Multi Drug Resistance on Cancer Cell Lines

Kunal Kumar Singh

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In Partial Fulfillment of the Requirements for

The Degree of Master of Technology

Department of Electrical Engineering

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Declaration

I declare that this written submission represents my ideas in my own words, and where ideas or words of others have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources that have thus not been properly cited, or from whom proper permission has not been taken when needed.

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(Signature)

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(Kunal Kumar Singh)

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(Roll No.)

Approval Sheet

This Thesis entitled Multi Drug Resistance on Cancer Cell Lines by Kunal Kumar Singh is approved for the degree of Master of Technology from IIT Hyderabad

(Dr. Subha Narayan Rath) Examiner
Dept. of Biomedical Engineering
IIT Hyderabad

(Dr. K. Sri Rama Murty) Examiner
Dept. of Electrical Engineering
IIT Hyderabad

(Dr. Ketan P. Detroja) Adviser
Dept. of Electrical Engineering
IIT Hyderabad

(Prof. M. Vidyasagar) Co-Adviser
Dept. of Electrical Engineering
IIT Hyderabad

(Dr. Soumya Jana) Chairman
Dept. of Electrical Engineering
IIT Hyderabad

Abstract

This document can be treated as Final Thesis Report for the project titled "Multi-Drug Resistance on Cancer Cell Lines". Drug is said to be resistant on cancer site if it doesn't bind the specified cancer tumor target or site. Drug Resistant mutant is a major obstacle in cancer treatment and a lot of research have been done to overcome this phenomenon with the present technology and also with limited success. My interests lies in understanding drug resistance or more specifically multi-drug resistance with the help of mathematical modeling through the lens of system biology. The processes and underlying experimental research is much applied than the simple mathematics which explains it here. You may find some experimental quotations and results which I have to believe to be true in order to address the problem. The central theme of this project is two-fold. First, we look at a stochastic processes describing cancer growth, mutant formation and treatment success or failure based on whether resistant mutants are created prior-to/after treatment or based on the rate of growth of cancer cells under simultaneous treatment with single/multiple drugs. Further refinement in the existing model is necessary to include biological complexity and realism. So, we incorporate cross-resistance effects of drugs which happens when multiple drugs are used for treatment. Result shows that cross-resistant two drugs are superior than single drug in use as most of the mutation confers resistant to that single drug in first line therapy. Adding second drug in combination with the first drug, despite of cross-resistance effect, improves the treatment success. We will also look into the aspects of quiescence effects and its relation to drug resistance. Finally, at the end we will review an optimal drug dozing regimen based on continuous and pulsed dosing scheme to delay the resistance formation to a maximum extent that arises due to single (epi)genetic alteration only. The stochastic process described here is muti-type branching process. We will calculate the resistance generation probability based on initial cancer tumor load and growth or death rate of cell colony. We will also find an average population size of resistant cells over time scale and other useful parameters defining the multi-type branching process.

In short, this report is a "review work" on drug resistance effect and treatment strategy with a viewpoint of adding layers of abstraction to the existing model in order to remain close to practical/realistic conditions. Two very interesting things focused my attention in this project initially. The first being the abrupt change in treatment response when single drug therapy is replaced with multiple drug treatment. In my view there is still no conforming answers about what combinations of drug is optimal for personalized treatment for various cancer types. Secondly, some explanations in the modeling takes measurements when cancer has grown to a substantial amount after the treatment had began but focus less on the cumulative growth. So I think that these two topics can invoke some interesting questions on which research can be carried out.

Contents

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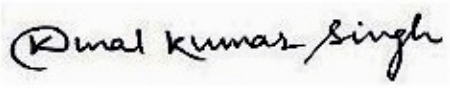

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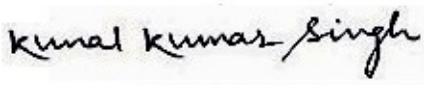

(Kunal Kumar Singh)

EE14MTECH11035

(Roll No.)

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(Dr. Soumya Jana) Chairman
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IIT Hyderabad

Chapter 1

Introduction to Cancer and Drug resistance

1.1 Introduction

The field of cancer research have seen a massive support from researchers and scientists from various domain of science and technology [2]. Cancer is a disease of abnormal genes. Alteration in one gene however, do not suffice to develop cancer but further hit of mutants, malignancy and invasion blows up the growth [3]. Cancer grows as a result of somatic evolutionary process. The change or alteration in normal regulation and cell-cycle advancement, division of cells and death results in tumor formation and cancer growth. Biological process of evolution such as cell mutation and selection can be described with the help of stochastic processes [4] and mathematical models can build a framework to predict and explain patterns in the success and failure of anti-cancer therapy. The molecular level of understanding of such processes can be useful in developing anti-cancer therapy. In traditional cancer treatment approach, chemotherapy and radiation therapy have been successful in past and is still considered as the standard treatment of cancer but, "targeted therapy" have gained the focus of attention in last few decades. A short history of "targeted anticancer therapies" is cited in [5]. The "targeted therapy" refers to those drugs which have a dedicated mechanism and are active on specific targeted proteins or biological pathways to alter the abnormal growth of cancerous cells. With the ongoing development of "targeted therapies" mathematical models can prove to be an effective tool to explore the combinational treatment approach and can tailor an effective personalized treatment of cancer. The biggest advantage of using targeted therapy as compared to other means of treatment like chemotherapy and radiation therapy is the minimal side effects due to specificity of targeted drugs. Chemotherapy or radiation therapy kills both the cancerous as well as healthy cells leading to severe side effects but targeted drugs do not harm healthy cells and kills only cancer cells thus, have a selective advantage over chemotherapy and radiation therapy. The first targeted drug which was developed and was used to block the activity of BCR-ABL kinase protein in chronic myeloid leukemia (CML) was Gleevec (imatinib) [6] followed by the development of other small molecule inhibitors or targeted therapies [5]. The biggest limitation of these drugs arises when the evolution of resistance takes place and tumor growth becomes uncontrolled, so, these drugs can no longer fight against the proliferating resistance mutants. There are two general mechanism for

resistance generation in cancer cells [7][8]. (i) *Host factors such as absorption and rapid metabolism, which forms intrinsic resistance and,* (ii) *Genetic alteration leading to escape of treatment.* There are other means of resistant generation, for example, MDR or *multi-drug resistance* [8]. In MDR, drugs which are structurally and functionally unrelated to each other when acted upon the cancer target becomes simultaneous resistant. Our first half of discussion will be centered around MDR in cancer and treatment using "targeted drugs". We will touch upon this topic in the next few chapters by looking through the lens of system biology and developing few tools using stochastic processes to build the foundation of cancer growth and its dynamics including the resistance generation and treatment effects. We will develop the framework of cancer treatment and drug resistance in context of chronic myeloid leukemia (CML). Subsequently we will look at quiescence effects and its relation to drug resistance. In the last chapter we will look at another process called as muti-type branching process which define the avalanche in cancer growth. We will use the parameters to define the events that happens in the process and design drug administration schedules optimally with the help of toxicity constraints family in order to avoid or maximally delay the emergence of resistance. We should keep in mind that the theoretical work that we describe here is only valuable to the field of cancer research if models are validated by experiment, predictions are tested, and models are revised in the light of empirical data.

1.2 What is Cancer?

The development of a healthy human requires the cooperation of more than ten million cells in the body. This cooperation is maintained by signals and cellular checkpoints which determine whether cells divide, die, or differentiate. Cancer is a result of the collapse of this cooperation [9] which results in the selfish, uncontrolled growth of cells within the body and eventually leads to the death of the organism. Cancers arise and progress by accumulating various mutations and epigenetic changes, which gives them a selective advantage over healthy cells, and lead to growth and pathogenesis. However, the evolution of primary cancer do not lead to the death of organism. Some Cancer cells acquire the ability to enter the blood stream, travel to a different site, and start growing unbounded if enough nutrient is available in vicinity. This process is referred to as metastasis. It is usually the metastatic growth which kills the organism.

1.3 Treatments of Cancer

Cancer treatment aims at stopping the uncontrolled growth of Cancer cells. The traditional therapies include chemotherapy and radiation therapy. The objective behind these therapy is to damage the genomes of cells such that they halt division and/or die. Dividing cells are targeted in this approach. However healthy dividing cells are also affected in this approach leading not only severe side effects but, can also induce the formation of new tumors. A better solution to avoid side effects is to target only Cancer cells and isolate healthy cells rather than targeting dividing cells in general. We call such approach as "targeted treatment approaches". Targeted drugs or small molecule inhibitors specifically target molecular defects in Cancer cells which eliminates their ability to grow out of control. The success behind these targeted drugs lies in halting the mechanism of growth and reducing the tumor load. The treatment of chronic myeloid leukemia (CML) with the targeted

inhibitor imatinib and subsequent inhibitors distinib and nilotinib has seen the greatest success so far [10]. Presently in many cancer types the defects that drive and maintain aberrant proliferation of cancer cells remain poorly defined, thus preventing the development of targeted inhibitors. However, new targeted drugs are in clinical trials and the scope of research on these classes of drugs has a well promising future.

1.4 Small Molecule Inhibitors

Small Molecule Inhibitors or "Targeteted therapies" are used for targeted treatment of cancer. These inhibitors counters specific cellular defects and resists the growth of cancer. Targeted therapies shows high effectiveness and a high specificity for cancer cells as compared to chemotherapy and radiation therapy. These drugs binds specific tumor targets and cures the tumor load. However drug resistance is a major problems in targeted treatment approach especially in the advanced phase of the disease, and can lead to treatment failure. The inhibitors which we will be focusing on in our cancer modeling exercise are imatinib, disatinib and nilotinib. We will discuss everything here in context of chronic myeloid leukemia (CML).

1.5 The Role of Mathematical Models

In addition to clinical and experimental research, the field of mathematical oncology, which uses mathematical and computational models can be very useful to study the treatment of CML with small molecule inhibitors. The concept of evolution plays a special role in this context. Mathematical models allow us to investigate the evolutionary dynamics of drug-resistance in cancer, which in turn can lead to the design of combinational drug treatment strategies aimed at overcoming treatment failure due to resistance. Moreover mathematical models provide insight and make predictions that would be impossible to achieve by experimental and clinical study alone. The biggest challenge faced by modelers is that the complexity of the process underlying tumor growth, progression, and therapy is the enormous, and excluding it makes the model very simplistic, which in turn increases uncertainty that arises when formulating biological processes in terms of mathematical models. We will start with a simple definition of biological processes underlying cancer growth and mutant generation and keep on refining the model by adding the complexity of the process and explaining it using mathematical equations.

1.6 Scope of the text

The contents presented in the subsequent chapters is a review work in order to understand the underlying principles of emergence of drug resistance in cancer and its treatment using multiple drugs. One reason for cancer generation is the development of multiple point mutations, others include gene amplification, chromosomal instability etc. We will focus on the former case in context of CML. It is widely accepted that first line imatinib therapy fails in many CML patients and calls for second generation inhibitors such as disatinib and nilotinib. We are interested to understand that how a combination of these three inhibitors are useful in the treatment of CML. In chapter 2 we will use some mathematical tools to model the growth of cancer, the generation of resistance, and the

effect of multidrug treatment. The methodology is stochastic, based on the description of a cellular colony as a birth–death process with mutations. We will analyze a simple model by assuming that there is no cross-resistance effects of these three drugs. In chapter 3 we will include cross-resistance effect to modify and extend the model developed in chapter 2 whereas, In chapter 4 we will focus on the effect of Cellular Quiescence on the evolution of drug resistance in CML. In the last chapter we will look at another process called as muti-type branching process which define the avalanche in cancer growth. We will use the parameters to define the events that happens in the process and design drug administration schedules optimally with the help of toxicity constraints family in order to avoid or maximally delay the emergence of resistance. It is very important to keep in mind that the experimental setups and results presented here is quoted with appropriate reference and this is something which is taken for granted in order to address the problem.

Chapter 2

Evolution of Resistance and Multi Drug treatment

2.1 Introduction

The mathematical framework of drug resistance was first elucidated by J.H. Goldie and A.J. Coldman who pioneered the field of mathematical modeling of drug resistance using stochastic processes. Their ground breaking work can be seen here [11]. The framework presented in this chapter follows the tradition of [11] and extends the work further. This chapter focuses on developing a stochastic framework of a process called "Death and Birth Process in a cellular colony" to address the treatment outcome using multiple drugs [10]. We will develop a combinatorial mutation network to analyze how drugs in combination becomes resistant simultaneously. We will frame the process dynamics and include drug induced effects on the proliferating cell population. Every events that goes into the process has certain probabilities of occurrence and we will accumulate all these probability to explain the events mathematically. Kolmogorov equation is useful to analyze the process in general but we will use it in the special case only, ie., for the case of one and two drug cases. Successful treatment guarantees complete extinction of cancer cells and we will show that it happens only when the probability of non-production of resistant mutant equals the probabilities of extinction. The analysis is taken in the simplistic case by considering symmetric relations in parameter assumptions. Birth and Death rates (L, D) of cancerous cells are two entities which gives a rough idea about the overall growth rate of cancer. This quantity is related through a term called turnover rate (D/L). We will see that the dependence of turnover rate on successful treatment vanishes when single drug is used whereas, there will be a severe dependence of (D/L) on treatment outcome as the number of drugs are increased. At the end we will frame an equation for threshold tumor size and say that if this limit or some fraction of it exceeds, it is very difficult to prevent the emergence of resistance and a person may die. Under such assumption we can fix few parameters for example, like observable mutation rates "u" and fix the number of drugs useful for successful treatment. The same goes when we fix other parameters like death rate of cells etc.

