Mathematical modeling of cancer progression and response to chemotherapy

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The complex, constantly evolving and multifaceted nature of cancer has made it difficult to identify unique molecular and pathophysiological signatures for each disease variant, consequently hindering development of effective therapies. Mathematical modeling and computer simulation are tools that can provide a robust framework to better understand cancer progression and response to chemotherapy. Successful therapeutic agents must overcome biological barriers occurring at multiple space and time scales and still reach targets at sufficient concentrations. A multiscale computer simulator founded on the integration of experimental data and mathematical models can provide valuable insights into these processes and establish a technology platform for analyzing the effectiveness of chemotherapeutic drugs, with the potential to cost-effectively and efficiently screen drug candidates during the drug-development process.


Fundamental considerations on cancer biology

The physiological processes underlying cancer are highly complex, spanning a wide range of interrelated temporal and spatial scales. The fundamental causes are believed to reside at the genetic level, where mutations enable cells to develop a selective advantage, allowing them to reproduce or prolong life in defiance of normal constraints. In time, these cells form avascular masses limited to approximately a few millimeters in diameter owing to the transport limitations of oxygen and nutrients into tissue [1]. As inner layers of the nascent tumor begin to necrose, tumor angiogenic regulators are released by the tumor mass, which diffuse through the surrounding tissue and trigger a cascade of events upon arrival at local vasculature, culminating in the recruitment of vessels that supply blood to the burgeoning tumor (i.e., angiogenesis). At this point, the vascularized tumor may remain compact and noninvasive (i.e., benign), in which case it can usually be successfully removed by surgical resection or treated with radiation. Conversely, upon receiving infusion of nutrients from its newly formed vasculature, a tumor may become malignant and rapidly invade local tissue, usually acquiring mutations enabling navigation through the bloodstream and lymphatics to metastasize to other locations in the body [2]. The nonlocalized nature of metastatic cancer limits the success of surgical and radiation treatment approaches; thus, systemically administered chemotherapy continues to be the standard option in spite of varied outcomes.

Tumor neovasculature plays an integral role in the administration of such treatment. It is the first tumor-level barrier that an administered drug molecule must navigate on its journey to its intended intracellular target, and its anatomical and functional irregularities are thought to significantly impair drug distribution to lesion tissue. For standard therapy, once drug molecules extravasate through vasculature, they must diffuse through interstitial space, permeate cell membranes, survive a gauntlet of intracellular mechanisms designed to detoxify cells and finally bind to subcellular targets at sufficient cytotoxic concentrations [3]. This series of
barriers combines to produce an overall reduction in the efficacy of many unrelated anticancer drugs, a phenomenon called multidrug resistance (MDR) [4], and cannot be overcome simply by administering more drug, as toxicity to host tissue presents a formidable challenge.

For conventional chemotherapeutic treatment, strategic dosing is used to maximize anticancer-drug effects while minimizing host toxicity. However, recent antiangiogenic treatment strategies center around targeting tumor neovasculature instead of the lesion itself. By destroying the vascular bed, the tumor’s source of oxygen and nutrients is reduced, leading to starvation of the mass. Furthermore, such therapies might reduce metastasis by eliminating the escape route into systemic circulation [1]. In another innovative strategy known as vascular normalization, the intent is not to destroy all the vasculature, but rather to prune it of inefficient branches, thereby normalizing the abnormal structure and function of tumor vasculature and improving delivery of oxygen, nutrients and drugs [5]. Another promising approach to cancer treatment is the use of nanovected therapy, employing nanoscale devices to specifically target and deliver drug payloads to cancer cells [6]. These therapies, along with conventional treatment, are ideal candidates for in silico (computer) modeling, which has the power to offer insight into their efficacy and potentially develop into a means for predicting patient-tailored therapeutic regimens [7,8].

Role of modeling in cancer research & drug development
Developing a detailed understanding of the underlying pathophysiology of cancer, its progression, mechanisms of drug resistance at various scales, as well as the optimization of drug dosing protocols, is the subject of vast amounts of research directed towards the development of effective treatment and prevention strategies. Owing to the complexity of this disease, it has proven difficult to assign quantitative weights to each component. This may be due, in part, to the nature of experimental investigation, where mechanisms are often studied in an isolated context. It has been suggested that a conceptual framework is necessary to fully understand the data produced in quantity by tumor biologists and clinical oncologists [9-11]. The challenges of better understanding the overall cancer phenomenon and its treatment might benefit from an evaluation of mathematical modeling and bio-computation. This approach can be integrated with biological experiments and clinical trials. Traditional clinical and biological experiments require costly investments in both time and materials, and are limited by equipment precision, human error and the inability to distinguish between various underlying mechanisms governing tumor growth [11,12]. In parallel, a critical weakness of theoretical models is their plasticity in uncritically recapitulating training data, without regard to the model’s actual validity and predictive capability. Nevertheless, modeling can provide investigators with tools to run computational experiments that would otherwise be very difficult or impossible to recreate in an experimental setting (e.g., varying adhesion forces between cells or varying membrane permeabilities of a particular cell line); accordingly, modeling can provide valuable information to plan effective biological experiments for testing theoretical hypotheses. Data from biological experiments provide necessary constraints for choosing appropriate model parameters. Therefore, although pure theoretical or experimental investigations alone have inherent flaws and limitations, an ideal synergy between the two can be approached by using a circular, recursive workflow methodology.

