Simulating the Hallmarks of Cancer

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Abstract

Cancer can be viewed as the loss of cooperative cell behaviors that normally facilitate multicellularity, including the formation of tissues and organs. Hanahan and Weinberg describe the phenotypic differences between healthy and cancerous cells in a paper titled "The Hallmarks of Cancer" [10]. The authors propose six phenotypic changes at the cellular level as the essential hallmarks of cancer. This paper investigates the dynamics and interactions of these hallmarks in a model known as `CancerSim`.

The paper describes how CancerSim implements the hallmarks in an agent-based simulation which can help test the hypotheses put forth in [10]. Experiments with CancerSim are described that study the interactions of cell phenotype alterations, and in particular, the likely sequences of precancerous mutations, known as "pathways." The experiments show that sequencing is an important factor in tumorigenesis, as some mutations have preconditions—they are selectively advantageous only in combination with other mutations. CancerSim enables a modeler to study the dynamics of a developing tumor and simulate how progression can be altered by tuning model parameters.

Keywords: Cancer, Agent simulation, Cellular automata

[1] Artificial Life (to appear)

1 Introduction

In 2002 an estimated 6.7 million people died from cancer worldwide, and 10.9 million cases were diagnosed [18]. Over the next 25 years, some expect the number of diagnoses to double to 20 million[19] as infectious disease is better controlled and longevity climbs in developing nations. Although the need for better cancer treatment is obvious and significant resources have been devoted to "winning the war on cancer," progress has been slow and treatments are generally limited to specific forms of the disease. Most cancer research focuses on particular reactions or signal transduction pathways, which may offer targets for therapy. At this level of detail each variety of cancer is a unique and complicated phenomenon, and it is difficult to assess the effects of individual genetic alterations on the cell or tissue as a whole.

A more general view of cancer is outlined in Hanahan and Weinberg's "The Hallmarks of Cancer" [10], which suggests that six cellular alterations are essential to malignant growth. These six "hallmarks" are believed to be common to most or all human tumors. Such a view offers the hope that our understanding of cancer may some day move beyond the cataloging of specific cellular processes to a more general and unified theory of how such processes result in disease and how they might be manipulated to control or halt progression to cancer. To achieve this, however, will require an understanding of dynamics—how the mutations leading to the phenotypic hallmarks interact with one another and their surrounding environment.

Cancer cannot be understood simply by considering individual mutations. The effect of a mutation often depends on the context of other mutations within the same cell, the context of other mutant cells within a tumor, and the context of the abiotic environment of the tumor and patient. Cancer is thus a complex evolutionary system in which tumors consist of phenotypically (and genetically) heterogeneous populations of cells that are competing for scarce resources. Cancer is especially interesting from an artificial-life perspective because evolutionary dynamics and ecological dynamics occur on similar time scales and involve many subtle interactions.

In this paper, we are concerned with the dynamics of how cell populations become heterogeneous and acquire the hallmarks of cancer. Thus, we are interested in the relative frequency of different mutational pathways (which sequences of mutations are most likely), how long the different pathways take, and the dependence of pathways on various parameters. The paper describes an agent-based simulation known as `CancerSim` that models the Hanahan and Weinberg hallmarks. It then describes experiments that explore the consequences of Hanahan and Weinberg's hallmarks in terms of the dynamical properties of tumor formation and growth. By studying these cell population dynamics we hope to better characterize the processes of cellular alterations that underlie tumorigenesis. In particular, we would like to determine how well Hanahan and Weinberg's hallmarks can account for the appearance and behavior of cancer. Because the hallmarks specify cell *behavior*, they have implications for cell population dynamics that are difficult to foresee without an analytical model. If an implementation of the hallmarks failed to reproduce patterns commonly observed in cancer, we could conclude that the set of hallmarks is incomplete or not implemented correctly. Discovering new hallmarks might lead to new targets for therapy. If, on the other hand, the hallmarks explain cancer reasonably well, phenomena arising in the model may give rise to

testable hypothesis about cancer.

CancerSim models cancer abstractly, at the level of cellular interactions within a solid animal tissue. The simulation takes place in a bounded three-dimensional Cartesian array representing a tissue volume of approximately $1mm^3$, and on a time scale of many cell lifetimes (around 60 years assuming each timestep represents 1 hour). Users can see how the hallmarks interact, and they can affect the simulation by changing model parameters (Figure 2). Cells differentiate by random phenotypic mutations that occur during mitosis. A mutation is likely to propagate through the cell population over time if the mutation is advantageous in the microenvironment formed by neighboring cells. Limited space and nutrient places selective pressure on the cell population, and interesting evolutionary behaviors emerge over time.

2 Modeling the Hallmarks of Cancer

Hanahan and Weinberg describe the phenotypic differences between healthy and cancerous cells [10]. The authors propose six phenotypic changes at the cellular level as the essential hallmarks of cancer: unlimited mitosis; ignoring growth-inhibition signals; escaping dependence on external growth stimulation; the ability to recruit new vascular structures; motility and invasion; and disabling the safety mechanisms that normally detect mutation and trigger apoptosis. Genetic instability is an additional factor which accounts for the high incidence of mutations in cancer cells. In the following, we describe these hallmarks briefly and summarize how we chose to model them in CancerSim.

2.1 Self-Sufficiency in Growth Signals

Normal cells divide when stimulated by growth signals from other cells. This occurs when signaling molecules are bound by transmembrane receptors, prompting cell growth and division. In cancerous cells, these receptors are over-expressed, causing hyper-sensitivity to otherwise ambient levels of growth signal [6], or the downstream pathways for these receptors are turned on inappropriately by mutation [16]

In CancerSim, cells undergo mitosis only if they are within a predefined spatial 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 overlaps with the volume in which angiogenic factor is plentiful (described in the next section), but neither volume contains the other. Figure 1 illustrates a two-dimensional cross section of the volumes. Cells can escape the tissue's natural extent through the "Growth Signal" mutation. This mutation causes cells to proliferate regardless of the concentration of growth factor.

