Gene Ther Mol Biol Vol 9, 247-256, 2005

Evolutionary dynamics of drug resistance in cancer

Review Article

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Key words: drug resistance, mathematical model, drug resistant mutants, cellular turnover

Abbreviations: chronic myeloid leukemia, (CML)

Received: 15 September 2005; Accepted: 4 October 2005; electronically published: October 2005

Summary

Detailed molecular research has advanced treatment options against cancers significantly. Targeted drugs are being developed which attack specific abnormalities in the cancer cells. An especially promising example of this is the treatment of chronic myeloid leukemia (CML) with Imatinib mesylate. While therapy is often successful in early CML stages, therapy fails during the advanced blast crisis stage. The reason for this failure is drug resistance. In order to design strategies to overcome the problem of drug resistance, it is important to understand the principles according to which drug resistant cancer cells evolve. This requires mathematical models. Here, such a mathematical approach is reviewed. The mathematical framework is applied to CML, and some preliminary predictions and insights regarding the prevention of resistance are discussed.

I. Introduction

One of the most important clinical problems in cancer research is deeply connected to the principles of evolutionary biology: the emergence and prevention of resistance against drug treatment. Cancer cells which are resistant to specific cancer therapies are generated by random mutations which develop as the cancer cells divide without control. In the presence of treatment, these resistant cells are selected, resulting in continued disease progression despite drug therapy. Computational analysis of the evolutionary dynamics of cancer cells *in vivo* can allow us to understand how drug resistance emerges, and thus how resistance can be prevented.

Several treatment options against cancers exist, depending on the specific type of cancer under consideration. Traditionally, a wide variety of cancers have been treated with chemotherapy (Lawrence et al, 2003). Chemotherapeutic agents are toxic to the cells in general (Simon et al, 2000). Cancer cells divide rapidly, and thus take up the agents and are killed preferentially. Because of the non-specific nature of these drugs, strong side effects are observed, especially in tissues which have relatively high turnover rates. Chemotherapeutic agents are thought to damage the genome of cells (Simon et al, 2000). This damage can lead to cell death, in part because of the induction of cellular checkpoints which induce apoptosis or senescence of the cancer cells (Simon et al, 2000). Drugs which induce DNA damage can also be dangerous because they cause mutations which can transform otherwise healthy cells, or make the existing cancer cells more malignant (Finette et al, 2000).

In recent years, significant advances have been made regarding the molecular biology of cancer cells. Genes and alterations in cellular signaling pathways have been identified which initiate and drive cancer progression (Guillemard and Saragovi, 2004). As detailed knowledge became available, drugs were developed which can correct the precise molecular abnormalities which drive tumor progression (Yee and Keating, 2003; Guillemard and Saragovi, 2004). Such drugs are called small molecule inhibitors or targeted drugs. The best example of this is the treatment of chronic myeloid leukemia (CML) with Imatinib mesylate (Imatinib) (Melo et al, 2003; Druker, 2004). CML is initiated and driven by the *BCR-ABL* fusion gene which encodes a cytoplasmic protein with constitutive tyrosine kinase activity. Imatinib is a small molecule inhibitor of the Bcr-Abl kinase and thus removes the cause for uncontrolled cellular proliferation (Goldman and Melo, 2003). Treatment with Imatinib has shown remarkable clinical success in patients which have early stages of the disease. However, treatment tends to fail in patients with advanced disease because of the occurrence of drug resistance (Blagosklonny, 2002; Shannon, 2002).

Computational models have been used to elucidate the evolutionary dynamics of drug resistant cancer cells,

and to suggest strategies for prevention in the context of targeted therapy with small molecule inhibitors. Several important questions have been addressed.

1. When do resistant cells emerge? Are resistant cells more likely to pre-exist before treatment, or are they more likely to emerge during the phase of therapy?

2. How does the turnover rate of the cancer influence the evolution of drug resistant cells?

3. Can combination therapy be used to prevent drug resistance?

We will start this review by describing the computational framework in simple terms. Then we will discuss the questions outlined above in turn. Finally, we will apply this general framework to the specific case of CML therapy with Imatinib.

II. The mathematical model

In order to understand how resistant mutants are generated during cancer progression and treatment, we have developed the following computational model (Komarova and Wodarz, 2005), summarized schematically in **Figure 1**. Cancerous cells are described by a stochastic birth-death process with a positive net proliferation rate. If we denote the growth rate of cells as L and the death rate as D, the condition $L>D$ corresponds to clonal expansion. We further assume that cancer is detected when the colony reaches a certain size, N, at which moment therapy starts (treatment size). The effect of therapy is modeled by the drug-induced death rate, H, which shifts the balance of birth and death such that the colony shrinks. That is, the net cell death rate is now larger than the birth rate, $D+H>L$. If all cancerous cells were susceptible to the drug, then therapy would inevitably lead to eradication of cancer. However, in the course of cancer progression, mutations can lead to the generation of cell types which are resistant to the drug. This is assumed to occur with a probability u upon cell division. Before the tumor is treated, the mutant will behave identically compared to the wild-type. During therapy, however, the resistant phenotype will proliferate while the wild-type will be killed with a rate H.

Later on we will discuss whether and how the combination of two or more drugs (combination therapy) can be used to prevent treatment failure as a consequence of drug resistance. As a first step we consider the simplest assumption that a mutation which confers resistance to one drug does not confer resistance to any of the other drugs in use. This may not be the case for all drugs/mutations, and these effects have to be accounted for once a thorough understanding of this simplified scenario is reached. With these assumptions, in order to become resistant to n drugs, the cell has to accumulate n mutations. As an example, the pathways by which resistance can evolve in the context of triple drug therapy is shown schematically in **Figure 2**.

