the most physiologically relevant results.

3.3.1 Parameter values

Parameters (Table 3.1) for the model were either chosen from the literature or by searches of the parameter space. Parameters used in the models by Abbott [1] and Santos [143] were held constant (except mutation rate which was selected to be between the two values used by Santos) and other parameters were varied iteratively. The output was examined for concordance with *in vivo* tumours. We examined the physical structure of the tumours, and found that the tumours resemble the classic solid mass tumour structure of a necrotic core with a quiescent rim and proliferating rim [61], [159]. We also examined the growth rates and sizes of the tumours to compare to *in vivo* tumours. The tumours grow to roughly 2mm to 2.5mm before overwhelming the nutrients available and needing their own vasculature [60] and then grow to a maximum of 5.5mm. All calculations were done using an average cell diameter of 25 μm [163]. The tumours grow to this final size over a period of approximately two years, assuming a cell division time of 16-24 hours. This is in line with growth times for fast growing tumours, which reach clinically detectable size (0.2 cm to 1 cm) within two years [111].

المنارة للاستشارات
www.manaraa.com

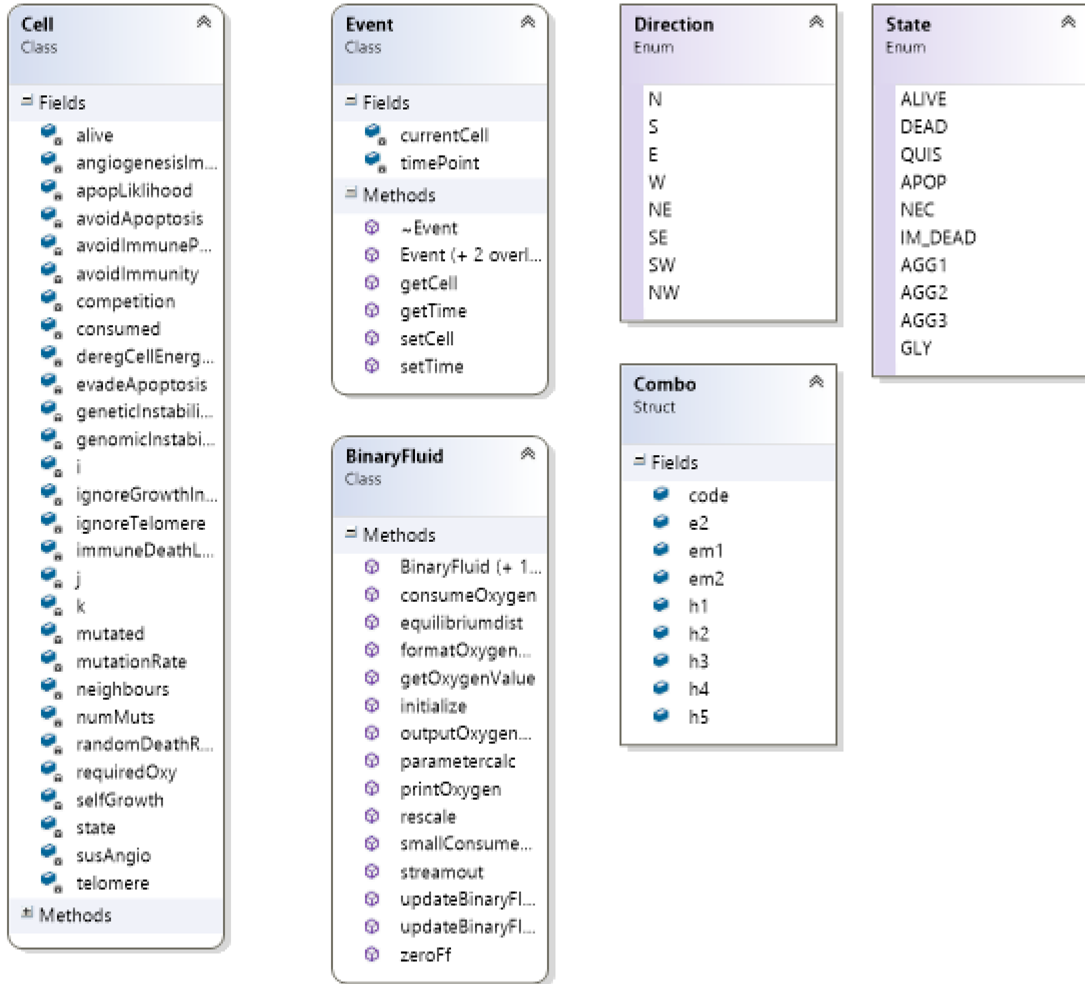

Figure 3.1: A simplified UML diagram showing the major objects involved in the simulation. This image was created using Microsoft Visual Studio.

3.3.2 Implementation details

The simulation software was implemented in C++ and compiled and run on the Sharcnet computing cluster at The University of Western Ontario. Figure 3.1 shows the main objects involved in the simulation.

3.4 Results

With all hallmarks active, every simulation run produced a tumour using parameters described in Table 3.1. A "tumour" is classified as a mass in which 99% or more of its alive cells have at least one mutation. The growth over time for a simulation with all hallmarks available can be seen in Figure 3.2.

المنارة للاستشارات
www.manaraa.com

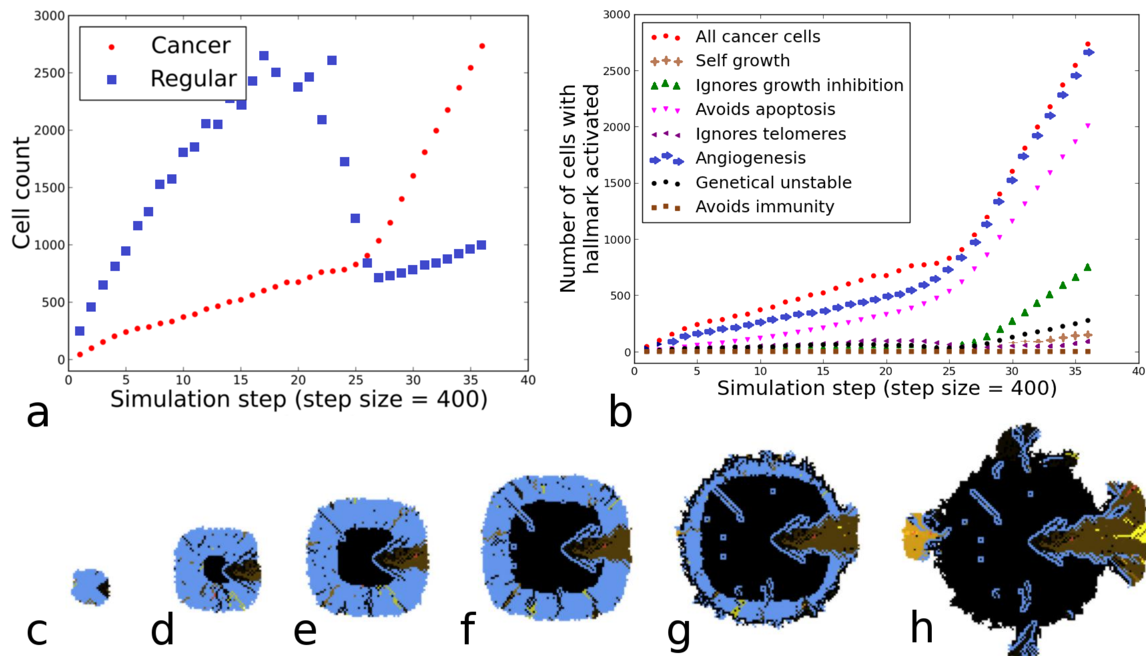

Figure 3.2: Cell counts and growth images for a tumour with all hallmarks available for activation. a and b: Total cell count for cancerous, non cancerous, and each hallmark is shown for an entire simulation. a) Regular verses cancerous cell growth b) Each individual hallmark growth and total cancer cell growth. c - h: Simulation of a colony of cells with all hallmarks available for activation at event steps 1 to 32. Dead cells are black, healthy cells are blue, all other colours represent some kind of unique cancer phenotype. Simulation steps: c) 1 d) 6 e) 12 f) 18 g) 24 h) 32

Figure 3.2a shows total cell counts throughout growth. Initially healthy cells grow rapidly, however around step 20 they sharply decline. Then, around step 25 cancer cells rapidly start to increase. This corresponds to a sharp increase in angiogenic cells as well as cells that avoid apoptosis, and relatively stable numbers of healthy cells. Figure 3.2c through h show the images produced from the same simulation. It can be seen in Figure 3.2c that initially healthy cells dominate the clone. Death is occurring, most likely due to random cell death or the initial fast killing of any cancerous cells by apoptosis and the immune system. By 3.2d we can already see the emergence of different cancer phenotypes. There are three major phenotypes present in the tumour from early on. The center of the tumour also begins to die at 3.2d. This is due to a lack of oxygen getting to the center of the tumour (causing necrosis). In 3.2g the outside of the tumour is also dying, as regular cells can go no further as they are outside the growth factor and blood range. In 3.2h we see the tumour is almost entirely cancerous, with a few different phenotypes protruding from the mass. This "fingering morphology", where the

المنارة للاستشارات
www.manaraa.com

border is not smooth but rough, is consistent with other models and histopathological observations [26], [18], [27]. It is believed that there are two forms of tumour invasion - either tumour cells outgrow normal tissue and expand as a bulk mass, or they form invasive contingents by intermingling with stromal cells. The fingering morphology is a consequence of this intermingling [88]. It has been noted that this fingering morphology looks like a crab, from which the word cancer was derived [88]. The fingering morphology is correlated with harsher microenvironments where only cells with particular phenotypes survive. This behaviour is evident in our model where certain subclones and phenotypes dominate the tumour.

To look at the impact of removing hallmarks, the total number of alive cancer cells at the end of the simulation when hallmarks were removed was compared to the total number of alive cancer cells at the end of a normal simulation, with all hallmarks available. Removing some hallmarks in pairs had very little effect on the growth of the tumour. In fact, knockout pairs SG & IGI, SG & AA, SG & IT, IGI & AA, IGI & GU, IGI & AI, AA & GU, AA & AI, IT & GU and GU & AI had no significant effect (all p values greater than 0.05 using Mann-Whitney U test) (see Figure 3.3 b and c for examples of final simulation image when cancer took over despite hallmark pair knockouts). Other pairs of hallmarks had such a large effect that a cancerous tumour never took over and the simulation ended prematurely as not enough cells survived. The normal cells continued to grow to the edge of the growth factor barrier, and then eventually consumed all of the oxygen in the system and the healthy cells died off. This can be seen in Figure 3.3 d and e. The following hallmark pairs significantly decreased cancer growth (as confirmed using Mann-Whitney U test): SG & A, SG & GU, SG & AI, IGI & A, AA & A, IT & A ($p=9.134\times10^{-5}$) and A & GU and A & AI ($p=9.083\times10^{-5}$). Other pairs had a smaller but still significant effect, using Mann-Whitney U test: AA & IT ($p=6.574\times10^{-4}$), SG & AA ($p=1.414\times10^{-3}$), IGI & AA ($p=3.642\times10^{-3}$), AA & AI ($p=5.665\times10^{-3}$) and GI & AI ($p=1.560\times10^{-2}$).

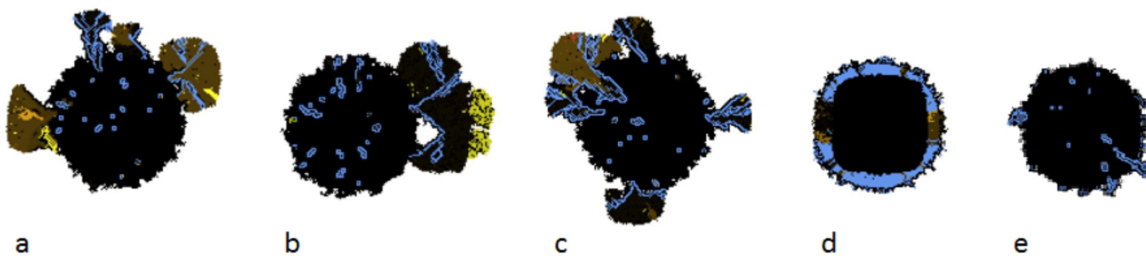

Figure 3.3: End of simulation images for 4 different hallmark-knockout pairs. a) no hallmarks knocked out b) IGI & AA c) IGI & IT d) SG & A e) IGI & A

المنارة للاستشارات
www.manaraa.com

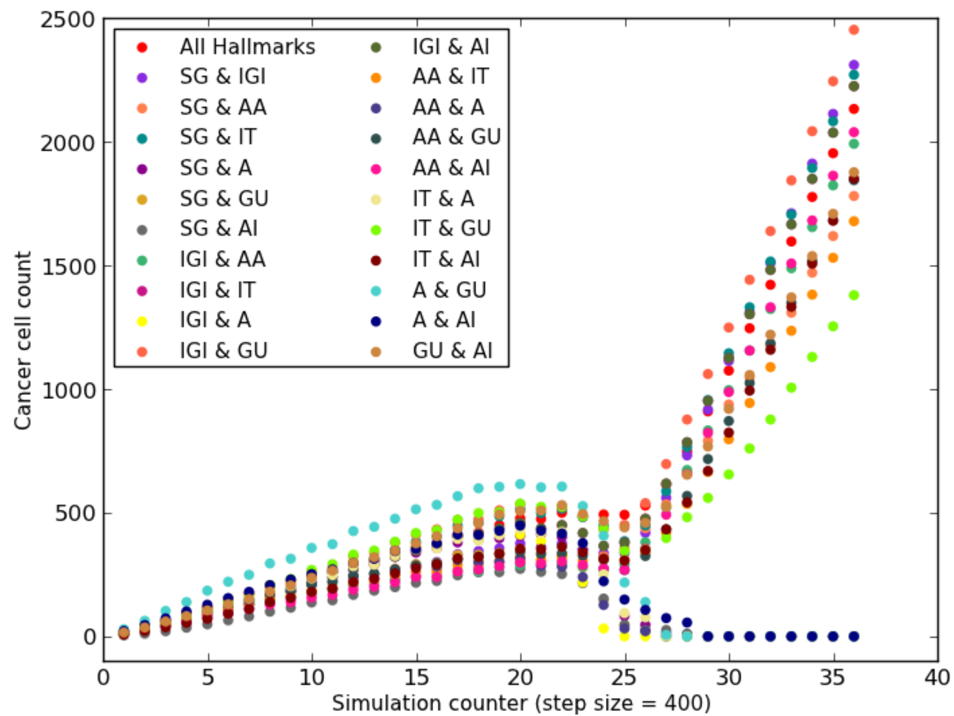

Figure 3.4: Total alive cancer cell count every 400 simulation steps is shown. Each hallmark-knockout pair was simulated and run 10 times. The average cell count from these runs was calculated and plotted

www.manaraa.com

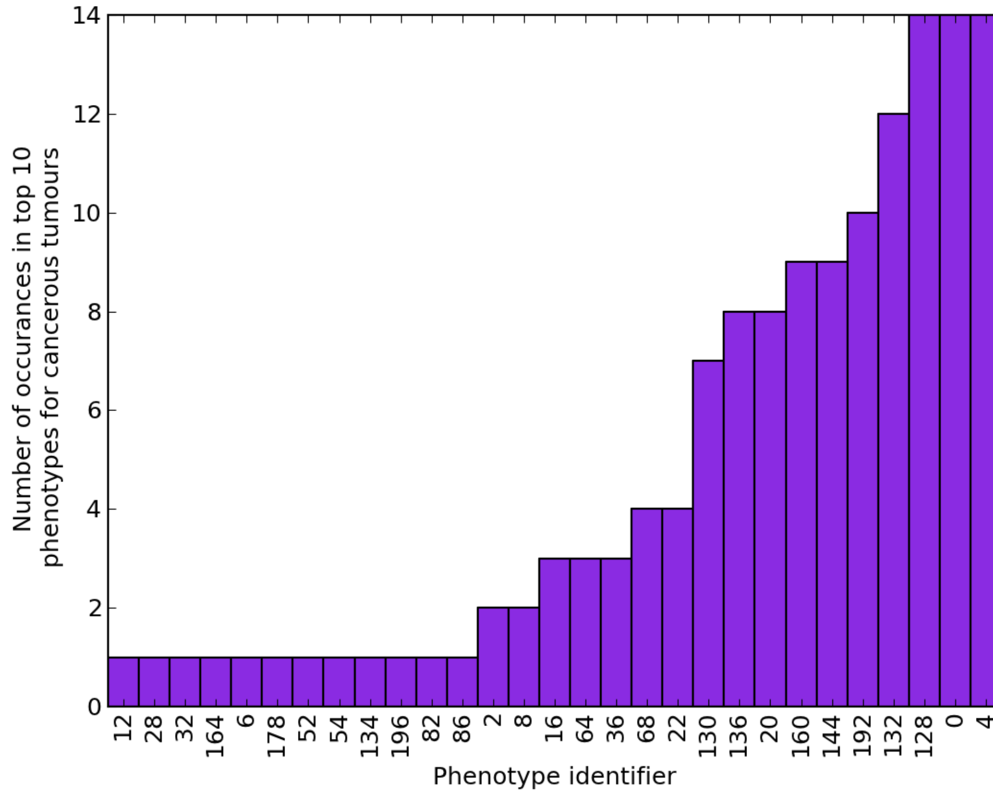

Figure 3.5: Each tumour at the end of the simulation had different phenotypes present. The top 10 phenotypes, by total number in the tumour, in each separate simulation were recorded at the end of simulation and totals were plotted

The effect of various hallmark pairs can be seen in Figure 3.4. This shows that some hallmark knockouts (A & GU, IGI & A, IT & A, SG & A, A & AI, SG & AI) do not result in a tumour. Cancer growth is fairly consistent across all of the simulations, regardless of knockout, until step 20. Here, all cell populations take a dip however certain simulations show strong growth after this point. The knockout pairs listed above however die off at this point, and these simulations do not result in a cancerous tumour.

Figure 3.5 shows a histogram of phenotypes that were in the top 10 phenotypes by cell count during the last stage of simulation for 14 unique simulations (knockout pairs that still resulted in a tumour). IDs can be mapped to phenotype using Table 3.2. While it is obvious that phenotype 0 (dead phenotype) will be present in large numbers in all runs, it is interesting that phenotype 4 and 132 are also present in every run in large numbers - these are cells with the ANGIOGENESIS hallmark activated and no other hallmarks, both alive and dead. Also, by the end of simulation almost all tumours have a large population of dead SELF GROWTH cells and AVOIDS

المنارة للاستشارات
www.manaraa.com

Table 3.2: Phenotype codes and the corresponding hallmarks present in the phenotype.