Objective
The objective of this article is to first present a brief overview of current mathematical and bio-computational modeling of cancer progression and therapy, followed by a description of the integrative approach that we envision towards the development of higher order bio-computational technology, centered about a simulator with the capacity to predict in vivo tumor growth and response to therapy. A virtual cancer simulator might provide a means to efficiently and cost-effectively screen drug candidates with the potential of significant savings in research and development. An additional, long-term goal of this research is to customize clinical cancer therapy by using cell-specific tumor information, thereby maximizing benefit to both patients and providers.

The endeavor to develop software packages capable of sophisticated, in vivo-like tumor simulation will be based on a modular, multiscale development process where individual components are built upon mathematical models simulating disease progression, anticancer drug pharmacology and drug resistance. These are then integrated to simulate the disease and possible therapies through a wide temporal and spatial range. This scalable scheme allows the simulator to be enlarged by adding and appropriately linking modules (FIGURE 1). This review is structured to familiarize readers with key modeling efforts over the past 30 years, inspiring the development of modules in a cancer simulator, examine the current status of cancer simulation, present applications of the simulator in the areas of drug development and optimization and indicate challenges for advancing cancer simulation towards higher levels of sophistication.

Fundamentals of cancer modeling
Mathematical models can provide biologists and clinicians with tools that might guide their efforts to elucidate fundamental mechanisms of cancer progression and either improve current treatment strategies or stimulate the development of new ones [13]. Many cancer models have been proposed that focus on one or more phases of cancer progression (i.e., avascular, angiogenesis or vascular) and can typically be categorized as either a discrete, continuum or hybrid approach [11,13,14]. Continuum models draw upon principles from fluid and continuum mechanics and describe cancer-related variables, such as cell population, nutrient concentration, oxygen distribution and growth factor concentrations as continuous fields by means of differential equations [13]. By contrast, cellular automaton (CA) models describe the dynamics of discrete elements (e.g., tumor cells) whose states...
are governed by a set of deterministic and/or probabilistic rules. The state evolution of these elements can be tracked through both space and time. Hybrid cancer modeling approaches combine continuum fields with CA descriptions. In particular, substances such as oxygen, nutrient, drug and growth factors can be described as a continuum in the tumor microenvironment, while individual CA elements dynamically evolve in response to local substance concentrations.

**Multiscale simulation**

The literature devoted to the theoretical investigation of solid tumor growth and angiogenesis using continuum and CA modeling approaches has been reviewed in depth by Araujo and McElwain [11], Moreira and Deutsch [14], and Mantzaris and colleagues [15]. While most of the models described within these reviews are able to provide useful insight into cancer-related processes occurring at a particular length and time scale, they do not address the fundamental problem of how phenomena at different scales are coupled [13]. The multiscale complexity of cancer progression warrants a multiscale modeling approach to be taken to produce truly predictive tumor simulators. Processes occurring at various length and time scales must be coupled appropriately in order to capture all the dynamics involved. Previous works have developed multiscale systems modeling complex biological processes, such as cancer [13,16–18], the heart and lung [19–22], and various phenomena related to developmental biology [23,24]. In particular, Jiang and colleagues [18] and Alarcon and colleagues [13,16] present frameworks for building multiscale cancer progression models capable of integrating a hierarchy of processes at varying length and time scales. Most cancer models and multiscale systems [11,13,16–18,25–30] primarily produce 1D and 2D simulations that are limited in their ability to capture complex in vivo tumor morphologies and microenvironment. The aim of this perspective is to describe strategies for overcoming these limitations by developing a framework focusing on accurately predicting the 2D and 3D distribution of oxygen, nutrients and growth factors in the microenvironment, evolution of complex tumor morphology, and tumor response to various treatment strategies. The underlying models of the multiscale system will

**Figure 1. Outlook for a multiscale cancer simulation under development in the mathematical oncology community.** A multiscale cancer simulation is founded on the integration of experimental data and mathematical models. It should provide valuable insights into the cancer phenomenon and establish a platform for analyzing the effectiveness of chemotherapeutic drugs with the potential to extend patients’ pain-free survival and cost-effectively and efficiently screen drug candidates during the drug development process. Modules are developed and coupled via sharing of information.

DC MRI: Dynamic contrast magnetic resonance imaging; EC: Endothelial cell; ECM: Extracellular matrix; RES: Reticuloendothelial system; TAF: Tumor angiogenic factor.
be founded on biological principles and controlling parameters will be adjusted according to experimental and clinical data, thus developing a tumor simulator capable of producing more in vivo-relevant predictions.

Modular development of a multiscale cancer simulation: earlier models

This section provides a brief overview of a select group of tumor growth, angiogenesis and pharmacology models serving as the inspiration and foundation for efforts to develop a multiscale cancer simulator.

Tumor growth

Cristini and colleagues were among the first to advance modeling of complex tumor morphologies beyond the limited capabilities of mathematical linear analyses and into the realm of nonlinear computer simulation [31]. The multifaceted nature of cancer requires sophisticated, nonlinear mathematical models to capture more realistic growth dynamics and morphologies. Boundary-integral simulations [31] of classic continuum-based tumor models [25–30] determined that a reduced set of two non-dimensional parameters (related to mitosis rate, apoptosis rate, cell mobility and cell adhesion) regulate morphology and growth (invasiveness) of avascular and vascularized tumors. In this model, there is no morphological representation of vasculature, rather the effect of vascularity is quantified by a parameter relating the concentration of nutrient in the blood, nutrient transfer rate from blood to tissue and nutrient consumption by the cells. Essentially, critical conditions were predicted that separate compact, noninvasive mass growth from unstable, fingering, infiltrative progression [31]. However, further analysis demonstrated that highly vascularized tumors tend to grow in compact, nearly spherical shapes showing little or no sign of invasiveness. This unexpected prediction suggests that tumors could maintain stable morphology under normoxic microenvironmental conditions. This result is supported by experimental observations indicating that hypoxia stimulates invasiveness and tumor fragmentation [32,33]. In later sections of the present article, tumor simulators built upon this framework and their importance in studying cancer therapy will be discussed, together with recent experimental confirmation.

