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How Should Cancer Models Be Constructed?

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Abstract

Choosing and optimizing treatment strategies for cancer requires capturing its complex dynamics sufficiently well for understanding but without being overwhelmed. Mathematical models are essential to achieve this understanding, and we discuss the challenge of choosing the right level of complexity to address the full range of tumor complexity from growth, the generation of tumor heterogeneity, and interactions within tumors and with treatments and the tumor microenvironment. We discuss the differences between conceptual and descriptive models, and compare the use of predator-prey models, evolutionary game theory, and dynamic precision medicine approaches in the face of uncertainty about mechanisms and parameter values. Although there is of course no one-size-fits-all approach, we conclude that broad and flexible thinking about cancer, based on combined modeling approaches, will play a key role in finding creative and improved treatments.

Keywords

mathematical oncology, cancer evolution, theoretical models, cancer ecology, cancer ecology and evolution

Introduction

Cancer is complex. The high-dimensional nonlinear dynamical system within each cell interacts through multiple chemical and physical pathways with hundreds of other cells both nearby and throughout the body. Especially in advanced adult-onset cancers, no two cancer cells are genetically identical, which shapes and further complicates their diverse interactions with each other and with host cells of many types. This presents great challenges both for cancer research and for clinical interventions. Treatment approaches seek to optimize patient survival by eliminating this entire complex system when possible and controlling it indefinitely otherwise. Any precision approach must address the high degree of heterogeneity of this disease both within and between patients.

This complexity makes traditional exploratory experimental approaches unfeasible with regard to both time and resources. We must turn to mathematical and computational methods to define and prioritize key hypotheses for experimental and clinical testing. Because cells and molecules can interact in counterintuitive ways, mathematics provides a rigorous tool to organize thinking about this unexpected complexity, and may suggest hypotheses or make predictions that are not obvious from intuition alone. As Einstein said: "Only theory can tell us what to measure and how to interpret it." Thus, we try to control cancer through quantitative understanding of individual patients, using models that capture the key components of cancer complexity while balancing useful accuracy with sufficient simplicity. But how can that balance be found?

We first point out that all mathematical models are based on a set of underlying assumptions. Often, biologists and mathematicians make assumptions without even realizing it, because they seem intuitive, obvious or conventional. The power of mathematical modeling lies in the ability to make those assumptions explicit, and to challenge them through

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comparison with experiments and predictions. It is important therefore to recognize as many of the assumptions in a model, biological or mathematical, as possible, and to explicitly state them so that they may be evaluated and challenged (see Box 1 for an overview of modeling).

There are two main approaches to mathematical modeling of biological processes: descriptive and conceptual. Conceptual models, which we discuss in detail next, are crucial for basic research and focus on relatively few biological mechanisms. In contrast, descriptive models, which are also very important for basic research, strive to take into account every available detail of the underlying biology, ideally providing a virtual replica of the biological system of interest. Descriptive models are appealing because one is less likely to miss an important player, and the hope is that simulations replicate the true biology closely. Such models have been used with some success to evaluate drug-drug interactions in preclinical drug analysis, with tools such as SimCyp,[1,2] or to predict drug metabolism during the drug development process (i.e., Gastroplus[3,4]). However, descriptive models are quite cumbersome, track hundreds or thousands of **state variables** with an equal number of equations, and impossible to analyze mathematically (Box 2 presents a glossary of key terms). Furthermore, descriptive models require even more **parameters** that can be unmeasurable or even unidentifiable, and these parameters potentially can be adjusted to fit almost any data. In this case, in contrast to the popular impression, fitting a model to experimental data does not confirm that the model correctly represents the underlying biology. For most descriptive models of cancer, the large number of parameters makes it computationally difficult to explore even a small fraction of possible parameter values.

Due to their size and complexity, these models may fail to pinpoint the key mechanisms to be targeted by therapy. For incompletely characterized systems, descriptive models require numerous assumptions that often lack experimental support, including the nearly invisible omission of unknown mechanisms or neglect of spatial interactions. Even in cases where there is experimental support, the results may be equally supportive of alternative models. Moreover, caution must be applied when extrapolating from laboratory or animal models to humans or between different tumor types. Even with human data, the accuracy of the experimental methods may affect the validity of the results. For example, a more accurate DNA sequencing method uncovered new findings concerning rare subclones.[5,6] Extrapolating parameters from laboratory or animal data is often necessary, but modelers must be aware of the risks. This applies to both conceptual and descriptive models, but is greater for descriptive models due to the number of details that the modeler must incorporate. In the many cases where new information is coming in every day, such as cancer-immune interactions, models must also change constantly, creating the potential for inconsistent results and challenges with version control. Finally, it is usually not possible, at least today, to collect the extensive data needed for descriptive models from patients, especially to support real time decision making.

