

**CancerSim:
A Computer-Based Simulation of
Hanahan and Weinberg's Hallmarks of
Cancer**

by

Robert Abbott

B.S., Brigham Young University, 1998

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science
Computer Science

The University of New Mexico

Albuquerque, New Mexico

May, 2002

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ABSTRACT OF THESIS

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Abstract

Hanahan and Weinberg’s important paper “The Hallmarks of Cancer” is a distillation of the immense body of cancer research into a small number of underlying principles. The paper poses six cellular alterations as essential to malignant growth. Cancerous cells defy the normal limits of mitosis, ignore growth-inhibition signals, escape dependence on external growth stimulation, cause extra vascularization, become mobile and invasive, and disable the safety mechanisms that normally detect mutation and trigger apoptosis. Genetic instability is an additional factor which may be necessary to account for the high incidence of cancer.

We implemented the minimal model of cancer proffered by the Hallmarks paper in CancerSim, a computer-based simulation. By implementing the Hallmarks, we

seek to determine whether the complex phenomena of cancer can indeed arise from just a handful basic principles.

To the extent that cancer is successfully simulated, we can examine the dynamics of a developing tumor and alter its progression by tuning the model parameters. CancerSim has command-line and graphical user interfaces to support both interactive investigation and off-line analysis of the model.

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Chapter 1

Cancer

Each year, there are 6 million deaths from cancer, and 10 million new cases diagnosed. Over the next 25 years, as deaths due to infection decrease, the number of diagnoses of cancer is expected to double to 20 million[11]. Over the last 30 years, the billions spent on cancer research and treatment have lead to better care overall, but breakthroughs for only a few types of cancer.

1.1 Hanahan and Weinberg's Hallmarks of Cancer

Hanahan and Weinberg's important paper "The Hallmarks of Cancer" suggests six cellular alterations as essential to malignant growth. These six hallmarks are believed to be common to most if not all human tumors. Genome instability is an additional factor which may be necessary to explain the high incidence of cancer.

1.2 CancerSim

To explore Hanahan and Weinberg’s proposal, we created a computer-based model. The program is named “CancerSim.” It allows the user to explore cancer by simulating and visualizing cell growth and phenotypic alteration (Figures 1.1, 1.2). Users can see how the hallmarks interact, and affect the simulation by changing model parameters. Each phenotype can be studied, as can the order in which mutations are most likely to take effect. CancerSim may help assign estimates of relative importance to the hallmarks, providing focus for further research.

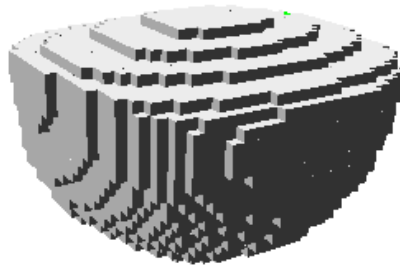


Figure 1.1: Healthy Tissue

CancerSim does not simulate every nuance of every form of cancer. Instead, our goal is to determine how well the hallmarks posited by Hanahan and Weinberg can account for the appearance and behavior of cancer. Cellular mechanisms are simulated only in the abstract, because our focus is on the high-level dynamics of tumorigenesis. CancerSim draws no distinction between genotype and phenotype; the “genotype” of a cell is simply a boolean vector whose values enable or disable certain behaviors.

Chapter 1. Cancer

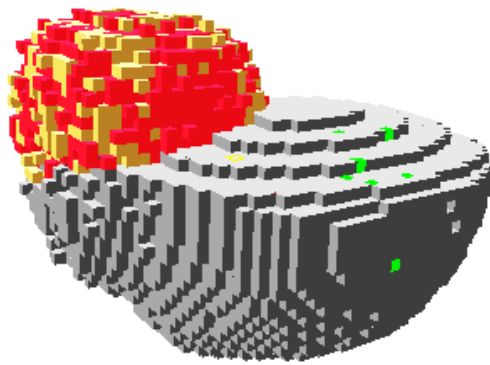


Figure 1.2: Cancerous Tissue

Chapter 2

Implementation

2.1 Fidelity vs. Runtime Efficiency

Although computers continually become faster and have more memory, it is not yet possible to simulate a realistic number of cells. CancerSim can comfortably simulate up to about 1,000,000 cells, but this falls far short of the human body, with its approximately $5 * 10^{13}$ cells. In contrast, recent computers have on the order of 10^9 bytes of memory, which is too little by 4 or 5 orders of magnitude. Computational speed is similarly inadequate.

Despite the limitations of computer hardware, we must try to simulate a large number of cells. Because CancerSim is driven by rare stochastic events (such as mutation), the law of averages demands a large sample size. For instance, the expected value of a \$1 lottery ticket might be 25 cents, but finding this value experimentally requires buying a huge number of tickets. Similarly, CancerSim must simulate a large number of cells to determine the true “expected” behavior.

Quicker execution also allows more exploration of parameter space.

Of course, running the simulation quickly is pointless if it fails to adequately model cancer phenotypes and their interactions. Therefore, the central issue in the implementation of CancerSim is the tradeoff between simulation fidelity and runtime efficiency.

2.2 Cellular Automata

CancerSim is a cellular automaton. Green[5] defines a cellular automaton as an array of elements on a grid. Each element follows the same rules in interacting with the others. (The elements are normally referred to as “cells” in the generic sense of the word, but here that usage is avoided to prevent confusion.) The cellular automaton consists of “state” describing the status of each element, a “neighborhood” defining the sphere of influence of each element, and a “program” defining the next state of an element based on its current state and the state of its neighbors. Each element is updated every timestep.

CancerSim varies from classical cellular automata in a couple of ways.

First, the “program” incorporates chance, so it is not strictly a mapping from element and neighbor states to successor states. (In particular, the additional factor is the state of the pseudo-random number generator).

Second, most elements do not change observably each time step. There are two reasons for this. First, most of the grid represents empty space most of the time. For instance, with a grid size of 125,000 elements, no more than about 16,000 can be occupied until several mutations have occurred. The tissue is bounded by growth factor concentration and vascularization (further explained below and illustrated in Figure 2.6.1). This allows room for the expansion of the tumor. Second, the only observable changes to cells are apoptosis and replacement (due to the mitosis of a

neighboring cell which ignores growth inhibit). In an actual tissue, only a fraction of all cells are undergoing such transitions at any given time. In CancerSim, the length of the cell cycle is a random variable distributed uniformly between 5 and 10 timesteps. This could be simulated in a classical cellular automaton by keeping a counter in each cell representing progression through the cell cycle, but performing such a large number of unobservable updates would be wasteful.

2.3 Event Model

CancerSim avoids unnecessary updates using a discrete event model. This means that each future update is represented by a data structure. The set of all future updates is stored in a priority queue, ordered on event time. Only observable updates are performed. Instead of decrementing a counter in each cell each timestep, the update representing mitosis is scheduled for execution several timesteps in the future. This minimizes the number of updates performed. However, the priority queue of updates must be maintained, consuming some memory and requiring some extra processing.

The simulation begins by initializing all elements to represent empty space. Then, the element at the center of the grid is changed to represent a cell having no mutations. Mitosis is scheduled for this initial cell, and this mitosis is the next event. After the new daughter cells are created, mitosis is scheduled for each of them, and so on. Each mitosis is carried out by copying the “genetic” information of the cell to an adjacent unoccupied space in the grid. Random errors occur in this copying process to simulate mutation. In CancerSim, these errors always add new mutations, and never “repair” previous ones. Frequently, cells are unable to replicate because of some limitation. For instance, there may be no free space adjacent to the cell, or insufficient growth signal. Cells overcome these limitations through mutation.

2.4 Vascularization

CancerSim’s vascular system develops according to signals sent by the cells. Each element in the grid may be occupied by a capillary segment (an element can contain both a cell and a capillary simultaneously). Nutrient diffuses from each capillary segment into the surrounding elements. The nutrient available to each cell is the sum of the contributions of all capillary segments, but the contributions from distant capillaries are vanishingly small.

The initial capillary segment is co-located with the initial cell. When a newly created cell has insufficient nutrient, a new capillary segment is created in the nearest candidate element. A candidate element is any element adjacent to an existing capillary segment; this ensures that the capillaries are continuous. However, if the new cell is outside a predefined boundary, no angiogenesis will occur unless the cell’s phenotype includes Cause Angiogenesis.

2.5 Completion Criteria

The simulation can conclude in three different ways. First, all the cells may die due to aging. Second, an arbitrary maximum number of timesteps may be reached. Without this time limit, CancerSim could continue forever due to the Evade Apoptosis mutation. Third, the simulation ends when cancer develops. In CancerSim, the criteria for “cancer” is the occupation of 90% of the grid. Healthy, non-cancerous cells can only fill a small portion of the grid because they are bounded by limited vasculature and growth factor (Figure 2.6.1).

2.6 Phenotypes

At the heart of CancerSim is its implementation of Hanahan and Weinberg’s hallmarks of cancer. Each hallmark is implemented as a cellular phenotype. Most of the phenotypes can be influenced by user-specified parameters.

2.6.1 Self-Growth

Normal cells proliferate only when stimulated by growth signals from other cells. The signal chemicals are bound by transmembrane receptors, prompting cell growth and division. In cancerous cells, these receptors are over-expressed, causing hypersensitivity to otherwise ambient levels of growth signal.

In CancerSim, cells undergo mitosis only if they are within a predefined boundary. This boundary represents a threshold in the concentration of growth factor; beyond this threshold, growth signals are too faint to prompt mitosis. The volume defined by this boundary intersects the volume in which angiogenic factor is plentiful (described in the next section), but neither volume completely encloses the other. Figure 2.6.1 illustrates a two-dimensional cross section of these volumes. Cells may escape the tissue’s natural extents through the “Growth Signal” mutation. This mutation causes cells to proliferate regardless of the concentration of growth factor.

