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Review

Evolution of acquired resistance to anti-cancer therapy

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HIGHLIGHTS

- Acquired drug resistance is a major limitation for the successful treatment of cancer.
- Evolutionary theory has contributed to understanding the dynamics of drug resistance in cancer.
- We review recent advances in evolutionary models of resistance in cancer.
- We outline how evolutionary thinking can contribute to outstanding questions in the field.

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ABSTRACT

Acquired drug resistance is a major limitation for the successful treatment of cancer. Resistance can emerge due to a variety of reasons including host environmental factors as well as genetic or epigenetic alterations in the cancer cells. Evolutionary theory has contributed to the understanding of the dynamics of resistance mutations in a cancer cell population, the risk of resistance pre-existing before the initiation of therapy, the composition of drug cocktails necessary to prevent the emergence of resistance, and optimum drug administration schedules for patient populations at risk of evolving acquired resistance. Here we review recent advances towards elucidating the evolutionary dynamics of acquired drug resistance and outline how evolutionary thinking can contribute to outstanding questions in the field.

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1. Introduction

The era of modern cancer therapy began with the discovery that nitrogen mustard, a chemical warfare agent, could be used in the treatment of human lymphomas (Goodman et al., 1946). Researchers working with the United States Department of Defense had observed that victims of mustard gas displayed myeloid and lymphoid suppression when autopsied, and began to investigate the effects of nitrogen mustards in a mouse model of lymphoma. When the first patients with non-Hodgkin's lymphoma were treated soon after obtaining positive results from the mouse model, scientists realized that human cancers may be successfully treated with pharmacological agents (Gilman, 1963). One of the first examples of rational drug design (rather than accidental discovery) followed soon afterwards, when Sidney Farber and colleagues at Harvard Medical School investigated the effects of folic acid on leukemia patients. They discovered that anti-folates could suppress the proliferation of malignant cells and re-establish normal bone marrow function (Farber et al., 1948). Anti-folates were later tested on germ cell tumors by researchers at the National Cancer Institute, leading to the identification of the first solid malignancy that responded to cancer chemotherapy (Li and Bergenstal, 1958). Research into pharmacological effectors of cancer cell proliferation soon took off and produced, among others, taxanes, platinum-based agents, and nitrosoureas. Most recently, the emerging molecular understanding of the processes driving tumorigenesis has led to the design of targeted anti-cancer agents – drugs that specifically act on well-defined protein targets or biological pathways that, when inhibited by those agents, impair the abnormal proliferation of cancer cells (Sawyers, 2004). Tyrosine kinase inhibitors such as Gleevec (imatinib) and Tarceva (erlotinib), and monoclonal antibodies like Herceptin (trastuzumab) serve as prime examples for this modern type of medicine.

Resistance against chemotherapy and targeted drugs is as old and as widespread as the use of these agents. The evolution of resistance represents a significant obstacle to the successful control of tumors since it abrogates the response to therapy. Resistance of cancer cells emerges due to two general mechanisms (Gottesman, 2002). Factors of the host organism such as poor absorption and rapid metabolism can reduce the total concentration of the drug in the gastrointestinal tract, blood stream, or the tumor itself; this mechanism is often referred to as intrinsic resistance. In addition, mechanical or biochemical factors may present challenges to the delivery of drugs into tumors. Alternatively, cancer cells may evolve specific genetic and/or epigenetic alterations that allow them to escape from treatment. Some of these alterations, such as loss of a cell surface receptor or transporter and overexpression or alteration in the drug target, lead to resistance against only a small number of related pharmacological agents. For example, overexpression of EGFR has been associated with resistance to the EGFR-inhibitor cetuximab (Schulte et al., 2013). Other factors result in simultaneous resistance to many structurally and functionally unrelated drugs – a phenomenon known as multi-drug resistance (MDR) (Gottesman, 2002; Gottesman et al., 2002; Clynes, 2000). MDR may stem from changes that limit the accumulation of drugs within cells by decreasing uptake, enhancing efflux, or affecting membrane lipids (Liu et al., 2001), block apoptosis (Lowe et al., 1993), induce mechanisms that detoxify drugs and repair DNA damage (Synold et al., 2001), and modulate the cell cycle (Shah and Schwartz, 2001) and checkpoints (Henning and Sturzbecher, 2003).

Mathematical models of these classes of resistance mechanisms have helped contribute to a better understanding of how drug resistance arises, the impact of current therapeutic protocols, as well as strategies for improving or optimizing treatment outcomes. For example, mathematical models of the tumor physiology have been developed to study the factors influencing drug delivery to tumors, predict spatiotemporal variation in drug distribution throughout tumors, and to design strategies for overcoming barriers to drug penetration (e.g. see Kim et al., 2013 and references therein). Here, we aim to provide a review of the mathematical modeling literature on another major class of resistance mechanisms in cancer: drug resistance due to the evolution of genetic or epigenetic alterations.

For many treatment and cancer types, the search is still on to elucidate genetic mechanisms of resistance; for other cases, important players of cellular drug resistance have already been identified (Clynes, 2000). Examples of the latter include amplification or overexpression of the p-glycoprotein family of membrane transporters (e.g. MDR1, MRP, LRP) which decrease intracellular drug accumulation; changes in cellular proteins involved in detoxification (e.g. glutathione S-transferase pi, metallothioneins, human MutT homolog, bleomycin hydrolase, dihydrofolate reductase) or activation of the chemotherapeutic drugs (DT-diaphorase, NADP:cytochrome P-450 reductase); changes in molecules involved in DNA repair (e.g. O6-methylguanine-DNA methyltransferase, DNA topoisomerase II, hMLH1, p21WAF1/CIP1); and activation of oncogenes such as HER-2/NEU, BCL-2, BCL-XL, c-MYC, RAS, c-JUN, c-FOS, and MDM2 as well as inactivation of tumor suppressor genes like p53 (El-Deiry, 1997). Treatment of chronic myeloid leukemia (CML) with the targeted agent Gleevec (imatinib) fails due to the emergence of point mutations in the BCR-ABL kinase domain (Gorre et al., 2001). To date, ninety different point mutations have been identified, each of which is sufficient to confer resistance to imatinib (Burgess and Sawyers, 2006). The second-generation BCR-ABL inhibitors dasatinib and nilotinib can circumvent most mutations that confer resistance to imatinib; the T315I mutation, however, causes resistance to all three of these BCR-ABL kinase inhibitors (Soverini et al., 2007). Recently, a new targeted agent, ponatinib, has been approved for use in patients with CML; ponatinib has been shown to be effective in overcoming the T315I mutation (Goldman, 2012). The T790M point mutation in the epidermal growth factor receptor (EGFR) and focal amplification of the MET proto-oncogene both confer resistance to the EGFR tyrosine kinase inhibitors Tarceva and Iressa (Pao et al., 2005; Engelman et al., 2007), which are used in the treatment of non-small cell lung cancer. Resistance to Herceptin, an agent targeting HER-2 that is widely used in breast cancer, has been associated with increased PI3K/Akt signaling as well as PTEN loss (Nahta and Esteva, 2006). Drugs that inhibit the activity of BRAF (a protein in the MAPK pathway) have been demonstrated to be highly effective at tumor reduction in BRAF-mutant melanomas; however, resistance usually emerges within six months. Recent investigations have suggested that activation of MEK may play a role in resistance by re-activating the MAPK pathway, and