Box 1: Introduction to Modeling

Mathematical models translate a set of biological assumptions into a quantitative form, generally in the form of equations or a computer simulation, that can then be analyzed and compared against biological data. If model predictions correspond to observations, then one can try to make additional predictions to guide further experiments; if predictions do not correspond to observations, then a gap in knowledge has been identified, and assumptions need to be revised. The assumptions encapsulate two key aspects: the choice of variables to include (**state variables)** and the rules by which those variables change and interact with each other. This paper contrasts descriptive models, which attempt to make the list of variables comprehensive, and conceptual models, which select key variables in advance.

For example, PKPD (pharmacokinetic/pharmacodynamic) models track the dynamics of a drug concentration through different compartments of the body (the PK part) and the effects of the drug on cells (the PD part). The PK component requires a choice of compartments, such as particular organ systems, along with rules for drug movement, degradation and excretion. The PD component tracks changes in cells or tissues, such as the altered protein levels or drug-dependent change in tumor growth, death, mutation, and metastasis, depending on dynamical rules of binding and reaction.

We focus on models based on explicit equations, although **agent-based models** or ABMs also span the range from conceptual to descriptive. A conceptual ABM includes only a small number of cell types and cell interactions in order to predict longer term dynamics with or without treatment.[40,41,91] A descriptive ABM seeks to include the full range of known cell types and their interactions.[92,93] A qualitative difference between equation and agent-based models lies in the fact that in equation-based models, we provide an explicit mathematical description of how variables interact with each other, and from that we calculate numerical solutions to these equations that describe the phenomena of interest. In ABMs, we define rules by which agents such as cells interact, and run computer simulations that keep track of each individual cell. ABMs allow incorporating spatial components into simulations, as well as a large degree of chance variation around average behavior. Output are analyzed as experimental data, and thus need to be replicated to obtain sufficient statistical power. As such, ABMs serve as an intermediate step between equation-based models and wet lab experiments.

Models can be **deterministic**, meaning that simulations or solutions provide the same output each time, describing the average behavior of the system, or **stochastic**, describing chance variations around average

(Continued)

Box 1. *(Continued)*

behavior, including some random number generation leading to different outputs in replicate runs. As with all modeling choices, there is a tradeoff between the simplicity and mathematical feasibility of deterministic models and the greater complexity and potential for realism of stochastic models. For small systems containing small numbers of agents (i.e. small groups of cells early in carcinogenesis), chance variations play a larger role than for larger systems.

Machine learning (ML) provides a fundamentally different quantitative approach to understanding data. Rather than describing specific mechanisms, ML algorithms use the data to find relationships between variables and identify correlations. There exist two key approaches within ML: supervised and unsupervised. Supervised learning involves using pre-defined categories into which data is sorted; the algorithm is trained on data for which an "answer key" is available, with the goal of then automatically sorting the data that do not have such an answer key into appropriate categories. An example of supervised ML in drug discovery would be assessment of drug properties of compounds that became successful drugs to predict which new compounds at pre-clinical stage may become successful drugs (i.e., sort data into "successful" and "unsuccessful" drug categories). Unsupervised ML in contrast involves finding such categories, into which data can be sorted. An example would be to find common properties of successful drugs within the "successful drug" category. Machine learning algorithms can be quite successful in, for instance, identifying patients who will respond best to particular treatment but as of now can do so only within the range of existing patients.

Mechanistic models have two important virtues. First, deviations from predictions can be linked to specific assumptions which can be corrected leading both to better prediction and greater understanding. Second, models that have been validated can be extrapolated beyond the range of existing data and make further testable predictions.

Given the tradeoffs between simplicity and complexity, the challenges of linking with data, and the difficulty of making and testing predictions, there is no single best choice for modeling. We argue that comparing a suite of models is the best strategy for making the choice of the most parsimonious model to capture data, coupled with critical tests that evaluate models using simulated data generated by other models. The ideal process works through constant interaction among modelers and empiricists.

Despite these challenges, descriptive models can provide information that allows more accurate parameter estimation in related conceptual models. For example, we might want to know all the ways a cell can become resistant to a drug to more accurately predict a single simple parameter of resistance kinetics. Descriptive models can be used to check the robustness of a conceptual model to changes in assumptions that address situations too complex to be represented in the more high-level conceptual model.