2.6.2 Sustained Angiogenesis

Cells cannot survive at distances of more than about 100um from blood supply. Despite this fact, cells are normally unable to induce angiogenesis (new capillary growth). Without such growth, tumors can only grow to about 0.5mm[4]. The importance of angiogenesis to tumorigenesis has been studied since the early 1970’s and

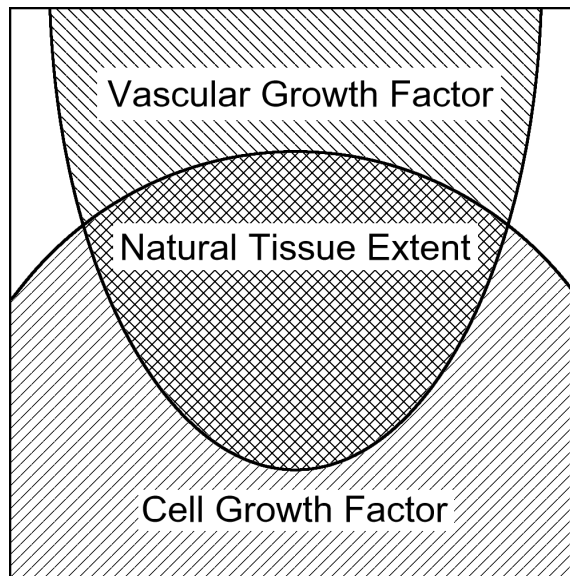


Figure 2.1: Tissue Growth Bounded by Growth Factor and Vascularization

has resulted in clinical trials of several angiogenesis inhibitors. Because cancerous cells are genetically unstable, they are highly adaptive and quickly develop resistance to chemotherapy. However, these cells cannot survive without the the local circulatory system (vasculature). Because the vasculature itself is not malignant, it should be less adaptive and more susceptible to sustained treatment[7]. However, such treatments alone only suppress (rather than eliminating) tumors. Another drawback is that angiogenesis is not specific to tumors; it is a normal part of homeostasis[4], so disabling angiogenesis may harm healthy tissue.

Angiogenesis is regulated by a variety of signals; over two dozen inducers (primarily bFGF and vascular endothelial growth factor, or VEGF) and a similar number of inhibitors are known. Some of the inducers are not specific to endothelial cells; they promote growth in other cells as well[4]. Cancerous cells may over-produce inducers, lack inhibitors, or both. The “angiogenic switch” is the threshold in the ratio of inducers to inhibitors sufficient to trigger angiogenesis.

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In CancerSim, angiogenesis is inhibited outside of a predefined area (Figure 2.6.1). Vasculature outside this area can be developed only in response to signals from cells with the “Sustained Angiogenesis” mutation. Cells signal for angiogenesis only when the concentration of nutrient is too low. This is a simple model of hypoxia (oxygen deficiency), “the most potent external stimulus of angiogenic factor” [4].

2.6.3 Insensitivity to Growth-Inhibitor Signals

Normal cells eventually enter a post-mitotic, differentiated state. In this state the cell carries out a specialized role beneficial to the organism as a whole, and no longer reproduces. By ignoring anti-growth signals, cancer cells forgo full maturity and normal functioning, thus maintaining replicative potential.

CancerSim models contact inhibition, which is a type of growth inhibition. Contact inhibition prevents overcrowding by arresting the growth and division of cells already in contact with many other cells. In the three-dimensional grid space of CancerSim, each cell has up to 26 neighbors (to be considered to be neighbors, cells need only make contact at a corner). Cells forgo mitosis if all of the neighboring grid locations are already occupied by living cells. The “Ignore Growth Inhibit” mutation allows cells to divide even when there is no empty location for the new daughter cell. In this case, the daughter cell competes for survival with a randomly chosen neighbor cell with a $1/g$ likelihood of success, where g is a tunable parameter.

2.6.4 Evasion of Apoptosis

Cells have mechanisms to initiate apoptosis (self-destruction). Normal cells express receptors for apoptosis signals. Such signals are sent by intra-cellular sensors which detect DNA damage, signaling imbalance, survival factor insufficiency, or hypoxia.

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Oncogene over-expression is among the factors that trigger early apoptosis. Tumor cells can be distinguished because they lack some of the surface features present on normal cells, and express some features not normally present on mature cells[15]. The immune system detects and responds to these cells as non-self. In this way, the body is protected from malfunctioning cells. To survive, tumor cells must disable either the effectors of mitosis or the receptors that trigger them. Over 50% of human cancers contain a mutated, nonfunctional P53 tumor suppressor gene. This prevents manufacture of P53 protein - a necessary component in some pathways of DNA damage detection. (P53 mutation is not the only way to evade apoptosis triggered by DNA damage, but it is probably the most important.)

Each cell in CancerSim is checked for genetic damage once per cycle. If mutation is detected, the cell undergoes apoptosis instead of mitosis. The probability of detection of damage in the cell is n/e , where n is the number of mutations carried by the cell and e is a tunable parameter. Thus, the probability of apoptosis due to genetic damage is proportional to the number of mutations. The “Evade Apoptosis” mutation causes cells to ignore apoptosis signals. This removes the selective disadvantage of genetic damage.

2.6.5 Limitless Replicative Potential

Cells normally become senescent after a fixed number of divisions, and are unable to further reproduce. In culture, various human cells are limited to 60-70 divisions, though most die before exhausting this potential. This limitation stops tumors from growing large enough to see without a microscope. Experiments show, however, that cells occasionally acquire limitless replicative potential. Such cells are said to be “immortal.”

The mechanism of limited replicative potential lies in the inability of DNA poly-

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merase to completely replicate chromosomes. The ends of chromosomes are called “telomeres.” With the duplication of each chromosome, 50-100 base pairs at the end of each telomere are lost. Eventually, the DNA of the chromosome is exposed. The unprotected DNA fuses together. Once the cell’s genetic material is in this deformed configuration, the death of the cell is imminent.

In cancerous cells, the telomeres are extended to compensate for what is lost by DNA polymerase. Usually this is accomplished by overproducing telomerase enzyme, which appends molecules to the telomeres. Alternately, telomeres may be lengthened by homologous recombination. This is usually prevented by mismatch repair machinery. (But this machinery may itself be defective, which is one form of genetic instability[2].)

CancerSim implements telomere shortening. The initial cell is created with telomeres of a length specified by the parameter t . With each mitosis, the telomeres are shortened by one unit length. When the telomere length falls to 0, the cell dies. The “Limitless Replicative Potential” mutation stops telomere shortening. Cells with this mutation can undergo mitosis any number of times.

2.6.6 Tissue Invasion and Metastasis

Metastasis - the spreading of cancer in the body - is the final stage of cancerous development. 90% of cancer fatalities are due to growths other than the original tumor. Localized cancer has a limited impact, while metastatic cancer takes root wherever possible within the body.

To circulate through the body, cells must decouple themselves from surrounding tissue. Cell adhesion molecules and integrins are disrupted by mutation, transcriptional repression, or proteolysis (the dissolution of protein into peptides and amino acids). Once free from their moorings, cancer cells escape the influence of regulatory

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signals. Cancer cells also increase protease production, perhaps to facilitate the destruction inherent in the invasion of tissue (a protease is an enzyme that catalyzes the breakdown of proteins).

CancerSim does not simulate metastasis, because only a localized area is simulated.

2.6.7 Genome Instability

Genetic transcription is a very accurate process. It seems unlikely that all the mutations preconditioning cancer should occur during a human lifetime, yet cancer is a common occurrence. Carcinogens cause genetic instability by altering the cellular environment. This selects cells for the ability to evolve quickly, which favors genetic instability[2]. In an environment absent of carcinogens, cells are already well adapted. Since most mutations are disadvantageous, genetic stability is beneficial when the environment is hospitable.

There are various enzymes in the body to detect and repair different kinds genetic damage. This indicates the possibility of various specific types of genetic instability[2].

Studies based on actual detection rates place the number of mutations in cancerous cells at more than 10,000, and mathematical models suggest that the actual number of mutations is probably 10^{12} or more[16]. However, the vast majority of these probably have no effect on the progression of the malignant growth, so they are not modeled. The genetic instability phenotype causes other mutations to occur with increased likelihood. For unstable cells, the base mutation rate specified by the parameter m is scaled by a factor of i , which is a tunable parameter.

2.7 Random Apoptosis

In CancerSim, the only effect of cell aging is telomere shortening, which is an artifact of mitosis. Under this model, a cell that never reproduces (because of contact inhibition) never ages, and will never die. Given sufficient initial telomere length, the tissue quickly fills all available space. Cell-cell contact then inhibits all mitosis, precluding genetic mutation. The simulation is at an impasse.

To ensure the progress of the simulation, an element of random apoptosis is added. Each cell cycle, every cell is subjected to a $\frac{1}{a}$ risk of death, where a is a tunable parameter. This is a simple, generalized model of mechanical, chemical, and radiological damage, aging, and collateral damage inflicted by the immune system.

2.8 Random Number Generation

Random number generation is an important issue in CancerSim. In fact, almost all the parameters of the model influence the frequency of random events. In the simulation, all random behavior is driven by a random number generator. In the same way that dice influence the progression of a board game, these random numbers are interpreted by CancerSim to determine, for instance, whether genetic mutation will occur during a given cellular mitosis.

The key fact of computer-generated random numbers is that they are actually not random at all. This extends from the deterministic nature of computers; they are designed to carry out instructions in a predictable way. Hardware dedicated to the random number generation exists, based on random physical processes such as resistance noise[8] and radioactive decay. However, such hardware is not generally available. Worse, providing truly random input to a program renders its results non-repeatable.