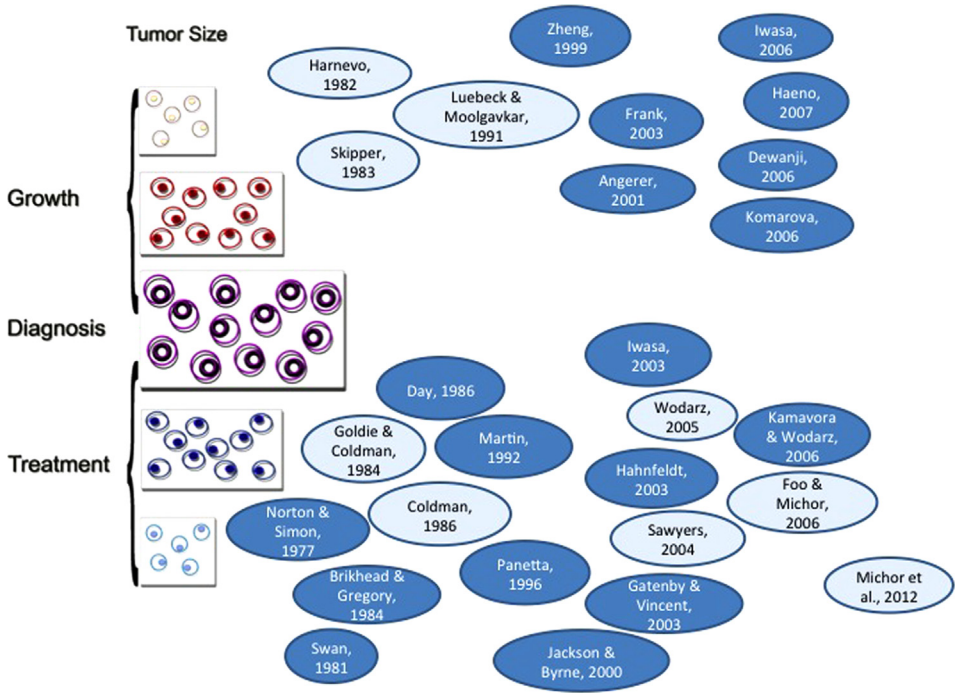

Fig. 1. Evolutionary modeling has contributed to elucidating the risk of pre-existing resistance, the probability that resistance arises during treatment, the effects of the choice of dosing strategies on the dynamics of resistant cells, and the optimal strategy to prevent or delay the onset of drug resistance. For details on the references, please see the list of citations.

combination therapies utilizing both a BRAF and MEK inhibitor seemed promising in preliminary studies (Villanueva et al., 2010). Specific genetic mechanisms of cellular drug resistance have also been identified for conventional cytotoxic therapies; for example, dihydrofolate reductase (DHFR) gene amplification causes resistance to methotrexate (Goker et al., 1995), while resistance against Taxol (paclitaxel) has been associated with specific tubulin point mutations which alter microtubulin dynamics and stability (Orr, 2003). Epigenetic mechanisms of drug resistance have also been observed; for example, dynamic chromatin modifications may act as an independent route to drug resistance of cancer cells and can be reversed with drugs that alter the epigenetic state of cells (Sharma et al., 2010).

2. Previous work

Evolutionary modeling of acquired resistance has contributed substantially to an understanding of this obstacle to the successful treatment of cancer patients. Evolutionary modeling, in combination with experimental approaches, can contribute to elucidating important clinical parameters such as the risk of pre-existing resistance, the probability that resistance arises during treatment, the effects of the choice of dosing strategies on the dynamics of resistant cells, and the optimal strategy to prevent or delay the onset of drug resistance. In this section, we review some key results in evolutionary modeling of drug resistance in cancer that have driven our progress in prognostic and preventative methods (Figs. 1 and 2). There has been a vast amount of work on this topic and thus we do not intend for this review to be comprehensive; instead we aim to provide the reader with a general overview of the major questions and historical developments in the field. For clarity, we have divided this section into three main areas: pre-existing resistance (which emerges prior to diagnosis and treatment), resistance emerging during the course of treatment, and lastly the design of optimal treatment schedules to maximally delay or prevent resistance.

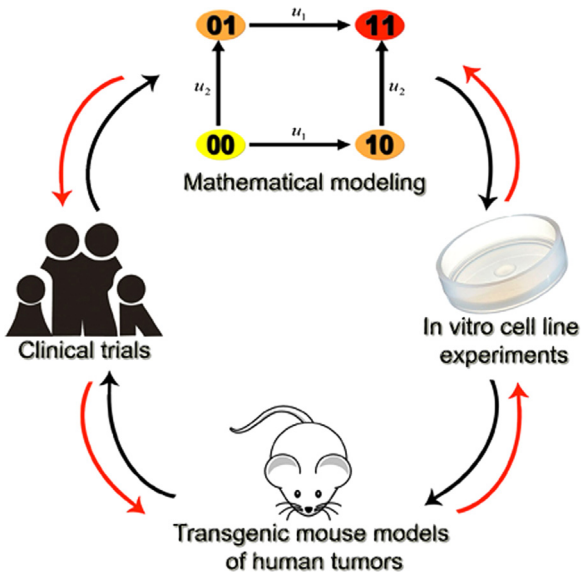

Fig. 2. Progress towards an understanding of the evolutionary dynamics of acquired resistance requires close collaboration between researchers performing mathematical modeling studies as well as cell line and mouse model experiments and clinical investigations.