Code	Hallmarks Present
0	Healthy
128	Healthy dead
4	Angiogenesis
132	Dead, angiogenesis
192	Dead, self growth
144	Dead, avoids apoptosis
169	Dead, ignores growth inhibition, ignores telomers, avoids immunity
29	Avoids immunity, angiogenesis, ignores telomere, avoids apoptosis
136	Dead, ignore telomeres
139	Dead, avoids immunity, genome unstable, ignores telomere
22	Genome unstable, angiogenesis, avoids apoptosis
68	Angiogenesis, self grows
36	Angiogenesis, ignores growth inhibition
64	Self growth
16	Avoids apoptosis
8	Ignores telomeres
2	Genome unstable
86	Genome unstable, angiogenesis, avoids apoptosis, self growth
82	Genome unstable, avoid apoptosis, self growth
196	Dead, angiogenesis, self grows, dead
134	Dead, genome unstable, angiogenesis
54	Genome unstable, angiogenesis, avoids apoptosis, ignores growth inhibition
52	Angiogenesis, avoids apoptosis, ignores growth inhibition
178	Dead, genome unstable, avoids apoptosis, ignores growth inhibition
6	Genome unstable, angiogenesis
164	Dead, angiogenesis, ignores growth inhibition
32	Ignores growth inhibition
28	Angiogenesis, ignores telomere, avoids apoptosis
12	Angiogenesis, ignores telomere

المنارة للاستشارات
www.manaraa.com

APOPTOSIS cells. It is interesting that alive ANGIOGENESIS cells dominate, but the SELF GROWTH and APOPTOSIS cell group that dominates is dead. Also present in the majority of simulations in large numbers are dead cells with IGNORES GROWTH INHIBITION activated and IGNORES TELOMERES activated, as well as alive cells that are both ANGIOGENIC and AVOID APOPTOSIS.

3.5 Discussion

As expected, when all hallmarks are available for activation the tumour grows to the largest extent, presumably as these tumours can take advantage of all hallmarks and the different abilities each confers. It was also expected that knocking out 2 hallmarks would significantly lower the growth of cancer. We hypothesized that certain pairs would perform better than others, and that knocking out hallmarks in pairs could have more than just an additive effect.

Half of all tumours with SELF GROWTH knocked out did not result in a tumour. SG allows a tumour to extend beyond the normal boundary of growth. In areas of the body where growth factor is limited, this would be a very important hallmark. However if a tumour is growing where there is ample growth factor the hallmark may be less effective as a drug target.

All simulations with ANGIOGENESIS knocked out failed to result in a tumour. Similar to SG, ANGIOGENESIS allows a cell to live outside the predefined blood boundary. One reason the ANGIOGENESIS hallmark is more powerful is because it conveys benefit to not just the cell with the mutation, but surrounding cells, as all nearby cells benefit from the new vasculature.

The last hallmark that was knocked out in more than one pair that did not lead to a tumour is GENOME INSTABILITY. Since GENOME INSTABILITY can lead to all of the other mutations being activated more frequently this is understandable.

As evident in Figure 3.4, there is a bifurcation in total cell count - either similar to both hallmarks or almost none. This is because cell populations which result in a tumour show almost exponential growth and are not limited by oxygen or space due to acquired mutations. Cell populations that do not result in a tumour are limited by both of these factors, and so eventually almost all cells die as this overpopulation cannot be sustained by the normal vasculature.

It is interesting that of the phenotypes that dominated clones at the end of simulation (shown in Figure 3.5), many cells with a single mutation grew quickly but died off. Single mutation

المنارة للاستشارات
www.manaraa.com

phenotypes were largely present, but in dead cells. In contrast, the phenotypes that dominated and were still alive had multiple mutations. While multiple mutations increase the chances of death by the immune system and apoptosis, this suggests it still conveys a very strong advantage overall. This supports the hypothesis that knocking out multiple hallmarks, if you can find the correct pairs, will be better than single-target treatments.

Many knockouts did not prevent the tumour from forming. For example, all knockouts that included IGNORING GROWTH INHIBITION still resulted in a tumour, except for one (IGI & A). In our model IGI allows cells to grow even when there is no space around them, but this only conveys an advantage to internal cells. Cells on the proliferating edge always have space, and therefore removing it does not seem to hurt growth to a significant degree. This could be a limitation of the model as in reality the proliferating rim of a tumour may have space constraints from surrounding tissue.

Other limitations of the model include the fact that ANGIOGENESIS only provides a benefit to itself or cells immediately around it. In addition, it only provides an advantage while the cell is living. In reality angiogenic cells start the creation of blood vessels and those remain even if the cells die.

3.6 Conclusions

We have modelled the impact of cancer hallmarks, as proposed by Hanahan and Weinberg, on early tumour growth using a cellular automaton model of cancer cells and lattice Boltzmann methods for two phase fluids (oxygen in the blood) [74], [75]. Our results show that knocking out pairs of hallmarks does not necessarily have an additive effect. Santos *et al.* found that AVOIDING APOPTOSIS and IGNORING GROWTH INHIBITION were the most critical hallmarks independently when cells had a high rate of mutation, and they also found that IGNORING TELOMERES and SELF GROWTH had a small impact [143]. Looking at the impact of knocking out both AVOIDING APOPTOSIS and IGNORING GROWTH INHIBITION, we did not see a significant decrease in tumour growth. This is interesting as it is not what would be expected from the findings of knocking out singular hallmarks if one assumes linear combination of knockout effects.

We found that knocking out the ability for a cancer cell to SELF GROW and AVOID IMMUNE SYSTEM, as well as SELF GROW and be GENETICALLY UNSTABLE, prevent a tumour from growing. Neither SELF GROWTH nor GENETIC INSTABILITY had a great effect in the simulations done by San-

المنارة للاستشارات
www.manaraa.com

tos *et al.* (the immune system was not modelled in this work) however in combination they had a strong and significant effect. This supports our hypothesis that knowing the impact of individual hallmarks, which can be extended to individual drugs, does not necessarily give insight into the impact of combining those knockouts and drugs.

Lastly, we found that knocking out the ability for cells to INDUCE ANGIOGENESIS combined with any other hallmark prevented tumour growth. Research has been done into anti-angiogenesis drugs however the conclusions were not always positive. Patients still died from small tumours throughout the body as opposed to one large tumour, which was seen without the drugs [55]. It is hypothesized that without angiogenesis, other factors became important, such as metastasis. Perhaps the key is preventing cells from inducing angiogenesis and limiting other cancerous abilities. Currently trials are underway to test pairing anti-angiogenesis drugs with various chemotherapy drugs for multiple types of cancer including breast, colon and medulloblastoma [43], [84], [81], [112], [77] - in other words, combination therapy trials.

We have found that the effect of knocking out cancer hallmarks in pairs can have varying levels of success. This suggests that clinical research should be done into combination drug treatment as not all drugs that are strong individually will necessarily be strong in combination. Since cancer treatments can be physically and emotionally challenging for patients, knowing in advance what combinations will not be successful could greatly enhance the quality of life of people undergoing cancer treatment.

A natural question that arose after this analysis was what would be the impact of knocking out hallmarks in triplets, or even groups of four? Cancer patients are often treated using combinations of two drugs, as shown above, however clinical trials have previously been conducted, and some are currently under way, investigating the safety and efficacy of using three and even four drugs at a time [167], [171], [125], [122]. The results of knocking out pairs of hallmarks was enough to show that knowledge of a drug's individual effect may not confer knowledge of drug effects in combination. This leads to the question of whether drugs (or in this case, hallmark knockouts) in triplets or quads would also have varying levels of effectiveness, and whether some would have a multiplicative effect or not effect at all.

المنارة للاستشارات
www.manaraa.com

Chapter 4

Impact of triplet and quadruplet knockouts of cancer hallmarks on tumour growth

المنارة للاستشارات
www.manaraa.com

4.1 Introduction

In 2012 Santos and Monteagudo set out to determine the relative importance of different cancer hallmarks on the overall growth of a tumour [143]. They concluded that parameters such as mutation rate and telomere length had an impact on the relative importance of hallmarks.

If it is the case that tumour composition factors, such as mutation rate and cell division rate, affect the relative importance of individual hallmarks, how much more might that be the case when looking at the combinations of hallmarks? As shown in the previous chapter, not all hallmarks that are strong individually are strong in combination; in fact, the key combinations involved ANGIOGNESIS with other hallmarks, not EVADE APOPTOSIS. With pairs, it seemed that being able to grow in more areas, with hallmarks such as ANGIOGENESIS and SELF GROWTH was more important than avoiding death mechanisms (through EVADES APOPTOSIS or IGNORE GROWTH INHIBITION).

Research in the medical field recognizes the need for combination therapy and many clinical trials are underway looking at pairs of drugs for various cancer types. Pancreatic cancer, a cancer with an 8% survival rate, is a common combination drug target [33]. Gemcitabine (the standard treatment for advanced pancreatic cancer) is a nucleoside analog (meaning it mimics one of the building blocks of DNA). It causes apoptosis by interfering with DNA construction. For pancreatic cancer unfortunately it does not appear to have curative effects, but rather is palliative, allowing patients a better quality of life as they die. A better quality of life is a relative term however as gemcitabine causes neutropenia (a reduction in the number of neutrophils, a type of infection-fighting white blood cells) which makes patients susceptible to life threatening bacterial or fungal infections. Gemcitabine also causes flu like systems, fatigue, nausea, vomiting, weakness, hair loss and more. However, clinical trials are under way to pair it with other chemotherapy drugs which could have the potential to actually stop cancer growth, not just slow it down.

Gemcitabine was tested in combination with oxaliplatin in a trial conducted in 2005 [102]. Oxaliplatin is a platinum-based antineoplastic agent that is believed to inhibit DNA synthesis by creating inter- and intra-strand cross links in DNA, preventing it from being replicated [34], [71]. The study found that the combination had a statistically significant effect on progression-free survival (9.0 months in combination vs 7.1 months without, p=.04) and clinical benefit (38.2% vs 26.9%, p=.03). Despite a seemingly small improvement in survival time, patients lived approximately 30% longer free of progression with the combination treatment [102].

المنارة للاستشارات
www.manaraa.com

Since then more combination trials have been completed, including one in 2007 looking at gemcitabine alone and in combination with erlotinib for pancreatic cancer – a disease which had not shown an improvement in survival since the introduction of gemcitabine in 1996 [116]. Erlotinib is a reversible tyrosine kinase inhibitor which binds to cell receptors usually bound by ATP, causing signalling cascades to not be initiated [35].

The study tested the combination on 569 patients (half of which received the combination, and half of which received gemcitabine plus placebo) and found that progression free survival, one-year survival and overall survival were all significantly improved when the combination treatment was administered [116].

These combinations showed promise; however, after completion of the work in chapter two, I wondered if three or even four drugs would be a possibility, and if it would have a better effect. In 2009, following the study described above, another study was initiated that looked at gemcitabine and erlotinib in combination with a third drug, bevacizumab [167]. Bevacizumab, sold under the trade name Avastin™, is an angiogenesis inhibitor (stops the growth of new blood vessels). It inhibits vascular endothelial growth factor, one of the chemicals that signals angiogenesis. Of the 607 participants in the trial, the triplet combination was tested on 306 patients, and 301 patients received just the double combination treatment of gemcitabine and erlotinib. Unfortunately this combination did not lead to a statistically significant overall survival, but progression free survival was improved significantly (p=0.002) [167]. Perhaps more importantly, the triplet was well tolerated by patients and safety data did not differ from previously described safety profiles of the individual drugs. This suggests that triplet combinations are possible, however it also underscores the need to find the correct triplets.

Currently, trials are under way to address the question of what triplets are best as well as safe. VU (Vrije Universiteit) University Medical Center currently has an ongoing study looking at the effect of chemoradiation with gemcitabine in combination with panitumumab (trade name Vectibix™) for patients with pancreatic cancer [171]. Panitumumab blocks one of the receptors commonly over expressed in cancer [35]. It has also been used in colorectal cancer. This trial is looking at combining three different treatments - a traditional chemotherapy drug, an immunotherapeutic agent, and radiation.

Another currently ongoing study looking at triplet combinations is being performed by OncoMed Pharmaceuticals, Inc, the creator of a novel anti-cancer stem cell antibody named

المنارة للاستشارات
www.manaraa.com

OMP-59R5 (tarextumab). This drug appears to have anti-tumour activity through various mechanisms including interfering with tumour angiogenesis and cancer stem cell growth [125].

Even more recent is a study being performed by New Mexico Cancer Care Alliance that is currently recruiting patients for a trial of three different drugs for metastatic pancreatic cancer [122].

Lastly, studies have even been conducted with up to four different treatments in combination. A study from 2007 looked at the effects of combining oxaliplatin, fluorouracil, leucovorin and bevacizumab on previously treated metastatic colorectal cancer. The quadruplet combination of these drugs found a statistically significant improvement in survival [70].

These studies highlight the benefit of combination treatment beyond just two drugs. They also show that it is hard to predict what triplet or quadruplet combinations will have a statistically significant improvement over the drugs alone or the drugs in pairs. In addition, it is critical to determine the safety of combinations, as it is possible that the drugs in combination will have more severe effects. Because of this risk, it is even more critical to eliminate combinations that would either be too toxic to be tolerable and/or ineffective from the pool of possible treatments, before testing on humans.

As there are over 160 different approved chemotherapy drugs [40], not to mention different forms of radiation, selecting possible combinations is a daunting task, and one that cannot be done by brute force trials. Combinations could include multiple chemotherapeutic agents or chemotherapy drugs with radiation. The choice of which drugs and modalities to pair together is challenging, and the repercussions of incorrect choices are great in the world of clinical trials. However, with computer modelling, it is possible to quickly and safely test many possible combinations.

This model seeks to assist in this task of determining what drug and treatment combinations are most effective for the treatment of solid mass tumours. This model is a high level, abstract model of cancer growth, modelling cancer as a collection of 8 hallmarks, as described by Hanahan and Weinberg [74], [75]. Instead of modelling individual drugs, we have chosen to model the impact drugs could have if they successfully stopped a hallmark's ability. For example, drugs that inhibit angiogenesis, such as bevacizumab, can be modelled by turning off the angiogenesis hallmark in the model. In this way, we classify all drugs by their impact on a hallmark, and are able to model multiple combinations by looking at the combinations

المنارة للاستشارات
www.manaraa.com

of hallmarks they impact. Following this heuristic we have created a model of early tumour growth that models knocking out hallmarks in triplets and quadruplets to see which have the greatest effect on overall tumour growth. For a list of the hallmarks modelled and drugs that are either currently available or in trials to target them, please see Appendix D.

4.2 Methods

This two dimensional cellular automaton simulation models early tumour growth of a solid mass tumour. There is a highly abstract model of the immune system as well as tumour induced vasculature. In combination with the cellular automaton model of the individual cells, we have included a two-phase fluid model of oxygen in the blood around the tumour. This is implemented using lattice Boltzmann methods. The oxygen modelling implementation was previously described in Section 3.3.

This version of the model builds on that described in Chapter 3. The hallmarks are modelled in the same way as described in Chapter 3, as is the event process. One major update has been included in this iteration of the model – the inclusion of the "emerging hallmark" REPROGRAMMING ENERGY METABOLISM via a new cellular automaton state "Glycolytic phenotype". Hanahan and Weinberg introduced REPROGRAMMING ENERGY METABOLISM as an emerging hallmark in their updated hallmark paper in 2011 [75]. Healthy cells usually rely on a form of metabolism that uses oxygen to create ATP, the energy molecule of the cell. Cells that are in a low oxygen environment can switch to a form of metabolism that is more heavily dependent on gylcolysis, a process of ATP creation that is 18x less efficient but has less dependency on oxygen. Cancer cells have been found to reprogram their glucose metabolism to rely largely on this alternative mechanism. This was first observed by Otto Warburg, and has since been termed "the Warburg effect" [173], [175]. There are many different theories as to why cancer cells would choose this alternative mechanism, see Chapter 2 Section 2.2.7 for a discussion of this effect.

Life cycle pseudocode

The lifecycle event process is similar to that of Chapter 3, however with the addition of the glycolytic phenotype. An updated version of the pseudocode is included here for the ease of the reader.

1: $firstCell \leftarrow$ Cell

المنارة للاستشارات
www.manaraa.com

2: $time \leftarrow 0$
3: $firstCell.scheduleMitoticEvent()$
4: $eventQueue.push(firstCell)$
5: $S \leftarrow 0$
6: **while** *eventQueue* **not** *empty* **do**
7: $currentCell \leftarrow eventQueue.pop()$
8: **if** *currentCell.isAlive* **then**
9: $time \leftarrow currentCell.time()$
10: $dead \leftarrow currentCell.died$ {check for random cell death.}
11: **if** *currentCell.isMutated* **then**
12: $apop \leftarrow currentCell.apoptosis$ {check for death via apoptosis if cell is mutated. Avoided if apoptosis hallmark is on}
13: **end if**
14: $cangrow \leftarrow$ **false**
15: **if** *selfGrowthHall* **or** *withinGrowthRange* **then**
16: $cangrow \leftarrow$ **true**
17: **end if**
18: **if** *spaceToGrow* **then**
19: $space \leftarrow$ **true** {If ignore growth inhibition is on, it can compete for space if no space available}
20: **end if**
21: $telo \leftarrow$ **false**
22: **if** $currentCell.getTelomere > 0$ **or** *currentCell.ignoresTelomereHallark* **then**
23: $telo \leftarrow$ **true**
24: **end if**
25: **if** *currentCell.withinBlood* **or** *currentCell.isOrWithinAngiogeneic* **then**
26: $blood \leftarrow$ **true**
27: **end if**
28: **if** $currentCell.killedByImmune\ != $ **true or** *currentCell.avoidsImmune* **then**
29: $stillAlive \leftarrow$ **true**
30: **end if**
31: **if** *currentCell.enoughOxygen* **or** *currentCell.glycolytic* **then**
32: $oxygen \leftarrow$ **true**
33: **end if**
34: **if** *cangrow* **and** *space* **and** *telo* **and** *blood* **and** *stillAlive* **and** *oxygen* **then**

المنارة للاستشارات
www.manaraa.com

35: *daugtherCell* ← *currentCell.mitosis* {daughter cell may be mutated during mitosis event}
36: *currentCell.mitosisOccured*() {opportunity for mutation during a mitosis event}
37: *currentCell.scheduleMitoticEvent*()
38: *daughterCell.scheduleMitoticEvent*()
39: *eventQueue.push*(*daughterCell*, *currentCell*)
40: **end if**
41: **end if**
42: **end while**

4.2.1 Oxygen modelling

Please see Section 3.3 for information on the oxygen modelling in this simulation.

