Resisting Resistance

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Abstract

Targeted therapies, immunotherapies, and improved chemotherapies are being developed to reduce the suffering and mortality that come from human cancer. Although these approaches, and in particular combinations of them, are expected to succeed eventually to a large degree, they all suffer one obstacle: Populations of replicating cells move away—typically in a high-dimensional space—from any opposing selection pressure they encounter. They evolve resistance. It is possible, however, to develop a precise mathematical understanding of the problem and to design treatment strategies that prevent resistance if possible or manage resistance otherwise. In this article, we present the fundamental equations that characterize the evolution of resistance. We provide formulas for the probability that resistant cells exist at the start of therapy, for the average number and sizes of resistant clones, and for the probability of successful combination treatment. We also demonstrate that developing new therapies that only maximize the killing rate of cancer cells may not be optimal, and that instead the parameters determining the fraction of resistant cells and their growth rate have a larger effect on the long-term control of cancer. These mathematical tools inform the search process for optimal therapies that aim to cure cancer.

PRE-EXISTING RESISTANCE

Despite the effectiveness of many cancer therapies in reducing tumor burden for significant amounts of time, ranging from several months to multiple years, acquired resistance to treatment remains one of the biggest challenges in the effort to cure cancer (Bozic et al. 2013, Chapman et al. 2011, Diaz et al. 2012, Engelman et al. 2007, Gorre et al. 2001, Katayama et al. 2011, Misale et al. 2012). Successful management, or prevention, of resistance requires deciphering the mechanisms by which it arises, and in particular whether it stems from pre-existing subclones or arises de novo during therapy. Although both of those mechanisms lead to acquired resistance in vitro (Hata et al. 2016), it is difficult to demonstrate, with the same level of certainty, which mechanism is responsible for acquired resistance in patients. This uncertainty primarily results from the inability to detect small resistant clones in vivo.

Using evolutionary principles and mathematical techniques, we have argued that pre-existing resistant subclones should generally be present in all patients with late-stage metastatic disease, for treatments for which there exists at least a single point mutation that can confer resistance (Bozic & Nowak 2014, Bozic et al. 2013, Diaz et al. 2012). For such treatments, this result does not imply that new resistant clones cannot also arise from the sensitive or persistor populations (Sharma et al. 2010) during treatment, but that optimal treatment strategies must take into account that resistant subpopulations of cancer cells are already present at the start of therapy.

From the perspective of mathematical analysis, the most important quantity determining if resistance is present in a cancerous lesion is its size M, representing the number of cancer cells (Goldie & Coldman 1979). To describe the intuition behind the argument that resistance is generally pre-existing, imagine a simple scenario in which a single cancer cell initiates a lesion. Its progeny cells are dividing until they reach M cells. Each division leads to an additional cell. Therefore, it takes $M - 1$ divisions to reach M cells. For large M we can neglect the term "-1." A lesion 1 cm in diameter contains roughly $M = 10^9$ cancer cells. In order to reach this size, the lesion has experienced 10^9 cell divisions.

Now let us assume that there is a single position in the genome that, if mutated, can provide resistance to a particular treatment. This means that at each division, a resistance mutation is produced with probability $u = 10^{-9}$, which is approximately the normal point mutation rate (Araten et al. 2005, Tomasetti et al. 2013). In other words, there are a billion attempts (divisions) to get an event that has a one in a billion chance of occurring (resistance mutation). The expectation is that one of those one billion divisions results in a resistance mutation.

In a more general scenario, there is also cell death in the growing lesion. In that case, it will take more than M divisions to reach M cells, and therefore even more resistant cells will be generated. However, many of them can be lost by cell death. Mathematical models for the evolution of resistance to single therapies typically consider a two-type branching process model (Athreya & Ney 1972, Kimmel & Axelrod 2015) in which a single sensitive cell initiates a lesion (Diaz et al. 2012, Iwasa et al. 2006, Komarova 2006, Komarova & Wodarz 2005). Cancer cells divide with rate b and die with rate d. If b is greater than d, we can have clonal expansion. In this model, although large populations of cancer cells grow inevitably, individual cells and small populations are subject to high probabilities of extinction. The death-birth ratio, $\delta = d/b$, is the probability that the lineage of any single cell will go extinct due to stochastic fluctuations. For realistic values of division and death rates in solid tumors, δ is typically larger than 0.5 and could be as high as 0.99 or higher (Beerenwinkel et al. 2007; Bozic et al. 2010, 2016). That means that the lineage of any individual cell is more likely to die out than to survive. For $\delta = 0.99$, even a population of 50 cells has a 60% chance of going extinct. The average number of divisions needed to reach M cells is $M/(1 - \delta)$. For example, if $\delta = 0.99$, then it takes 100 billion cell divisions to generate a lesion that contains one billion cells.

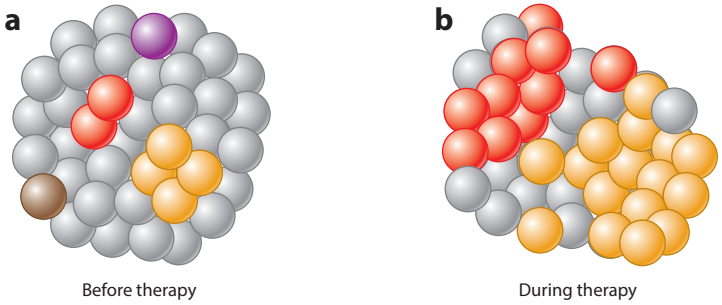

Figure 1

Resistance to therapy. (*a*) Mathematical modeling predicts that radiographically detectable tumors contain multiple resistant subclones (*colored spheres*). For parameters that may be typical for targeted therapies (Diaz et al. 2012), we expect that one in a million cells is resistant prior to treatment. Cells sensitive to treatment are depicted in gray. (*b*) During treatment, sensitive cells (*gray*) decline, and some small resistant subclones are lost due to stochastic drift. Successful resistant clones (*red* and *yellow*) expand during therapy.