Tumor-induced angiogenesis

Angiogenesis is the process by which cancers recruit enhanced blood supply to provide the oxygen and nutrients that are commonly considered necessary to support growth into larger, more invasive tumor masses. As is the case with tumor growth, tumor-induced angiogenesis is a topic receiving considerable attention from the biological modeling community and has been extensively reviewed (e.g., Mantzaris and colleagues [15]).

Mathematical model for tumor-induced angiogenesis

Angiogenesis is believed to be initiated by proangiogenic proteins (PAP), such as vascular endothelial growth factor (VEGF), that have been induced by a lack of oxygen and nutrients to be released from the necrotic tissue of a tumor lesion into surrounding tissue [34]. These proteins create a chemical gradient that triggers endothelial cells (ECs) from parent vessels in the pre-existing vasculature to migrate towards the tumor (chemotaxis). Eventually, through a number of complex mechanisms, the accumulation of ECs forms finger-like capillary sprouts extending from a parent vessel. Analogous to plant growth, these sprouts extend and grow towards the tumor along the chemical gradient guided by the migration of the sprout-tip. The interaction between ECs and the extracellular matrix (ECM) itself is also significant in directing the sprout-tips. Fibronectin is generated and adheres to the matrix, serving to guide the direction of endothelial cell progression via a process called haptotaxis, similar and complementary to chemotaxis. Once capillary sprouts from the parent vessel extend far enough towards the tumor, they tend to lean towards each other and form tip-to-tip and tip-to-sprout fusions called anastomoses (thought to be caused by haptotaxis) [35,36]. Through this process of anastomosis, an initial network of poorly perfused, interconnected immature vessels is formed. The previously described process of angiogenesis and subsequent anastomoses occurs in a repetitive fashion using the initial network as parent vessels, thereby producing an extended capillary bed concentrated in the tumor. However, the neovasculature is irregular and poorly perfused in comparison with normal tissue vasculature and will be portrayed as a biological barrier to anticancer treatment efficacy in upcoming sections.

Anderson and Chaplain developed a tumor-induced angiogenesis model with the ability to follow the motion of ECs at the capillary tips and control important processes, such as proliferation, branching and anastomosis [35]. Their model uses a hybrid approach (i.e., both continuum and discrete modeling) and focuses on three significant variables related to angiogenesis: EC density, and PAP and fibronectin concentrations.

The continuum component of the Anderson and Chaplain model describes the evolution of EC, PAP and fibronectin distributions using a reaction-diffusion system of three partial differential equations. The equation describing EC distribution accounts for three major components: small amounts of random motion possibly dependent on PAP concentration (i.e., diffusion); general EC migration owing to chemotaxis; and EC adhesion to ECM owing to an interaction with fibronectin (i.e., haptotaxis). The equation describing the evolution of PAP concentration uses an uptake term representing the binding of PAP to EC as they migrate through the tissue. The equation describing the evolution of fibronectin concentration includes terms representing fibronectin synthesis by endothelial cells as they migrate through the ECM, and a presumed degradation. Fibronectin exists in the ECM in bound form and is not freely diffusible, therefore no diffusion term is included.

The discrete component of the angiogenesis model uses a random-walk model, which governs the movement of individual ECs at the capillary sprout tips. The model essentially tracks the migration of sprout tips and presumes the shape of
the full sprouts based on the sprout tip paths. Using a discretized version of the continuum model, the individual motion of the EC sprout tips is governed by probabilities of the EC being stationary or moving left, right, up or down; these probabilities are functions of local fibronectin and PAP concentrations [35,36]. Predetermined rules for branching, anastomosis and cell proliferation produce the overall morphology of realistic tumor neovascularure.

Extension of Anderson & Chaplain angiogenesis model: blood flow

The abnormal nature of tumor vasculature compared with healthy tissue vasculature has been addressed [5]. Irregular tumor vasculature leads to restricted and inhomogeneous drug and nutrient extravasation to tumor tissue, which may exacerbate the situation by selecting for highly resistant clones. Anderson and Chaplain’s angiogenesis model appears to capture the irregularity of tumor vasculature through appropriate adjustment of the governing mathematical parameters. However, their model only describes the physical structure of the capillary network. Nutrient, oxygen and drug distributions in a tumor can be modeled in a simplified fashion by using the Anderson and Chaplain vasculature as a source boundary condition in a diffusion–reaction system. In reality, nutrient, oxygen and drug delivery depends on blood flow through the vasculature; therefore using the entire vasculature as a uniform source with blood–tissue transfer proportional to local pressures is a rather elementary description.

McDougall and colleagues developed a direct extension of the Anderson and Chaplain angiogenesis model [35] by describing the generated vascular networks as a series of straight, rigid cylindrical capillaries that join adjacent nodes [36]. Blood flow is modeled through the cylindrical vascular network by modeling the elemental flow rate in each segment with Poiseuille’s Law, which describes flow rate as a function of capillary lumen, fluid viscosity, capillary length and pressure drop. Using this simple flow model, McDougall and colleagues identified tumor neovascularization as a biobarrier to chemotherapy [36]. Results of their simulations indicated that the highly interconnected nature of irregular vasculature produced by tumor-induced angiogenesis could cause low rates of drug delivery to the tumor with the potential for the drug to actually completely bypass the entire mass depending on the tumor shape and consequent proangiogenic protein distribution. Additionally, the simulation results suggest that drug delivered by bolus injection suffers from severe dilution, thereby reducing drug efficacy.