An alternative approach encodes the assumptions and parameters into a simulation, sometimes called an Agent-Based Model or ABM. These models can easily incorporate factors like space, intrinsic differences among individuals (agents), spatial interactions with the tumor microenvironment, as well as mutations of particular traits, such as the rate of resource consumption or likelihood of migration. However, the challenges of complexity, parameter estimation, and testing are similar to those of equation-based descriptive models. In the case of cancer, an ABM could consist of multiple cells, where each cell is an "agent." These cells would proliferate, mutate, and interact with each other according to pre-specified rules, with varying degrees of experimental support. ABMs are computationally expensive, and this problem rapidly worsens as the number of "agents" increases. However, sometimes relevant biology emerges only at very large numbers of agents, and these may be approximated more efficiently with other techniques that describe their average behavior as a group rather than tracking them individually. For instance, a recent ultra-deep sequencing study and accompanying theoretical analysis using a conceptual equation-based model revealed new phenomena concerning mutational burden and drug resistance occurring between 1 million and 1 billion or more cells, a finding that may have been very computationally expensive to find using ABMs.[5,6] As ABMs can serve as an "intermediate approach" between wet lab and mathematical models, it is important to remember that these computational experiments also have hidden assumptions that must be carefully considered.

In contrast to descriptive models, conceptual models attempt to capture simple mechanistic explanations for patterns in the data by incorporating basic biological knowledge.[7] Building these models relies on modelers to understand the system well enough to distill key mechanisms that may be driving its dynamics. These models help to infer basic biological mechanisms and ideally generate testable critical predictions.[7] Other conceptual models lend themselves to application of advanced methods, such as optimal control or branch and bound, that compute schedules and drug dosing subject to constraints such as toxicities to both normal and immune cells.[8-10] In our view, one main strength of conceptual models lies in forcing modelers to question every aspect of their understanding of the biology, crystallizing what we think we know, and testing this understanding against experimental observations to identify gaps in knowledge.

Although not strictly dynamical models in the sense we use here, machine learning (ML) algorithms can be used to understand the complexities of cancer, and can be applied to drug discovery and development.[11] Machine learning approaches do not aim to uncover mechanisms but allow for empirical sorting of data into categories that can then be analyzed more scrupulously, enabling one to generate additional hypotheses about

Box 2: Glossary

Agent-Based Model (ABM): Model that tracks the movement, state changes and interactions of individual agents (such as cells) according to a set of rules described in a computer program and studied through simulation. Sometimes called an individual-based model (IBM).

Conceptual Model: Relatively simple set of mathematical equations or computer models that seek to capture key behaviors of a system.

Degrees of Freedom: Number of values in a model that are free to vary.

Descriptive Model: Set of mathematical equations that seek to capture the full behavior of a system.

Deterministic Model: Mathematical model without chance elements, meaning that the solutions provide identical outputs for identical inputs, representing the average behavior of a system for a given set of initial conditions.

Exponential Growth: Model for growth of a tumor (or other entity) that is proportional to current size, and thus continues to accelerate and grow without bound.

Gompertz model: The Gompertz model describes growth of a tumor (or other entity) that approaches a limiting size called the carrying capacity, assuming that the growth rate slows exponentially with time.

Logistic model: Model for growth of a tumor (or other entity) that approaches a limiting size or carrying capacity, assuming that the growth rate slows linearly with time. Also called the Verhulst model.

Nash equilibrium: In game theory, a set of strategies where no single player can benefit by unilaterally changing its strategy, resulting in a stable unchanging state. In evolutionary game theory of cancer, the cancer burden and relative abundance of subclones would remain stable at such an equilibrium.

Parameter: A quantity describing a rate or other biological value that does not change during the course of an experiment, but might differ in different circumstances or patients.

State variable: A quantity that changes during the course of an experiment (in contrast with a parameter). The choice of state variables defines the scope of a model, with conceptual models using few state variables and descriptive models many.

Stochastic model: A model, in contrast to a deterministic model, where outputs differ in different runs due to the inclusion of some randomness, and can be described with a distribution rather than a single number i.e. both average behavior and variation around the average.

mechanisms that can then be tested experimentally and with mathematical or computational models.

To illustrate how to choose a modeling strategy, we lay out a series of approaches of increasing complexity, linked with data of increasing resolution, and with different strengths for developing mechanistic understanding or for guiding therapy. Our goal is not to provide detailed recommendations on specific choices, but a more high-level "conceptual" guide to think about the goals and complexity of models. We also do not attempt to comprehensively review the many different approaches to modeling. Such comprehensive reviews are available.[12-15] We critically evaluate several specific approaches as examples and propose new directions for integrating models with research data and treatment.