If there is a single position in the genome that can lead to resistance if mutated, then a resistance mutation is produced at every cell division with probability $u = 10^{-9}$. Once a resistance mutation is produced, it will survive with probability $1 - \delta$. If $M = 10^9$, the expectation is again that one division will result in a resistance mutation with a surviving lineage because $1/(1 - \delta)$ and $(1 - \delta)$ cancel out.

The above result can be generalized to the case of multiple resistance mutations. Let n be the number of positions in the genome that can provide resistance if any single one of them is mutated. Then the probability that "successful" resistant cells (**Figure 1**), i.e., those whose progeny will not be lost due to stochastic drift, are present in a lesion of size M is (Komarova 2006, Komarova & Wodarz 2005)

$$P_M \approx 1 - e^{-Mnu}. \quad (1)$$

If there is a single position in the genome that can provide resistance if mutated, $n = 1$, then the probability that a lesion containing one billion cells has resistant cells that lead to relapse is $P_M = 0.63$. Thus, the majority of such lesions will relapse. If there are $n = 10$ positions in the genome that provide resistance if mutated, then almost 100% of such lesions will relapse.

Whereas the size of the lesion and the resistance mutation rate determine the probability that resistant cells capable of causing relapse are present in a lesion, the size of the pre-existing resistant subpopulation also depends on the ratio of death and division rates of cancer cells, δ (Bozic et al. 2013, Iwasa et al. 2006, Komarova et al. 2007, Michor et al. 2005, Tomasetti & Levy 2010a). The average number of resistant cells is given by (Bozic et al. 2013)

$$y \approx Mnu\frac{\log(M\ (1-\delta))}{1-\delta}. \quad (2)$$

In a lesion with $M = 10^9$ cancer cells, if there are $n = 10$ positions in the genome that can cause resistance if any one of them is mutated, the expected number of resistant cells is approximately 200 if there is no cell death ($\delta = 0$) and approximately 16,000 if the death-birth ratio is close to 1 ($\delta = 0.99$).

For parameter values that are typical for single targeted therapies, it is expected that approximately one in a million cells in a lesion is resistant to therapy at the start of treatment (Diaz et al. 2012). These estimates assume that the mutations that provide resistance do not alter the fitness of the cell carrying them prior to therapy. Therefore, the above calculations assume that resistance

mutations are neutral prior to treatment. They accumulate as passenger mutations (Bozic et al. 2016, Iwasa et al. 2006, Jones et al. 2008, Ling et al. 2015, Sottoriva et al. 2015, Waclaw et al. 2015, Williams et al. 2016, Yachida et al. 2010).

Conversely, some mutations responsible for resistance to targeted therapies may not be neutral, but act as drivers of the cancer themselves. Because they provide a growth advantage to cells carrying them, these driver-resistance mutations are able to reach much higher frequencies in the population. If present at very high frequencies, they can also lead to primary resistance. Examples of such mutations are *KRAS* mutations in colorectal cancer, which lead to primary resistance to EGFR inhibitors if they are detectable in the untreated tumor. In this case, the mathematical models predict that there is heterogeneity among patients concerning eligibility for treatment. Some patients may not respond at all because the resistant mutation is already clonal prior to treatment. The evolution of resistance in the case when resistant mutants are advantageous has been studied by Bozic & Nowak (2014), Durrett & Moseley (2010), Haeno et al. (2007), and Iwasa et al. (2006).

Finally, mutations responsible for resistance to therapy may also be deleterious and result in reduced fitness of resistant cells compared to sensitive cells. For example, Leder et al. (2011) measured the fitness of 11 point mutations providing resistance to the tyrosine kinase inhibitors imatinib, dasatinib, or nilotinib used in the treatment of chronic myeloid leukemia. Three mutations resulted in increased fitness compared to the sensitive population (they included the T315I mutation, which was the most fit and conferred resistance to all three drugs). However, eight resistance mutations resulted in decreased fitness, with net growth rates 5–40% lower than the sensitive population. Even if all resistance mutations are as deleterious, they are still expected to be present in late-stage metastatic cancers (Bozic & Nowak 2014).

In some patients, point mutations may not be the dominant mechanism of resistance to targeted therapies. Acquired resistance to anti-EGFR agents in colorectal cancer occurs through the emergence of *KRAS* mutations in approximately 50% of the patients (Bardelli et al. 2013). Bardelli et al. reported that *MET* amplification is responsible for resistance to anti-EGFR treatment in three out of four colorectal cancer patients whose relapsed tumors did not harbor *KRAS* mutations. Similarly, *MET* amplification causes resistance to EGFR blockade in approximately 20% of relapsed non-small-cell lung cancers (Turke et al. 2010).

The rate of gene amplification in some cancer cells can be as high as 10^{-4} per gene per cell division (Tlsty 1990, Tlsty et al. 1989). This corresponds to the average resistant cell fraction as high as one in a thousand (for no cell death occurring in the lesion, $\delta = 0$) to one in six cells (when the death-birth ratio is $\delta = 0.99$). These numbers suggest that, if resistance is mediated via gene amplification, then resistant cells can be present at detectable frequencies in patients prior to treatment. Indeed, in the two out of three colorectal cancer patients (Bardelli et al. 2013) and four out of four lung cancer patients (Turke et al. 2010) in which MET amplification was responsible for acquired resistance to anti-EGFR agents, small pre-existing subpopulations with *MET* amplification were detectable in pretreatment samples.