2.1. Resistance emerging before diagnosis and treatment

The evolution of resistance mutations before a tumor is diagnosed and treatment is initiated has been of considerable interest both to the cancer research community and to evolutionary biologists. The presence of pre-existing resistance often determines the success of therapies and influences treatment choices; in leukemias, for example, pre-existing resistance increases the likelihood that allogeneic bone marrow transplants will be chosen over chemotherapeutic interventions (Kaeda et al., 2006).

2.1.1. Luria–Delbrück models of bacterial resistance

The investigation of the dynamics of resistance mutations emerging during exponential expansion of a cell population was initiated by Luria and Delbrück in 1943 (Luria and Delbrück, 1943). Their work was aimed at settling a fundamental issue in bacteriology: the question whether phage-resistant bacteria arise by spontaneous random mutation or from adaptation – i.e. directed mutation (Zheng, 1999). They performed a series of experiments that suggested a constant rate of random mutations in each generation of bacteria. Based on this principle, the authors formulated a mathematical model to determine the rate at which such mutations emerge during bacterial growth. They considered a deterministic exponential growth model for both normal and mutant bacteria, assuming that both types grow at the same rate, in conjunction with a probabilistic mutation model. Their analytical results describing the distribution of the number of resistant bacteria in an exponentially growing population became known as the Luria–Delbrück distribution. Many subsequent contributions and extensions to the Luria–Delbrück theory have since been made, e.g. Lea and Coulson, 1949; Armitage, 1952; Kendall, 1952; Mandelbrot, 1974; Bartlett, 1966; Crump and Hoel, 1974; Koch, 1982; Stewart et al., 1990; Sarkar et al., 1992; Goldie, 1995; Angerer, 2001; Frank, 2003; Dewanji et al., 2005; Zheng, 2008, 1999). Many of these contributions are discussed in reviews by Zheng (1999) and Skipper (1983).

2.1.2. Applications of Luria–Delbrück models to understanding drug resistance in cancer

During the last half-century, models based on the theory suggested by Luria and Delbrück have also attracted the interest of cancer researchers. Several investigators studied this model with the aim of generalizing the results or applying them to specific situations arising in tumorigenesis, e.g. Frank (2003), Dewanji et al. (2005), Angerer (2001), Iwasa et al. (2006), Haeno et al. (2007), Komarova and Wodarz (2005) and Komarova and Mironov (2005). Some of these models are based on pure birth processes and do not include the possibility of cell death (Zheng, 1999). However, in most situations of cancer growth, cell death cannot be neglected; thus, several authors introduced extensions of the Luria–Delbrück process that explicitly incorporate cell death. Using stochastic processes with a differentiation hierarchy to represent the sensitive and resistant cells, Coldman and Goldie were the first to observe that a higher rate of cell death results in a larger number of resistant cells for a given total population size (Coldman and Goldie, 1986). Angerer (2001) later investigated the influence of cell death on the Luria–Delbrück distribution and derived limit laws for this distribution as the population size tends to infinity.

In 2006, Iwasa et al. designed a two-type branching process model representing sensitive and resistant cancer cells to calculate the risk of pre-existing resistance at the time of tumor diagnosis. The authors determined the expected number of resistant cells as a function of the tumor detection size, the fitness values of sensitive and resistance cells, and the mutation rate. In this model, the growth of the sensitive cancer cell population was represented by a linear birth and death process, and during each sensitive cell division a resistant cell could arise with a probability given by the mutation rate. The resistant population was also modeled as a linear birth and death process, where the growth and death rates could differ from those of sensitive cells depending on the fitness effect of resistance mutations in the absence of therapy. The authors found that the probability of resistance increases with the detection size and the mutation rate, and that a tumor with larger apoptosis rates has a higher incidence of resistance. Using a similar two-type birth and death process model, Komarova et al. (2007) studied the dynamics of resistance at the time of detection for the special case of neutral mutations – resistance mutations that do not alter the fitness parameters of cells. They also presented an efficient computational method to estimate the expected number of resistant cells at the time at which the total population reaches a fixed size. This computational method was based on a boundary layer treatment of a continuous PDE approximation to the recursive Kolmogorov equation for the moments. These works have contributed to a theoretical understanding of the dynamics of drug resistance arising from a single point mutation in an exponentially growing tumor population (up to and including the time of tumor detection).

2.1.3. Non-exponential tumor growth

While the assumption of exponentially growing tumor populations is reasonable in early phases of tumor growth, its validity can become compromised as resource limitation effects set in at higher population levels. Several studies have been undertaken to explore the impact of non-exponential growth dynamics on the evolution of mutation-induced resistance (Dewanji et al., 2005; Tomasetti, 2012). For example, Dewanji et al. (2005) developed an extension of the Luria–Delbrück model that considered non-exponential growth dynamics, following the development of the tumorigenesis model by Luebeck and Moolgavkar (1991). They assumed arbitrary but deterministic growth of normal cells, while resistant cells were considered to grow according to a linear (constant-rate) birth and death process with either mean exponential or Gompertzian growth. They showed that the inclusion of the possibility of cell death, as well as the assumption of Gompertzian growth of mutant cells, led to larger variations in the number of cells harboring resistance mutations as compared to earlier growth models. More recently, Tomasetti (2012) compared the impact of various growth laws on the dynamics of resistance. He showed that the probability that a given random mutation will be present by the time a tumor reaches a certain size is independent of the type of curve assumed for the average growth of the tumor, for a general class of growth curves.

However, note that in these models of resource-limitation, the growth law is externally imposed, in contrast to models where growth is naturally limited 'from within', i.e. when the cell birth and death rates are dependent on the current population size and/or resource levels, so that the resulting growth law is a consequence of this dependence. The latter situation is more difficult to analyze due to the loss of independence between different cell lineages in the branching process, but might represent a more realistic model of resource-limited growth. In a step towards an understanding of such models, Sorace and Komarova (2012) recently analyzed the accumulation of neutral mutations in state-dependent, controlled-growth branching process models. The dynamics of non-neutral mutations (such as mutations conferring drug resistance) in this setting of resource limitation, however, remains an important open question.

