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Modeling genetic heterogeneity of drug response and resistance in cancer

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Abstract

Heterogeneity in tumors is recognized as a key contributor to drug resistance and spread of advanced disease, but deep characterization of genetic variation within tumors has only recently been quantifiable with the advancement of next generation sequencing and single cell technologies. These data have been essential in developing molecular models of how tumors develop, evolve, and respond to environmental changes, such as therapeutic intervention. A deeper understanding of tumor evolution has subsequently opened up new research efforts to develop mathematical models that account for evolutionary dynamics with the goal of predicting drug response and resistance in cancer. This study describes recent advances and limitations of how models of tumor evolution can impact treatment strategies for cancer patients.

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Introduction

Heterogeneity in cancer refers to the many ways that tumors can vary between and within patients [1]. Within an individual, intertumor heterogeneity describes the difference between spatially separated tumors, whereas intratumor heterogeneity is the variation seen within an individual tumor. Heterogeneity is observed at the genetic and phenotypic level, where genetic heterogeneity captures the diversity of genetic alterations in tumor cells (e.g., point mutations, indels, structural variants), and phenotypic heterogeneity refers to how cells from the same genetic background can present different phenotypic states (e.g., morphology, differentiation, biomarker expression, the likelihood of therapeutic response). For the purpose of modeling therapeutic response, the study authors draw a distinction in that selection of cells in response to treatment occurs at the phenotypic level (e.g., rapidly growing cells are killed by chemotherapeutic agents), where tumor evolution occurs at the genetic level with clonally expanding populations passing on somatic alterations (e.g., loss of TP53 allowing damaged cells to expand). For a complementary opinion of how phenotypic heterogeneity affects drug treatment and response, see Heiser and Meyer [56]. Here, we will focus on how mathematical models can account for genetic heterogeneity in predicting therapeutic response.

Mathematical modeling has long been used as a way to capture the essential elements and interactions of a biological system [2–6]. The goal is to link the dependent variables or outputs (e.g., growth, invasion, response to treatment) via mathematical equations to the inputs of the model (e.g., transcript level, protein abundance). If properly constructed, the model should faithfully recapitulate the biological system. In turn, the model can be used to simulate how perturbations to the system will affect outputs. Here, we will explore different mathematical modeling approaches that account for tumor heterogeneity and how different theories of tumor evolution can be used to design treatment regimens.

Measuring genetic heterogeneity

Our ability to capture the resolution needed to characterize *genetic* tumor heterogeneity has been driven by advances in next-generation sequencing technology, which can produce output for tens of billions of nucleotides, and our ability to isolate different genetic elements to be sequenced (e.g., exome capture, chromatin immunoprecipitation). Bulk RNA and DNA sequencing, where a tumor resection/biopsy consists of hundreds to millions of cells is sequenced in aggregate, has been the dominant form of sequencing used to date. Bulk sequencing has been a boon to cancer research and has been used to characterize the genomic landscape of all major cancer types [7]; however, bulk sequencing has limitations. Although there are computational methods to successfully "deconvolute" different immune and other stromal cell populations from the sample [8], along with estimating tumor clonality and ploidy [9–11], subpopulations of cancer cells are simply missed because they are at a lower fraction of the tumor than can be detected. Targeted, deep sequencing methods have been developed to resolve cells at a 1/10,000 fraction of the population, but these approaches can only be applied to a limited number of predefined genomic regions [12]. More recently, technologies have been developed to generate RNA and DNA sequencing at single-cell resolution [13]. These methods provide the single-cell measurements that are needed to truly identify rare subpopulations of cells, but these technologies are very costly, present new statistical and bioinformatic challenges [14] and remain almost exclusively used in the research setting, with limited clinical applications [15].

Although technological advancements in measuring tumor -omics are valuable, even more fundamental experimental factors can limit our ability to properly sample tumors spatially and temporally. A needle biopsy, for example, will only provide a small cross-section of a solid tumor, which can miss entire subpopulations of cells because of sampling bias [14]. Multiregion sequencing is an approach to sample independent regions of the tumor to increase spatial resolution and reduce bias. However, multiple biopsies of a tumor (or multiple tumors) in a person is highly invasive and impractical for tumors in sensitive regions, such as the brain. Temporal resolution of genetic heterogeneity can also be a challenge. Serial biopsies of solid tumors during or after treatment can be highly invasive and cause more harm to patients, yet knowing how subpopulations of cancer cells change over time is important for adjusting treatments to how the tumor evolves. Liquid biopsies have been proposed as an approach to serially sample tumor content from a simple blood draw. It has been reported that over 50% of mutations found in primary and metastatic tumors can be identified by sequencing circulating tumor cells [16–18]. These results are promising and liquid biopsies could potentially be used to identify when different subpopulations arise in the tumor during treatment, yet it remains unclear what the relationship is between the growth of subpopulations in tumors and when these subpopulations can be detected through circulating tumor cells.