The complete description of the size of the resistant population would require knowledge of its full probability distribution. However, an explicit solution for the resistant mutant size distribution in a tumor containing M cells has proven elusive in the general, fully stochastic setting that includes cell death (Zheng 1999). This problem is a generalization of the famous Luria-Delbruck model for the evolution of resistance in bacterial populations (Luria & Delbruck 1943). Recently, there has been significant progress in the study of the resistant mutant distribution in some limits of the fully stochastic setting (Kessler & Levine 2013, 2015) and in a semistochastic setting (Dewanji et al. 2005, Keller & Antal 2015). Because the expressions for the probability distribution for the

Table 1 Formulas for the evolution of resistance to single therapies

Quantity	Formula[a]	Reference(s)
Probability resistant cells are present	$1 - \exp(-\frac{Mub}{d}\log\frac{1}{1-d/b})$	Iwasa et al. 2006, Michor et al. 2005
Probability successful resistant cells are present	$1 - e^{-Mnu}$	Komarova 2006, Komarova & Wodarz 2005
Probability resistant stem cells are present[b]	$1 - \exp(-\frac{Mu'b'}{d'}\log\frac{1}{1-d'/b'})$	Tomasetti & Levy 2010b
Expected number of successful resistant clones	Mu	Bozic & Nowak 2014
Median size of k-th resistant clone	$\frac{Mu}{1-d/b}(2^{1/k} - 1)$	Bozic & Nowak 2014

[a] M is the number of cancer cells in a lesion, b is the division rate of cancer cells, d is the death rate of cancer cells, and u is the resistance mutation rate.
[b] b', d', and u' are the effective division, death, and resistance mutation rates of stem cells, respectively, as described in the text.

size of the resistant population in the stochastic case with cell death are generally very technical, we restrict our attention to describing the probability that (successful) resistance is present at all (**Table 1**), and the average number of resistant cells. The average is biased by rare realizations of the stochastic process in which the number of resistant mutants is very large, and tends to overestimate the typical number of resistant cells. We use it because it is a simple description of the full probability distribution, and because formulas for the average are available also in the general case of any number of drugs (Bozic et al. 2013).

COMBINATION THERAPY

Cross-Resistance

Combination therapies of two or more targeted agents have been proposed as a means to combat acquired resistance (Bhang et al. 2015, Bozic et al. 2013, Glickman & Sawyers 2012, Sawyers 2013). The crucial parameter determining the success of combination therapy is the number of mutations that can provide resistance to all drugs in the combination simultaneously (cross-resistance). The reason is that the same basic evolutionary principles hold in the case of a drug combination: If there is even a single position in the genome that can provide resistance to the combination if mutated, the combination will not be curative in the majority of patients with late-stage metastatic disease. Thus for a combination to have the potential to be curative, it should not be opposed by cross-resistance.

For a combination of two drugs, let n_1 and n_2 denote the number of positions in the genome that can provide resistance to drug 1 and 2, respectively. The number of positions that can provide resistance to both drugs is $n_{1,2}$. Even if two drugs in a combination have some level of cross-resistance ($n_{1,2} > 0$), the combination can still be beneficial over single therapies as there might be a reduced number of resistance mechanisms operational against both drugs, compared to each individual drug. The combination with $n_{1,2}$ positions that can provide resistance to both drugs if mutated is expected to behave comparably to a monotherapy opposed by $n_{1,2}$ resistance mutations. If $n_{1,2}$ is significantly lower than the number of mutations opposing individual drugs, then combination therapy is beneficial. In line with these arguments, Komarova et al. (2009) studied the combination of two versus three existing drugs for the treatment of chronic myeloid leukemia, in a setting in which there exists a mutation that provides resistance to all (two or three) drugs. Despite cross-resistance, they found that combining two drugs led to prolonged response

Table 2 Expected number of cells resistant to the combination of k drugs with no cross-resistance

Number of drugs	M[a] $= 10^5$	$M = 10^6$	$M = 10^7$	$M = 10^8$	$M = 10^9$	$M = 10^{10}$	$M = 10^{11}$
$k = 1$	0.9	12	138	1,600	18,400	207,200	2.3×10^6
$k = 2$	$\ll 1$[b]	$\ll 1$	0.002	0.026	0.34	4.3	53
$k = 3$	$\ll 1$	$\ll 1$	$\ll 1$	$\ll 1$	$\ll 1$	$\ll 1$	0.001

[a] M is the number of all cancer cells in the lesion. For each drug, there are 100 positions in the genome that provide resistance if mutated. The point mutation rate is $u = 10^{-9}$, and the death-birth ratio is $\delta = 0.9$.
[b] $\ll 1$ denotes <0.001.

compared to treatment with the single drug, but adding a third drug did not lead to a significant benefit.

The benefits of combining targeted therapies over monotherapies have been demonstrated both in preclinical models of lymphoma and colorectal cancer (Choi et al. 2014, Misale et al. 2015) and in clinical trials of *BRAF*-mutated melanoma (Larkin et al. 2014, Long et al. 2014, Robert et al. 2015). Combination of two anti-HER2 monoclonal antibodies, pertuzumab and trastuzumab, together with the chemotherapy drug docetaxel was more effective than trastuzumab and docetaxel alone for treatment of HER2-positive breast cancer (Baselga et al. 2012, Swain et al. 2015).

Two Steps to Resistance

Only a combination of two drugs with no cross-resistance is predicted to lead to long-term control in the majority of late-stage metastatic patients (Bozic et al. 2013) (**Table 2**). Combining two drugs with no cross-resistance forces the cancer cells to acquire resistance via two steps, which is significantly harder to achieve than acquiring resistance with a single point mutation. These two steps represent obtaining resistance to one drug first and then to the other. For example, let n_1 be the number of positions in the genome that can provide resistance to drug 1 if any of the n_1 positions is mutated, and n_2 be the corresponding number of such positions for drug 2. There is no mutation that provides resistance to both drugs, which means $n_{1,2} = 0$. Then the average number of cells resistant to both drugs in a lesion of size M is given by (Bozic et al. 2013)

$$y_{12} \approx M n_1 n_2 u^2 \left[\frac{\log(M(1-\delta))}{1-\delta}\right]^2. \tag{3}$$

We recall that u is the point mutation rate, and $\delta = d/b$ is the ratio of death and birth rates of cancer cells.