2.1.4. Considerations of multiple genetic alterations necessary for resistance

The models outlined above in the exponential growth setting have been extended to investigate situations in which several genetic alterations must be accumulated in a single cell for resistance to emerge (Frank, 2003; Haeno et al., 2007; Komarova and Mironov, 2005; Harnevo and Agur, 1992; Komarova and Wodarz, 2005, 2007a,b; Durrett and Moseley, 2010). Such scenarios arise when multiple mutations are required to confer resistance to a single drug, or when multiple drugs are used that necessitate an independent resistance mutation for each drug. In the 1990s, Harnevo and Agur studied drug resistance emerging

due to oncogene amplification using a stochastic branching process model (Harnevo and Agur, 1992; Harnevo, 1991). In this model, cells were able to accumulate multiple copies of an oncogene, and each subsequent amplification event was considered as a probabilistic event. The authors studied the conditions for the emergence of drug-resistant mutants both prior to and during treatment with one or two drugs. In 2003, Frank designed a three-type branching process model to describe cells with zero, one, or two mutations (Frank, 2003), and found that the distribution of cells with two mutations informs about the effective time of occurrence of the first mutation; the latter – depending on its frequency in different tissues in the body – could even have arisen during embryonal development. Komarova and Wodarz later considered the emergence of cells resistant to multiple drugs (Komarova and Wodarz, 2005; Komarova, 2006). The authors assumed that cells must accumulate m mutations to become resistant to m drugs, and also allowed for the generation of intermediate resistance phenotypes. They calculated the probability of resistance arising before the initiation of therapy for the limiting assumption that all resistance mutations are neutral, and concluded that resistance predominantly arises prior to treatment; this situation was in contrast to resistance generated during continuous therapy with one or multiple drugs, in which case resistance could also arise after the initiation of treatment. The authors also applied their mathematical framework to study imatinib resistance arising in chronic myeloid leukemia (CML) patients (Komarova and Wodarz, 2005; Wodarz and Komarova, 2005) and to address the effects of cellular quiescence on the likelihood of pre-existing resistance (Komarova and Wodarz, 2007a,b).

The stochastic model presented by Iwasa et al. (2006) was later extended to incorporate resistance due to the accumulation of two mutations (Haeno et al., 2007). The authors derived the probability that a population of sensitive cancer cells has evolved a cell with both mutations before the entire population reaches detection size as well as the expected number of cells carrying both mutations at that time. Durrett and Moseley (2010) considered the first time a resistant cell with m mutations arises in an exponentially expanding population of sensitive cancer cells. The authors considered a multi-type linear birth and death process wherein cells with k mutations give rise to cells with $k+1$ mutations at a given rate. They estimated the arrival times of clones with a certain number of mutations by approximating the sensitive cell population growth with its asymptotic limit. The authors furthermore derived a limiting distribution for the ratio between the number of cells harboring one resistant mutation and the sensitive cells at the time when the latter reaches detection size.

2.1.5. Recent clinical applications

In recent years, these types of models have been utilized to quantify the risk of pre-existing resistance in various cancer types. For example, Diaz et al. (2012) and Leder et al. (2011) studied the relative benefits of first-line combination therapy with multiple BCR-ABL kinase inhibitors to treat CML, using a model in which a spectrum of resistant mutants can arise due to various point mutations in the kinase domain of BCR-ABL. Diaz et al. (2012) also utilized a branching process model of mutation accumulation prior to treatment to analyze the probability of rare KRAS-mutant cells existing in colorectal tumors prior to treatment with EGFR blockade. The authors fit the model with clinical observations of the timing of detected resistance and concluded that the mutations were present prior to the start of therapy. These studies are part of a more widespread effort to apply such models to clinically useful situations.

2.2. Resistance emerging during treatment

In a seminal paper published in 1977, Norton and Simon proposed a model of kinetic (not mutation-driven) resistance to cell-cycle specific therapy in which tumor growth followed a Gompertzian law. The authors used a differential equation model in which the rate of cell kill was proportional to the rate of growth for an unperturbed tumor of a given size. Their model predicted that the rate of tumor regression would decrease during treatment. They suggested that one way of combating this slowing rate was to increase the intensity of treatment as the tumor became smaller, thereby also increasing the chance of curing the disease. Predictions of an extension of this model were later validated with a clinical trial comparing the effects of a dose-dense strategy and a conventional regimen for chemotherapy (Citron et al., 2003). Their model and its predictions have become known as the Norton–Simon hypothesis and have generated substantial interest in mathematical modeling of chemotherapy and kinetic resistance (D'Onforio et al., 2012; Rodriguez-Brenes et al., 2013; Agur, 2012).

2.2.1. Stochastic models of anti-cancer therapy

Evolutionary theorists started thinking about the emergence of resistance during cancer treatment after Goldie and Coldman published their seminal results in the 1980s (Coldman and Goldie, 1986; Goldie and Coldman, 1983, 1984). First, the authors designed a mathematical model of cancer treatment to investigate the risk of resistance emerging during the course of therapy with one or two drugs (Goldie and Coldman, 1983). Sensitive cancer cells were assumed to grow according to a pure birth process, while resistance mutations arose with a given probability per sensitive cell division and then grew according to a stochastic birth process. The administration of a drug was considered to cause an instantaneous reduction in the number of sensitive cells. The authors derived the probability of resistance emerging during the sequential administration of two drugs, concluding that the probability of resistance at any given time depends on the total number of cancer cells and the mutation rate(Foo et al., 2012; Norton and Simon, 1977). The authors proposed two strategies to maximize the probability of successful therapy: (i) treatment should be started as soon as possible since the probability of cure decreases with increasing size of the tumor and since larger tumors present a higher level of heterogeneity, which is anti-correlated with the chance of curing the disease (Goldie and Coldman, 1984) and (ii) multiple drugs should be used in combination whenever possible, or in an alternated manner otherwise. This strategy allows the drugs to exert the maximum effect against heterogeneous cell populations. The idea that early initiation of therapy and combination therapy with multiple drugs could reduce the risk of resistance became known as the Goldie–Coldman hypothesis.