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For these reasons, uncertain events in CancerSim are driven by a *pseudo*-random number generator, or “PRNG,” as is common in computer simulation. Each invocation of the PRNG transforms its internal state by a simple function and returns a number which appears to be random, but which is actually just a portion of the new internal state. Because the internal state of the PRNG is finite in size, it has only a finite set of configurations. Once some state is entered for the second time, the PRNG is in a cycle and will generate the same sequence of “random” numbers indefinitely. The length of the cycle is the “period” of the PRNG. A good PRNG must have a long cycle.

CancerSim’s PRNG is adapted from the GNU standard C library, which was in turn adapted from BSD (Berkeley Systems Distribution) UNIX. The formula used in this PRNG is $x_n = x_{n-1}^{31} + x_{n-1}^3 + 1$, and x is a 112 bit number. This permits a maximum period of ($2^{112} \approx 10^{33}$), which is not of practical concern, but the actual period depends on the start state, and is almost sure to be much shorter than this maximum. The least significant bit, in particular, has a very short period ($2^7 - 1 = 127$), but entropy from lower-order bits propagates to higher-order bits. Of the 112 internal bits, only the 31 most significant are output in each pseudo-random number.

The start state of a PRNG is created from a “seed” value provided by the user. For each seed, the PRNG generates a different sequence of numbers. The user must be allowed to specify this value to make runs variable, yet repeatable. In CancerSim, the user influences the random number seed through the parameter r . Rather than directly specifying the random seed, r is combined with the other starting parameters using a hash function to determine the random seed. This way, pseudo-random number sequences are re-used only between runs with identical parameter sets.

The key random events in CancerSim are genetic mutations. The most straightforward way to test for such an event is to generate a random number in the interval

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$[0, 1)$ and call it a “success” if the random number is less than p , the probability of the event. Genetic mutations, however, are very rare. Instead of generating a random number each time the unlikely event *might* occur, we instead generate a number which represents the number of failures before the next success. This value initializes a counter, which is decremented each time the event might occur. When the count falls to 0, the event occurs, and the number of trials until the next success is recalculated. The number of trials between each success in such an event is given by the geometric distribution. Knuth[8] gives a very convenient formula for generating a number from the geometric distribution. Here, the *rand* function is expected to return a number in the interval $[0, 1)$ from the uniform distribution.

$$n = \lceil \frac{\log(\text{rand}())}{\log(1-\frac{1}{p})} \rceil$$

2.9 Summary Of CancerSim Parameters

Each parameter can be specified either by a command-line parameter switch (Table 2.9) or from the ‘Settings’ dialog box in the GUI. Switches are used in this way:

```
GUI -n1000000 -r123 -t30
```

2.10 Representation

The basic data structures in CancerSim are three-dimensional arrays representing cells. There is an array for each piece of information maintained on a per-cell basis. A more object-oriented approach would have been to create a data structure (or class) representing the cell, and instantiate one three-dimensional array of these. However, that approach requires more memory due to memory alignment of the

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Parameter Name	Switch	Description
Grid Size	-n	The computational grid contains n cell locations
Mutation Rate	-m	Each allele is copied with a $\frac{1}{m}$ chance of mutation
Random Apoptosis	-a	Each cell cycle exposes every cell to a $\frac{1}{a}$ chance of death from non-specific causes
Telomere Length	-t	Telomere shortening limits the initial cell to t replications
Evade Apoptosis	-e	A cell with n mutations has an extra $\frac{n}{e}$ likelihood of dying each cell cycle, unless one of its mutations is “Evade Apoptosis”
Genetic Instability	-i	Increase the likelihood of mutation by a factor of i for cells with this mutation
Ignore Growth Inhibit	-g	Cells with this mutation have a $\frac{1}{g}$ chance of killing off neighbor to make room for mitosis
Random Seed	-r	Seed value for the pseudorandom number generator

Table 2.1: CancerSim Parameters

fields within the data structure. The issue is analogous to packing boxes of various sizes into several trunks. If each trunk is filled with boxes of only a single size, the boxes pack tightly and there is no wasted space in the trunk. If boxes of various sizes are placed against each other, the trunk will contain pockets of air, which are wasted space. Maintaining a separate array for each field also decreases execution time. In the few cases where a traversal of all cells is required, only a single field is examined. Keeping a separate array for each field places these values next to each other in memory, which decreases the time required to traverse all of them. Table 2.2 shows the information maintained for each cell.

Name	Datatype	Notes
Genetic Instability	boolean	
Ignore Growth Inhibit	boolean	
Evade Apoptosis	boolean	
Limitless Replication	boolean	
Sustained Angiogenesis	boolean	
Self Growth	boolean	
Telomere Length	integer	Replicative potential of this cell
Mutation Pathway	Phenotype_History	Records the order in which mutations were obtained
Capillary	boolean	Whether there is a capillary at the cell's location
Nutrient	real-valued	Concentration of oxygen etc. at the cell's location
Sequence Number	integer	Tag to distinguish between cells reusing the same grid location

Table 2.2: CancerSim Cell State

2.11 Visualization

CancerSim models a tissue in three dimensions, but must display it in two dimensions (on a monitor). In this situation, it is greatly helpful to be able to rotate the tissue smoothly and interactively. Rendering 1,000,000 cells quickly requires a bit of technique.

To save time, the cells are displayed as cubes. A cell could be represented by a more organic shape such as a sphere, but drawing cubes is advantageous in several ways.

First, rendering hardware draws polygons, and triangles in particular. Organic-looking curved surfaces can be approximated, but this requires piecing together a large number of triangles. A rectangle can be drawn with only two triangles.

Second, cubes pack together tightly, completely occluding the tissue's interior.

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This means that only the *exterior surface* of the tissue needs to be rendered. This is crucial, since most cells are in the interior of the tissue.

Finally, cubes can be rendered efficiently because their faces can often be coalesced into larger rectangles. For instance, while a single cube has 6 faces, any number of cubes of the same size lined up against each other form a rectangular volume which still has only 6 faces. Figure 2.2 is a special rendering of a tissue in which every rectangle is outlined in black. If the cells' faces were not coalesced, every face of every cell would be displayed individually as a small square, increasing rendering time.

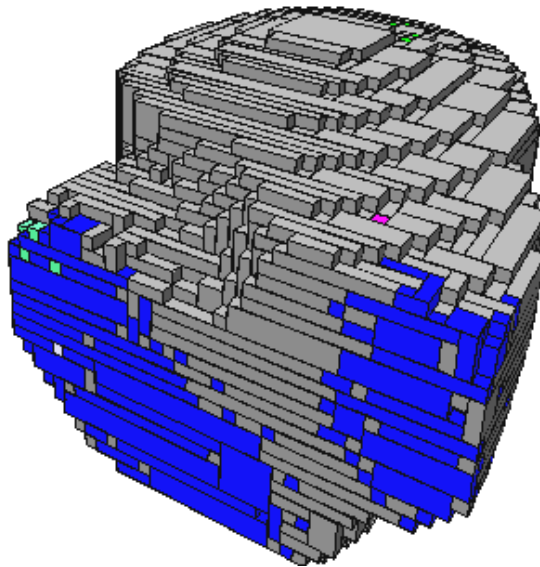


Figure 2.2: Illustration of Rectangle Aggregation

2.12 Languages, Tools, Libraries, Platforms, and Licensing

CancerSim is freely available on the World Wide Web under the terms of the GNU General Public License. The source code is available at <http://www.cs.unm.edu/~rabbott/CancerSim>. This means that anyone can use CancerSim as a basis for further development so long as the modified source code is also released under the GPL (see [14] for details). Moreover, all the tools and libraries used by CancerSim are also free.

Portability to various platforms is desirable. The main difficulty in accomplishing portability is the graphical user interface (GUI). CancerSim uses the wxWindows[12] cross-platform GUI library, which is available on Linux, various flavors of UNIX, MacOS, and all versions of Microsoft Windows starting with Windows 3.1. CancerSim is written in C++ and should be portable to any of these platforms. To date it has been compiled on Linux using the GNU [13] g++ compiler, and on Microsoft Windows using g++ under the Cygwin UNIX Environment [10]. Importantly, wxWindows supports platform-independent use of OpenGL, a portable application programming interface for 3d graphics used by CancerSim.

The data in the Results section was extracted using the Perl scripting language. CancerSim Review (see below) relies on Perl and GNU's plotting program "gnuplot."

Chapter 3

How to Use CancerSim

CancerSim offers three user interfaces: graphical (GUI), command-line (CLI), and a post-processor called CancerSim Review.