Three (and More Steps) to Resistance

Toxicity can be a formidable problem with combination therapies. But if toxicity can be tolerated, it would be advantageous to increase the number of drugs in the combination especially if there is no cross-resistance between individual agents. In the case of k drugs without cross-resistance, the tumor would have to develop resistance via (at least) k steps to overcome the combination.

The expected number of cancer cells resistant to the combination of k drugs with no cross-resistance in a lesion with M cancer cells is given by (Bozic et al. 2013)

$$y_{1\ldots k} \approx M n_1 \cdots n_k u^k \left[\frac{\log(M(1-\delta))}{1-\delta}\right]^k. \tag{4}$$

Here $n_1, n_2, \ldots, n_k$ are the number of positions in the genome that provide resistance to drug $1, 2, \ldots, k$ if mutated, respectively. As before, δ is the death-birth ratio of cancer cells, and u is the point mutation rate. The expression in Equation 4 is dominated by the term u^k, and decreases with k (**Table 1**). In general, combination therapies with two drugs with no cross-resistance would be enough to control metastatic cancer in most patients, and triple therapy with no cross-resistance is predicted to lead to cancer control in virtually all metastatic patients (see **Table 1**) (Bozic et al. 2013). For example, let us assume that each of the three potential therapies is opposed by 100 point mutations (i.e., $n_1 = n_2 = n_3 = 100$), but there is no overlap between mutations providing resistance to these therapies (i.e., $n_{1,2} = n_{2,3} = n_{3,1} = n_{1,2,3} = 0$). A lesion 1 cm in diameter (containing roughly $M = 10^9$ cells) is expected, according to Equation 4, to contain on average between 10^{-8} (for no cell death in a lesion) and 10^{-3} (for a death-birth ratio $\delta = 0.99$) cells resistant to the combination of these three therapies. In other words, at least a thousand such lesions are needed to find a single cell resistant to this triple therapy.

EVALUATING THE EFFECTIVENESS OF ANTICANCER DRUGS

It is likely that pre-existing resistance is the reality for many, if not most, currently available systemic cancer therapies. These therapies are not curative, but can still provide a benefit to the patient in the form of reduced tumor burden for a certain amount of time. Although cancer eradication remains a veritable task, significant improvement in the management of cancer can be obtained by devising treatments that provide the greatest long-term benefit to the patient.

Some of the critical parameters that determine the potential of a drug for long-term control of cancer are the fraction of pre-existing cells resistant to the drug and their growth rate during treatment. However, parameters describing the resistant population are usually not studied in the development of new drugs. Often only the short-term effect of the drug on the sensitive population is being optimized in the initial preclinical stages. Although drugs need to be able to reduce the size of the sensitive population or at least keep it in check, critical parameters for evaluating the long-term benefit of systemic cancer therapies are dependent on the resistant population.

Let us consider a deterministic model in which f denotes the fraction of cells resistant to a particular therapy that are present in a cancerous lesion prior to the start of therapy. Furthermore, let d be the net death rate of sensitive cancer cells and g the net growth rate of resistant cells in the presence of therapy. Then the volume of the cancer at time t after initiation of therapy, relative to the volume at the start of therapy, is given by

$$v(t) = (1 - f)\, e^{-dt} + f e^{gt}. \tag{5}$$

This functional form was shown to be a good description of the change in tumor size during therapy in in vitro and in vivo models of targeted therapy in colorectal cancer (Misale et al. 2015) and of measures that correlate with tumor volume in prostate cancer (serum prostate-specific antigen) and multiple myeloma (M protein levels) (Blagoev et al. 2014).

Equation 5 can be used to quantify the concept of primary resistance. In this case, the volume of cancer cells, v, increases even when therapy starts. This means the slope of $v(t)$ at time $t = 0$ is positive, which occurs for $fg > (1 - f)d$ (therapy 1 in **Figure 2*a***). If, conversely, $fg < (1 - f)d$, then the tumor initially does respond to treatment (therapy 2 in **Figure 2*a***).

The dynamics of relapse is primarily determined by the initial fraction of resistant cells, f, and their growth rate during treatment, g. For example, therapies with a higher fraction of resistant cells relapse earlier, all else equal. This difference in relapse time can be significant if the resistant cell fraction varies by one to several orders of magnitude (**Figure 2*b***). In **Figure 2*b***, one in a thousand cells is resistant to therapy 1 prior to treatment, whereas one in a million cells is resistant

