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The Dynamics of Drug Resistance: A Mathematical Perspective

Orit Lavi[1], **Michael M. Gottesman**[1,*], and **Doron Levy**[2,*]

[1]Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD

[2]Department of Mathematics, University of Maryland, College Park, MD

Abstract

Resistance to chemotherapy is a key impediment to successful cancer treatment that has been intensively studied for the last three decades. Several central mechanisms have been identified as contributing to the resistance. In the case of multidrug resistance (MDR), the cell becomes resistant to a variety of structurally and mechanistically unrelated drugs in addition to the drug initially administered. Mathematical models of drug resistance have dealt with many of the known aspects of this field, such as pharmacologic sanctuary and location/diffusion resistance, intrinsic resistance that is therapy independent, therapy-dependent cellular alterations including induced resistance (dose-dependent) and acquired resistance (dose-independent). In addition, there are mathematical models that take into account the kinetic/phase resistance, and models that investigate intra-cellular mechanisms based on specific biological functions (such as ABC transporters, apoptosis and repair mechanisms). This review covers aspects of MDR that have been mathematically studied, and explains how, from a methodological perspective, mathematics can be used to study drug resistance. We discuss quantitative approaches of mathematical analysis, and demonstrate how mathematics can be used in combination with other experimental and clinical tools. We emphasize the potential benefits of integrating analytical and mathematical methods into future clinical and experimental studies of drug resistance.

Keywords

drug-dependent/independent resistance; drug scheduling and sequencing; evolution of resistance; mathematical modeling; multidrug resistance

1. Introduction

Resistance to chemotherapy is a key impediment to successful cancer treatment that has been intensively studied for the last three decades. Understanding the biological mechanisms of drug resistance and developing agents to target those mechanisms are important steps in the design of new therapies. Several central genes and pathways have been identified as contributing to the resistance of cancer cells to chemotherapy. Theoretically, abnormalities could develop from point mutations, gene amplification or other genetic or epigenetic changes that affect biological functions. Penetration of antineoplastic agents into the cancer

[*]Address correspondence to: 1) Michael M. Gottesman at the Laboratory of Cell Biology, National Cancer Institute, National Institutes of Health, 37 Convent Dr., Room 2108, Bethesda, Maryland, USA. GottesmM@mail.nih.gov. 2) Doron Levy at the department of Mathematics, University of Maryland, College Park, MD, 4146 CSIC Building #406, Paint Branch Drive, College Park, MD 20742-3289, USA. dlevy@math.umd.edu.

cell induces their lethal pharmacological effect by interaction with target molecules. Altered activity of membrane-embedded drug uptake and efflux pumps can inhibit this effect by reducing intracellular drug accumulation, thereby preventing drug-target interactions. The primary effect of anticancer drugs is to inflict damage to target molecules, thereby triggering various cellular signal transduction pathways, leading to cell death or cell cycle arrest. These secondary effects result in apoptosis or other types of cell death including autophagy, mitotic catastrophe, necrosis and senescence. Therefore, modifications of these genes and pathways can mediate anticancer drug resistance. Resistance mechanisms can affect single drugs or drug targets or multiple drugs simultaneously (MDR). In the case of MDR, the cell becomes resistant to a variety of structurally and mechanistically unrelated drugs in addition to the drug initially administered (Figure 1A, (Gillet and Gottesman, 2010; Teicher, 2006)).

Mechanisms of drug resistance are demonstrated in Fig. 1A (Gillet and Gottesman, 2010), and Fig. 1B shows various means by which these mechanisms might be activated. Cells, illustrated at the bottom of this figure, can exist in three states: normal, sensitive cancer cells, and resistant cancer cells. Resistance can be produced either by intrinsic causes (such as by mutant genes) or by external causes (such as by signaling from the microenvironment). Resistance can develop as a single step or as multiple steps of random genetic mutations or any other abnormality occurring in gene products. Such changes can be the consequence of drug administration, or can be acquired independently of any drug. Furthermore, resistance to multiple drugs can be achieved by cell location and by drug properties. This situation is depicted in the lower part of Fig. 1B. A sensitive cancer cell is effectively resistant if a drug cannot reach it. Fig. 1B includes four scenarios, which can be thought of in terms of four different initial configurations. Case 1 refers to diffusion resistance in the interior of the tumor. In contrast, cases 2–4 in Fig. 1B illustrate advanced cancers. Case 2 assumes the occurrence of independent resistant cells. Case 3 demonstrates a group of resistant cells in which certain cells may have favorable micro-environmental conditions. This situation will depend on the tumor's topology, and local regions may develop different properties when it comes to drug resistance. In Case 4 of Fig. 1B, we show completely resistant cancer cells. These cells are either intrinsically resistant or have acquired resistance due to prior chemotherapy.

