What Tumor Dynamics Modeling Can Teach us About Exploiting the Stem-Cell View for Better Cancer Treatment

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ABSTRACT: The cancer stem cell hypothesis is that in human solid cancers, only a small proportion of the cells, the cancer stem cells (CSCs), are self-renewing; the vast majority of the cancer cells are unable to sustain tumor growth indefinitely on their own. In recent years, discoveries have led to the concentration, if not isolation, of putative CSCs. The evidence has mounted that CSCs do exist and are important. This knowledge may promote better understanding of treatment resistance, create opportunities to test agents against CSCs, and open up promise for a fresh approach to cancer treatment. The first clinical trials of new anti-CSC agents are completed, and many others follow. Excitement is mounting that this knowledge will lead to major improvements, even breakthroughs, in treating cancer. However, exploitation of this phenomenon may be more successful if informed by insights into the population dynamics of tumor development. We revive some ideas in tumor dynamics modeling to extract some guidance in designing anti-CSC treatment regimens and the clinical trials that test them.

KEYWORDS: cancer stem cells, tumor heterogeneity, branching processes, clinical trials

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Introduction

Intra-tumor heterogeneity. Intra-tumor heterogeneity is the phenomenon where the cancer cells in a single human tumor are not identical but highly diverse. Intra-tumor heterogeneity has received considerable attention over a half century, but the past decade has seen a dramatic shift in the focus. Originally, cancer biologists and cancer modelers were intrigued by the possibility that different cell subpopulations within the cancer had different sensitivities to treatments, acquired by somatic mutations and selected for during treatment. This would mandate that combinations of multiple treatments were needed for each patient. Over the decades from the 1960s, clinical research has developed standards of care requiring combination chemotherapy in many types of cancer, ranging from modestly to wildly successful.

This focus on heterogeneity faded as new high-throughput assay systems, such as expression microarrays and deep sequencing, developed. "Personalized medicine" would discover each patient's magic bullet treatment. Tumor heterogeneity can interfere with this vision, since it results in sample-to-sample variation. The higher that variation is, the more limited is the potential for predictive power. Diaz-Cano[1] provides a detailed analysis of these considerations. These assays require homogenized samples, where the individual identity of the cancer cells is lost as the sample is prepared. The gain in vast numbers of molecular features obtained from these assays tends to obscure the absence of knowledge about individual tumor cell subpopulations. Testing multiple specimens in a patient's primary tumor is not common in practice. While techniques have arrived to assess individual cells, tumor heterogeneity implies that their use will not mitigate the need for multiple sampling.

Cancer stem cells. What revived interest in tumor heterogeneity was the rapidly mounting evidence of a tiny but critically important subpopulation, namely the cancer stem cells (CSCs). Several seminal papers convinced many leading researchers that CSCs are real in human cancers, notably breast,[2] ovarian,[3] brain metastases of lung cancer,[4] pancreatic cancer,[5] and glioblastoma.[6] Now there is vigorous activity in identifying or developing agents specifically capable of killing or suppressing CSCs and in moving these agents through the clinical trial system rapidly. Excitement is rising that this new focus will lead to rapid advances in cancer treatment, including the most common, deadly, and refractory cancers.

The possibility that only a tiny proportion of tumor cells are capable of indefinite self-renewal has major implications for developing better anticancer agents and treatment strategies. The main challenges are

- to identify or develop effective agents against CSCs;
- to choose the most promising treatment regimens in strategic combination with existing agents; and
- to test those regimens in clinical trials designed with clinical endpoints useful for assessing treatments against CSCs.

This article focuses on the latter two challenges, using modeling as a tool to develop and critique ideas for clinical testing. It examines some ideas on cancer treatment strategy that arose decades ago from the tumor heterogeneity paradigm, when the heterogeneity of concern was about subpopulations with drug resistance mechanisms rather than cancer stem cells. These ideas have potentially serious implications for designing treatment regimens that combine multiple agents and for choosing clinical endpoints by which to assess the success of these regimens. In a different era of cancer research, Von Hoff[7] decried the potential loss of valuable anticancer agents to a clinical trial system inflexibly following the standard cytotoxic drug model. The same issues are germane in this new CSC setting, and deserve wider recognition.

Developments in Cancer Stem Cell Science

The breadth of research into cancer stem cells is extensive. In place of a complete review, this section provides an overview sufficient to discuss the clinical trials design issues further on.

Definitions. The starting point must be terminology. There is still considerable controversy about the definition of CSCs, and whether their presumed key properties are coexistent in a well-delineated subset of cells.[8–10] Sakariassen et al.[11] provide an excellent review of the controversies and evidence. Some researchers prefer alternative terms like tumor-initiating cells, tumorigenic cells, or tumor-propagating cells[12,13] as less prejudicial or more operational. Likewise, terminology also differs somewhat among investigators with regard to the cancer cells that are not stem cells. Progenitor cells refer to non-CSCs with the ability to divide but not for self-renewal, while terminal cells cannot divide. Together, they constitute what some investigators call "derived cells". However, these terms are not universal; one also sees the term "differentiates cells". Herein, the term "non-CSCs" will represent cancer cells that are not CSCs.

In the common view, the tumor originally initiated from a normal stem cell and inherited a repertoire of important behaviors, especially self-renewal and strong self-defense mechanisms; furthermore, all CSCs descend from a cell that transformed to a cancerous phenotype. Alternative views are widely discussed: A pre-cancerous cell originally without stem cell characteristics might have stumbled upon the "on" switch[14] through genomic or other instability, and derived cells might acquire CSC characteristics. For purposes of the arguments in this article, we will assume tentatively that a cancer stem cell subpopulation exists in many or most human cancers, with the following properties.

1. CSCs alone are self-renewing, and in that sense, constitute an "immortal" population;
2. CSCs give rise to "derived cells" (non-CSCs), which may divide a limited number of times and give rise to the bulk of the tumor. Non-CSCs may further divide into those that are still capable of proliferation and the terminal cells, those that intrinsically cannot proliferate;
3. Non-CSCs do not acquire stem cell self-renewal properties;
4. CSCs are resistant to most standard chemotherapy; in fact, some standard agents might stimulate division of CSCs.

Isolating and testing CSCs. Operationally, CSCs are concentrated from tumors by selecting certain cell surface markers, and the success of the selection is assessed by some assay. The assay could simply be examining cell morphology (spheroid formation) or through observing the number of cells needed to form a cancer cell colony in a xenograft. Nevertheless, they provide a means to test agents against selected cells, thereby offering the exciting possibility of an entirely new and biologically compelling avenue for anticancer drug development. The development of molecular markers and signatures for CSCs promises to yield improved ways of monitoring the effects of treatment earlier and more precisely.