Stephanou and colleagues [37] extended the work of McDougall and colleagues [36] by developing an algorithm that normalizes vasculature produced by Anderson and Chaplain’s angiogenesis model [35]. They examined how pruning vessels by antiangiogenic drugs might affect blood flow distribution and consequently drug delivery to tumors. Their work included blood flow simulations in fully 3D vasculature. Stephanou and coworkers later included vascular adaptation effects [38], due to shear stress generated by flowing blood [39,40], to the angiogenesis model to investigate how adaptive remodeling affects oxygen and drug supply to tumors. Alarcon and colleagues also modeled the vascular adaptation effects in an effort to study inhomogeneity of oxygen distribution in tumors and the consequential role of hypoxic cells in tumor invasion [13]. More recently, McDougall and colleagues modified their angiogenesis model to simultaneously couple vessel growth with blood flow to dynamically include the effects of vascular adaptation [41], rather than adapt the vasculature a posteriori like Stephanou and colleagues [36] and Alarcon and colleagues [13].

Pharmacology & drug efficacy

In the event that a cancer has metastasized, systemic treatment is generally necessary in the form of chemotherapy delivered to the primary and secondary tumors through the bloodstream. Drugs must overcome various resistance mechanisms and barriers that affect their efficacy en route to their respective targets, thus producing the overall MDR phenomenon. Individual mechanisms and barriers occur at different scales. At the subcellular and cellular scale, there exists a range of drug influx/exflux pumps, changes in the expression of topoisomerases and alterations in metabolic pathways (e.g., influencing drug metabolism, DNA repair and/or apoptosis). At the tumor and body scale, resistance can be due to normal clearance mechanisms (e.g., urinary system, reticuloendothelial system or the blood–brain barrier), abnormal tumor vasculature, tumor microenvironment, and tumor 3D structure [5]. Consequently, these biobarrriers impede the delivery of chemotherapeutic drugs at effective concentrations to all cancer cells.

Pharmacokinetics

In the study of drug delivery, it is common to conceptualize the organism as a system of interconnected pools called compartments. The investigation of the properties of these compartments and the material fluxes between them is termed ‘compartment modeling’ [42]. Conventional pharmacokinetic (PK) models use compartment modeling to investigate cellular drug-uptake and intracellular drug interactions, as well as provide insight into modeling cellular-scale mechanisms contributing to drug resistance. For example, a standard threecompartment model was used by Dordal and colleagues to investigate cellular drug uptake [43]. Their objective was to quantify increased efflux, decreased intracellular sequestration and decreased membrane permeability as they relate to a reduction in drug effectiveness. Using flow cytometry, they assessed the cellular uptake of doxorubicin and fluorescent rhodamine-123 in drug-resistant and -sensitive cancer cells. By fitting the experimental data to the compartmental model, kinetic parameters for both inward and outward transport were obtained and used to quantify the relative importance of the previously mentioned cellular mechanisms. Specifically, their results indicate that of the three cellular mechanisms modeled, decreased intracellular sequestration in a nonexchangeable compartment is quantitatively the most significant contributor towards drug resistance [43]. Similarly,
compartment modeling can be applied to investigate additional components affecting drug delivery, such as extracellular drug binding and target repair mechanisms.

Pharmacodynamics
While PK describes drug penetration, pharmacodynamics (PD) describes drug cytotoxicity. Although the mechanisms contributing to drug effects are incompletely understood, several phenomenological models adequately yield fractional cell survival, $S$, as a function of concentration–time exposure history. The Hill-type model $S = (1 + Ax^m)^{-1}$ is often used, where $x$ is a measure of cellular damage, such as extra- or intracellular area under the curve (AUC). In turn, AUC is given as the integral of concentration with respect to time $\int C dt$. Another possibility is the exponential kill model $S = e^{-kx}$, where $x$ again is a suitable measure of damage and $k$ is a constant. While these are perhaps the simplest PD models in use, other equations can be employed.

A study by El-Kareh and Secomb investigated several measures of cellular damage in conjunction with the Hill-type model given above to determine which provided the best fit to experimental data [44,45]. Their investigation was prompted by the observation that models employing extracellular AUC consistently overestimated cytotoxicity in cases of extended exposure to the drugs cisplatin and doxorubicin. Experimentally, toxicity would achieve a plateau, above which continued exposure, even to continually refreshed drug, would have no effect. To explain this, they hypothesized that it was not the time of exposure per se that correlated with cytotoxicity, but rather the peak level of DNA-bound drug [44]. Accordingly, they used this measure and showed that for short exposure times, the delay in achieving DNA-bound drug equilibrium could explain increasing cytotoxicity in time. Various experimental cell survival data were fit to determine appropriate values for the constants $A$ and $m$. The new model was compared with previous models describing the relationship between cytotoxicity and exposure time. El-Kareh and Secomb’s model consistently proved to be the best fit even for long exposure in *in vitro* datasets [46], establishing that peak DNA-bound cisplatin is a stronger indicator of cytotoxicity than extra- or intracellular concentrations. Later, they extended the model to doxorubicin [45]. Experimental evidence suggests that doxorubicin has two cytotoxic mechanisms, one involving topoisomerase II inhibition by intracellular drug and the other involving apoptosis induction via extracellular drug. El-Kareh and Secomb proposed a model that combines the effects of both mechanisms into the cellular damage by summing peak intra- and extracellular drug concentrations. Similar to the cisplatin model, their doxorubicin PD model provides better fits to *in vitro* cytotoxicity datasets than previous models [45].