Along with biological and clinical research, mathematical approaches have been developed to model development of drug resistance. Mathematical modeling has a different perspective from experimental laboratory research and depends on different assumptions, tools, and methods. All such models are based on experimental or clinical data. Conclusions from these models can guide researchers to develop new experiments with a more refined focus, and in some cases can lead to new clinical trials.

Scientists who are unfamiliar with contemporary research in the mathematical sciences may wonder about the contribution of theoretical modeling to MDR research. More specifically, one might ask what aspects of MDR can be (and indeed are) studied mathematically? What can mathematics potentially contribute to the study of MDR? How well is mathematics integrated into the study of MDR?

While one of the main goals of this review paper is to demonstrate the aspects of MDR that have been mathematically studied, an even more important goal is to explain how, from a methodological perspective, mathematics can be used to study drug resistance. We would also like to emphasize the potential benefits of integrating analytical and mathematical methods into future clinical and experimental studies of drug resistance. Mathematics should be viewed as a research tool that can be used to complement other tools in the study of MDR.

From an experimental point of view, tremendous progress has been made over the past decade, and in view of that, a lot of what was done historically with mathematical models could be considered as outdated. Most of the mathematical studies were based on ABC transporters as the main mechanism of resistance. Contemporary biological knowledge shows a substantially more complex picture of drug resistance. Still, we think that there is great value in discussing the methodologies and the research questions that have been studied in order to encourage experimentalists to consider mathematics as yet another viable and even indispensable research methodology. In addition to the somewhat obvious use of mathematics as a language for analytically quantifying biological processes, it can be used to compensate for certain shortcomings of experimental techniques. In certain cases, mathematical analysis can be used as a tool to guide the experimental design.

Several review papers have been published on mathematics and drug resistance (Agur, 2010; Chapman et al., 2007; Clare et al., 2000; Fister and Panetta, 2000; Foo and Michor, 2010; Gardner, 2002a; Gardner and Fernandes, 2003; Goldie and Coldman, 1998; Kufe et al., 2003; Michelson, 1993; Michor et al., 2006; Panagiotopoulou et al., 2010; Piccart-Gebhart, 2003; Retsky et al., 2005; Simon and Norton, 2006; Swierniak et al., 2009; Wodarz and Komarova, 2005). In view of the present understanding of MDR, it turns out that most of the review papers have focused on a subset of the issues related to drug resistance. From that perspective, this paper could be viewed as a “review of reviews” in which we make an attempt to combine different views into a unified document and present a broader view of MDR modeling. When considering mathematical modeling, one standard approach to reviewing models is to discuss different approaches based on their mathematical frameworks. Since our main goal in this paper is to present a mathematical approach to the study of MDR, we decided not to focus on the specifics of the mathematical tools. Instead, we write from the point of view of the biological issues – and accordingly, comment on some of the ideas that mathematicians have been considering.

Mathematical models for drug resistance have employed methods that span from deterministic to stochastic, from discrete (agent-based) to continuum models (ordinary differential equations (ODE), partial differential equations (PDE), delayed differential equations (DDE), etc.). In deterministic models, the dynamics of a system follow a set of known rules without any room for random variation. In contrast, with stochastic models the future evolution is described by random events, and the initial configuration does not completely determine the future state of the system. Agent-based models provide a description of a system as a collection of rules of interaction between autonomous agents. Continuum models provide a more computationally manageable tool when the number of elements being modeled is very large. In such cases, instead of considering individual agents, populations that are modeled are typically described as concentrations.

Beyond the specific method that is used, studies can be motivated by specific biological questions. Other studies focus on mathematical analysis important to theoreticians that deal with the mathematical nature of the mathematical models, without any particular connection to a specific biological problem. In any event, the mathematical tool is based on certain assumptions and therefore is not the issue of importance in this case. We emphasize the importance and uniqueness of the process in mathematical modeling, starting with assumptions that are being made prior to modeling. How are these assumptions then converted into a mathematical model? How is data incorporated into the model? How can a complex biological structure be captured using a compact set of ideas? How can the biological question, the data, the assumptions, and the mathematical formulation be connected? It is equally important to ask what we can learn from a mathematical model as opposed to a specific experiment.

In this review, we distinguish between mathematical modeling and statistical modeling. We also distinguish between mathematical modeling and certain algorithmic approaches (such as are used for analysis of genomic data). We refer the reader to related reports and reviews (Crivori et al., 2006; Demel et al., 2009; Woodahl and Ho, 2004). It is important to note that much of what has been said about mathematical models of MDR is also true of general aspects of mathematical approaches to address other biomedical problems, not necessarily for cancer. In the following section, we present several questions that have been addressed with mathematical and computational methods related to MDR complexity.