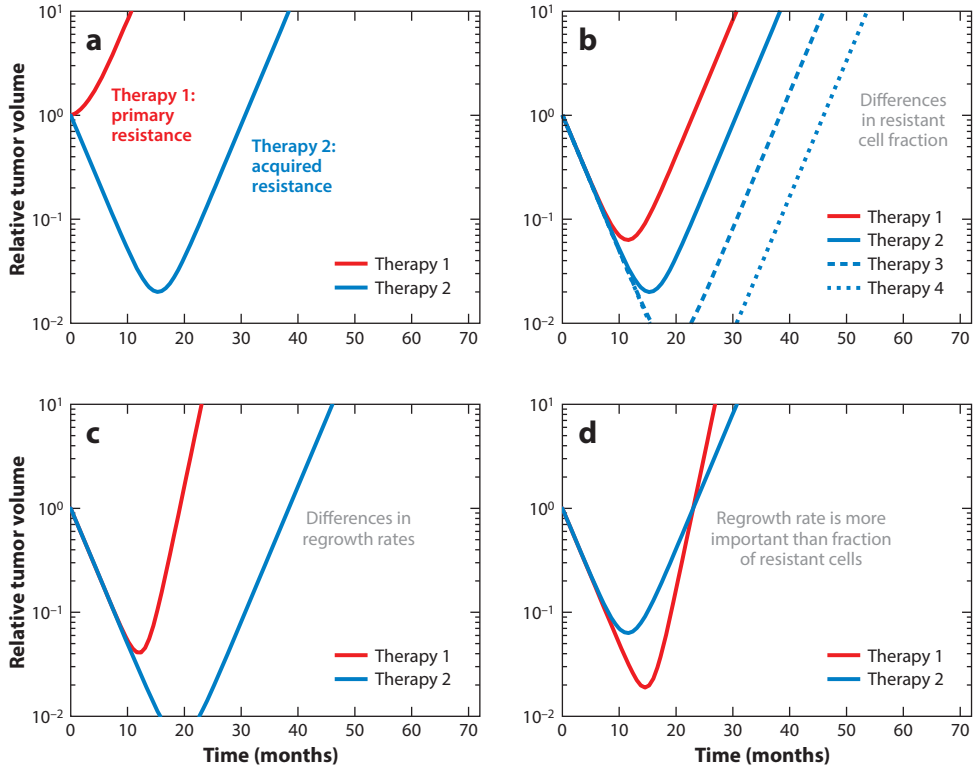

Figure 2

Dynamics of cancer under therapy. We use a simple model in which a fraction f of cancer cells is resistant prior to the start of therapy (Equation 5). Therapy is initiated at time 0. Under therapy, sensitive cells decline at rate d, and resistant cells grow at rate g. (*a*) Primary resistance occurs if $fg > (1 - f)d$ (therapy 1), and acquired resistance occurs if $fg > (1 - f)$ (therapy 2). (*b*) Differences in the pre-existing resistant cell fraction lead to differences in the effectiveness of therapy. The fraction of cells resistant to therapy 1 is $f_1 = 10^{-3}$, and the fractions of cells resistant to therapies 2–4 are 10- to 1,000-fold lower ($f_2 = 10^{-4}, f_3 = 10^{-5}$, $f_4 = 10^{-6}$). The death rate of sensitive cells and the growth rate of resistant cells under therapy are the same for all therapies: $d = 0.01$ and $g = 0.01$. (*c*) Differences in the growth rates of resistant cells lead to differences in the effectiveness of therapy. The growth rate of cells resistant to therapy 1 is $g_1 = 0.02$, and the growth rate of cells resistant to therapy 2 is $g_2 = 0.01$. The fraction of cells resistant to both therapies and the death rates of sensitive cells under both therapies are equal: $f_1 = f_2 = 10^{-5}$ and $d_1 = d_2 = 0.01$. (*d*) Differences in the growth rate of resistant cells have a larger effect on the effectiveness of therapy compared to differences in the initial resistant fraction. The fraction of cells resistant to therapy 1 is 1,000-fold lower than the fraction of cells resistant to therapy 2 ($f_1 = 10^{-6}, f_2 = 10^{-3)}$. The growth rate of cells resistant to therapy 1 is twofold higher than the growth rate of cells resistant to therapy 2 is ($g_1 = 0.02$, $g_2 = 0.01$). The death rates of sensitive cells under therapy are equal: $d_1 = d_2 = 0.01$. All rates are per day.

to therapy 4. If we assume that the measure of the effectiveness of therapy in controlling cancer is the time until the tumor reaches 10 times its initial size (measured in the number of cells), then for therapy 1 it takes 30 months to reach 10 times the initial size, whereas for therapy 4 it takes more than 50 months.

Similarly, therapies with higher growth rates of resistant cells relapse earlier compared to therapies with similar fractions of pre-existing resistant cells that are less fit (Stein et al. 2009, 2011). In **Figure 2*c***, both therapies are opposed by the same initial fraction of pre-existing resistant

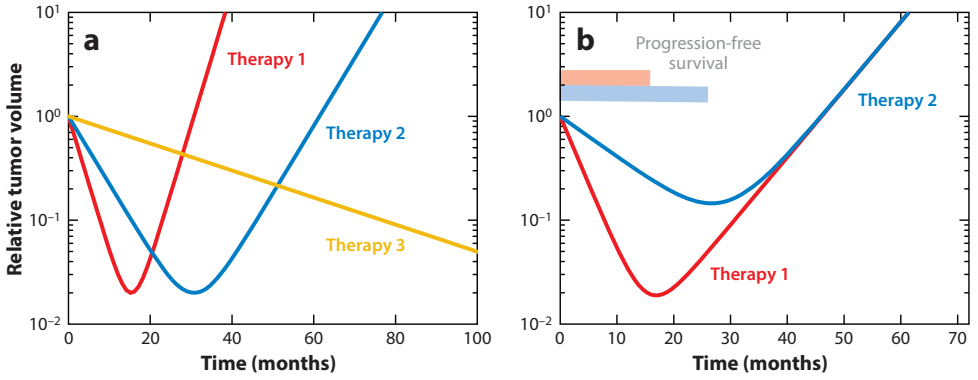

Figure 3

Killing rate and progression-free survival are not sufficient measures of long-term cancer control. (*a*) Even though therapy 1 (*red*) seems more effective in the first year of treatment due to its higher killing rate of sensitive cells, therapy 2 (*blue*) leads to longer control of cancer. The parameters are the fraction of pre-existing resistant cells, $f_1 = f_2 = 0.0001$; death rates of sensitive cells during treatment, $d_1 = 0.01$ and $d_2 = 0.005$; and growth rates of resistant cells during treatment, $g_1 = 0.01$ and $g_2 = 0.005$ per day. Therapy 3 (*yellow*) has the lowest killing rate of sensitive cells, $d_2 = 0.001$, but leads to tumor eradication as it is not opposed by resistance, $f_3 = 0$. (*b*) Therapy 1 (*red*) leads to much lower tumor burden during therapy, but it has shorter time to progression compared to therapy 2 (*blue*). The parameters are the fraction of pre-existing resistant cells, $f_1 = f_2 = 0.001$, and the death rates of sensitive cells during therapy, $d_1 = 0.01$ and $d_2 = 0.003$.

cells, but cells resistant to therapy 1 grow at a faster rate. A tumor undergoing therapy 1 will reach 10 times its initial size more than 20 months before the same tumor would undergoing therapy 2.