**Integrated tumor simulation:**
**foundations for a multiscale simulator**
Thus far, this perspective has focused on providing a fundamental overview of key cancer modeling efforts in the areas of tumor growth, tumor-induced angiogenesis, blood flow and pharmacology. The focus will now be on strategies for the development of a higher order computer simulator capable of customizing cancer drug therapy based on cancer-specific information and built upon the fundamental framework described in the preceding sections. Here, we present current developments in simulation technology, including the determination of model parameter values and validation of their performance based on experimental *in vitro* and *in vivo* data.

**Model descriptions**
Zheng and colleagues produced a first-generation multidimensional tumor simulator employing a sharp-interface (level-set) finite-element numerical method for tracking the tumor boundary [47]. This model is capable of simulating 2D tumor evolution through the major phases of growth, including avascular dormancy, neovascularization and subsequent rapid expansion and infiltration of host tissue. Wise and colleagues recently produced a second-generation 3D tumor simulator employing a more physically accurate diffuse-interface formulation on a finite-difference framework, which can more realistically represent tissue interfaces and clonogenic heterogeneity [48]. Both simulators not only serve to model tumor progression, but also provide test-beds for therapeutic strategies and hypotheses (FIGURES 2 & 3) [49,50].

These models build on the continuum-based approach used by Cristini and colleagues, which considers tumor mass as an incompressible and viscous material that locally expands and contracts in correspondence to variable rates of cell mitosis and apoptosis [31]. Tumor cells themselves are not individually represented. In Zheng and colleagues’ formulation, local lesion environment is modeled as three sharply demarcated nonintersecting domains: viable tumor, necrotic tissue and host tissue [47]. Although Wise and colleagues’ diffuse-interface formulation uses a similar partitioning of the lesion environment into tissue domains, boundaries are not as strictly defined [48]. Instead, at a given location, intermixing of several cancerous clones along with necrotic and host tissue can be represented by specifying their relative mass fractions. This cannot be done in the sharp interface model and is a critical improvement towards realistically simulating mutation-driven heterogeneity. Please and colleagues were amongst the first to apply multiphase modeling to tumor growth by capturing both tumor cells and extracellular fluid as separate continuum phases [51,52]. Work by Ward and King [53,54] and Brewd and colleagues [55] followed suit by also modeling avascular cancer growth as a two-phase description comprised of tumor tissue and dead tissue (extracellular space). This multiphase modeling is needed to capture avascular tumor growth as live tumor cells proliferate into the dead tissue space. Brewd and colleagues extended their avascular model [54] to describe vascular tumor growth, thus incorporating a third phase to describe the spatial and temporal distribution of blood vessels [56]. Along similar lines, Wise and colleagues also used a multiphase modeling approach by capturing the evolution and interactions between intermixing multiple tumor species, necrotic tissue, host tissue and interstitial...
Araujo and McElwain recently proposed a multiphase model of tumor growth that includes a solid phase representing the extracellular matrix, in an effort to more accurately capture residual stresses [57].

While it is the growth and regression of lesion tissue that is of primary interest, other processes support and interact with this growth, necessitating a modular design in which simulator components are dedicated to process management. The major components essential to basic lesion simulation are specific to growth and regression, nutrient delivery and angiogenesis. Beyond these, modules pertaining to genetic mutation, cell-cycle and phenotype specifics, and therapy can be added to provide pertinent information from smaller spatial and temporal scales. The growth and regression component postulates that cell velocity is proportional to pressure gradient (Darcy’s law), which is commonly used to model motion through porous substrates (i.e., a continuum of cells flowing through the extracellular matrix). Morphology, especially as it pertains to invasiveness, is affected by parameters that model cell adhesion, for instance through the definition of an equivalent surface tension at the tumor boundary [27]. Based on inputs from other components, the growth module produces a cell displacement (velocity) field and advects the tumor boundary in the case of Zheng and colleagues’ method [47], and the species mass fractions in the case of Wise and colleagues’ method [48].

Vasculature is incorporated into these simulators as an angiogenesis module inspired by Anderson and Chaplain’s angiogenesis model [33,47], linked to tumor growth through the release of tumor angiogenic regulators by necrosing tumor tissue. The transition between avascular and vascular tumor growth is marked by the recruitment of microvasculature from local...
blood vessels (i.e., angiogenesis). The governing processes of angiogenesis are still very much in debate, but one proposed mechanism followed by Anderson and Chaplain’s model grows new vessels from parent vessels due to chemotaxis of endothelial cells along an angiogenic regulator gradient towards the tumor [33]. ECs also interact with the extracellular matrix in a process known as haptotaxis.

Underlying these components are sophisticated numerical algorithms, including adaptive computational meshes [58,59], that enable high-resolution rendering of complex tumor morphologies, including fingering, invasion and reconnection, across multiple length scales at the minimum computational expense.

Simulation as an investigative tool: integration of experimental data with theoretical modeling

A key aspect of the successful development of an advanced simulator of in vivo tumor growth is to both derive fundamental parameter values from, and validate simulator performance through, in vitro and in vivo experimental (and clinical) results. Often, the process begins with elementary properties of cells growing in monolayer, such as doubling time, oxygen and glucose consumption, response to confluence, and uptake and response to drugs. Maintaining awareness that these properties probably change in vivo, it is believed that when enough information is known regarding the mechanisms that give rise to a cell’s behavior, whether that be in a dish or in a living organism, the appropriate interplay of these mechanisms via a computer model can predict not only individual cell behavior, but also that of cellular aggregates within a given set of environmental conditions. This line of reasoning is justified by the success of cell automaton models in predicting cell and tissue behavior, suggesting that gene expression information and cell-signaling mechanisms involved in determining cell behavior can be reduced to a subset of governing rules [60–62].

