Minireview

New tools for cancer chemotherapy: computational assistance for tailoring treatments

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Abstract

Computational models of cancer chemotherapy have the potential to streamline clinical trial design, contribute to the design of rational, tailored treatments, and facilitate our understanding of experimental results. Mechanistic models based on functional data from tumor biopsies will enable physicians to predict response to treatment for a specific patient, in contrast to statistical models in which the probability of response for a given patient may differ substantially from the population average. While microarray analyses of gene expression also show promise for guiding individualized treatments, it may be difficult to link statistical mining of microarray data with mechanistic, tailored treatments. Furthermore, gene expression does not identify how drugs should be scheduled. This review summarizes mechanistic mathematical models developed to improve the design of chemotherapy regimens. Mechanistic models that incorporate both genetic resistance and cell cycle-mediated resistance during treatment with multiple drugs will be most useful in designing treatment regimens tailored for individuals. Because there are already a number of papers that address the applications of microarray technology, we will limit our discussion to the contrasts between mechanistic computational models and microarray technology, and how these two approaches may complement one another. (Mol Cancer Ther. 2003;2:1079–1084)

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Introduction

A New Strategic View

New anticancer treatment strategies must weave together the following aspects: choices of drug combinations that are tailored for a given patient; early treatment with cytostatic drugs applied concurrently with cytotoxics; and mechanistic modeling of cell kill to facilitate the predictive optimization of individualized treatment regimens. Mechanistic modeling serves as a tool that incorporates patient-specific cell-kinetic parameters, such as proliferative and apoptotic indices, enabling physicians to predict heterogeneous outcomes across patients. Otherwise, physicians usually regard heterogeneity as noise that dilutes the power of clinical trials. An alternative avenue of active anticancer research is microarray technology. While microarrays show great promise for identifying a suite of mutations characterizing a given tumor, which ultimately may help to guide the choice of treatments, this technology is in the exploratory stage of data mining. Although there are difficulties in collecting and stably preserving RNA and achieving adequate sample sizes to differentiate truly significant genes from the substantial noise present in microarray data, impressive advances have been made in the development of statistical methodology (1, 2).

Countering Drug Resistance

Genetic resistance and kinetic (cell cycle-mediated) resistance both influence outcome (3). While genetic resistance requires treatment with a new, non-cross-resistant drug, kinetic resistance to a cell cycle phase-specific drug is reversible when a cell enters the susceptible phase of the cell cycle. Consequently, cell cycle kinetics of tumor and normal cells affect response rates and host toxicity, respectively. Empirically, aspects of cell kinetics, for example, the quiescent fraction, the S-phase fraction, and apoptotic rates, have a large influence on prognosis and response to treatment (4–6). Moreover, individual variation in cell kinetics can be substantial: cell cycle times in tumors range from 30 to 60 h; apoptotic indices between 0.1% and 4%; proliferative indices from 1% to 70%; and S-phase fractions from 1% to 40% (7–9).

Computational Guidance

The complex interactions of cell kinetics, pharmacodynamics, pharmacokinetics, and drug scheduling can best be understood and predicted with the aid of a model (10–12). Mechanistic models generate testable predictions and identify treatments most likely to improve outcome, and thus help to limit the number of experiments to be performed. Finally, formalizing a hypothesis into a computational model that is consistent with many laboratory

and clinical trial results encourages conceptual thinking (13). Although some computational approaches to allow tailoring of dosing to individual patients are currently used in the clinic, they are not mechanistic, and so they will not be described in this review. However, because these methods are already in use, their results may be helpful for parameter estimation and guiding the application of the models discussed in this review.

Although even the most complex mathematical models simplify the actual process being modeled, they serve as invaluable tools to investigate how changes in basic assumptions are likely to affect outcomes. Evaluation of models demands caution, however, as many make too many simplifications and omit key processes, such as genetic drug resistance or cell cycle-mediated resistance.

Statistical models describe data, but fail to generate predictions. Accordingly, one cannot advise a regimen tailored for an individual patient based on statistical models. As Goldie (14) observes, ''In rare instances, the best average treatment may be the poorest option for a particular patient.''