In general, because the size of the resistant population depends linearly on the initial fraction of resistant cells f and exponentially on the growth rate of resistant cells g, differences in the growth rate of resistant cells have a more pronounced effect on the effectiveness of therapy than differences in the fraction of resistant cells. In **Figure 2*d***, therapy 1 has a 1,000-fold higher fraction of resistant cells compared to therapy 2. The growth rate of cells resistant to therapy 2 is only twofold higher compared to cells resistant to therapy 1. However, tumors undergoing therapy 2 will reach 10 times their initial size earlier compared to tumors receiving therapy 1.

Despite that most preclinical efforts aim to maximize the short-term effect of the drug on sensitive cells, the killing rate of sensitive cells does not significantly affect the long-term control of cancer. In **Figure 3*a***, therapy 1 has a higher killing rate compared to therapy 2, and seems significantly more effective in the first year of treatment. However, therapy 2 leads to longer control of cancer, due to the lower growth rate of cells resistant to it. The initial fraction of resistant cells is the same for therapies 1 and 2. The most successful treatment, however, is therapy 3, which has the lowest killing rate but is not opposed by resistance and hence leads to tumor eradication.

The initial effectiveness of a drug in treating a disease (i.e., its killing rate or rate of replication inhibition) and the strength of resistance that oppose it are not necessarily coupled. This can be seen in the example of anti–hepatitis C virus treatments, in which some therapies have modest efficacy in inhibiting new virion production and high barrier to resistance (mericitabine; Gane et al. 2015, Guedj et al. 2012), some have high efficacy and low barrier to resistance (lomibuvir; Di Bisceglie et al. 2014, Reddy et al. 2012), and some have high efficacy and high barrier to resistance (sofosbuvir; Lawitz et al. 2013). The barrier to resistance depends on the total mutation rate giving rise to resistance and the growth rate of resistant mutants prior to and during therapy. Even in

cancer, one can think of most targeted therapies as having high effectiveness in killing cancer cells but low barrier to resistance, and immunotherapy as being slower in killing cancer cells but having a high barrier to resistance.

Additionally, some therapies may even accelerate the growth of resistant cells. For example, several mutations in the androgen receptor have been shown not only to provide resistance to the anti-androgens nilutamide and flutamide, used in the treatment of prostate cancer, but also to be in fact activated, rather than inhibited, by these therapies (Watson et al. 2015).

Progression-free survival is used as a measure of drug efficacy in patients, and therapies are often changed at progression. We recall that the tumor is initially sensitive to treatment if $fg < (1-f)d$. The minimal tumor volume during therapy is achieved at time

$$t_{\min} = \frac{1}{d+g} \log\left[\frac{(1-f)\,d}{fg}\right].$$

Here $t_{\min}$ is the time until progression. As expected, the time until progression is inversely proportional to the pre-existing resistant fraction, f, and the growth rate of resistant cells under therapy, g. However, as seen in **Figure 3*b***, drugs that have higher killing rates of sensitive cells can result in shorter times to progression, everything else equal. Even though the total tumor burden remains lower at all times in the case of the stronger drug, this drug would be deemed inferior if compared to the weaker drug on the basis of time to progression. This scenario is particularly worrisome as progression is often deemed to necessitate the switching of drugs.

Potential benefits in the form of prolonged patient survival may be obtained by optimizing dosing strategies of existing therapies (Basanta et al. 2012, Foo et al. 2012, Foo & Michor 2009). Chmielecki et al. (2011) used a combination of mathematical modeling and in vitro experiments to predict that high-dose pulses combined with a continuous low dose of EGFR inhibitors gefitinib or erlotinib would lead to a prolonged benefit in treating non-small-cell lung cancer. Das Thakur et al. (2013) studied intermittent dosing of the BRAF inhibitor vemurafenib in in vivo models of melanoma. They showed that intermittent dosing carried out on a 4-weeks-on drug and 2-weeks-off drug schedule leads to significant survival advantage. Leder et al. (2014) developed a mathematical model for gliomas undergoing radiotherapy, in which tumors contained a mix of stem-like resistant and differentiated sensitive cells. The authors also account for the dynamic plasticity of these two cell types and use it to mathematically predict the optimal treatment strategy, which showed increased survival benefit in mice. Most recently, Enriquez-Navas et al. (2016) used an adaptive approach to the dosing of chemotherapy. After the initial dose, each subsequent dose was inversely proportional to the response of the tumor to the previous dose: If the tumor shrank significantly, the dose was halved, and doubled if the tumor grew. They demonstrated that this approach can significantly prolong progression-free survival in in vivo models of breast cancer. Further insights into the parameters describing the division and death rates of sensitive and resistant populations in patients' tumors with and without therapy, and in particular their interactions (Marusyk et al. 2014), will aid in the development of mathematical models for the optimization of treatment strategies that may lead to sustained benefit in patients.

HETEROGENEITY OF RESISTANCE

Resistance mechanisms against a single therapy vary across patients. For example, ~100 different resistance mutations opposing imatinib therapy in chronic myeloid leukemia have been described to date (Gambacorti-Passerini et al. 2003, Leder et al. 2011, Shah et al. 2002). There is also increasing evidence that multiple resistance mechanisms can be responsible for resistance to therapy in a single patient's cancer (Burger et al. 2016; reviewed in Burrell & Swanton 2014).