3.1 Command Line Interface

The command-line interface is for non-interactive use. It is normally run with a set of parameters to influence the simulation, and its output is typically redirected to a file. At intervals of 100 timesteps, information about the state of the simulation is output for later analysis. It is either run from a controlling program or using a shell, i.e.:

```
CLI -n27000 -i100 -g10 -t100 -m1000000 -e20 -a1000 -r1 > output
```

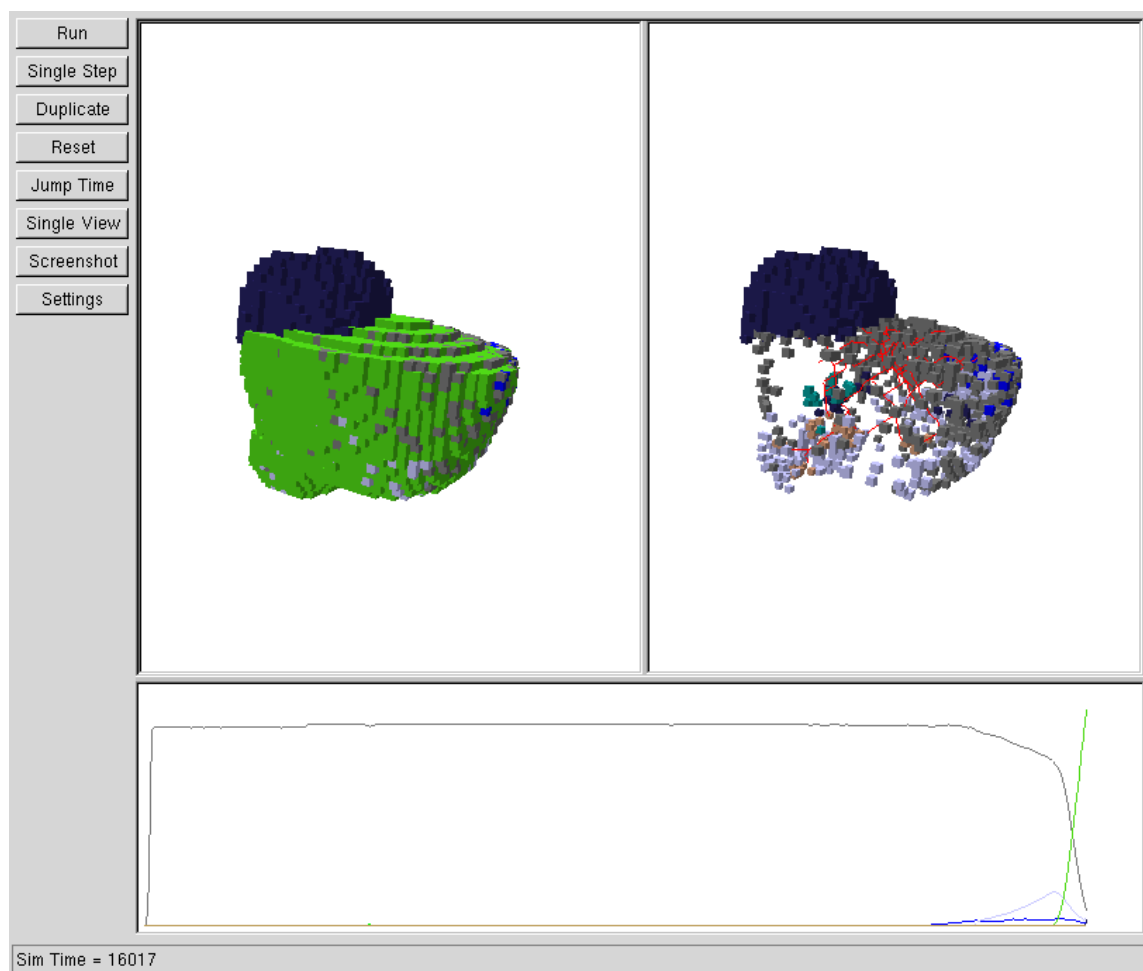



Figure 3.1: CancerSim GUI

3.2 Graphical User Interface

The graphical user interface (Figure 3.1) is for interactive use. The GUI can be run from a command-line allowing starting parameters to be specified, just as with the CLI. This is a convenient way to initialize the simulation when the parameters are known in advance.

3.2.1 Commands

The left portion of the CancerSim GUI window is a column of command buttons.

The “Run” button is pressed to begin the simulation and again to pause it.

The “Single Step” button takes the simulation through one timestep. This does not imply that only one event is processed; generally, many events occur on each timestep.

The “Duplicate” button creates a new CancerSim window. The simulation state of the new window is identical to the original, but proceeds forward independently. This functionality is used either to create a snapshot of a simulation at a certain timestep or to test the effect of a parameter by altering it in a duplicate window and then continuing the simulation.

The “Reset” button resets CancerSim to its starting configuration of a single cell. This button brings up a dialog box requiring confirmation, since resetting the simulation discards its previous state. In this dialog box is the grid size specification. The maximum number of cells, n , is specified here. The CancerSim grid is always cubic. The side length of the cube is $\lfloor n^{\frac{1}{3}} \rfloor$, so the n specified is reduced to the next lowest perfect cube if necessary.

The “Jump Time” button brings up a dialog for the specification of a new sim time. If the user confirms this action, the simulation is “jumped” (without the normal 3-D view updates) to the specified time, just as if the user had run the simulation until the specified time and pressed the “Stop” button. If the new sim time is in the past, the result is as if the user had stopped the simulation at that time.

The “Dual View” button divides the 3-D view into two panes. Both panes show the tissue from the same angle. However, the two views may have different cell visibilities. (See below for more information on cell visibility).

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The “Screenshot” button allows the user to save an image of the 3-D view in Microsoft’s “Bitmap” (.bmp) format. This format is simple and widely supported in imaging and word processing software.

The “Settings” button summons a dialog for the editing of all simulation parameters (except grid size; see “Reset,” above). These parameters are defined near the beginning of this chapter. The initial values of these parameters can be specified from the command line.

3.2.2 3-D View

The 3-D view initially shows just a dot representing the first cell in the tissue. As the simulation is run, the 3-D view is updated every 100 timesteps, or whenever the simulation is paused.

In the 3-D view, each cell is rendered as a small cube. The cube is colored according to the phenotype of the cell it represents. The phenotype of any cell can be displayed by left-clicking on the cell. This reports the cell’s phenotype in the upper-left-hand corner of the 3-D view, and outlines the cell in yellow. The mutations listed for the cell are displayed in the same order as they were obtained by the cell’s ancestors.

To view the tissue from a different angle, the user may left-click on a cell, then move the mouse while still holding the left button down.

To see inside the tissue, or simply to declutter the display, the user can hide all cells of any phenotype by right-clicking on a cell of that phenotype. When cells are hidden, the vasculature of the tissue becomes visible (as branching structure drawn in red). To make all cells visible, right-click anywhere but on a cell.

3.2.3 History View

The 2-D graph below the 3-D view shows information about the history of the simulation. It plots the number of cells of each phenotype against time. In this view, cell populations are not differentiated by the order in which their ancestors acquired mutations. As time passes (by running the simulation) the graph becomes more compressed.

When the user left-clicks within the History View, the coordinate on the curve nearest the mouse cursor is displayed, e.g: “ $t = 87, n = 1501.$ ” Here, t is the sim time, and n is the number of cells of that phenotype living at that time. When a point of interest is located on this curve, it can be visualized using the “Jump Time” button (see above).

3.2.4 CancerSim Review

Running CancerSim to completion can take a significant amount of time, depending mainly on the grid size and the speed of tumor progression. This makes it difficult and time-consuming to compare the results of several runs, or to quickly locate a set of parameters that will generate interesting behavior.

To address this issue, a coarse parameter sweep of CancerSim has been performed (comprising 4374 runs and over 1.3 *trillion* cell events in total). Data from the output of these runs can be quickly visualized using the CancerSim Review GUI (Figure 3.2). The tradeoff of using this ready-made data is that only a few settings of each parameter are available. The available settings are shown in Table 3.1.

CancerSim Review presents a graph of three curves. Each curve corresponds to a run of CancerSim. The three runs vary only by random seed; all other parameters are identical. Each curve shows how the number of cells varied over time during the

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course of a run. If the curve reaches the top of the graph, the run ended with cancer.

The CancerSim Review Settings Window selects the information to be displayed on the graph. First is a drop-down box designating the type of cell to be counted. By default, all cells are counted. Any of the mutations can be selected, creating a graph of the prevalence of that phenotype. The radio buttons in the Settings Window select runs with various parameters. At the bottom of the settings window is a button entitled “CancerSim.” Pressing this button launches the CancerSim GUI preloaded with the parameters from CancerSim Review. The user can then use the GUI to visualize the run summarized by CancerSim Review. Naturally, the parameters may be fine-tuned (or changed completely) once the GUI is launched; in this case the interactive simulation will not correspond to the plot in CancerSim Review.

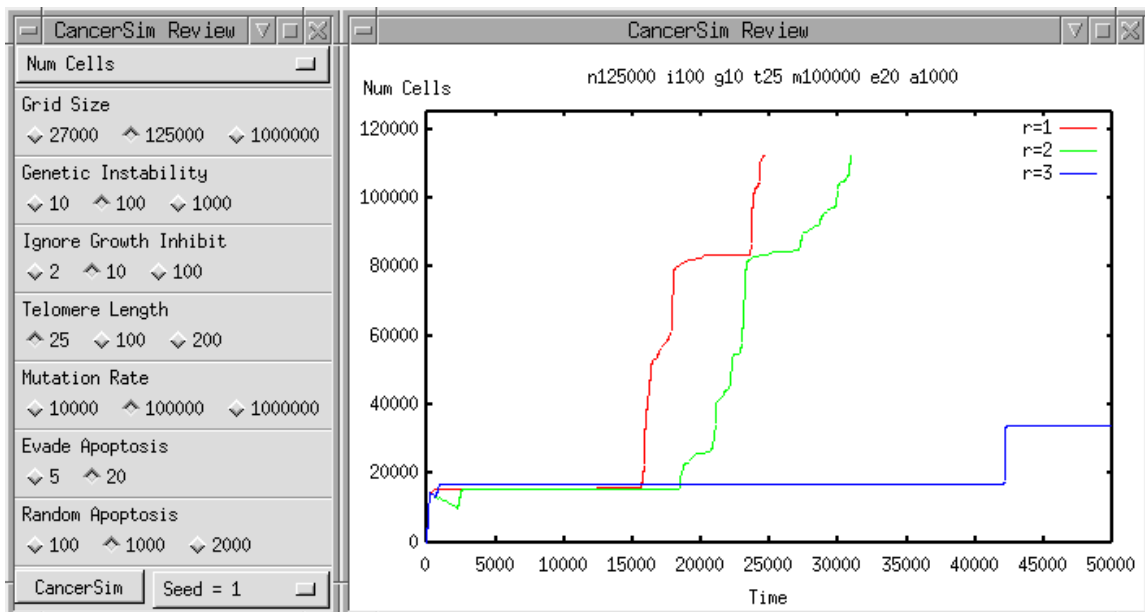


Figure 3.2: CancerSim Review GUI

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Setting	Values
Grid Size	27000, 125000, 1000000
Mutation Rate	10^4 , 10^5 , 10^6
Random Seed	1, 2, 3
Random Apoptosis	100, 1000, 2000
Telomere Length	25, 100, 200
Evade Apoptosis	5, 20
Genetic Instability	10, 10^2 , 10^3
Ignore Growth Inhibit	2, 10, 100

Table 3.1: CancerSim Review GUI Parameter Settings

Chapter 4

Results

The primary “result” of CancerSim is the degree to which its simple implementation of Hanahan and Weinberg’s Hallmarks of Cancer realistically models cancer. This assessment can be made only by medical experts. It is bound to be subjective, and will depend on the user’s interests. If nothing else, though, CancerSim provides a frame of reference for more precise discussion of the Hallmarks and a useful starting point for higher fidelity simulations of specific phenomena.