Microarrays: Promising, but Patience Needed

Currently, analyses of microarray data fit in the statistical model category, because methods of both supervised (*e.g.*, neural net) and unsupervised (clustering) learning for analyzing microarray data do not identify mechanisms or pathways, but rather statistical associations that are obscured by noise from processes such as nonspecific hybridization. High costs per sample for many commercially available gene chips (*e.g.*, $\$300 - \$500$) and nonreusability mean that sample sizes are small. This, combined with the fact that one is calculating thousands of statistics (gene presence or absence) per sample, mean that one risks a high false-positive identification of genes as present. Statistical methods are being developed to deal with these and other challenges such as data normalization and choosing an appropriate clustering method (2). For example, analyses based on clustered groups of genes, rather than single genes, to interpret biological activity may allay some of the problems of multiple testing and false-positive results. Standards for recording and reporting microarray data also must be agreed on and widely followed (15). Research that will further an accurate physical understanding of sequence-specific hybridization and fluorophore binding will also improve microarray analysis (16).

In addition, microarray experiments require approximately 100 mm^3 of tissue to extract 10–40 μg of high-quality RNA (1). The tissue should be snap-frozen within 30 min of resection and stored at −80°C or lower to prevent RNA degradation. Changes in some mRNAs have been measured even a few minutes after surgical disturbance and devascularization (17). Such stringent requirements for RNA currently limit the feasibility of using microarray data as a matter of course in the clinic.

Microarrays allow us to evaluate the expression level or presence/absence of thousands of genes simultaneously. Thus, their current value is as a data-mining tool. Adequate models of gene regulatory networks have yet to be developed, and it may take some time before *mechanistic*, predictive models based on microarray experiments can be used in the clinic (18, 19). Perhaps a more immediate benefit of microarray technology will be in prospectively identifying tumors resistant to particular drugs, allowing alternatives to be used before patients are needlessly exposed to toxic drugs (20), or in classifying tumors as to type or level of aggressiveness (21, 22).

In contrast, computational models already exist (10, 12). Kinetic model input can be obtained rapidly, accurately, and inexpensively through assays/kits for measuring such features as proliferative fractions, S-phase fractions, cell cycle times, and apoptotic indices from tumor biopsies (5, 23), and clinical oncologists often request this information.

Microarrays and mechanistic computational models may be applied synergistically, so that they complement one another. For example, key genetic pathways can be indicated by microarray analyses to be involved in pathogenesis or drug response. Then detailed computational models of those pathways can be constructed, and *in silico* experiments performed to predict the effects of specific changes in gene expression, or combinations of such changes. The mechanistic models can be used to design specific laboratory experiments that will discriminate between alternative hypotheses regarding gene interactions/regulation. In this way, the advantages of microarrays in pointing to genes or clusters of genes that are involved in specific pathways can complement the advantages of mechanistic computational models for understanding cell cycle-specific effects of drugs, and together both approaches may be used to optimize drug scheduling and drug combinations for a given individual's genetic profile.

This review will briefly summarize some of the mechanistic computational models for cancer chemotherapy. Here it is only possible to cover a small subset of the many models in this field. Most of these models have investigated the impact of drug scheduling or dosing of a single drug. Only a few have modeled treatment with multiple drugs. Most of these models make too many simplifications to be used in the clinic for tailoring chemotherapy regimes. To our knowledge, only two models have incorporated sufficient complexity to potentially be useful in the clinic for optimizing and individualizing cancer chemotherapy regimens. These two models include kinetic drug resistance, the evolution of genetic drug resistance, application of multiple drugs, and treatment with cytostatics (10, 12).

Computational Models

General Issues

Which Toxicity Should One Choose? Initially it may seem advantageous to *simultaneously* consider toxicity and tumor cell kill in a single optimization model. This is the approach of optimal control and resonance effect models, which are discussed below. A disadvantage to this simultaneous optimization, however, is that toxicity to only one cell population, usually blood stem cells, is modeled. Actually, neurological, cardiac, gastrointestinal, and/or other toxicities may limit treatment. These may occur

simultaneously as disparate functions of schedule and dose (*e.g.*, AUC, peak drug concentration, time above a threshold concentration, dosing rate, cumulative dose, etc.). All factors cannot be optimized simultaneously. Thus, treatment effects on the tumor should be modeled separately from, not simultaneously with, host toxicity. This allows physicians to evaluate the balance of toxicity and tumor control independently in a given patient who faces unique health concerns and objectives (*i.e.*, cure or palliation).