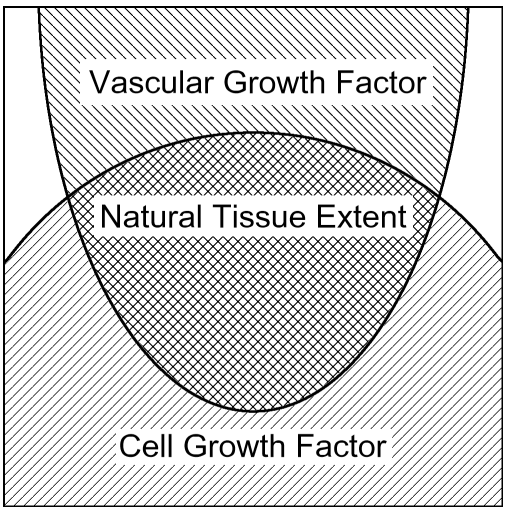


Figure 1: Tissue Growth Bounded by Growth Factor and Vascularization

2.2 Sustained Angiogenesis

Cells cannot survive at distances of more than about 100 μm from blood supply. Despite this fact, cells are normally unable to induce angiogenesis (new capillary growth). Without such growth, tumors can grow only to about 0.5 mm [7]. The importance of angiogenesis to tumorigenesis has been studied since the early 1970's and has resulted in clinical trials of several angiogenesis inhibitors [29].

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 [8]. Some of the inducers are not specific to endothelial cells; they promote growth in other cells as well [7]. 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 [9].

In CancerSim, angiogenesis is inhibited outside of a predefined spatial region (Figure 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 roughly models hypoxia (oxygen deficiency), arguably the most important external stimulus of angiogenic factor [7].

2.3 Insensitivity to Growth-Inhibitor Signals

In nature, 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 it 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 (neighbors 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 with a $1/g$ likelihood of success, where g is a tunable parameter.

2.4 Evasion of Apoptosis

Cells have mechanisms to initiate apoptosis (programmed cell death). Normal cells express receptors for extracellular and intracellular apoptosis signals. Such signals are sent by intracellular sensors which detect DNA damage, signaling imbalance, survival factor insufficiency, or hypoxia. Oncogene over-expression is among the factors that trigger early apoptosis. Tumor cells lack some of the surface features present on normal cells and express some features not normally present on mature cells [25]. The immune system detects and responds to these cells as non-self. In this way, the body is protected from malfunctioning cells. To become cancerous, cells must disable either the effectors of apoptosis 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. The P53 mutation is not the only way to evade apoptosis triggered by DNA damage, but it is probably the most important.

Before undergoing mitosis, each cell in CancerSim is checked for genetic damage. If mutation is detected, the cell undergoes apoptosis instead of mitosis. The probability of detection of damage in the cell is $\frac{n}{e}$, 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.

2.5 Limitless Replicative Potential

Cells normally become senescent after a fixed number of divisions and are unable to continue dividing. In culture, human cells are limited to 50-70 divisions (the "Hayflick Limit" [11]), although most die before exhausting this potential. This limitation prevents many tumors from growing large enough to be seen without a microscope. Experiments show, however, that cells can acquire limitless replicative potential. Such cells are said to be immortal.

The mechanism of limited replicative potential arises from the inability of DNA polymerase to replicate chromosomes completely. 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, and the unprotected DNA fuses together. Once the cell's genetic material is in this deformed configuration, the cell dies quickly.

In cancerous cells, 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. Alternatively, telomeres can be lengthened through homologous recombination. Under normal circumstances, such an outcome is prevented by mismatch repair machinery. However, this machinery may itself be defective, inducing one form of genetic instability [2].

Telomere shortening is simulated by CancerSim. The initial cell is created with telomeres of length t, a parameter of the simulation. With each mitosis, the telomeres are shortened by one unit length. When the telomere length falls to 0, the cell dies. The *limitless replication* mutation stops telomere shortening. Cells with this mutation can undergo mitosis any number of times.

2.6 Tissue Invasion and Metastasis

The final stage of cancerous development is known as metastasis, the spreading of cancer to new locations in the body. 90% of cancer fatalities are caused by these secondary metastatic lesions because they cannot be cured by local therapies such as surgery or radiation [23].

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. Once freed from their moorings, cancer cells escape the influence of regulatory 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).

In its current form, CancerSim does not simulate metastasis, because the simulation represents only a single tissue. We assume that metastasis can occur once cells reach the outer boundary of the simulation.

2.7 Genetic Instability

A tumor is not simply a homogeneous clone of cells. Tumor cell heterogeneity arises from multiple clones of cancer cells created through mutation. Genetic transcription is generally a highly accurate process, but cytotoxic carcinogens can cause genetic instability by altering the cellular environment. This alters selective pressure, favoring clones of cells that can evolve quickly, i.e. those with the greatest genetic instability [2]. In an environment without carcinogens, cells are better off with low mutation rates because most mutations are deleterious. However, genetic instability may be advantageous to the individual cells in unstable and hostile carcinogenic environments. There are several enzymes in the body to detect and repair different kinds of genetic damage, and loss of these may correspond to specific types of genetic instability[2].

Experimental evidence suggests that the number of acquired genetic mutations in cancerous cells is greater than 10,000 [24, 13], although mathematical models suggest that the actual number of mutations is probably 10^{12} or more [26]. However, the vast majority of these likely have no effect on the progression of the malignant growth, so they are not modeled.

The exact mechanism of genetic instability is unknown, but the rate at which mutations must occur suggests a clonal phenotype that allows rapid accumulation of genetic damage.

Although the cause of genetic instability in cancer is not fully understood, there is little doubt that it exists as tumors exhibit a wide variety of cell phenotypes, collectively referred to as cell heterogeneity. In CancerSim, genetic instability is modeled as a "mutator phenotype" which causes other mutations to occur with increased likelihood. Cells that have become genetically unstable have their base mutation rate scaled up by a tunable parameter.

3 CancerSim

CancerSim implements the hallmarks described above in a three-dimensional agent-based simulation that resembles a cellular automaton. The simulation consists of cells and a circulatory system (vasculature), both of which grow according to their own rules. Each cube in the three-dimensional simulation contains either one cell or empty space, although the vascular structure may pass through a cube (with or without an occupying cell). CancerSim has both command-line and graphical user interfaces to support interactive investigation and batch execution. The graphical interface is illustrated in Figure 2.