2.2 Model: Birth-Death Process and Mutant generation

There are two kinds of cells in a cell colony namely, sensitive cells upon which the drug that we use is effective and, the resistant/mutant cells which are inactive to respond to any drug. We will assume that the only events that goes in the cellular colony to form cancer tumor are the following, also refer figure 2.1.

- *Faithful reproduction:* Increases the number of cells by one.
- *Death:* Reduces the number of cells by one.
- *Reproduction with a mutation:* Increases mutant type cell by one.
- *Transformation:* Decrease sensitive cells and, simultaneously increase mutant cell.

Figure 2.1: Birth & Death Process along with Mutations in a cell colony. [10]

The population of the cell colony becomes heterogeneous when cell mutates and give rise to a different kind of cell or mutant cell. The cells colony comprises of various "cell type" namely a combination of sensitive cells and resistant/mutant cells. When a drug is administered then sensitive cells dies while resistant cell remains intact. So, a cell can have two possible states namely *resistant state* or *susceptible state*. Let us say that the number of sensitive and resistant cells are denoted by integers i and j respectively. lets say that vectors (i, j) forms a state space. Sensitive cell mutates with rate u, looses its property and gains a resistance property. We show this change in property through a combinatorial mutation diagram as $X_0 \xrightarrow{u} X_1$. In the notation X_k the subscript k differentiates between cell types. Let us say that $k = 0$ represents susceptible property of the drug, and $k = 1$ represents resistant property of the drug. If we extend the same formulation for the case of two drugs in use then the network will look look a square with arrows coming out from any of the one node and resting on the diagonal node. If we incorporate one more drug then the combinatorial mutation network for the case of three drugs looks like the one as shown in figure 2.2 [12, 13]. Each mutation rates u_1, u_2, u_3 are taken different in general. If we look at the diagram and for example we see that state "000" is fully susceptible and state "111" has become fully resistant while mutating through various path with different mutation rates and resting to fully resistant state. There can be multiple pathways for a cell to get converted from fully susceptible to complete resistant state. Note that the state has three positions corresponding to drug number. Similarly we can extend the same network to accommodate more number of drugs and analyze the model. In the next section we will represent the model in general but analyze it in special case of two drugs only in order to understand the methodology in simple form.

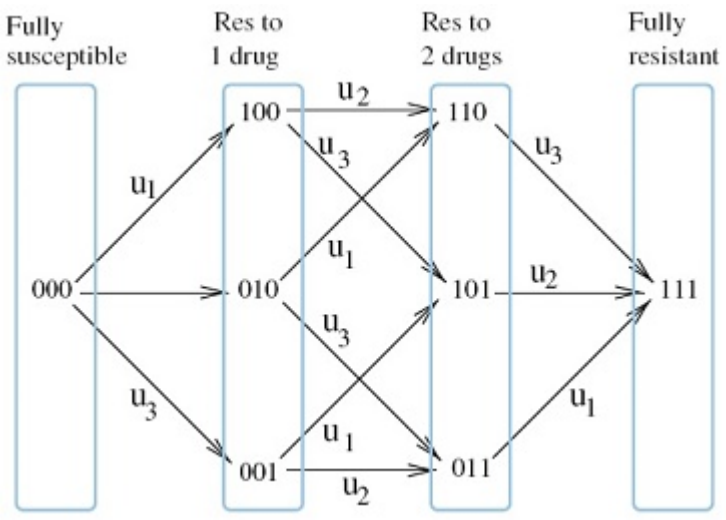

Figure 2.2: Combinatorial Mutation Network for three drugs in use. [10]

2.3 Process Dynamics and Modeling

The dynamics of the model is a Markov birth–death process, continuous in time and have discrete state-space as (i, j). Four events namely *(faithful division, death, mutation, and transformation)* occurs in the cell colony and their probability of occurrence is proportional to the infinitesimal time increment, Δt in which either of one of the four events occurs one at a time. Each event may change the resistance phenotype. For example, in figure 2.2 for $m = 3$ drugs in use there are eight different resistant phenotype given by (000, 001 ..... 111). As said the time of occurrence of one event at a time Δt is very small, the probability associated with each of the four events where resistance phenotypes changes from one type to another through *(faithful division, death, mutation, and transformation)* is given below. Figure 2.3 shows an example of node "A^s" in a mutation network where $0 \leq s \leq 2^{m-1}$, $u_{1,2}^{s,in}$ is the rate of mutation rate needed to produce resistance phenotype "A^s" whereas, $u_{1,2,3,4}^{s,out}$ defines the mutation rate needed to produce other different resistance phenotypes. Consider the following events with associated probabilities,

- **Faithful division:** The probability associated with identical creation of a cell of type "A^s" is $L_s(1 - \sum_j u_j^{s,out})\Delta t$.
- **Mutation:** The probability that a cell type "A^s" mutates to produce another cell type "$A_j^{s,nxt}$"; $\forall\ j$, is $L_s u_j^{s,out}$.
- **Cell Death:** With probability associated with cell death is $d_s \Delta t$.
- **Cell Transformation:** The probability that a cell type "A^s" transforms to another cell type "$A_j^{s,nxt}$"; $\forall\ j$, is $K_s \alpha_j^{s,out}$. In our exercise we will consider no transformation, or $K_s = 0$.

NOTE: The quantity L_s is the birth rate, and $d_s = D_s + H_s$ is the death rate of type s. D_s is the natural death rate and H_s is the drug induces death rate of various cell type. The growth of wild type cells are generally greater than its natural death rate ($L_0 > D_0$) or $0 \leq D_0/L_0 < 1$. The term D_0/L_0 is the turnover rate of wild type cells and it points the slow-growth or high-growth cancer. On the application of drug the cell colony decreases in size due to the high death rate of wild type

cells only. The quantity H_s is associated with drug induced death rate. If any cell responds to H_s then we assume $H_s = H = constant$.

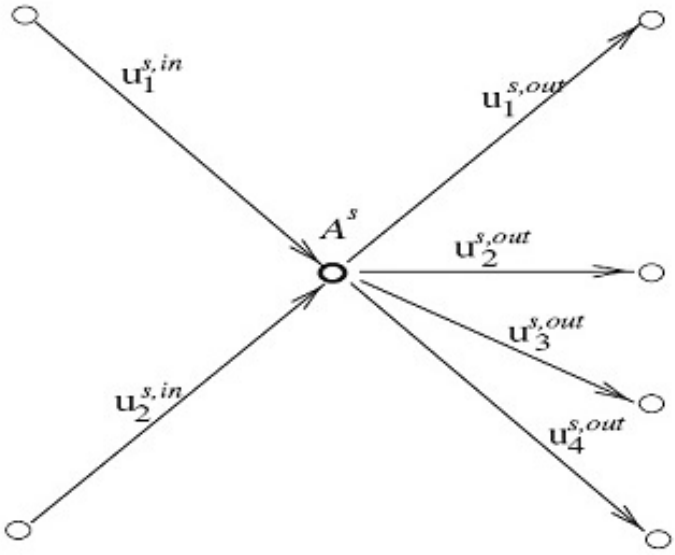

Figure 2.3: An example of a vortex in a mutation diagram

2.3.1 Process Analysis with one drug only

The stochastic process analysis done here is applicable for one drug treatment only. In further section extension is made to accommodate multiple drugs in general. System state integer vectors (i_0, i_1) comprises of sensitive cells and resistant cells respectively. The probability of these two cell type to be in state (i_0,i_1) at time t is given by the quantity $\varphi_{i_0,i_1}(t)$. According to Kolmogorov forward equation $\varphi_{i_0,i_1}(t)$ is given by,

$$\begin{aligned}\varphi_{i_0,i_1} = &\Big[L_0(1-u)(i_0-1)\varphi_{i_0-1,i_1} + L_1(i_1-1)\varphi_{i_0,i_1-1}\Big] \\ &+ \Big[L_0 u i_0 \varphi_{i_0,i_1-1}\Big] \\ &+ \Big[d_0(i_0+1)\varphi_{i_0+1,i_1} + d_1(i_1+1)\varphi_{i_0,i_1+1}\Big] \\ &- \Big[\varphi_{i_0,j_0}(L_0+L_1+d_0+d_1)\Big]\end{aligned} \tag{2.1}$$

The term in the first square bracket represent faithful division of cells, followed by division with mutation in the second bracket. The term in the third bracket is for death of cells and lastly a negative term represents no change in the system state. We introduce a new quantity here called as probability generating function given by,

$$\Psi(\xi_0,\xi_1;t) = \textstyle\sum_{i,j} \varphi_{i_0,i_1}\xi_0^{i_o}\xi_1^{i_1} \tag{2.2}$$

Solving equation 2.1 and 2.2 we can obtain a closed form PDE given by,

$$\frac{\partial \Psi}{\partial t} = \frac{\partial \Psi}{\partial \xi_0}\Big(L_0(1-u)\xi_0^2 + d_0 - \xi_0(L_0 + d_0 - L_0 u \xi_1)\Big) + \frac{\partial \Psi}{\partial \xi_1}\Big(L_1\xi_1^2 + d_1 - \xi_1(L_1 + d_1)\Big) \tag{2.3}$$

The solution of the above partial differential equation (PDE) can invoke many interesting questions. We will use this PDE to address the problem of success or failure of therapy.

2.3.2 Process Analysis with multiple Drug

In this subsection a similar analysis as seen previously can be done to incorporate multiple drugs. In general consider that we use m number of drugs. The probability mass function is written as $\varphi_{i_0,\ldots,i_n}(t)$. Similarly, the probability generating function be given by,

$$\Psi(\xi_0, \ldots, \xi_n; t) = \textstyle\sum_{i_0,..,i_n} \varphi_{i_0,..,i_n}(t) \prod_{s=0}^{n} \xi_s^{i_s} \tag{2.4}$$

If we solve equation 2.4 using probability mass function $\varphi_{i_0,\ldots,i_n}(t)$ then we will obtain a hyperbolic partial differential equation as,

$$\frac{\partial \Psi}{\partial t} = \sum_s \frac{\partial \Psi}{\partial \xi_s}\Big[\xi_s^2 L_s(1-u^{s,out}) + d_s + \xi_s L_s \sum_j \xi_j u_j^{s,out} + K_s \sum_j \xi_j \alpha_j^{s,out} - \xi_s(L_s + d_s - K_s\alpha^{s,out})\Big] \tag{2.5}$$

where the notation $u^{s,out}$ and $\alpha^{s,out}$ is given as,

$$u^{s,out} = \sum_j u_j^{s,out} \qquad \alpha^{s,out} = \sum_j \alpha_j^{s,out}$$

2.3.3 Solution of system of PDE and HPDE

Till now we have obtained a closed form solution of kolmogorov forward equation for single drug treatment as well as multiple drug treatment. We need the closed form of equation 2.3 and 2.5 to address the problem of treatment success or failure. We can solve the system of PDE with the help of standard method of characteristic [10]. The characteristics equation obtained after solving equation 2.5 is given by,

$$\dot{\xi_s} = L_s(1-u^{s,out})\xi_s^2 + \Big[L_s \textstyle\sum_j \xi_j u_j^{s,out} - (L_s + d_s + K_s\alpha^{s,out})\Big]\xi_s - K_s \textstyle\sum_j \xi_j \alpha_j^{s,out} + d_s \tag{2.6}$$

If M_0 cells are not resistant any drugs at $t = 0$ then $\xi_{M_0,0,\ldots,0}(0) = 1$ and,

$$\Psi(\xi_0, \ldots, \xi_n; 0) = \xi_n(0)^{M_0}$$
$$\Psi(\bar{\xi_0}, \ldots, \bar{\xi_n}; \bar{t}) = \xi_n(\bar{t})^{M_0} \qquad \textit{and } t = \bar{t} > 0$$

Consider the simplest two stage treatment process:

- $0 < t < t_*$: Pretreatment phase, No drugs in use such that $H_s = 0$, $d_s = D_s$.
- $t \geq t_*$: Treatment phase, Includes drug induced death of cells such that $d_s = D_s + H_s$.