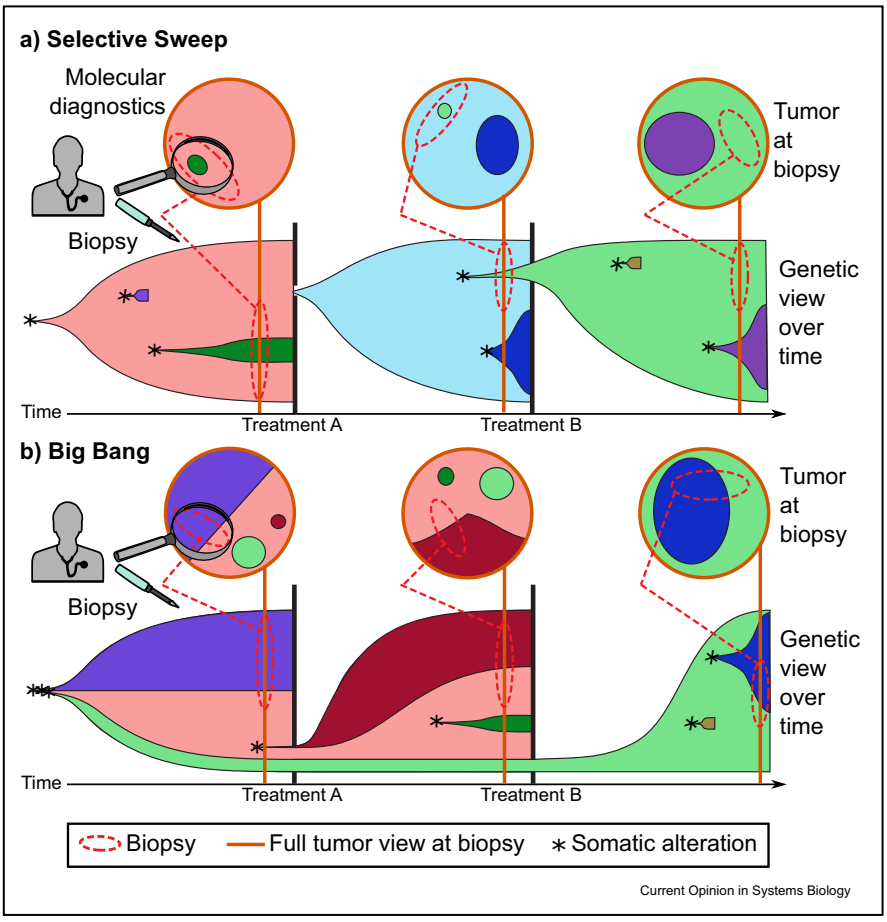
Models of genetic heterogeneity

To develop rationally designed and molecularly targeted treatment strategies, we must understand *when* genetic heterogeneity develops in tumors and how tumors evolve under the selective pressures of drug treatment. If, for example, the tumor evolves from a single clone and gains somatic alterations in a sequential manner through growth and in response to treatment, then this sets up a constant arms race between the patient's cancer and the oncologist where treatments select for certain genotypes. If, on the other hand, the actionable somatic alterations are present from tumor initiation, then it would stand to reason that the full array of alterations can be identified and a treatment strategy can be designed to hit the actionable targets or treatment can be adjusted based on which subpopulation is more prevalent.

A variety of models that describe the evolution of tumors and capture the timing of when somatic alterations arise have been presented [19–21]. Here, we will focus on two prevalent theories of tumor evolution. First, the selective sweep model, posits that cells arise in the tumor because they have a selective growth advantage and the subsequent progeny expand to sweep across the population. These cells will expand until another genetic alteration arises that provides a new selective growth advantage. The growth advantage is specific to the local tumor environment and if that environment changes, with the treatment of a drug, for example, a different cell population will then have the growth advantage [22]. More recently, an alternative model of tumor evolution has been proposed, where the mutations central to progression occur very early in tumor development. This "Big Bang" model of tumor evolution suggests that tumors are genetically heterogeneous from initiation and subsequent genetic alterations are modifications that build on the original ancestral cancer-driving alterations [23].