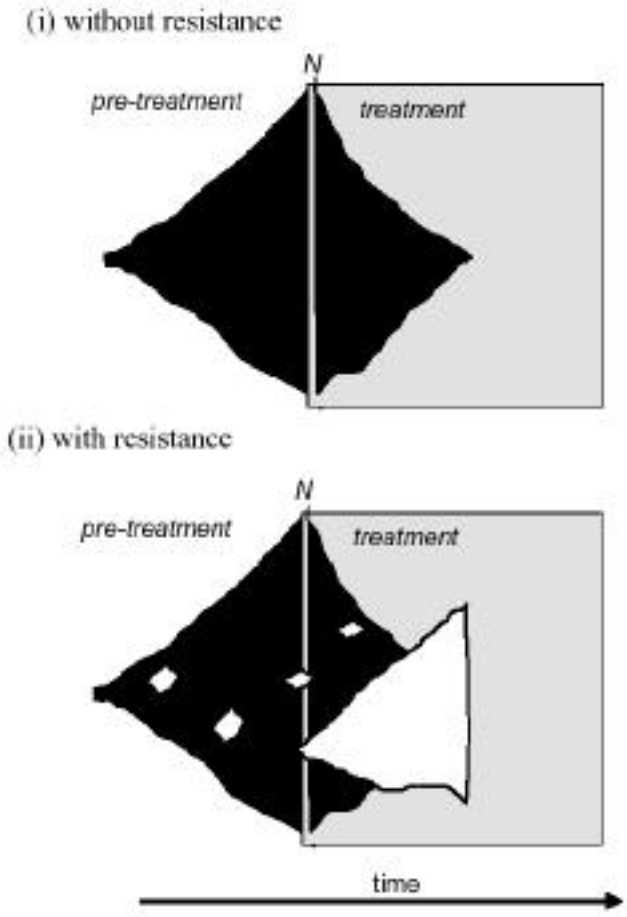

Figure 1. Schematic representation of the assumptions which underlie the modeling framework. (**i**) Without resistance, the cancer grows exponentially, and treatment starts at tumor size N. Upon treatment, the tumor size shrinks until it is driven extinct. (**ii**) If mutations can occur, resistant cell clones are generated. This can occur both before and during treatment. As therapy is applied, a resistant cell clone can expand while the wild-type declines. Therefore, treatment fails to drive the cancer extinct. Treatment phases are indicated by shading.

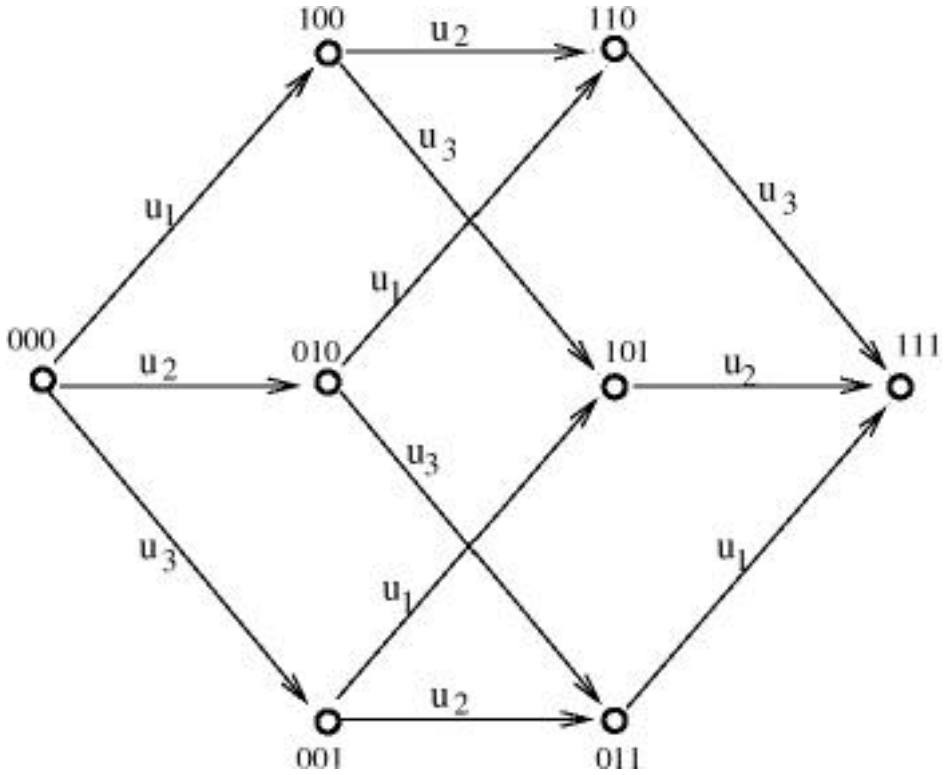

Figure 2. Schematic diagram showing pathways to the evolution of resistance against three drugs. Each phenotype is characterized by a binary number which encodes its resistance properties with respect to each drug. "0" means susceptible, and "1" means resistant. For example, "011" means that the corresponding cell type is susceptible to drug 1 and resistant to drugs 2 and 3. The arrows indicate mutations, and the mutation rates are marked above each arrow. The leftmost type is fully susceptible to all drugs. The rightmost type is fully resistant to all drugs.