In 1984, the authors extended their earlier work by incorporating a differentiation hierarchy into the branching process model of tumor growth to study the emergence of resistance to one or two equivalent chemotherapeutic drugs (Coldman and Goldie, 1986). In this paper, the birth and death rates of cells were modeled as time-independent constants; cells were assumed to divide with a fixed and common interdivision time and each sensitive cell division gave rise to a resistant cell with a certain probability. Treatment was considered to occur at a series of fixed time points, at which either of the drugs was administered at a constant dose. The effect of treatment was modeled as an additional probabilistic cell kill law on the existing population. A generalization of this approach was later studied by Day (1986), who relaxed the assumption of equivalence or "symmetry" between the two drugs. This approach provided the basis of OncoTCap, a

software developed by Day and colleagues (http://www.oncotcap.pitt.edu/2000/) that performs simulations of the treatment outcome of a single patient. Later on, Goldie and Coldman (2009) published a book summarizing these results as well as the fundamental principles of mathematical modeling of drug resistance in cancer.

In the past decade, there has been renewed interest in evolutionary modeling approaches to cancer therapy, e.g. Komarova and Wodarz (2005), Komarova (2006), Iwasa et al. (2003) and Komarova et al. (2009). Iwasa et al. used a multi-type branching process model to study the probability of resistance emerging during treatment of a heterogeneous cell population (Iwasa et al., 2003). They considered resistance emerging due to one or several genetic alterations and calculated the probability of success or failure of treatments consisting of one or more drugs exerting diverse effects on the population of cancer cells. They also determined the risk of pre-existing resistance. Consistent with earlier findings of Goldie and Coldman, they determined that the chance of cure is largest when many drugs are administered simultaneously since in this situation, tumor cells must evolve many mutations to escape from eradication.

Later on, Komarova and Wodarz developed a stochastic mathematical model to investigate multi-drug resistance to anti-cancer therapies (Komarova and Wodarz, 2005). They considered situations in which k mutations are required to confer resistance against k drugs, and modeled the effect of therapy by increasing the ratio of cell death to cell birth in cancer cells. They found that the chance of resistance increases with enhanced turnover rates independently of the number of drugs administered, and determined that the treatment phase is unimportant for the production of resistant cells since the majority of resistance mutations arises before the tumor is diagnosed. The model was also used to find the optimum number of drugs such that both toxicity and the evolution of resistance are minimized. When the model was applied to chronic myeloid leukemia (CML) to investigate the optimum number of drugs for this disease, they found that administration of three chemotherapeutics minimizes the risk of resistance. Later on, these authors studied the importance of stem cell quiescence in the evolution of resistance against anti-cancer treatment (Komarova and Wodarz, 2007a,b). They designed a stochastic process model to investigate the dynamics of a population of cancer stem cells which cycle between an active, proliferative state and an inactive, quiescent state. The authors determined that the risk of resistance is independent of the presence and extent of quiescence if therapy involves only a single drug. Once two or more drugs are used, the risk of resistance increases with the fraction of quiescent stem cells. The risk of resistance emerging during therapy was again found to be negligible as compared to resistance arising before the start of treatment. These findings have important implications for the design of treatment strategies since they show that the use of drugs reducing the extent of quiescence among cancer stem cells cannot effectively decrease the chance of resistance. Komarova et al. used their earlier mathematical model (Komarova, 2006) to computationally investigate the probability of treatment success when combining one to three drugs, with and without cross-resistance (Komarova et al., 2009). The authors assumed that many mutations can confer resistance to one drug, but only one mutation confers resistance to multiple drugs; this assumption mirrors the scenario of known resistance mechanisms to the BCR-ABL kinase inhibitors imatinib, dasatinib, and nilotinib used in the treatment of CML. When the authors applied their framework to CML, they concluded that a combination of two, but not three, drugs improves the therapeutic outcome for this disease.

One common feature of these models of genetic resistance is that the treatment effect was formulated as an additional probabilistic cell death rate of sensitive cells, separate from the underlying birth and death process model with constant rates of cell growth and death. When using such assumptions, a drug cannot alter the proliferation rate of either sensitive or resistance cancer cells. A main effect of targeted therapies (e.g., imatinib, erlotininb, gefitinib), however, is the inhibition of cancer cell proliferation. Reduced growth rates of cancer cells in turn lead to a diminished probability of resistance since there is less chance that resistant cells arise during sensitive cell divisions. To relax this assumption, we utilized a non-homogenous multi-type birth-death process model wherein the birth and death rates of both sensitive and resistant cells are dependent on a temporally varying drug concentration profile, and studied resistance dynamics under general time-varying treatment schedules (Foo and Michor, 2009, 2010). Later on this stochastic framework was coupled with pharmacokinetic models incorporating drug absorption and elimination processes within the body (Foo and Michor, in preparation). This approach allows for a consideration of the effects of metabolic processes and patient-level variability in pharmacokinetics on the evolution of drug resistance.

2.2.2. Deterministic models of anti-cancer therapy

So far, we have discussed models in which the process of acquiring resistance mutations is stochastic and, in many cases, the growth of sensitive and resistant cell populations is also governed by a stochastic process. However, several deterministic mathematical models of resistance have also been proposed in the literature (Swan, 1981; Birkhead and Gregory, 1984; Panetta, 1996; Jackson and Byrne, 2000; Gatenby and Vincent, 2003; Michor et al., 2005). In 1981, Swan introduced a model of radiotherapeutic resistance using first order linear kinetics to describe the sensitive and resistant cell populations. In 1984, Birkhead and Gregory introduced a model consisting of a system of difference equations to investigate the dynamics of resistance to a single cytotoxic agent as well as to multiple drugs. These authors later proposed a multi-compartment model describing both cycling and active sensitive and resistant cell populations (Birkhead et al., 1987). In both models, the authors assumed that chemotherapy reduces the sensitive cell population by a constant fraction, and provided a means of evaluating the effectiveness of treatment strategies by predicting tumor response and the evolution of resistance. In 1996, Panetta introduced a competition model that incorporated the effect of tumor-normal cell interactions during periodically pulsed chemotherapy. This author also extended his model to describe a resistant tumor subpopulation and discussed strategies to prevent disease recurrence when resistant cells are not cross-resistant to multiple drugs. In particular, he considered the question of when to switch therapies if there are two non-cross-resistant drugs available. The author concluded that treatment regimens should be switched sooner if the rate of induction of resistance to the second drug is large or if the second drug is very effective.