Diaz et al. (2012) and Bettegowda et al. (2014) performed "liquid biopsies" of colorectal cancer patients receiving anti-EGFR therapies panitumumab or cetuximab. Sequencing of circulating tumor DNA fragments revealed multiple resistance mutations in three out of nine patients with acquired resistance in the Diaz et al. (2012) study and in 17 out of 24 patients in the Bettegowda et al. (2014) study. Diaz et al. searched only for resistance mutations in the *KRAS* gene, whereas Bettegowda et al. additionally probed mutations associated with resistance in *NRAS*, *BRAF*, and *EGFR* genes. Misale et al. (2014) found that multiple resistant clones often coexist in colorectal cancer cells that developed acquired resistance to EGFR blockade in vitro, as well as in the plasma of colorectal cancer patients who developed resistance to these therapies.

Although the studies using liquid biopsies point to the existence of coexisting polyclonal resistance mechanisms in patients with acquired resistance, they do not reveal the lesions in the patients that carry specific resistance mutations, and whether acquired resistance in single lesions results from monoclonal or polyclonal resistance. Recently, Juric et al. (2015) studied the mechanisms of acquired resistance to the PI(3)Kα inhibitor BYL719 in a patient with metastatic breast cancer bearing an activating *PIK3CA* mutation. They sequenced samples from 14 different lung metastases from this patient and found six different resistance mechanisms, all including biallelic inactivation of the *PTEN* gene. In this study, the authors do not report single lesions with multiple resistance mechanisms. In contrast, several studies (Burrell & Swanton 2014, Romano et al. 2013, Shi et al. 2014) routinely found multiple coexisting resistance mechanisms to the BRAF inhibitors vemurafenib and dabrafenib within the same biopsy in melanomas. This is in line with our prediction using mathematical modeling and clinically relevant parameter values that patients with metastatic disease will harbor multiple resistant subclones, even within a single radiographically detectable lesion (Bozic & Nowak 2014). More precisely, the expected number of resistant subclones in a lesion with M cells is given by

$$N_{\text{cl}} = Mu, \tag{6}$$

where u is the resistance mutation rate. Equation 6 includes only "successful" resistant subclones, i.e., those destined to survive stochastic drift (**Figure 1**). Assuming $M = 10^9$ and a point mutation rate of 10^{-9}, the expected number of resistant subclones is exactly the number of positions in the genome that provide resistance to the drug if mutated. The sizes of individual resistant clones have also been derived (Bozic & Nowak 2014). The median number of cells in the k-th successful resistant clone is given by $\text{Med}(Y_k) = \frac{Mu}{1-\delta}(2^{1/k} - 1)$. This result means that the ratio of the median sizes of the second and the first resistant subclone is $\sqrt{2} - 1$, and that the second resistant clone is about half the size of the first.

An intriguing emerging strategy in the management of metastatic cancer is trying to detect the individual mechanisms responsible for resistance in individual lesions, using a combination of liquid and traditional biopsies (Russo et al. 2016). However, we expect that even in single lesions the mechanisms of resistance will be diverse, and that chasing the dominant ones would not lead to long-term control, as new ones will reappear eventually (Burrell & Swanton 2014). A better strategy would be using a drug cocktail that can work against all known resistance mutations upfront. This is in line with recent work suggesting that optimal drug combinations should be based on the consideration of the entire tumor heterogeneity instead of just the dominant subpopulation (Zhao et al. 2014).

NO PRE-EXISTING RESISTANCE

In this section, we describe the scenarios in which there may be no pre-existing resistance at the start of therapy. In this case, effective cancer therapies need to prevent or delay the emergence of

de novo resistance. We have already described one such scenario earlier: the case of combination therapy with no cross-resistance.

In tumors driven by a small number of stem cells, such as chronic myeloid leukemia, resistant cancer stem cells may not be present in patients with early stage disease (Michor et al. 2005). The relevant number of cells that determines whether resistance is pre-existing in that case is the number of cancer stem cells, which may be many orders of magnitude lower than the number of all cancer cells (Michor et al. 2005, Werner et al. 2016). This results from the fact that only resistance mutations that occur in cancer stem cells would be able to remain in the tumor indefinitely, whereas resistance mutations that appear in differentiated cells would be lost from the population (Werner et al. 2011). Stem cell division can result in symmetric renewal (two stem cells), asymmetric division (one stem and one differentiated cell), or symmetric differentiation (two differentiated cells) (Dingli et al. 2007, Tomasetti & Levy 2010b). Let α be the probability of asymmetric division, β the probability of symmetric differentiation, and $1 - \alpha - \beta$ the probability of symmetric self-renewal. Tomasetti & Levy (2010b) showed that this model, in which relevant resistance can only appear in cancer stem cells, is equivalent to the model without a differentiation hierarchy, with some parameter adjustments. Let b be the division rate of cancer stem cells, d their death rate, and u the resistance mutation rate. Then $b' = b(1 - \alpha - \beta)$ is the effective division rate of stem cells, adjusted to only account for symmetric self-renewal. $d' = d + b\beta$ is the adjusted death rate of stem cells, which includes loss due to symmetric differentiation. Finally, $u' = u(1 - \alpha/2 - \beta)/(1 - \alpha - \beta)$ is the adjusted resistance mutation rate to account for mutations that occur during asymmetric divisions, which do not lead to the change in the number of stem cells. Similarly as before, the probability that resistant stem cells that will not be lost due to stochastic drift are present in a population of M cancer stem cells is $P_M \approx 1 - e^{-Mu'}$.

Another scenario in which resistance may not be pre-existing is encountered in in vitro experiments, which typically use a much smaller number of cells compared to the number of cancer cells in a lesion. Very recently, Hata et al. (2016) cultured 1,200 small pods, each containing 5,000 EGFR-mutant non-small-cell lung cancer cells. Pods were treated with the EGFR inhibitor gefitinib. The authors found that the large majority of pods (>90%) did not contain pre-existing resistant cells. This is in accordance with predictions based on the small number of cells in each pod (**Table 2**). The pods without pre-existing resistant cells seemed to have developed resistance through de novo evolution of drug-tolerant (persistor) cells. The emergence of drug tolerance in cancer has recently been characterized using a mathematical evolutionary model (Chisholm et al. 2015).