Antiangiogenic therapies target tumor neovasculature with the intention of disabling a tumor’s source of oxygen and nutrients. However, a recently proposed concept termed ‘diffusional instability’ suggests that antiangiogenic therapies may actually trigger tumor instability and tissue invasion [49]. Based on simulations of tumor growth, Cristini and colleagues hypothesized that complex tumor morphologies leading to local tissue invasion are caused by oxygen and nutrient gradients [49]. These gradients result in regions of hypoxia and acidosis, which directly increase tumor invasiveness by increasing cell motility through the production of autocrine motility factor, expression of tumor urokinase.

Figure 3. Correspondence between in silico tumor progression and in vitro tumor spheroid growth. (A) Example of a fairly compact in vitro glioma spheroid with high levels of cell adhesion. (B) An in silico representation of a compact tumor based on appropriate input parameters. (C) Example of an in vitro glioma spheroid with low levels of cell adhesion. The formation of subspheroids indicates an overall morphological instability. (D) An in silico representation of an unstable tumor with low cell adhesion. Bar: 130 mm.

Adapted from [49] with permission from the American Association for Cancer Research.
plasminogen activator receptor, production of cathespin B and upregulation of hepatocyte growth factor [63–65]. Friebøes and colleagues tested the hypothesis using experimentally derived parameters as inputs to the simulator [66]. The experiments varied glucose and serum conditions for in vitro human and rat glioblastoma spheroids, yielding parameters relating tumor shape and relative nutrient concentration. Subsequently, simulations were used to predict and interpret in vitro tumor spheroid morphology and invasiveness. Accordingly, simulations using the experimentally determined parameters suggested that tumors might form shapes that maximize cellular exposure to oxygen, nutrients and growth factors by correspondingly adjusting cell proliferation and adhesion. Macklin and Lowengrub further supported these predictions in their simulations [67].

Results of these three studies exemplify the usefulness of integrating experimental and theoretical research to develop valuable insight into explaining biological phenomena. Experimentally derived parameters were used to drive the realism of the tumor simulators, while also interpreting nutrient gradients, resulting from the presence of multiple cell species, inherent diffusion limits and therapy as a mechanism for local tumor infiltration.

Predictive modeling of chemotherapy & antiangiogenic treatment: implications for drug development

Chemotherapy simulations using a 2D tumor model

Therapy simulations performed recently by Sinek and colleagues using Zheng and colleagues’ tumor simulator [47] along with Anderson and Chaplain’s vasculature model [33] were among the first to be performed in a true multidimensional (rather than cylindrically or radially symmetric) and vascularized setting. While these simulations used an elementary one-compartment model (lesion mass with no cellular resolution) with the vasculature serving as the nutrient and drug source, they were nevertheless useful in highlighting potential adverse characteristics of tumor response to intravenously supplied cytotoxic drug [58]. Among these were some of the previously mentioned biobarriers, including the susceptibility of lesion vasculature to collapse under pressure generated by cellular proliferation and its consequent contribution to nutrient and drug heterogeneity. More importantly, it was demonstrated that these heterogeneities may impair therapeutic efficacy by precipitating invasive morphology, even in a best-case scenario assuming uniform drug delivery from vasculature (e.g., by using constant-release ‘smart’ nanovectors uniformly extravasated along vessels), a single, drug-sensitive cell-type and negligible host toxicity. Other simulations in this study demonstrated how angiogenic normalization might be of benefit [5,50]. The first two researchers closely examined cellular behavior with regard to drug, such as the fraction and sensitivity of cycling versus quiescent cells, response to cycle phase-specific and nonspecific drugs, and response to cytotoxic and cytostatic drug combinations. The latter two are more concerned with lesion scale PK effects, such as drug exposure of inner regions of spheroids and cancer islets, and the dynamics of tumor response to various dosing regimes. While the model per se developed by Cristini and coworkers is not necessarily an extension or refinement of these previous efforts, its coupling with Zheng and colleagues’ tumor growth and angiogenesis simulator represents advanced modeling technology, enabling the simulation and analysis of chemotherapy applied to vascularized in vivo tumors [47].

While this PKPD model was designed explicitly for cisplatin and doxorubicin, since their primary mechanisms of action have been well characterized and both drugs are widely used, the intention is that it may be adapted to simulate therapy using other drugs. During a simulated intravenous drug bolus administration, concentration is assumed constant along the vasculature, which acts as a source throughout the tumor. Concentration subsequently decreases as drug diffuses through interstitium and is taken up by cells. The model accounts explicitly for tissue- and cell-level biobarriers, tracking drug from the point of extravasation through lesion interstitium and cell membranes, and into intracellular organelles and target. The amount and time of exposure of DNA-bound drug then determines its effect throughout the lesion. Each element of the drug pathway contributes a measure of transport resistance, the end result being diminished and nonuniform drug-to-target presentation and consequent compromised efficacy. By providing precise control over parameters corresponding to PKPD elements, the chemotherapy model can deliver powerful and flexible hypothesis-testing capability. More strategically, it is envisioned as part of a larger scheme intended to optimize established clinical therapies and to serve as a screening phase towards improving overall efficiency and cost effectiveness during the drug development process.