4.2.2 Knockouts

All eight hallmarks modelled were knocked out in all possible groups of three or four. All combinations of triplet knockouts (58) and quadruplet knockouts (70) were analyzed. Each simulation started with one healthy cell that had the ability to sustain a mutation. Only mutations not knocked out were able to be sustained by the cell. The full simulation, from initial healthy cell to death or tumour takeover, was completed 10 times for every triplet and quadruplet hallmark in order to perform statistical analysis.

4.2.3 Parameters

The parameters used in this version of the simulation are the similar to those described in Table 3.1 with the addition of the glycolytic phenotype oxygen requirement of zero and different, more aggressive levels of oxygen intake.

Parameters for the model were chosen from the literature and those that were not found in previous publications were chosen via a parameter search looking for strong concordance with *in vivo* tumours (please see Table 4.1 for values). Parameters used in the models by Abbott [1] and Santos [143] were held constant (except mutation rate which was selected to be between the two values used by Santos) and other parameters were varied iteratively. The output was examined for concordance with *in vivo* tumours. The tumours all resemble the classic spherical solid mass tumour structure consisting of a necrotic core with a quiescent rim and proliferating rim [61, 159]. The tumours also grow to roughly 2mm to 2.5mm before

المنارة للاستشارات
www.manaraa.com

Table 4.1: Parameters used in simulations.

Description	Symbol	Value	Ref
Initial telomere length	t	60	Similar to [153]
Evade apoptosis factor	ev	10	[1]
Mutation rate	m	300	Chosen to lay between two used in [143]
Random death rate	d	10000	Simulation
Competition likelihood	c	10	[1]
Angiogenesis immunity	ai	10	Simulation
Avoid immunity	aip	10	Simulation
Immunity death	i	1000	Simulation (equal to random cell death in [143])
Genetic instability factor	gif	5	Simulation
Blood density	ρ_B	300 mol/m^3	[57]
Boundary oxygen density	ρ_O	9 mol/m^3	[111]
Thermal diffusivity	D	0.134 $mm^2 * s^{-1}$	[166]
Oxygen requirement for glycolytic cells	Og	0.0	[127]
Oxygen requirement for healthy cells	Oh	0.015	Simulation
Oxygen requirement for quiescent cells	Oq	0.0075	[63]
Oxygen requirement for aggressive level 1 cells	$Oa1$	0.0136	Simulation
Oxygen requirement for aggressive level 2 cells	$Oa2$	0.0125	Simulation
Oxygen requirement for aggressive level 3 cells	$Oa2$	0.0115	Simulation

www.manaraa.com

overwhelming the nutrients available and needing their own vasculature [60] and then grow to a maximum of 5.5mm. All calculations were done using an average cell diameter of 25 μm [163]. The tumours grow to this final size over a period of approximately two years, assuming a cell division time of 16-24 hours. This is in line with growth times for fast growing tumours, which reach clinically detectable size (0.2 cm to 1 cm) within two years [111].

4.3 Results

When all hallmarks were available for activation (that is, a mutation could potentially give a cell the abilities conferred by every hallmark), cancer took over in 92% of simulations with a mutation rate of 1 in 300. In the cases when cancer did not take over, there were cancer cells but they were not able to overcome the growth factor limitations or the oxygen limitations imposed by the healthy cells in the area. In cases when cancer did take over, the simulation was stopped after an arbitrary number of steps because the tumour had begun to grow uncontrollably. The cancer cells represented greater than 95% of the alive cells in the simulation, which has been previously used as a measure for when cancer has officially "taken over" [1].

Figure 4.1 shows the growth progression over time for the simulation with all hallmarks available for activation. Figure 4.4a also shows the cell count for cancerous cells, healthy cells, and all mutations over time for this growth. Initially the simulation starts with one healthy cell that does not have any mutations. This cell has a mitosis event scheduled for some point in the future, and the event queue begins processing events.

It can be seen in Figure 4.1a that from one cell the group of cells has continued to grow into a spheroid with a necrotic core, quiescent rim (grey) and proliferating rim. A few pockets of cancer (green colours) are already visible in the proliferating rim. Dead cells are seen both in the necrotic core (making up 88% of the dead cell content of the tumour) and in the proliferating rim (here cells have died from apoptosis as they have acquired mutations). A few cells have died from the immune system, but only 3. The majority (68%) of alive cells are still healthy cells at this point.

The tumour continues to grow in this way with cancer cells on the rim pushing forward and the immune system and cell systems trying to keep them at bay via apoptosis. By time 403, in Figure 4.1b, the central necrotic core is growing and fewer healthy cells are surviving (56% of alive cells). More pockets of cancer have developed and at this point the heterogenous mix of cells contains over 115 unique phenotypes (combinations of mutations).

المنارة للاستشارات
www.manaraa.com

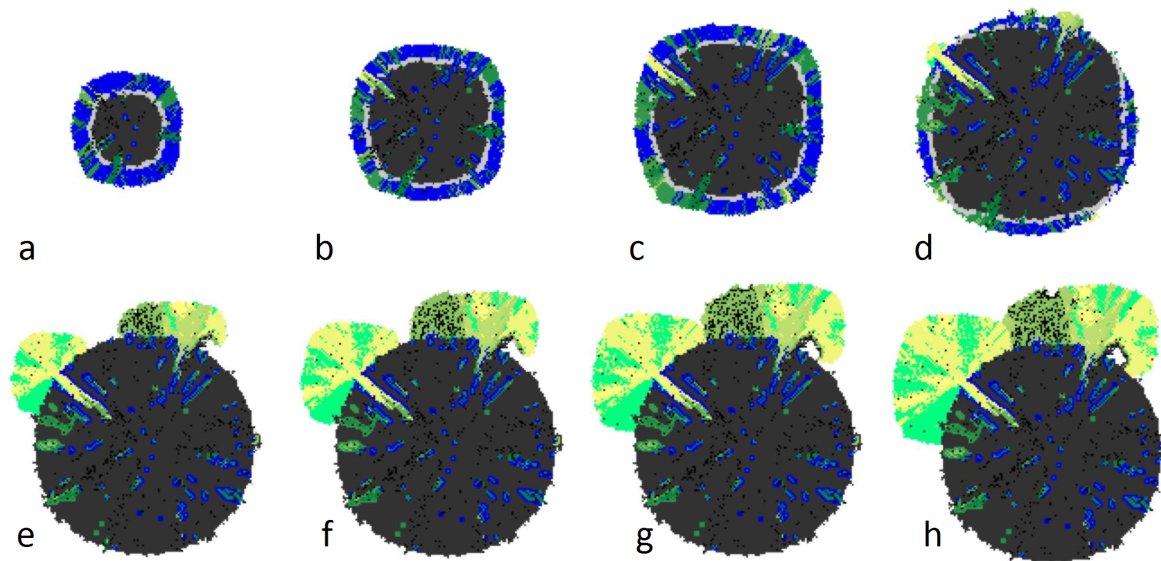

Figure 4.1: A sample simulation of tumour growth when all hallmarks are available for activation. This simulation shows the tumour at events 5000 through 40000 (increasing by 5000 steps per image), which corresponds to timepoints 292 to 864. Different colours represent different cell phenotypes. Black cells are dead (necrotic, apoptotic, random death or due to the immune system), blue cells are non-cancerous and alive, grey cells are quiescent (cancerous or healthy) and all other colours are some set of mutations (of which there are over 40,000 possible combinations). Time points are: a) 292 b) 403 c) 487 d) 590 e) 739 f) 795 g) 834 h) 864.

As growth continues it reaches a point where the healthy cells can no longer proliferate actively (between Figure 4.1d and 4.1e). At this point the actively dividing areas are only the two cancer subclones that are on the outer edge. Healthy cells remain in the center, those that are along a blood system provided by angiogenic cancer cells, however they have no space to divide. At this point the number of healthy alive cells plummets, making up only 18% of the total alive cell fraction. Cancerous cells make up the remaining 82%, with over 160 different phenotypes present. At this point, a few distinct phenotypes are dominating the tumour. The two most prominent phenotypes are: AI, GU, A, IT, AA, IGI, SG and GP, AI, GU, A, IT, AA, IGI, SG.

These clones continue to dominate until the simulation ends with two large subclones emerging from the mass of dead, healthy cells. In Figure 4.1h cancer cells make up 93% of the total alive cells, and almost all of the proliferating cells.

المنارة للاستشارات
www.manaraa.com

Growth of this tumour is considered baseline – a tumour growing with no "treatments" applied. At the last time point shown above, this tumour is roughly 3.875 mm using an average cell size of 25 μm [163]. This tumour takes roughly two years to get to this size. We compared the growth of tumours grown in an environment where some hallmarks are removed (in groups of three or four) to the growth of this ideal growth.

In order to simulate combination targeted drugs, the tumour was grown without the ability to ever activate groups of three and four hallmarks. Many drugs specifically target behaviours of cancer cells, such as their ability to self grow or induce angiogenesis, and so we have modelled the impact of these drugs by removing that hallmark from the system. Figures 4.2 and 4.4 show sample final growth images and the cell growth numbers over time for representative hallmark groups. Figure 4.3 shows the overall success of the hallmark knockouts with respect to stopping cancer growth.

For the triplet knockouts, 66% of them halted tumour growth. An example of what this looks like can be seen in Figure 4.2f. In these cases, some cancer cells would still appear but would not manage to dominate the growth. The simulation would end prematurely as there were no actively proliferating cells. Cancer and healthy cells may have been alive at the end, however either they did not have enough oxygen or space to be actively dividing. To determine the statistical significance of the changes seen when treatments were applied, the total number of alive cancer cells at the end of the simulations with treatments present were compared to the total number of alive cancer cells at the end of a regular simulation. Each treatment was simulated 10 times, as was the normal tumour simulation, and these sets of 10 numbers were compared. The number of alive cancer cells at the end of simulation was statistically decreased from that at the end of simulation will all hallmarks active (all 36 groups had $p < 0.01$, and 10 combinations had $p < 0.00009$ using the Mann-Whitney-U test).

Other groups of triplets caused a statistically significant decrease in cancer growth but did not completely stop it (see Figure 4.2d for an example of this growth). There were 10 combinations of three hallmarks removed that caused a decrease in growth however still resulted in cancer in some iterations of the simulation. These combinations had cancer in 60% of simulations or less with one exception having cancer in 90%. When the total numbers of alive cancer cells at the end of simulation were compared with those at the end of simulation with all hallmarks active, there was significantly less in these combinations ($p < 0.01$ using Mann-Whitney-U test).

المنارة للاستشارات
www.manaraa.com

Six sets of triplet knockouts had no significant effect on growth and cancer still took over to the same effect. An example of how the tumour looked at the end of simulation can be found in Figure 4.2b.

Lastly, four sets of hallmark knockouts caused a statistically significant increase in growth. These hallmark knockouts were: IGI, IT, GLY; IGI, IT, GU; IGI, GLY, AI and IGI, GU, AI. In these cases cancer still arose in 90% of runs, the same number of events progressed further in time, and ended with larger numbers of alive cancer cells ($p < 0.01$ using Mann-Whitney-U test). An example of the growth that resulted in these cases can be found in Figure 4.2h.

For the quadruplet knockouts, 79% of the combinations (55 of the 70 possible combinations) resulted in a complete halting of cancer growth (this can be seen in Figure 4.2g). None of these simulations resulted in a tumour and each was run 10 different times. Similar to the triplets, some alive healthy and cancerous cells existed but were not actively proliferating. This was significantly different than the growth with all hallmarks active ($p < 0.01$ using Mann-Whitney-U test).

Another 14% of knockout combinations resulted in a significant decrease in cancer growth. In these, cancer still dominated some simulations (at most 70% of the simulations run, as few as 20% in some cases) but the final amount of cancer present was significantly different than that present with all hallmarks available for activation ($p < 0.01$ using Mann-Whitney-U test). Figure 4.2e shows an example of a quadruplet knockout at the end of simulation with statistically less alive cancer cells than baseline.

One combination actually increased the amount of cancer in the simulation by a statistically significant amount ($p = 0.0086$ using Mann-Whitney-U test). The final growth of this quadruplet can be seen in Figure 4.2i. This was the knockout combination of IGI, IT, GLY, AI, meaning that the hallmarks present were SG, AA, A, GU. This tumour also grew to approximately 6 mm, a 33% increase in tumour size over baseline growth. Lastly, a few combinations had no statistically significant effect on the growth (4/70).

المنارة للاستشارات
www.manaraa.com

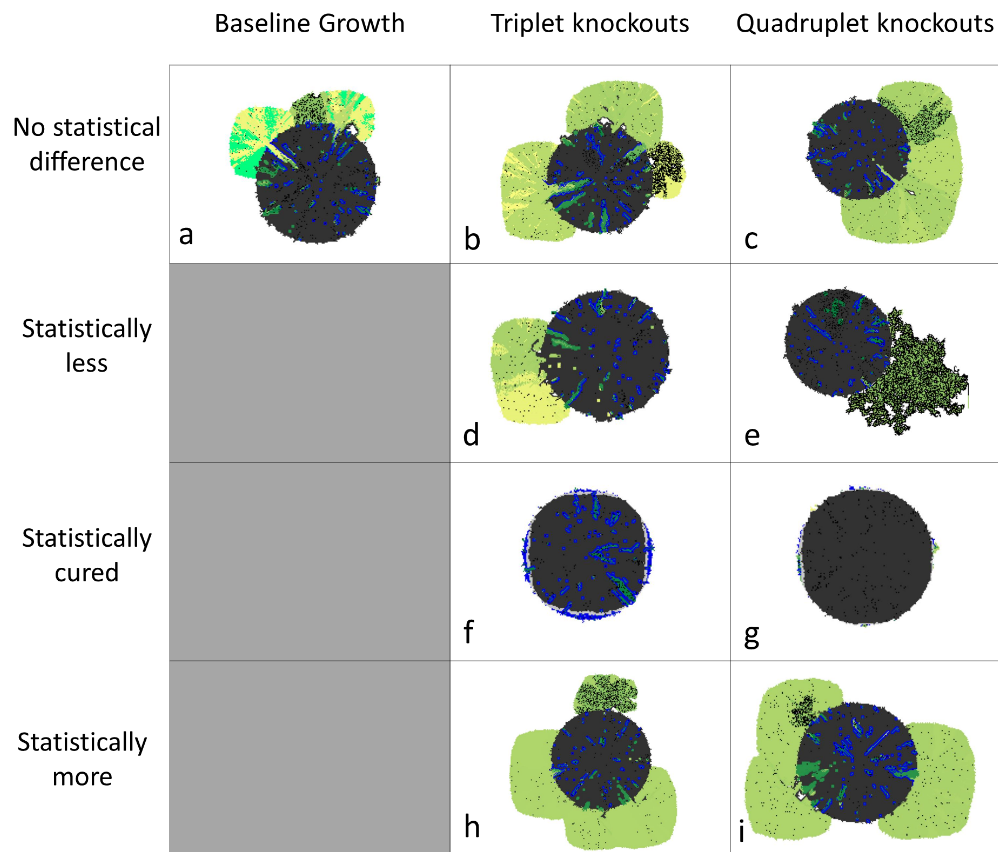

Figure 4.2: Examples of final growth images for a variety of growth outcomes. Each image is the last image produced by the simulation. a) all hallmarks available for activation; b) a triple knockout with no effect on final growth; c) a quadruple knockout with no effect on growth; d) a triple knockout with less total cancer than baseline; e) a quadruple knockout with less total cancer than baseline; f) a triple knockout that resulted in the simulation ending prematurely when no proliferating cells remained; g) a quadruple knockout that ended prematurely when no proliferating cells remained; h) a triple knockout that resulted in more overall cancer; i) a quadruple knockout that resulted in more overall cancer.

المنارة للاستشارات
www.manaraa.com

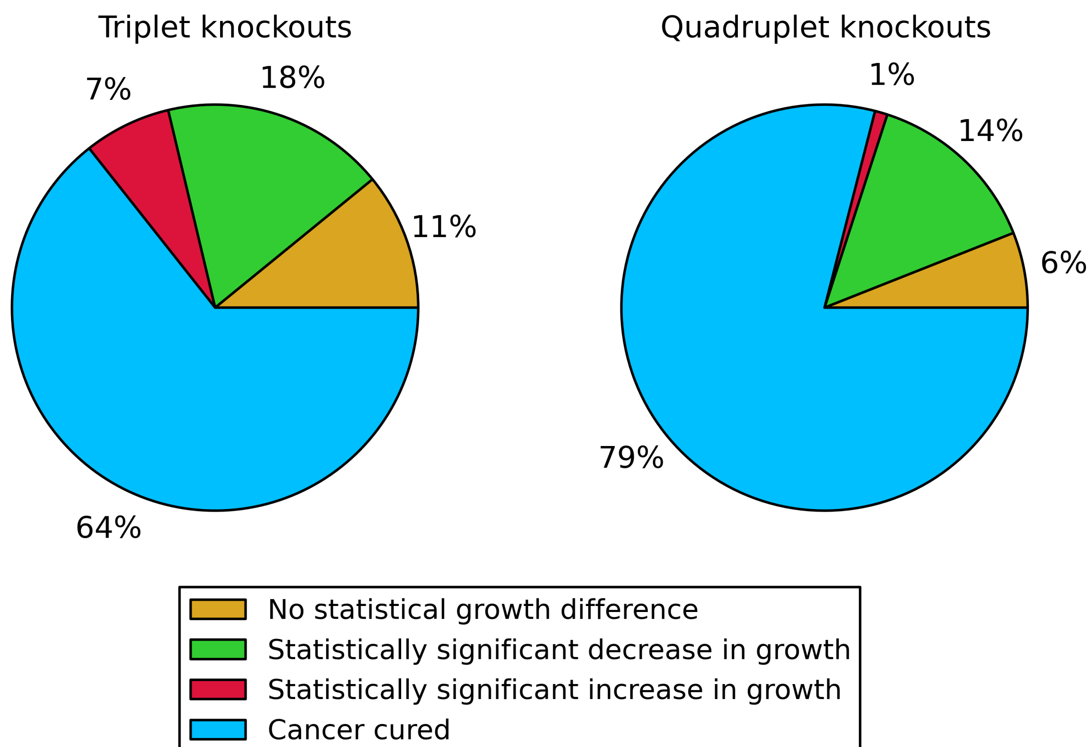

Figure 4.3: Comparisons of success of hallmark knockouts in triplets and quadruplets.