For mathematical details, the interested reader is referred to (Komarova and Wodarz, 2005). For simplicity, we assume that mutant cells which are not resistant to all drugs in use are killed with the same rate as wild-type cells. Alternatively, it can be assumed that such mutants are partially resistant (i.e. are affected less than the wild-type but more than the fully resistant phenotype (Schabel FM, Jr, et al, 1979, 1983)). However, analysis indicates that this does not alter our results significantly.

III. Timing of the emergence of drug resistant mutants

This is a fundamental question which has important implications for the development of treatment strategies with the aim to prevent failure due to resistance. On the one hand, the growth of a tumor involves many cell divisions during which mutations can occur. In addition, many cancers are characterized by some form of genetic instability which accelerates the rate at which cells acquire mutations during this growth phase. Hence, it is possible that by the time the tumor reaches a certain size, there will be at least one cell which is resistant to therapy. Upon start of treatment, this cell will proliferate and grow to large numbers, preventing the eradication of the tumor. The computational framework has allowed us to quantify the probability that at least one cell is resistant before the start of treatment. On the other hand, once therapy starts, cells can also acquire mutations. While the death rate of the tumor cells is now larger than their rate of division, cell divisions, and thus mutations, can still occur. The probability that at least one resistant mutant is generated during the phase of treatment can also be calculated in the context of our computational framework.

The upshot of these calculations is that the treatment phase does not contribute significantly to the generation of drug resistance and thus can be ignored in this context. If resistance poses a problem during treatment, the model suggests that resistant cells must have pre-existed before the start of therapy. The situation is slightly different depending on whether treatment occurs with a single drug, or whether several drugs are used in combination:

1. If the cancer is treated with a single drug, then there is a parameter region in which the generation of resistant cells is more likely to occur during the treatment phase than during the growth phase before therapy. This occurs if the efficacy of treatment is weak relative to the growth rate of the cancer. In our symbolical notation explained above, it occurs if $H < 2(L-D)$. However, we argue that this is not relevant for practical purposes. This condition means that the number of cell divisions during treatment is higher than the number of cell divisions during the growth phase before treatment. In other words, the time it would take to eradicate the tumor by drugs in the absence of resistance is larger than the age of the

tumor upon start of therapy. This seems like an unrealistic parameter regime.

2. If two or more drugs are used, the treatment phase becomes completely insignificant with respect to the generation of resistant cells. That is, in all parameter regions, resistance is more likely to pre-exist than to be generated during therapy. The reason lies in the dynamics of the intermediate mutants. During the growth phase, a cell with a single mutation will undergo clonal expansion and this facilitates the generation of further mutations. During the treatment phase, a cell with a single mutation has a negative growth rate (as it is susceptible to one or more drugs). This makes it unlikely that additional mutations can be attained before the clone is extinct.

Because the treatment phase can be ignored, we note that the chances of treatment failure due to resistance are not influenced significantly by the efficacy of treatment (assuming that treatment is strong enough to remove the cancer in the absence of resistance).

IV. Effect of cellular turnover on the rise of resistance

Let us consider the number of cell divisions which occur during the growth phase until the tumor has reached size N. This is roughly given by $v=NL/(L-D)$. We can see that if $D=0$ or $D << L$, the number of cell divisions is approximately given by $v \approx N$. On the other hand, if D is close to L ($D \simeq L$), many more cell divisions are required to reach size N, since a high death rate cancels the effect of cell divisions. For convenience, we will call the scenario where $D \approx L$ a high-turnover cancer. In contrast, we will call the scenario where $D=0$ or $D << L$ a low-turnover cancer.

How does the turnover rate of the cancer influence the emergence of resistant cells during the growth phase and thus the pre-existence of resistance? The answer depends on how many drugs are used to treat the cancer.

1. If the cancer is treated with a single drug, then the probability that a resistant mutant pre-exists before therapy is not dependent on the turnover rate of the cancer. That is, high-turnover and low-turnover cancers behave in exactly the same way as far as the pre-existence of mutants is concerned. An intuitive explanation is as follows. A higher turnover cancer requires more cell divisions to reach size N, and thus more mutants are created. At the same time, however, the death rate of the mutants is also increased. The two effects cancel each other out. Similar results were also observed in related and earlier mathematical models by Goldie and Coldman who did pioneering work in this field of research (Coldman and Goldie, 1986).

2. If two or more drugs are used, the probability that resistant mutants pre-exist does depend on the natural death rate of the tumor cells, D. In other words, the dynamics are different for high-turnover and low-turnover cancers. The higher the turnover rate of the cancer cells, the higher the probability that a resistant mutant exists when the cancer has reached size N. The larger the number of drugs used, the stronger this dependency. To explain this, consider the process of mutant generation. In the case of one drug, the increase in mutant production is canceled out exactly by the increase in mutant death as the turnover rate of the tumor cells is increased. This does not hold for two or more drugs. Now, an increase in the turnover rate of the tumor cells increases the production rate of resistant mutants more than it increases the death rate of the mutant cells. The net effect is that a resistant mutant is more likely to be present at the time of treatment if the turnover rate of the tumor cells is higher. In general, if the number of drugs is increased, a higher natural death rate of tumor cells, D, contributes increasingly to the production of resistant mutants and thus to treatment failure.

This gives rise to the important insight that cancers which are characterized by a high turnover rate (i.e. the death rate of cells is close to their division rate) might be difficult to control with combination therapy. This is discussed further in the next section.