Catch 22: Scheduling for Genetic *versus* Kinetic Resistance. Many models using various mathematical approaches predict that continuous infusion is more effective than short bolus pulses, particularly of cell cycle phase-specific drugs (12, 24–26). Continuous infusion addresses kinetic resistance by exposing more cells to the drug while those cells are in the sensitive phase of the cell cycle, and minimizes the time for tumor regrowth between treatments by prolonging drug exposure. Many clinical trials found that continuous infusion of cell cycle phase-specific drugs increases response rates or survival (27, 28). Some models suggest that the schedule modification of prolonged drug exposure is more effective than dose escalation, even if genetically resistant cells are present (12, 29). Clinical trial results also support this prediction (30, 31).

Prolonged continuous infusion, however, may facilitate the evolution of drug resistance by gradual processes such as gene amplification (32). A model of this double bind, that is, of contrasting pressures of kinetic *versus* genetic resistances, predicted that fewer, higher dose-rate, short-term infusions for cell cycle-nonspecific drugs or many, lower dose-rate, long-term infusions for cell cycle phase-specific drugs may maximize the chances of cure (33).

Each of the rates of apoptosis and cell division, not just the net balance of the two (*i.e.*, the ''growth rate'' parameter in many models), must be included in models of cancer chemotherapy. Both of these processes influence the response to cell cycle phase-specific chemotherapy and the rate of evolution of drug resistance. A tumor with a high proliferative fraction and a high apoptotic index may respond better to cell cycle phase-specific drugs, but may also evolve drug resistance sooner, than a tumor with the same net growth rate but with a low proliferative fraction and a low apoptotic index. Clinical trial results are consistent with this logic: patients who have higher proliferative indices respond better to cell cycle-specific cytotoxic chemotherapy, but also have higher recurrence rates and shorter relapse-free survival (6). Prognoses as a function of apoptotic rates are more complex (23), and it is likely that complex models that discriminate cell division and cell death, not only the net balance of the two, are needed to make quantitative predictions based on the interaction of apoptotic and proliferative rates for an individual patient.

Simple Models: Single Drug, Limited (or No) Consideration of Resistance

Dose Response and Scheduling. Commonly, the Hill model (34) is used to describe dose response curves. However, it is a statistical model that is neither mechanistic nor predictive, and provides no insight into how cell cycle dynamics or exposure time affects the shape of the dose response (*e.g.*, slope and saturation point). Several researchers developed mechanistic models of exposure time-dependent dose response after a single exposure (as in cell culture) for cell cycle-specific and cell cycle-nonspecific drugs (11, 35, 36). They linked pharmacokinetic models of drug distribution with simple pharmacodynamic models distinguishing tumor cells as proliferative (and therefore drug sensitive) or non-proliferative (and therefore kinetically drug resistant). These models accurately fit *in vitro* and *in vivo* data, and highlight the importance of considering changes in drug concentration over time (pharmacokinetics) and the proliferative fraction of the target cell population (pharmacodynamics).

Many models investigate scheduling and/or dosing of multiple exposures of a single drug, as occurs in treating patients. Norton and Simon (37) modeled kinetic resistance to a cell cycle-specific drug but ignored genetic (biochemical) resistance evolution. They suggested that moderate early doses followed by later dose intensification would kill more tumor cells and assure a higher chance of cure than early intensification or constant doses. Coldman and Murray (38) took the opposite approach, modeling genetic drug resistance but not kinetic resistance. Their models predicted the opposite strategy, that dose intense regimens of early chemotherapy cycles would be superior because they would reduce the likelihood of the evolution of drug resistance and thus increase the probability of cure.

In fact, treatment designs must combat both genetic and kinetic resistances (39). Nonetheless, most models incorporate only one of these aspects. Many optimal control models account for genetic resistance and host toxicity but not cell cycle phase-specific effects of drugs (24), while resonance effect models consider cell cycle phase-specific effects and host toxicity, but not biochemical resistance (40, 41). These approaches are briefly reviewed below, as are works that incorporate both genetic and kinetic resistances (10, 12, 42).