Size of tumor at the start of treatment, ie., at $t = t_*$ can be written as,

$$N = M_0 \exp\Big((L_0 - D_0)t_*\Big) \tag{2.7}$$

We are interested to calculate the function $\Psi(\bar{\xi_0}, ..., \bar{\xi_n}; t_* + \bar{t})$, where $\bar{t} \geq 0$ to obtain the information at time ahead of the start of treatment. The intuitive idea for solving this problem is as follows:

- We know the initial conditions namely $\xi_0(0) = \bar{\xi_0}, ..., \bar{\xi_n}(0) = \bar{\xi_n}$ apriori. We need to solve the equation 2.6 using *treatment coefficients* and the initial conditions. The solution obtained would be $\xi_0(\bar{t}), ..., \xi_n(\bar{t})$.
- Next, $\xi_0(\bar{t}), ..., \xi_n(\bar{t})$. becomes the initial condition. Use *pretreatment coefficients* to obtain the solution $\xi_0(t_*), ..., \xi_n(t_*)$ at time $t = t_*$ which is $\Psi(\bar{\xi_0}, ..., \bar{\xi_n}; t_*) = \xi_n(t_*)^{M_0}$

2.3.4 Treatment Success Vs. Treatment Failure

An intuitive idea says that if the fully resistant mutant are never produced in the process then there is a fair chance that the cell colony gets extinct as a result of multiple drugs in use. We formulate the idea into the mathematics with following few notations,

- $\Psi(0, 1, ..., 1; t)$: Probability of non-production of fully resistant mutant or cell type "A^n" at time t under the initial conditions $\xi_0 = 0 \ \ , \ \ \xi_i = 1 \ \ ; \ \ 1 \leq i \leq n$.
- $\Psi(0, 0, ..., 0; t)$: Probability that all cells (sensitive/resistant) of all type have got extinct under the initial conditions $\xi_0(0) = \xi_1(0) = ... = \xi_n(0) = 0$.

If we put the arguments of $\Psi(0, 1, ..., 1; t)$ and $\Psi(0, 0, ..., 0; t)$ in equation 2.4 and if the condition in equation 2.6 is satisfied then under the above initial condition as time $t \to \infty$ we get,

$$P_{nonprod}(t) = \Psi(0, 1, ..., 1; t) = \xi_0(t)^{M_0}$$
$$P_{ext}(t) = \Psi(0, 0, ..., 0; t) = \xi_0(t)^{M_0}$$

$$\lim_{t\to\infty} P_{ext} = \lim_{t\to\infty} P_{nonprod}$$

The probability of treatment failure can be defined as,

$$P_{failure}(t) = 1 - P_{ext} = 1 - P_{nonprod} = 1 - \xi_0^{M_0} \tag{2.8}$$

The successful extinction of cell colony can be achieved if it get extinct spontaneously or when tumor grows but treated successfully by the therapy. The former being the transient solution for the quantity $\Psi(0, 0, ..., 0; t)$ and the later reflects the steady state behavior as shown below.

$$\Psi_{0,0,...,0}(t) = \Psi(0, 0, ..., 0; t) = \xi_0(t)^{M_0} = (D_0/L_0)^{M_0} + (1 - (D_0/L_0)^{M_0})P_{success}(t)$$

$$P_{success}(t) = \frac{\xi_0(t)^{M_0} - (D_0/L_0)^{M_0}}{(1-(D_0/L_0)^{M_0})} \tag{2.9}$$

2.4 Simplifying calculations using symmetrical coefficients

If we look again at the figure 2.2, we can see a vertical strip of classes of phenotype which are either fully susceptible to drug, resistant to a single, multiple or all drugs. If we can group such resistant types into classes such that in each class the cell types are resistant to k drugs and susceptible to $(m-k)$ drugs. The number of such classes are k, and $0 \leq k \leq m$. Also, ξ_s reduces to ξ_k only with $0 \leq k \leq m$. If we further let the birth and death rates in each class to be equal, along with all mutation rates equal we can make further simplification. In this way we achieve dimension reduction from $n = 2^m + 1$ to $m + 1$ and equation 2.6 reduces to a simplified form as,

$$\dot{\xi_k} = L_k(1-(m-k)u)\xi_k^2 + \Big[(m-k)L_k u\xi_{k+1} - (L_k + d_k)\Big]\xi_k + d_k \qquad 0 \leq k \leq m \tag{2.10}$$

2.4.1 Process Analysis with two drugs

A boolean combination of two different drug type produces four phenotypes of resistance; as compared to eight phenotypes in case of three drugs as shown in figure 2.2. For two drugs (m=2) the four phenotypes are "A^{00}", "A^{01}","A^{10}", "A^{11}". As we observed the case of one drug in section 2.2 we can similarly extend the same idea to accommodate two drugs. The function $\varphi_{i_{00},i_{10},i_{01},i_{11}}$ satisfies the following Kolmogorov forward equation.

$$\begin{aligned}
&\dot{\varphi_{i_{00},i_{10},i_{01},i_{11}}} = \\
&\Big[L_{00}(1-u_1-u_2-u_{12})(i_{00}-1)\varphi_{i_{00}-1,i_{10},i_{01},i_{11}} + L_{10}(1-u_2-u_{12})(i_{10}-1)\varphi_{i_{00},i_{10}-1,i_{01},i_{11}} \\
&+ L_{01}(1-u_1-u_{12})(i_{01}-1)\varphi_{i_{00},i_{10},i_{01}-1,i_{11}} + L_{11}(i_{11}-1)\varphi_{i_{00},i_{10},i_{01},i_{11}-1}\Big] \\
&+ \Big[i_{00}\big(u_1\varphi_{i_{00},i_{10}-1,i_{01},i_{11}} + u_2\varphi_{i_{00},i_{10},i_{01}-1,i_{11}} + u_{12}\varphi_{i_{00},i_{10},i_{01},i_{11}-1}\big) \\
&+ i_{10}\big(u_2\varphi_{i_{00},i_{10},i_{01},i_{11}-1} + u_{12}\varphi_{i_{00},i_{10},i_{01},i_{11}-1}\big) + i_{01}\big(u_1\varphi_{i_{00},i_{10},i_{01},i_{11}-1} + u_{12}\varphi_{i_{00},i_{10},i_{01},i_{11}-1}\big)\Big] \\
&+ \Big[d_{00}\varphi_{i_{00},i_{10}+1,i_{01},i_{11}} + d_{10}\varphi_{i_{00},i_{10}+1,i_{01},i_{11}} + d_{01}\varphi_{i_{00},i_{10},i_{01}+1,i_{11}} + d_{11}\varphi_{i_{00},i_{10},i_{01},i_{11}+1}\Big] \\
&- \Big[\Big(i_{00}(L_{00}+d_{00}) + i_{10}(L_{10}+d_{10}) + i_{01}(L_{01}+d_{01}) + i_{11}(L_{11}+d_{11})\Big)\varphi_{i_{00},i_{10},i_{01},i_{11}}\Big]
\end{aligned} \tag{2.11}$$

2.4.2 Equations for the Characteristics

If we look at earlier formula of probability generating function and we substitute $m = 2$ to consider only two drugs then the probability generating function is given by,

$$\Psi(\xi_{00},\xi_{10},\xi_{01},\xi_{11};t) = \sum_0^n \varphi_{i_{00},i_{10},i_{01},i_{11}}(t)\xi_{00}^{i_{00}},\xi_{10}^{i_{10}},\xi_{01}^{i_{01}},\xi_{11}^{i_{11}} \tag{2.12}$$

By solving equation 2.11 and 2.12 we obtain the equation of characteristics as,

$$\begin{aligned}
\dot{\xi_{11}} &= \left[L_{11}\xi_{11}^2 - (L_{11}+d_{11})\xi_{11} + d_{11}\right] \\
\dot{\xi_{10}} &= \Big[L_{10}\xi_{10}^2(1-u_2) - \big[(L_{10}u_2\xi_{10} - (L_{10}+d_{10})\big]\xi_{10} + d_{10}\Big] \\
\dot{\xi_{01}} &= \Big[L_{01}\xi_{01}^2(1-u_1) - \big[(L_{10}u_1\xi_{01} - (L_{01}+d_{01})\big]\xi_{01} + d_{01}\Big] \\
\dot{\xi_{00}} &= \Big[L_{00}\xi_{00}^2(1-u_1-u_2) - \big[(L_{00}(u_1\xi_{10}+u_2\xi_{01}) - (L_{00}+d_{00})\big]\xi_{00} + d_{00}\Big]
\end{aligned} \tag{2.13}$$

Under appropriate initial conditions, we can solve the systems of equations in 2.13 to calculate the probability of treatment success recursively. We will look into the solution later in the section.

2.4.3 Statistical count of cells in various classes

If one wishes to calculate the *Mean numbers of cells in each class* as grouped using symmetric coefficient constraints (Section 2.4) then we can obtain all the moments as shown below,

$$\begin{aligned}
x_{00}(t) &= \sum i_{00}\varphi_{i_{00},i_{10},i_{01},i_{11}}(t) \qquad x_{10}(t) = \sum i_{10}\varphi_{i_{00},i_{10},i_{01},i_{11}}(t) \\
x_{01}(t) &= \sum i_{01}\varphi_{i_{00},i_{10},i_{01},i_{11}}(t) \qquad x_{11}(t) = \sum i_{11}\varphi_{i_{00},i_{10},i_{01},i_{11}}(t)
\end{aligned}$$

where the summation is performed over all the four indices's. We obtain,

$$\begin{aligned}
\dot{x_{00}} &= \big[L_{00}(1-u_1-u_2) - d_{00}\big]x_{00} \\
\dot{x_{10}} &= \big[L_{10}(1-u_2) - d_{10}\big]x_{10} + L_{00}u_1x_{00}, \\
\dot{x_{01}} &= \big[L_{01}(1-u_1) - d_{01}\big]x_{01} + L_{00}u_2x_{00}, \\
\dot{x_{11}} &= \big[L_{11} - d_{11}\big]x_{11} + L_{10}u_2x_{10} + L_{01}u_1x_{01}.
\end{aligned} \tag{2.14}$$

Under the initial conditions: $x_{00}(0) = M_0 \quad x_{10}(0) = x_{01}(0) = x_{11}(0) = 0$. we can obtain the *Mean numbers of cells in each class* by solving the above state space equation.

2.5 Resistant Mutant comes in picture again

Resistance mutant if happens to be present forms a major obstacle in treatment. Drugs used in treatment fails and there is nothing that we can do. We have to know that is it before the treatment that it formed or during the process of treatment? Again to make life simple we will use only symmetric coefficients described in section 2.4. Consider two new notations,

- $P^{\uparrow}(N)$: Probability to develop resistance as $t \to \infty$, if mutation happens iff before treatment.
- $P^{\downarrow}(N)$: Probability that at least one fully resistant mutant exist as $t \to \infty$ and no mutation happens after treatment begins. Colony size is N in both cases.

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2.5.1 Presence of resistant mutants before treatment phase only

If resistant mutant exists before therapy begins then let us consider that no mutants are generated during therapy. Do the colony ever vanishes as $t \to \infty$ or in other words what is the *probability of non-extinction* of colony?

- *Characteristic equation for system before treatment*

$$\begin{aligned} \dot{\xi_i} &= L(1 - i\,u)\xi_i^2 + \Big[i\,L\,u\,\xi_{i+1} - (L + D)\Big]\xi_i + D \\ \dot{\xi_m} &= L\xi_m^2 - (L + D)\xi_m + D \end{aligned} \tag{2.15}$$

- *Characteristic equation for system as and after treatment starts*

$$\begin{aligned} \dot{\xi_i} &= L(1 - i\,u)\xi_i^2 + \Big[L + D + H\Big]\xi_i + D + H \\ \dot{\xi_m} &= L\xi_m^2 - (L + D)\xi_m + D \end{aligned} \tag{2.16}$$

As explained in section 2.3.3 we can solve the above two set of system of equation by first obtaining the steady state variable of system of equation 2.16 and then substituting the value as an initial condition in system of equation 2.15. Preexistence of resistant mutants thus obtained is,

$$\boxed{P^{\uparrow}(N) = \lim_{t\to\infty} \Psi(0, 1, ..., 1, t_0 + \bar{t}) = 1 - \xi_0(t_*)^{M_0} \quad ; \quad t_* = \tfrac{1}{L-D} ln(N/M_0)} \tag{2.17}$$

2.5.2 Mutants generation during treatment phase only

We assume that there is no mutant cell initially and is grown during treatment phase only. The colony comprises of N susceptible cells with no resistant cells. In this case we assume that no mutants are present beforehand and are only generated after the start of therapy. The *Systems of equation* for this case is,

$$\begin{aligned} \dot{\xi_i} = L(1 - i\,u)\xi_i^2 + \Big[i\,L\,u\,\xi_{i+1} - (L + D + H)\Big]\xi_i + D + H \quad 0 \le i \le m. \\ \dot{\xi_m} = L\xi_m^2 - (L + D)\xi_m + D \\ \xi_i(0) = 0 \quad 0 \le i \le m. \end{aligned} \tag{2.18}$$

We solve the system of equation similarly under the limiting case $u \ll (L - D)$ and $t \to \infty$ then,

$$\begin{aligned} \lim_{t\to\infty} \xi_i &= \frac{i!(L - D)L^{i-1}u^i}{(D + H - L)^i} \qquad 0 \le i < m. \\ \lim_{t\to\infty} \xi_m &= \frac{D}{L} \end{aligned} \tag{2.19}$$

The probability to develop resistance as $t \longrightarrow \infty$ is given by,

$$P^{\downarrow}(N) = 1 - \lim_{t\to\infty} \xi_0(t)^N$$

Suppose that the number of drugs used is m then from equation 2.19 we get

$$P^{\downarrow}(N) = 1 - \left[1 - \frac{m!(L-D)L^{m-1}u^m}{(D+H-L)^m}\right]^N \tag{2.20}$$

Observation: Compare Equation 2.17 and Equation 2.20

- If tumor size N is large probability of success is low.
- If $P^{\uparrow}(N) > P^{\downarrow}(N)$, Fully resistant mutants are created before therapy and treatment phase is less useful.
- If $P^{\uparrow}(N) < P^{\downarrow}(N)$, Most mutants are generated after treatment starts.