المنارة للاستشارات
www.manaraa.com

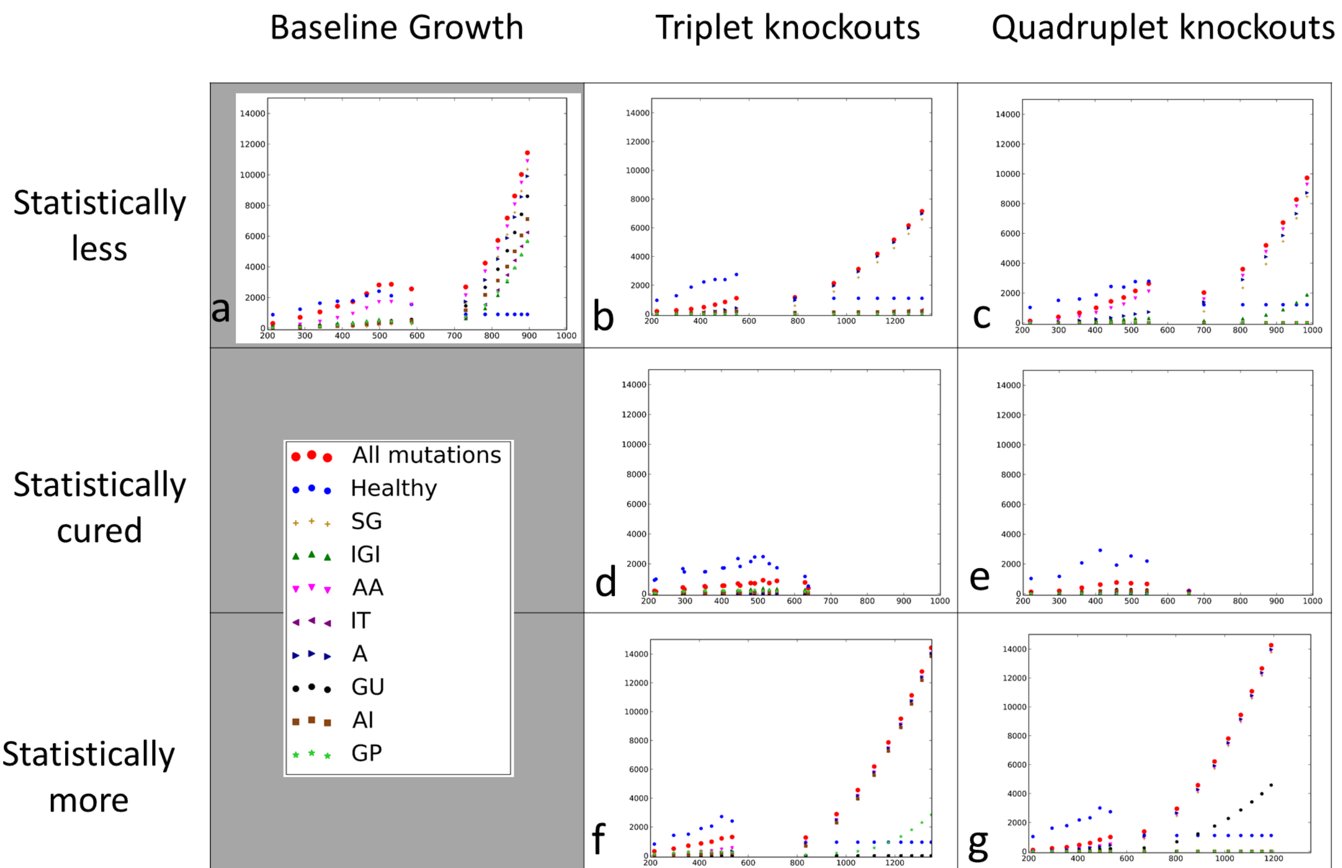

Figure 4.4: Comparisons of total growth over time of all hallmarks for triplet and quadruplet knockouts. For all graphs, the left axis is total cell count and the bottom is time step. a) growth of all cancer cells, healthy cells, and all mutations over time when all hallmarks are available for activation. b and c) examples of growth charts for hallmark knockouts which resulted in significantly less cancer, but often still some cancer. d and e) examples of growth charts for hallmark knockouts which resulted in "cured" tumours. f and g) examples of growth charts for hallmark knockouts that resulted in more cancer.

www.manaraa.com

4.4 Discussion

As expected, the addition of more "treatments" was correlated with less overall cancer growth. The triplets combinations effectively stopped the growth of cancer in 69% of cases, and the quadruplet combinations stopped growth in 79% of cases. There were fewer combinations that had no effect with the quadruplets as well (6% of quadruplet knockouts had no effect while 11% of triplets had no effect). It is expected that combination treatments will be better than individual, and, in general, this held true. However, interestingly, both the triplet groups and quadruplet groups had some combination(s) that statistically increased the cancer growth.

In the triplet groups, the hallmark knockout groups of: IGI, IT, GLY; IGI, IT, GU; IGI, GLY, AI and IGI, GU, AI all caused a significant increase in cancer growth. For the quadruplets, the knock out of IGI, IT, GLY, AI caused an increase. All of these groups share the IGNORE GROWTH INHIBITION hallmark; three of the five have the IGNORE TELOMERE hallmarks; three out of five have the GLYCOLYTIC PHENOTYPE and three out of five have AVOIDS IMMUNE SYSTEM.

While Hanahan and Weinberg identified 10 hallmarks and characteristics, they are not all necessarily equal in their ability to spur on cancer growth. In this simulation, IGNORE GROWTH INHIBITION appears to be less necessary for growth. The way this hallmark works is it allows cells to grow even when there is no space immediately around them, effectively ignoring the "contact inhibition" normally seen in healthy cells. This does not convey any benefit to cells on the outside proliferating rim of the tumour, where they always have space, by definition. Since the outside rim is usually the area with the most proliferation, it is understandable that IGNORE CONTACT INHIBITION would not be as critical to growth. However, *in vivo* the tumour may not have space on the proliferating rim once it bumps into other organs and internal structures. In this case, the IGNORE GROWTH INHIBITION hallmark might become much more important. In this simulation the tumour did not get to that stage and as such it appears to be a less critical hallmark.

The IGNORE TELOMERE hallmark allows cells to survive for much longer than a cell normally could, giving them effective immortality. However this immortality is only with respect to age, not surroundings. Cells with this hallmark can still be killed due to lack of oxygen, the immune system, apoptosis and random cell death. Cells with naturally short telomeres might benefit more from this, and it might allow them to propagate more mutations, however in this simulation it appears it is not critical to rapid cancer growth. The telomere length was chosen based on average life span of a cell, however depending on the cell type of a specific tumour

المنارة للاستشارات
www.manaraa.com

this hallmark may become more or less critical.

The glycolytic phenotype hallmark allows cells to survive in low oxygen environments. However, this mutation only benefits the cell that has the mutation and not the surrounding cells. The angiogenesis mutation conveys the same ability, living in an area of low oxygen as provided by the existing vasculature, however that mutation benefits the cell with it and all neighbouring cells, since new vasculature would provide oxygen to surrounding areas. Perhaps because of this weakness the glycolytic phenotype appears to be less necessary for growth, if cells can attain the angiogenesis hallmark. This model of glycolytic phenotype is very high level and ignores the side benefits of the phenotype, including the production of acid that may make an unfavourable environment for healthy cells, making more room for cancerous cells. In the future adding this layer to the model may show glycolytic phenotype to be more critical for cancer growth. However, in this simulation it appears to be slightly less necessary than others.

Lastly, present in 3 of the 5 knockout pairs that increased growth was avoids immune system. This hallmark decreases the probability a cell will die via the immune system. The immune system can kill any cancerous cell, however it is more deadly to cells that are along the tumour vasculature, as the immune cells travel through this. The probability of a cell dying via the immune system increases with increased mutations. This hallmark would not be as beneficial to cells with fewer mutations, so perhaps as more hallmarks are removed from the simulation, the benefit of this hallmark is reduced.

In contrast to examining what hallmarks were removed in stronger tumours, looking at what hallmarks were left leads to even more insight about the relative strength of hallmarks and their communal behaviour. In all five hallmark groups left after knock outs that resulted in more cancer growth there were the following hallmarks: self growth, angiogenesis and avoids apoptosis. Genetic instability was present in three of the five as well, and glycolytic phenotype was present in two of the five.

Self growth and angiogenesis both have very critical roles in this simulation, and in cancer in general. Healthy cells can only grow where there is enough growth factor. Cancer cells often acquire the ability to grow without this growth factor present, and in fact, it is such a strong influence in cancer growth that most chemotherapy drugs target the result of this ability (the result being rapid cell growth). It has been stated that self sufficiency in growth signals is the most fundamental trait of cancer cells [75]. In this simulation there was a boundary of growth factor. Beyond this healthy cells could still grow but with decreasing likelihood

www.manaraa.com

as the growth factor dropped off, and eventually it was not possible. In order to grow large enough to take up the vast majority of the simulation space the tumour would need some SELF GROWTH cells. Similarly, the existing "vasculature" in the system only delivered a sufficient amount of blood to a predefined area and dropped off outside of this. Cells therefore needed ANGIOGENESIS to continue getting large amounts of oxygen for survival. ANGIOGENESIS also is the only hallmark which conveyed a benefit to not only the cell with the mutation but surrounding cells, which may have made it even more beneficial to cancer growth (as would be the case *in vivo*). In reality, anti-angiogenesis drugs are looking promising in combination treatments. Bevacizumab was the first approved by the FDA, and since then more have been approved for various cancers (including sorafenib, sunitinib, pazopanib, and everolimus). Some trials have already been conducted pairing anti-angiogenesis drugs (most commonly bevacizumab) with other chemotherapy agents [70], [167]. The success of these and other trials has spurred more trials, and currently trials are underway pairing anti-angiogenesis drugs with one and up to five other drugs [81], [77] and [112].

AVOIDS APOPTOSIS was also a strong influencer of cancer growth. This hallmark prevents a cell from dying via apoptosis, regardless of the number of mutations the cell has sustained. Cells can still die via the immune system or lack of oxygen, but this in combination with angiogenesis (which was in all of the groups causing increased growth) would allow a cell to be close to immortal. Apoptosis is one of the cell's only built in safety mechanisms to stop the propagation of mutations and as such it is not surprising that removing it causes much damage.

All groups that still had cancer, whether decreased, increased or significantly unchanged, had both SELF GROWTH and ANGIOGENESIS hallmarks. Most had AVOIDS APOPTOSIS and many had IGNORE GROWTH INHIBITION. Since IGNORE GROWTH INHIBITION was knocked out in groups that lead to increased growth this is somewhat surprising. It underscores that fact that not all hallmarks always behave the same way. Clearly their pairing is crucial to their importance in the growth over time. In some cases and some situations (mentioned earlier) IGNORES GROWTH INHIBITION appeared to not be very beneficial. However, it was present in 10 out of 16 triplets that resulted in cancer growth.

While SELF GROWTH was present in all groups that still resulted in tumour growth, it was not necessary to knock it out in order to stop tumour growth. For the triplets, 15 out of 36 treatment combinations did not have SELF GROWTH knocked out and still completely halted growth. For the quadruplets, 20 of the 55 did not have SELF GROWTH and stopped growth. The exact same number for both groups did not have ANGIOGENESIS knocked out and still stopped growth. All

المنارة للاستشارات
www.manaraa.com

36 triplet and 55 quadruplet knock outs that stopped cancer growth were missing either self growth or angiogenesis however. According to this simulation, these are very powerful knock-outs however not the "magic bullet" of curing cancer either.

It was hypothesized that not all groups would be equal in their effect, and that more drugs would not necessarily be beneficial. Some triplet and quadruplet pairs had no significant change in the growth at all. What was more surprising was that some triplet and even quadruplet knock outs resulted in an increase in the cancer growth. One would assume that more treatments against a cancer would result in increased success, and in the vast majority of cases this is true, however this is not always the case.

4.5 Conclusions

This work shows that combination treatments, in this high level simulation, are beneficial for stopping cancer growth. It also underscores the critical point that not all combinations have this effect. This simulation showed that some hallmarks are stronger than others and removing the ability for weaker mutations to occur self-selects for only the strongest mutations possible to occur. This does not guarantee that the strongest phenotypes will arise, but it limits the possibility for lesser combinations. This has been seen *in vivo* as well when cancer recurs after treatment. For example, the childhood brain cancer medulloblastoma has very low survival after recurrence [23]. The initial treatment includes surgery, radiation and often chemotherapy, however if there is recurrence there is very little that can be done and very few documented survivors [23]. It is hypothesized that this is because the cells that survived the initial onslaught of treatments either were or are now resistant to those treatments, and so there is nothing more that can be done. The litany of treatments given selects for the strongest cells, and if they repopulate the tumour, it will be even stronger. However, Koschmann *et al.* suggest that retreatment with chemotherapy and radiation can be helpful in these patients and also recommends that novel combinations should be investigated for these cases [96].

This work found that both self growth and angiogenesis were strong hallmarks. Anti-angiogenesis drugs have been investigated for use in combination therapy, and many combinations are still undergoing clinical trials. A 2013 study looked at anti-angiogenesis clinical trials in stage II of the testing process [174]. The authors looked at 5 year reports of anti-angiogenesis trials in gynecological cancers and concluded:

> These anti-angiogenic drugs while used either alone or in combination with chemotherapy, presented mixed results in treating gynecological cancers. The real challenge

www.manaraa.com

> is how to take best advantage of the anti-angiogenesis hypothesis for therapeutic benefit. Much remains to be done before these molecules work efficaciously in treating gynecological cancer. [174]

This simulation clearly emphasis this point. While knocking out angiogenesis was often beneficial (similar to SELF GROWTH) the real key is combining it properly. It was not sufficient to knock out either of these two hallmarks, and some combinations that were successful in halting cancer growth did not involve them. Overall however, they appear to be quite strong, and when paired correctly in a combination, resulted in decreased growth.

In addition to combining the hallmarks correctly, it is important to consider the role of more specific details than this high level simulation shows, such as tissue type, patient genetics and history. In some circumstances, for example a tumour growing in a tissue-dense area, other hallmarks may be more beneficial (like IGNORE GROWTH INHIBITION). In areas that are oxygen rich, ANGIOGENESIS may be less beneficial. All of these factors underscore the need for research into what combinations are best and when, and point away from a "one-sized fits all" approach to medicine. With increasing interest in "individualized medicine" there should be more focus on modelling and detecting, for a particular case, what combinations are best. This simulation acts a proof of concept that not all combinations are created equal, and that different circumstances (in this high level case, oxygen availability) could change the efficacy of a treatment course.

المنارة للاستشارات
www.manaraa.com

Chapter 5

Conclusions

المنارة للاستشارات
www.manaraa.com

It is known that cancer is a leading cause of death and suffering throughout the world, with much money, time and energy going towards the search for a “cure” [119], [33]. However, there exists no single cure for this group of diseases, and as such, it is critical that researchers look into multiple treatment options, building an arsenal against cancer consisting of different chemotherapy drugs, radiation, surgery, hormone therapy and any other individually-successful treatment types. In the 1969 Cancer Research paper *Evidence that drugs in multiple combinations have materially advanced the treatment of human malignancies*, Henderson and Samaha state:

> In the most general sense, combinations of therapies, whether drugs and/or other modalities, will always play an important role in the management of diseases for which there exists no single specific and totally effective treatment [78].

For over 40 years we have known that combination therapy is critical for the treatment of this group of diseases, however much more needs to be done to identify which combinations are likely to be most effective with the least toxicity. Clinical trials are continually evaluating this question however they are lengthy (taking up to 15 years to go from idea conception to drug combination approval), costly (estimates range from US$1.3 billion to US$1.7 billion [44]) and potentially dangerous to patients [102], [70], [167], [125],[122], [171], [81]. In 2012, Rejniak and Anderson published a paper on the current state of the art of cancer modelling, and outlined why we need to move beyond the traditional reductionist approach of studying cancer, and look at modalities that can model cancer across scales [140]. Computer simulations of cancer have existed for over 50 years, however it is only recently they have gained in popularity as computer power and memory have begun to match researchers ambitions for simulations. Now it is clear that these computer simulations of cancer growth can yield accurate results and be a strong additional tool in the fight against cancer.

This work aimed to bridge two scales – the microscopic scale of the cell and the mesoscopic scale of blood flow around a tumour – in order to make a biologically relevant model of cancer growth that could be used for cancer treatment investigations. Using cellular automata as cancer cells and lattice Boltzmann methods for fluid dynamics, this simulation proved it could grow a tumour from one healthy cell in a reasonable amount of time, up to a biologically consistent size. Once a working model existed, I wanted to answer the question of how combinations of treatments would impact growth. Specifically, were good individual treatments always better together? Was a combination always the best from a strictly cellular growth level? In order to address these questions, it was necessary to model the treatments in a broad way so the model stayed high level and applicable. As such, “treatments” consisted of removing from

المنارة للاستشارات
www.manaraa.com

the simulation various cancer hallmarks, the critical cell changes that make cancer what it is, as proposed by Hanahan and Weinberg in 2000 and revised in 2011 [74], [75]. These hallmarks are often targeted using drugs (for example, many chemotherapies target the ability for a cell to SELF GROW) and so represent a good high level approach to modelling various cancer treatments. Information on existing drugs that target each individual hallmark is available in Appendix D.

I used this concept of removing hallmarks to model the impact of over 150 different combinations of abstract "treatments" to show the challenges and opportunities inherent to combination treatment of cancer. Building on the work of knocking out individual hallmarks presented by Santos *et al.* [143], paired knockouts were initially investigated, and revealed that individual knockouts in combination are not necessarily additive, but can be much more powerful when paired correctly, having a multiplicative, and significant, impact on overall growth. This lead to the natural question of "is more better"? Triplet and quadruplet hallmarks were then knocked out in combination. These groups revealed even more interesting results. They validated the earlier work, that some combinations are more than additive, but also revealed that some combinations are detrimental, causing more harm than leaving the tumour completely untreated.

The end question after applying a treatment was always did the treatment stop cancer growth, lessen it, have no effect, or enhance it. The overall results of these questions for all pairs, triplets and quadruplets can be seen in Figure 5.1. As more treatments were added, more were successful at completely eliminating the cancer. This is to be expected as we would hope removing many of cancer's abilities would weaken it. However, as more treatments were added the potential for the treatments to have negative interactions grew. Paired treatments never allowed the cancer to grow more than it did without any treatment, however some triplets and one quadruplet actually caused an increase in overall cancer growth.

In addition to looking at overall growth, I wanted to know what hallmarks occurred together most often in "curative" treatments. In order to determine this, I assigned scores to all pairs of hallmarks in every treatment. Any pair of two hallmarks that occurred together in a treatment group (whether it be a pair, triplet or quadruplet) that cured cancer was given a score of two. Any pair that occurred together in a treatment that significantly reduced cancer, but did not stop it, was given a score of 1. Any pair that occurred together in a treatment that had no effect was given a score of 0. Any pair that occurred together in a treatment that led to a statistically significant increase in cancer growth was given a score of -5. Scores for all pairs were summed and the results can be found in Figure 5.2.