The selective sweep model suggests that a bottleneck event, such as a drug treatment, would select for existing subclones and potentially induce novel mutations that have a fitness advantage in the presence of treatment. In the context of precision oncology, and illustrated in Figure 1A, a tumor biopsy, which will be biased and sample only a subsection of the tumor, is collected to generate a molecular diagnostic report. A treatment is then selected based on the molecular features of the biopsy. This treatment then selects for the resistant populations that allows the tumor to bypass treatment and resume growth. Under this

Figure 1



Two models of tumor evolution under selective treatment pressure. A precision oncology approach where a tumor biopsy (red dashed ovals) is taken and a molecular diagnostic report is generated to select treatments based on the limited view to the overall disease (large red circle). (**a**) The selective sweep model allows for genetic alterations (*) to arise, which present the cells with a growth advantage and these subpopulations sweep through the population. Under treatment, genetic alterations can arise because of the drug directly, or through selection for a pre-existing subclone. (**b**) In the Big Bang model, driving alterations are present when the tumor arises with resistant populations being present at low frequencies until the drug treatment selects for that pre-existing population.

model, subsequent treatments would select for subclones in the population that are resistant to the treatment. This model suggests that the tumor is constantly adapting to treatment and new subclones will arise that provide a growth advantage over other cells under treatment. Molecular diagnostic reports must be run to identify the new genetic alterations and knowing the genetic make-up of the ancestral tumor will not aide in developing a mathematical model for treatment.

The Big Bang model offers an alternative view of tumor evolution. Under this model, tumor driving alterations and treatment resistance clones arise at or near tumor initiation, implying that resistant cell subpopulations are present from the beginning and it is only through selective treatment pressure that these cells are given the opportunity to expand. Evidence for this model has recently found in colorectal cancer [23] and supported in other cancer types [24–26]. Applying the same treatment paradigm (Figure 1B), a tumor biopsy is collected, molecular diagnostic generated, and treatment selected. Under the Big Bang model, although cells can acquire mutations over time, subpopulations of cells that will arise after treatment are present from tumor initiation and treatment only selects for pre-existing populations. This model suggests that all potentially therapeutically targetable populations of tumor cells are present when the tumor is first identified in the clinic. Therefore, if we know the complete genetic content of the ancestral tumor, we can design treatment strategies that will target the appropriate cell lineages and potentially avoid selecting for treatment resistance. This study describes a clinical trial in

prostate cancer by Zhang et al. [27] that successfully applies these concepts in the following section.

Mathematical models of treatment response that incorporate genetic heterogeneity

A major ambition of cancer systems biology is to develop mathematical models that can predict long-term treatment response for patients. To faithfully recapitulate tumor development and response to treatment, these models must consider genetic heterogeneity and tumor evolution [28,29]. Here, the study authors focus on the recent efforts that apply principles of game theory as a framework to capture genetic heterogeneity and predict corresponding drug response [30,31].