2.5.3 Special case: One Drug

Generation of mutants during treatment: If one drug ($m = 1$) is used for treatment then the probability to create resistance during therapy (Equation 2.20) is,

$$P^{\downarrow}(N) = 1 - \left[1 - \frac{(L-D)u}{(H-(L-D))}\right]^N \tag{2.21}$$

Under a certain threshold value of drug induced death rate H as $H = H_c = 2(L - D)$ "which we can set by using appropriate drug concentration" we get,

$$P^{\downarrow}(N) = 1 - (1-u)^N \approx 1 - exp(-Nu) \approx Nu \tag{2.22}$$

Generation of mutants before treatment: If we set m=1 and solve equation 2.15 then we will end up getting the following result. Note that we are looking for a mutant to be created and survived in order to detect it at diagnosis.

$$\xi_0(t) = 1 - \frac{(exp[(L-D)t] - 1)Lu}{L-D} \approx 1 - \frac{NLu}{M_0(L-D)} \tag{2.23}$$

The probability of mutant creation from the susceptible cell when the colony size reaches N is,

$$P_{create} = 1 - \xi_0^{M_0} \approx \frac{NLu}{(L-D)}$$

The probability for survival of at-least one mutant cell as $t \to \infty$

$$P_{survive} = 1 - \frac{D}{L}$$

$$P_{failure} = P^{\uparrow}(N) = P_{create}P_{survive} = Nu \tag{2.24}$$

Conclusion: One Drug

- The probability of failure is dependent on colony size N and u and depends on the presence

of resistant mutant before the treatment. Mutant generation before and after treatment is independent of turnover rate of cancer.

- If $H = H_c = 2(L - D)$ then $P^{\uparrow}(N) = P^{\downarrow}(N)$
- For the realistic case ie., If $H > H_c = 2(L - D)$ then $P^{\uparrow}(N) > P^{\downarrow}(N)$, or resistance is mostly generated before the start of therapy.

2.5.4 Special case: Two Drug

Generation of mutants during treatment: If two drugs ($m = 2$) are used for treatment then the probability to create resistance during therapy is given as,

$$P^{\downarrow}(N) = 1 - \left[1 - \frac{2(L-D)u^2}{(H-(L-D))^2}\right]^N \tag{2.25}$$

$$P^{\downarrow}(N) = \left[\frac{2(L-D)u^2 N}{(H-(L-D))^2}\right] \quad ; \quad Nu^2 \ll 1 \tag{2.26}$$

Generation of mutants before treatment: In case of two drugs (m=2) "we will make the approximation of a doubly stochastic process, whereby generation of each one-hit mutant leads to a birth–death process, all of which are independent and identically distributed" [10]. The probability of mutant creation and survival is,

$$P_{create} = 2\left(\frac{Lu}{L-D}\right)^2 N\left(ln\frac{N}{M_0} - 1\right) \quad and \quad P_{survive} = 1 - \frac{D}{L}$$

$$P^{\uparrow}(N) = P_{create}P_{survive} = \frac{2Lu^2N}{L-D}\left(ln\frac{N}{M_0} - 1\right) \tag{2.27}$$

We see that if the number of drugs is increased the term L and D appears which defines the turnover rate of cancer. $P^{\uparrow}(N)$ now depends on the turnover rate, D/L. From equation 2.27 we can observe that if the natural death rate of tumor cells D is high then resistant mutants are produced at a higher rate and treatment fails. See Figure 2.4. Also if $D \to L$ then there is no solution. This is an inherent problem with this analysis. Taking the ratio of equation 2.26 and 2.27 for $M_0 = 1$ we get,

$$\frac{P^{\downarrow}}{P^{\uparrow}} = \left[\frac{H}{(L-D)} - 1\right]^{-2}\Big[ln(N) - 1\Big]^{-1}$$

$$\frac{P^{\downarrow}}{P^{\uparrow}} < 1 \quad \Longleftrightarrow \quad H > (L-D)(1 + (ln(N) - 1)^{-0.5})$$

Conclusion : Two Drugs

- For two drugs, the generation of resistant mutants in the pre-treatment phase depends on the turnover rate, D/L.

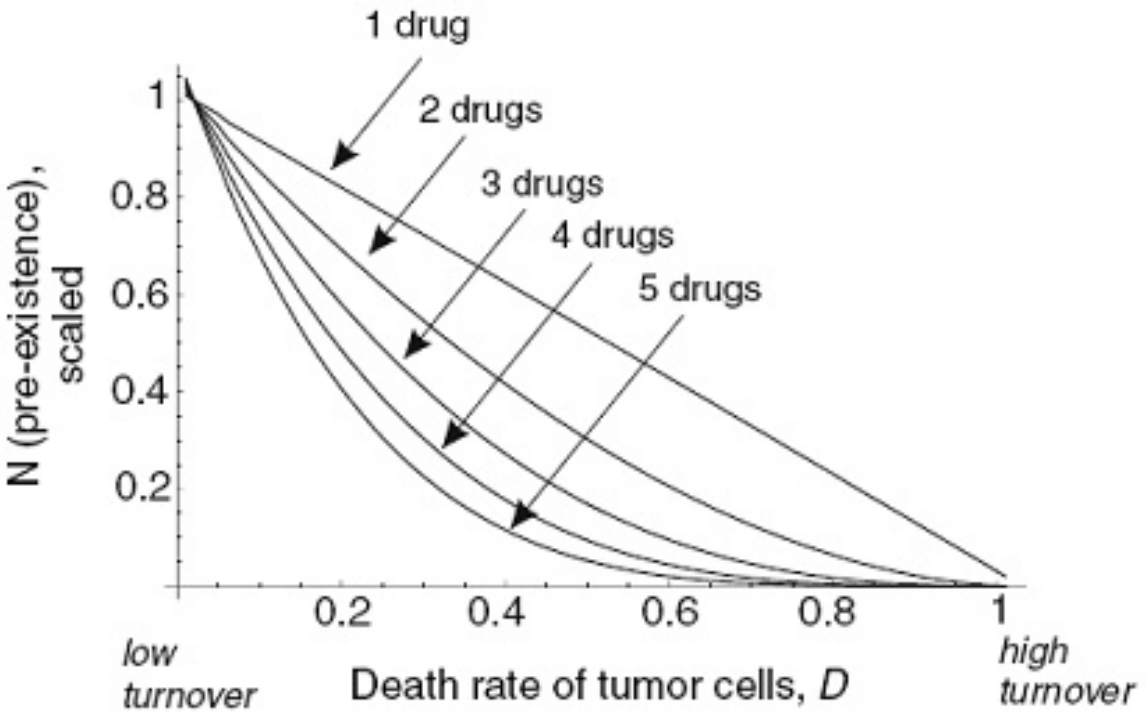

Figure 2.4: Generation of mutant before treatment, Tumor size vs. death rate (D) of tumor [10].

2.6 Quantifying Treatment Success

Probability of successful treatment: Probability of treatment success [10] can be written as,

$$P_{success}(N) \approx \left(1 - \frac{HNu}{(H+D-L)M_0}\right)^{M_0} \tag{2.28}$$

If therapy is very strong such that $H \gg L - D$, then probability of successful therapy is independent of turnover rate D/L of cancer,

$$P_{success}(N) \approx \left(1 - \frac{N}{M_0}u\right)^{M_0} \tag{2.29}$$

2.7 Summary

In this chapter formulation and analysis of a stochastic model of death-birth process with mutation under multi-drug treatment was carried out to investigate the dependence of treatment outcomes on the initial tumor load (N), mutation rate (u) and the turnover rate (D/L) of cancerous cells. We saw the importance of pretreatment phase in successful treatment of cancer and related mutant production. Two special cases namely the use of one/two drug apart from general case is set as an example to demonstrate the process underlying growth of cancer.
We observed that in case of one drug:

- The probability of failure in the treatment of a given cancer size (N) is independent of turnover

rate (D/L) of cancer,

- If $H = H_c = 2(L - D)$ then $P^{\uparrow}(N) = P^{\downarrow}(N)$
- For the realistic case ie., If $H > H_c = 2(L - D)$ then $P^{\uparrow}(N) > P^{\downarrow}(N)$, or resistance is mostly generated before the start of therapy.

In case of two or more drug:

- Pre-treatment phase plays the dominant role in treatment failure and; generation of resistance in treatment phase is less important,
- High-turnover cancers have a higher probability of treatment failure (for the same size N) than low-turnover cancers;
- Both of these effects become stronger for larger numbers of drugs.

Chapter 3

Re-Modeling and Cross Resistance of Drugs

3.1 Goal

The first half of this chapter has the following goal: To understand the reason for the difference between the single drug treatment and multi-drug treatment cases as observed on the basis of turnover rate dependence. The paper [14] is based on deterministic framework in contrast to the earlier work which were stochastic to mainly address the problem of parameter dependence of success under single drug treatment.
The second half of the chapter has the following goal: To study the optimum use of cross-resistant drugs instead of non-cross resistant drugs when mutation (here T315 mutation) confers complete resistance to a number of drugs (here drugs in use are imatinib, disatinib and nilotinib) in the treatment of chronic myeloid leukemia (CML).

3.2 Introduction

Earlier work on the process modeling can be explained alternatively in a deterministic framework as well. In this section we will formulate a deterministic model of the process where two cell types grow, die and mutates as seen earlier. We will focuses our attention to address the problem of dependence of treatment success/failure on turnover rate (D/L) of the cancer under the use of single drug. The result says that the treatment success always depends on the turnover rate of cancer.

3.2.1 One drug treatment: An Elementary Model

Let us assume that the population comprises of two kinds of cells namely, Wild-type cancer cells denoted by $N(t)$ and Resistant cells denoted by $R(t)$ at some point in time. We will consider two phases namely pre-treatment and treatment phase. Other symbols have their usual meaning.

Pretreatment phase, $t \leq t_*$

$$\begin{aligned} N^{'}(t) &= (L-D)N(t) \\ R^{'}(t) &= (L-D)R(t) + uN(t) \\ N(0) &\neq 0, R(0) = 0 \end{aligned} \tag{3.1}$$

Treatment phase, $t \geq t_*$

$$\begin{aligned} N^{'}(t) &= (L-D-H)N(t) \\ R^{'}(t) &= (L-D)R(t) + uN(t) \\ & N(t_*), R(t_*) \end{aligned} \tag{3.2}$$

$$t_* = \frac{1}{L-D} ln\left(\frac{N(t_*)}{N(0)}\right) \tag{3.3}$$

Solving equation 3.1 using equation 3.2 and related treatment phase conditions we get the total amount of resistant cells at the time of diagnosis which is,

$$R(t_*) = N(0)ut_* exp\big[(L-D)t_*\big] \approx \frac{N(t_*)\ u\ ln\big[N(t_*)/N(0)\big]}{L\big[1-(D/L)\big]} \tag{3.4}$$

3.2.2 Observations

- The amount of resistant mutants generated before the beginning of the treatment clearly depends on the turnover rate.
- The slower the growth of the cancer (i.e., $D/L \approx 1$) the larger is the larger is the number of resistant mutant.
- Conversely, faster the tumor grows (i.e., $D/L \approx 0$) smaller is the resistance generation prior to treatment. See equation 3.4.

3.2.3 Resistance progeny

Now, assume that as soon as therapy begins at $t = t_*$ no mutants are allowed to be created. So, the only resistance that remains are the one created beforehand and we call it as "resistance progeny" and denote it by $R^p(t)$. $R^p(t)$ is given by

$$R^p(t) \approx \frac{M_0\ u\ ln(M_0/N_0)}{L\big[1-(D/L)\big]} exp\big[(L-D)t\big] \tag{3.5}$$

The additional term $exp\big[(L-D)t\big]$ accounts for the growth of the resistance progeny during treatment. Similarly such dependence can be shown to be present also in the case of a multi-drug therapy.

3.2.4 Conclusion and Discussion

- If we look at the equation 3.5 then we will see that the resistance progeny vanishes only when $t \to \infty$. So it is only at $t = \infty$ the result shows a lack dependence of the resistance on the turnover rate.
- The analysis done above is deterministic in nature and is based on an elementary compartmental system of linear ordinary differential equations, rather than on stochastic processes as seen in previous chapter.
- Irrespective of the number of drugs used the a related quantity $R^p(t)$ shows that treatment outcome always depends on the turnover rate D/L of cancer.

3.3 Cross Resistance of drugs: In-Vitro Experiment

Our earlier understanding of the process was based on the assumption that no cross resistance is observed in drugs. But, now we will dig deeper to understand the phenomenon of cross resistance of drug and its implications. when resistance against one drug implies resistance to other drugs in use than we encounter cross resistance effect. To uncover the principles of drug resistance in cancer, "drugs in different combinations and different concentrations have been used in In-Vitro experiment". Now I quote the following from [10].