Examples of What Models Can Address

Tumor Growth Models

Historically, the first observable quantity was of course the tumor itself, and the simplest models focus on describing tumor growth dynamics. The general shape of tumor growth curves is obtained both from in vitro and in vivo studies, showing initial **exponential growth** that, after a time, either slows or saturates as the population reaches some limits; the dynamics are captured well by **logistic** or **Gompertz** models, although discussion continues as to which provides a better mechanistic explanation for the dynamics.[16,17] In pharmaceutical modeling, the Simeoni model appears to describe tumor growth in xenograft models well. This model predicts exponential growth in initial stages, followed by linear growth[18,19]; the saturation stage is often not reached in vivo and thus need not be included. While mouse models involve a single lesion that is permitted to grow until the animal must be euthanized for humane reasons (thus artificially tying survival with the growth of a single large lesion), in humans large lesions are often surgically removed or irradiated if present in isolation, and death in the metastatic phase is generally associated with a large number of (micro)metastases infiltrating vital organs. These micrometastases are likely still growing according to an exponential growth curve given their small size. Radiologic data from clinical trials also follows large lesions visible on CT, and these, like the large lesions in mouse models, may have different growth properties than the more numerous small lesions that likely have a larger influence on survival. They are also less likely to exhibit competitive dynamics between tumor cells because oxygen and nutrients can readily diffuse in.[6]

In formulating models of tumor growth, one must give careful thought to eventual clinical application. It may be that our most commonly available data sources, i.e. mouse models and radiology, point us in the wrong direction,[20] because, as outlined above, mouse models artificially tie survival to the size of single large lesion, and radiology can only quantify lesions above a certain size. Historically, failure of treatments in mouse models has portended failure in the clinic, but success in mouse models has often not translated to clinical success. Similarly, taking tumor shrinkage and growth of large lesions as endpoints has enabled some life extension; however, in many cases long term results remain elusive.

Drug Dynamics and PKPD Models Including Intratumoral Biodistribution

To include therapeutic interventions, growth models are often coupled with well-developed pharmacokinetic (PK) models that describe drug absorption, distribution, metabolism and elimination to establish a relationship between drug dose and tumor regression. Such PK models can be descriptive, taking into account various compartments in the body, such as physiologically based pharmacokinetic (PBPK) models,[21-23] or more conceptual, focusing primarily on the change in drug concentrations over time and its impact on tumor volume.[24] The relationship between drug concentration and its effects (pharmacodynamics, or PD) is established through coupling tumor cell growth and death rate with drug concentration,[18,19,25] and can contribute to the analysis used to choose human doses to be taken forward in clinical trials. In larger lesions, we may need to consider non-uniform drug distribution within the tumor mass, especially for large molecule therapeutics such as monoclonal antibodies. Cells within the diffusion range of nutrients in relation to capillaries but beyond the diffusion range of large molecule therapeutics may escape therapy. Equation-based models of such diffusion phenomena may improve optimal antibody dosing.[8,26-28]

Modeling Tumor Heterogeneity

In ecology, genetic differences among individuals are the raw material for evolution, and many other forms of heterogeneity affect population dynamics and individuals' interactions with other species and the environment. Such heterogeneity includes differences among individuals based on size, sex, age, mode of metabolism, spatial distribution or other important aspects of phenotype. Heterogeneity in cancer parallels its role and types in ecology. The first level of heterogeneity is generated both by genetic and long-term epigenetic changes that are largely irreversible and by reversible phenotypic plasticity. This cell heterogeneity is in turn shaped by ecological heterogeneity because selection operates differently in different environments.[29] For example, phenotypic plasticity allows melanoma cells with the bRAF V600E mutation to be highly sensitive to bRAF inhibition while colorectal cells with the same mutation are not.[30,31]

To study mutation, Beckman[7] described an approach to conceptual modeling of carcinogenesis and tumor growth termed focused quantitative modeling (FQM).[7] In FQM, conceptual modeling is focused on answering a single key question, and only details deemed relevant to that question are supplied; this approach has also been referred to as "fit for purpose" modeling. Generally, in complex biological systems, even these details may not be available, requiring reformulation of research questions and assessment of answers in terms of quantities that can be estimated, such as ratios relative to a reference value rather than absolute quantities. In some cases, this may be sufficient, i.e. when some quantities cancel out, revealing key parameters of the model. An example is a relatively simple model,[32] where the authors not only answered the key question about the mutator hypothesis (showing that carcinogenesis is indeed accelerated by genetic instability at the level of mutations or DNA rearrangements) but also predicted a number of phenomena, such as high mutational burden in cancers and intra-tumoral diversity over and above the mosaicism in normal tissues,[5] high prevalence of mutations that affect genome integrity,[33,34] lower mutational burden of tumors like retinoblastoma,[35] and convergent evolution of tumors.[7,36]

Bayesian modeling approaches have been used to support conceptual models based on complex experimental datasets. They compute the probability or likelihood that the observed data would have been obtained with a particular set of assumptions and parameter values for the model. The higher the likelihood, the more experimental support for the model. This can be used to compare the likelihood of different models. For example, Beckman and Loeb[32] postulated a mechanism of carcinogenesis in which normal cells successively acquired positively selected driver mutations that progressively increased cellular fitness, and subsequent growth and random mutation was "neutral," i.e. having little effect on fitness, analyzing a conceptual model using FQM. Sottoriva et al.[37] utilized an extensive multiparameter multi-lesion experimental dataset from colorectal cancer and a Bayesian approach to validate a similar conceptual model, which they termed the Big Bang model of tumor growth.