It is controversial whether such observations adequately validate a conclusion that the molecular expression pattern selected does reveal a CSC subpopulation.[9] Studies of molecular markers claimed for CSCs reveal details that argue against an overly simplified view. Different marker patterns arise in studies of different types of cancer. Even the directionality can differ; for example, in breast cancer, a pattern frequently associated with tumorigenicity is low expression of CD24, with high expression of CD44 ($CD44^{high}/CD24^{low}$), but in ovarian cancer the signature $CD44^{high}/CD24^{high}$ is reported, so that high rather than low CD24 expression marks the CDCs. Jaggupilli and Elkord[15] review the unsettled nature of our understanding in the roles of these markers in indicating CSCs. Marker patterns may change with ongoing research. For example, after Wei et al[16] defined a CD44+CD24+Epcam+ signature for ovarian cancer most consistently enriched for a population capable of colony growth, the same team[17] augmented this signature for CSCs to require also a deficit in E-cadherin expression. The proportion of CSCs in tumors seems to vary widely. The tightness with which various CSC signatures correlate with tumor initiation potential is somewhat murky.[8,18] One would also expect that markers truly indicative of CSCs would collocate reasonably well in the same cells in a tumor, in niches associated with CSCs, but some studies provide

contrary evidence.[19] These dilemmas and more are reviewed by Clevers.[20]

Despite these persistent quandaries, for present purposes we tentatively accept the idea that the screening methods do identify treatments with specific efficacy against CSCs.

Developing CSC-specific agents and molecular targets. There is considerable excitement about methods to identify candidate agents that can kill CSCs.[21,22] These agents, whether new or old, could potentially end the depressing log-jam in the treatment of metastatic solid tumors. If the cells responsible for the indefinite self-renewal of tumors could be eliminated, the tumor would over time melt away as the non-CSCs reach the limits of their finite proliferation potential.

Candidate agents targeting CSCs have been discovered by empirical sensitivity testing utilizing the molecular signatures discussed above to select and expand cells for testing, or by activity on actors in important stem-cell-related pathways. Some of the targets and pathways expected to be useful for killing or suppressing CSCs relate to Notch, Frizzled-Wnt, Hedgehog, PI3K, AKT, mTOR, Twist, focal adhesions, CXCR, and interleukin 6 (IL-6).[22–29] Some of the agents in clinical testing[30] include investigational products of companies such as OncoMed, Verastem and LCLabs: demcizumab, vantictumab (OMP-18R5), tarextumab, ipafricept, anti-notch1, VS-4718, VS-5584, defactinib, vismodegib (GDC-0449), and vorinostat, as well as established drugs for other diseases like metformin that could be repurposed. Another strategy under development is to target the immune system against CSCs. For example, recent reports show[31,32] that non-killer (NK) cells preferentially target CSCs, though there are apparently contradictory results.[33] The complexities for cell population dynamics are enormous when interactions with the immune system are important; there is even evidence in favor of a role for immune cells in generating CSCs from non-CSC tumor cells.[34–36] For this reason, this article omits immunological treatments and modeling details from the scope.

The increasing resistance to chemotherapy in metastatic disease relative to primary lesions suggests that CSCs may themselves have high levels of resistance to the standard chemotherapy drugs. Considerable evidence points to specific mechanisms of resistance, most prominently the high activity of efflux pumps in CSCs.[37,38] A prominent speculation is that stem cells must be highly self-protective to defend their crucial role in responding to tissue needs, and CSCs inherit these defenses. Treatments that do well in Phase II trials manifest cytotoxic or cytostatic effects on the bulk of the tumor cells, or else their effects would not be detectable. If these treatments nevertheless have no effect on CSCs, then one would expect eventual tumor recurrence with resistant disease, and limited potential for curing patients.

It is even possible that agents that perform well in Phase II trials are counterproductive due to contrary effects on CSCs. For example, the commonly used chemotherapy, doxorubicin, stimulated the growth of CSCs in the ovarian cancer model, OVCAR5, xenografted in mice, while suppressing other cells.[17] Anticancer agents directly affecting non-CSCs may also have indirect effects on CSCs. For example, induction of IL-6 may stimulate CSCs to divide more.[29] This could lead to counter-productive effects that would not manifest immediately but only later in the clinical course. Phenomena like this will affect which treatment strategies are most likely to succeed.

Some surprisingly positive effects of established targeted therapies may also owe their existence to CSC biology. HER2 drives luminal breast CSCs in the absence of HER2 amplification. Ithimakin et al[39] note an interesting implication for the efficacy of the adjuvant trastuzumab: Herceptin and other antagonists of HER2 could have positive therapeutic benefits outside of its traditional realm of HER2 amplification, simply because of effects on CSCs.

Whether CSCs are more sensitive or less sensitive to radiotherapy compared to non-CSCs seems unclear. Most published papers assume or conclude that CSCs are less sensitive, for example Bao et al,[40] but work pointing in the opposite direction comes from Kim et al,[41] who conclude that low DNA repair capacity may characterize some CSC populations and confer sensitivity to ionizing radiation. The settings differ in regard to types of cancer; that may be the cause of the discrepancy.

Clinical Challenges in Testing Agents Which Target CSCs

Strategies for exploiting anti-stem-cell agents need to address two goals at once: reduction in CSCs, and minimization of morbidity from the non-stem-cell bulk of tumor in primary and metastases. In anticancer medicine development, new agents pass through the Phase II clinical trial with "clinical response" as the primary endpoint. Clinical response is a fairly easily detected signal, usually finalized in a time frame of a very few months. Since it primarily reflects changes in bulk tumor, effects on a tiny subpopulation of CSCs would be invisible. A partial or complete clinical response could occur with no decrease in CSCs, or even an increase. The evidence cited above concerning doxorubicin suggests that agents successful by traditional Phase II criteria can even stimulate CSCs. Thus standard RECIST-criterion[42] clinical response is not likely to be an adequate clinical endpoint for measuring the efficacy of a CSC-targeted agent.

"Effectiveness", the long-term success of tumor treatment in preventing or delaying recurrence and death, is often poorly correlated with tumor response; for example, Han et al[43] found an R^2 of 0.22 between objective response rates and overall survival across 91studies. Since Phase II trials perform a triage function, no agent that fails on the short-term response outcome is likely to make it into the definitive Phase III trials.