2. Mathematical modeling of MDR

Every mathematical model is based on a set of assumptions, in the same way that any diagram of metabolic pathways is based on experimental data and its interpretations. Mathematical models rarely include a full description of every component that is involved in a given process. An educated choice has to be made in terms of what should be included in the model and what should be left out. An even more fundamental choice is the *resolution* at which the mathematical model is written. For example, should the mathematical model describe the molecular level or is it enough to describe the phenomenon at the cellular level? Since the first goal of a mathematical model, in many cases, is to capture basic principles underlying the biological complexity, it is common to see different, yet related, biological elements, combined into one group.

For example, many mathematical studies have aimed at modeling multidrug resistance, but in practice, accounted only for resistance to a single drug. A typical assumption is that this drug represents a family of drugs with the same targets (e.g., drugs related to the cell cycle). The model is then used to calculate the potential of these drugs to eliminate resistant cancer cells, or to study the differences between two types of drugs. Another example of simplification, which is commonly used, is the use of an ABC transporter as a critical component in the dynamic of resistant cell. Since this transporter effluxes many drugs and its effect remains several weeks after treatment, the assumption is that this efflux-transporter family represents multidrug-resistant cells or at least a common type of resistance. In addition, it is important to note that a single tumor can be thought of being composed of many sub-populations and several stages of sensitivity can be associated with cells. Most models consider tumors as composed of two groups, sensitive or resistant. But there are models in which partial resistance and its relationship to the concentration of the drug is being addressed (Gardner, 2000; Swierniak et al., 2009).

Mathematical models of drug resistance have dealt with many of the known aspects of the field. The list includes *pharmacologic sanctuary* and *location/diffusion* resistance, *intrinsic* resistance (therapy-independent), therapy-dependent cellular alterations including *induced* resistance (dose-dependent) and *acquired* resistance (dose-independent). In addition, there are mathematical models that take into account *kinetic/phase* resistance (i.e., resistance that is based on the phase of the cell cycle/G0), and mathematical models that investigate *intra-cellular mechanisms* that are based on specific biological functions (such as ABC transporters, apoptosis and repair mechanisms).

In this section we provide a snapshot of the questions mathematicians study with relation to drug resistance. Given the enormous activity in the field, such a list cannot be considered comprehensive. Instead, it should be considered as a guideline to the potential of mathematical modeling and analysis in the field.

Given the complexity of the mechanisms that cause MDR, it is not surprising that mathematical models do not incorporate everything that is biologically and clinically known about the problem. Mathematical models of drug resistance typically focus on one (or more)

of the underlying mechanisms. This will be discussed in some detail when addressing specific models. Moreover, there are various fundamental questions that are related to mathematical modeling in cancer research. Some of the problems that fall under this category are: mono/multi cellular layer culturing and the differences of drug transport (Venkatasubramanian et al., 2008), cancer initiation (Michor et al., 2004), cancer growth (Chapman et al., 2007), metastasis {Clare, 2000 #10, angiogenesis {Mantzaris, 2004 #91}, cancer dormancy (Demicheli et al., 1997; Retsky et al., 1997), tumor-immunology and immunotherapy, microenvironment, etc. Such problems have been extensively studied by the mathematical modeling community, in most cases without making any direct connection with drug resistance mechanisms.

2.1. What is the optimal protocol for drug scheduling in terms of dose and timing?

Scheduling protocols are determined by a range of dependent variables, and studied as such. The main topics relate to dose intensification, dose densification (continuous or discrete), protocol escalation, timing and duration. Some models also include different population dynamics between resistant vs. sensitive cells or cancer vs. normal cells or cancer cells vs. drug. The timing function may include the cell cycle phase, cancer stages and even surgery.

2.1.1. Kinetic vs. mutation resistance—When considering optimal therapy, the goal is to maximize the control of the tumor while minimizing toxicity. Norton and Simon proposed a model (Norton and Simon, 1986, 1977) in which a particular chemotherapeutic treatment results in a rate of regression in tumor volume that is proportional to the rate of growth for an unperturbed tumor of that size. They also used the concepts of dose intensification and especially dose densification. The chance of eradicating the tumor is maximized by delivering the most effective dose level of drug over as short a time as possible. Thereby, tumors given less time to grow between treatments are more likely to be eradicated. Norton and Simon based their theory solely on kinetic resistance, that is, based on the phase of the cell cycle/G0. In parallel, Goldie and Coldman proposed a different protocol based on different assumptions and methodology (Goldie and Coldman, 1979; Goldie et al., 1982). They modeled chemotherapy scheduling with the objective of minimizing the development of drug resistance based on the occurrence of mutations (Fig. 1B, case 2). When more than one non-cross-resistant drug is used, it was expected that the treatment should alternate between drugs as quickly as possible in order to reduce the occurrence of resistant cells, thus maximizing the probability of cure.