المنارة للاستشارات
www.manaraa.com

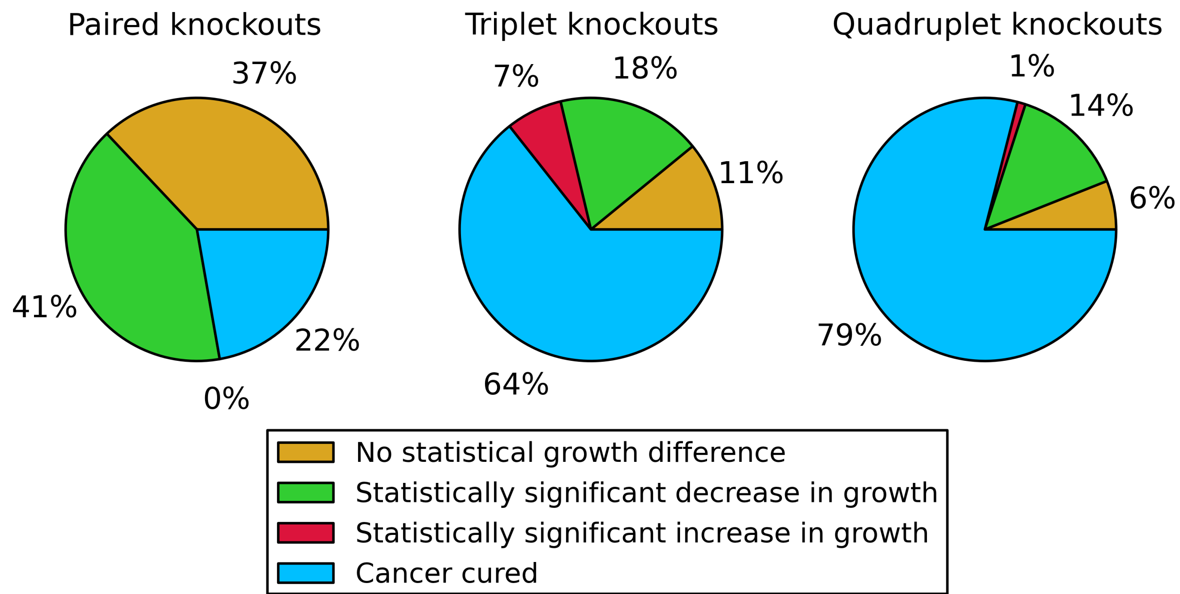

Figure 5.1: Comparisons of hallmark knockout success for doublet, triplet and quadruplet knockouts (treatments). Significant decreases in growth were those treatments that lowered cancer growth with a $p < 0.05$, as compared to growth with all hallmarks available. Cancer was considered cured if it did not take over in any of the 10 replicate simulations. Significant increases in growth were those treatments that resulted in more growth than tumours grown without any knockouts, with a $p < 0.05$. All p-values were calculated using Mann-Whitney U test.

Interestingly, the strongest score was AVOIDS APOPTOSIS and ANGIOGENESIS. However, the pairs that were most often knocked out in successful cures were ANGIOGENESIS and SELF GROWTH. It is clear that both of these (SELF GROWTH and ANGIOGENESIS) are very successful pairs (all pairs involving either of these hallmarks are quite "hot" with scores at 30 or above), but they are not the strongest pair together. This suggests that they are strong together, but that there exists tumour conditions where they are not the optimal combination. It reveals as well that pairs that did not necessarily seem obviously important could potentially be very strong because overall they had the most positive impact. This diagram also shows clearly that certain pairs (such as IGNORES GROWTH INHIBITION with GENETICALLY UNSTABLE, AVOIDS IMMUNE SYSTEM with GLYCOLYTIC PHENOTYPE as well as AVOIDS IMMUNE SYSTEM with GENETICALLY UNSTABLE and AVOIDS IMMUNE SYSTEM with GLYCOLYTIC PHENOTYPE) are not very strong overall, regardless of what treatment combination they are included with. This kind of analysis could potentially eliminate pairs from clinical treatment options for certain growth environments or tumour types if repeated with a simulation programmed for those environments.

المنارة للاستشارات
www.manaraa.com

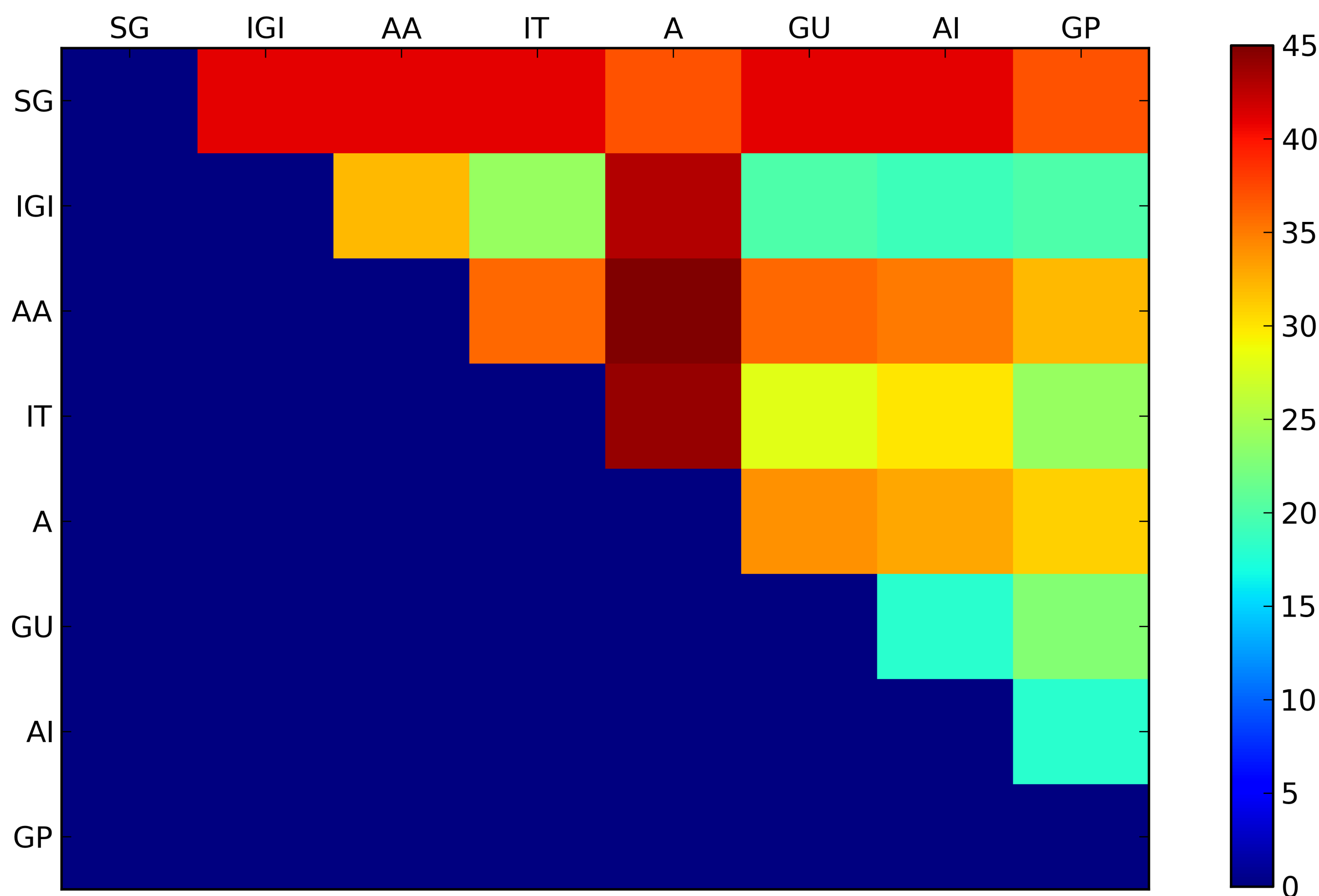

Figure 5.2: A symmetric heat map of co-occurrences of hallmarks in knockout treatments. Hallmarks that appeared together more often in cures are hotter. Values were calculated using the following heuristic: pairs present in a treatment that cured (stopped) cancer growth were assigned a score of +2; pairs present in a treatment that significantly lessened cancer growth were assigned a score of +1; pairs present in a treatment that had no significant effect were assigned a score of 0; pairs present in a treatment that made cancer growth significantly worse were assigned a value of -5.

www.manaraa.com

While it might seem strange that knocking out two, three, or even four hallmarks could result in no statistically significant change to cancer growth, in reality, it is not unusual for a drug, or combination of drugs, to have no effect. Many clinical trials are terminated when it is found that the treatment has no improvement over current available treatments [105], [169], [99]. It sometimes even occurs that treatments or combinations of treatments have no impact and in fact are more dangerous to a patient. Clinical trials have been halted when the study shows no improvements but increased death or toxicity in patients as compared to traditional treatments [132], [142], [149]. It does happen that a drug fails clinical trails for a particular type of cancer, however proves to be effective and safe for others. In fact there is currently an effort to reinvestigate "failed" trails where some small number of patients greatly benefited from the "failed" treatment [97]. What used to be considered an anecdote, when one patient would greatly benefit from a treatment that failed overall, is now being seen as an "n-of-1" study with the potential for great success for a subgroup of patients. Perhaps that one patient had a particular genetic makeup making them highly responsive to a treatment. Determining what is unique to that patient may open the drug up to all patients with similar genetic mutations in their tumour, allowing the drug to be beneficial to some subgroup. Since we know cancers can vary greatly between patients, this idea of investigating the outliers could lead to more "individualized" medicine that is highly effective in the right cases.

The fact that drugs are sometimes dangerous or useless in one case, only to be highly effective in others, underscores the need for determining in what situations drugs are going to be successful far in advance of costly clinical trials. This simulation was able to determine that for a general, non-specific cancer, some combinations of treatments that were effective individually are in fact useless or even detrimental when applied together. It is not obvious a priori what combinations will be successful. However, models such as this can help shed light on what combinations perhaps can be avoided all together, or which are most likely to have a strong impact. Since we cannot test all possible drug combinations on every individual looking for general effects or those specific "n-of-1" cases, we see the use and importance of cancer simulation, with accurate oxygen modelling and cancer representations. Similar models in the future could be more personalized in order to model treatments at the individual level. Indeed, there are a variety of ways this model could be extended in the future to be even more beneficial:

- Using more tunable parameters in order to simulate cancer growth with different initial conditions and parameters.
- Adding more microenvironmental variables, such as glucose transport, growth factor

المنارة للاستشارات
www.manaraa.com

diffusion and acid production.

- Providing more granular control over the cellular automata rules to model more individualized mutations.
- Knocking out hallmarks at different points in the simulation
- Incorporating a highly accurate vascular growth to model next phase of cancer progression.

A strength of this model is the generality of it, however this is also a weakness. Cancer grows in a variety of environments and a great future step would be making the model more highly tunable to simulate this. Redoing the analysis of combination treatments in changing situations, such as cells with higher mutation rates, lower cell turnover, surrounding tissue constraints, etc, would allow for more information on what combinations might be beneficial in what circumstances. For example, perhaps increasing available oxygen to model cancers growing in highly vascularized locations would reveal highly successful treatments not involving anti-angiogenesis.

This model was highly abstract, which is good for an initial high-level simulation, however in the future the addition of more microenvironmental factors would be beneficial. For example, adding in a fluid model of acid around the tumour would allow for the glycolytic phenotype to be modelled more precisely, including both its ability to allow cancer cells to survive with low oxygen and also the production of a highly acidic environment. In addition to modelling acid, other cell types could be added to simulate the tumour microenvironment, including things such as immune cells, cells involved in inflammation response, and epithelial cells.

Mutations in this model impact the rules of the cellular automata, however it would be beneficial to allow more control over the changes to these rules. Many hallmarks were changed in binary fashion, being either on or off. It may be beneficial to put these on a sliding scale so hallmarks can be reduced or increased depending on mutations and treatments. Since it is known treatments in reality are rarely 100% successful, this would allow for a more realistic modelling of cancer treatment.

Hallmarks in this model were removed at the beginning of simulation. This is consistent with the previous work looking at cancer hallmark's relevance by Santos *et al.* [143]. However, it would be better in the future to allow hallmarks to be removed at different points in the

المنارة للاستشارات
www.manaraa.com

system. For example, a hallmark could be removed early on, and then later a second hallmark removed, to model the case where one drug is applied and later it is realized a second is needed.

Lastly, the vascular model in this system was quite abstract and only provided oxygen to angiogenic cells or their direct neighbours. It would be more accurate to decouple cancer cells from the vasculature and model oxygen diffusing from the new vasculature points. A separate model of the vasculature would allow for new branches to be added to existing vasculature when a cell acquires the ability to stimulate angiogenesis. Modelling the oxygen diffusing out of this would allow cells farther away from the new vasculature to benefit as well.

To summarize my contribution: this work uniquely pairs a lattice Boltzmann model (LBM) of a two phase fluid, oxygen in the blood, with a seven-state cellular automata (CA) model of cancer. To our knowledge, this is the first time such a two-phase fluid model has been paired with a multi-state cellular automata model in order to simulate early growth with more accurate oxygen availability. In addition, this work is the first to examine knocking out hallmarks in pairs, triplets and quadruplets in order to create a highly abstract simulation of combination cancer treatment. The model showed that firstly this pairing of LBM and CA can grow a realistically shaped tumour on a biologically realistic time scale. Secondly, it sheds light on the complicated, potentially counterintuitive, reality that individual drugs are not necessarily better together. It emphasizes the need for critical investigation as to what pairs are best when and in what situations. It also serves as a launching point for further study into what pairs are best by having the ability to be extended in a personalized way. Variables, initial conditions and rules can be altered to model more specific cancers or individual situations in order to be a first line test for treatment ideas. Alexander Anderson from the Moffitt Cancer Research Center, home to some of the most well known cancer simulation researchers, stated in their Integrated Mathematical Oncology Newsletter "We ultimately see *in silico* models as a pre-treatment protocol to suggest the best therapeutic regime or to indicate which should be avoided." [16]. This model has added to the current array of cancer models in order to assist in this ultimate goal and eventually increase survival for people diagnosed with this complicated group of diseases we call cancer.

المنارة للاستشارات
www.manaraa.com

Bibliography

[1] RG. Abbott, S. Forrest, and KJ. Pienta. Simulating the hallmarks of cancer. *Artificial Life*, 4(12):617–34, 2006.

[2] Abby Siegel. Siliphos in advanced hepatocellular carcinoma, 2013.

[3] TH Adair and JP Montani. *Angiogenesis*. Morgan and Claypool Life Sciences, 2010.

[4] JM Adams and S Cory. The Bcl-2 apoptotic switch in cancer development and therapy. *Oncogene*, 26(9):1324–1337, 2007.

[5] Julio A Aguirre-Ghiso. Models, mechanisms and clinical evidence for cancer dormancy. *Nature Reviews Cancer*, 7(11):834–846, 2007.

[6] KGMM Alberti and C Cuthbert. The hydrogen ion in normal metabolism: a review. *Metabolic acidosis*, pages 1–15, 1982.

[7] Davide Alemani, Francesco Pappalardo, Marzio Pennisi, Santo Motta, and Vladimir Brusic. Combining cellular automata and lattice boltzmann method to model multiscale avascular tumor growth coupled with nutrient diffusion and immune competition. *Journal of Immunological Methods*, 376:55–68, 2012.

[8] American Cancer Society. Pancreatic cancer survival rates. `http://www.cancer.org/cancer/pancreaticcancer/overviewguide/pancreatic-cancer-overview-survival-rates`, 2013.

[9] American Cancer Society. Breast cancer survival rates. `http://www.cancer.org/cancer/breastcancer/detailedguide/breast-cancer-survival-by-stage`, 2014.

[10] American Cancer Society. Cancer facts and figures 2014. `http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151.pdf`, September 2014.

المنارة للاستشارات
www.manaraa.com

[11] American Cancer Society. Cervical cancer. http://www.cancer.org/cancer/cervicalcancer/detailedguide/cervical-cancer-survival, September 2014.

[12] American Cancer Society. The history of cancer. http://www.cancer.org/cancer/cancerbasics/thehistoryofcancer/index, 2014.

[13] American Cancer Society. Liver cancer survival rates. http://www.cancer.org/cancer/livercancer/detailedguide/liver-cancer-survival-rates, 2014.

[14] American Cancer Society. Prostate cancer survival rates. http://www.cancer.org/cancer/prostatecancer/detailedguide/prostate-cancer-survival-rates, 2014.

[15] A. R. A. Anderson, K. A. Rejniak, P. Gerlee, and V. Quaranta. Modelling of Cancer Growth, Evolution and Invasion: Bridging Scales and Models. *Mathematical Modelling of Natural Phenomena*, 2(3):1–29, June 2008.

[16] Alexander Anderson, Robert Gatenby, David Basanta, and Ariosto Silva. Integrated mathematical oncology newsletter.

[17] Alexander R A Anderson and Vito Quaranta. Integrative mathematical oncology. *Nature reviews. Cancer*, 8(3):227–34, March 2008.

[18] Alexander R A Anderson, Alissa M Weaver, Peter T Cummings, and Vito Quaranta. Tumor morphology and phenotypic evolution driven by selective pressure from the microenvironment. *Cell*, 127(5):905–15, December 2006.

[19] Alexander RA Anderson. A hybrid mathematical model of solid tumour invasion: the importance of cell adhesion. *Mathematical Medicine and Biology*, 22(2):163–186, 2005.

[20] Steven E Artandi and Ronald A DePinho. Telomeres and telomerase in cancer. *Carcinogenesis*, 31(1):9–18, 2010.

[21] Anna Azvolinsky. Most cited cell article of all time, hallmarks of cancer gets an update, 2011.

[22] Natalia Bailón-Moscoso, Juan Carlos Romero-Benavides, and Patricia Ostrosky-Wegman. Development of anticancer drugs based on the hallmarks of tumor cells. *Tumor Biology*, 35(5):3981–3995, 2014.

المنارة للاستشارات
www.manaraa.com

[23] Judith Balter-Seri, Celia Mor, Avinoam Shuper, Rina Zaizov, and Ian J Cohen. Cure of recurrent medulloblastoma. *Cancer*, 79(6):1241–1247, 1997.

[24] Dalit Barkan, Jeffrey E Green, and Ann F Chambers. Extracellular matrix: a gatekeeper in the transition from dormancy to metastatic growth. *European Journal of Cancer*, 46(7):1181–1188, 2010.

[25] David Basanta, Benjamin Ribba, Emmanuel Watkin, Benoit You, and Andreas Deutsch. Computational analysis of the influence of the microenvironment on carcinogenesis. *Mathematical Biosciences*, 229(1):22 – 29, 2011.