Even with modern processor speeds, it is still not feasible to simulate a realistic number of cells using an individual-based approach in which each cell is represented explicitly. On a 2 GHz PC, CancerSim can comfortably simulate up to about 1,000,000 cells, representing approximately 1 cubic millimeter of tissue. A tumor that is detectable by standard radiographic techniques is approximately a cubic centimeter in size and represents 1 billion cells, while the entire human body has approximately $5 * 10^{13}$ cells. Modern computers have on the order of 10^9 bytes of memory, which is too little storage to simulate all cells in the body by 5 orders of magnitude (assuming 10 bytes of memory for each cell), and computational speed is similarly inadequate. Thus, a parallel version of CancerSim would require about $100,000$ nodes to simulate the number of cells in a human body. Despite these limitations, it is important to simulate a large number of cells in order to observe the evolutionary trajectories and interactions in which we are interested. And, it is important to run repeated experiments in order to observe the distribution of outcomes. Thus, CancerSim's representation of cells and cell dynamics is quite abstract in order support these requirements.

The simulation begins with a single cell. As the simulation proceeds, cells replicate, die (apoptose), or signal for angiogenesis (request blood supply). During mitosis, cells acquire phenotypic mutations probabilistically. Normal cells divide only within a confined region of the simulated space. The simulation terminates when the tissue occupies 90% of the simulated space, when all cells die, or after an arbitrary number of steps. Cells are stationary and do not move from their place of origin.

Cells are displayed according to a color scheme, in which the color of a cell depicts which hallmarks it has acquired. There are $2^6 = 64$ possible mutational states that a cell can be in, so there are 64 different color assignments representing these possibilities. Normal cells are coded gray and cells with all six hallmarks are coded black.

CancerSim is implemented in C++ and is available under the terms of the General Public

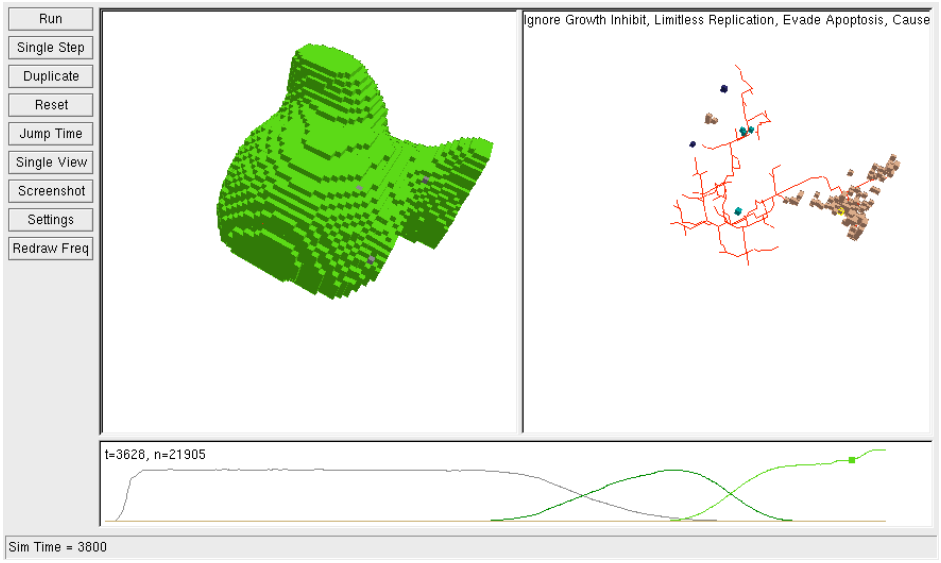


Figure 2: Cancerous tissue displayed by the CancerSim visualization package. The display is divided into three regions, menu buttons for controlling the simulation and setting parameters; time-series plots of cell population levels for each phenotype present in the tissue; and a real-time display of growing tissue. In this example, the display is in "dual view" mode in which two different views of the same tissue are displayed simultaneously. The left-hand view shows the exterior of the growing tissue. In the right-hand view, the green cells have been selected away, so the underlying vascular structure and less frequent cell phenotypes can be seen.

License (GPL) from `http://www.cs.unm.edu/~forrest/software/cancersim/` . CancerSim uses the wxWindows[21] 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.

3.1 Cells

CancerSim's model of cellular mechanisms is highly simplified, which allows us to simulate large cell populations and to focus on inter-cellular interactions. Cell properties are expressed as phenotypic traits rather than genes, and the particular traits of a cell are represented as a boolean vector whose values enable or disable the six hallmark phenotypes. For convenience, we refer to this vector as a "genotype." A small amount of additional state information is maintained for each cell: telomere length (controls how many times a cell line is allowed to divide), mutation history, unique identification tag, capillary information (is there a capillary at this cell's location?), and nutrient level (is the cell supplied with oxygen and energy?). Table 1 summarizes this information.

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 and energy at the cell's location
Sequence Number	integer	Tag to distinguish between cells reusing the same grid location

Table 1: CancerSim: Information contained in every cell

3.1.1 Cell Lifecycle

In CancerSim each cell goes through a primitive cell cycle. The G0 phase is triggered by contact inhibition. Thus, cells with a full set of neighbors normally do not attempt to replicate. Cells next to empty space within the tissue's natural extent automatically enter the G1 phase. G1 is modeled by the passage of time; each cell occupies equal volume and the physical enlargement of cell growth is not simulated. DNA replication occurs in the S phase. Daughter cells normally inherit the genotype of their parent, but the replication of DNA occasionally introduces a mutation. Then, at the G2 checkpoint, cells undergo a check for genetic damage. Apoptosis is triggered in cells found to contain genetic defects. (The G1 checkpoint is not modeled separately because it is similar). Finally, the cell undergoes mitosis in the M phase. One of the daughter cells occupies the grid location of its parent, while the other fills an empty adjacent location.

In CancerSim, cells undergo random apoptosis with low probability. On each cell cycle, each cell is subjected to a $\frac{1}{a}$ chance of death, where a is a tunable parameter. This might be due to mechanical, chemical, or radiological damage, aging, or the immune system.

3.2 Vascularization

All cells require blood flow to provide nutrients. CancerSim's vascular system develops according to signals sent by the cells. Nutrient diffuses from each capillary segment into the surrounding elements of the grid. The nutrient available to each cell is the sum of the contributions of all capillary segments, but the contribution from distant capillaries is

vanishingly small, as nutrient levels decrease as a power law function of the distance from their source.