Although most models focus on cure, there are many cases in which tumor eradication does not occur, either in the context of palliation or failure to cure. In those cases, the goal of chemotherapy is to extend survival and improve the quality of life. On this subject, Monro and Gaffney (Monro and Gaffney, 2009) asked whether an intermediate level of chemotherapy would restrict tumor growth and increase the time of survival in a palliative setting. Their populations model based on ODEs predicted that reduced chemotherapy protocols could lead to longer survival times due to competition between resistant and sensitive tumor cells (Fig. 1B, case 3). Very early treatment was also predicted to quickly lead to the resistance of most tumor cells, reducing survival time. Also, they claimed that the common protocol escalation strategy of dose densification could reduce survival times.

To investigate the influence of cancer heterogeneity on treatment impact, Castorina *et al.* (Castorina et al., 2009) reported the dynamic relationship between two populations, primary tumor cells and a secondary, faster replicating cancer cell population that emerged from the first population. This dynamic led to growth instability at a certain time point and the timing of this was key to developing a chemotherapeutic schedule. They suggested a modification of the “Norton-Simon late intensity” schedule when tumor time evolution is non-uniform.

They stated that optimal chemotherapeutic dose should be determined by the balance of the two populations and their specific growth rates.

2.1.2. Optimal control theory—Fister and Panetta (Fister and Panetta, 2000) used methods of optimal control to maximize the normal bone marrow as well as the dose of the drug. They predicted that optimal drug delivery would be by periodic continuous infusions. Swierniak and coauthors (Swierniak et al., 2009) have also studyied the question of optimal drug delivery. The mathematical models developed in these works have predominantly focused on modeling *gene amplification* and considering a stochastic approach to modeling the evolution of cancer cells (Kimmel et al.; Swierniak et al.). These studies, conducted from the point of view of control theory, provide a way to determine the minimal dose of the drug that will guarantee the asymptotic decay of the tumor population. These results were obtained by using optimization methods on the mathematical models (Smieja et al., 2000; Swierniak and Smieja, 2005). The calculations of Swierniak *et al.* were done in order to minimize the total cancer mass at the end of a specified time interval while minimizing the total dose of the drug. Methods of optimal control were used to study optimal timing and doses of the treatment.

2.1.3. Continuous infusion—Several models predict that continuous infusion (in particular of cell cycle phase specific drugs) is more effective than short pulses (Gardner, 2002a; Gardner, 2000; Murray, 1990; Panetta, 1997; Shochat et al., 1999; Swan, 1990; Swan and Vincent, 1977). This is because continuous infusion prevents tumor re-growth between treatments, and exposes more cells to the drug when they are in the sensitive phase of the cell cycle. An obvious problem with a continuous infusion is the following: if the drug is applied too quickly, then cells that are in an invulnerable part of their cycle may escape lethal exposure. If, on the other hand, the drug is applied too slowly by continuous infusion, drug resistance may develop. Gardner (Gardner, 2000) modeled this tradeoff and used his model to provide insight on how the chance of a cure is connected with the dose and the type of infusion. The optimal therapy depends on many variables, including the patient's tumor cell kinetics. Adaptive therapy, adjusted to the parameters of individual patients, can be advantageous when based on analytical tools, as provided by mathematical models.

2.2. When several drugs are available, how many drugs should be used? Should they be used in combination or sequentially?

When several drugs are available to treat a cancer patient, how many drugs should be used to prevent treatment failure? What are the properties needed for this decision? Should the drugs be administered simultaneously or sequentially? What is the optimal drug administration in this case? Are the drug effects synergistic or sub-additive? These questions have been extensively studied in the mathematical literature and partly relate to schedule protocol (see (Kufe et al., 2003) and the references therein).