[26] Lorenzo Bello, Valeria Lucini, Francesco Costa, Mauro Pluderi, Carlo Giussani, Francesco Acerbi, Giorgio Carrabba, Marilou Pannacci, Dario Caronzolo, Silvia Grosso, Svetlana Shinkaruk, Federica Colleoni, Xavier Canron, Giustino Tomei, Gerard Deleris, and Andreas Bikfalvi. Combinatorial administration of molecules that simultaneously inhibit angiogenesis and invasion leads to increased therapeutic efficacy in mouse models of malignant glioma combinatorial administration of molecules that simultaneously inhibit angiogenesis. *Clinical cancer research*, 2004.

[27] Nicola Bellomo and Elena De Angelis. *Selected topics in cancer modeling: genesis, evolution, immune competition, and therapy*. Springer, 2008.

[28] Katie Bentley, Paul Bates, and Holger Gerhardt. Artificial life as cancer research: embodied agent modelling of blood vessel growth in tumours. In *Proceedings of Artifical Life XI*, 2008.

[29] María Berdasco and Manel Esteller. Aberrant epigenetic landscape in cancer: how cellular identity goes awry. *Developmental cell*, 19(5):698–711, 2010.

[30] E Bhatnagar, EP Gross, and M Krook. A model for collision processes in gases. i. small amplitude processes in charged and neutral one-component systems. *Phys. Rev.*, 94(3), 1954.

[31] Maria a Blasco. Telomeres and human disease: ageing, cancer and beyond. *Nature reviews. Genetics*, 6(8):611–22, August 2005.

[32] Baste R C, Kufe D W, Pollock R E, Weichselbaum R R, Holland J F, and Frei E. *Holland-frei cancer medicine*. B.C. Decker, 2000.

[33] Canadian Cancer Society's Advisory Committee on Cancer Statistics. Canadian cancer statistics 2014, 2014.

المنارة للاستشارات
www.manaraa.com

[34] Cancer Alliance for Research, Education and Survivorship. Chemocare. http://chemocare.com/chemotherapy/drug-info/Oxaliplatin.aspx#.VK7LyCvF-wU, 2014.

[35] Cancer Research UK. Cancer drugs. http://www.cancerresearchuk.org/about-cancer/cancers-in-general/treatment/cancer-drugs/, 2014.

[36] Cancer Research UK. Leukaemia survival statistics. http://www.cancerresearchuk.org/cancer-info/cancerstats/types/leukaemia/survival/leukaemia-survival-statistics, September 2014.

[37] Cancer Research UK. Lung cancer survival statistics. http://www.cancerresearchuk.org/cancer-info/cancerstats/types/lung/survival/lung-cancer-survival-statistics, September 2014.

[38] Cancer Research UK. Pancreatic cancer survival statistics. http://www.cancerresearchuk.org/cancer-info/cancerstats/types/pancreas/survival/pancreatic-cancer-survival-statistics, 2014.

[39] CDC. Leading causes of death. *Centers for Disease Control*, 2009.

[40] CenterWatch. Fda approved drugs for oncology. https://www.centerwatch.com/drug-information/fda-approved-drugs/therapeutic-area/12/oncology, 2015.

[41] Shiyi Chen and Gary D Doolen. Lattice boltzmann method for fluid flows. *Annuual review of fluid mechanics*, 30:329–64, 1998.

[42] Koei Chin, Carlos Ortiz de Solorzano, David Knowles, Arthur Jones, William Chou, Enrique Garcia Rodriguez, Wen-Lin Kuo, Britt-Marie Ljung, Karen Chew, Kenneth Myambo, et al. In situ analyses of genome instability in breast cancer. *Nature genetics*, 36(9):984–988, 2004.

[43] Chinese Academy of Medical Sciences. Efficacy and safety study of recombinant endostatic combined with chemotherapy to treat advanced colorectal cancer. ClinicalTrials.gov Bethesda (MD): National Library of Medicine (US), 2014.

[44] Roger Collier. Rapidly rising clinical trial costs worry researchers. *Canadian Medical Association Journal*, 180(3):277–278, 2009.

[45] Francesco Colotta, Paola Allavena, Antonio Sica, Cecilia Garlanda, and Alberto Mantovani. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*, 30(7):1073–1081, 2009.

المنارة للاستشارات
www.manaraa.com

[46] John Condeelis and Jeffrey W Pollard. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell*, 124(2):263–266, 2006.

[47] Lisa M Coussens, Wilfred W Raymond, Gabriele Bergers, Marion Laig-Webster, Ole Behrendtsen, Zena Werb, George H Caughey, and Douglas Hanahan. Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes & development*, 13(11):1382–1397, 1999.

[48] Chi V Dang and Gregg L Semenza. Oncogenic alterations of metabolism. *Trends in biochemical sciences*, 24(2):68–72, 1999.

[49] Michele De Palma, Mary Anna Venneri, Rossella Galli, Lucia Sergi Sergi, Letterio S Politi, Maurilio Sampaolesi, and Luigi Naldini. Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer cell*, 8(3):211–226, 2005.

[50] Ralph J DeBerardinis, Julian J Lum, Georgia Hatzivassiliou, and Craig B Thompson. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. *Cell metabolism*, 7(1):11–20, 2008.

[51] R Demicheli, MW Retsky, WJM Hrushesky, M Baum, and ID Gukas. The effects of surgery on tumor growth: a century of investigations. *Annals of Oncology*, page mdn386, 2008.

[52] David G DeNardo, Pauline Andreu, and Lisa M Coussens. Interactions between lymphocytes and myeloid cells regulate pro-versus anti-tumor immunity. *Cancer and Metastasis Reviews*, 29(2):309–316, 2010.

[53] Katharina M Detjen, Svenja Rieke, Antje Deters, Petra Schulz, Annett Rexin, Sonja Vollmer, Peter Hauff, Bertram Wiedenmann, Marianne Pavel, and Arne Scholz. Angiopoietin-2 promotes disease progression of neuroendocrine tumors. *Clinical Cancer Research*, 16(2):420–429, 2010.

[54] Sabine Dormann and Andreas Deutsch. Modeling of self-organized avascular tumor growth with a hybrid cellular automaton. *In silico biology*, 2(3):393–406, January 2002.

[55] John M L Ebos, Christina R Lee, William Cruz-Munoz, Georg a Bjarnason, James G Christensen, and Robert S Kerbel. Accelerated metastasis after short-term treatment with a potent inhibitor of tumor angiogenesis. *Cancer cell*, 15(3):232–9, March 2009.

المنارة للاستشارات
www.manaraa.com

[56] Elsevier. Most cited cell articles since 2010. http://www.journals.elsevier.com/cell/most-cited-articles/.

[57] K. Erbertseder, J. Reichold, B. Flemisch, P. Jenny, and R. Helmig. A coupled discrete/continuum model for cancer-therapeutic transport in the lung. *PloS One*, 7(3), 2012.

[58] Gerard Evan and Trevor Littlewood. A matter of life and cell death. *Science*, 281(5381):1317–1322, 1998.

[59] J Ferlay, HR Shin, F Bray, D Forman, CD Mathers, and D Parkin. Globocan2008 cancer incidence and mortality worldwide: Iarc cancerbase no. 10, 2008.

[60] J. Folkman. Tumor angiogenesis: therapeutic implications. *Nature Engl. J. Med.*, 285:L529–L533, 1971.

[61] Judah Folkman and Mark Hochberg. Self-regulation of growth in three dimensions. *The Journal of experimental medicine*, 138(4):745–753, 1973.

[62] J P Freyer, E Tustanoff, a J Franko, and R M Sutherland. In situ oxygen consumption rates of cells in V-79 multicellular spheroids during growth. *Journal of cellular physiology*, 118(1):53–61, January 1984.

[63] Christian Frezza, Liang Zheng, Daniel a Tennant, Dmitri B Papkovsky, Barbara a Hedley, Gabriela Kalna, David G Watson, and Eyal Gottlieb. Metabolic profiling of hypoxic cells revealed a catabolic signature required for cell survival. *PloS one*, 6(9):e24411, January 2011.

[64] Lorenzo Galluzzi and Guido Kroemer. Necroptosis: a specialized pathway of programmed necrosis. *Cell*, 135(7):1161–1163, 2008.

[65] Robert A Gatenby and Robert J Gillies. Why do cancers have high aerobic glycolysis? *Nature Reviews Cancer*, 4(11):891–899, 2004.

[66] P Gerlee and A R A Anderson. An evolutionary hybrid cellular automaton model of solid tumour growth. *Journal of theoretical biology*, 246(4):583–603, July 2007.

[67] P. Gerlee and A R A Anderson. A hybrid cellular automaton model of clonal evolution in cancer: the emergence of the glycolytic phenotype. *Journal of theoretical biology*, 250:705–722, 2008.

المنارة للاستشارات
www.manaraa.com

[68] P Gerlee and A R A Anderson. Evolution of cell motility in an individual-based model of tumour growth. *Journal of theoretical biology*, 259(1):67–83, July 2009.

[69] Jana L Gevertz, George T Gillies, and Salvatore Torquato. Simulating tumor growth in confined heterogeneous environments. *Physical biology*, 5(3):036010, January 2008.

[70] Bruce J Giantonio, Paul J Catalano, Neal J Meropol, Peter J O'Dwyer, Edith P Mitchell, Steven R Alberts, Michael A Schwartz, and Al B Benson. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *Journal of Clinical Oncology*, 25(12):1539–1544, 2007.

[71] Joanne Graham, Mohamed Muhsin, and Peter Kirkpatrick. Oxaliplatin. *Nature Reviews Drug Discovery*, 3(1):11–12, 2004.

[72] Sergei I Grivennikov, Florian R Greten, and Michael Karin. Immunity, inflammation, and cancer. *Cell*, 140(6):883–899, 2010.

[73] D Hanahan and J Folkman. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell*, 86(3):353–64, August 1996.

[74] D. Hanahan and R.A. Weinberg. The hallmarks of cancer. *Cell*, 100(1):57–70, 2000.

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

[76] Donna E Hansel, Alan K Meeker, Jessica Hicks, Angelo M De Marzo, Keith D Lillemoe, Richard Schulick, Ralph H Hruban, Anirban Maitra, and Pedram Argani. Telomere length variation in biliary tract metaplasia, dysplasia, and carcinoma. *Modern pathology*, 19(6):772–779, 2006.

[77] Hellenic Oncology Research Group. Paclitaxel plus bevacizumab for older patients with breast cancer. ClinicalTrials.gov Bethesda (MD): National Library of Medicine (US), 2014.

[78] E.S. Henderson and R.J. Samaha. Evidence that drugs in multiple combinations have materially advanced the treatment of human malignancies. *Cancer Research*, 29(12):2272–2280, 1969.

[79] Susanne Herwig and Michael Strauss. The retinoblastoma protein: a master regulator of cell cycle, differentiation and apoptosis. *European Journal of Biochemistry*, 246(3):581–601, 1997.

المنارة للاستشارات
www.manaraa.com

[80] Yoshito Hirata, Nicholas Bruchovsky, and Kazuyuki Aihara. Development of a mathematical model that predicts the outcome of hormone therapy for prostate cancer. *Journal of theoretical biology*, 264(2):517–27, May 2010.

[81] Hoffmann-La Roche. A study of Avastin (bevacizumab) in combination with chemotherapy in patients with breast cancer progressing after first-line therapy with avastin and chemotherapy (TANIA). ClinicalTrials.gov Bethesda (MD): National Library of Medicine (US), 2014.

[82] Peggy P Hsu and David M Sabatini. Cancer cell metabolism: Warburg and beyond. *Cell*, 134(5):703–707, 2008.

[83] National Cancer Institute. Cancer trends progress report. `http://progressreport.cancer.gov/doc_detail.asp?pid=1&did=2009&chid=95&coid=927&mid`, 2013.

[84] International breast cancer study group. Combination chemotherapy after surgery in treating patients with stage i, stage ii or stage iii breast cancer. ClinicalTrials.gov Bethesda (MD): National Library of Medicine (US), 2014.

[85] Arnaud Jacquel, Magali Herrant, Laurence Legros, Nathalie Belhacene, Frederic Luciano, Gilles Pages, Paul Hofman, and Patrick Auberger. Imatinib induces mitochondria-dependent apoptosis of the Bcr-Abl-positive K562 cell line and its differentiation toward the erythroid lineage. *The FASEB journal*, 17(14):2160–2162, 2003.

[86] Peter A Jones and Stephen B Baylin. The epigenomics of cancer. *Cell*, 128(4):683–692, 2007.

[87] Melissa R Junttila and Gerard I Evan. p53—a jack of all trades but master of none. *Nature Reviews Cancer*, 9(11):821–829, 2009.

[88] Yoonseok Kam, Katarzyna A Rejniak, and Alexander R A Anderson. Cellular modeling of cancer invasion: integration of in silico and in vitro approaches. *Journal of cellular physiology*, 227(2):431–8, February 2012.

[89] A R Kansal, S Torquato, I V Harsh GR, E A Chiocca, and T S Deisboeck. Simulated brain tumor growth dynamics using a three-dimensional cellular automaton. *Journal of theoretical biology*, 203(4):367–82, April 2000.

[90] Philip W Kantoff, Celestia S Higano, Neal D Shore, E Roy Berger, Eric J Small, David F Penson, Charles H Redfern, Anna C Ferrari, Robert Dreicer, Robert B Sims, et al.

المنارة للاستشارات
www.manaraa.com

Sipuleucel-t immunotherapy for castration-resistant prostate cancer. *New England Journal of Medicine*, 363(5):411–422, 2010.

[91] Michael B Kastan. DNA damage responses: mechanisms and roles in human disease: 2007 G.H.A. Clowes Memorial Award Lecture. *Molecular cancer research : MCR*, 6(4):517–24, April 2008.

[92] Toshiaki Kawai, Sadayuki Hiroi, Kuniaki Nakanishi, and Alan K Meeker. Telomere length and telomerase expression in atypical adenomatous hyperplasia and small bronchioloalveolar carcinoma of the lung. *American journal of clinical pathology*, 127(2):254–262, 2007.

[93] Candia M Kenific, Andrew Thorburn, and Jayanta Debnath. Autophagy and metastasis: another double-edged sword. *Current opinion in cell biology*, 22(2):241–245, 2010.

[94] Ryungsa Kim, Manabu Emi, and Kazuaki Tanabe. Cancer immunoediting from immune surveillance to immune escape. *Immunology*, 121(1):1–14, 2007.

[95] James Korkola and Joe W Gray. Breast cancer genomes—form and function. *Current opinion in genetics & development*, 20(1):4–14, 2010.

[96] C Koschmann, KL Schmidt, JR Geyer, and S Leary. Survival after recurrence of medulloblastoma in the contemporary era. *Journal of clinical oncology*, 29(15), 2011.

[97] Heidi Ledford. Cancer researchers revisit 'failed' clinical trials, 2013.

[98] Yu-Zhen Li, Dao-Yuan Lu, Wei-Qi Tan, Jian-Xun Wang, and Pei-Feng Li. p53 initiates apoptosis by transcriptionally targeting the antiapoptotic protein arc. *Molecular and cellular biology*, 28(2):564–574, 2008.

[99] Lilly. Lilly announces Enzastaurin Phase III study did not meet primary endpoint in diffuse large B-Cell lymphoma. `https://investor.lilly.com/releasedetail.cfm?ReleaseID=763858`, 2013.

[100] Bryn A Lloyd, Dominik Szczerba, Markus Rudin, and Gábor Székely. A computational framework for modelling solid tumour growth. *Philosophical transactions. Series A, Mathematical, physical, and engineering sciences*, 366(1879):3301–18, September 2008.

[101] Wing-Cheong Lo, Edward W Martin, Charles L Hitchcock, and Avner Friedman. Mathematical model of colitis-associated colon cancer. *Journal of theoretical biology*, 317C:20–29, September 2012.

المنارة للاستشارات
www.manaraa.com

[102] C Louvet, R Labianca, P Hammel, G Lledo, MG Zampino, T Andre, A Zaniboni, M Ducreux, E Aitini, J Taieb, et al. Gemcitabine in combination with oxaliplatin compared with gemcitabine alone in locally advanced or metastatic pancreatic cancer: results of a GERCOR and GISCAD phase III trial. *Journal of Clinical Oncology*, 23(15):3509–3516, 2005.

[103] Scott W Lowe, Enrique Cepero, and Gerard Evan. Intrinsic tumour suppression. *Nature*, 432(7015):307–315, 2004.

[104] Zhen Lu, Robert Z Luo, Yiling Lu, Xuhui Zhang, Qinghua Yu, Shilpi Khare, Seiji Kondo, Yasuko Kondo, Yinhua Yu, Gordon B Mills, et al. The tumor suppressor gene arhi regulates autophagy and tumor dormancy in human ovarian cancer cells. *The Journal of clinical investigation*, 118(12):3917–3929, 2008.

[105] John S Macdonald, Sheryl McCoy, Robert P Whitehead, Syma Iqbal, James L Wade III, Jeffrey K Giguere, and James L Abbruzzese. A phase II study of farnesyl transferase inhibitor R115777 in pancreatic cancer: a Southwest oncology group (SWOG 9924) study. *Investigational new drugs*, 23(5):485–487, 2005.

[106] Paul Macklin, Mary E Edgerton, Alastair Thompson, and Vittorio Cristini. Patient-calibrated agent-based modelling of ductal carcinoma in situ (DCIS) I: Model formulation and analysis. *Journal of Theoretical Biology*, 2011.

[107] C Maley and Stephanie Forrest. Exploring the relationship between neutral and selective mutations in cancer. *Artificial Life*, 6(4):325–345, 2000.

[108] Marcos Malumbres and Mariano Barbacid. Milestones in cell division: to cycle or not to cycle: a critical decision in cancer. *Nature Reviews Cancer*, 1(3):222–231, 2001.

[109] David A Mankoff, Janet F Eary, Jeanne M Link, Mark Muzi, Joseph G Rajendran, Alexander M Spence, and Kenneth A Krohn. Tumor-specific positron emission tomography imaging in patients:[18f] fluorodeoxyglucose and beyond. *Clinical Cancer Research*, 13(12):3460–3469, 2007.

[110] Mayo Clinic. Cancer surgery: physically removing cancer. `http://www.mayoclinic.org/diseases-conditions/cancer/in-depth/cancer-surgery/art-20044171`, 2014.

[111] W. D. McArdle, F. I. Katch, and V. L. Katch. *Essentials of Exercise Physiology*. Lippincott Williams & Wilkins, third edition, November 2005.

المنارة للاستشارات
www.manaraa.com

[112] Medical University of Vienna. Metronomic and targeted anti-angiogenesis therapy for children with recurrent/progressive medulloblastoma (MEMMAT). ClinicalTrials.gov Bethesda (MD): National Library of Medicine (US), 2014.