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. This may create a branch in the vasculature if the newly created cell is not in a direct line with existing capillaries. In principle, a branch can be formed anywhere in the vascular tree and not just at the leaves, which is a departure from reality. However, even with this simple growth rule, natural looking fractal-like structures emerge endogenously, as illustrated in Figure 1. If the new cell is outside the predefined angiogenic boundary, no angiogenesis will occur unless the cell's phenotype includes *cause angiogenesis*.

3.3 Event Model

CancerSim varies from classical cellular automata in a couple of ways. The update rules are associated with the cells of the simulation (not to be confused with "cells" in a classical cellular automaton), and the update rules incorporate probabilities, which makes them nondeterministic. Also, 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, no more than about 16,000 elements can be occupied unless several mutations have occurred. The tissue is bounded by growth factor concentration and vascularization (described earlier and illustrated in Figure 1). This allows room for the expansion of the tumor. Second, the only observable changes to cells are apoptosis and replacement. 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 is computationally inefficient.

CancerSim avoids unnecessary updates by 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 single normal cell (no mutations). Mitosis is scheduled for this initial cell, and this mitotic division is the next event. After the new daughter cells are created, mitosis is scheduled for each of them, and so on. Each mitotic division is carried out by copying the "genetic" information of the cell to an unoccupied adjacent 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, such as contact inhibition or insufficient growth signal. Cells overcome these limitations through mutation.

3.4 Completion Criteria

The simulation can terminate in three different ways: All the cells might die off due to aging; the simulation might continue until a fixed maximum number of timesteps has completed (without such a limit, CancerSim could continue indefinitely if its cells had acquired immortality through the *evade apoptosis* phenotype); the simulation reaches a cancerous state. In CancerSim, the criterion for "cancer" is the occupation of 90% of the grid. Healthy, non-cancerous cells can fill only a small portion of the grid because they are bounded by limited vasculature and growth factor. When the simulation is run with the typical parameters listed below, the simulation reaches the cancerous state on the vast majority of runs.

3.5 CancerSim Parameters

In CancerSim, each of the hallmark mutations occurs with equal probability. This probability is determined by the Base Mutation Rate parameter. An additional parameter for each hallmark controls the affect of the associated phenotype as described by Table 2.

In CancerSim, parameter selection is not straightforward for several reasons. First, parameters such as the number of cells in a sizable tissue cannot be set to realistic values due to computational limitations. Second, CancerSim is necessarily an abstraction of real cell dynamics, so some parameters do not have direct analogues in nature. For instance, the Telomere Length parameter controls programmed cell destruction, which is actually a complex, environment-dependent interaction of several factors including initial telomere length, telomerase activity, oxidative stress, and even emotional stress [5]. Finally, all parameters interact in complex ways. For instance, the smaller the cell population size, the larger the mutation rate must be to obtain the expected incidence of cancer.

Our choice of parameter values was guided by the observation that hallmarks only have meaningful interactions within some region of interest. At an extreme parameter setting, a phenotype may have no noticeable effect, demoting it from hallmark status. At the other extreme, a hallmark could dominate, becoming in effect the *only* hallmark of cancer. To find regions of interest in parameter space, we conducted an 8-dimensional parameter sweep with 3 runs for each of 1458 parameter settings (4374 runs in total).

Table 2 lists CancerSim parameters and typical values. Each of the cell phenotypes except for *self-sufficiency in growth signals* and *sustained angiogenesis* have an associated parameter.

3.6 Visualization

CancerSim models a tissue in three dimensions, but must display it in two dimensions (on a monitor). In this situation, it is helpful to be able to rotate the tissue smoothly and interactively. Rendering 1,000,000 cells efficiently involved several decisions.

Parameter Name	**Typical Value**	**Description**
Cell Phenotype Parameters		
Telomere Length	100	Telomere shortening limits the initial cell to t replications
Evade Apoptosis	10	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	100	Increase the likelihood of mutation by a factor of i for cells with this mutation
Ignore Growth Inhibit	10	Cells with this mutation have a $\frac{1}{g}$ chance of killing off neighbor to make room for mitosis
Other Parameters		
Grid Size	125000	The computational grid contains n cell locations
Base Mutation Rate	100000	Each allele is copied with a $\frac{1}{m}$ chance of mutation
Random Apoptosis	1000	Each cell cycle exposes every cell to a $\frac{1}{a}$ chance of death from non-specific causes
Random Seed	(any)	Seed value for the pseudo-random number generator

Table 2: CancerSim Parameters

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. Although rendering hardware draws polygons, and triangles in particular, organic-looking curved surfaces must be approximated, by piecing together a large number of triangles which is computationally expensive. In addition, cubes pack together tightly, completely occluding the tissue's interior. This means that only the exterior surface of the tissue need be rendered. This is crucial, because most cells are in the interior of the tissue and therefore do not need to be rendered. Note that the CancerSim interface provides a nice option for users wishing to view the interior of the tissue, illustrated in Figure 1.

4 Experimental Results

In this section we describe initial experiments that we conducted with CancerSim, first to calibrate the model and then to begin studying what the hallmarks model implies about how cancers develop.

We first identified a region of interest for each parameter by experimenting with different values. An "interesting" region is a range of parameter values that have a noticeable effect on the outcome of the runs. Then we performed 100 runs varying only the random seed. For this set of runs, each parameter was held at a constant value, selected from its region of interest. Thus, we chose parameter values that gave every hallmark a chance to affect the simulation. Using these data, we then studied the relative frequency of alternate "pathways to cancer."

Hanahan and Weinberg argue that "virtually all cancers must acquire the same six hallmark capabilities." However, this does not imply that the progression from normal tissues to cancer is inflexible and predictable. Instead, the hallmark phenotypes can have varied causes and can be acquired in different sequences. CancerSim records the history of each cell's phenotype. This is simply the order in which the cell's mutations were acquired by its ancestors, known as its "pathway." Only the current phenotype of the cell affects its behavior, but the pathway is recorded for later analysis.

In CancerSim, as in vivo, cancerous tissue is almost never homogeneous at the end of a run; it contains cells with heterogeneous phenotypes. Moreover, cells with identical phenotypes may have obtained them by different pathways, and these different pathways may affect the time it takes to develop cancer. Even if the tissue consists only of cells with all six of the phenotypes, up to $6! = 720$ different pathways are possible. Finally, cells with identical pathways are probably, but not necessarily, related (aside from the common descent from one ancestral cell at the beginning of the simulation); it may be coincidence that two distinct cell lines receive mutations in the same sequence.