V. How can the emergence of resistance be prevented?

The combination of several drugs together seems like an obvious strategy to prevent treatment failure as a result of resistance. If a one cell has to accumulate a sufficient number of mutations in order to become fully resistant, it is less likely that a resistant cell will exist upon start of treatment. If there is no cross-resistance between different drugs, then a cell has to acquire n mutations in order to become resistant to n drugs (**Figure 2**). Combination therapy has shown great success in the context of HIV infection (Bonhoeffer et al, 1997; Ribeiro and Bonhoeffer, 2000). In a typical HIV infected patient, we can expect that viruses are present which are resistant to one or two drugs. However, it is extremely unlikely that a virus exists which is resistant to three drugs (Ribeiro et al, 1998). This provides the rational for why a combination of three drugs was required to achieve long-term suppression of the virus by drug therapy. In the following, we discuss how combination therapy affects the chance of treatment failure in the context of cancers treated with targeted small molecule drugs.

This is addressed in the following way. We ask at which tumor size N the probability of treatment failure reaches a threshold value, which we denote by δ. This means that if we start treatment at tumor size N, failure will be observed in a fraction δ of the patients, while treatment will be successful in a fraction $1 - \delta$ of patients. For now we assume that an acceptable goal is to treat 99% of patients successfully, that is $\delta = 0.01$. In other words, if more than 1% of patients shows resistance, we consider the treatment strategy a failure. So we ask at which tumor size treatment failure is expected to occur. In particular, we ask how the number of drugs used in combination influences the tumor size when resistance is observed. According to the model, this depends on the mutation rate, u, and the turnover rate of the tumor cells (value of D relative to L) (**Figure 3**).

1. The higher the rate at which resistance mutations are acquired, u, the less the effect of adding another drug, and the more difficult it becomes to treat (**Figure 3**). Consider the most optimistic scenario when $D=0$ (**Table 1**). Assume that cancers can reach up to sizes of 10^{13} cells

(McKinnell et al, 1998). Then, the physiological point mutation rate, $u=10^{-9}$, requires two drugs, $u=10^{-7}$- $u=10^{-8}$ requires three drugs, $u=10^{-5}$- $u=10^{-6}$ requires four drugs, and $u=10^{-4}$ requires six drugs (**Table 1**). By extrapolation, 10 drugs are needed if $u=10^{-3}$, and about 30 drugs are needed if $u=10^{-2}$. Therefore, if resistance mutations can occur at levels which are significantly higher than the physiological mutation rate (e.g. because genetic instability promotes the generation of resistance mutations), combination therapy is unlikely to be advantageous.

2. A high turnover rate of cancer cells also abolishes benefits which can be obtained from combination therapy (**Figure 3b**). In the context of combination therapy, resistance arises at lower tumor sizes as the death rate of tumor cells, *D*, is increased. In fact, if the death rate of tumor cells, *D*, comes close to their division rate, *L* (high