[113] A Monteagudo and J Santos. A cellular automaton model for tumor growth simulation. *Advances in Intelligent and Soft Computing*, 154:147–155, 2012.

[114] A Monteagudo and J. Santos. Cancer stem cell modeling using a cellular automaton. *IWINAC LNCS*, 7931:21–31, 2013.

[115] A Monteagudo and J Santos. Studying the capability of different cancer hallmarks to initiate tumor growth using a cellular automaton simulation. Application in a cancer stem cell context. *BioSystems*, 115:46–58, 2014.

[116] Malcolm J Moore, David Goldstein, John Hamm, Arie Figer, Joel R Hecht, Steven Gallinger, Heather J Au, Pawel Murawa, David Walde, Robert A Wolff, et al. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *Journal of clinical oncology*, 25(15):1960–1966, 2007.

[117] J Naidoo, DB Page, and JD Wolchok. Immune modulation for cancer therapy. *British journal of cancer*, 2014.

[118] Loretta J Nastoupil, Adam C Rose, and Christopher R Flowers. Diffuse large B-cell lymphoma: current treatment approaches. *Oncology*, 26(5):488–95, 2012.

[119] National Cancer Institute. Cancer research funding. `http://www.cancer.gov/cancertopics/factsheet/NCI/research-funding`, 2014.

[120] National Cancer Institute. Targeted cancer therapies. `http://www.cancer.gov/cancertopics/factsheet/Therapy/targeted`, 2014.

[121] Simona Negrini, Vassilis G Gorgoulis, and Thanos D Halazonetis. Genomic instability—an evolving hallmark of cancer. *Nature reviews Molecular cell biology*, 11(3):220–228, 2010.

[122] New Mexico Cancer Care Alliance. Combination of irinotecan, oxaliplatin and cetuximab for locally advanced or metastatic pancreatic cancer, 2014.

[123] Hiroaki Nozawa, Christopher Chiu, and Douglas Hanahan. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proceedings of the National Academy of Sciences*, 103(33):12493–12498, 2006.

المنارة للاستشارات
www.manaraa.com

[124] Kenneth P Olive, Michael A Jacobetz, Christian J Davidson, Aarthi Gopinathan, Dominick McIntyre, Davina Honess, Basetti Madhu, Mae A Goldgraben, Meredith E Caldwell, David Allard, et al. Inhibition of hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science*, 324(5933):1457–1461, 2009.

[125] OncoMed Pharmaceuticals, Inc. A phase 1b/2 study of omp-59r5 in combination with nab-paclitaxel and gemcitabine in subjects with previously untreated stage iv pancreatic cancer (alpine), 2014.

[126] Enzo Orlandini, Michael R Swift, and JM Yeomans. A lattice Boltzmann model of binary-fluid mixtures. *EPL (Europhysics Letters)*, 32(6):463, 1995.

[127] A R A Anderson P Gerlee. A Hybrid cellular automaton model of clonal evolution in cancer: The emergence of the glycolytic phenotype. *Journal of Theoretical Biology*, 250(4):705–722, 2010.

[128] F Pagés, J Galon, MC Dieu-Nosjean, E Tartour, C Sautes-Fridman, and WH Fridman. Immune infiltration in human tumors: a prognostic factor that should not be ignored. *Oncogene*, 29(8):1093–1102, 2009.

[129] Zhaohui Peng. Current status of gendicine in china: recombinant human Ad-p53 agent for treatment of cancers. *Human gene therapy*, 16(9):1016–1027, 2005.

[130] S. Pennacchietti, P. Michieli, M. Galluzzo, M. Mazzone, S. Giordano, and P.M. Comoglio. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell*, 2:347–361, 2003.

[131] Rosario Perona. Cell signalling: growth factors and tyrosine kinase receptors. *Clinical and Translational Oncology*, 8(2):77–82, 2006.

[132] Pfizer. Pfizer discontinues a phase 3 trial of figitumumab in non-small cell lung cancer (NSCLC) for futility. `http://www.pfizer.com/news/press-release/press-release-detail/pfizer_discontinues_a_phase_3_trial_of_figitumumab_in_non_small_cell_lung_cancer_nsclc_for_futility`, 2009.

[133] Monika Joanna Piotrowska and Simon D Angus. A quantitative cellular automaton model of in vitro multicellular spheroid tumour growth. *Journal of theoretical biology*, 258(2):165–78, May 2009.

المنارة للاستشارات
www.manaraa.com

[134] Van R Potter. The biochemical approach to the cancer problem. *Federation proceedings*, 17(2):691–697, 1958.

[135] An-Shen Qi, Xiang Zheng, Chan-Ying Du, and Bao-Sheng An. A cellular automaton model of cancerous growth. *Journal of theoretical biology*, 161:1–12, 1993.

[136] Marius Raica, Anca Maria Cimpean, and Domenico Ribatti. Angiogenesis in pre-malignant conditions. *European journal of cancer*, 45(11):1924–1934, 2009.

[137] Ignacio Ramis-Conde, Mark A.J. Chaplain, and Alexander R. A. Anderson. Mathematical modelling of cancer cell invasion of tissue. *Mathematical and Computer Modelling*, 47(5-6):533–545, March 2008.

[138] Christophe M Raynaud, Juana Hernandez, Frédérique P Llorca, Paolo Nuciforo, Marie-Christine Mathieu, Frederic Commo, Suzette Delaloge, Laure Sabatier, Fabrice André, and Jean-Charles Soria. DNA damage repair and telomere length in normal breast, preneoplastic lesions, and invasive cancer. *American journal of clinical oncology*, 33(4):341–345, 2010.

[139] Katarzyna A Rejniak and Alexander R A Anderson. Hybrid models of tumor growth. *Wiley interdisciplinary reviews. Systems biology and medicine*, 3(1):115–25, 2011.

[140] Katarzyna A Rejniak and Alexander R A Anderson. State of the art in computational modelling of cancer. *Mathematical medicine and biology: a journal of the IMA*, 29(1):1–2, March 2012.

[141] B Ribba and T Alarc. The use of hybrid cellular automaton models. *Training*, pages 444–453, 2004.

[142] Everardo D Saad, Paulo M Hoff, et al. Molecular-targeted agents in pancreatic cancer. *Cancer Control*, 11(1):32–38, 2004.

[143] J. Santos and Á. Monteagudo. Study of cancer hallmarks relevance using a cellular automaton tumor growth model. *Parallel Problem Solving from Nature-PPSN XII*, pages 489–499, 2012.

[144] Andrew M Scott, James P Allison, and Jedd D Wolchok. Monoclonal antibodies in cancer therapy. *Cancer immunity*, 12, 2012.

[145] S.F. Sener, A. Fremgen, H.R. Menck, and D.P. Winchester. Pancreatic cancer: a report of treatment and survival trends for 100,313 patients diagnosed from 1985–1995, using

المنارة للاستشارات
www.manaraa.com

the national cancer database. *Journal of the American College of Surgeons*, 189(1):1–7, 1999.

[146] J W Shay and W E Wright. Hayflick, his limit, and cellular ageing. *Nature reviews. Molecular cell biology*, 1(1):72–6, October 2000.

[147] Charles J Sherr. Cancer cell cycles. *Science*, 274(5293):1672–1677, 1996.

[148] Sachin Man Bajimaya Shrestha, Grand Roman Joldes, Adam Wittek, and Karol Miller. Cellular automata coupled with steady-state nutrient solution permit simulation of large-scale growth of tumours. *International journal for numerical methods in biomedical engineering*, 29(4):542–559, 2013.

[149] Sidney Kimmel Comprehensive Cancer Center. Ipilimumab +/- vaccine therapy in treating patients with locally advanced, unresectable or metastatic pancreatic cancer, 2013.

[150] J Smolle. Cellular automaton simulation of tumour growth – equivocal relationships between simulation parameters and morphologic pattern features. *Analytical cellular pathology : the journal of the European Society for Analytical Cellular Pathology*, 17(2):71–82, January 1998.

[151] Mark J Smyth, Gavin P Dunn, and Robert D Schreiber. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. *Advances in immunology*, 90:1–50, 2006.

[152] S. L. Spencer, M. J. Berryman, J. A. Garcia, and D. Abbott. An ordinary differential equation model for the multistep transformation to cancer. *Journal of Theoretical Biology*, 231:515–524, 2004.

[153] Sabrina L Spencer, Ryan A Gerety, Kenneth J Pienta, and Stephanie Forrest. Modeling somatic evolution in tumorigenesis. *PLoS computational biology*, 2(8):e108, 2006.

[154] Statistics Canada. Leading causes of death, by sex. *Statistics Canada*, 2009.

[155] Dirk C Strauss and J Meirion Thomas. Transmission of donor melanoma by organ transplantation. *The lancet oncology*, 11(8):790–796, 2010.

[156] S Succi. *The Lattice Boltzmann Equation for Fluid Dynamics and Beyond*. Oxford University Press, 2001.

المنارة للاستشارات
www.manaraa.com

[157] Xiaoqiang Sun, Le Zhang, Hua Tan, Jiguang Bao, Costas Strouthos, and Xiaobo Zhou. Multi-scale agent-based brain cancer modeling and prediction of TKI treatment response: Incorporating EGFR signaling pathway and angiogenesis. *BMC Bioinformatics*, 13(1):218, 2012.

[158] Surveillance, Epidemiology, and End Results Program. National Cancer Insitute. Cancer Stat Facts Sheet. `http://seer.cancer.gov/statfacts/`, 2014.

[159] RM Sutherland, B Sordat, J Bamat, H Gabbert, B Bourrat, and W Mueller-Klieser. Oxygenation and differentiation in multicellular spheroids of human colon carcinoma. *Cancer research*, 46(10):5320–5329, 1986.

[160] Robert M Sutherland. Cell and environment interactions in tumor microregions: the multicell spheroid model. *Science*, 240(4849):177–184, 1988.

[161] Mr Swift, E Orlandini, Wr Osborn, and Jm Yeomans. Lattice Boltzmann simulations of liquid-gas and binary fluid systems. *Physical review. E, Statistical physics, plasmas, fluids, and related interdisciplinary topics*, 54(5):5041–5052, November 1996.

[162] Michele WL Teng, Jeremy B Swann, Catherine M Koebel, Robert D Schreiber, and Mark J Smyth. Immune-mediated dormancy: an equilibrium with cancer. *Journal of leukocyte biology*, 84(4):988–993, 2008.

[163] Baldwin H Tom, Lynne P Rutzky, Milda M Jakstys, Ryoichi Oyasu, Celia I Kaye, and Barry D Kahan. Human colonic adenocarcinoma cells. *In vitro*, 12(3):180–191, 1976.

[164] U.S. Food and Drug Administration. The FDA's drug review process: Ensuring drugs are safe and effective. `http://www.fda.gov/Drugs/ResourcesForYou/Consumers/ucm143534.htm`, 2014.

[165] Claire M Vajdic and Marina T van Leeuwen. Cancer incidence and risk factors after solid organ transplantation. *International journal of cancer*, 125(8):1747–1754, 2009.

[166] J. W. Valvano, J. R. Cochran, and K. R. Diller. Thermal conductivity and diffusivity of biomaterials measured with self heated thermistors. *International Journal of Thermophysics*, 6(3), 1985.

[167] Eric Van Cutsem, Walter L Vervenne, Jaafar Bennouna, Yves Humblet, Sharlene Gill, Jean-Luc Van Laethem, Chris Verslype, Werner Scheithauer, Aijing Shang, Jan Cosaert, et al. Phase iii trial of bevacizumab in combination with gemcitabine and erlotinib in

المنارة للاستشارات
www.manaraa.com

patients with metastatic pancreatic cancer. *Journal of clinical oncology*, 27(13):2231–2237, 2009.

[168] Matthew G Vander Heiden, Lewis C Cantley, and Craig B Thompson. Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science*, 324(5930):1029–1033, 2009.

[169] Vical. Vical Phase 3 Trial of Allovectin Fails to Meet Efficacy Endpoints. `http://www.vical.com/investors/news-releases/News-Release-Details/2013/Vical-Phase-3-Trial-of-AllovectinR-Fails-to-Meet-Efficacy-Endpoints/default.aspx`, 2013.

[170] Bert Vogelstein and Kenneth W Kinzler. The multistep nature of cancer. *Trends in Genetics*, 9(4):138–141, 1993.

[171] VU University Medical Center. Chemoradiation with gemcitabine in combination with panitumumab for patients with locally advanced pancreatic cancer (vectibix), 2014.

[172] Zhihui Wang, Christina M Birch, Jonathan Sagotsky, and Thomas S Deisboeck. Cross-scale, cross-pathway evaluation using an agent-based non-small cell lung cancer model. *Bioinformatics (Oxford, England)*, 25(18):2389–96, September 2009.

[173] Otto Heinrich Warburg and Frank Dickens. *The metabolism of tumours: investigations from the Kaiser Wilhelm Institute for Biology, Berlin-Dahlem*. Constable & Company Limited, 1930.

[174] Xia-wei Wei, Zhi-rong Zhang, and Yu-Quan Wei. Anti-angiogenic drugs currently in phase ii clinical trials for gynecological cancer treatment. *Expert opinion on investigational drugs*, 22(9):1181–1192, 2013.

[175] Sidney Weinhouse, Otto Warburg, Dean Burk, and Arthur L Schade. On respiratory impairment in cancer cells. *Science*, 124(3215):267–272, 1956.

[176] Esther Witsch, Michael Sela, and Yosef Yarden. Roles for growth factors in cancer progression. *Physiology*, 25(2):85–101, 2010.

[177] World Health Organization. The global burden of disease: 2004 update, 2008.

[178] Jennifer A Woyach, Gerard Lozanski, Amy S Ruppert, Arletta Lozanski, Kristie A Blum, Jeffrey Alan Jones, Joseph M Flynn, Amy J Johnson, Michael R Grever, Nyla A Heerema, et al. Outcome of patients with relapsed or refractory chronic lymphocytic

المنارة للاستشارات
www.manaraa.com

leukemia treated with flavopiridol: impact of genetic features. *Leukemia*, 26(6):1442–1444, 2012.

[179] Jeffrey Wyckoff, Weigang Wang, Elaine Y Lin, Yarong Wang, Fiona Pixley, E Richard Stanley, Thomas Graf, Jeffrey W Pollard, Jeffrey Segall, and John Condeelis. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer research*, 64(19):7022–7029, 2004.

[180] Xiao-Ming Yin and Zheng Dong. *Essentials of apoptosis: a guide for basic and clinical research*. Springer, 2009.

[181] Hua Yu, Drew Pardoll, and Richard Jove. Stats in cancer inflammation and immunity: a leading role for stat3. *Nature Reviews Cancer*, 9(11):798–809, 2009.

[182] Wei-Xing Zong and Craig B Thompson. Necrotic death as a cell fate. *Genes & development*, 20(1):1–15, 2006.

المنارة للاستشارات
www.manaraa.com

Appendix A

Glossary

Aerobic Metabolism Oxygen-using metabolism that extracts energy (ATP molecules) from carbohydrates, such as glucose

Agent Based Model A type of computational model which simulates the actions of autonomous agents

Anastomosis Connection of separate branches of the vascular system to form a network

Angiogenesis The formation of new blood vessels

Apoptosis Programmed cell death that occurs as a natural part of tissue life and can occur as the result of damage to a cell

Basal level The base or minimum level

Cellular automata (CA) A discrete model where individual autonomous units exist in a finite number of states and can change states based on rules and their environment

Capillary The smallest blood vessels in the body

Caspase Cystine-dependent aspartate-specific proteases

Cell surface receptors Proteins on the surface of a cell that help the inside of the cell communicate with the environment on the outside

Chemotaxis Movement by a cell or organism in reaction to a chemical stimulus

Endothelial cells The cells lining the walls of blood vessels. These cells migrate towards tumours when tumour cells produce TAF

Epigenetic Genetic influences that are not related to the actual sequence of DNA

المنارة للاستشارات
www.manaraa.com

Extracellular Matrix All connective tissue and fibres that are not inside the cell but provide support for the environment outside of the cell

G0 Phase A resting phase of the cell cycle

G1 Phase A step in the cell cycle where the cell grows and creates building blocks needed for DNA replication

G2 Phase A gap time in the cell cycle where the cell grows and when it is ensured the cell is ready to divide

Glioblastoma multiforme A highly malignant brain tumour

Haematocrit The ratio of the volume occupied by packed red blood cells to the volume of the whole blood

Haptotaxis Migration of a cell down a concentration gradient

Homeostasis The balance maintained in the body e.g. regulating temperature

Hypoxia Oxygen deficiency causing a very strong drive to correct the deficiency

Ligand A molecule that can bind to another molecule

M Phase A part of the cell cycle when cell growth stops and the cell splits in two

Metabolism The breakdown and creation of metabolites in order to power the cell

Metastasis The spreading of a disease (especially cancer) to another part of the body

Matrix metalloproteinase Enzymes that degrade the matrix

Mesenchymal cell A type of cell that can easily migrate through the body

Mitosis Cell division

Necrosis The localized death of living cells (as from infection or the interruption of blood supply)

Neoplasm An abnormal growth of tissue, often a characteristic of cancer

Oncogene A gene with the potential to cause cancer

Oncoprotein A protein encoded by an oncogene that has the potential to cause cancer

المنارة للاستشارات
www.manaraa.com

Perfusion Pumping a liquid into an organ or tissue (especially by way of blood vessels)

Phenotype The set of observable characteristics of an organism that are the result of its environment and genetics interacting. In this model this is specifically the result of all hallmarks, rules and parameters for each cellular automata

Protease An enzyme that breaks down proteins and peptides

Proteolysis The process of breaking down proteins or peptides

Retinoblastoma A rare malignant tumour of the retina

S Phase A part of the cell cycle where DNA replication occurs

Senescence A cell state that is viable but non-proliferative

TAF Chemicals secreted by tumours, frequently when the tumour is experiencing hypoxia

Telomere Short, repeating cap of DNA on the ends of chromosomes

Telomerase An enzyme that builds telomeres

Tumour An abnormal mass of tissue

Tumour supressor gene A gene that protects a cell from some step on the path towards cancer

Vascular Relating to the system of blood vessels

Vascularizattion The organic process whereby body tissue becomes vascular and develops capillaries

المنارة للاستشارات
www.manaraa.com

Appendix B

Abbreviation List

A Angiogenesis hallmark

AA Avoid apoptosis hallmark

AI Avoid immune system hallmark

ASMR Age-standardized mortality rates

CA Cellular automata

ECM Extracellular matrix

IGI Ignore growth inhibition hallmark

IMS Intracellular mechanical stress

GBM Glioblastoma multiforme

GP Glycolytic phenotype hallmark

GU Genetically unstable hallmark

IT Ignores telomere hallmark

LBM Lattice Boltzmann Methods

MMP Matrix metalloproteinases

MTS Multicellular tumour spheroid

p53 Protein 53

المنارة للاستشارات
www.manaraa.com

RAS Rat sarcoma

RSA Random sequential addition process

RB Rentinoblastoma

SG Self growth hallmark

TAF Tumour angiogenic factor.