We call a pathway "dominant" if it is shared by more than 50% of the cells in the tissue at the conclusion of the simulation. By this definition, a tissue might not have any dominant pathway. Figure 3 shows how the probability of finishing with some dominant pathway varies according to the base mutation rate, taking the average over all other parameters. Only runs ending with cancer are included.

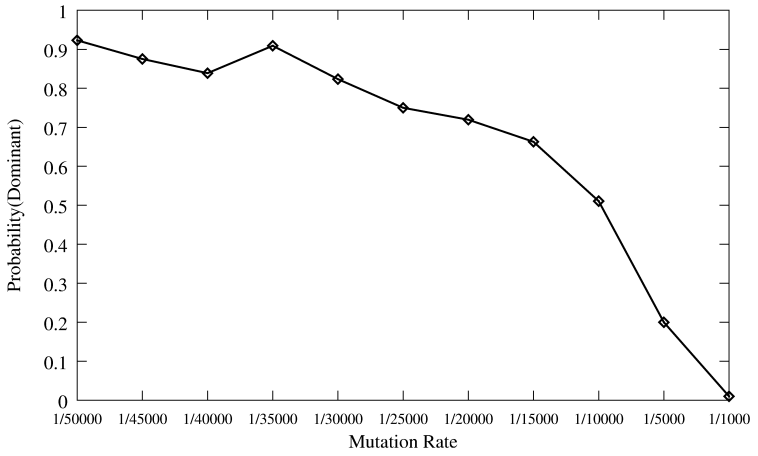
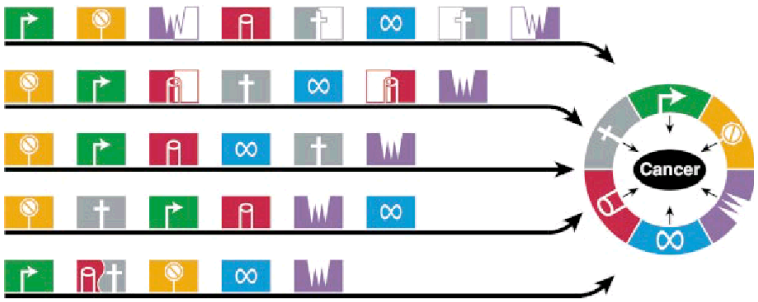


Figure 3: Low mutation rates lead to tumor homogeneity. The figure plots likelihood of a dominant pathway vs. mutation rate. Each data point represents the average of 100 runs at the specified mutation rate. Runs not resulting in cancer were discarded. For each run resulting in cancer, the variable **Dominant** is *true* if over half the cells at time of death share a genetic pathway; *false* otherwise.

When the mutation rate is low (towards the left side of Figure 3), the simulation trajectory has its own logic. If the first mutation conveys a selective advantage, descendants of the mutant cell multiply until the selective advantage is fully exploited. For example, the *self growth* mutation allows its cells to grow into the region lacking growth factor. It provides no selective advantage within the tissue's natural extent, where growth factor is already plentiful, nor outside the vascularized region. Once these ecological dynamics have played out and the mutation has been fully exploited, a second selectively advantageous mutation can gain a foothold and expand to its natural extent. Thus, low mutation rates allow ecological dynamics to play a more important role in the simulation and (as we see in Figure 3) make it more likely that one phenotype will dominate the population. On the other hand, when the mutation rate is high, the effect of a single mutation is less independent. Descendants of a mutant cell are likely either to be hampered by other more degenerate cells, or else develop their own additional mutations.

Having established that some pathways will dominate under low (and biologically plausible) mutation rates, we can ask which ones are the most likely. We analyze the random seed parameter sweep, which consists of 100 runs varying only the random seed. Cancer arose in 96 of 100 runs, and of the 96, 90 runs completed with some dominant pathway. Figure 4(b) shows how many times each dominant pathway occurred. All but one of the 90 runs resulted in dominant pathways formed by permutations of the same four mutations. In all, only 7 different pathways dominated, with the single most common pathway accounting for 48% of cancer cases. Thus, all pathways to cancer are not equally likely in CancerSim.

Perhaps the most striking feature of these results is the absence of *sustained angiogenesis*. This does not mean that *sustained angiogenesis* was unimportant in these runs—just that



(a) Theoretical pathways of cancer, reproduced from Hanahan [10].

(b) Pathways of CancerSim. The number of occurrences of each pathway is indicated.

Evade Apoptosis
Ignore Growth Inhibit
Tissue Invasion / Metastasis
Self-Sufficiency In Growth Signals
Limitless Replication
Sustained Angiogenesis
Genetic Instability

(c) Legend

Figure 4: Pathways to Cancer

the dominant phenotype at the end of the run did not have this mutation. Figure 5 shows the population of angiogenic cells during a representative simulation. A significant number of

angiogenic cells was produced. But after the resulting vascularization, *sustained angiogenesis* no longer offered a selective advantage; all cells took advantage of the extra nutrient (as shown by the increased total cell population in Figure 5), and the angiogenic cells were out competed by other cells..

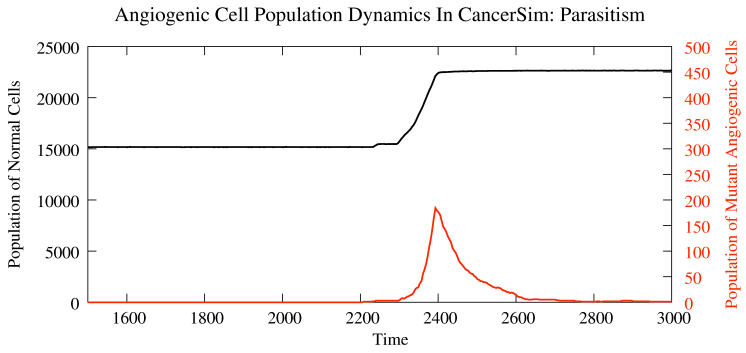


Figure 5: A Small, Short-Lived Population of Angiogenic Cells

Evade apoptosis, however, appears in every pathway. It never occurs first in the sequence because it conveys no selective advantage until the cell has at least one other mutation. This is because apoptosis responds to genetic damage (in our model, a phenotypic change is a proxy for genetic change), so the ability to evade apoptosis is not advantageous until there are some mutations to evade.