Special attention is paid to intracellular compartments and associated fluxes so that resistance mechanisms can be accurately modeled. In the case of cisplatin, glutathione conjugation and removal from cytosol, as well as DNA repair, are key mechanisms. The model for doxorubicin includes an additional lysosomal compartment owing to the affinity of these organelles and their hypothesized role in expelling drug via exocytosis. Intercompartmental fluxes are governed by rates (the k’s in FIGURE 4), which are themselves derived from underlying tissue and cell parameters governing the transport of drug molecules. Some of the more important parameters are drug diffusivity, membrane permeability,
was due to a resistant fraction of cells (\(F\)). However, Cristini and coworkers hypothesized that this effect into account the extended exposure plateau of cytotoxicity.

Parameters can be tuned not only to drug temporally through multiple compartments based on govern-
ing rate parameters. Parameters can be tuned not only to drug transmembrane carrier activity (e.g., PGP and MRP), organelle sequestration and DNA repair. The PDE system of equations modeling PK (e.g., cisplatin model in FIGURE 4) is capable of tracking the amount of drug both spatially and temporally through multiple compartments based on governing parameters. Parameters can be tuned not only to drug and cancer type, but also to cancer grade as well as cellular subtleties of individual patients. Initially, parameter values were obtained through experimental data reported in published literature; however, it is intended that more targeted histological, cellular and genetic analysis of tissue biopsies will provide information necessary to dynamically determine model parameter values, yielding a truly predictive tool.

The PD component is a phenomenological model along the lines of El-Kareh and Secomb’s work \([44,45]\), which also takes into account the extended exposure plateau of cytotoxicity. However, Cristini and coworkers hypothesized that this effect was due to a resistant fraction of cells (\(F\)), and that, for a given DNA-bound level of drug, the remaining sensitive fraction, \(S - F\), would follow classical exponential kill kinetics. The resulting PD model for both cisplatin and doxorubicin describing the surviving fraction of cells \(S\) is as follows:

\[
\left(\frac{dS}{dt}\right) = -\lambda_s \max \{S - F, 0\}
\]

\[
\lambda_s = \frac{M}{(1 + A - 1)^{1/n}}
\]

\[
F = \frac{1}{1 + Be^{\frac{s}{g}}}
\]

where \(\lambda_s\) is the effect rate of DNA-bound drug \(s\), \(M\) is the maximum possible effect rate of the drug and \(A, B, n\) and \(m\) are parameters fit specifically to each drug/tissue. The model has been compared with various experimental cytotoxicity data available in the literature and proves to be an acceptable method for modeling cell death. This PD model, in combination with the previously mentioned cisplatin and doxorubicin PK models, integrates as a chemotherapy module in a tumor simulator to describe drug delivery from the vasculature to its target, and corresponding tumor regression. Specifically, the drug effect rate \(\lambda_s\) links the PD model with the tumor growth (and regression) module, which tracks local cell densities and resulting cell velocities, and consequently determines whether a simulated tumor grows or regresses.

**Chemotherapy simulations**

FIGURES 5 & 6 are typical chemotherapy simulations using the model in FIGURE 4, demonstrating some recent findings of Cristini and coworkers (UNPUBLISHED DATA) regarding heterogeneous nutrient and drug distribution, corresponding cell survival, nonuniform tumor regression and stabilization at a mass far above detectable levels \([50]\). In particular, PKPD input parameters for doxorubicin and cisplatin determined through literature and experiments have been used to compare the performance of these two drugs. Doxorubicin is known to quickly penetrate cell membranes and avidly bind to intracellular organelles and DNA, whereas cisplatin enters cells much more slowly and exhibits far less binding \([72,73]\). These differences reveal themselves dramatically in FIGURE 5, where strong gradients of doxorubicin stand in contrast to minor gradients of cisplatin, a behavior governed by the ratio of cellular uptake to diffusive flux. Furthermore, the level of DNA-bound doxorubicin declines almost immediately after the 2-h bolus infusion, whereas cisplatin within the cytosol continues to accrue on DNA for several hours longer, only showing a marked decline 8 h after the cessation of infusion. The PD model furthermore predicts a survival gradient of approximately 10% from vessel source to distal tissue face in both cases. Taking advantage of the precise control of experimental parameters available in silico, the model suggests that this is due to the decrease in nutrients and oxygen, which penetrates...
approximately 150 µm into tissue before being fully consumed. Hypoglycemia and hypoxia affect cells in ways that may create drug resistance [74] and these substrate gradients proportionally decrease cell cycling in the model [47,50]. Simulation can be used to investigate a tumor’s morphological response to chemotherapy, as well as quantify drug, nutrient and pressure distributions within a tumor (FIGURE 6).

**Antiangiogenic therapy simulations**

As mentioned earlier, antiangiogenic therapies target tumor neovasculature with the intention of disabling a tumor’s source of oxygen and nutrients. However, these therapies may trigger tumor instability and tissue invasion through a recently proposed concept termed diffusional instability [49]. Using 2D simulations of tumor growth linked to angiogenesis, Cristini and colleagues hypothesized that complex tumor morphologies leading to local tissue invasion are caused by oxygen and nutrient gradients [49]. Frieboes and colleagues tested this hypothesis through *in vitro* experiments using simulations to predict and interpret *in vitro* tumor spheroid morphology and invasiveness [66]. Results from this (simplified) model indicated that diffusion gradients of nutrients and growth factors could indeed influence tumor morphology and invasiveness (FIGURE 3). Simulations predicted tumor shape as a function of cell proliferation and adhesion based on cellular access to oxygen, nutrients and growth factors: low proliferation rates and high cell adhesion are sufficient to maintain compact tumor shapes, whereas high proliferation rates and
low cell adhesion promote tumor fingering and eventual formation of separate tumor clusters. Additionally, there is computational evidence that the presence of multiple tumor cell species with varying nutrient uptake and death rates can also lead to aggressive fingering into surrounding tissue \textit{in vivo} as a result of therapy [67].
Conclusion
Cancer is a multifaceted disease with complex governing processes occurring at a wide range of temporal and spatial scales. Biological barriers and MDR mechanisms, such as body clearance systems, tumor neovasculature, drug influx/efflux pumps, DNA-repair mechanisms, tumor microenvironment and 3D structure, exist at each of these scales and hinder the efficacy of anticancer therapies. Biosimulation based on mathematical modeling of cancer-related phenomena might prove to be a valuable tool for integrating the vast amount of data produced by experimental and clinical cancer specialists, thus providing a method to analytically investigate cancer progression and drug resistance. Multiscale tumor simulation work by Arakelyan and colleagues [13,16] has examined effects of oxygen/nutrient development [50].