In papers [15],[16] a quantitative analysis of mutations has been performed. In In-Vitro experiments described in these papers, CML cancer cells, Ba/F3, $p210^{bcr-able}$ were exposed to a minimally cytotoxic agent, N-ethyl-N-nitrogenous (ENU), a potent inducer of point mutations. The cells were then cultured in 96-well plates supplemented with graded concentrations of inhibitors. After some time (about 28 days), wells with positive outgrowth were expanded and then sequenced for mutations. Three different inhibitors namely imatinib, dasatinib, and nilotinib, were used, in different combinations and solo. Inhibitor concentrations used for the three inhibitors are listed in Table 3.1. The noted concentrations were motivated by the fact that nilotinib is at least 20-fold and dasatinib at least 300-fold more potent than imatinib [15]. After analysis of the total of 768 wells, there were 726 mutations. Of the 30 specific point mutations that had been previously identified in imatinib resistant patients, 25 were recovered in this experiment. In total, 26 point mutations were identified.

In vitro experiments suggest that,

1 Different resistant mutant types are produced when concentrations of drugs are used alters.
2 Mutants are resistant to more than one drug and shows cross resistance effects.

	Low Dose (nM)	Medium Dose (nM)	High Dose (nM)
Imatinib	2000	4000-8000	16000
Dasatinib	5	10-25	100-500
Nilotinib	50-250	500-1000	2000-50000

Table 3.1: Categorization of the doses of each inhibitor, as used in [16]

The summary of the experiment is shown in figure 3.1(a)-(b). In particular we can see there is only one mutation that confers resistance to all the three drugs. This mutation is termed as T315 mutation which shows resistance to all the three drugs used.

3.4 Methodology

We incorporate cross-resistance effects of drugs under multi-drug treatment as shown in figure 3.2(b). The same combinatorial mutation network under no cross resistance as analyzed in in chapter 2 is also shown to the left in the figure 3.2(a). Let $u_{i,j}$ be the mutation rate, which is nothing but weighted mutation rate $k_{i,j}u$. The subscript i, j, k are the three drugs in used and the term u is the basic mutation rate. For two drugs, there are three such rates, $u_{1,2}$, $u_{1,3}$, $u_{2,3}$. For three drugs $k_{1,2,3} = 1$ when all three drugs are given in high concentration, refer figure 3.1(a)-(b). We just need to add the weighting factor $k_{i,j,k}$ to our previous model to include cross-resistance effect in our new model. The factor $k_{i,j,k}$ are the integers represented in the boxes in figure 3.1.

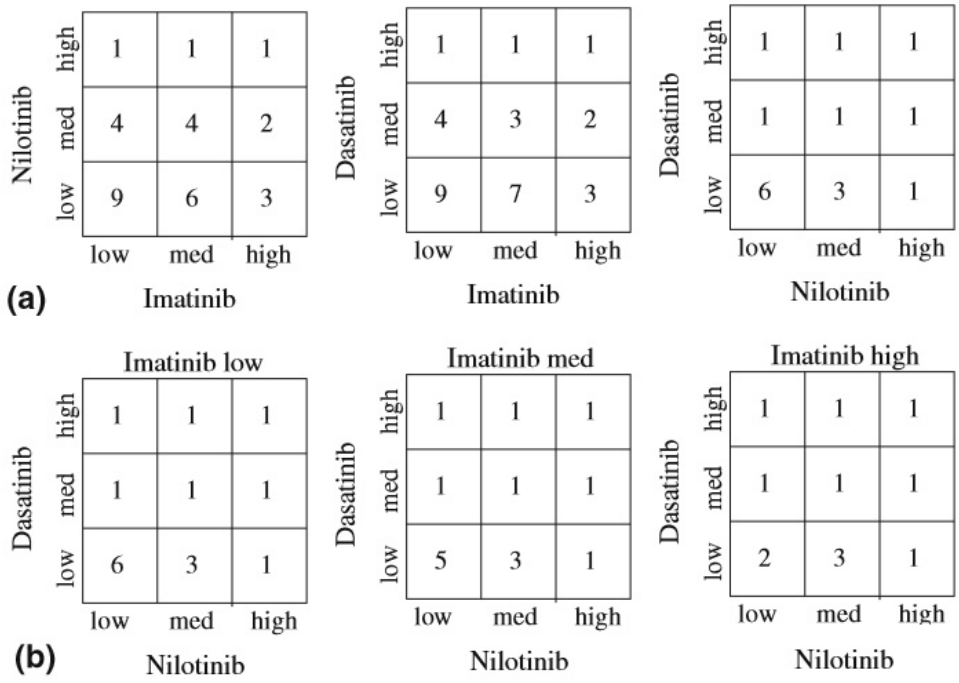

Figure 3.1: (a) Number of doubly resistant mutants for different combinations of drug doses. (b) Number of triply resistant mutants [15].

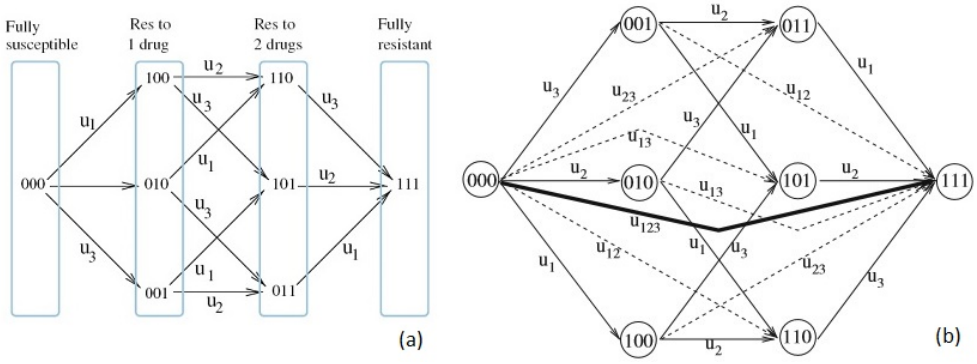

Figure 3.2: (a) Combinatorial mutation diagram for different resistance classes under no cross-resistance. (b) Under cross-resistance drugs. Treatment is done using m = 3 drugs [10]

3.4.1 Drug Combination vs. Treatment Success

The degree of cross-resistance varies from one drug to another. For example, for three drugs combinations there can be five different possibilities, based on pairwise combinations of cross-resistance properties and the triple-resistance property of drugs in use as shown in the figure 3.3. Let $N = 10^8 - 10^{13}$ and drug induced death rate of sensitive cells be ten times the growth rate of cancer ($d = D + H = 10L$), under no treatment let $D = 0 - 70\%$ of L. Also let $k_i = k = 10 - 100$ & $k_{123} = 1$. Observation gained from figure 3.4 implies that,

- Under no cross resistance, treatment success increases with number of drugs m.
- Two drugs with cross resistance is better than single drug for treatment in CML.
- Additional third drugs do not make significant difference to the treatment success.
- Result obtained in figure 3.4 is independent of kinetic parameter, [10][16]

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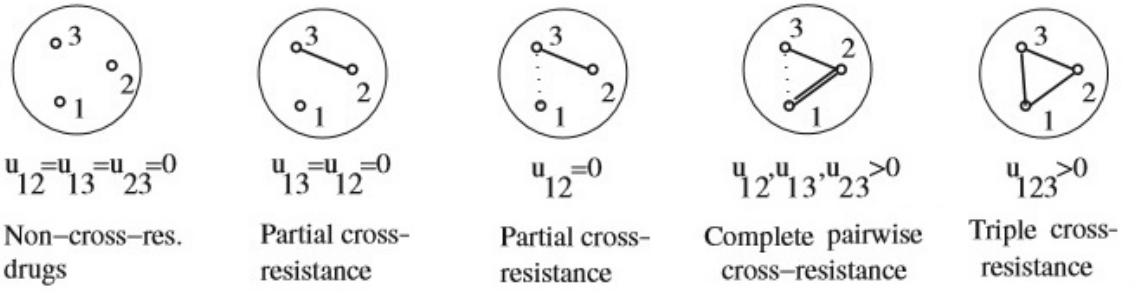


Figure 3.3: Possible combinations of three drugs cross resistance network [10].

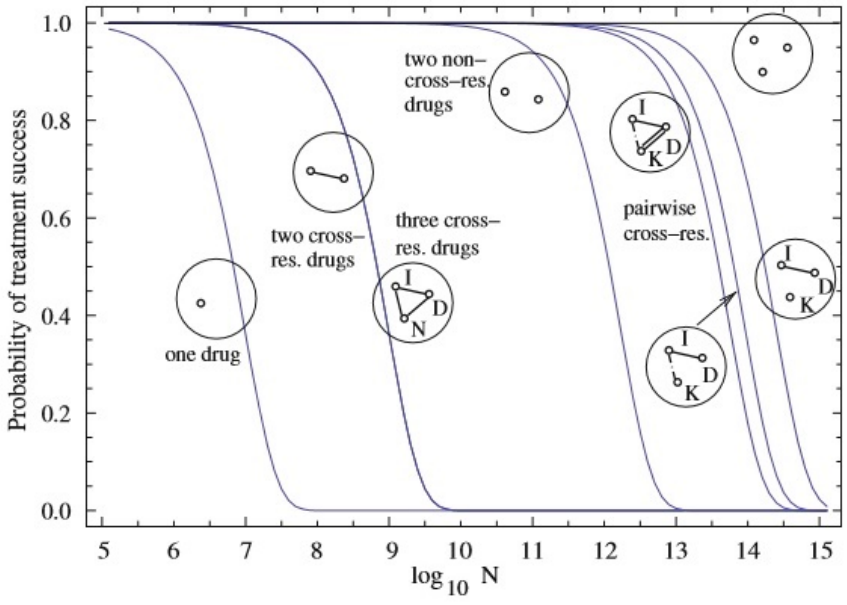


Figure 3.4: The probability of successful therappy vs. colony size, $log_{10}N$. Parameters values are $u = 10^{-9}$, $k = 100$, $M_0 = 100$, $D/L = 0.5$, $H/L = 3$. "I","D", "N", "K" stands for imatinib, dasatinib, nilotinib & future drugs to bind T315I mutant respectively [10].

By looking at the figure 3.5(A)) we can say that two drug combination become insignificant if mutant resistant to one drug only is very. If mutation rate for only one drug (say $k = 1000$) is unrealistically high (figure 4.5(B)) then, combining third drug may be advantageous. On a qualitative level, treatment success is independent of parameters like death rates or growth rates etc. Refer figure 3.6.

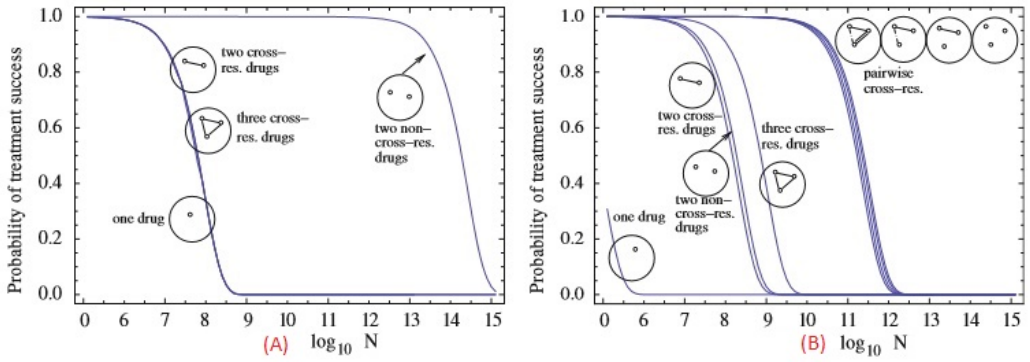


Figure 3.5: The probability of successful treatmet vs. colony size, $log_{10}N$. (A) Here $k = 10$. (B) Here $k = 10^4$ [10].

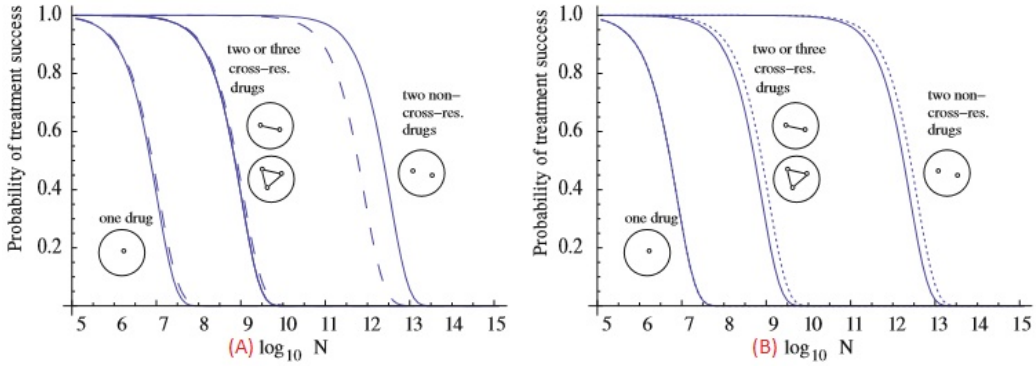


Figure 3.6: Parameter dependence on the treatment success/ failure. (A) Natural Death rate dependence: Parameters values are $D/L = 0$ (solid lines) and $D/L = 0.8$ (dashed lines); $H/L = 10$. (B) Drug-induced death rate dependence: $H/L = 3$ (solid lines) and $H/L = 400$ (dotted lines); $D/L = 0$. Other parameters are same as in Figure 3.4 [10].