Only two pathways include *genetic instability*. This does not agree with studies suggesting that genetic instability is an important hallmark of cancer and that it appears early in carcinogenesis [2]. In CancerSim genetic instability is only a weakly selective mutation. This is because *genetic instability* in itself has no effect on the phenotype; it only increases the likelihood of future advantageous mutations. Thus, in the short run it is a selectively neutral mutation [14]. This is an example of "associated selection" [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 and would model the effect of mutagenic carcinogens, whose effect is never localized to just a single cell.

Limitless replication usually occurs first. This is surprising because our data were collected from runs using an initial telomere length of 100, which permits a total clonal expansion of 2^{100} (about 10^{30}) total cells—far more than the simulation can accommodate. This means that the average cell is under little pressure to escape apoptosis due to telomere shortening. However, cells with mutations have high turnover because detected faults trigger apoptosis (unless the affected cell has the *evade apoptosis* mutation). A cell with a highly selective mutation could thus have many opportunities for replication, potentially depleting its telomeres.

The *ignore growth inhibit* mutation is consistently near the end of the pathway. It may be

that other mutations are more important until most of the empty space (initially inaccessible due to insufficient growth factor and vascularization) is occupied.

4.1 Example Simulation Run

The interaction of cell phenotypes in CancerSim gives rise to many interesting cell population dynamics. Here, we describe one example run of CancerSim illustrated in Figure 6.[2]

By $t = 150$, the tissue reaches its natural extent, bounded by low concentrations of cell growth factor and angiogenic factor. As time passes, a number of mutations occur, primarily deleterious. For example, at $t = 585$ a cell line obtains the *cause angiogenesis* mutation. Because the cell acquiring this mutation is on the interior of the tissue, it has an adequate blood supply and the mutant cell has no advantage. At $t = 636$ the abnormal cell is caught by the G2 check for genetic damage, and is destroyed. At $t = 4260$, a cell obtains the *ignore growth inhibit* mutation (Figure 6(a)). This time, the cell (by chance) passes the G2 checkpoint, and the mutation confers an immediate selective advantage because the cell can divide at the expense of its neighbors. By $t = 4376$ there are 28 cells in this line. However, the G2 check often detects the mutation, causing high turnover and rapid telomere shortening. Before long, the mutant cells exhaust their replicative potential, and at $t = 4558$ the last *ignore growth inhibit* cell dies, leaving the tissue free of this mutation.

The tissue continues with a steady population of cells for a long time. Cellular turnover is low. By time $t = 12500$, 15 separate mutation events have occurred, including (by chance) at least one occurrence of each possible mutation. Through a combination of mutation detection and limited replication, all mutant cells are eliminated and the tissue is healthy. However, the tissue has aged. Many cells exhaust their telomeres and die (Figure 6(b)). Other cells, having fewer opportunities for mitosis, have ample remaining replicative potential and quickly replace the dying cells. However, the increased cell turnover requires more opportunities for mutation. The mutation rate per DNA replication remains the same, yet the mutation rate for the tissue as a whole increases sharply.

At $t = 13563$, a cell acquires the *limitless replication* mutation (Figure 6(b)). It is not the first cell with this mutation, but this time the cell is near the edge of the tissue where telomere shortening is an important factor. The new cell line grows rapidly until $t = 14313$, when a cell from this line acquires the *ignore growth inhibit* mutation. Although this mutation alone was previously unsuccessful (Figure 6(a)), the combination of *ignore growth inhibit* and *limitless replication* quickly spreads throughout the tissue.

At time $t = 15441$, a cell near the tissue boundary obtains the *cause angiogenesis* mutation. Because nutrient is inadequate near this cell, it sends a signal prompting the growth of a new blood vessel. The increased nutrient allows the *cause angiogenesis* cell line to grow beyond the previous extent of the tissue. However, other cell lines benefit equally from the new supply of nutrient, and almost immediately begin to compete for the new habitable region (Figure 6(c)). At $t = 15571$, the last remaining angiogenic cell is supplanted by another mutant (but non-angiogenic) cell. Tumorigenesis is suspended because no new vasculature

[2]The parameters used are: -n 125000 -i 100 -g 4 -t 40 -m 100000 -e 20 -a 400 -q 50000 -r 3.

can be formed. Here, the precancerous cells' lack of coordination postpones the onset of cancer.

At $t = 16377$ a cell obtains the *evade apoptosis* mutation (Figure 6(d)). Avoiding DNA damage detection benefits this cell line greatly because it already has three other mutations. This line lacks only two of the mutations modeled in CancerSim: *cause angiogenesis* and *genetic instability*, (which is now unnecessary because the cell already has all the selective mutations). By $t = 19000$, the cells are limited by lack of nutrient. A sharp increase in the only remaining angiogenic cell line allows the dominant cell line to escape the boundaries of healthy tissue, bringing an end to the simulation at $t = 21526$.

5 Discussion

5.1 Pathways to Cancer

Hanahan and Weinberg propose that cancer arises through various pathways by sequencing the mutations at the phenotypic level. However, CancerSim's pathways to cancer are quite different from those presented in the Hallmarks paper (compare figures 4(a) and 4(b)). 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 could be that CancerSim models the phenotypes incorrectly or insufficiently. Cancer is not fully understood, so implementing a model requires many generalizations, interpretations, and best-guesses. We have greatly simplified both cell and vascular dynamics, both for computational efficiency and for conceptual clarity. There may be more efficacious ways of modeling the hallmarks that would change our experimental results.

Second, our choice of parameters could have been biologically unrealistic and affected the outcome of the simulations. For instance, *limitless replication* might have been overly favored because the telomere length specified for the cells was too short. A simulation as powerful as CancerSim can produce a wide variety of outcomes simply through changing key parameters. Although we performed sensitivity studies in order to choose parameter values for the experiments, an important area of future work is to perform more detailed explorations of parameter space and to reconcile our parameter choices more directly to the biological literature. It is difficult to choose model parameters correctly. The real-world values of many parameters are unknown, and some parameters cannot be modeled accurately due to computational constraints, e.g., the number of cells in an organ or human body. This constraint caused us to alter other parameters to compensate. For example, we wanted a realistic amount of mutation in our tissue even though we were modeling a relatively small number of cells. Although we chose parameter values that we felt were biologically appropriate, an important area of future investigation is to determine the parameter choices more carefully. It is important to note that the simulation's parameter values are not always directly comparable to the biological realm. For example, mutation rates in CancerSim should not correlate directly with genetic mutation rates because they measure the propensity

for phenotypic change, a measure that aggregates many mutational changes in a nontrivial way.