(Sub-) Optimal Control. Optimal control theory from engineering applied to cancer chemotherapy seeks to maximize tumor control while minimizing toxicity, with a cost function determining the balance of each factor (26). Unfortunately, the predictions of various optimal control models differ depending on the competing maximizations and minimizations chosen, the selection of which is ambiguous. Should one aim to maximize normal bone marrow and also the amount of drug given? This is the approach taken by Fister and Panetta (43), who predicted that periodic continuous infusions (lasting 7 days) are better than the other scenarios that they consider. Or should one seek to minimize the total cancer mass at the end of some specified time interval while minimizing the total drug used? Swierniak *et al.* (44) took both of the above approaches, predicting that treatments at periodic intervals are close to optimal solutions, but that there are many optimal solutions, both long-term continuous infusions and periodic short-term infusions or pulses.

Also, equations for optimal control can be difficult to solve. Consequently, such models employ a suite of

simplifications to arrive at solutions. To our knowledge, optimal control models have not simultaneously incorporated cell cycle phase specificity and evolution of drug resistance or treatment with multiple drugs.

Dissonance about Resonance. Some researchers optimize cell cycle phase-specific drug schedules based on a ''resonance effect'' (40, 41, 45). Periodic drug administrations by bolus, at intervals lasting an integer multiple of the mean cell cycle time of normal cells (resonance), are predicted to limit toxicity without compromising tumor cell kill. They predict a resonance effect based on the logic that normal cells in a vulnerable phase of the cell cycle are killed by the first drug pulse. One cell cycle later, few normal cells reenter that vulnerable phase, limiting normal cell kill by subsequent drug pulses. Tumor cells often have a different mean and a more heterogeneous distribution of cell cycle times than normal cells, so they will not be sheltered by the resonance effect. Resonance models predict that continuous infusion is very toxic.

Although limited *in vitro* and *in vivo* evidence exists in support of the resonance effect (45), no clinical trials have been designed to test this hypothesis. The facts that many drugs persist for hours at high levels in the body and that various toxicity-limiting tissues have different cycling times would make such a trial challenging to design. One prediction of the resonance effect contradicted by clinical trials is for continuous infusion to be more toxic than bolus doses. In fact, decreased toxicity of continuous infusion relative to bolus or short infusion is often observed, sometimes allowing a higher total dose to be used (28, 46). Andersen and Mackey (45) recently developed a more realistic resonance effect model, including features such as G_0 and apoptosis, and predicted that no optimal schedule could be found for their example using acute myelogenous leukemia. No resonance effect models have considered treatment with multiple drugs.

Complex Models: Multiple Drugs, Genetic, and Kinetic Resistances

OncoTCap: Advantages and Disadvantages. OncoTCap, The Oncology Thinking Cap Software version 2.1, is a software developed by R. S. Day and colleagues at The University of Pittsburgh Cancer Institute [http://www.oncotcap.pitt.edu/2000/ (47)] based on a continuous-time, stochastic, birth-death, multitype branching process model by Day (48). OncoTCap examines the effects of different drug combinations and schedules, adapting simpler models by Goldie and Coldman (49). OncoTCap uses stochastic (probabilistic) dynamics, which are particularly appropriate when only a small number of cancer cells are present, and thus captures the random, unpredictable nature of tumor mutations and tumor heterogeneity. This software enables one to simulate the treatment outcome of an individual patient or a patient population, so that the probability of cure can be predicted. For exponentially growing tumors (*i.e.*, those that do not experience a density-dependent reduction in growth), OncoTCap also implements a deterministic (as opposed to a stochastic) solution that may be used to computationally validate the stochastic predictions.

OncoTCap has many advantages. It incorporates metastasis and potential interactions with tumor micro- and macro-environments such as blood supply and body organ. In addition, it includes the features of tumor cell heterogeneity, a quiescent cell population, resistance evolution, and options to model tumor growth that is either exponential or decelerating as a tumor becomes larger (Gompertzian growth). OncoTCap has a graphical user interface with a wide variety of options that the user may modify, and a Clinical Trial Wizard is available as an educational tool for planning clinical trials and exploring potential outcomes.

OncoTCap has several disadvantages that hinder its immediate application in the clinic. It uses unrealistic pharmacodynamics and pharmacokinetics for both drug activity and concentration. For example, for a drug pulse, cell kill is assumed to occur immediately on drug application. Thus, drug half-life, cell cycle phase specificity, and prolonged, concentration-dependent kill as the drug is excreted are not incorporated. In addition, the required inputs for characterizing tumor growth rates are theoretical, and not easy to estimate in the clinic. For example, it is not clear how one would estimate the ''Gompertz split,'' which characterizes the reduction in the rate of growth that can be attributed to a decrease in mitotic rate as opposed to an increase in cell death rate, for a given patient, particularly when the tumor is first detected. Consequently, many required input parameters are difficult to estimate from tumor biopsies, demanding guesswork by the user. If these factors can be overcome, OncoTCap may provide valuable assistance in predicting individual response to treatment with multiple drugs.