Cancer clinical trialists occasionally find the choice of clinical endpoints to be inadequate and revisit approaches to detecting efficacy. A similar crisis arose earlier concerning how to evaluate agents intended to achieve long-term stable

disease without necessarily inducing response. Long-term observation is required. A notable example was in the development of gemcitabine for pancreatic cancer. Von Hoff[7] noted that poorly chosen clinical trial designs can cause a useful agent to be abandoned. One conclusion was that tumor response as a Phase II clinical endpoint would sometimes be an inferior measure compared to time to progression. Agents with primarily cytostatic effects, such as kanamycin,[44] would be impossible to develop using tumor response as the primary endpoint.

There is always the risk of toxicity from these agents. Just as chemotherapy interfering with proliferation causes harm to normal proliferative cell tissues, agents targeting CSCs may harm normal stem cells. An extra challenge is that damage of normal stem cells might manifest only after substantial delay.

For all these reasons, time-to-event endpoints like progression-free survival (PFS) would be more likely to fulfill the role of efficacy endpoint in Phase II trials for anti-CSC agents.

Tumor Dynamics and Cancer Heterogeneity

I now review some work from decades ago attempting to apply mathematical modeling of human tumor heterogeneity to guide treatment regimen design. For many years, interest in tumor heterogeneity focused on the development of resistance mechanisms, with an eye to developing strategies using multiple agents. Each agent would have its own spectrum of activity on a different subset of cancer cells. This thinking was instrumental in developing some successful treatment regimens, including childhood acute lymphocytic leukemia, breast cancer, and Hodgkin Disease.

The Goldie–Coldman model. The rise of combination chemotherapy in the 1960s received its biggest boost from the extraordinary conversion of childhood acute leukemia from nearly uniformly fatal to routinely curable. Initially, the success was thought to be due to hitting cancer cells in different phases of the cell cycle. Later, techniques for growing cell lines led to numerous investigations into acquired drug resistance mechanisms, and gave prominence to the idea that somatic mutations during tumor development lead to these mechanisms in distinct subpopulations. Suppose there are two agents and two mutation-induced cellular mechanisms conferring drug resistance to each drug, respectively. Each drug has a critical task: eliminate the cells sensitive to it but not the other drug. To cure the patient, at minimum, both tasks must complete successfully. A subpopulation resistant to one chemotherapy agent would be killed by a different agent if that different agent is introduced soon enough, before the subpopulation would grow too large. Thus Goldie and Coldman[45–47] proposed a treatment strategy of two alternating non-cross-resistant drugs in successive treatment courses to optimize the chance of eliminating each cancer cell subpopulation.

The worst drug rule. The modeling strategy used an assumption of symmetry between the drugs: equivalent potency, identical mutation rates to resistance, and so on. Day[48,49] relaxed the assumption of symmetry, allowing one of the drugs to be substantially more potent than the other. Here, potency means the log reduction in the size of the subpopulation of sensitive cells when delivered at the feasible dose as determined by the toxicity constraint. One might suppose that a weaker drug should be used less. Instead, the resulting optimal strategy was a surprise. This strategy is called the "worst drug rule" (WDR), since it calls for treating with the worse of the two drugs initially and/or for more extended time than the better drug. A typical demonstration is in Figure 1, which displays the probability of "cure", a final cancer cell count of zero, as a function of the treatment schedule. The 16 white boxes represent 16 treatment sequences of two agents **a** ("Worse-Agent") and **b** ("Better-Agent") with a total of 12 courses. The boxes are placed along the vertical dimension according to the number of courses of **a** versus **b** in the schedule, and along the horizontal axis according to the temporal placement of the courses. The numbers in the white boxes are the probabilities of zero cancer cells at the end of treatment. These calculations are performed by evaluating probability generating functions, iterating solutions to partial differential equations.[49] Details of the modeling system are in Appendix A.

The middle row shows that for fixed and equal numbers of courses (6 each for **a** and **b**), the best outcome is achieved by starting with Worse-Agent, whose potency is only half the log-kill parameter as Better-Agent. The left side shows schedules that start with Better-Agent; it compares the timing of switching to Worse-Agent. Indeed, the earlier the switch occurs, the better, as illustrated by the highest cure probability, 82% (yellow box: **3a9b**). Thus, both the "worst first" and "worst more" strategies do much better than the Goldie–Coldman alternating strategies in the middle, **(ab)x6** and **(ba)x6**. The magnitude of the effect is larger than the one found by Goldie and Coldman, and it robustly survived considerable sensitivity analysis.[48,50]

The reason for this somewhat paradoxical result is clear upon reflection. The worse of the drugs, being of lower potency, has more difficulty achieving its unique responsibility, namely the removal of cells resistant to the more potent drug. The regimen must make allowance for this in scheduling, or else there will be a high risk that the worse drug will fail at its task. This phenomenon is named the "worst drug rule".

Further mathematical study and development of the WDR by Katouli and Komarova[51] led to an independent recommendation: the "best-drug-first, worst-drug-longer" strategy, consistent with **3a9b** Figure 1, with a finding that the optimality is robust to levels of cross-resistance.

Though many years have passed from the discovery of the WDR principle to the resurgence of CSC biology, the fit between the cancer stem cell story and the WDR is quite close. To translate these ideas into the context of CSC biology and regimen development, we consider an agent StandardChemo (log-kill = 2) that does not affect CSCs,