A third possibility is that the pathways suggested by Hanahan and Weinberg are incorrect or insufficient, for example, reflecting gaps or errors in our understanding of the processes of cancer. It is possible that the six hallmark phenotypes are not enough to characterize cancer completely and that new hallmarks will be identified in the future. It is striking that both CancerSim and a similar ODE model (discussed below) found different pathways from Hanahan and Weinberg, suggesting that the discrepancy does not arise from trivial implementation or modeling details.

5.2 Modeling the Hallmarks Using Ordinary Differential Equations

Anther approach to studying the dynamical interactions of cancer is based on differential equations, as in [27, 20, 22]. Similar to the work reported here, Spencer et al. [22] use a differential-equation model to study the hallmarks proposed by Hanahan and Weinberg. Their model resembles CancerSim in several ways. Like CancerSim, the ODE model represents phenotypic properties of cells and describes how they change over time. However, it groups some mutations into a single category. In particular, self-sufficiency in growth signals and insensitivity to anti-growth signals are grouped into a single *increased replicative rate* mutation (R in their notation). Likewise, evasion of apoptosis and limitless replication are modeled as a single *immortality* mutation (D in their notation). Further, the ODE model of immortality is slightly different from CancerSim. In CancerSim, cells with the *limitless replication* or *evade apoptosis* mutations can still die from random apoptosis. Similarly to CancerSim, metastasis is modeled as the final mutation after the other four mutations have occurred.

The ODE model consists of seven differential equations that model a heterogeneous population of cells undergoing the multi-step progression to cancer. Each equation describes how the size of one cell population (e.g., the population of cells containing the genetic instability and sustained angiogenesis mutations) changes over time. The model was used to explore certain questions about the hallmarks, including: The kinetics of various paths to cancer, the effect of inherited mutations on cancer development (i.e., the effect of all cells in a tissue inheriting a hallmark), and a sensitivity analysis of variations in the parameters. They found the sequence DRAG (immortality, increased replicative rate, angiogenesis, and genetic instability) to be the pathway with the shortest expected time to cancer. This is a similar result to that found by CancerSim in that both models find that cell death drives the progression to cancer rather than genetic instability. As we mentioned above, this result bears further exploration.

There are some limitations of the ODE model, some of which are addressed by CancerSim. In the ODE approach, it is difficult to represent how cells spread through space, which is important, especially for understanding metastasis, while space is an integral part of the CancerSim model. Likewise, it is impossible to follow the fate of a single cell using the

ODE approach—the actual mutational pathways are more easily studied in CancerSim. And, an ODE model such as this cannot reveal in detail the interesting interactions and competitions among phenotypically heterogeneous populations of cells. An important area of future research is to compare the two models more closely and compare the results they produce. To do this will require careful calibration of the various parameter values and assumptions.

5.3 Model Extensions

Since the Hanahan and Weinberg paper was published in 2000, research in cancer has continued to progress. Several recent developments are relevant to the CancerSim model. Importantly, it appears that while virtually all cancers exhibit tumor cell heterogeneity, there may exist in each tumor a small clonal population of tumor stem cells [1]. These stem cells, representing approximately 1-5% of the tumor mass have the ability to recreate the tumor cell heterogeneity of the original tumor mass if passaged into an immunocompromised animal. It is postulated that these cells are the ones that must be destroyed by therapy to truly eradicate a cancer and may be the basis of the inability to cure many advanced cancers. As more is learned about stem cells, it may be that CancerSim will need to model this population of cells.

In addition, each of the characteristics of cancer cells outlined by Hanahan and Weinberg can be broken into subcharacteristics or substeps. For example, the process of invasion and metastasis can be broken down into the cell phenotypes necessary for cell invasion, cell survival in the circulation as it travels to distant sites, cell movement into the new tissue, and cell growth in the new microenvironment of the target organ [12]. The current version of CancerSim does not model invasion and metastasis. More sophisticated versions could be developed that model these crucial steps in cancer development.

5.4 Future Directions

In nature, normal tissue growth rates fall off as the size of the tissue increases, a feature that is lacking in CancerSim and often modeled using the Gompertz Equation [17]:

$$V(t) = V_0 e^{\frac{\beta}{\alpha}\left(1-e^{-\alpha t}\right)} \tag{1}$$

where $V(t)$ is the volume at time t, and α and β are growth parameters.

Growth curves are also well approximated by a power law [28]:

$$\left(\frac{m_v}{M_v}\right)^{\frac{1}{4}} = 1 - \left[1 - \left(\frac{m_0}{M_v}\right)^{\frac{1}{4}}\right] e^{-at/4M^{1/4}} \tag{2}$$

where m is the mass of the tissue/organism at time t, M is the asymptotic size, and a is a growth parameter related to the metabolic rate, cell size and energy to create a cell.

It is believed that these growth curves result from constraints on how cells can acquire nutrients and oxygen from vasculature [3]. In CancerSim, the rules for vascular recruitment

and growth result in stable growth rates over time—the tissue continues growing until it reaches the artificial boundaries of the simulation. More realistic growth dynamics could be achieved by reprogramming the rules for vascular growth to more closely mimic what is observed in nature. For example, CancerSim does not distinguish between veins and capillaries and it does not respect what is known about how the lengths of each link in the network increase through time. These are not trivial additions, however, because it is not known exactly which mechanistic rules produce the globally observed growth curves.

From an artificial life perspective, the hallmarks correspond to properties that must have been acquired through evolution in order to support multi-cellularity. Traits like "listening to your neighbors" and "suicide in response to genetic damage" are clearly crucial for multi-cellular systems. Although there have been some limited successes in developing artificial-life models that can evolve and sustain multi-cellularity, true multi-cellular artificial life systems have been difficult to implement. It would be interesting to take the hallmarks (actually the inverse of each hallmark) as a set of hypotheses about what properties are necessary to support multi-cellularity and to test the hypotheses in an artificial-life simulation such as Echo or Avida. An important component of such a study would be identifying what properties an environment must have in order for the hallmarks to be relevant. Finally, we can imagine studying the hallmarks in wider settings. For example, trying to identify natural analogs to the hallmarks in social systems.