Additionally, the behavior of CancerSim itself can be studied. However, the significance of these results hinges on the larger question of whether CancerSim resembles reality at all.

In the spirit of optimism, but with these significant disclaimers never out of mind, this chapter analyzes CancerSim’s output.

For each parameter, CancerSim Review was used to locate a region of interest. An “interesting” region is one in which only the parameter in question varies, and changing the parameter has a noticeable effect on the development of cancer. These single-parameter sweeps are intended to indicate the parameter’s general ef-

fect, though in fact the parameter might have a different effect with another set of fixed parameters. Finally, 100 runs were performed varying only the random seed in order to examine the stability of model. Using these data, studies of the “pathways to cancer” and “longevity” were performed.

4.1 Pathways To Cancer

An issue raised by the Hallmarks paper is the sequence of the changes leading to cancer. The authors believe that “virtually all cancers must acquire the same six hallmark capabilities [6].” Does this imply that the progression from normalcy to cancer is inflexible and predictable? Not according to Hanahan and Weinberg. Instead, the hallmark behaviors may have varied causes, and may be obtained in varying orders. CancerSim records the history of the phenotype for each cell. This is simply the order in which the cell’s mutations were acquired by its ancestors, or its “pathway.” Only the current phenotype of the cell affects its behavior, but the pathway is recorded for the purposes of this analysis.

In CancerSim, as in vivo, the tissue is practically never homogeneous at the time of death; it contains cells with various phenotypes. Moreover, cells with identical phenotypes may have obtained them by different pathways. In fact, even if the tissue consists *only* of cells with all six of the phenotypes, up to $6! = 720$ different pathways are theoretically possible. In such a tissue, all cells would have identical phenotypes, so continuing the simulation would not be informative. Finally, cells having taken the same pathway are *probably* but *not necessarily* related (other than through the original cell); it may be coincidence that two distinct cell lines receive mutations in the same sequence.

We call a pathway “predominant” if it is shared by more than 50% of the cells in the tissue at the time of death. By this definition, a tissue might not have any

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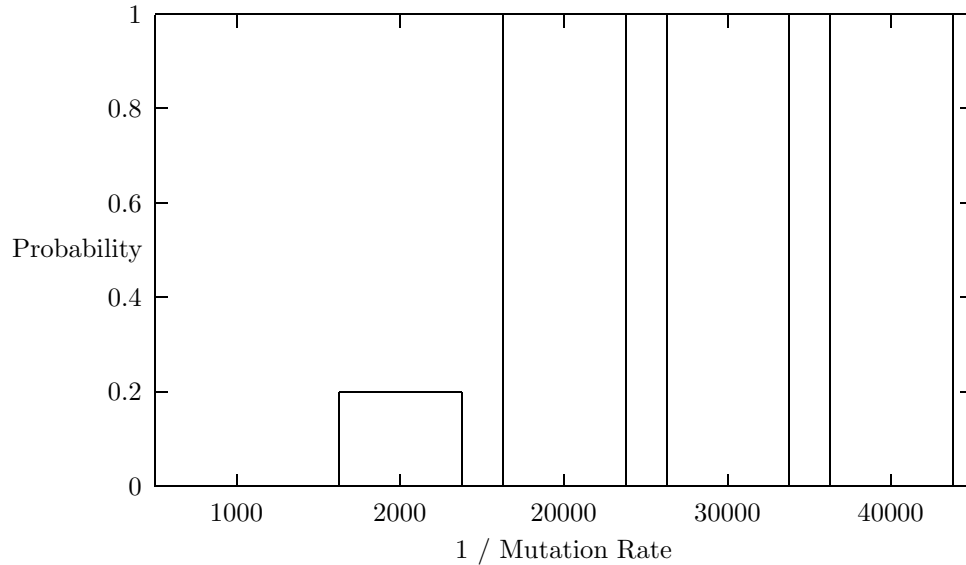


Figure 4.1: Likelihood Of Predominant Pathway Vs. Mutation Rate

predominant pathway. Figure 4.1 shows how the probability of finishing with some predominant pathway varies according to the base mutation rate, taking the average over all other parameters. Only runs ending with cancer are included.

When the mutation rate is low (towards the right end of Figure 4.1), the simulation proceeds in an orderly manner. First, some mutation occurs. If the mutation conveys a selective advantage, descendants of the mutant cell multiply until the selective advantage is fully exploited. (For instance, the “Self Growth” mutation allows growth into the area lacking only growth factor. It provides no selective advantage within the region where growth factor is already plentiful). If the cell has all the necessary mutations, this means filling all available space, causing the simulation to end. Otherwise, the simulation proceeds until the next mutation. Thus, low mutation rates raise the probability of some pathway predominating.

When the mutation rate is very high, mutations are not given time to play out independently. Descendants of a mutant cell are likely either to be hampered by

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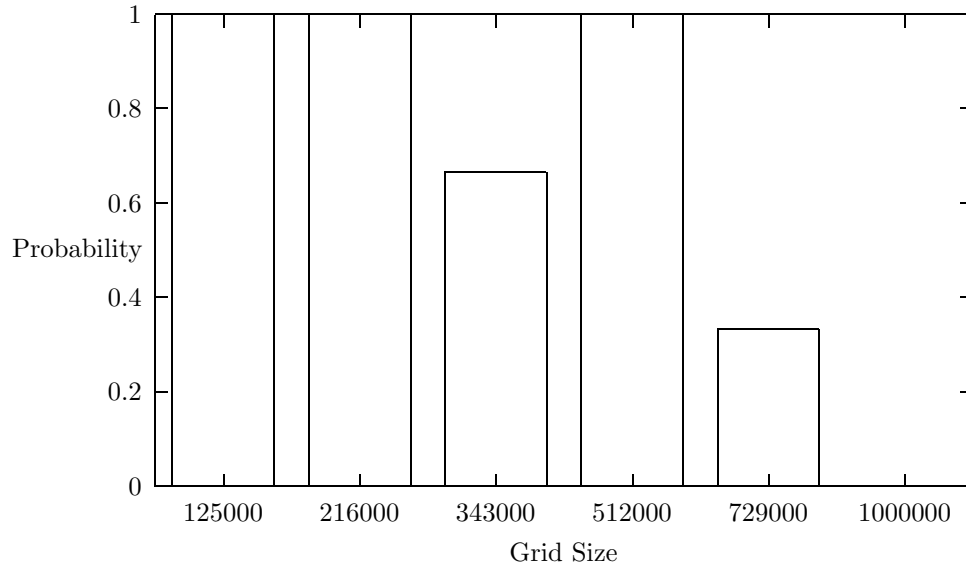


Figure 4.2: Likelihood Of Predominant Pathway Vs. Grid Size

other more degenerate cells, or else develop their own mutations.

Figure 4.2 indicates that running on a larger computational grid also decreases the likelihood of some pathway predominating. This may be because it takes longer for each advantageous mutation to sweep the larger tissue, which allows more time for additional mutations. This effect is similar to the case in which the grid is smaller, but the mutation rate is higher.

Having examined the likelihood that some pathway will predominate, we can ask which pathways are the most likely to do so. We analyze the random seed parameter sweep, which consists of 100 runs varying only by random seed. Cancer arose 96 of 100 runs. Of those 96 runs, 90 completed with some predominant pathway. Of these, all but one were permutations of the same 4 mutations. In all, only 7 different pathways predominated, with the top 2 accounting for over 50% of the runs. Clearly, in CancerSim, all pathways to cancer are not equally likely.