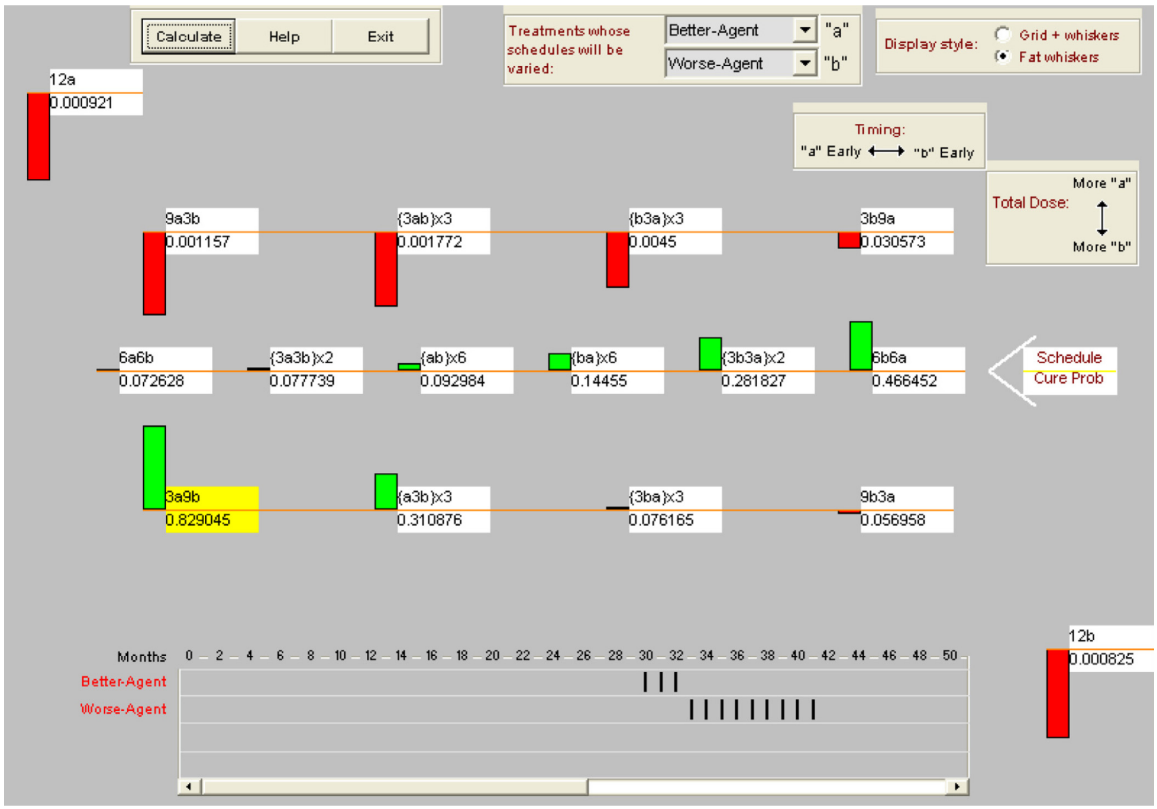

Figure 1. The Worst drug rule. Probabilities of zero cells, by treatment schedule.
Notes: The 15 white boxes and one yellow box represent 16 treatment sequences of two agents **a** ("Worse-Agent") and **b** ("Better-Agent") with a total of 12 courses. For example, the schedule in the yellow box is **aaabbbbbbbbb**, three courses of **a** followed by nine of **b,** while (a3b)x3 represents **abbbabbbabbb**. The number in each box is the probability of "cure", defined as a final cancer cell count of zero using that schedule. The boxes are placed along the vertical dimension according to the number of courses of **a** versus **b** in the schedule, and along the horizontal axis according to the temporal placement of the courses. The vertical rectangles are visual representations of the cure probabilities, transferred to a logit scale centered at the mean of the twelve logits: logit(0.07). Green rectangles flag the schedules better than this average; red indicates worse than average.

and agent StemCellActive (log-kill = 1) that only affects CSCs. The "log-kill" parameter represents the number of logs (base 10) in reduction of the sensitive subpopulation from a single course. Thus, StemCellActive is the "worst drug". Figure 2 shows simulation runs demonstrating several phenomena related to treatment strategies in a CSC biology context. All runs are begun with the same random number seed. (Unlike the classic WDR scenarios, chance plays little part, since rare mutations to drug resistance is not the issue.) Details of the population kinetics parameter settings are in the figure legend.

These demonstrations are not predictions; they are intended to illustrate and provide some intuition for some of the potential consequences of treatment strategies that are not so obvious. The true consequences of different strategies will present themselves only in clinical trials.

In panel A, the treatment plan is six courses of StemCellActive, an agent that affects only the CSCs. The tumor continues to grow initially, possibly recorded as a progression, and possibly causing morbidity. Then the tumor melts away gradually. The reduction of bulk tumor is delayed as the consequences pass through the non-CSC generations. Time to response is very long. Because the CSCs were not eliminated (logkill = 1 per course; six courses given, total logkill = 6), the tumor will eventually recur.

In panel B, the treatment is extended with three more courses of StemCellActive. The tumor is eliminated eventually (total logkill = 9). However, the initial course as clinically observed has not changed. Thus, again the patient might be assessed initially as progressive disease, even though the patient has been cured.

In panel C, in place of the three extra courses of StemCellActive, three courses of StandardChemo (logkill = $3 \times 2 = 6$) are given, doubling the total additional logkill. The time to response is greatly hastened, but the eventual recurrence is not affected. (The terminal cells, in gold, are assumed partially resistant to StandardChemo; this assumption has no effect on the outcome.) A Phase II trial with response as the endpoint would judge strategy C to be a success, and strategy B, which is curative, a failure.

In panel D, the "best drug" StandardChemo is the initial treatment, and not until relapse does the strategy switch drugs to StemCellActive. This strategy aligns with what may appear as common sense. The outcome is poor. Though there is a