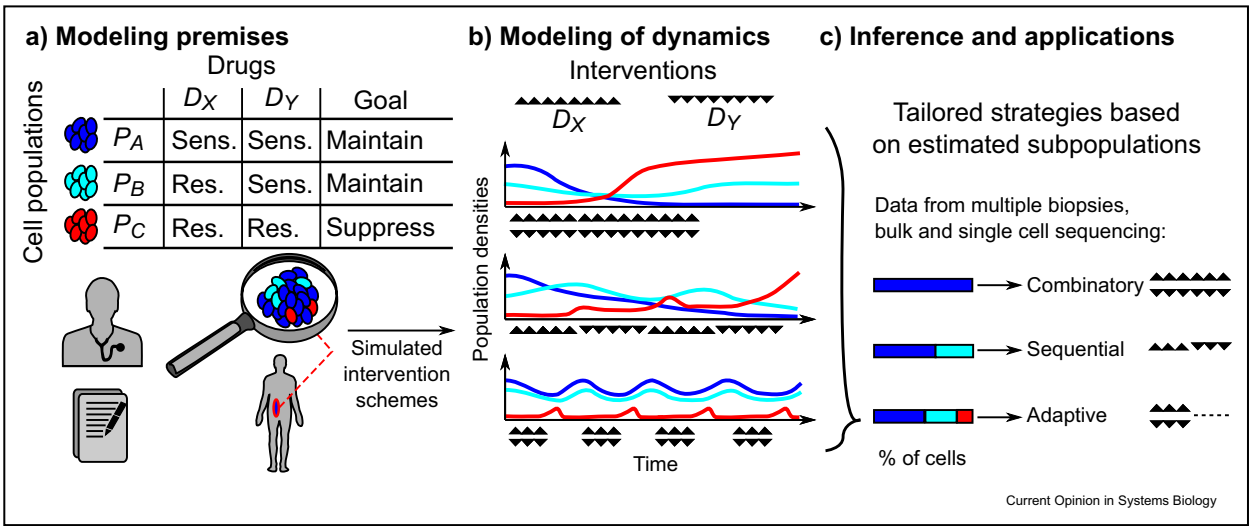
Game theory uses mathematical models to describe the interactions and strategic responses between rational decision-making entities [32]. This approach has been used to model cooperation or competition among cancer cells [30]. Of course, cancer cells are not making rational decisions, but respond according to deterministic cellular mechanisms [31]. Nevertheless, an oncologist will make rational decisions based on the fullest set of information about the patient's cancer and the cells comprising tumors will respond accordingly. Knowing how different cells will respond to treatment and knowing the cellular composition of tumors will allow the oncologist to make the best treatment decisions. A key assumption is that genetic alterations arising to exhibit a complex trait, such as resistance to a treatment, comes at some fitness cost to the cell. Tumor cells are competing with each other for local resources and a genetic alteration that leads to treatment resistance is likely to present a growth disadvantage under nontreatment, neutral conditions, but an advantage under the selective pressure of drug treatment. There are always tradeoffs in carrying different genetic alterations and it is the environment combined with exogenous stimulus that determine which genetic alterations are more fit. Applying game theory principles to simulate population dynamics requires assumptions about the types of subclonal populations, their interactions, the microenvironment [33], time and space [34], and the initial relative quantities of key subpopulations [30]. Careful formulation of these assumptions is essential, as some are counter-intuitive, such as the observation that the immune system promotes tumor growth under certain circumstances [35] and that optimal drug combinations do not necessarily hit their corresponding target subpopulations in the sample directly [34].

The appeal of game theory for cancer treatment lies in the asymmetry of the game from the perspective of the oncologist, who has the opportunity to intervene with treatments that changes the tumor environment. The oncologist can make treatment decisions based on prior knowledge and the information measured from a patient's cancer (Figure 2A). Without knowing the genomic landscape of the patient's cancer, the oncologist is limited to their prior medical training and the advantage is tilted only slightly in favor of the oncologist. However, with a more complete picture of the genetic composition of the tumor, along with an understanding on how mechanisms of resistance arise, the chances are increased that the oncologist will choose a strategy that will more effectively treat the cancer. This information can be used to build mathematical models, and then run simulations to evaluate treatment strategies *in silico* (Figure 2B) to identify the most effective treatment strategy given the composition of the tumor (Figure 2C). In the ideal scenario, the oncologist would have perfect knowledge of every cell in the tumor and surrounding stroma, and have a complete understanding of all mechanisms of treatment response and resistance. Although this scenario is clearly aspirational, as we described in the previous section, technological advances are allowing researchers to measure molecular features of tumor cells at an even increasingly fine resolution [36–38]. Major efforts, such as the Human Tumor Atlas are generating robust, detailed molecular measurements across an array of tumor types [39].

As researchers gain functional knowledge of tumor development and treatment response, the advantage increasingly shifts to the oncologist. We see examples of such insights impacting clinical practice today. A recent and successful clinical application of applying game theory principles can be found in an adaptive clinical trial for treatment of metastatic, castration-resistant prostate cancer. Adaptive or flexible clinical trials allow for modification of treatments based on patient response [40]. Zhang et al. [27] designed a treatment schedule based on a mathematical model of how three proposed prostate cancer cell subpopulations grow and interact with each other under androgen stimulation and deprivation (i.e., treatment with abiratirone). The authors were able to simulate different treatment scenarios using prostate specific antigen (PSA) levels as a proxy for tumor growth/response. They arrived at a treatment schedule where a patient was given abiraterone until PSA levels drop to 50% of the patient's PSA level before treatment. The patient was taken off abiraterone until their PSA levels rose again. The results of this clinical trial showed that one of the 11 patients in the adaptive arm of the trial progressed, whereas 14 of 16 patients progressed using standard of care treatment [27].