In addition to the schedule question, the two hypotheses of Goldie and Coldman (Goldie and Coldman, 1979) and Norton and Simon (Norton et al., 1976; Simon and Norton, 2006) also have different conclusions about drug combinations and sequential strategies. Bonadonna et al. (Bonadonna et al., 2004; Bonadonna et al., 1995) proposed two dose schedules: alternating and sequencing of adjuvant cyclophosphamide, methotrexate, and fluorouracil (CMF) given simultaneously in combination with doxorubicin (A) for patients with high-risk stage II breast cancer. The alternating regimen consisted of two courses of CMF followed by one course of A, repeated for four cycles ([CMF2→A] × 4), while the sequential regimen consisted of four courses of A followed by eight courses of CMF (A4→CMF8). The dose levels, interval lengths, and total treatment duration were identical in both arms. The Goldie and Coldman theory supported alternating schedules, while the

Norton and Simon theory supported sequential schedules. A series of clinical trials by the Cancer and Leukaemia Group B (CALGB) and the American Breast Intergroup (Citron et al., 2003) were conducted and confirm the hypothesis of Norton and Simon.

Another important question is when treatment should be switched from one type of drug to a second non-cross-resistant drug. Panetta (Panetta, 1998) defined a model that describes a heterogeneous tumor population and the effects of chemotherapy. The model specified conditions that were related to the ratio of resistant to sensitive cells. The model indicated that the more effective the treatment, the sooner it would be necessary to switch to the second non-cross-resistant treatment. Birkhead and Gregory (Birkhead and Gregory, 1984) came to a similar conclusion.

Day and colleagues (Day, 1986) developed a software, named The Oncology Thinking Cap Software (OncoTCap, http://www.oncotcap.pitt.edu) that performs simulations of the treatment outcome of a single patient to assess the probability of cure. They examined the effects of different drug combinations and schedules using continuous-time, stochastic, birth-death and branching process methods. The model was based on the theory of Goldie & Coldman (Goldie and Coldman, 1998). Another computational tool was developed by Gardner (Gardner, 2002b) called Kinetically Tailored Treatment (KITT). This model also predicts drug combinations, doses, and schedules likely to be effective in reducing tumor size and prolonging patient life. Treatment strategies may be tailored to individuals based on tumor cell kinetics. The model incorporates intra-tumor heterogeneity and evolution of drug resistance, apoptotic rates, and cell division rates.

Komarova and Wodarz (Komarova and Wodarz, 2005) addressed the question of how many drugs should be used to prevent treatment failure depending on the size of the tumor. Based on the time that resistance arises (“before the start of treatment”) and the level of turnover rates (specifically “high”), one of their conclusions was that combination therapy is less likely to yield an advantage over single-drug therapy. They also demonstrate how their stochastic mathematical framework can be applied to the treatment of a specific cancer (chronic myeloid leukemia) with small molecule inhibitors.

Most studies differentiate between cross or non-cross-resistant drugs, while the definition of *cross-resistant* does not necessarily specify the mechanistic resistance but only the outcome. The outcome is the response of a cell/patient to a second drug when the cell/patient is already resistant to the first drug, regardless of the pathways or biological functions that cause the resistance. Araujo *et al.* (Araujo et al., 2005) focused on a specific biochemical network, the EGFR signaling pathways, and used chemical kinetics equations describing the changes in concentration of the components over time. They used mathematical modeling to investigate combination therapy in which multiple nodes in the network are targeted simultaneously with specific inhibitors. It was demonstrated that the reduction of signaling is significantly enhanced when several upstream processes are inhibited. It was also suggested that this strategy could be used with lower doses and consequently would reduce toxicity.

Recently, Roe-Dale and colleagues (Roe-Dale et al., 2011a, b) proposed two models for sequential regimens of CMF and doxorubicin used in breast cancer and for gastric cancer chemotherapy involving a taxane (either paclitaxel or docetaxel) coupled with flavopiridol. Their models incorporate cell cycle specificity and resistance to study why doses of the same drugs given in different orders result in different clinical outcomes. In the breast cancer model, they suggest that without any assumptions regarding dose density, it is resistance rather than cell cycle specificity that is responsible for the superiority of the

sequential regimen. As for the gastric cancer model, they indicated that for an enhanced synergistic effect, flavopiridol must be administered following taxane therapy.

2.3. What is the likelihood of the interaction between the drug and an efflux transporter?

Location Resistance (also referred to as *Diffusion Resistance*) occurs when certain cells are not exposed to the drug due to their location within the tumor (Fig. 1B, case 1). Drugs and biologics that are large molecules may have a rather limited perfusion capability and location resistance is expected to occur in cells that are located away from the capillary bed (Minchinton and Tannock, 2006; Tredan et al., 2007).