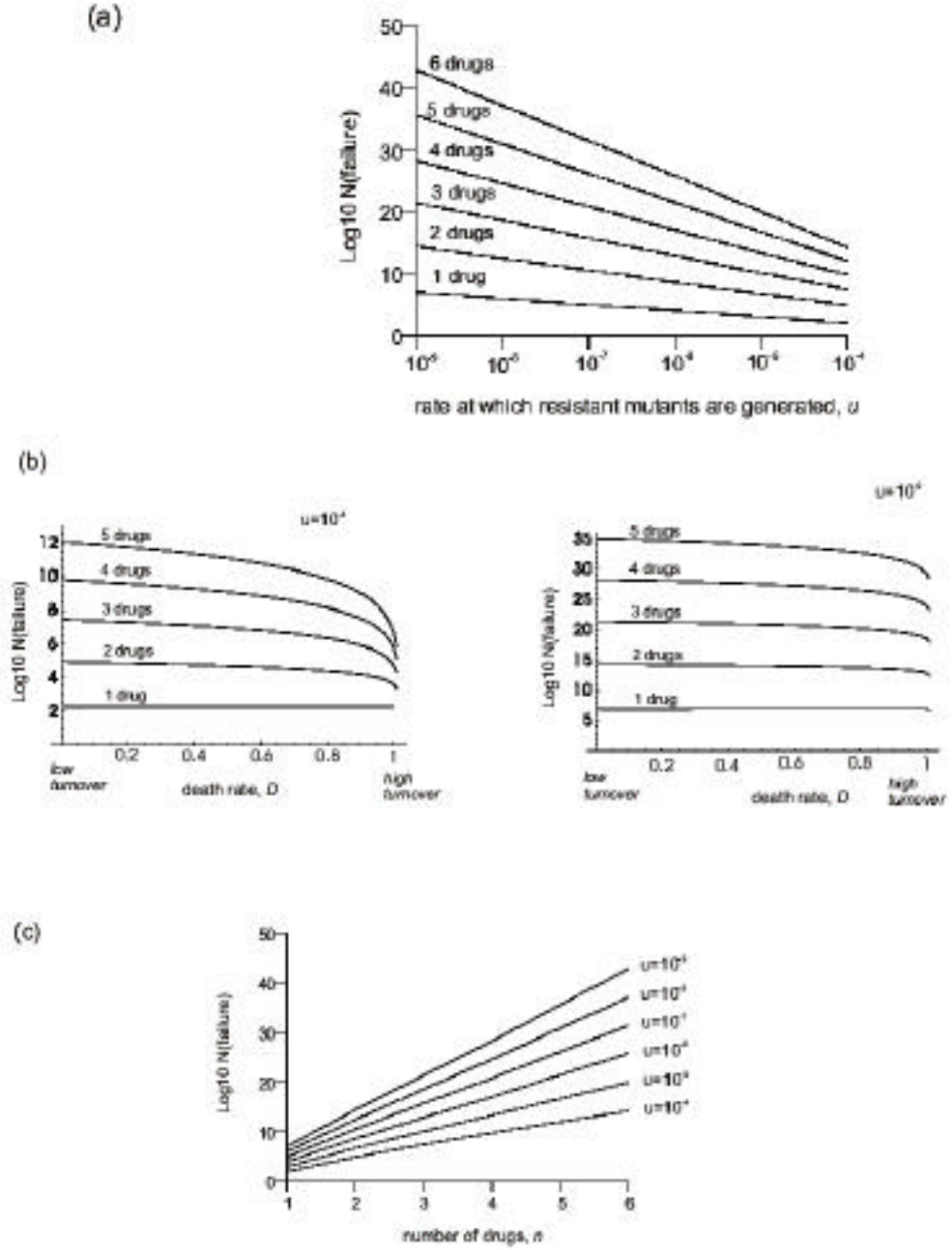

Figure 3. Log tumor size, *N*, at which treatment failure is observed, depending on the parameters of the model. (**a**) Dependence on the rate at which resistant mutants are generated, *u*. The higher the value of *u*, the lower the tumor size at which treatment fails. The larger the number of drugs, the stronger this dependency. (**b**) Dependence on the natural death rate of tumor cells, *D*. The higher the value of *D* (i.e. the higher the turnover of the cancer), the lower the tumor size at which treatment fails. The higher the number of drugs, and the higher the rate at which resistant mutants are generated, *u*, the more pronounced this trend. (**c**) Dependence on the number of drugs, *n*. Increasing the number of drugs increases the tumor size at which treatment fails. The higher the mutation rate, however, the lower the advantage gained from adding further drugs. Baseline parameter values were chosen as follows: $L=1$, $\delta=0.01$.

Table 1. The $\log_{10}$ size at which resistance becomes a problem (i.e. treatment failure in more than 1% of patients), depending on the number of drugs and the rate at which resistant mutants are generated, *u*. If we assume that the cancers cannot grow beyond 10^{13} cells without causing death, a treatment regime can be considered acceptable if resistance only becomes a problem at sizes which are greater than 10^{13} cells (i.e. $\log_{10}$ of the size > 13). The parameter regimes where this occurs and treatment is expected to be successful are indicated by shading in the table. The calculations assume *L=1, D=0.*

	1 drug	*2 drugs*	*3 drugs*	*4 drugs*	*5 drugs*	*6 drugs*
$u=10^{-4}$	2.01	4.95	7.46	9.81	12.06	14.23
$u=10^{-5}$	3.01	6.73	10.13	13.36	16.70	20.02
$u=10^{-6}$	4.01	8.61	12.91	17.04	21.49	25.83
$u=10^{-7}$	5.01	10.53	15.75	20.8	26.17	31.43
$u=10^{-8}$	6.01	12.47	18.62	24.6	30.90	37.10
$u=10^{-9}$	7.01	14.42	21.36	28.23	35.61	42.86

turnover cancer), then the effect of combining multiple drugs disappears (**Figure 3b**). The size at which resistance arises converges to the same value, no matter how many drugs are used. In this case, the frequency with which cancers arise is low because they have a high chance to go extinct spontaneously, but when they do arise, the chances of complete tumor eradication are very slim. Because high turnover cancers are likely to grow very slowly, however, drug therapy could still increase the life-span of the patient by reducing the number of tumor cells for a prolonged period of time. Re-growth of resistant cells to large sizes would take a long time.

This leaves us with the following message: combination therapy will provide benefits for preventing treatment failure due to resistance, except in the following cases: (i) The cancer has a high turnover rate. Such cancers are likely to grow slowly. (ii) Resistance mutations against the drugs in use can be generated at rates which are several orders of magnitude higher than the physiological mutation rate.

VI. Application of the framework to CML

The best case study of cancer therapy with targeted small molecule inhibitors is the treatment of CML with imatinib (Calabretta and Perrotti, 2004). CML is a disease which progresses in three stages (Melo et al, 2003): the chronic phase, the accelerated phase, and blast crisis. These stages are characterized as follows.

1. During the chronic phase, the tumor remains at relatively low levels, and there is an expansion mostly of terminally differentiated cells.

2. The accelerated phase is characterized by the expansion of a higher fraction of undifferentiated cells.

3. During blast crisis, undifferentiated cells undergo massive expansion. This phase is also characterized by the presence of genomic instability.