We can use Hata et al.'s results to estimate the fraction of cells resistant to gefitinib that are present in the parental cell population. The authors report that ~100 pods contained pre-existing resistant cells. Assuming that each such pod contained a single resistant cell, the fraction of resistant cells in the parental population is $100/(1{,}200 \times 5{,}000) \sim 10^{-5}$. All resistant clones harbored the same $EGFR^{T790M}$ mutation. Their estimated fraction (1 in 10^5 cells) in the parental population is higher than would be expected for a single point mutation, suggesting that the $EGFR^{T790M}$ mutation either is affected by a higher mutation rate or provides additional fitness to cells carrying it.

DISCUSSION

The problem of resistance to therapy is a general one, affecting the success of antiviral, antibacterial, and anticancer treatments (Abel Zur Wiesch et al. 2015, Aktipis et al. 2011, Bonhoeffer & Nowak 1997, Bonhoeffer et al. 1997, Bozic et al. 2012, Chang et al. 2011, Coldman & Goldie 1983, Fu et al. 2015, Glickman & Sawyers 2012, Greulich et al. 2012, Iwasa et al. 2003, Kepler & Perelson 1998, Lipsitch & Levin 1998, McLean & Nowak 1992, Moreno-Gamez et al. 2015, Nichol et al. 2015,

Nowak et al. 1997, Regoes & Bonhoeffer 2006, Ribeiro et al. 1998, Rosenbloom et al. 2012). In HIV, the rapidly evolving virus is controlled in the majority of patients using a cocktail of multiple drugs that act on different viral targets (Nowak & May 2000). The hope for cancer is that similar combination therapies, which will prevent cross-resistance, will be available for the majority of patients. The situation in cancer, compared to HIV, is complicated given the heterogeneity of different cancers, and even the heterogeneity among cancers of the same type (De Sousa et al. 2013, Lawrence et al. 2014, Vogelstein et al. 2013, Wood et al. 2007).

Another very exciting avenue in the fight against cancer is immunotherapy. These drugs do not kill cancer themselves, but induce the immune system to recognize and eliminate cancer cells (Hoos et al. 2010). The mode of action of immunotherapies seems very different from other systemic therapies. Even though currently only a minority of patients respond, their responses are generally long lasting (Callahan & Wolchok 2013), compared to more transient responses obtained with many targeted therapies. Mathematical modeling of these therapies must take into account the dynamic interaction between the cancer and the immune system, and requires approaches not covered in this review. Interesting parallels can be drawn to the mathematical modeling of immune responses to persistent virus infections (Nowak & Bangham 1996, Nowak & May 2000).

In this article, we primarily study genetic mechanisms of resistance. Other possible mechanisms of resistance include adaptive resistance, in which the presence of the drug leads to the change in feedback regulation of cancer cells, rendering them resistant to the drug. Examples of adaptive resistance include *BRAF(V600E)* mutated colon cancers, which develop resistance to BRAF(V600E) inhibition through a rapid feedback activation of EGFR (Prahallad et al. 2012).

Mathematical models of cancer have addressed a variety of problems, including quantifying cancer initiation (Nowak et al. 2002, 2003), progression (Altrock et al. 2015, Antal et al. 2015, Beerenwinkel et al. 2007, Bozic et al. 2010, Durrett et al. 2010, Waclaw et al. 2015), epidemiology (Armitage & Doll 1954, Luebeck & Moolgavkar 2002, Meza et al. 2008, Michor et al. 2006, Tomasetti et al. 2015), the growth and invasion of tumors (Harpold et al. 2007, Swanson et al. 2003), the effect of angiogenesis on cancer progression (Alarcon et al. 2005, Byrne et al. 2006, Chaplain et al. 2006), and the role of microenvironment (Anderson et al. 2006, Gerlee & Anderson 2007, Greaves & Maley 2012, Merlo et al. 2006).

Here we have focused on those models that specifically speak to the evolution of resistance. We have provided key formulas for the probability of pre-existing resistance, as well as the size and composition of the resistant population. We have differentiated between resistant clones that will survive and those that are present in the tumor but are unable to lead to relapse, therefore characterizing the probability of relapse. The fundamental finding is that combination therapy will not succeed (or will succeed only temporarily) if there is a single step, such as a point mutation, that provides cross-resistance to all drugs in the cocktail. Only if two steps with low probability of occurrence, such as two point mutations, are needed for resistance to a combination, does the combination have a chance of controlling cancer in patients. If one of the two steps has a higher probability, such as a gene amplification or an epigenetic modification, then success is much less likely.

Mathematical models of cancer treatment (Equation 5) can lead to important insights that should be taken into account in the development of new therapies. Using the model, we demonstrate that drugs for long-term control of cancer should optimize parameters of the resistant cancer cell population, including the fraction of resistant cells and especially the growth rate of the resistant population (**Figures 2** and **3*a***). These parameters have a stronger effect on overall survival than the parameters describing the sensitive population, such as the killing rate of cancer cells, which is the parameter usually optimized in drug discovery. (The underlying assumption is, of course, that the drug is sufficiently effective against the sensitive population so that it goes

into exponential decline.) We also show that progression-free survival is an imperfect measure of long-term control, as it negatively correlates with the drug killing rate; thus a more effective drug has a shorter progression-free survival compared to a less effective one, all else being equal (**Figure 3*b***). The formulas presented here provide the basis for an engineering-like understanding of the problem faced by the biomedical and pharmaceutical communities in the attempt to cure cancer.

DISCLOSURE STATEMENT

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