Panagiotopoulou *et al.* (Panagiotopoulou et al., 2010) developed a spatiotemporal mathematical model in order to study the likelihood of the interaction between the drug and a transporter, specifically P-glycoprotein (P-gp). They then used their model to show that these interactions are driven by the mechanical interaction between drug molecular weight and the membrane mechanical properties based on random diffusion of the drug in the membrane. Such results can potentially assist in designing a type of mechanical control for drug delivery. The authors listed several future challenges, including pumping kinetics (non-instantaneous). Therefore, considering drug-pumping kinetics allows for higher concentrations of drugs, the design of new therapeutic strategies is based on molecular weight and the ability to move within the membrane. The second challenge mentioned was to capture the true complexity of MDR by adding to their model other drug resistance related transporters. Panagiotopoulou and colleagues also were interested to determine how a higher pH, as observed in resistant cells, can influence the transverse movement of drugs as a function of size. This interesting relationship between drug diffusion and tumor microenvironment was also observed by Venkatasubramanian and colleagues (Venkatasubramanian et al., 2008) using a wider definition of microenvironment. They integrated the intracellular metabolism, nutrient and drug diffusion, cell-cycle progression, cellular drug effects, and drug pharamacokinetics. They raised the important issue of cellular culturing (monolayer vs. three-dimension cultures) and its impact over the results and predictions.

For more than twenty years, the P-gp pump has been studied at the molecular and tumor levels from a mechanistic perspective. Demant *et al.* (Demant et al., 1990) developed a model of drug transport (P-gp) on the molecular level. They asked, “Could endosomal transport of drug under varying levels of pH account for a major portion of drug efflux in MDR cell lines?” Their model described three compartments: the extracellular medium, the cytoplasm, and the endosomal vesicles. From their model they concluded that active transport is the primary efflux mechanism in MDR cell lines, and that diffusion and exocytosis are not fast enough to account for the rapid efflux observed experimentally. Michelson and Slate (Michelson and Slate, 1994, 1992) expanded the model of Demant and colleagues to incorporate diffusion, the energy dependence of the pump and an inhibitor (to model MDR reversal). Other molecular models deal with the kinetics of P-gp. The models of Spoelstra *et al.* (Spoelstra et al., 1992), Horio *et al.* (Horio et al., 1990), and Michelson and Slate are all variations upon the Michaelis-Menten transport theme. The differences between the three models were mainly in their experimental designs and in the detailed descriptions of diffusion, energy dependence, etc.

The models that have been developed thus far can be used to make simple predictions about how MDR reversal agents could be optimally employed to block pumping activity. However, in order to create a more realistic model, one must consider other complexities of P-gp functions (e.g., binding sites and the affect on ATPase activity).

On the tumor level, Michelson and Slate (Michelson and Slate, 1989; Slate and Michelson, 1991) developed a mathematical model that describes drug resistance from the tumor level (population dynamic) to the cellular level. They used a more mechanistic approach, by defining it as one or all of the following physiologic pathways: decreased drug uptake, increased drug efflux, increased degradation/metabolism of drug, increased drug-target concentration, and altered drug-target properties. They showed that any cell that pumps out enough drug such that its concentration at the target site remained “low enough”, significantly enhanced its chances for survival.

2.4. How effective is chemotherapy in eradicating a tumor?

Pharmacological sanctuary occurs when a tumor develops in a site where drug access is limited by biological barriers such as the blood brain barrier. Tumors sometimes develop elsewhere and then metastasize to an area of sanctuary. In such cases, it is important to consider the potential effectiveness of chemotherapy. To address this subject, Wein *et al.* (Wein et al., 2002) developed a mathematical model for the spatiotemporal dynamics of a brain tumor treated with a specific cytotoxic agent. Their study provided a prediction for the probability of curing the tumor that requires estimating parameters that are related to the characteristics of the tumor, to the drug design, and to the drug delivery. With such a model it is then possible to determine the required circumstances within which such targeted therapy can be effective. Further general examples of modeling the brain tumors and treatments reviewed by Deisboeck and colleagues (Deisboeck et al., 2009).

2.5. How is early detection and early therapy connected with the development of drug resistance?

Among the better known mathematical models of drug resistance are the models of Goldie and Coldman (Goldie and Coldman, 1998) mentioned earlier. They developed a probabilistic model of cell mutations. In their model, the mutations were assumed to be related to the dose of the drug. Such models can then be used in order to study, for example, the probability that mutations will result in drug resistance. Based on their calculations, Coldman and Goldie showed that early detection and early therapy can decrease the chances of developing resistance (the probability of developing resistance increases as the tumor mass increases). Another outcome of their calculations is that alternating doses of non-cross-resistant drugs may be better than sequential chemotherapy.