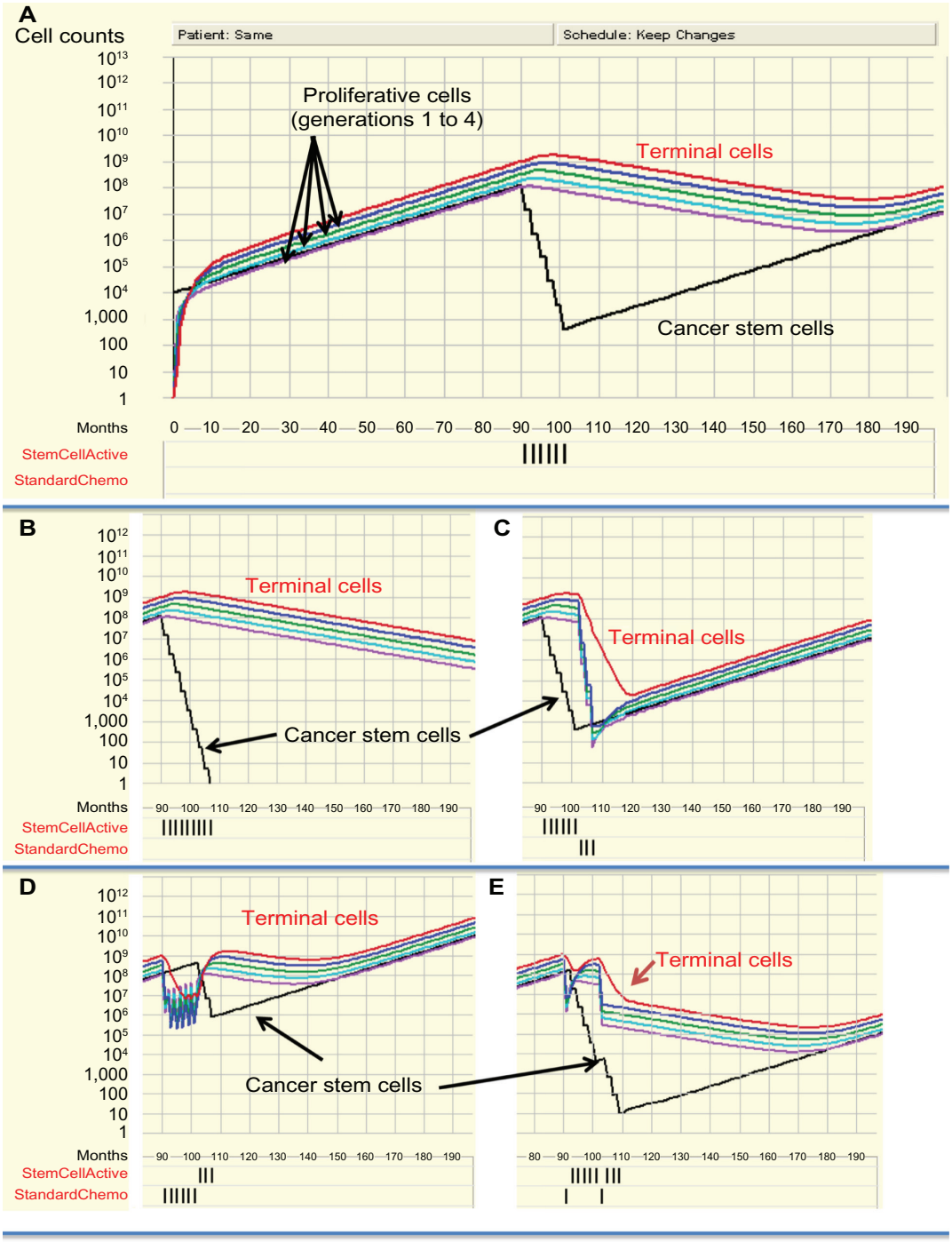

Figure 2. Simulations of treatment scheduling strategies.

Notes: Stochastic simulations initialized with 10^4 cancer stem cells, and the same random number seed throughout. Tumor cell counts are along the vertical axis logarithmically spaced. The treatment schedules are along the bottom, indicated by vertical ticks where treatments are given. The agents are labeled StandardChemo and StemCellActive. Parameters (all times are means): For CSCs, mitosis time = 2.7, cell death time = 30, time to progenitor = 0.5. For proliferative cells, mitosis time = 0.9, cell death time = 10, mean time between progenitor generations = 0.5, mean time from final generation to terminal cells = 0.5. For terminal cells, mitosis time = 90, cell death time = 1. Up to the initiation of treatment at 90 months, the curves are identical; thus panels B through E begin shortly before 90. (**A**) Treatment with StemCellActive, 6 courses. The tumor continues to grow initially, possibly recorded as a progression. Then the tumor melts away gradually. Time to response is very long. Because the CSCs were not eliminated (logkill = 1 per course; 6 courses given, total logkill = 6), the tumor will eventually recur. (**B**) With three more courses of StemCellActive, the tumor is eliminated eventually (total logkill = 9). The initial course as clinically observed is not changed. (**C**) With three courses of StandardChemo (logkill = $3 \times 2 = 6$) in place of the three extra courses of StemCellActive, the time to response is greatly hastened, but the eventual recurrence is not affected. StemCellActive is the "worse drug" (comparing the slopes of the CSC line, in black, and the non-CSC lines). (The terminal cells, in red, are assumed partially resistant to StandardChemo; this assumption has no effect on the outcome.) (**D**) Introducing StandardChemo first and waiting until relapse to switch drugs to StemCellActive has a bad outcome. Though there is a rapid response, it is short-lived. The StemCellActive courses are too little, too late. (**E**) To eliminate the tumor while controlling morbidity from non-CSCs, the StandardChemo treatment should be introduced only when symptom control is needed.

rapid response, it is short-lived. The StemCellActive courses are too little, too late.

In panel E, the strategy is to avoid morbidity from the cancer without compromising long-range curative potential. StandardChemo treatment should be introduced only when symptom control is needed. As a proxy for morbidity, here a cell count in excess of 10^9 is used as a signal to introduce a course of StandardChemo. In this run, one more course of StemCellActive would have eliminated the tumor permanently. This treatment regimen used two courses of StandardChemo and eight of StemCellActive.

Challenges in Clinical Trial Design for CSCs

These simulations highlight some unique challenges in evaluating anti-CSC strategies in clinical trials: choice of regimens to test and choice of clinical endpoints for assessment.

The primary risk for a patient from non-CSCs is morbidity and mortality from the disruption of normal tissue function. The primary risk from CSCs is the ongoing and indefinite production of new tumor cells. Control of CSCs without control of non-CSCs will fail as the patient gets sicker and dies. Control of non-CSCs without control of CSCs has been a common result of the familiar sequence: primary treatment, tumor reduction, improvement in symptoms, recurrence locally or in metastases, refractoriness to treatment, mortality.

Treating patients successfully requires control of morbidity by control of the visible non-immortal bulk of tumor, and also control of the cancer stem cell population. Neither of these two tasks is sufficient, and they present different challenges and opportunities. This observation stimulates thinking about applying the WDR. One strategy that leverages the power of the WDR in the context of CSCs would be to administer the CSC-active agent until the short-term risk of unacceptable morbidity is high, and then provide standard chemotherapy to control morbidity. As an immediate consequence, in the adjuvant setting the CSC-active agent would be strongly preferred. Upon tumor recurrence, the choice becomes difficult. Morbidity risk increases, rapidly in some cases and slowly in others. It is plausible that even then the CSC-active agent could be a good choice. An alternative CSC-active agent, if available, could be a better choice; but standard chemotherapy effective on the primary, which has at best diminished efficacy on metastases, would be least favored. Conversely, in the neoadjuvant setting, since the main clinical purpose is to de-bulk the tumor prior to primary surgery, CSC-targeted therapy would be a low priority at best.