The initiation and further progression of CML is driven by a chromosome translocation, resulting in the *BCR-ABL* fusion gene which encodes a cytoplasmic protein with constitutive tyrosine kinase activity (Goldman and Melo, 2003). The drug Imatinib is a small molecule inhibitor of the Bcr-Abl kinase and can achieve sustained hematologic and cytogenetic responses in chronic phase disease. Treatment of blast crisis, however, often fails because of drug resistance (McCormick, 2001). In accordance with our framework it has been reported that mutants might pre-exist the initiation of treatment rather than being generated during the treatment phase (Gambacorti-Passerini et al, 2003; Nardi et al, 2004). Data suggest that two main types of mutations confer resistance to the cells (Gorre et al, 2001; McCormick, 2001; Gambacorti-Passerini et al, 2003): the amplification of *BCR-ABL*, or a point mutation in the target protein. Genetic instability (Loeb, 1998) is likely to promote the occurrence of gene amplifications which have been measured to occur in cancer cells at a rate of 10^{-4} per cell division (Tlsty et al, 1989). On the other hand, the point mutation rate is about 10^{-9} per base per cell division (Loeb et al, 19). However, the frequency of gene amplifications is much less than that of point mutations among patients (Gambacorti-Passerini et al, 2003). Part of the reason might be that *BCR-ABL* amplifications are costly to the cells in the absence of treatment (Tipping et al, 2001). Including this assumption into the modeling framework, however, shows that even if this fitness cost is very significant, amplifications should still be observed more often than point mutations. However it is thought that the level of resistance is a function of the number of extra copies of the *BCR-ABL* gene. Therefore, if a significant degree of resistance requires 2 or more amplification events (but only one point mutation event), we expect that a resistant mutant is generated faster by point mutation than by gene amplification, explaining the observed frequencies.

Thus, for prevention of drug resistance we assume that resistant mutants are generated maximally with a point mutation rate of $u=10^{-8}$-10^{-9}. Experiments with susceptible CML cell lines have shown viability measurements (in the absence of treatment) of about 90% (Tipping et al, 2001). From this we can roughly calculate that the relative death rate of cancer cells is in the range of *D/L=0.1-0.5.* In this parameter region, we find that a combination of three drugs should prevent resistance and ensure successful therapy even for advanced cancers (**Table 2a**). This assumes that the size of advanced cancers is less than 10^{13} cells, which derives from white blood cell count measurements which range from 10^5-10^6 per microliter of blood in blast crisis. Recent findings (Nowicki et al, 2004) indicate that *BCR-ABL* might

Table 2. Application to the treatment of CML blast crisis with imatinib. We give the $\log_{10}$ size at which resistance becomes a problem, depending on the number of drugs and the turnover rate of the cancer cells (value of *D/L*). From published data, we estimated that the value of *D/L* must lie between 0.1 and 0.5, and we also present calculations for *D/L=0.9*. We consider treatment robust if resistance only arises at tumor sizes which are larger than 10^{13} cells (i.e. the value 13 in the table). In this case, the combination of three drugs is expected to result in the prevention of resistance and successful treatment. ***(a)*** Calculations assuming that resistant mutants are generated with a rate of $u=10^{-8}$. The reason for this parameter choice is as follows: while the point mutation rate is around $u=10^{-9}$, several point mutations can lead to resistance and this increases the rate. ***(b)*** Calculations assuming that resistant mutants are generated with an elevated rate of $u=10^{-6}$, i.e. a 100 fold increase. This represents the borderline where three drugs will not prevent resistance anymore. Thus, as long as the point mutation rate is elevated less than 100 fold by *BCR-ABL*, triple drug therapy should prevent resistance.

(a)

	1 drug	*2 drugs*	*3 drugs*	*4 drugs*	*5 drugs*
D/L=0.1	5.95	12.34	18.45	24.38	30.19
D/L=0.5	5.95	12.13	17.99	23.69	29.26
D/L=0.9	5.95	11.48	16.70	21.74	26.66

(b)

	1 drug	*2 drugs*	*3 drugs*	*4 drugs*	*5 drugs*
D/L=0.1	4.00	8.55	12.80	16.89	20.86
D/L=0.5	4.00	8.31	12.37	16.20	19.93
D/L=0.9	4.00	7.68	11.07	14.40	17.40

increase the amount of reactive oxygen species and thus the rate of point mutations. As long as the elevation of the mutation rate is less than a hundred fold, our results remain robust (**Table 2b**).

In summary these calculations provide optimistic results for the treatment of CML with a combination of different targeted small molecule inhibitors. This is especially interesting at this stage because research has given rise to additional small molecule inhibitors which are different from Imatinib, and which might also be effective at treating CML. However, these drugs show a degree of cross resistance with Imatinib, and this weakens the power of combining these drugs (Shah et al, 2004; Yoshida and Melo, 2004). These and other limitations of our framework are discussed in the next section.

VII. Extensions of the model

We have reviewed a first framework in order to study and understand the principles according to which drug resistant mutants arise in the context of targeted small molecule therapy, and to explore implications for prevention strategies. At this stage, we do not aim to provide exact predictions because more biological detail needs to be incorporated into the model backbone discussed here. This can be done rather easily as more information becomes available. In the following, we mention some obvious first steps in this respect.

1. We assumed that cancer cells grow exponentially. This is a good assumption for blast crisis in CML. In general, however, the growth patterns of cancers are more complicated. Not much information is available. There might be multiple rounds of exponential growth, separated by periods of stasis where the cancer fails to grow. During these static periods, the cancer might either be dormant (i.e. cells do not divide or die), or the cancer might turn over at a high rate. The exact growth pattern of cancer cells over time is determined by many factors which include the requirement for blood supply (angiogenesis), growth factors, the requirement to accumulate further mutations, etc. Such details can be easily incorporated into the mathematical framework if they become available for specific cancers.