In a recent paper, Tomasetti and Levy (Tomasetti and Levy, 2010) developed a model that describes how the probability of developing drug resistance depends on the number of long-lived cancer cells at the time of detection, on the probability of mutations, and on the turnover rate of the cancer cells. They derived the mathematical model using ODEs for the wild-type cancer population and branching processes for the mutant cells. By combining the theoretical results from Tomasetti and Levy, and clinical data on CML (Hochhaus et al., 2009), Tomasetti (Tomasetti, 2011) showed that early detection and early therapy may reduce the chances of developing drug resistance.

2.6 What is the probability that at the time of diagnosis resistant cancer cells are already present?

Michor *et al.* (Michor et al., 2006) used the mathematical framework of branching processes to describe the accumulation of mutations in independent lineages. One of the main questions investigated in their model was the estimation of the risk of developing drug resistance as a function of the tumor size at the time of diagnosis. They concluded that if the number of replicating cancer cells exceeds a critical threshold, then the therapeutic outlook is dim. The therapy is likely to succeed if the number of cancer cells is well below this

threshold. The chance of successful therapy is much lower for cancers with genetic instability.

2.7 How fast does the subpopulation of cells that develop drug resistance grow?

Swierniak, Kimmel and Smieja (Swierniak et al., 2009) used their mathematical models to demonstrate that even though the mutation probability is very low, the resistant population may grow exponentially. They proved that the population decays only if the average proliferation time of the resistant subpopulation is sufficiently long compared with the difference between the de-amplification and amplification probabilities.

2.8 What function best describes the “growth law” of cancer and what are the consequences of having different growth descriptions?

In spite of the fundamental importance of this question, it still has no agreed-upon answer. Three commonly used functions for cancer growth are exponential, logistic, and the Gompertz law (Clare et al., 2000; Guiot et al., 2003; Hart et al., 1998; Kufe et al., 2003; Retsky et al., 1990; Simon and Norton, 2006; Spratt et al., 1993). Many variations of these functions appear in the literature. While exponential growth may fit early stages of some tumors, a function that describes a slower rate of growth, as the tumor size increases, is expected to provide a better description of the dynamics of tumor growth. This explains why logistic and Gompertzian laws have been considered as candidates for the solution of this problem. While Gompertzian growth is the standard function that is the basis for much of cancer chemotherapy, the goodness of its fit to data is questionable. A major problem with Gompertzian growth is that it does not allow for temporary dormancy of a tumor. Each growth description can consequently suggest different treatment strategies (Clare et al., 2000).

3. Discussion

This paper was written in order to provide an overview of the kind of questions that mathematicians study in the area of drug resistance. Not every work in the field was mentioned. We also did not attempt to review all the questions that were addressed in the literature. Instead, we tried to focus on the quantitative approach of mathematical analysis, and to demonstrate how mathematics can be used in combination with other experimental and clinical tools.

When compared with alternative approaches, mathematical modeling has a number of strengths. Mathematical modeling provides an analytic way of integrating and synthesizing individual components into a comprehensive picture in order to understand how the system works as a whole. This procedure falls under what is commonly referred to, these days, as “systems biology”. It may provide an analytical understanding of a specific mechanism and can be used as a way to interpret the meaning of experimental data that goes beyond heuristic arguments. Mathematical modeling may also provide insight into the dynamics of the system beyond what is available using current experimental methodologies. A good, validated mathematical model can potentially guide future experiments in terms of the important parameters that control the dynamics of the problem and therefore what should be experimentally measured. Such a model can be also used to determine the timing of certain a measurement. For example, when should a blood sample be collected? More generally, a mathematical model can be used to choose which experiment should be conducted.

In many cases, the most important contribution of a mathematical model is not just the validation of the model (based on the data, etc.). It is actually the next step, in which the mathematical model is used to extrapolate the present knowledge and provide guidance in terms of what should be the next step. Mathematical modeling does not end with fitting

curves, or statistically significance results. The dynamic understanding of the system may help to choose the experimental targets.

One of the main challenges with a simplified mathematical model is to interpret the results and transform the conclusions to clinical relevance. In numerous cases, close collaborations among researchers from interdisciplinary backgrounds have overcome this challenge. A recent interdisciplinary study was reported by Mikkelsen and colleagues (Mikkelsen et al., 2011). They conducted a clinical trial and showed that infusion duration is an important determinant of the intracellular accumulation of active methotrexate in acute lymphoblastic leukemia (ALL) cells in vivo, with more prominent effects in certain subtypes of ALL, indicating that this must be considered when contemplating changes in treatment to reduce costs or toxicity. This study was based on a pharmacokinetic model of systemic and cellular disposition of methotrexate (Panetta et al., 2010; Panetta et al., 2002).