Later on, Jackson and Byrne (2000) studied the effect of the vasculature on the response of tumor cells to therapy. The authors considered a deterministic PDE model describing the intratumoral drug concentration and density of cancer cells, and treated the tumor as a continuum of two cell types (sensitive and resistant), which may differ in both their proliferation rate and their response to therapy. In this study, the authors investigated the tumor response to continuous infusion and bolus injection of chemotherapeutic drugs in the presence and absence of drug-resistant subpopulations. Interestingly, they found that when the tumor contains a drug-resistant population, continuous infusion significantly increases the time to cure, thus rendering bolus injection the preferred strategy. This work fits into a large body

of literature on PDE models of tumor growth (not necessarily focused on the problem of drug resistance), e.g. Adam (1987), Byrne and Chaplain (1995) and Greenspan (1972).

In 2003, Gatenby and Vincent designed a Lotka–Volterra model of tumor and healthy cells to study the effects of treatment with cytotoxic drugs. The authors found that in general, cytotoxic drugs alone are insufficient to eradicate the tumor, since there are two main barriers to achieving a complete remission of the tumor: evolving populations tend to produce resistant clones which are able to drive the system back to an equilibrium abundance, and drug-induced alterations of the tumor microenvironment change the selection pressure and select for tumor cells with larger evolvability. In 2009, Monro and Gaffney modeled the dynamics of chemotherapy resistance in a model of palliative treatment, when tumor eradication is not feasible. In such cases, the goal of treatment is to prolong survival and improve the quality of life. Using a simple ODE-based model of tumor growth with Gompertzian dynamics, the authors predicted that reduced chemotherapy treatment can prolong survival times due to the effects of competition between the sensitive and resistant cells.

2.2.3. Alternative approaches to therapy

Most of the evolutionary mathematical models discussed so far have focused on cytotoxic or targeted drugs whose objective is to kill cancer cells or inhibit their proliferation. However, there have also been some efforts to model alternative approaches to treatment, such as the use of viruses as anti-tumor agents, modification of the tumor microenvironment, and altering the behaviors of stromal or immune cells (e.g. Wodarz, 2001; Maley et al., 2004; Silva and Gatenby, 2010). For example, Maley and colleagues studied an alternative evolutionary approach; they proposed to use "boosters" of benign cells to increase their fitness (Maley et al., 2004). They designed a computational model to investigate the dynamics of mutations emerging in cells that proliferate on a two-dimensional lattice and harbor a small number of oncogenes, tumor suppressor genes, genes preventing mutator phenotypes, and genes conferring sensitivity to drugs. To investigate the dynamics of competition, they assumed a constant population size, and showed that therapies that increase the fitness of benign cells are effective in all stages of the disease when combined with traditional chemotherapy. In another study, Silva and Gatenby (2010) introduced a two-dimensional cellular automaton model to study the effects of a combined treatment strategy of glucose restriction and chemotherapy aimed at stabilizing tumor size and minimizing chemoresistance. The authors assumed that phenotypic chemoresistance and proliferative capacity are inversely related – resistance to cytotoxic drugs requires expenditure of resources and energy which reduces cell proliferation rates – and that increased robustness to chemotherapy generated by the hypoxic core of a tumor confers fragility to glucose restriction due to the Warburg Effect. The authors found that sequential administration of glucose competitors and chemotherapeutics provide the best outcome when the goal is to stabilize tumor size and minimize resistance. In general, mathematical modeling of non-cytotoxic, alternative approaches to therapy remains an area for greater development, as discussed below.

2.3. Optimal dosing strategies

The design of optimal drug administration schedules to minimize the risk of resistance represents an important issue in clinical cancer research. If drugs are administered at sufficiently low doses, no drug holidays are necessary to limit the side effects and reduce patient toxicity; if drugs are administered at more concentrated doses, however, then rest periods are needed to limit toxicity. Such drug holidays can lead to an exponential rebound of the tumor cell population and hence pose a significant risk for the emergence of resistance. Several clinical studies have been performed to identify optimum dosing frequencies, concluding in some cases that a low-dose continuous strategy for chemotherapy is more effective (Hryniuk, 2001). Other studies have found that more concentrated dosages are beneficial (Lake and Hudis, 2004). The advantage of a low-dose continuous approach is often attributed to its ability to prevent angiogenesis rather than leading to low rates of resistance (Hahnfeldt et al., 2003) Such strategies are often implemented as combination therapy, sometimes including a second drug administered at a higher dose in a pulsed fashion.

A significant amount of research effort has been devoted to developing mathematical models to identify the most effective chemotherapeutic administration regimens using optimization and control techniques (see Martin and Teo, 1993 and references therein). While many models are aimed at rapidly minimizing the total tumor burden, long-term treatment success may require a more nuanced approach. Long-term patient survival hinges upon the control of both drug-resistant subpopulations within the tumor as well as the total tumor burden. The simultaneous control of these two aspects of tumor growth is difficult, as they are often accomplished by exerting opposing evolutionary selection pressures. In addition, these goals must be achieved under the constraints of avoiding long-term toxicity and dose-limiting side effects. Such constraints vary between patients as well as between drug and tumor types, and are often difficult to ascertain from limited clinical data available. Thus, the mathematical problem of therapeutic optimization is complicated by two issues: the formulation of an objective function in terms of model variables that realistically represents patient survival, and dealing with often incomplete or uncertain constraints on the optimization space.