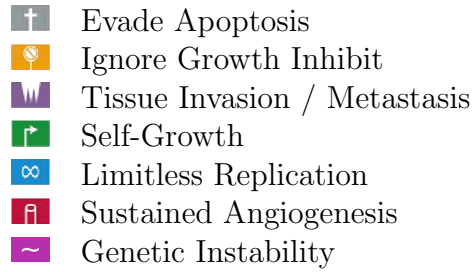


Figure 4.3: Pathways of Cancer Legend

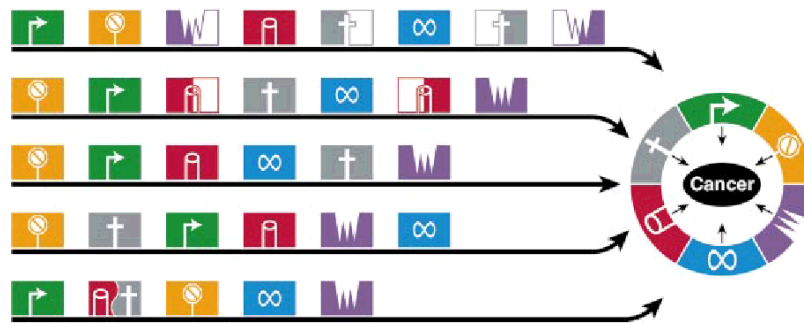


Figure 4.4: Pathways of Cancer (Reproduced From Hanahan [6])

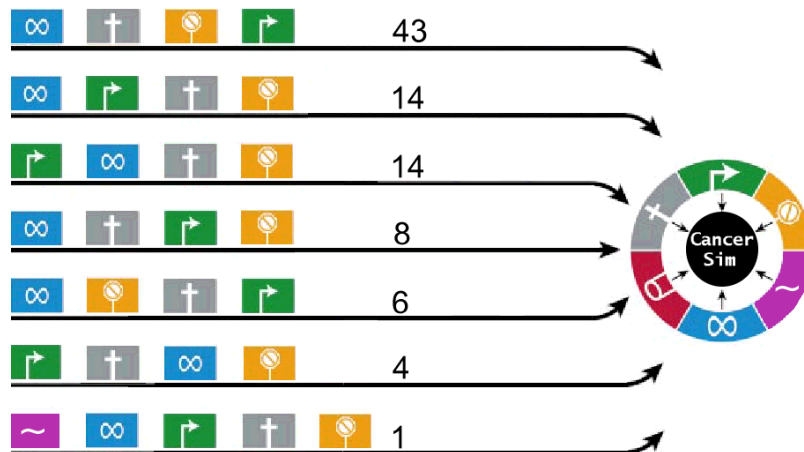


Figure 4.5: Pathways of CancerSim. The number of occurrences of each pathway is indicated.

Surprisingly, the Sustained Angiogenesis mutation appears in only one pathway. This does *not* mean that Sustained Angiogenesis was unimportant in these runs, only

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that the predominant phenotype at the end of the run did not have this mutation.

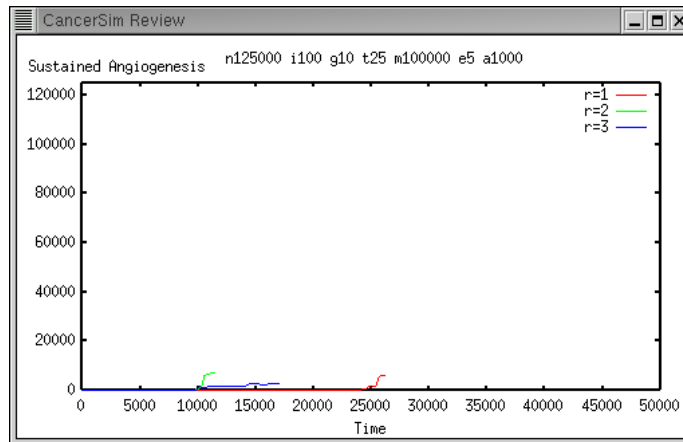


Figure 4.6: Consistent Low-Level Angiogenesis

Figure 4.6 shows the population of angiogenic cells during three simulations that resulted in cancer. In each case, there is a significant number of angiogenic cells. However, the number of such cells is small, and not part of the predominant pathway. Still, these cells cause the angiogenesis necessary to support the growth of the tumor. Ultimately, “Cause Angiogenesis” is a rare mutation because its effects can be shared.

Evade Apoptosis, on the other hand, appears in every pathway. However, it never occurs first in the sequence. This makes sense, because it conveys no selective advantage until the cell has at least one other mutation.

Only two pathways involve the Genetic Instability phenotype. This does not agree with studies indicating that genetic instability is truly a hallmark of cancer, and that it appears early in carcinogenesis[2]. It seems that in CancerSim, genetic instability is a very weakly selective mutation.

Limitless Replication usually occurs first. This is a bit surprising since this data was collected from the random seed parameter sweep, which used an initial telomere length of 100, and 2^{100} is about 10^{30} - an enormous number of cells. This means

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that the *average* cell is under little pressure to escape apoptosis due to telomere shortening. However, to benefit from highly selective mutations and sweep the tissue, precancerous cells must be able to replicate many times.

The “Ignore Growth Inhibit” mutation is consistently near the end of the pathway. It may be that other mutations are more selective until most of the space initially inaccessible due to insufficient growth factor and vascularization is occupied.

4.2 Longevity

We define longevity as the amount of time taken for the tissue to develop cancer. To prevent any run from continuing forever, we impose an arbitrary cutoff of 50,000 time steps. If the tissue dies before the maximum number of time steps (due to telomere shortening), the longevity is simply the maximum number of time steps; that is, only cancer decreases longevity.

Each data point in the longevity plot represents the average longevity of the tissue over several runs with a fixed set of parameters (other than the random seed). The number of trials for each data point is indicated on the plot. The total number of runs contributing to the plot is the number of data points times the number of trials per data point.

4.2.1 Grid Size

In CancerSim, the natural extent of the tissue (as defined by growth factor concentration and vascularization) is proportional to the grid size. The larger the grid, the more cells in the tissue. This seems to generally increase the prevalence of cancer (Figure 4.7). In the model, more cells provide more opportunity for mutation and

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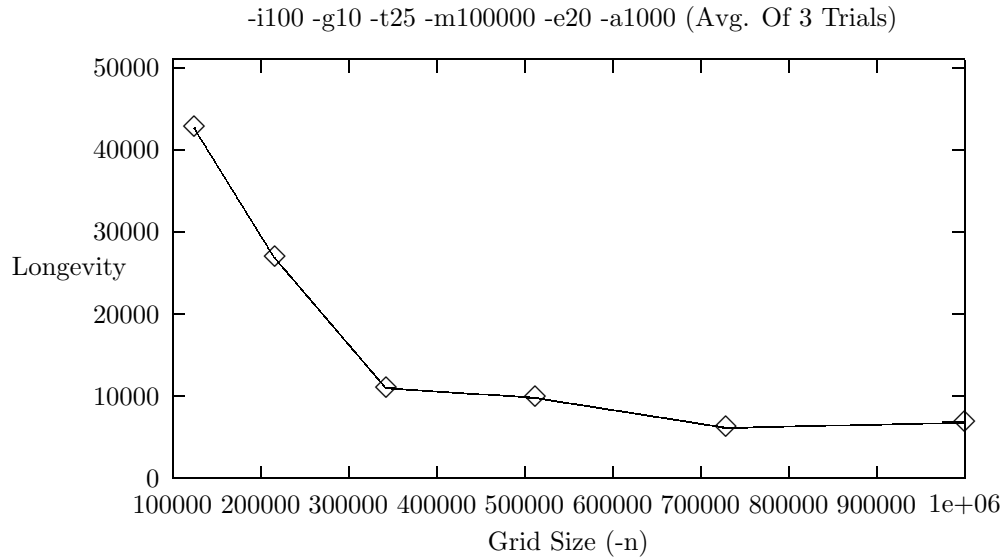


Figure 4.7: Longevity Vs. Grid Size

the onset of cancer.

This leads to a hypothesis about cancer in large vs. small organisms. An occurrence of cancer anywhere in the body will metastasize and invade other tissue. Ultimately, it will kill the organism. In effect, each cell in a multicellular organism puts the others at risk. But because cancer is not contagious, the risk is limited to the individual. Thus, a given number of cells will be more resistant to cancer when divided among a larger number of individuals. The joint liability of multicellularity seems to favor smaller organisms and exert more evolutionary pressure on large ones. For this reason, organisms with more cells (or more cellular turnover) probably have more highly evolved cancer defense mechanisms.

Rodent cells (often used in cancer models) are more sensitive to carcinogens than human cells. This is due to the relative sophistication of the human mechanisms of cell growth, programmed apoptosis, and differentiated function; all of these counteract oncogenes[17]. CancerSim indicates that this relation may hold generally for

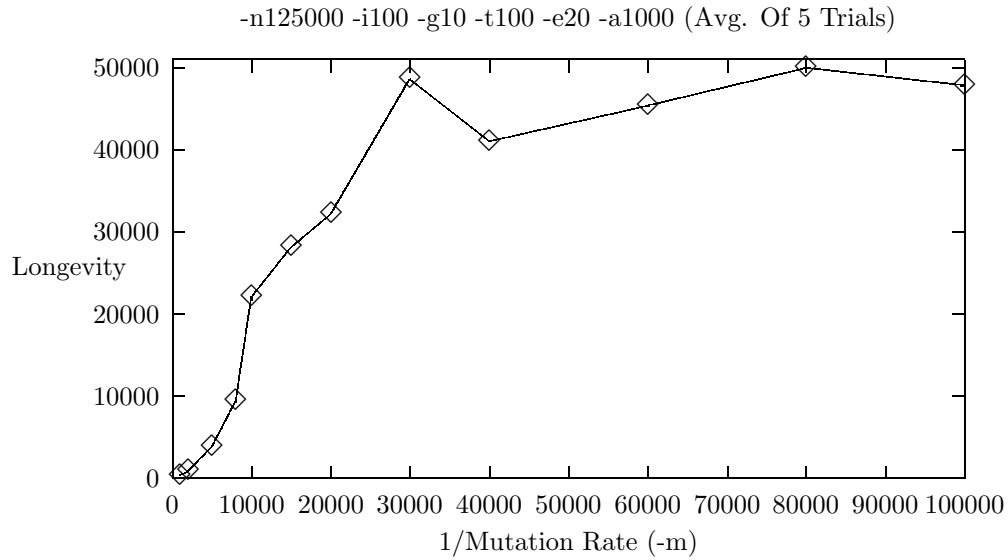


Figure 4.8: Longevity Vs. Mutation Rate

large animals relative to smaller ones.