A second example was given by the studies of Citron and co-authors on breast cancer (Citron, 2008; Citron et al., 2003). These studies were based on the Norton-Simon dose density hypothesis. Their results indicated improved disease-free and overall survival. Dense dose adjuvant chemotherapy improves clinical outcomes without increasing toxicity.

Another interdisciplinary approach was the ‘Virtual R&D’ (‘virtual patient’), a clinically validated modeling system that accurately predicts the efficacy and toxicity of various drug combinations in individuals and in populations. The use of this model in clinical research is expected to shorten the development time of new drugs. (Agur, 2010).

Clearly, the mechanisms that control the emergence and the evolution of drug resistance are very complex. The scientific knowledge in this area is rapidly evolving. Deriving mathematical models to address drug-resistance related questions have been conducted in parallel with the evolution of understanding on the biomedical side. Existing mathematical models incorporate some of the known elements of drug resistance.

While the mathematical community has focused on studying certain aspects of the problem, several other aspects have remained unexplored. In general, most mathematical models do not address the mechanism of resistance (specific pathways) and its implications. The microenvironment is routinely studied as part of studying the problem of cancer growth, without any emphasis (or consideration) of the resistance mechanisms. It is well known that not all patients that relapse have resistant cells as some sensitive cells may manage to avoid being exposed to the drug due to their location in the tumor or in the body. Perhaps they even have the ability to migrate to other physical locations.

It is evident that mathematical models that study the problem of scheduling do not include different types of resistance. It is also known that not all relapses are caused by mutations. Diffusion resistance is one of the main reasons for a treatment failure. Modulating the drug dose is an indirect way to challenge the problem of drug delivery, but this strategy can still fail in cases where the drug is unable to enter the cells (in a specific location in the tumor/body). Such limitations imply that it would be helpful to model the drug sequence by their penetrability and only then by their biological functions.

We would like to emphasize that there is no perfect model that can integrate everything. Any optimal mathematical model should be adapted to the initial configuration of the tumor and the specific characteristics of the patient.

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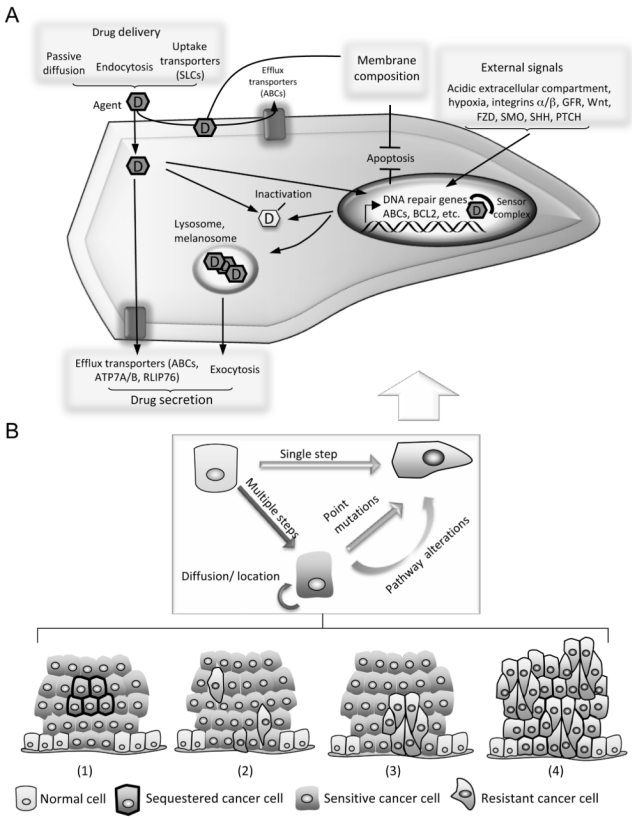

Figure 1.

MDR mechanisms. Cellular mechanisms of drug resistance are demonstrated in Fig. 1A, which illustrates many of the known molecular mechanisms of drug resistance. Fig. 1B illustrates the resistance in relation to the population dynamic. Cells can exist in three states: normal, sensitive cancer cells, and resistant cancer cells. Resistance can be produced either by inner mechanisms (e.g., mutations) or by external mechanisms (e.g., microenvironment signals). Resistance can develop as a single step or as multiple steps of random genetic mutations or any other abnormality occurring in gene products. Such changes can be the consequence of drug administration, or can be acquired independently of any drug. Furthermore, resistance to multiple drugs can be achieved by cell location and by drug properties. Fig. 1B includes four scenarios: diffusion resistance, intrinsic resistance, induced resistance by the micro-environmental conditions, and completely resistant cancer cells. The completely resistant cells are either intrinsically resistant or have acquired resistance due to prior chemotherapy.