The concept of neutral evolution of cancer after initial driver selection was further validated using a combination of conceptual and Bayesian modeling applied to a public database of human tumors by Williams et al.,[38] and again by Loeb et al.[5] using an exceptionally accurate DNA sequencing technique and a related conceptual model. Notably, this new conceptual model recognized that a key assumption of prior models, the popular "infinite sites assumption,"[39] i.e. that a new mutation at a particular DNA base arises in one cell only at any given instant, would no longer hold when the total cancer contained over a million cells. This conceptual insight and the new experimental data in turn suggested a much greater level of mutational diversity in clinically diagnosable cancers of at least a billion cells than previously suspected, a phenomenon which would only increase with tumor burden during a patient's clinical course.[6] These phenomena were not anticipated by ABMs used to validate earlier Bayesian and conceptual models, as these ABMs had only 1 million cells.[38]

Many other forms of tumor heterogeneity can alter tumor evolution: metabolic heterogeneity,[40] where differences in glucose metabolism can contribute to immune evasion; heterogeneity with respect to resource supply,[41] where chronically elevated energy supply can lead to evolution of cell characteristics such as hyperproliferation and tissue invasion; phenotypic switching between proliferative and migratory states[42]; and heterogeneity with respect to fitness strategy.[43-46]

Tumor Microenvironment and Immune Interactions

The above models are predicated on the notion that cancer cells are the key determinants of tumor growth, which can arguably

be a reasonable assumption in immune deficient mice. However, it is increasingly recognized that cancer cells are not the only key players in tumor growth, and that tumor-stromal interactions may not be by-products of tumor growth and adaptation but sometimes its drivers. These drivers include cancer associated fibroblasts,[47] physical cues, such as pore size of the extracellular matrix,[48] or activated stroma in pancreatic cancer.[49] Moreover, stromal elements can alter the sensitivity of malignant cells to therapy. As shown in microfluidic co-culture, the presence of osteoblasts greatly reduces the sensitivity of myeloma cells to the proteasome inhibitor bortezomib.[50]

Evolution of immune evasion has been considered in several models, such as Bayer et al.[46] which looks at a public goods game between selfish and immunosuppressive cooperative cancer cells, predicting the impact of transient dynamics of therapy outcome. Wilkie and Hahnfeldt[51,52] model immune-induced dormancy, where cancer cells that have been suppressed by the immune system for longer may evolve greater resistance to immune-mediated T cell killing; model analysis reveals that a decline in immune cell recruitment is a stronger predictor of tumor escape from the immune system than decrease in cell kill rate, a hypothesis that can be evaluated experimentally and harnessed for therapeutic purposes. Both examples focus on how tumor-immune interactions evolve, which can give insights into when immune-based therapy could fail and when it may be more likely to succeed.

Furthermore, as the success of immunotherapy in many cancer types has revealed over the last decade, there is great benefit to targeting not only cancer cells themselves but also their natural "predators," cytotoxic immune cells.[53] Large descriptive models are being developed to capture the increasing knowledge about specifics of cancer-immune interactions,[54] but there arguably also exists a need for smaller conceptual models that may lend themselves to analysis and hypothesis testing. Such models may help answer questions that pertain to timing and scheduling of immunotherapy[55,56] as well as try to generate testable hypotheses underlying difference in response subject to changing order of therapy administration.[57-59] A descriptive multi-scale model may provide too many **degrees of freedom** to generate testable hypotheses, which may be less of an issue with a smaller conceptual model that can act as an experimental system that requires many fewer resources and less time to investigate compared to an experimental setup.

Examples of Specific Modeling Strategies

Predator-Prey Models

One possible starting point for studying tumor-immune interactions comes directly from ecology: predator-prey models that capture interactions between predator (immune cells) and prey (cancer cells). The analogy is not perfect, and not all assumptions that are characteristic of this class of models may be directly applicable to cancer-immune interactions. For instance, there is no direct conversion of prey biomass (cancer) into predator biomass (immune cells), and the prediction of oscillatory behavior is typically not observed in mature tumors. The presence of a larger population of prey does not make it easier for the predator in these systems. Nevertheless, modifications of this class of models serve as a starting point for developing minimal conceptual models for capturing various types of interactions that occur in larger ecological systems and evaluating their applicability to cancer, expanding our list of useful hypotheses and mechanisms that may then become targets for treatment. Examples include immune evasion through competition for shared nutrients between predator and prey,[60] study of tumor-promoting inflammation,[51] and immune (predator) suppression through downregulation of recognition mechanisms, such as antigen-presenting cells.[61] Theoretical studies of predator-prey systems in ecology through conceptual models can generate insights of where to look for targetable mechanisms in cancer-immune interactions.