The set of regimen choices originally studied by Goldie and Coldman expressed a constraint that the two hypothetical agents A and B could not be given in the same cycle due to toxicity limitations. Thus, for each cycle there was a choice, A or B. This artificial setting nevertheless receives some echo from the study of Schott et al, where the CSC-active agent and non-CSC-active agent were not concurrent, in order to minimize risk of toxicity. Going forward in future trials, it will be desirable to begin by deciding what toxicity constraints should apply when combining a stem-cell-active treatment with a standard treatment, listing a few feasible combination schedules acceptable by these constraints, and designing a trial to compare these schedules. Then the clinical trial can help to answer a biological question as well as a clinical question.

Having found via the WDR that the "obvious" way to combine multiple anticancer agents and modalities is often exactly the worst way (Fig. 1), a failure to design trials informed by tumor dynamics is risky; early clinical failures may kill an entire promising stream of treatment development.

Biological Complications

Modeling tumor dynamics with CSCs involves complications tied with aspects of the biology for which we have at best hints up to now. We do not understand if, or how, the CSCs and non-CSCs communicate. How do CSCs "know" when to divide asymmetrically and when symmetrically? Does a CSC respond to its immediate microenvironment, or systemic signals affecting all CSCs in concert?

A simplifying assumption made in discussions of CSCs is that their differentiated descendants are indeed permanently not self-renewing. However, there is some evidence that a proliferative non-CSC cancer cell, supposedly with potential for only small numbers of cell divisions, might occasionally obtain the CSC phenotype. If so, then this can radically change the balance in comparing treatment strategies. There is evidence that hypoxic conditions, typically found in the interior of tumor primaries and advanced metastases, can induce cells to regain self-renewing capability.[14] Some evidence in favor of this possibility appears, for example, in neuroblastomas[52] and breast cancer.[53] This biological possibility complicates the adjuvant setting. If proliferative non-CSC cells can gain CSC-like self-renewing capability, if the rate is not too low, this could argue against continued use of CSC-active agents. Recruitment into the CSC subpopulation could exceed the ability of the CSC-active agent to reduce the subpopulation.

Another complication involves the recently discovered biology: there appear to be two major populations of CSCs. An intriguing set of observations have recently appeared connecting CSCs with transitions of cancer cell phenotype between epithelial and mesenchymal forms.[54,55] The epithelial–mesenchymal transition (EMT) is a phenomenon identified from embryology, giving rise to the loss of cell-to-cell contact and polarity.[56] Epithelial cells tend to be polar, nonmotile, and relatively sensitive to chemotherapy agents. They generally express E-cadherin. Mesenchymal cells are motile, and generally do not divide; they generally express N-cadherin. An association between CSCs and EMT is widely acknowledged. Experimental induction of EMT in mammary-derived immortalized cells by the embryogenesis-associated transcription factors Snail and Twist generates characteristics of stem cells, such as the $CD44^{high}/CD24^{low}$ expression pattern.

A study by Tsuji et al from 2008[25] examined requirements for establishing lung metastases (albeit from hamster cheek pouch carcinoma cells), and found a requirement for two distinct CSC populations, one EMT-like and the other, termed non-EMT or MET, representing the switch from mesenchymal to epithelial characteristics phenotype. A mixture infused subcutaneously work in tandem to establish metastases; the EMT cells appear to be responsible for local invasion, with non-EMT cells involved in proliferation at a metastatic site. Some took this study as evidence that the EMT state and CSC are mutually exclusive rather than strongly correlated. An intriguing recent report[57] reinforces and expands upon the Tsuji et al results. Liu et al state that in human breast cancer there are two classes of CSCs working in tandem; those in EMT and those transitioning in the reverse direction: MET. The MET takes place in embryogenesis to develop organs (kidney, ovaries) distant from the epithelial tissue surfaces created earlier in an embryogenesis. Liu et al.[57] review evidence that the $CD24^{low}CD44^{high}$ markers identify CSCs with mesenchymal-like properties that are motile and invasive, while aldehyde dehydrogenase (ALDH) expression identifies epithelial-like CSCs that are proliferative. The tentative picture is that the interior of a primary tumor is the home of the MET epithelial-like cells, where cell division takes place, while the periphery is the home of the EMT mesenchyme-like CSCs, characterized by motility, invasiveness, and metastatic potential. A new metastasis would require a transition back to the epithelial CSC form.

Thus, the spatial dimensions of invasion and metastasis, in conjunction with stem cell plasticity between the EMT and MET states, appear to be indispensible components of the full picture. Mathematical modeling aiming at understanding how anti-CSC agents might be best used should ideally take into account this very complex level. That would require far more detailed information than is available at this early stage.

Discussion

The study of CSCs is in flux, and replete with controversies. The identification of CSCs patterns relies heavily on functional assays displaying physical characteristics such as nonpolarity, or tumor-forming potential in xenografts. Cells passing these tests may not be entirely identical with cells playing the CSC role in human tumors in patients. We have seen that the identity of the putatively best CSC molecular signatures varies across tumor types. Just a few of the controversies are: whether CD24 is expressed or not, whether CSCs are insensitive or sensitive to radiotherapy, whether CSCs originate from normal stem cells or not, and whether non-self-renewing cancer cells have the ability to regain self-renewing capacity. Clevers also notes findings of plasticity of CSCs, and warns: "Only if the CSC phenotype is a stable trait will it be advantageous to selectively target CSCs".[20]

One must ask which of the apparent differences in these answers across tumor types are truly biological, or artifacts of experimental systems. Another caution is that the subpopulations selected for markers correlating with tumor initiation potential might still be quite heterogeneous, running a risk that chemosensitivity testing on those cells could be partially or largely due to effects on a subset of cells not responsible for tumor initiation. Lehman et al.[10] provides a good review of some basic gaps in current understanding, together with some provocative negative experimental results that strongly suggest caution as the theory develops. Modelers would like good answers for key parameters, none so key as the proportion of CSCs in a primary tumor,[58] yet the methods for estimating these parameters from experiments do not warrant great confidence in the accuracy of the estimates, which range widely.

If CSCs really are the key to long-term behavior of tumors in response to treatment, then the cautionary implications of tumor heterogeneity for the value of high-throughput assays are probably magnified by considerations of CSC biology. The assays presumably measure primarily the properties of the derived cells, forming the bulk of tumors and hence of the samples, while missing crucial properties of the more important CSCs.[9] This phenomenon might explain why the cancer biomarker enterprise has not been as successful as hoped in clinical application.[59,60] However, this need not be the case; the CSCs and derived-cell populations may share important properties, allowing the assay results for bulk tumor to detect characteristics of the CSCs.