Expected to help optimize treatment strategies and new drug utilization of a cancer simulator processes occurring at a wide range of temporal and spatial scales, in silico implementation & utilization of a cancer simulator

Technically, the design and in silico implementation of a cancer simulator is a monumental task and one that has been tacitly ongoing for the past few decades. Beyond this, the question of its use in clinical practice and pharmaceutical development is open.

To say that cancer is a complex disease is an understatement. With an estimated 30,000 genes, each comprised of 3000 bases on average, the human genome is a fantastically complicated enigma. Its physical description has only recently been elucidated and, while some of the functions of genes or the proteins they encode are known, the magnitude of their innumerable potential interactions is certainly overwhelming. A model that attempts too boldly to include everything biologists know regarding the cell and tissue would be far too intractable to either analyze or simulate, the lightening pace of computational improvement notwithstanding. Of course, this has never stopped scientific inquiry from succeeding, to a very high degree, in situations that may be somewhat analogous. After all, one need not fret over the exact shape of an apple, nor the positions and quantum states of each of its electrons, in order to predict its trajectory to earth.

Thus, the challenge in designing a cancer simulator is in choosing appropriate scales of resolution and subsuming unnecessary detail within the chosen level. Since cancer is inherently multiscalar, this must be done at each scale of interest. At the level of the genome and its protein products, for example, one could find duplication of pathways, or could find that a set of pathways converges on a particular phenotypic result. Thus, it would not be necessary to model each gene and each protein; rather, a surrogate functional token might represent the net function of a related set of genes. For instance, with respect to the apoptotic program, recognizing that a cell's final commitment to apoptosis is ultimately determined by mitochondrial pore disposition of Cytochrome C, the openers, including Bax and Bid, could be represented by a single token, while the closers, including Bcl-2 and Bcl-xL, could be represented by another [76]. In this way, numerous and similarly functioning pathways could be braided into far fewer and more easily managed strands. Another device to reduce complexity while maintaining accuracy in cell repertoire is to employ stochastic modeling. For example, while it may be asking for too much to track all the chemical reactions giving rise to a glial cell's cytoskeletal flexions, its migratory trajectory may be adequately represented by a biased random walk.

The use of a cancer simulator in clinical practice or pharmaceutical development is a more difficult question. In the clinic, it must first be decided what role such a simulator would play. Cancer is not a deterministic disease; its driving force, after all, is genetic mutation. One role currently envisioned – that of individually tailored therapy – might be a difficult one, even if very specific information on a given patient's cancer is extracted through genetic or proteomic analysis. Perhaps the statistical predictions would not be substantially better than today's empirically based prognoses. The history of using in vitro chemosensitivity assays to assist in patient therapy selection reminds us to be cautious [77].

More promising is the use of simulation to enhance pharmaceutical and novel therapy development. If drug/cell characteristics could be determined adequately through traditional wetlab techniques, such as cytotoxicity assays, uptake/efflux assays and blot analyses, the resulting functional parameters could be used as simulation input. The resulting simulation output would therefore carry weight, at least in terms of hypothesis generation, and guide the next phase of inquiry. It is important to note that the power of this method lies in the relative ease with which simulation could be carried out, employing parameter perturbation to thoroughly explore the response space before any cells, animals or humans are ever needed. The savings in resources and lives is certainly powerful motivation in pursuing this line of development.

Five-year view
The pharmaceutical industry spends an average of US$500–800 million over a span of 10–15 years per drug developed and released into the market [78]. The overall drug-development process is highly inefficient due to the high failure rate (>75%) of the majority of drug candidates in costly clinical trials [79]. These considerations are apparently having an impact on the industry, as important players, such as Novartis and Glaxo-SmithKline, are devoting increasingly greater resources to computational modeling. While the informational aspects of modeling, such as gene sequencing, expression and the statistical inference of

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their effects, have dominated this discipline for the last decade, the importance of more mechanistic modeling, linking subcellular signaling with cellular phenotype and gross tissue response, is coming to light [80]. The results described in this review exemplify the power of this approach in isolating and explaining determinants underlying cancerous progression and therapeutic success. Simulation can provide a powerful hypothesis test-bed, examining and clarifying recurring patterns critical to the growth of cancer, shedding light on the most promising avenues towards successful anticancer therapies. This translates into an effective tool to improve drug development by preclinically screening drugs in silico, predicting response before a single subject has been recruited and assisting in the efficient designs of clinical tests. Therefore, it is probable that the rational development of anticancer drugs will depend heavily on the type of computational modeling described in this paper, and that no serious pharmaceutical contender will be without some level of in silico research.

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Papers of special note have been highlighted as:
• of interest
•• of considerable interest

•• Comprehensive review of mathematical models.
• First simulations of solid tumor morphology in the full nonlinear regime.


Connection between morphology and invasion (49) is demonstrated in vitro.


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