Evolutionary Game Theory

Predator-prey models provide one example of an ecological interaction that proves a useful simplification of cancer. Evolutionary Game Theory examines this interaction along with competition and cooperation in an explicitly fitness maximizing framework. For instance, Gatenby and colleagues have developed an approach to managing emergence of therapeutic resistance called adaptive therapy that personalizes care based on an individual's response to therapy.[62] This model relies on a key assumption that the population of cancer cells consists of therapy-resistant cancer cells that compete with more sensitive cells that have higher fitness in the absence of treatment.[63] However, this assumption may not always hold, as cases of greater fitness of therapy-resistant cells, even in the absence of therapy, have been documented,[64,65] and thus generalizing the notion of fitness cost of resistance may be a risky default assumption. Costs of resistance are highly sensitive to environmental conditions like nutrient availability, and thus interact with other aspects of the ecology of the tumor. For example, as stated above, competitive interactions and reduced fitness for resistant cells, the fundamental assumptions behind adaptive therapy, are far less likely to apply to diffuse micrometastases that tend to be central to morbidity and mortality in patients, because nutrients diffuse readily into these micrometastases. Nonetheless, if these two key assumptions are granted, it can imply, in analogy with pest control,[66] that drug-sensitive cells can serve as competitors and thus control the emergence of resistant cells. Consequently, drug holidays may be recommended, because drug-sensitive cells are thought in this model to hold drug-resistant cells in check in a competitive equilibrium.

However, there are cases where the key role of competition may not hold. For instance, cooperative interactions have been shown in breast cancer[67] and quite extensively in pancreatic cancer, where a variety of different cell lines support both each other's growth and resistance to chemotherapy in a complex

web of cooperative interactions.[68] Models are essential to unravel the structure of the web of interactions. The treatment strategy for cooperative webs might favor reducing tumor heterogeneity to undermine positive feedback loops, in stark contrast to the maintenance of diversity that should inhibit tumors structured by competition. Questioning the underlying assumptions, which is necessitated by the modeling process, is thus critical for selection of a therapeutic approach. The same evolutionary principles apply in both cases, but play out qualitatively differently, and models need to be ecumenical and flexible to evaluate and respond to ever-changing realities. While applying evolutionary principles to managing population heterogeneity is undeniably critical in devising therapeutic regimens, it is important to evaluate the assumptions and corresponding predictions against experimental data.

Dynamic Precision Medicine (DPM)

Another applied approach to dealing with extreme genetic diversity and non-equilibrium dynamics of cancer during treatment is called dynamic precision medicine (DPM).[9] DPM introduced an evolutionary dynamic approach to multi-drug therapy, and, like adaptive therapy, is adaptable in a personalized fashion. It does not a priori assume a single model of competition or cooperation between cancer cells, although the underlying mathematics is flexible enough to allow for either of these phenomena to be introduced when justified by experimental or clinical data. Instead, the DPM approach focuses on estimated prevalence of various subclones and their respective drug sensitivity properties, net growth rates, and mutation rates, which determine the probability of emergence of variant cells that can be acted on by selection. That is, the dynamics are accepted "as is" without assumptions from other disciplines such as ecology, but with a simple default if no measurement can be made. Variation in mutation rates plays a key role in this approach. While cancer cells on average may have an increased mutation rate according to the mutator hypothesis,[32,69] the mutation rate may further vary between cancer cells within a single patient as they may have different random mutations in the many proteins necessary for genome maintenance, as shown in yeast[70-72] and in human cancers.[34] Beckman and Loeb (2017) term the cells and subclones that have these additional "mutator mutations" above and beyond those present in the bulk tumor as *hypermutators*,[73] and simulations show that they can influence predictions of what can constitute an "optimal therapy" even when they are present as rare cells.[9] Hypermutator cells can rapidly acquire simultaneous resistance to multiple elements of a combination therapy.