Prior mathematical models focusing on CSCs have included the work of Ganguly and Puri[61] and Molina-Peña and Álvarez.[62] Both approaches utilize ordinary differential equations describing compartmental models. Ganguly and Puri conclude with six inferences from the model observations relating to treatment strategies. They are difficult to translate into guidance for treatment regimens and trial designs. Molina-Peña and Álvarez provide constraints to match known behaviors of human tumors. In regard to treatment prescription, they conclude that "encouraging CSC differentiation could be an effective therapeutic strategy". These models are ambitious efforts, but inevitably have many parameters poorly known (apparently 23 and 8, respectively, somewhat larger if counting initial conditions and simplifying assumptions). Observations backing those parameters and assumptions will be indirect at best, for example, taking a given CSC signature assay seriously as providing an accurate estimate of the proportion of CSCs in the tumor. Thus, sensitivity analysis to critique results is essential, and both studies do provide sensitivity analyses. A number of other modeling studies for tumors with CSCs develop the cellular automata approach, incorporating spatial aspects of tumor growth.[63–66]

One notable observation from the modeling efforts of Enderling et al is that increasing apoptosis among the non-CSC cells can paradoxically increase tumor growth by reducing competition with CSC cells.[66] Mathematical modeling of tumor biology can make discoveries like this despite overwhelming

complexity, facets too poorly known or unknown, and vast numbers of parameters whose values would have to be known for quantitative predictions. Despite all the limitations, observations from mathematical modeling can generate new and relevant hypotheses. When these observations are of a surprising nature, they can build better intuitive understanding. Biological and clinical researchers can entertain possibilities that otherwise would not be on the table.

The OncoTCap platform used here for demonstrating the WDR has capabilities to account for local and systemic spatial inhibition through Gompertzian growth rules and nested spatial properties (Appendix A). The WDR has fared well in previous sensitivity analyses.[48,51] Exploring the WDR's range of validity and limitations in the CSC context would have utility through a comprehensive sensitivity analysis incorporating maximal details and complexities. Highly elaborate models do run risks: implying unwarranted precision and interfering with discovering useful new general ideas obscured in an avalanche of details.

Concepts on the nature of tumor heterogeneity development tend to three types of explanations: stochastic evolution under mutation and competition; hierarchical evolution resembling the structures of normal tissues; and heterogeneity from variations across the microenvironments within tumors, at their boundaries, and at potential metastatic sites.[67,68] The original WDR emerged from studies of drug resistance, with a stochastic viewpoint for the evolution of tumors through random mutations. The CSC picture is more hierarchical, with CSCs giving rise to a series of descendants terminating in cells with no proliferative capacity. The simulations presented in Figure 2 follow a hierarchical model, yet also demonstrate a WDR phenomenon.

The approach presented here is much less ambitious from the modeling perspective, but may in its modesty contribute to imaginative strategic thinking as treatment regimens and clinical trials are designed with CSCs in mind. In the demonstrations we have shown above, every assumption and parameter setting can be justly criticized. However, in this setting of very imperfect biological knowledge, mathematical modeling can perform a constructive role as an assistant for creative thought as clinical experiments are planned.[69] It is then part of the responsibility of clinical trial designs with CSC-targeted agents to follow a principle promoted by Bernard Fisher through decades of breast cancer research[70,71]: every clinical trial should test a biological hypothesis as well as a treatment.

The simulations illustrate several kinds of patient fates depending on treatment strategy. These are not all immediately obvious; modeling helps understand how these possibilities may arise. They also highlight the extreme risks of missing an invaluable treatment if the clinical trials of CSC-targeting agents proceed with business as usual. I do not have a simple solution, but no solution can emerge unless we are aware of the problem.

Appendix A: Oncology Thinking Cap

Overview of the Oncology Thinking Cap (OncoTCAP). The Oncology Thinking Cap (OncoTCAP) computer program (Version 2) is an extensive cancer modeling laboratory for conducting thought experiments in cancer biology and treatment. This facility is in active use by several cancer researchers to answer practical questions about treatment optimization. The conceptual basis is that tumor heterogeneity underlies most of the central themes of cancer biology and cancer treatment.[8] These include: apoptotic mechanisms, cell cycle control, repair mechanisms, and mutational processes that may disrupt normal mechanisms, as well as tumor growth kinetics, sensitivity to treatments, treatment resistance mechanisms, local spread, and metastasis. All of these phenomena can be specified in terms of variations in properties of cancer cells, properties that are heritable in a genetic, positional, or other sense.

OncoTCAP lets users describe biological and chemical relationships as experienced by each individual tumor cell. It then maps accurately to the macroscopic behavior of the tumor in regard to growth kinetics and response to treatment. Computational engines and graphic displays incorporate all these aspects to produce individual patient simulations or calculate cure probabilities. Thus OncoTCAP provides the means to synthesize these details into a model for calculating predictions about cancer patients and the success of treatment plans. The modeling framework strives toward a hypothesis-neutral ideal, to encourage the challenging of assumptions, and to be fully responsive to new developments in cancer biology.[3–5,13] OncoTCAP is publicly available for download to Windows 95 platforms from http://www.pci.upmc.edu/tcap.

Branching process concepts. Suppose there are K cell types, and let the numbers at time t be represented by the cell count vector written as $\underline{N}(t) = (N_1(t), ..., N_K(t))$. Part of a cell's behavior is its "kinetics", its propensity to undergo a change without any episodic external intervention. These changes include change of location (metastasis and local migration), mitosis, cell death, intra-mitotic mutation, and mutation during mitosis. They may also represent the passage of a cell through cell cycle phases or through other status groups. An event is characterized and implemented by the change in the vector of cell counts:

$\underline{e} = \underline{N}(t + dt) - \underline{N}(t)$. Here are some examples with three cell types A, B, and C, and $\underline{N}(t) = (N_A(t), N_B(t), N_C(t))$:

$\underline{e} = (-1,0,0)$	**Death of an A:**	**A→ {nothing}**
$\underline{e} = (-1,1,0)$	**"Conversion":**	**A→ B**
(eg, migration or cell cycle traverse)		
$\underline{e} = (1,0,0)$	**Mitosis:**	**A→ A A**
	Mutation:	**B→ B A**
$\underline{e} = (2,-1,0)$	**Mutation:**	**B→ A A**
$\underline{e} = (-1,1,1)$	**Mutation:**	**A→ B C**

Let $f_{k,\underline{e}}(t)dt$ be the probability that the event corresponding to change vector $\underline{e}$ occurs to a cell of type k during

the infinitesimal interval ***dt***. The key assumptions are the "Markov" property and the independence of contemporaneous cell fates, leading to

$$\frac{1}{dt} E\{\underline{N}(t+dt) - \underline{N}(t)\} = \sum_{\underline{e}} \underline{e} \sum_{k} f_{k,\underline{e}} N_k(t).$$

Cell types, properties, levels, and rules. The central concept in OncoTCAP is that each cancer cell has a set of attributes described by selecting, for each "heritable property", a particular "level". These levels represent information such as physical location in a particular organ, portion of an organ or type of microenvironment, and presence of specific mutations or variations in gene dosage or gene expression affecting key mechanisms. The roster of distinct types of cells that may appear in a tumor is obtained by considering all possible combinations of these properties.