Thus, we first focus specifically on previous attempts to identify schedules that minimize the size of drug-resistant subpopulations, e.g. Costa et al. (1992, 1994). In 1992, Martin et al. used optimal control techniques to maximize host survival time – the time during which the total tumor size can be constrained below a specified level. The authors used a deterministic differential equation model representing sensitive and fully resistant cell populations following Gompertzian, logistic or exponential growth laws. The total tumor cell population was modeled as a control variable, and the optimal tumor burden was identified as a function of the initial number of resistant cells. The authors found that, assuming Gompertzian growth, a large tumor burden can significantly increase survival time. Similarly, Costa et al. (1992) used a deterministic differential equation model to describe the evolution and treatment of a tumor containing drug-resistant cells. The authors used the concentration of the drug at the tumor site as the control variable, and defined the optimal schedule as the one minimizing the total tumor size at a given time. They assumed that the cell kill rate is linearly proportional to the drug concentration, and showed that the optimal strategy was the administration of the maximally allowed drug concentration. In a subsequent study, the authors extended this model to include pharmacokinetic effects to study the interplay between drug decay and drug resistance (Costa et al., 1994). In this paper, they used the concentration of the drug injection instead of the concentration at the tumor site as the control variable, and concluded that the strategy corresponding to the maximum rate of drug injection is optimal under the exponential model of cell growth, but suboptimal for other models of cell growth.

In 2003, Kimmel and Swierniak described an optimal control approach to identify administration schedules for cell cycle-specific chemotherapeutic agents (Kimmel and Swierniak, 2005). The authors aimed to minimize the total number of cancer cells at the end of treatment while incorporating a toxicity constraint on

normal tissues. They formulated an optimal administration problem in the context of drug-resistance due to gene amplification, using an infinite system of differential equations based on a branching random walk model of the gene amplification dynamics. Another optimal control problem addressing drug resistance due to gene amplification in a non-cell-cycle specific model was investigated by Ledzewicz et al. in 2006 (Ledzewicz and Schattler, 2009).

In contrast to the above approaches to optimal chemotherapy, Coldman and Murray (2000) used a model in which tumor growth was governed by a multi-type stochastic pure birth process. The authors considered situations in which two drugs were administered to a heterogeneous cell population consisting of cells sensitive to one or both of these drugs. They incorporated toxic effects of chemotherapy on normal tissues and formulated an optimal control problem to maximize the probability of tumor cure times the probability of no toxicity. The authors concluded that early intensification of therapy is beneficial in situations in which resistance is likely. The authors additionally applied the model to clinical trial data for adjuvant treatment of breast cancer and described the optimal treatment regimen for this situation. The authors later extended their framework to investigate the effect of heterogeneity in model parameters across a population of patients on the optimal strategy (Murray and Coldman, 2003). Later on, we designed a stochastic process approach to identify optimum dose administration strategies where the birth and death rates of both sensitive and resistant cells are dependent on a temporally varying drug concentration profile (Foo and Michor, 2009, 2010). Katouli and Komarova (2010) developed a methodology for identifying the optimal scheduling for cyclic treatment strategies – alternating administration of two drugs. The authors utilized their framework published earlier (Komarova and Wodarz, 2005) and incorporated the phenomenon of cross-resistance, in which one mutation confers simultaneous resistance to two drugs. Using a deterministic simplification of this framework, the authors designed a systematic method for identifying the optimal timing and administration order of two drugs with given characteristics.

Recently, we applied the methodology developed in Goldie and Coldman (1983, 1984) to identify optimal dosing strategies to prevent or delay development of drug resistance in EGFR-mutant lung cancer under various treatment regimens of erlotinib (Foo et al., 2012; Chmielecki et al., 2011). Using combined toxicity data from various Phases I and II clinical trials of erlotinib to inform constraints, we found that high-dose pulses with low-dose continuous therapy impede the development of resistance to the maximum extent, both pre- and post-emergence of resistance. An optimized strategy was tested in vitro and was found to delay the emergence of resistance significantly as compared to current standard dosing procedures. This strategy is currently being implemented as a clinical trial at the Memorial Sloan-Kettering Cancer Center. In addition, using our model, we found that the probability of resistance is greater in fast versus slow drug metabolizers, suggesting a potential mechanism, unappreciated to date, influencing acquired resistance in patients. We have also recently extended this model to evaluate the impact of combinations of EGFR-targeted therapeutics (e.g., erlotinib) and cytotoxic therapeutics (e.g., paclitaxel) and identify strategies that impede the outgrowth of primary T790M-mediated resistance in NSCLC populations (Mumenthaler et al., 2011). In this approach, we systematically explored the space of combination treatment strategies and demonstrated that optimally timed sequential strategies yielded large improvements in survival outcome relative to monotherapies at the same concentrations. Our investigations revealed regions of the treatment space in which very low-dose sequential combination strategies, after preclinical validation, may lead to a tumor reduction and improved survival outcome for patients with T790M-mediated resistance with minimal toxicity. These strategies will also be tested in the clinic in the future.

3. Short-term open questions

Despite the significant research effort devoted to the elucidation of the evolutionary dynamics of resistance to anti-cancer therapy, many open questions remain. In this section, we provide our perspective on the short-term open questions in the field. In particular, we first discuss the limitations of the existing models and several areas in which refinement to these models is needed to more accurately reflect the biological processes.

Several studies have already been undertaken to apply models of pre-existing resistance to clinical data and predictions (Diaz et al., 2012; Chmielecki et al., 2011; Mumenthaler et al., 2011). One theme emerging from these studies is the need for a stronger linkage between models of pharmacokinetic processes (e.g. drug absorption and metabolism kinetics) and evolutionary models of drug resistance. To date, we have utilized only basic pharmacokinetic models to describe the connection between administration schedules and the amount of drug available to cancer cell populations at any given time (Foo et al., 2012).This study has demonstrated the impact of pharmacokinetic processes (and inter-patient variation in them) on clinical resistance outcomes. However, these processes are highly complex in nature – for example, it is known that metabolites (intermediate products of drug metabolism) are associated with some targeted cancer therapies, and that these metabolites are also likely play a role in changing evolutionary pressures on tumors (e.g. Togashi et al., 2010). Therefore, the incorporation of more detailed and accurate descriptions of pharmacokinetic processes is crucial for evolutionary modeling of resistance dynamics during treatment. The development of these models of course intricately depends on the availability of accurate data upon which the models can be based; such data needs to become more freely available for the development of the field. This issue is discussed in more detail below.