Rather than optimizing short term tumor size reduction, something that computer scientists would term a "greedy algorithm," the algorithm focuses on long term survival optimization by preventing future relapse (to quote hockey great Wayne Gretzsky: "Skate to where the puck is going, not where it has been.") Hypermutator cells are frequently a therapeutic priority: dynamics do not necessarily impose a constant rate of change for all the cells as with some other evolutionary models.[62,74,75] Simulations of DPM with over 3 million virtual patients suggest that application of DPM could double median survival and dramatically increase long term disease control rates.[9] Long-range planning of up to 5 years at a time further improves results, provided the plan is continuously adapted based on new data.[10] Such long-range planning with up to three drugs is computationally feasible in part because the model does not include all the known complexities of cancer but is instead a conceptual model. These ideas are not easily tested using conventional experimental approaches, and new techniques intended to facilitate experimental refinement and validation are under development.

DPM, like adaptive therapy, remains difficult to prove or refute for several reasons.[20] First, mouse models may not reflect the human on a variety of levels, especially tumor growth dynamics (exponential or Gompertzian) and the presence or absence of competitive dynamics between subclones. Mouse models have historically overpredicted efficacy in humans even for individual therapies. Second, it is very challenging and expensive to measure subclones directly in patients. Cell free DNA techniques lack the sensitivity required for DPM, where as few as one resistant hypermutator cell in 100,000 may ultimately change the optimal therapy significantly.[9] Cell-free DNA measurements do not allow one to determine which cells if any harbor resistance mutations to two elements of a therapeutic cocktail simultaneously, a key parameter for DPM. Circulating tumor cells can give this information, but the sensitivity of these measurements is even lower. Moreover, the resistance mutations are not identified for many important therapies, meaning actionable DNA changes are not presently known. We have not seen a validation of competitive dynamics in adaptive therapy based on direct serial measurements of subclones for example. Radiologic evidence for "competitive release" of resistant subclones is flawed in that it measures bulk tumor size and growth of minor subclones cannot be seen until they constitute a sufficient percentage of the bulk tumor. Thus, while increasing tumor size due to growth of a resistant subclone can be seen after many of the sensitive cells have been eradicated and the tumor size reaches a nadir, those cells may have been growing all along at the same rate as a minority in the bulk tumor without only a minor impact on the bulk measurements. The "competitive release," implying increased growth rate of resistant cells due to removal of sensitive cells (i.e. competitive dynamics) may simply be an unmasking of a steady growth rate of resistant cells that was occurring all along. Despite these difficulties, bioinformatic information about resistance mutations and the cost and sensitivity of cell free and single cell DNA sequencing technologies continue to improve.

Coping With Uncertainty

Models, both conceptual and descriptive, must cope with the challenge of uncertain, unmeasured, and even unmeasurable parameters. A focus on measuring or estimating kinetic parameters and rate constants can preclude using the model to

describe complex interactions, such as with the immune system. These challenges are addressed in part by explicitly including stochasticity in the model, which makes comparisons with data more direct because these models generate their own error distributions, but at the cost of making models more complex, slower to simulate and more difficult to analyze. Pharmacokinetic modeling relies heavily on estimating and predicting parameters that govern dynamics of drug in the body, because these values can dramatically affect dose and scheduling predictions. Thus, in order for a coupled PK-tumor-immune model to be practically useful, parameters, as well as variability in parameters associated with immune dynamics, typically need to be measured or estimated accurately, with appropriate translation between such parameters and processes from animal models to humans.

This requirement can occasionally be partially circumvented by a well-constructed empirical model, such as the one developed by Tran et al.,[56] where the authors develop a PKPD model aimed at finding the optimal dose-schedule relationship to maximize cancer cell kill while preserving anti-cancer immunity. In this model, only 2 of 5 variables can be measured experimentally; the relationship between other variables is essentially an educated guess. Nevertheless, this empirical model reproduced the available mouse experimental data with a single set of parameters, and even rediscovered data not used in fitting, suggesting that it is possible for a conceptual model to capture complex dynamics even when not all pieces can be measured experimentally.

Given the difficulty of translating parameters even between different patients, the challenge of generalizing across species or from lab to patient is fundamental. This gap needs to be filled to truly enable the use of conceptual modeling in preclinical development of immuno-oncology drugs and other complex questions such as evolutionary guidance of therapy. Some of the efforts from descriptive modeling of laboratory systems may provide estimates for conceptual models, that is, conceptual models may be linked to a variety of information sources in a modular fashion.[9,76,77]