3.5 Conclusion and Discussion

- Combining two drugs out of imatinib, nilotinib & dasatinib is advantageous over only one.
- Results are independent of natural/drug-induced death rate of cancer cell colony[8].
- Treatment success is high $iff\ N \leq 10^{10}$. However the figure is higher than this at diagnosis.
- Three drug combination doesn't improve treatment success.

Chapter 4

Quiescence Effect vs. Drug Resistance

4.1 Introduction

Tumor stem cell dynamics affects the emergence of resistant mutant. In this chapter we will focus our attention on the dynamics of tumor stem cell quiescence. We will look at two new events along from those described in death birth process in previous chapters namely, cells entering quiescence state and cells coming out of the quiescence state or moving into active state. When cells become inactive they are said to be in quiescence state. The result shows that when treated with single drug, the treatment failure is independent of quiescence parameters which is not the case when two or more drugs are used for treatment. Al the analysis carried out is in context of treatment of CML.

4.2 Model: Cellular Quiescence vs. Drug Resistance

Stochastic model comprises of Active CML cells and quiescent CML cells. Active CML cells responds to mutations and drug activity while Quiescent cells are inactive to any drug activity. Before we move on to modeling exercise, Let us describe few parameters with the following notations,

- N : Tumor size at which cancer is detected and imatinib therapy starts.
- u : Mutation rate.
- L : Rate of division of active CML cells.
- d : Natural as well as drug induced death rate of active CML cells.
- α : Rate at which an active cell enter quiescent/inactive state.
- β : Rate at which quiescent cells enters active state.

GOAL: We will calculate the probability that all the cancer have died out as a result of applied therapy and no resistant mutant is present or is spreading out during the cell extinction. We will also look at the parameter dependence like cellular growth, mutations, quiescence, and death and its relation to probability of successful therapy. We will re-use the same model as developed in chapter 2 but now we include two additional parameters α and β as defined above.

4.2.1 The framework of Quiescence effect in CML

Under the absence of resistant mutants the system state (i, j) comprises of number of cycling cells denoted by i and the number of quiescent cells given by j. In an infinitesimal time instent Δt various events that happens in the colony of active and quiescent cells can be briefly described as follows,

- Cell Division: Active cell increases, $(i, j) \to (i + 1, j)$ with probability $i\, L\, \Delta t$;
- Cell Death: Active cell dies, $(i, j) \to (i - 1, j)$ with probability $i\, d\, \Delta t$;
- Quiescent effect: Active cell transforms to Inactive cell, $(i, j) \to (i - 1, j + 1)$ with probability $i\, \alpha\, \Delta t$;
- Reverse Quiescent effect: Quiescent cell turns active $(i, j) \to (i + 1, j - 1)$ with probability $j\, \beta\, \Delta t$;
- No Change: State floats, $(i, j) \to (i, j)$ with probability $1 - (i[L + d + \alpha] + j\beta)\Delta t$.

The events and associated probability can be formulated in a combinatorial network and can be presented in a similar way as seen earlier in chapter 2 using Kolmogorov forward equation as,

$$\begin{aligned}\dot{\varphi_{i,j}} =& L(i-1)\varphi_{i-1} + d(i+1)\varphi_{i+1} + \alpha(i+1)\varphi_{i+1,j-1} \\ &+ \beta(j+1)\varphi_{i-1,j+1} - \varphi_{i,j}\big[(L+d+\alpha)i + \beta j\big]\end{aligned} \tag{4.1}$$

We are interested in the statistical behavior, as statistical behavior captures better insight into the process of quiescence. The statistical namely, mean number of active & quiescent cells denoted by (x, y) is shown below.

$$x = \sum_{i,j=1}^{\infty} i\varphi_{i,j} \quad and \quad y = \sum_{i,j=1}^{\infty} j\varphi_{i,j} \tag{4.2}$$

Solving Eq.4.1 and Eq.4.2 we get,

$$\dot{x} = (L - d - \alpha)x + \beta y \quad ; \quad \dot{y} = \alpha x - \beta y \quad where \quad x(0) = I_0, y(0) = J_0 \tag{4.3}$$

We can extend the system to accommodate multiple drugs "m". We will assume symmetrical parameters value in use namely, $L_s = L$, $\alpha_s = \alpha$, $\beta_s = \beta$, $d_s = 0$. The following system is same as equation 4.3 but considered for m number of drugs now under symmetric coefficient.

$$\begin{aligned}\dot{x_0} &= (L(1-u_0) - \alpha)x_0 + \beta y_0 \\ \dot{y_0} &= \alpha x_0 - \beta y_0 \\ &\cdots\cdots \\ \dot{x_k} &= Lu_{k-1}x_{k-1} + (L(1-u_k) - \alpha)x_k + \beta y_k \\ \dot{y_k} &= \alpha x_k - \beta y_k \qquad 0 < k \leq m \\ where \;\; u_k &= (m-k)u\end{aligned} \tag{4.4}$$

4.3 Statistical Results under $\beta = 0$

Let us consider that the factor $\beta = 0$ and analyze the results obtained after solving the above systems of equation. We can obtain an analytical form of equation. Later we will see the case where rate of cell awakening β is a non zero quantity.

4.3.1 The expected number of mutants

The expected number of mutants for any class k can be given by,

$$\sum_{k=0}^{m}(x_k + y_k) = \frac{L\, exp[(L-\alpha)t]-\alpha}{L-\alpha} \tag{4.5}$$

If the expected number reaches size N then the time taken to reach the size is,

$$t_* = \frac{ln\left[\frac{N(L-\alpha)+\alpha}{L}\right]}{L-\alpha}$$

4.3.2 Cycling cells fraction at size N

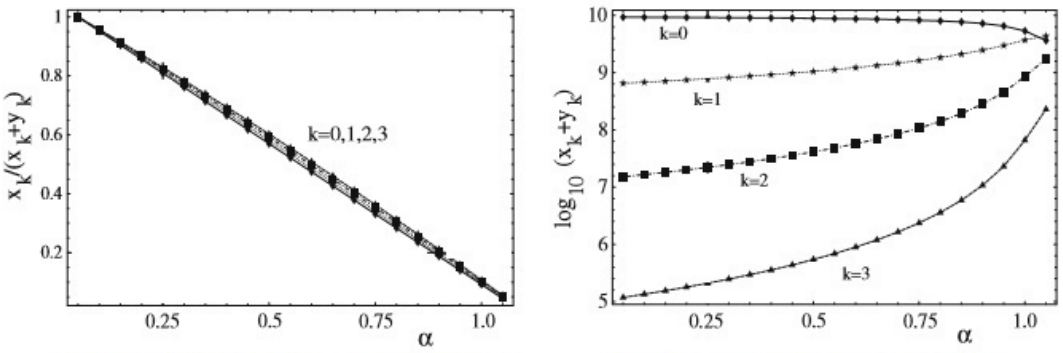
The fraction of cycling cells [10] of class k at size N is,

$$\frac{x_k}{x_k+y_k} = \left(1-\frac{\alpha}{L}\right)(1+O(1/ln\, N)) + O(u) \tag{4.6}$$

4.3.3 Mean number of mutants resistant to m drug

The mean number of mutants resistant to all m drugs [10] can be written as,

$$x_m(t_*) + y_m(t_*) = N\left[\frac{u\, lnN}{(1-\frac{\alpha}{L})}\right]^m + \quad h.o.t \tag{4.7}$$



a) The fraction of cycling mutants of class k b) Total number of mutants of class k

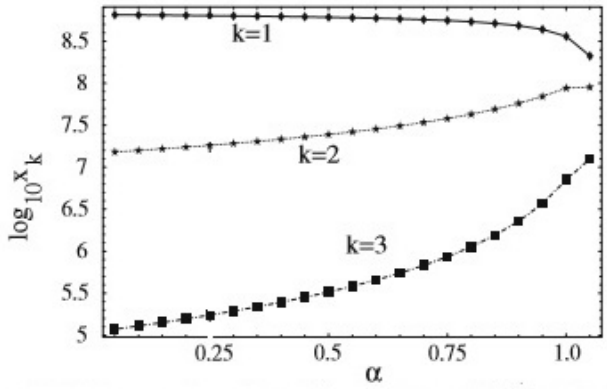
$N = 10^{10}, m = 3,\ L = 1, u = 10^{-3}$

Figure 4.1: Plot of statistical parameters as described in Eq.4.6 and Eq.4.7. [10]

Lets plot Eq.4.6 and Eq.4.7 w.r.t α while neglecting higher order terms. We observe that if m increases the dependence of total number of mutants becomes stronger, figure 3.1(b). If we use only one drug, $m = 1$, then we see that the mean number of cyclic mutants of class $k = 1$ is given by,

$$x_k = N\, u\, lnN \tag{4.8}$$

The quantity x_k is independent of α, but depends on colony size N and mutation rate u. figure 4.2.



The number of cycling mutants of class k, x_k. $N = 10^{10}$, $m = 3$, $L = 1$, $u = 10^{-3}$

Figure 4.2: Numerical Solution for system described in Eq.6. [10]

4.3.4 Total number of fully resistant mutant

$$W_{m-1} = mNu\left[\frac{u\, lnN}{(1-\frac{\alpha}{L})}\right]^{m-1}$$

For $m = 1$, W_{m-1} is independent of α while for $m > 1$ the quantity depends on α.

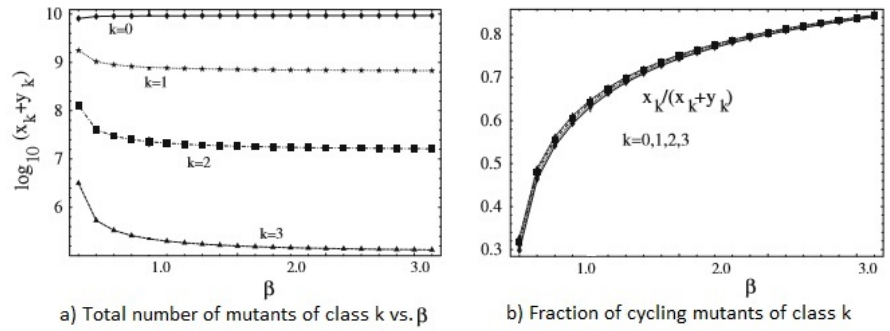
4.4 Numerical Results: The case $\beta > 0$

If we says that the quiescent cells turns active or the value of $\beta > 0$ then, a closed form of analytical solution cannot be obtained. However, numerical results can be obtained with the aid of graphical representation as shown in figures below.

- The total mutant amount of class k decays with β, figure 4.3(a).
- Cycling cell fractions increases with β, figure 4.3(b).
- Cell awakening fraction β is opposite to quiescence rate α.

4.5 Parameter α and β dependence on mutant elimination

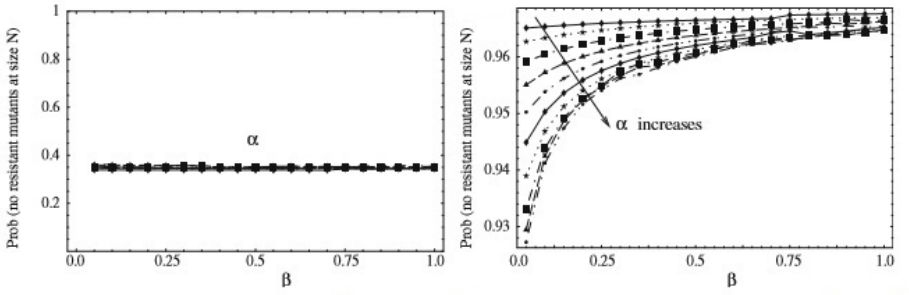
In most cases the tumor growth phase before treatment invokes resistance evolution rather than the treatment phase, we calculate the probability of successful therapy under quiescence parameter α and β. Several scenarios are considered in the figures below for different number of drugs used,



$N = 10^{10}, m = 3,\ L = 1, u = 10^{-3}, \alpha = 0.\dot{7}$

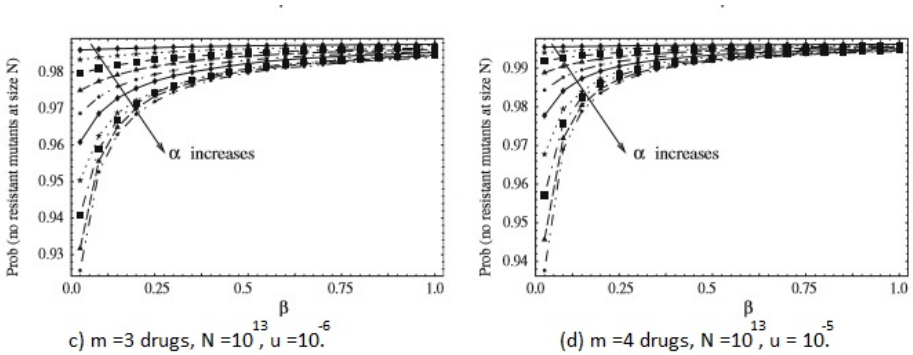
Figure 4.3: System dependence on β. [10]

mutation rates and the cancer size. Figure 4.4 and 4.5 illustrates the dependence of quiescence parameter α and β when resistant mutants vanishes completely under varying parameter values.