There is also a need for more models aimed at understanding the role of the tumor microenvironment on the evolution of resistance. Tumors frequently display a substantial amount of spatial heterogeneity – both in terms of cell type population composition as well as microenvironmental factors such as drug, oxygen and glucose concentration (Hsu and Sabatini, 2008). Recent studies have suggested that in many cancers, the emergence of drug resistance is driven by the tumor microenvironment. For example, heterogeneity in drug concentration can create niches within tumors that enable novel modes of adaptation, and cellular response to therapies may be inhibited or enhanced based on the local nutrient density (Anderson et al., 2006; Tredan et al., 2007; Wu et al., 2013). Spatial population heterogeneity can also be expected to modulate the nutrient microenvironment. Drug-resistant cells may uptake and metabolize drug and nutrients at differing rates (or even using different processes) than their drug-sensitive counterparts, thus, contributing further to the establishment of environmental heterogeneity. Therefore, more explicit spatial models of the tumor microenvironment and the feedback between heterogeneous cell populations and their environment must be developed to fully understand the spatial dynamics of resistance in tumors. Again, the generation of this literature depends on the as-of-yet limited availability of accurate clinical and preclinical data.

Depending on the context of the specific drug and cancer type being addressed, various other model refinements may be necessary to gain a full understanding of resistance dynamics. For example, many anti-cancer therapies are specific in their mode

of action to a particular stage of the cell cycle; such situations will require more detailed models of the population dynamics to reflect the stage-specificity in reproduction, mutation and death events. Alternatively, to accurately capture the dynamics of certain cancers, such as leukemias, a refined model of the population structure (i.e. cell differentiation hierarchy) is necessary, especially since current therapies may be specific to certain levels of the differentiation hierarchy. For certain drugs, a wide variety of resistance mechanisms have been reported; for considering these as well as combination therapies it is important to also incorporate multiple cell types and mechanisms of resistance. In addition, recent single cell profiling studies have revealed an extraordinary extent of heterogeneity in phenotype even in genetically identical cell populations (Elowitz et al., 2002; Becskei and Mattaj, 2005; Kaern et al., 2005; Feinerman et al., 2008). In the future, it may be necessary to consider this widespread heterogeneity and include fitness distributions and stochasticity in growth and death rates. As above, the availability of appropriate data will be crucial for developing these models.

4. Long-term open questions

We have so far discussed a large and growing body of work aimed at developing an understanding of the evolutionary progression that leads to resistance in tumors. These approaches have led to a more complete picture of how various factors (e.g. patient pharmacokinetics, cancer and drug type, resistance mechanism and tumor microenvironment) impact the dynamics of drug resistance. Even more importantly, they have led to a greater appreciation of the culpable role of various treatment strategies in selecting for drug resistance. A long-term goal of these efforts is the following: can we utilize these models and the insights they provide to delay or prevent the evolution of resistance? To put it another way, can we design effective strategies to harness or direct the inevitable evolution of cancer cell populations into phenotypic states in which either eradication or chronic control of tumors is possible?

A first approach towards that goal is to utilize evolutionary models for optimal selection of drug scheduling and combination strategies. Several studies along these lines have already been published, but much more work is required to be able to provide general guidelines for the design of treatment schedules and combination strategies. Many questions still remain: for example, is patient outcome generally better if targeted therapies are administered in combination with generic cytotoxic drugs or other targeted therapies? If second-line targeted therapies that overcome resistant strains to first-line therapies are available, should they be administered at the start of treatment, or only after failure of the first therapy? What is the best administration schedule for these types of combinations, and what are the toxicity constraints? Should we administer long course chemotherapy and keep patients on the drug beyond progression of their disease? Recent studies have shown that drug antagonism between antibiotics may slow the evolution of bacterial resistance (Yeh et al., 2009). Along similar lines, it may be possible to utilize suppressive drug combinations to prolong the time until cancer progression.

Although evolutionary models can greatly aid in the identification of optimal drug scheduling and combination schedules to delay disease progression due to resistance, this approach may often result in only temporary prolonged patient survival. Most tumors at diagnosis are heterogeneous and often harbor drug-resistant subpopulations. Under conventional therapies aimed at killing drug-sensitive cells, basic evolutionary theory predicts that these resistant subpopulations will be selected for and their outgrowth will become inevitable. Radically targeting any cell type (via maximal kill) can promote the rate of evolution of resistance; thus, a constant search for n^{th}-generation therapies against the $(n-1)$th-generation resistant mutants does not yield permanent solutions either.

An alternative, perhaps longer-term approach is to identify treatment strategies that do not rely upon cytotoxic action against a specific cell type or utilize current therapies at sufficiently low doses so as not to impose any stronger selection than is necessary within the tumor. Under this framework, modifications to desired clinical outcomes should be considered. In particular, chronic tumor control and minimization of the resistant fraction of tumors may be better endpoints than rapid tumor debulking or maximal cell kill. In recent work, Pepper has argued that targeting "public goods" within a tumor may provide a good alternative strategy (Gioeli, 2011). Public goods refer to external products secreted into the microenvironment by cancer cells, which benefit both themselves and other nearby cells. Drugs that target these public goods may be less vulnerable to resistance since they do not impose strong selective pressures on any specific cell type. A similar approach would be to target limited resources within the microenvironment with the goal of simply preventing unbounded growth or metastasis of the tumor, rather than tumor reduction. The microenvironment has become an attractive target in cancer therapy with the design of drugs that modify the current state of the tumor microenvironment, such as angiogenesis inhibitors, hypoxia-activated pro-drugs, and immunotherapy agents; here, evolutionary theory can serve as a tool to help understand and improve the design and use of such therapies.

The final long-term question we focus on is biomarkers. In particular, can we use evolutionary theory and modeling to develop useful biomarkers to help guide treatment and prognosis prediction? For example, sensitive cell turnover and death rates play an important role in determining the probability of developing resistance. This observation leads to interesting clinical questions – can the frequency of apoptosis in a tumor, along with tumor size, be used as a prognostic biomarker of resistance? Alternatively, can we utilize tumor shape or its microenvironmental profile as predictors of tumor aggressiveness or resistance development? Once resistance has been detected clinically, are there aspects of the tumor (e.g. size at recurrence or growth rate) that can be utilized to predict the diversity or aggressiveness of the recurrent tumor? If so, these markers could be used to guide treatment decisions post-tumor recurrence. An investigation of these types of questions is only just beginning and we believe that evolutionary modeling may provide many insights into the design of useful biomarkers to help guide treatment in the clinic.

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