4.2.2 Mutation Rate

CancerSim is highly sensitive to the mutation rate parameter (Figure 4.8). This confirms all indications that a mutagenic environment is likely to cause cancer.

4.2.3 Genetic Instability

Genetic instability has some effect, though it is not very pronounced (Figure 4.9). Considering the dramatic effect of the base mutation rate (see Figure 4.8), this deserves examination.

Unlike the other mutations, genetic instability is of no *direct* benefit to the cell because it does not increase the likelihood of survival or of reproduction. In the short

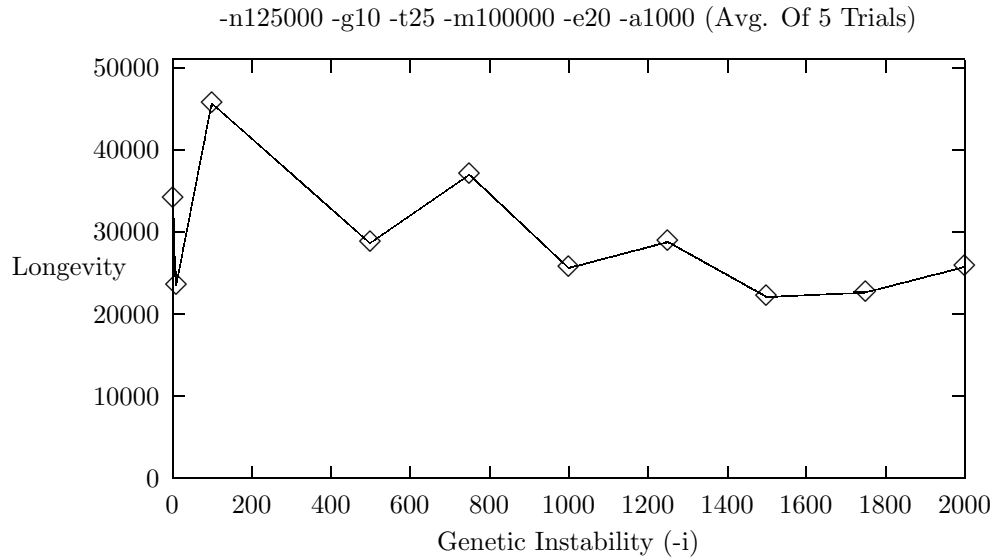


Figure 4.9: Longevity Vs. Genetic Instability

run, it is a neutral mutation [9]. Genetic instability does make future mutations more likely, but apparently this is only enough to decrease longevity slightly. This is the “associated-selection model” [2], whereby an allele is selected only through its relation to some other selective allele.

Other models of genetic instability would have a much larger effect. To simulate environmental effects, all cells might obtain genetic instability together. This would be equivalent to raising the base mutation rate. This might better model the effect of mutagenic carcinogens, whose effect is never localized to just a single cell.

4.2.4 Random Apoptosis

High rates of random apoptosis promote cancer (Figure 4.10). Increased cell turnover provides more opportunity for mitosis, and therefore for mutation[16].

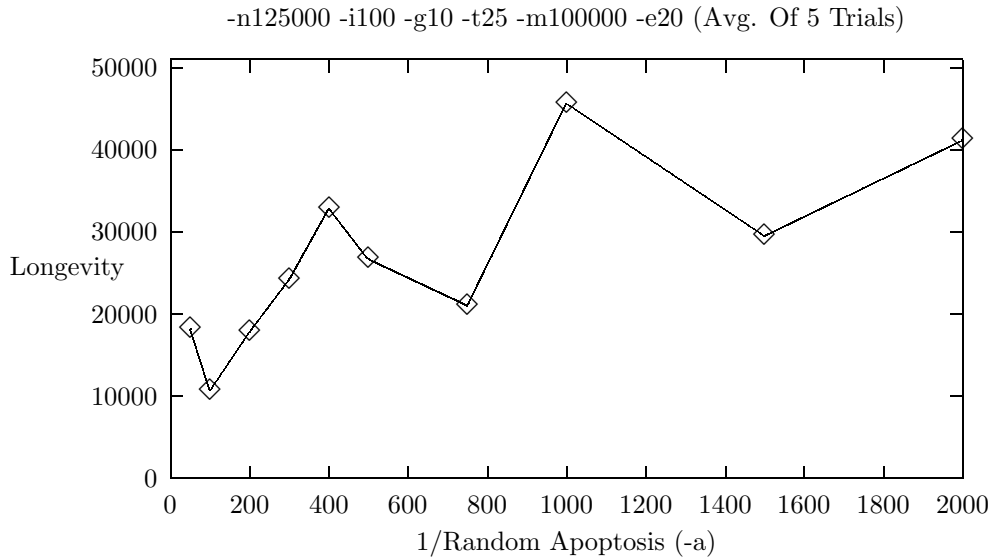


Figure 4.10: Longevity Vs. Random Apoptosis

4.2.5 Telomere Length

The length of telomeres (t) in the initial cell places a limit of 2^t on the size of the cell population. This limit clearly manifests itself for extremely small t . Figure 4.11 shows the total number of cells created during runs with very small values of t .

For any reasonable telomere length, though, 2^t is very large. Typically, at least one cell will acquire the “Limitless Replicative Potential” phenotype. Thus, unless t is very small, the number of cells created doesn’t noticeably correspond to the theoretical limit.

For moderate values of t (approximately 50 to 150 in Figure 4.12), telomere shortening appears to increase the risk of cancer. Even though there is ample replicative potential to fill available space, the increase in cell turnover due to telomere exhaustion provides more opportunity for mutation.

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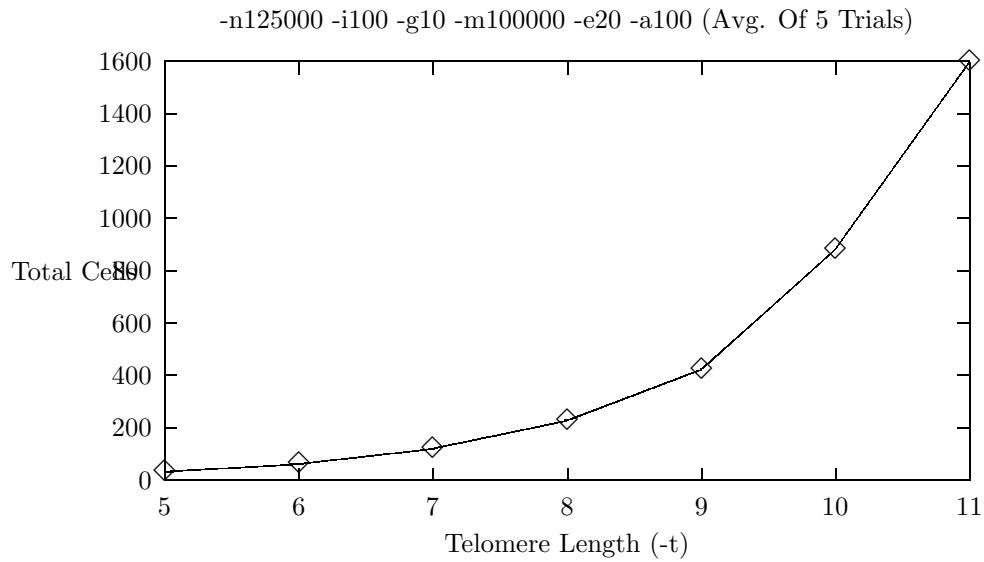