6 Conclusions

In conclusion, CancerSim is a model of Hanahan and Weinberg's proposed hallmarks of cancer, which allows us to study the dynamics and interactions of the hallmarks. Models such as CancerSim are relevant to the study of cancer and possible therapies, and they are interesting examples of artificial-life phenomena.

Understanding the pathways to cancer and, more broadly, the cellular interactions of tumorigenesis might lead to earlier detection and new strategies to effectively combat cancer. Although the CancerSim model is still in a preliminary stage, the experiments reported in this paper point to several interesting phenomena. First, is the discrepancy between the Hanahan and Weinberg results and those obtained both with CancerSim and the ODE model reported by Spencer et al. A second interesting phenomenon was illustrated by the role the *cause angiogenesis* mutation played in our simulations. Cancer is impossible without angiogenesis, yet *cause angiogenesis* is often not in the dominant phenotype at the end of the simulation. This is because the beneficial effects of angiogenesis are shared with other, non-angiogenic cells. Although this may not be completely realistic, it raises the question of why not all cells are cancerous, reproducing whenever possible without regard to the welfare of other cells and the organism as a whole [4]. A third phenomenon is illustrated by the role of the *limitless replication* mutation, which appears early in many of our runs because cells that have acquired mutations turn over quickly. This points to 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 nutrient for the elites which remain [15]. This underscores the importance of carefully targeting appropriate cells in therapy. Tools such as CancerSim have the potential to help study the likely consequences of various targeting strategies.

From an artificial-life perspective, the hallmarks provide an interesting set of hypotheses about the requirements for successful multi-agent behavior, whether at the cellular level (as we have studied in this paper) or perhaps at higher levels of organization, such as social systems. CancerSim generates a wide range of interesting phenomena, in large part because the ecological and evolutionary dynamics of the model play out on similar time scales. Social systems also have the property that learning at the individual level can occur at similar time scales to those on which cultural dynamics occur. As mentioned earlier, it would be interesting to consider whether or not there are natural analogs to the hallmarks in social systems. And, it would be interesting to characterize the properties that an environment must have in order for the hallmarks to be effective.

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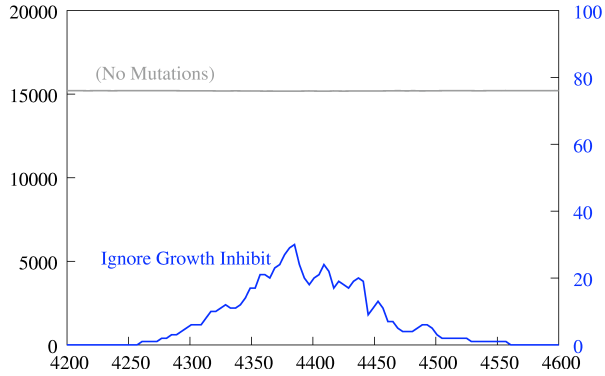
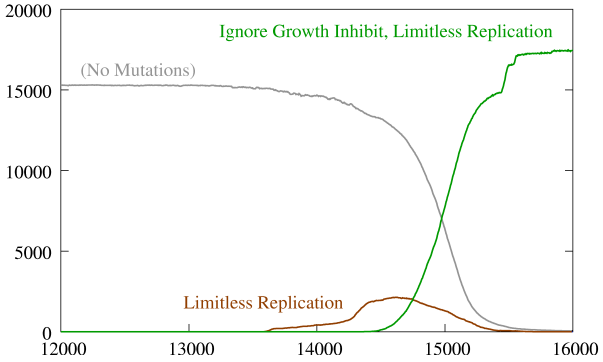
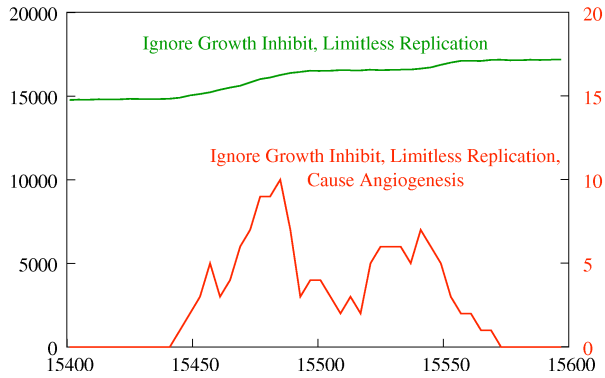


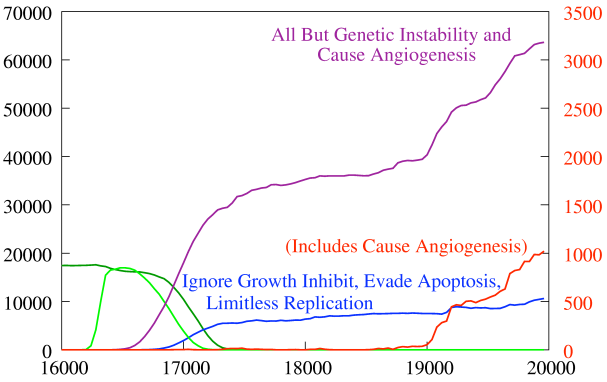
(a) *Ignore growth inhibit* conveys selective advantage, but DNA damage detection causes high cell turnover, leading to rapid telomere shortening and extinction of the cell line.



(b) As the healthy cells age, telomere shortening raises the cell death rate. There is low-level instability in the healthy cell population starting at $t = 13250$. High cell turnover raises the overall amount of DNA replication, thus raising the risk of mutation. The healthy cells are eradicated by mutant cells by $t = 16900$.



(c) *Cause angiogenesis* conveys only a short-term selective advantage because the new vasculature is exploited by all cells. Angiogenic cell generation (at most 10 cells in this example) corresponds to a much larger increase (2414 cells) in the dominant cell line.



(d) The simulation ends when a highly mutant cell line escapes the tissue's natural bounds and fills the computational grid.

Figure 6: Snapshots of cell populations at several times during an example run.