2. In our framework, populations of wild type and resistant cells do not interact with each other in any form. In reality, however, they might be in competition with each other. It is unclear at the moment whether cancer cells compete with each other or not. On the one hand, there is plenty of space for them to grow and this should reduce the effect of competition. On the other hand, it seems that only a relatively small fraction of the cancer actually contributes to growth at the edge of the tumor, and these cells might very well compete for nutrients and blood supply. If it turns out that competition between cancer cells does play an important role in the tumor growth kinetics, this can be accommodated in the model. Competition would influence the rate at which resistant cells rise to high numbers upon start of treatment. This would in turn be influenced by the strength of drug therapy. These concepts would be interesting to explore in model extensions.

3. Another important issue is the existence of partial cross resistance in combination therapy. For example, the most promising new CML drugs show some degree of cross resistance with Imatinib (Shah et al, 2004; Yoshida and Melo, 20). If this is the case, our framework still applies, but the calculations would have to be modified in the following way. Suppose drug X possesses cross-

resistance with imatinib. This means that a part (or all) of the mutants resistant to imatinib will also be (partially) resistant to drug X. In the case where they are fully resistant to both drugs, treating with the two drugs will not be more effective than treating with just one of the drugs, and the clinical strategy will have to be developed using other important considerations such as toxicity etc. However if resistance to drug X is partial, then the resistant mutants will have a slower growth rate under a two-drug therapy compared to that under a single-drug therapy. In this case, we can calculate the advantage of a two-drug therapy, for instance in terms of a reduction of the tumor load. The occurrence of cross-resistance is discussed in (Daub et al, 2004; Burgess et al, 2005; von Bubnoff et al, 2005).

4. Another important issue is the heterogeneity of tumors. In CML (as well as AML, and several solid tumors including breast and central nervous system tumors (Faderl et al, 1999; O'Dwyer et al, 2002; Al-Hajj and Clarke, 2004)), there is evidence for the existence of cancer stem cells, comprising a fraction of the total tumor burden. For CML, the fraction of stem cells in blast crisis is more than 30%, and it is much smaller in the chronic phase (Faderl et al, 1999; O'Dwyer et al, 2002). It has been proposed that these cancer stem cells, which are the only tumor cells that have potential for self-renewal, may account for drug resistance after an initial response to therapy. This circumstance can be taken into account by using the present framework. As resistance is mainly a problem in blast crisis and usually does not arise in the chronic phase, we performed our calculations for the latter phase of the disease. During this phase, the blasts undergo a phase of rapid exponential growth, and therefore the quantitative results of our present calculations apply. However, it would be an interesting extension to consider heterogeneous populations of the chronic and accelerated phases of CML. There, stem cells constitute a smaller fraction of the total population, and the predominant division pattern is asymmetric, so one would have to make two modifications in the model: (i) the total (effective) population of dividing cells is smaller, (ii) resistant mutants may appear by two mechanisms: as a result of a mutation upon a symmetric division of a stem cell, and as a result of an asymmetric division. It can be checked that this will lead to a lower chance of the generation of resistance compared to the blast crisis.

5. In the present calculations we assumed that resistant mutants behave in the same way as the wild type tumor cells before treatment starts. This may not be the case. If one can establish that resistant mutants possess a fitness advantage in the absence of treatment, this will definitely make the estimate of the probability of resistance generation higher. Indeed, resistant mutants will grow faster and reach higher numbers (and a larger fraction of the total tumor load) before the treatment starts. On the other hand, if resistant mutants are at a disadvantage before the beginning of therapy, this would make generation of resistance less likely. This information (as it becomes available) can be very naturally incorporated in the model by including a different growth rate (L) and death rate (D) of the mutants compared to the wild type.

VIII. Conclusions

In this review, we have discussed a mathematical framework which helps us to understand the principles which underlie the emergence of resistance in cancers treated with targeted small molecule drugs, and explored prevention strategies. The point of the CML calculations is to illustrate how the mathematical framework can be applied to the targeted treatment of a specific cancer, based on experimental observations. While improved predictions will require that a higher degree of complexity is included in the model, as discussed above, the basic framework can accommodate this easily. In addition, it will be interesting to take into account the many new and controversial concepts which are being discovered and discussed in the literature. The strength of our framework is that it can be used to study many complex scenarios. New information can be incorporated as it becomes available from experiments and clinical trials.

References

Al-Hajj M and Clarke MF (**2004)** Self-renewal and solid tumor stem cells. **Oncogene** 23, 7274-82.

Blagosklonny MV (**2002)** STI-571 must select for drug-resistant cells but 'no cell breathes fire out of its nostrils like a «dragon». **Leukemia** 16, 570-2.

Bonhoeffer S, May RM, Shaw GM and Nowak MA (**1997)** Virus dynamics and drug therapy. **Proc Natl Acad Sci U S A** 94, 6971-6976.

Burgess MR, Skaggs BJ, Shah NP, Lee FY and Sawyers CL (**2005)** Comparative analysis of two clinically active BCR-ABL kinase inhibitors reveals the role of conformation-specific binding in resistance. **Proc Natl Acad Sci U S A** 102, 3395-400.

Calabretta B and Perrotti D (**2004)** The biology of CML blast crisis. **Blood** 103, 4010-22.

Coldman AJ and Goldie JH (**1986)** A stochastic model for the origin and treatment of tumors containing drug-resistant cells. **Bull Math Biol** 48, 279-92.

Daub H, Specht K and Ullrich A (**2004)** Strategies to overcome resistance to targeted protein kinase inhibitors. **Nat Rev Drug Discov** 3, 1001-10.

Druker BJ (**2004)** Imatinib as a paradigm of targeted therapies. **Adv Cancer Res** 91, 1-30.