$M_0 = 10^3,\ L = 1, d = 0.$

Figure 4.4: probability of having no fully resistant mutants at size N for different quiescence parameters



$M_0 = 10^3,\ L = 1, d = 0.$

Figure 4.5: probability of having no fully resistant mutants at size N for different quiescence parameters

Chapter 5

Drug Administration Regimens

5.1 Introduction

It has now been established that drug resistance is the central cause of failure of may cancer targeted drugs. If such resistance is inevitable or inherent to many cancer then can we delay its emergence once it is detected? Can we do it in an optimal fashion such that the delay of resistance emergence is maximum? What are the drug administration regimens which can achieve our target with minimum side effects? We will review these topics in this chapter. The drug administration strategy at present is continuous in time meaning that drugs are administered at very low dozes on daily basis without any drug holidays to limit the side effects but still effective on killing malignant cells in cancer. There can be an alternative strategy where drug can be administered in a pulsed fashion meaning that a high doze of drug pulse for a fixed number of days followed by complete rest to the person in order to cope up the side effects of the drug. It is well understood that there is a tolerance limit of a person to withstand the side effects of drugs. High concentration of drug can lead to large scale killing of cancer cells but with severe side effects while low dozes of drugs cannot compensate the rate of growth of cancer with the drug induced death rate of the cells. However, low concentration of dugs can have little side effects on persons day-to- day life. We ask ourselves that is there any intermediate solution between these two regimens? If it exist then, can it be administered in in optimum sense?

Some investigation on this issue claims that low doze continuous dozing strategy is effective in treatment [17] while some claims that high doze drug administration is fruitful [18]. Earlier a model of kinetic resistance to cell-cycle therapy was propose by Norton and Simon [19] for chemotherapeutic treatment. They suggested that if intensity of treatment was increased then one can combat the slow rate of tumor regression and increase the chances of cure. The model is named after them as Norton-Simon hypothesis [19]. In past there have also been a substantial effort to model the genetic resistance [11]. Iwasa et.al. used a multi-type birth-death process model to study the genetic resistance [20][21]. A model for multi-type birth-death process using multi-drug was also seen in the work of Komarova and Wodarz [12][13]. In this chapter we will develop a stochastic framework of a non-homogeneous multi-type birth-death process. The drug concentration profile which is administered in a pulsed fashion over time determines the growth parameters of cancer [1]. In the end, an optimal control problem is formulated to delay the resistance emergence maximally.

5.2 General Pulsed Administration Drug Dozing Schedule

In this section we will look at another stochastic process of evolution of sensitive and resistant cells according to a process called as multi-type branching process [4]. The growth and death rate of cancer is determined by the drug doses administered in a pulsed fashion whereby, high doze of drugs administration is followed by drug holidays for recovery. During drug therapy the cell population reduces in size due to high death rate of sensitive cell given by c_1 as compared to birth rate as q_1. The corresponding rates for resistant cells are c_2 and q_2. During drug holidays the resistant cells emerge with birth and death rates as r_2 and d_2. Also the birth and death rate of sensitive cells during drug holidays are r_1 and d_1. The genetic alteration conferring resistance is due to single mutation only. If we look at the figure 5.1 we can see that the length of pulse T_{on} for treatment phase is different from drug holiday which is T_{off}, also assume $K = T_{on} + T_{off}$, $\alpha_{on} = q_1 - c_1$,, $\alpha_{off} = r_1 - d_1$, and $\gamma = \alpha_{on}T_{on} + \alpha_{off}T_{off} < 0$. We also see that when $T_{off} = 0$ then the regimen becomes continuous dosing scheme, which reduces to a special case of pulsed dosing scheme. From now on we will treat continuous drug dozing strategy as the special case of general pulsed drug dosing strategy. In figure 5.1 when growth rate of sensitive cell is low (0.5 here) then the population of sensitive cell decreases over time in pulsed dosing regimen. But, when the growth rate of sensitive cell is high (1.8 here) then the population of sensitive cell increases over time. In the absence of treatment cell colony evolves at a higher rate ie., $r_1 > d_1$ and $r_2 > d_2$. If $r_2 = d_2$ and $d_2 = c_2$, then drug in use is ineffective against resistant cancer cells and confers *complete resistance*. It is useful to calculate the probability of resistance and optimize the dosing schedule in such a way that this quantity is minimum. Let us look at how we can achieve this. If complete resistance has occurred then the probability of the absolute extinction of resistance cells starting from one is $\frac{d_2}{r_2}$ [4] irrespective of drug dosing schedules. To looker deeper into the process dynamics it its essential to calculate few parameterized quantity under a certain time frame [0,T] like:

- Average number of sensitive cell at any instant of time.
- Sensitive cell division count as a result of branching in time interval [0,T].
- The number of resistant mutant produced in time interval [0,T].
- Expected count of sensitive cell birth until it gets extinct.
- The number of regimen cycle for sensitive cell extinction.
- Probability to produce one fully resistant clone before sensitive cell population extincts.
- Expected number of resistant cell at time T.
- Compute the above quantities under partial resistance and also for continuous dozing regimen.

[*i*] **The Average number of sensitive cell at any instant of time is approximated as,**

$$p(t) = M\exp(\alpha_{on}t) \qquad M : Number\ of\ sensitive\ cells.$$

[*ii*] **The sensitive cell division count produced in branching process in time interval [0,T]**

$$\begin{aligned} b(T, q_1, c_1, M) &= \int_0^T Mq_1\exp(\alpha_{on}s)ds \\ &= \left[\frac{Mq_1}{q_1 - c_1}\right]\exp\Big([\alpha_{on}T] - 1\Big) \end{aligned} \tag{5.1}$$

[*iii*] The resistant cell clones are produced from the sensitive cells as a result of mutation. The number of such resistant cells in the time interval [0,T] is distributed binomially. We can write the distribution law as,

$$Bin\Big(b(T, q_1, c_1, M), u(1 - \frac{d_2}{r_2})\Big).$$

[*iv*] **The expected number of sensitive cell birth**

The sensitive cells produced at the beginning of i_{th} treatment cycle and; i_{th} holiday is $p_{i,0}$ and $p_{i,1}$ respectively. The quantity is given as,

$$\begin{aligned} p_{i,0} &= Mexp\Big((i-1)\gamma\Big) \\ p_{i,1} &= Mexp\Big((i-1)\gamma\Big)exp\Big(\alpha_{on}T_{on}\Big) \end{aligned} \tag{5.2}$$

[*v*] **The number of total birth of sensitive cells until the cell colony gets extinct is,**

We use equation 5.1 and 5.2 under the binomial distribution law to arrive at the following equation.

$$\mathbf{B} = \frac{1-exp(W\gamma)}{1-exp(\gamma)}\left[\frac{q_1}{q_1-c_1}exp\Big(\alpha_{on}T_{on}-1\Big) + \frac{r_1}{r_1-d_1}\exp\Big(\alpha_{off}T_{off}-1\Big)\exp\Big(\alpha_{on}T_{on}\Big)\right] \tag{5.3}$$

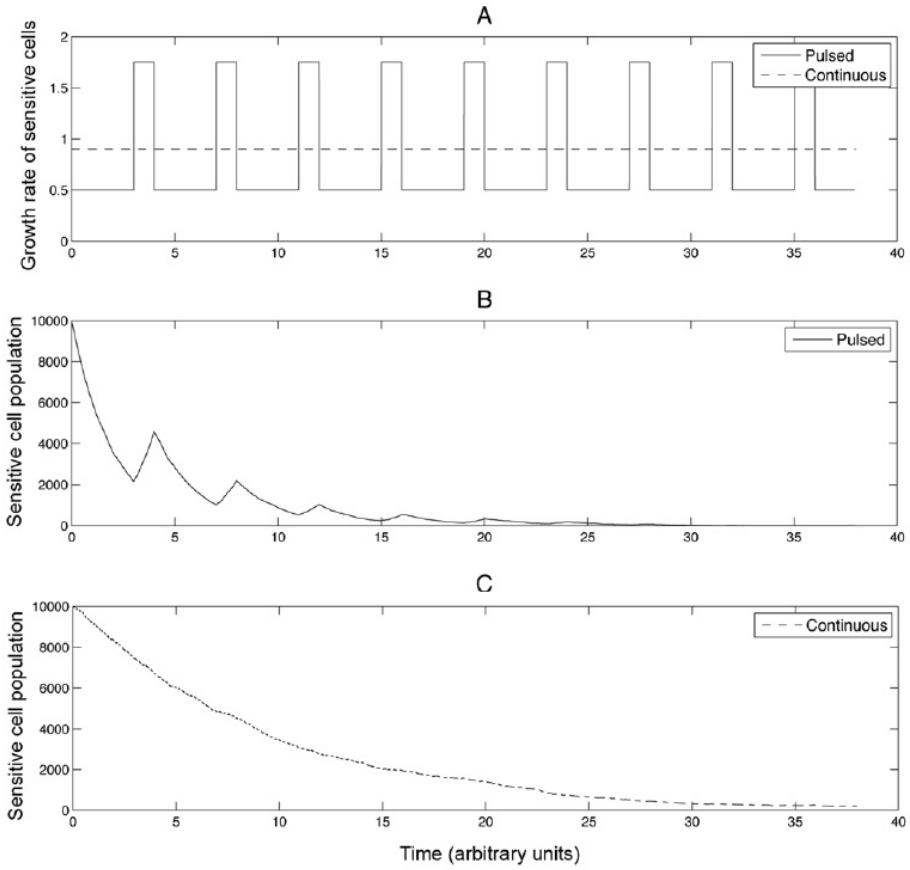


Figure 5.1: The growth dynamics of sensitive cancer cells during various treatment regimens.

[*vi*] **Total number of cycles W needed for sensitive cell population to extinct**

$$W \approx \frac{1}{2}\Big[log_e \frac{1}{M}\Big]\gamma^{-1} \tag{5.4}$$

[*vii*] **Probability to produce one fully resistant clone before sensitive cells extinct.**
The surviving resistant cell count produced from the sensitive cell clones is binomial distributed as,

$$Bin\Big(\mathbf{B}, u(1-\frac{d_2}{r_2})\Big).$$

The Poisson approximation of the above binomial distribution is done and the probability to produce one fully resistant clone obtained is,

$$P = 1 - exp\Big[-\mathbf{B}u(1-\tfrac{d_2}{r_2})\Big] \tag{5.5}$$

[*viii*] **The expected number of resistant cells at time $t = T$.**
If a resistance clone is produced then it keeps on replicating identical resistant cell clones. To estimate the number of such cells at any given time $t = T$ we need to estimate the production of resistant cell in any given small time interval and then estimate its growth till time $t = T$. We need convolution operator to achieve this.

$$\begin{aligned} R(T) &= u\int_0^T B^{'}(t)exp\big[(T-t)(r_2-d_2)\big]dt \\ &\cong \frac{Mu\gamma - exp(T(r_2-d_2)) + exp(T\gamma/(T_{on}+T_{off}))}{\big[(exp(\gamma)-1)(d_2-r_2)(T_{on}+T_{off})+\gamma\big]} \end{aligned} \tag{5.6}$$

5.3 Continuous Dosing Regimen as a special case

Of we just include $T_{off} = 0$ in the analysis that we did in previous section we can obtain all the parameters for continuous dosing regimen. Thus, continuous dozing regimen becomes a special case of general pulsed dosing regimen. The quantities obtained from previous analysis under $T_{off} = 0$ can be given as,

$$\mathbf{B} \cong \tfrac{(1-M)q_1}{(q_1-c_1)} \tag{5.7}$$

$$P = 1 - exp\Big[-\mathbf{B}u(1-\tfrac{c_2}{q_2})\Big] \tag{5.8}$$

$$R(T) = Muq_1\tfrac{exp[(q_1-c_1)T]-exp[(r_2-d_2)T]}{(q_1-c_1)-(r_2-d_2)} \tag{5.9}$$

5.4 The case when resistance exist prior to treatment

It is generally seen that there exist a small amount of resistant cells before therapy begins. This result is also presented in previous chapters. So what do we need to do in our existing result in

order to accommodate the change made in assumption of pre-existance of resistant mutants? We can intelligently classify the population of size M into two classes such that the first class comprises of $M(1-s)$ *sensitive cells* and the other class comprises of Ms *resistant cells*. In order to avoid the emergence of resistance the following conditions should satisfy,

- Resistant mutant which exist prior to treatment should get extinct and,
- No more resistant clone are produced from sensitive cell division during treatment.