There is little published at this time describing specific results of OncoTCap, indicating that this software is ripe for additional generation of predictions. Currently, an investigation is under way to simulate the optimal duration of tamoxifen treatment in early breast cancer. The results published at this point are still in early development, and indicate that a tamoxifen-stimulated phenotype plays a role in the time to breast cancer recurrence (50). A prediction made by the original model on which OncoTCap is based is the ''Worst Drug Rule'' (48). This rule states that the optimal treatment schedules of multiple drugs tend to use more of the less effective agent and/or to use the less effective agent earlier in the treatment schedule compared to the more effective drug. The reasoning is the following: when a patient receives an alternating treatment and the tumor recurs, the cause of failure is predicted to be the growth of cells resistant to the more effective drug but sensitive to the ''worst'' drug.

Kinetically Tailored Treatment—KITT: Mechanistic and Predictive. A model called kinetically tailored treatment, or KITT, incorporates the complex interactions of proliferative and apoptotic rates on tumor growth and evolution, the differential response to cell cycle phase-specific, cell cycle phase-nonspecific, and cytostatic drugs (which may be given concurrently according to different schedules), and the evolution of drug resistance (12). KITT aims to tailor chemotherapy regimens for individuals based

on their tumor cell kinetics. It uses a system of deterministic differential equations. The evolution of resistance is modeled both via incremental processes like gene amplification and via mutations of large effect like changes in a target enzyme. It simulates the accumulation, or clonal dominance, of resistant cells over susceptible cells through the course of treatment with multiple drugs. KITT also incorporates sub-models to capture realistic drug pharmacokinetics and pharmacodynamics, time-dependent dose response, and intratumor heterogeneity.

KITT uses input parameters specific to a given patient's tumor that can be measured from tumor biopsies: apoptotic index; proliferative fraction; S-phase fraction; cell cycle time; and drug resistance. All of these features can be feasibly and relatively inexpensively measured (5, 23), and are often requested by clinical oncologists. These input variables are preferable to transition rates or ''growth rate'' and ''size plateau'' which are difficult to estimate and are required by many of the models discussed above.

These kinetic parameters also capture and summarize the outcome of many interacting mutations, the suite of which probably occurs uniquely in every patient. This observation points to another difficulty inherent in using microarray data to predictively design treatments for the clinic: First, a meaningful grouping of patients based on their amalgam of mutations will be difficult. Second, the sample size for any particular assemblage of mutations will be so small that statistically showing that one treatment results in a survival advantage over another for a specific mutation-based grouping of patients may be difficult.

Results of the KITT model are consistent with published outcomes of clinical trials. However, more testing is needed, particularly in assessing data from individual patients in which all the necessary input variables (listed above) are known. Predictions of KITT indicate that including cytostatic drugs early in treatment and concurrently with cytotoxic drugs substantially increases the probability of cure and prolongs survival. This result concurs with suggestions that the way in which clinical trials of new cytostatic, or ''target-based'' drugs, are performed must be rethought (51–53).

KITT predictions also suggest that altering drug scheduling may be more effective but not necessarily more toxic than dose escalation. In addition, drug combinations predicted to be more effective in a patient with a high proliferative index may differ from those for a patient whose tumor has a low proliferative index. This model produces results consistent with outcomes of clinical trials investigating the interaction of cell cycle kinetics and the alternating *versus* sequential scheduling of CMF plus doxorubicin chemotherapy (54). Ultimately, it is hoped that this software will assist in the design of individually tailored treatments.

Conclusion

Computational tools show promise for tailoring cancer treatments for individuals. Such tools exist and the necessary input data can be feasibly collected from assays on tumor biopsies. OncoTCap and KITT, the most promising models that have been developed for predicting outcomes, must still be validated in the clinic. These models help elucidate complex interactions among multiple drugs, tumor cell kinetics, and resistance evolution. Microarrays, while promising in the long term, still must overcome substantial obstacles before they can be used to identify individualized treatment protocols.

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