Figure 4.11: Cell Count Vs. (Short) Telomere Length

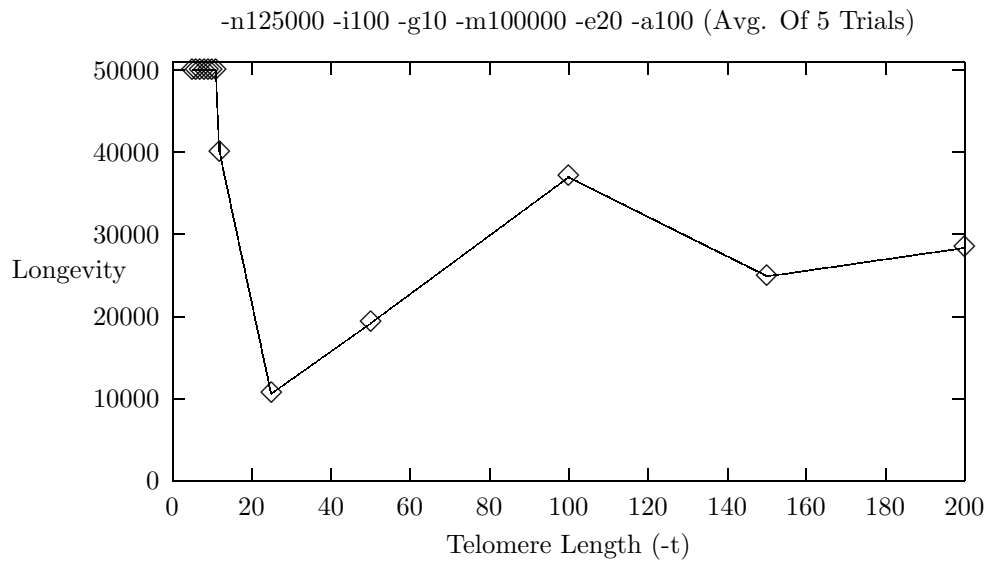


Figure 4.12: Longevity Vs. Telomere Length

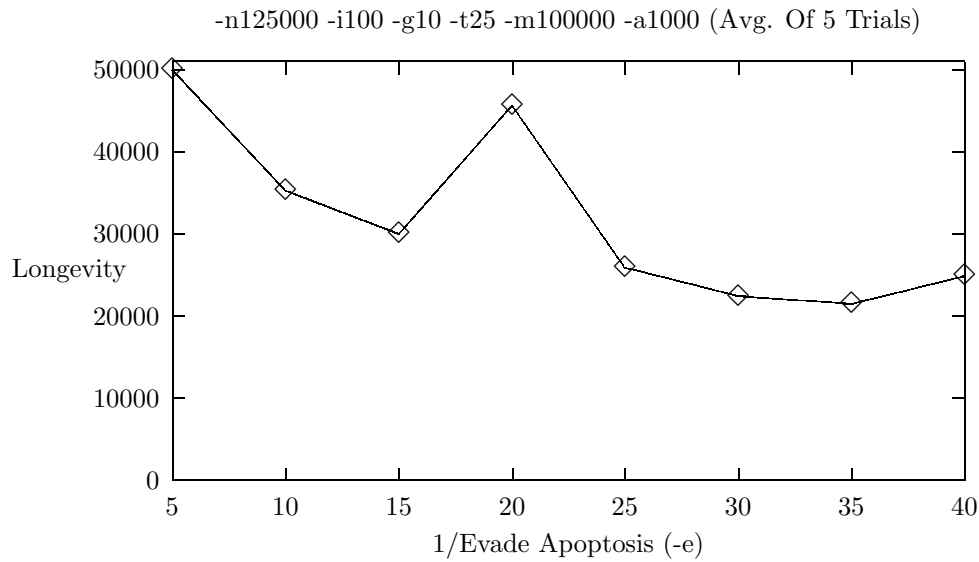


Figure 4.13: Longevity Vs. Apoptosis Evasion

4.2.6 Evade Apoptosis

The body's efficiency in eliminating defective cells has a significant impact on longevity (Figure 4.13). Mutant cells can sometimes be killed before proliferating. But if the cell is able to make enough redundant copies of itself before being destroyed, it is too late. Any mutant cells killed are quickly replaced, perhaps with additional mutations.

4.2.7 Ignore Growth Inhibit

In CancerSim, the tissue quickly reaches its natural extent. Without mutation, new cell growth is possible only when apoptosis frees up space. The "Cause Angiogenesis" and "Self-Growth" phenotypes allow cells on the surface of the tissue to extend out into unused space beyond the natural extent of the tissue. However, most of the cells are interior to the tissue, not on its surface. The "Ignore Growth Inhibit" phenotype

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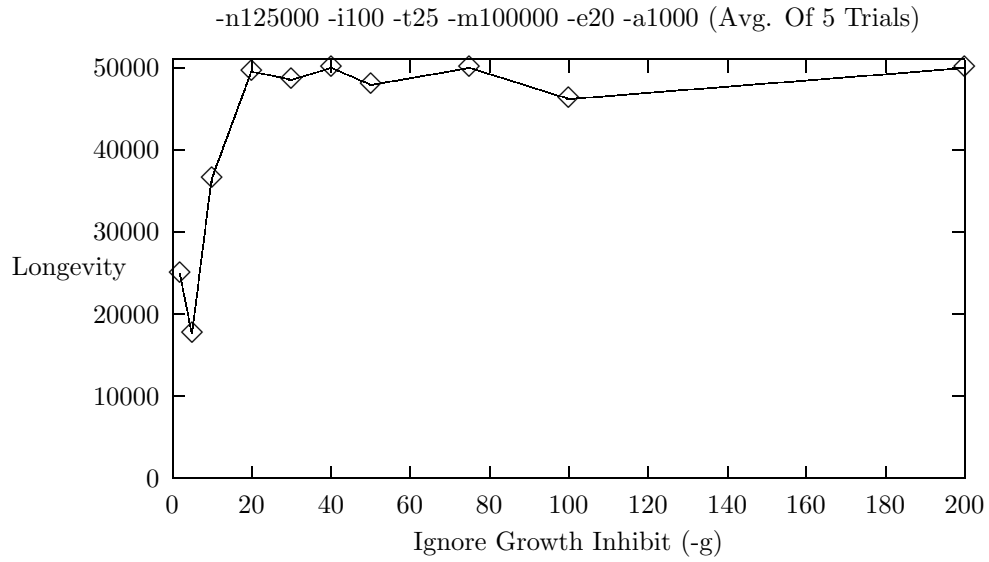


Figure 4.14: Longevity Vs. Ignore Growth Inhibit

allows cells inside the tissue to reproduce even when there is no room available (by killing a neighbor). Because this mutation benefits every space-constrained cell, it is strongly selective, and sharply decreases longevity (Figure 4.14). However, it has little effect when the odds of displacing the incumbent cell are less than about 5 percent.

Chapter 5

Conclusion

At several points during the development of CancerSim, seemingly small alterations to the mechanics of the simulation had large effects on the simulation's dynamics. For instance, the affect of the Genetic Instability phenotype was greatly magnified by applying it immediately instead of only to future mitoses. In another case, an error in the initialization of a random number generator obscured the effect of telomere shortening on tumorigenesis, even though the numbers generated were not regular in any noticeable way. Unfortunately (for the analyst), the dynamics of cancer probably *are* very nonlinear; in some cases, the difference between life and death might be the survival of a certain precancerous cell.

In such circumstances, the key to creating a useful simulation is ongoing dialog between the implementer and a domain expert. CancerSim would not have been possible without input and feedback from Kenneth Pienta, M.D. of Internal Medicine. Reading about cancer is no substitute for laboratory and clinical expertise.

Due to CancerSim's high level of abstraction, care must be taken not to draw conclusions which depend on phenomena simulated insufficiently or not at all.

Chapter 5. Conclusion

An analogy may be made to flight simulation. The “Blue Box” flight trainer was used to train over 500,000 airmen during World War II [1]. This simulator was mounted on four pneumatic bellows to roughly approximate the motion of flight, and its interior featured a control column, foot pedals, and various flight and navigational instruments. Yet the Blue Box was fully enclosed and give the pilot no outside view. It was useful for instrument training even though it failed to simulate most other aspects of flight. In contrast, the recent Boeing 777 airliner was fully designed and tested as a computer model before ever being built [3]. This feat of modeling and simulation hinged on the science of computational fluid dynamics, and in particular the identification of useful abstractions (such as the Navier-Stokes equations) to describe the flow of fluids without tracing the paths of individual molecules. Effective simulations model valid abstractions of important factors, while excluding unnecessary detail.

In the continuum of simulator fidelity, CancerSim may be closer to the Blue Box than to Boeing’s digital model of the 777 airliner. Still, CancerSim’s implementation of cancer phenotypes permits many interesting observations.

5.1 Disagreement About the Pathways to Cancer

CancerSim’s pathways to cancer are very different from those presented in the Hallmarks paper [6] (compare figures 4.4 and 4.5). Hanahan and Weinberg place Insensitivity to Anti-Growth Signals near the beginning of each pathway, and Limitless Replication towards the end. In CancerSim, just the opposite is true. There are several possible explanations for this discrepancy.

First, it may be that CancerSim models the phenotypes incorrectly or insufficiently. Creating a working implementation requires many interpretations and best-guesses. There might be errors in CancerSim’s implementation. However, it may also

Chapter 5. Conclusion

be that such a small number of phenotypes fail to sufficiently characterize cancer. If so, some important cancer phenotypes are being overlooked and deserve more study.

Second, CancerSim may have been run with incorrect parameters. For instance, Limitless Replication might have been overly favored because the telomere length specified for the cells was too short. Further parameter studies could reveal whether the pathways encountered by CancerSim are stable under various parameters.

Finally, the pathways suggested by Hanahan and Weinberg might be incorrect. There may be errors in our understanding of the hallmarks cancer. CancerSim is a useful tool for re-examining the cancer phenotypes and their interactions.

5.2 The Mutation Rate Isn't Everything

The largest determinant of longevity in CancerSim is the number of mutant cells created. Predictably, cancer develops more quickly when the mutation rate is high. However, the mutation rate is only one factor contributing to the total number of mutations. The size of the organism and the expected lifespan of its cells must be factored in, too. These determine how often opportunities for mutation arise.

This indicates a dilemma in cancer treatment. On one hand, the cancerous cells must be killed. On the other hand, trying to kill them may simply cull the weaker or less adaptable cell lines, leaving more space and sustenance for the elites which remain. This underscores the importance of carefully targeting only malignant cells in therapy.

Furthermore, evolution may have optimized DNA transcription to maximize genetic adaptation, subject to some low rate of cancer given the expected lifespan of the species. If this is true, efforts to incrementally extend lifespan may be progressively hindered by cancer.

5.3 Cancer and Multicellularity

Cancer is impossible without angiogenesis, yet Cause Angiogenesis is often not in the predominant phenotype at the end of the simulation. This is because the beneficial effects of angiogenesis are shared with other, non-angiogenic cells. This may not be completely realistic, but it raises the question of why all cells aren't "cancerous," reproducing whenever possible without regard to the welfare of other cells and the organism as a whole.

The mutations causing cancer are all selective at the cellular level, so cellular "evolution" towards cancer is inevitable given enough time. How, then, did multicellularity arise? How did single-cell organisms evolve into such complex arrangements of differentiated cells? The interactions of multicellularity are more than symbiosis; noncancerous cells go so far as to obey signals prompting apoptosis. Further research is needed to elucidate the forces causing multicellularity. Such insight could suggest more effective methods of cancer treatment or avoidance, which is the ultimate aim of CancerSim.

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