The work by Zhang et al. [27] represents an alternative approach to treating certain cancers as a chronic disease, rather than with the objective of eliminating the entirety of a patient's cancer. Prostate cancer is a multifocal disease and the heterogeneity it displays might

Figure 2



Overview into population dynamics driven modeling. Modeling several subpopulations and their dynamics allows one to simulate effects arising from genotypic variation across different treatment scenarios, including different drug targets, dosage, and scheduling. (**a**) In this example, there are three cell populations that vary in their response to two drugs (D_X and D_Y). The clinical aim is to suppress the growth of population P_C, while maintaining P_A and P_B. (**b**) Dynamic models can be used to simulate the impact on the different cell populations in response to potential intervention strategies. (**c**) Based on measurements of tumor heterogeneity and subpopulation composition from a patient's tumor, the most effective treatment strategy can be identified. In this example, an adaptive treatment strategy, where the drugs are adjusted based on how the subpopulations change over time would accomplish the objective to suppress P_C and maintain P_A and P_B.

preclude it from being completely eliminated. PSA levels are also an easily measured biomarker of disease progression, so prostate cancer is a particularly appealing cancer for this approach. Similarly, cancers that have advanced to a point where rational therapeutic efforts to cure the disease will likely not be successful are also potential candidates for this approach. Although speculative, it is likely that for more heterogenous cancers with many more subpopulations to model and target, the shift to treating it as a chronic disease could be the better approach. A major caveat to Zhang et al. [27] is that PSA levels are a proxy for the three-cell-type model. The model itself cannot be directly tested because it would require frequent, invasive sampling of a patient tumors, which would cause unnecessary harm to the patient. Despite these caveats, the results presented by Zhang et al. [27] demonstrate that alternative treatment strategies can be based on mathematical models with great success.

Future challenges

The underlying premise of precision oncology is that molecular profiling of one's cancer holds the key to designing the most effective treatment. The data generated from bulk, multiregional, single cell and deep, targeted sequencing suggest that subpopulations of cancer driving and treatment resistance cells are in fact present from the early stages of cancer development [14,23–26]. These findings are encouraging from the perspective that if we are able to characterize the genetic content of a tumor, then we will have the necessary information to design highly effective treatment strategies. However, current clinical practice, for example, in sampling the tumors, has not yet fully reached this point. Here, the study authors discuss some of the major challenges in adopting such approaches to practical cancer treatment.

Although there are tremendous success stories [41–43], current molecular diagnostics reveal actionable targets in a limited number of patients [44,45], and even then, the cancer often develops resistance to treatment. Current molecular diagnostic tests simply do not have the resolution to properly sample genetic heterogeneity from a tumor biopsy [46]. In addition, a biopsy is an incomplete snapshot of an evolving tumor, thus the ancestral state, spatial distribution of heterogeneity, and trajectory of tumor development is unknown. Each of these points presents major challenges for technologies aimed at measuring genetic heterogeneity.

Major international, collaborative efforts (e.g., TCGA, ICGC) have generated petabytes of bulk sequencing data across tens of thousands of tumors. A technical challenge that can be addressed today is the harmonization of existing, large-scale -omics data sets and associating these data sets with clinical outcomes from patient medical records. A great deal of knowledge can be gained from studying multiple independent patient cohorts [47–49], yet most cancer types do not have a

comprehensive and harmonized set of data to derive novel insights and test new hypotheses. There is a treasure trove of cancer -omic data already generated and a concerted effort to harmonize these data sets would provide some of the data necessary to develop more robust mathematical models of tumor evolution and treatment response and we can begin to address some fundamental questions. For example, it remains an open question whether bulk sequencing is sufficient to infer ancestral states, subclonal selection, and evolutionary dynamics in the context of treatment [50–52].

Other challenges will require the generation of new data. Deep -omic profiling of even a few patients can reveal a great deal about genetic heterogeneity and tumor development. Because of undersampling of intratumoral heterogeneity [53], sampling multiple tumor foci and also serially sampling tumors during treatment are needed before, during, and after treatment. Although a challenge to collect, these data will be critical for not only testing mathematical models, but also being able to adjust the model based on a patient's response to treatment [4]. There are fascinating possibilities in applying mathematical modeling to immunotherapies, considering the interactions with tumor, stromal, and immune cells [54,55]; samples are now being collected and the associated data being generated to address this challenge.

Finally, we must get to the point where clinical trials will be open to incorporating mathematical models to aide in treatment decisions. The work by Zhang et al. [27] is an excellent demonstration of what can be gained by incorporating treatment strategies developed from mathematical models into clinical trials. Future work should continue on this trajectory with the goal of improving patient survival and quality of life. As our knowledge improves, so will the models of treatment resistance, and subsequently how we treat patients. The studies presented here represent the start of novel approaches to treat cancer and we foresee mathematical modeling playing major roles in future clinical applications.

Conflict of interest statement

Nothing declared.

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* of special interest
** of outstanding interest

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