TP53 Tumour protein 53

www.manaraa.com

Appendix C

Raw p-values for all knockout data

C.1 Doublet knockout data

Hallmarks knocked out	p-value	Additional notes
SG, IGI	2.137×10^{-1}	No change
SG, AA	1.414×10^{-3}	Less cancer
SG, IT	1.061×10^{-1}	No change
SG, A	9.134×10^{-5}	Cancer cured
SG, GI	9.134×10^{-5}	Cancer cured
SG, AI	9.134×10^{-5}	Cancer cured
IGI, AA	3.642×10^{-3}	Some cancer still grew
IGI, IT	2.363×10^{-1}	No change
IGI, A	9.134×10^{-5}	Cancer cured
IGI, GI	4.250×10^{-1}	No change
IGI, AI	3.669×10^{-1}	No change
AA, IT	6.574×10^{-4}	Some cancer still grew
AA, A	9.134×10^{-5}	Cancer cured
AA, GU	7.010×10^{-3}	No change
AA, AI	5.665×10^{-3}	Some cancer still grew
IT, A	9.134×10^{-5}	Cancer cured
IT, GU	1.805×10^{-3}	No change
IT, AI	3.201×10^{-2}	No change
A, GU	9.083×10^{-5}	Cancer cured

المنارة للاستشارات
www.manaraa.com

A, AI	9.083×10^{-5}	Some cancer still grew
GI, AI	1.560×10^{-2}	Some cancer still grew

Table C.1: Hallmark paired knockout data. Each hallmark knockout was simulated 10 times. The final cancer growth was compared to growth when all hallmarks were available for activation using the Mann-Whitney-U test.

C.2 Triplet knockout data

Hallmarks knocked out	p-value	Additional notes
GI, GLY, AI	3.388×10^{-1}	Cancer in 7/10
IGI, IT, AI	1.923×10^{-1}	Cancer in 6/10
SG, IGI, GU	2.898×10^{-3}	Cancer cured
A, GLY, AI	9.1336×10^{-5}	Cancer cured
IGI, IT, GLY	4.554×10^{-3}	Cancer in 9/10
		Significantly more growth
SG, IGI, A	2.914×10^{-4}	Cancer cured
A, GU, AI	2.914×10^{-4}	Cancer cured
IGI, IT, GU	8.629×10^{-3}	Cancer in 10/10
		Significantly more growth
SG, IGI, IT	2.898×10^{-3}	Cancer cured
A, GU, GLY	1.230×10^{-4}	Cancer cured
IGI, IT, A	1.649×10^{-4}	Cancer cured
SG, IGI, AA	2.198×10^{-4}	Cancer cured
IT, GLY, AI	2.137×10^{-1}	Cancer in 10/10
IGI, AA, AI	2.293×10^{-3}	Cancer in 4/10
IT, GU, AI	2.363×10^{-1}	Cancer in 9/10
IGI, AA, GLY	1.413×10^{-3}	Cancer in 5/10
IT, GU, GLY	4.849×10^{-1}	Cancer in 9/10
IGI, AA, GU	8.629×10^{-3}	Cancer in 9/10
IT, A, AI	6.574×10^{-4}	Cancer cured
IGI, AA, A	9.1336×10^{-5}	Cancer cured
IT, A, GLY	9.1336×10^{-5}	Cancer cured

المنارة للاستشارات
www.manaraa.com

IGI, AA, IT	2.898×10^{-3}	Cancer in 6/10
IT, A, GU	3.843×10^{-4}	Cancer cured
SG, GLY, AI	7.010×10^{-3}	Cancer cured
AA, GLY, AI	5.665×10^{-3}	Cancer in 7/10
SG, GU, AI	4.554×10^{-3}	Cancer cured
AA, GU, AI	8.531×10^{-4}	Cancer in 3/10
SG, GU, GLY	4.554×10^{-3}	Cancer cured
AA, GU, GLY	1.805×10^{-3}	Cancer in 4/10
SG, A, AI	3.843×10^{-4}	Cancer cured
AA, A, A	9.1336×10^{-5}	Cancer cured
SG, A, GLY	9.1336×10^{-5}	Cancer cured
AA, A, GLY	1.231×10^{-4}	Cancer cured
SG, A, GU	2.198×10^{-4}	Cancer cured
AA, A, GU	2.198×10^{-4}	Cancer cured
SG, IT, AI	2.898×10^{-3}	Cancer cured
AA, IT, AI	4.554×10^{-3}	Cancer in 7/10
SG, IT, GLY	5.665×10^{-3}	Cancer cured
AA, IT, GLY	4.554×10^{-3}	Cancer in 7/10
SG, IT, GU	1.101×10^{-3}	Cancer cured
AA, IT, GU	3.642×10^{-3}	Cancer in 6/10
SG, IT, A	6.575×10^{-4}	Cancer cured
AA, IT, A	9.0826×10^{-5}	Cancer cured
SG, AA, AI	8.531×10^{-4}	Cancer cured
IGI, GLY, AI	5.665×10^{-3}	Cancer in 9/10
		Significantly more growth
SG, AA, GLY	5.040×10^{-4}	Cancer cured
IGI, GU, AI	8.629×10^{-3}	Cancer in 9/10
		Significantly more growth
SG, AA, GU	3.843×10^{-4}	Cancer cured
IGI, GU, GLY	0.4849	Cancer in 6/10
SG, AA, A	9.1336×10^{-5}	Cancer cured
IGI, A, AI	9.1336×10^{-5}	Cancer cured
SG, AA, IT	3.843×10^{-4}	Cancer cured
IGI, A, GLY	9.1336×10^{-5}	Cancer cured
SG, IGI, AI	2.293×10^{-3}	Cancer cured
IGI, A, GU	9.1336×10^{-5}	Cancer cured

المنارة للاستشارات
www.manaraa.com

SG, IGI, GLY	3.642×10^{-3}	Cancer cured

Table C.2: Hallmark triplet knockout data. Each hallmark knockout was simulated 10 times. The final cancer growth was compared to growth when all hallmarks were available for activation using the Mann-Whitney-U test.

C.3 Quadruplet knockout data

Hallmarks knocked out	p-value	Additional notes
A, GU, GLY, AI	2.198×10^{-4}	Cancer cured
IGI, AA, GU, AI	1.805×10^{-3}	Cancer in 5/10
SG, AA, IT, GLY	1.649×10^{-4}	Cancer cured
IT, GU, GLY, AI	4.251×10^{-1}	Cancer in 8/10
IGI, AA, GU, GLY	3.642×10^{-3}	Cancer in 6/10
SG, AA, IT, GU	1.649×10^{-4}	Cancer cured
IT, A, GLY, AI	1.230×10^{-4}	Cancer cured
IGI, AA, A, AI	1.649×10^{-4}	Cancer cured
SG, AA, IT, A	9.1336×10^{-5}	Cancer cured
IT, A, GU, AI	1.230×10^{-4}	Cancer cured
IGI, AA, A, GLY	9.1336×10^{-5}	Cancer cured
SG, IGI, GLY, AI	1.414×10^{-3}	Cancer cured
IT, A, GU, GLY	1.230×10^{-4}	Cancer cured
IGI, AA, A, GU	9.1336×10^{-5}	Cancer cured
SG, IGI, GU, AI	1.805×10^{-3}	Cancer cured
AA, GU, GLY, AI	1.414×10^{-4}	Cancer in 5/10
IGI, AA, IT, A	1.414×10^{-4}	Cancer in 5/10
SG, IGI, GU, GLY	5.040×10^{-4}	Cancer cured
AA, A, GLY, AI	9.1336×10^{-5}	Cancer cured
IGI, AA, IT, GLY	2.898×10^{-3}	Cancer in 6/10
SG, IGI, A, AI	2.914×10^{-4}	Cancer cured
AA, A, GU, AI	9.1336×10^{-5}	Cancer cured
IGI, AA, IT, GU	2.293×10^{-3}	Cancer in 4/10
SG, IGI, A, GLY	1.231×10^{-4}	Cancer cured

المنارة للاستشارات
www.manaraa.com

AA, A, GU, GLY	9.1336×10^{-5}	Cancer cured
IGI, AA, IT, A	9.083×10^{-5}	Cancer cured
SG, IGI, A, GU	1.649×10^{-4}	Cancer cured
AA, IT, GLY, AI	6.574×10^{-4}	Cancer in 2/10
SG, GU, GLY, AI	3.642×10^{-3}	Cancer cured
SG, IGI, IT, AI	1.805×10^{-3}	Cancer cured
AA, IT, GU, AI	6.575×10^{-4}	Cancer in 3/10
SG, A, GLY, AI	1.649×10^{-4}	Cancer cured
SG, IGI, IT, GLY	3.642×10^{-3}	Cancer cured
AA, IT, GU, GLY	3.843×10^{-4}	Cancer in 2/10
SG, A, GU, AI	3.843×10^{-4}	Cancer cured
SG, IGI, IT, GU	8.531×10^{-4}	Cancer cured
AA, IT, A, AI	9.1336×10^{-5}	Cancer cured
SG, A, GU, GLY	1.649×10^{-4}	Cancer cured
SG, IGI, IT, A	1.641×10^{-4}	Cancer cured
AA, IT, A, GLY	9.1336×10^{-5}	Cancer cured
SG, IT, GLY, AI	4.554×10^{-3}	Cancer cured
SG, IGI, AA, AI	2.198×10^{-4}	Cancer cured
AA, IT, A, GU	9.1336×10^{-5}	Cancer cured
SG, IT, GU, AI	1.805×10^{-3}	Cancer cured
SG, IGI, AA, GLY	1.640×10^{-4}	Cancer cured
IGI, GU, GLY, AI	1.723×10^{-1}	Cancer in 7/10
SG, IT, GU, GLY	6.574×10^{-4}	Cancer cured
SG, IGI, AA, GU	5.040×10^{-4}	Cancer cured
IGI, A, GLY, AI	9.1336×10^{-5}	Cancer cured
SG, IT, A, AI	1.649×10^{-4}	Cancer cured
SG, IGI, AA, A	9.1336×10^{-5}	Cancer cured
IGI, A, GU, AI	1.231×10^{-4}	Cancer cured
SG, IT, A, GLY	1.231×10^{-4}	Cancer cured
SG, IGI, AA, IT	3.843×10^{-4}	Cancer cured
IGI, A, GU, GLY	1.231×10^{-4}	Cancer cured
SG, IT, A, GU	6.575×10^{-4}	Cancer cured
IGI, IT, GLY, AI	8.629×10^{-3}	Cancer in 9/10 Significantly more growth
SG, AA, GLY, AI	1.231×10^{-4}	Cancer cured
IGI, IT, GU, AI	3.116×10^{-1}	Cancer in 6/10

المنارة للاستشارات
www.manaraa.com

SG, AA, GU, AI	3.842×10^{-4}	Cancer cured
IGI, IT, GU, GLY	1.923×10^{-1}	Cancer in 7 /10
SG, AA, GU, GLY	2.914×10^{-4}	Cancer cured
IGI, IT, A, AI	9.1336×10^{-5}	Cancer cured
SG, AA, A, AI	9.1336×10^{-5}	Cancer cured
IGI, IT, A, GLY	9.1336×10^{-5}	Cancer cured
SG, AA, A, GLY	9.1336×10^{-5}	Cancer cured
IGI, IT, A, GU	1.649×10^{-4}	Cancer cured
SG, AA, A, GU	9.1336×10^{-5}	Cancer cured
IGI, AA, GLY, AI	2.989×10^{-3}	Cancer in 6/10
SG, AA, IT, AI	1.649×10^{-4}	Cancer cured

Table C.3: Hallmark quadruplet knockout data. Each hallmark knockout was simulated 10 times. The final cancer growth was compared to growth when all hallmarks were available for activation using the Mann-Whitney-U test.

المنارة للاستشارات
www.manaraa.com

Appendix D

Drugs that target hallmarks

An example of a drug that targets each of the hallmarks of cancer simulated in this paper. For more examples and information on the development of cancer drugs based on the hallmarks of cancer please see [22].

Drug	Hallmark targeted	Notes
Bevacizumab	Angiogenesis	Blocks VEGF to stop the angiogeneic ability of cancer
Paclitaxel	Self growth	Inhibits mitosis to stop cancer cells from dividing
Interleukin-2	Avoid immunity	Boosts the natural immune response so the immune system can attack the cancer
GV1001	Ignore telomeres	A telomerase inhibitor vaccine currently in development and trials
Imatinib	Avoids apoptosis	Used in leukemia where a chromosomal abnormality stimulates abnormal proliferation of hemopoietic precursor cells that are inhibited from dying via apoptosis [85]
Silybin	Glycolytic phenotype	Clinical trial was recently completed investigating using this in liver cancer to stop glycolysis. No results yet posted [2]
Gendicine	Genome Unstable	A drug that activates normal p53, this is used to treat head and neck cancers by activating normal p53. p53 then guards the genome properly, initiating apoptosis in cells with too

المنارة للاستشارات
www.manaraa.com

		much damage stopping the propagation of damage and therefore stabilizing genomes of remaining cells [129]
Flavopiridol	Ignores Growth Inhibition	Useful in treating relapsed or refractory chronic lymphocytic leukemia (CLL); targets over expressed cyclins that allow cells to evade RB signals and over grow [178]

المنارة للاستشارات
www.manaraa.com

Curriculum Vitae

Name:	Jenna Butler
Education	Honours Bachelor of Science, 2005-2009 Doctorate of Philosophy, 2009-2015 University of Western Ontario London, ON
Honours and Awards:	NSERC PGS M, 2011 - 2015 3 Minute Thesis Ontario Provincial Competition 2nd Place 2015 USC UWO Award of Excellence in Teaching, 2014 UWO Faculty of Science Excellence in Undergraduate Teaching Award, 2013 3 Minute Thesis Competition, 2nd Place, 2012 Biology Graduate Research Forum Best Talk, 2012 Best Talk Award, Western Research Forum, 2012 Society of Graduate Students Teaching Assistant Award, 2010 Ontario Graduate Scholarship, 2009, 2010, 2011 UWO Gold Award for highest average in graduating program, 2009 Google Anita Borg Scholarship, 2009 NSERC Undergraduate Research Awards, 2008, 2009 Computer Research Association Honourable Mention, 2008
Related Work Experience:	<u>Limited Duties Lecturer, Computer Science Department</u> CS1026 - Fundamentals of Computer Science, Fall 2010 CS1027 - Fundamentals of Computer Science II, Summer 2011 CS1026 - Fundamentals of Computer Science, Spring 2012 CS2120, CS9642 - Computing for the Sciences, Fall 2013 <u>Teaching Assistant, Computer Science Department</u> CS1026 - Fundamentals of Computer Science, Fall 2009, 2014 CS2212 - Software Engineering, Spring 2010, Spring 2011, Spring 2012 <u>Software Development Engineer In Test Intern, Microsoft</u> Developed internal tools for Microsoft, Redmond, Summer 2012, 2013, 2014

المنارة للاستشارات

www.manaraa.com

Publications:

Jenna Butler, Marjorie Elizabeth Osborne Locke, Kathleen A. Hill, Mark Daley. “HD-CNV: hotspot detector for copy number variants.” *Bioinformatics* 29, no. 2 (2013): 262-263.

Jenna Butler, Frances Mackay, Colin Denniston, Mark Daley. “Simulating Cancer Growth Using Cellular Automata to Detect Combination Drug Targets.” In *Unconventional Computation and Natural Computation*, pp. 67-79. Springer International Publishing, 2014.

Jenna Butler, Frances Mackay, Colin Denniston, Mark Daley. “Simulating Cancer Growth Using Cellular Automata to Detect Combination Drug Targets.” Invited Paper in *Natural Computing*. *Out for review.*

Presentations and Posters:

Poster. Jenna Butler*, Frances Mackay, Colin Denniston, Mark Daley. “Detecting combination drug targets using cellular automata and lattice Boltzmann methods”. Grace Hopper Celebration 2014. *Accepted but unable to attend.*

Poster. Jenna Butler*, ST Eitutis, MEO Locke, AE Wishart, M Daley, KA Hill. “HD-CNV: Hotspot Detector of Copy Number Variants”. Ontario Celebration of Women in Computing. October 2012.

Presentation. Jenna Butler*, F. Mackay, C. Denniston, M Daley. “Modelling the impact of oxygen availability and mutation on solid mass tumour growth”. Ontario Celebration of Women in Computing. October 2012.

Presentation. Jenna Butler*, F. Mackay, C. Denniston, M Daley. “Simulating avascular cancer progression and mutation using cellular automata and Lattice Boltzmann methods”. Biology Graduate Research Forum. October 2012.

Poster. Wishart AE*, Eitutis ST, Butler J, Locke B, Daley M, Hill KA. “Patterns of copy number changes between spleen and cerebellum differ in the harlequin mouse model of mitochondrial dysfunction”. Environmental Mutagen Society Annual Meeting, Bellevue, WA. August 2012.

Presentation. Jenna Butler*, M Daley. Using cellular automata and “Lattice Boltzmann meth-

المنارة للاستشارات
www.manaraa.com

ods to model the impact of oxygen on solid tumour growth". University of Western Ontario Research in Computer Science. April 2012.

Presentation. Jenna Butler*, M Daley. "Modelling the impact of oxygen availability on solid tumour growth". Western Research Forum. March 2012.

Poster. Jenna Butler*, ST Eitutis, MEO Locke, AE Wishart, M Daley, KA Hill. "HD-CNV: Hotspot Detector of Copy Number Variants". Western Research Forum. March 2012.

Poster. Jenna Butler*, ST Eitutis, MEO Locke, AE Wishart, M Daley, KA Hill. "HD-CNV: Hotspot Detector of Copy Number Variants". London Health Research Forum. March 2012.

Poster. Jenna Butler*, M Daley. "Modelling solid tumour growth using cellular automata and Lattice Boltzman methods". Grace Hopper Celebration of Women in Computer Science. November 2011.

Poster. Jenna Butler*, ST Eitutis, MEO Locke, AE Wishart, M Daley, KA Hill. "HD-CNV: Hotspot Detector of Copy Number Variants". Biology Graduate Research Forum. October 2011.

Presentation. Jenna Butler*, M Daley. "Modelling tumour growth with Lattice Boltzmann methods and cellular automata". Western Research Forum. March 2011.

Invited Talk. Jenna Butler*, M Daley. "A hybrid cellular automaton model of tumour growth". University of Western Ontario Biology Graduate Research Forum. October 2010.

المنارة للاستشارات
www.manaraa.com