Faderl S, Talpaz M, Estrov Z and Kantarjian HM (**1999)** Chronic myelogenous leukemia: biology and therapy. **Ann Intern Med** 131, 207-19.

Finette BA, Homans AC and Albertini RJ (**2000)** Emergence of genetic instability in children treated for leukemia. **Science** 288, 514-7.

Gambacorti-Passerini CB, Gunby RH, Piazza R, Galietta A, Rostagno R and Scapozza L (**2003)** Molecular mechanisms of resistance to imatinib in Philadelphia-chromosome-positive leukaemias. **Lancet Oncol** 4, 75-85.

Goldman JM and Melo JV (**2003)** Chronic myeloid leukemia--advances in biology and new approaches to treatment. **N Engl J Med** 349, 1451-64.

Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN and Sawyers CL (**2001)** Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. **Science** 293, 876-80.

Guillemard V and Saragovi HU (**2004)** Novel approaches for targeted cancer therapy. **Curr Cancer Drug Targets** 4, 313-26.

Komarova NL and Wodarz D (**2005)** Drug resistance in cancer: principles of emergence and prevention. **Proc Natl Acad Sci U S A** in press.

Lawrence TS, Blackstock AW and McGinn C (**2003)** The mechanism of action of radiosensitization of conventional chemotherapeutic agents. **Semin Radiat Oncol** 13, 13-21.

Loeb LA (**1998)** Cancer cells exhibit a mutator phenotype. **Adv Cancer Res** 72, 25-56.

Loeb LA, Springgate CF and Battula N (**1974)** Errors in DNA replication as a basis of malignant changes. **Cancer Res** 34, 2311-21.

McCormick F (**2001)** New-age drug meets resistance. **Nature** 412, 281-2.

McKinnell RG, Parchment RE, Perantoni AO and Pierce GB (**1998**) The Biological Basis of Cancer (Cambridge University Press, Cambridge).

Melo JV, Hughes TP and Apperley JF (**2003)** Chronic myeloid leukemia Hematology. **Am Soc Hematol Educ Program,** 132-52.

Nardi V, Azam M and Daley GQ (**2004)** Mechanisms and implications of imatinib resistance mutations in BCR-ABL. **Curr Opin Hematol** 11, 35-43.

Nowicki MO, Falinski R, Koptyra M, Slupianek A, Stoklosa T, Gloc E, Nieborowska-Skorska M, Blasiak J and Skorski T (**2004)** BCR/ABL oncogenic kinase promotes unfaithful repair of the reactive oxygen species-dependent DNA double-strand breaks. **Blood** 104, 3746-53.

O'Dwyer ME, Mauro MJ and Druker BJ (**2002)** Recent advancements in the treatment of chronic myelogenous leukemia. **Annu Rev Med** 53, 369-81.

Ribeiro RM and Bonhoeffer S (**2000)** Production of resistant HIV mutants during antiretroviral therapy [see comments]. **Proc Natl Acad Sci U S A** 97, 7681-6.

Ribeiro RM, Bonhoeffer S and Nowak MA (**1998)** The frequency of resistant mutant virus before antiviral therapy. **Aids** 12, 461-5.

Schabel FM, Jr, Skipper HE, Trader MW, Laster WR, Jr, Griswold DP, Jr and Corbett TH (**1983)** Establishment of cross-resistance profiles for new agents. **Cancer Treat Rep** 67, 905-22.

Schabel FM, Jr, Trader MW, Laster WR, Jr, Corbett TH and Griswold DP, Jr (**1979)** cis-Dichlorodiammineplatinum(II): combination chemotherapy and cross-resistance studies with tumors of mice. **Cancer Treat Rep** 63, 1459-73.

Shah NP, Tran C, Lee FY, Chen P, Norris D and Sawyers CL (**2004)** Overriding imatinib resistance with a novel ABL kinase inhibitor. **Science** 305, 399-401.

Shannon KM (**2002)** Resistance in the land of molecular cancer therapeutics. **Cancer Cell** 2, 99-102.

Simon JA, Szankasi P, Nguyen DK, Ludlow C, Dunstan HM, Roberts CJ, Jensen EL, Hartwell LH and Friend SH (**2000)** Differential toxicities of anticancer agents among DNA repair and checkpoint mutants of Saccharomyces cerevisiae. **Cancer Res** 60, 328-33.

Tipping AJ, Mahon FX, Lagarde V, Goldman JM and Melo JV (**2001)** Restoration of sensitivity to STI571 in STI571-resistant chronic myeloid leukemia cells. **Blood** 98, 3864-7.

Tlsty TD, Margolin BH and Lum K (**1989)** Differences in the rates of gene amplification in nontumorigenic and tumorigenic cell lines as measured by Luria-Delbruck fluctuation analysis. **Proc Natl Acad Sci U S A** 86, 9441-5.

von Bubnoff N, Veach DR, van der Kuip H, Aulitzky WE, Sanger J, Seipel P, Bornmann WG, Peschel C, Clarkson B and Duyster J (**2005)** A cell-based screen for resistance of Bcr-Abl-positive leukemia identifies the mutation pattern for PD166326, an alternative Abl kinase inhibitor. **Blood** 105, 1652-9.

Yee KW and Keating A (**2003)** Advances in targeted therapy for chronic myeloid leukemia. **Expert Rev Anticancer Ther** 3, 295-310.

Yoshida C and Melo JV (**2004)** Biology of chronic myeloid leukemia and possible therapeutic approaches to imatinib-resistant disease. **Int J Hematol** 79, 420-33.

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