Example of a cell type

ORGAN	= Lung_Primary
MACRO-ENVIRONMENT	= (27,55,92)
MICRO-ENVIRONMENT	= perivascular
RAD-SENSITIVITY	= somewhat-radiation-sensitive

"Property" **"Level"**

Each "level" has one or more "rules" associated with it. The parameters governing the behavior of an individual cell are automatically generated by applying all the rules associated with the cell's properties. In this way, considerable economy of description is achieved. Users specify isolated aspects of cancer biology by creating, for each property, level the rules describing the modification of cancer cell parameters. These rules modify the kinetics parameters (eg, rates for mitotic, mutational, apoptotic or necrotic events, or Gompertz-like density dependence), and also modify the treatment sensitivities. (The rules are restricted to be commutative, so that the order in which the rules are applied will not matter.)

Kinetics rules. Kinetics rules affect kinetics parameters thus:

$$f_{k,\underline{e}} = f^0_{\underline{e}} \prod_{property} ruleval_{\underline{e}}(level(property,k)),$$

where $f^0_{\underline{e}}$ is the kinetics parameter for a "reference" cell type.

Gompertz growth control. Growth of solid tumors is generally subject to growth slowing, or plateauing, often referred to as Gompertz growth. For a single cell type, this model is

$$\frac{dN/dt}{N(t)} = K \log \frac{N(\infty)}{N(t)}$$

Primary tumors may slow down due to interstitial pressure, systemic antiangiogenic factors, or competition for growth factors. Metastases may slow down due to insufficient blood supply or systemic antiangiogenic factors. OncoTCAP has pioneered the application of Gompertz kinetics in a multiple-cell-type setting. Gompertz rules can be defined at any location scale. Thus $N(t)$ in the equation above refers to the cell count of a particular *Gompertz Region* (GR). (Whole Body, Organ, Macroenvironment, or Microenvironment).

These rules can even be combined across nested levels of location:

$$\xi_X(t) = \xi_1 \prod_{j:X\in GRj} \left(1 - \left\{1 - \frac{\gamma_{1X}}{\xi_{1X}}\right\}^+ \left\{\log\left(\sum_{Z\in GRj} N_Z(t)\right)\right\} GS_j / \log(GP_j)\right)$$

$$\gamma_X(t) = \gamma_1 \prod_{j:X\in GRj} \left(1 + \left\{1 - \frac{\gamma_{1X}}{\xi_{1X}}\right\}^+ \left\{\log\left(\sum_{Z\in GRj} N_Z(t)\right)\right\} (1 - GS_j) / \log(GP_j)\right)$$

Here X is the cell type, ξ is the birth rate, γ is the death rate, j indexes Gompertz rules, *GR = Gompertz Region, GP = Gompertz Plateau* = $N(\infty)$, *GS = Gompertz Split* allocates the slowdown to ξ and γ, and the "+" superscript takes negative numbers to zero. For example, a Gompertz rule based on total tumor cells, representing a tumor-produced serum angiostatic factor, can be combined with a Gompertz rule based on tumor cells in local microenvironments, representing spatial competition or restricted access to serum growth factors and oxygen.

Cancer treatments. *"Episodic" ("cytotoxic") treatments.* Part of a cell's behavior is its propensity to respond to a killing episode, generated by primary surgery or by a cytotoxic agent. These effects are modeled with binomial distributions for each cell type; the probability of survival is modified by the properties through application of the rules. Dosage modification is managed by applying the Skipper log-kill hypothesis separately for each cell type.[21] Thus the cell killing of a drug d administered at time t is implemented separately on each cell type k as

$$N_k(t+dt) \sim binom(N_k(t), q_{k,d}(t))$$

where $q_{k,d}(t)$ = pr(a cell of type k survives the episodic treatment d administered at time t)

$$= q^0_d \prod_{property} rule_d(level(property,k)).$$

Thus, in addition to kinetics rules, levels can also have rules that modify treatment sensitivity.

For ease of use, treatments can be packaged into combinations (for simultaneous application of several agents), or into treatment courses (for repeated administration of an entire treatment sequence).

"Continuous" ("cytostatic") treatments. Extended treatments such as hormone therapies, angiogenesis modulators, and cytostatic agents are modeled as cell-type-specific modulations in the kinetic parameters over the period of the treatment. Therefore, their effects on cell types are described similar to the effects of property-level rules. A drug d administered over an interval $[a,b]$ may act on a particular cell type k by further modifying the kinetics event rate $f_{k,e}(t)$ for $t \in [a,b]$, multiplying it by $\prod_{property} rule_{e,d}(level(property,k))$. This treatment effect model would be appropriate for hormonal therapies and anti angiogenesis agents.

Toxicity Model

The adverse effects of specific treatments are modeled probabilistically in a Markovian fashion, with either cumulative or absolute jumps. The state space corresponds to the standard Common Toxicity Criteria, a set of five-grade toxicity scales for different toxicity types. Resolution of the toxicity can be rapid, gradual, or disallowed.

Solutions. OncoTCap 2 provides two types of solutions. The joint probability generating function (jpgf) for the joint distribution of cell counts is obtained from iterated solutions to partial differential equations.[49,50] Evaluating the jpgf at the vector of all zeros yields the probability of no cancer cells at the end of the time horizon. Figure 1 was generated by these jpgf solutions.

These solutions are available only when parameters are fixed through time, or changed in a deterministic way. This limitation rules out Gompertzian growth and changes in treatment plan responsive to events in a patient's course, such as toxicity and recurrence. To complement this computation, a stochastic simulation engine operates on the vector of cell counts. Figure 2 was generated by these simulations.

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Author Contributions

Executes all aspects of this review and study: RSD. The author reviewed and approved of the final manuscript.

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