More importantly, even fundamental assumptions about cell phenotype and other fundamental model assumptions often cannot be measured directly in vivo. What are the prevalences of sensitive and resistant subclones, as well as their relative fitness levels in the absence of treatment? How many changes are epigenetic and potentially reversible? High depth sequencing may answer these questions if the phenotype can be simply linked to a genotype but can be challenging when faced by copy number variants and extrachromosomal DNA, as well as epigenetic changes and other forms of rapid cellular plasticity. Is competition among cancer cells crucial? How much and for how long can cells alter behavior to adapt to a niche based on reversible phenotypic plasticity? Furthermore, as outlined above, while large lesions may dominate mouse and radiologic data, they are not necessarily the most pertinent when considering cancer as a systemic disease in human patients. Systemic markers such as prostate-specific antigens are useful,[78] but when the marker is a secreted protein regulated by a signaling pathway inhibited by one of the drugs, the likelihood that total marker secretion is proportional to cell number is suspect. The apparent sudden growth of resistant clones upon treatment of sensitive clones, termed "competitive release" is often cited as evidence for competitive dynamics.[78] However, this may be explained equally well by sensitivity limits for detecting low prevalence subclones, both by radiologic and sequencing methods, which upon treatment of the predominant clone, may suddenly increase in relative prevalence without change in absolute cell number, as outlined above for the radiologic case. Careful experiments need to be set up to discriminate between the two possible mechanisms, as they may have different implications for how a more optimal treatment would be designed. Positive clinical results for evolutionary-guided therapies remain confounded by other potential mechanisms driving them, including the effect of intermittent scheduling on the intratumoral vasculature,[79,80] reversible gene expression profiles affecting drug resistance of a single subclone,[81] or the immune system.[82-85] The uncertainty about correct model assumptions is termed uncertainty about "model topology." FQM attempts to do a sensitivity analysis across multiple model topologies. However, due to the large number of possible assumptions and topologies, such a survey cannot be comprehensive.

Despite these seeming shortcomings, modeling to estimate kinetic parameters and rate constants can be a powerful and perhaps indispensable tool in making predictions about therapy optimization. When considering targeted therapy in the clinical setting, the current precision medicine model, while simple, recognizes that optimal therapy will vary between individuals. We argue that models are necessary to include two fundamental and tightly linked aspects of cancer: 1) heterogeneity within patients both within and between tumors, and 2) evolutionary dynamics.

Currently, precision medicine assigns drugs to patients based on the tumor's predominant or consensus molecular properties, despite the fact no two tumor cells are genetically identical, frequently leading to emergence of therapeutic resistance. Indeed, deep sequencing of colorectal cancer provides clear evidence that in any tumor large enough to be clinically visible, at least one cell will be present that is resistant to any single agent, with simultaneous resistance to more than one non-cross resistant therapy being acquired as the total cancer burden increases in the patient.[5] Current precision medicine is thus essentially a static "lock and key model," where the patient is treated with the best "key" for the predominant cell "lock" until the tumor progresses, at which point the process is repeated. This approach results in eventual emergence of therapeutic resistance due to intrinsic tumor heterogeneity.

More broadly, while many insights can be obtained from ecology, it is important to be aware of differences between species ecology and cancer ecology as it presents in human patients, and specifically, the differences between species evolution and cancer evolution. First, we must take into account not only the role of selection but also of mutation, including the likelihood that the optimal mutation rate for tumor evolution

exceeds that of species evolution. A very high mutation rate in tumors was predicted[86] and subsequently confirmed using highly accurate high depth DNA sequencing.[5] It was shown to be 2-4 orders of magnitude greater than the apparent mutation rate of germ cells based on a variety of methods, including epidemiologic analysis of human cohorts.[87] Moreover, because any cell that contributes to cancer progression can invade normal tissues, a more realistic competition model would consider that the resources available to cancer cells are not fixed but are in fact continually increasing as the cancer spreads and exploits more of the host without necessarily having to compete with itself. Therefore, some of the key assumptions of evolutionary game theory that have been finding application in cancer biology,[88-90] such as establishment of a **Nash equilibrium**, may not be applicable for an invading cancer that through the metastatic process is effectively increasing the size of the metaphorical resource pie, or mathematically, is increasing its carrying capacity or limiting population size. Such a process is non-equilibrium in nature, putting into question the applicability of the notion of a Nash equilibrium in this context, which may be applicable to dynamics of large lesions at their carrying capacity but not to metastatic cancers driven by diffuse infiltration responsible for clinical morbidity and mortality.

In summary, conceptual models of cancer are important in both basic cancer research and in applied models of therapy. They can help formalize understanding of key mechanisms and assumptions of underlying biology, which in itself is a critical step prior to running scenario analysis in an attempt to find optimal therapeutic approaches that could increase the likelihood of long-term patient survival. Descriptive models of cancer can then provide information to, and therefore refine, conceptual models of therapy in a modular fashion. Most importantly, model complexity depends on the underlying question and the intended application. Conceptual models will often include additional detail in areas deemed central to the questions under investigation, while utilizing simpler assumptions in other areas. Parsimonious models that have the flexibility to adjust to emerging data in both populations and the individual under treatment may be preferred. There is no one "correct" way to construct mathematical models of cancer, but flexible conceptual models, when used appropriately, can be indispensable in driving both basic and applied research forward.

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