The probability associated with avoiding resistance is nothing but the product of these two probabilistic event. The probability to avoid resistance is,

$$\begin{aligned} P_r^e &= 1 - (q_2/c_2)^{Ms} \qquad if\ q_2 < c_2 \\ P_r^e &= 1 \qquad\qquad\qquad\qquad otherwise. \end{aligned} \tag{5.10}$$

In a similar analogy the expected number of resistant cell population at time $t = T$ under pre-existing mutant is,

$$R(T) \equiv R_s(T) + Ms\ exp[(q_2 - c_2)T] \tag{5.11}$$

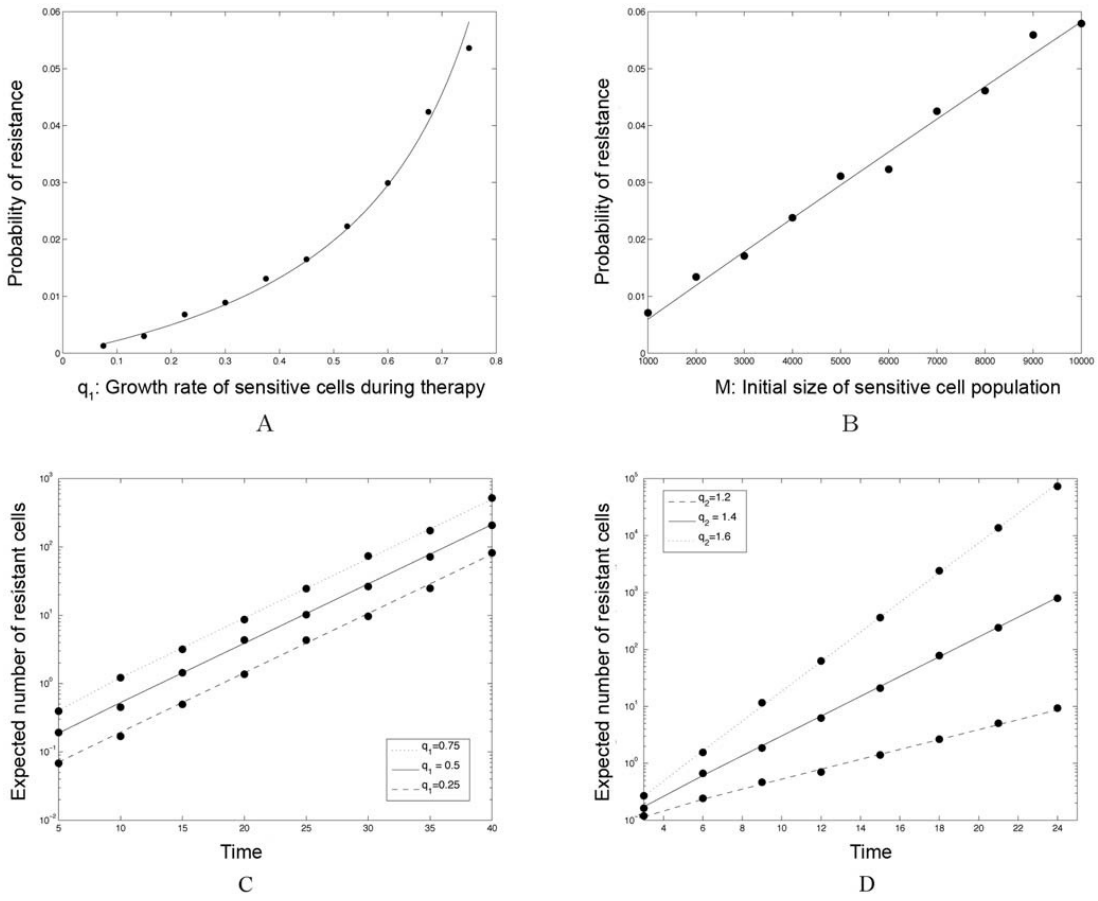


Figure 5.2: The Probability of resistance and expected number of resistant cells during continuous dosing scheme. (A) Parameters: $M = 10000$, $q_2 = 1.25$, $u = 10^{-5}$, and $c_1 = c_2 = 1.0$ (B) Parameters: $q_1 = 0.75$ and others as (A). (C) Parameters: $M = 1000$, $q_2 = 1.2$, $u = 10^{-4}$, and others as (A). (D) Parameters: $q_1 = 0.5$ and others as (C). [1]

5.5 Analytical Results vs. Numerical Simulation [1]

5.5.1 Continuous Administration Strategies

Let us first look at the numerical computation and parameter dependence on the probability of resistance under continuous dosing scheme which is nothing but a special case of general pulsed dosing scheme. It can be seen from figure 5.2(A) that as the rate of growth of sensitive cell population increases the chances of resistance formation accelerates. In figure 5.2(B) the relation is linear for the initial sensitive cell colony size M vs. the probability of resistance. There is a constant upward drift in the expected number of resistance cells w.r.t time under increasing growth rate q_1 of sensitive cell colony in figure 5.2(C) whereas, in figure 5.2(D) the expected number of resistant cell as a function of time is much more sensitive to the increase in the growth rate q_2 of resistance cell population.

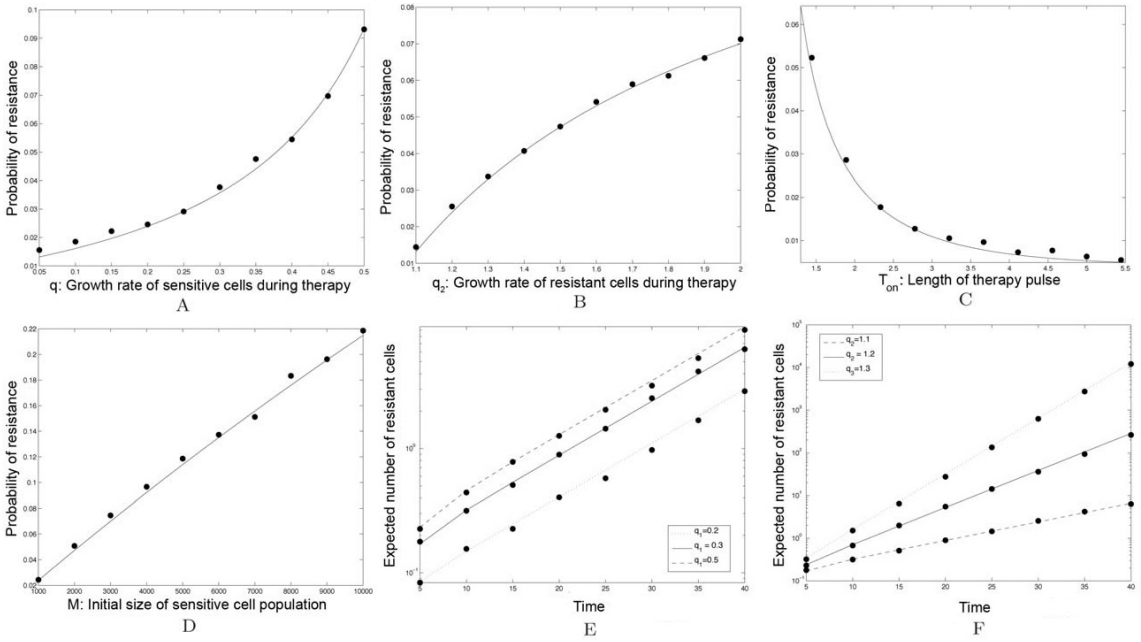


Figure 5.3: **The probability of resistance and expected number of resistant cells during pulsed therapy.** Parameters: $M = 1000, r_1 = 1.3, r_2 = q_2 = 1.1, u = 10^{-4}, d_1 = d_2 = c_1 = c_2 = 1.0$, *and* $q_1 = 0.5$ [1]

5.5.2 Pulsed Administration Strategies

The numerical computation and parameter dependence on the probability of resistance under pulsed dosing scheme is shown in the figure 5.3. It can be seen from figure 5.3(A) that as the rate of growth of sensitive cell population increases the chances of resistance formation accelerates where in figure 5.2(B) the relation is opposite when growth rate of resistant cell population is taken into account. In figure 5.3(D) the relation is linear for the initial sensitive cell colony size M vs. the probability of resistance whereas, the probability of resistance decreases exponentially w.r.t the drug dosing period T_{on} as seen in figure 5.3(C). There is a constant upward drift in the expected number of resistance cells w.r.t time under increasing growth rate q_1 of sensitive cell colony in figure 5.3(E) and in figure 5.3(F) the expected number of resistant cell as a function of time is much more sensitive to the increase in the growth rate q_2 of resistance cell population.

5.6 Optimizing dosing strategies

Finally we have reached to the end of our topic where we will discuss about the optimization of various treatment regimens in order to minimize the probability of generating resistance or delaying it to maximum extend in worst case. In an optimal control problem there are certain constrains. In our case we will have toxicity constrains determined through experimental data for each drug types. If we change the treatment schedules then the parameters which gets affected are as follows,

- On / Off treatment pulse duration, T_{on} / T_{off},
- Dose intensity affects the rate of growth q_1 and the rate of death c_1 of sensitive cell.
- Growth rate r_1 and death rate d_1 of the resistant cells are unaltered under complete resistance,
- All other parameter remains unaffected.

The quantity T_{on} and T_{off} are related as $K = T_{on} + T_{off}$ while q_1 and c_1 are drug concentration dependent parameter. So, toxicity constraints can be a function of two parameters only, We can see an example toxicity constraint (obtained through experiments) in figure 5.4(A). It defines the maximum tolerated time duration of treatment T_{on}, based on the death rate of sensitive cancer cells, for a 28-day treatment cycle [1]. Other such constraints family curve are shown in figure 5.4(B) and 5.4(C). We can use as many toxicity constraints families in order to test the probability of resistance.

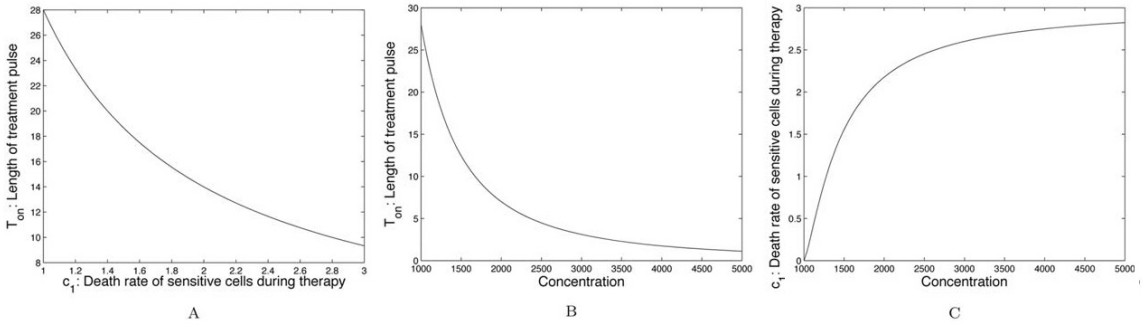


Figure 5.4: Toxicity constraint curves needed for optimization [1]

One such curve as an example is shown in figure 5.5. We can adjust the parameter in order to fall within the constraint limits and design the optimal drug dosing schedules. Consider the following flexible constraint of three constraints families as shown in the figure 5.5.

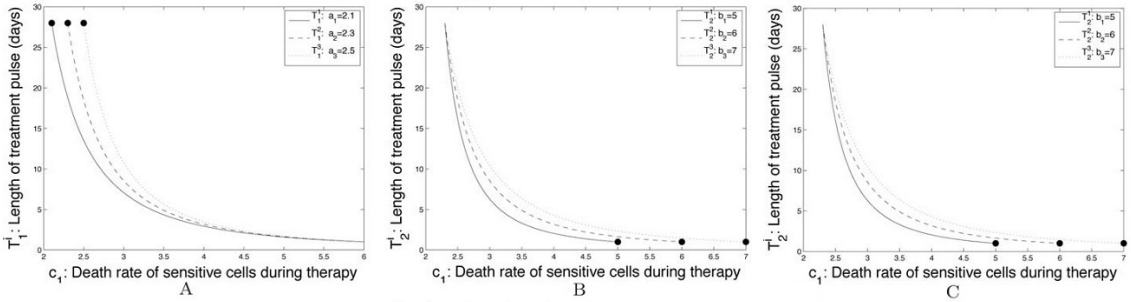


Figure 5.5: Toxicity constraint curves needed for optimization [1]

- Family [1] Vary maximum dose that can be tolerated for K(=28) days,
- Family [2] Vary maximum dose that can be tolerated for just one day,
- Family [3] Vary initial rate of decrease in T_{on} by increasing concentration.

The three constraints family curve is denoted by T_1^1, T_2^i and T_3^i. In the notation, T_j^i, j denotes the constraint family ($1^{st}, 2^{nd}$ *and* 3^{rd}) and the superscript i specifies the function they perform in the family. The function defining the first toxicity constraints family as shown in figure 5.5(A) is,

$$\begin{aligned} T_1^i(c_1) &= \frac{(6 - B_i)^2}{(c_1 - B_i)^2} \\ B_i &= \frac{a_i\sqrt{K} - 6}{\sqrt{K} - 1} \end{aligned} \tag{5.12}$$

The variable parameter for this family is is a_i. We set the value for the variable parameters as $a_1 = 2.1, a_2 = 2.3, a_3 = 2.5$ to obtain the three curves in each family as can be seen in the figure for each family. Similarly second family toxicity curve is shown in figure 5.5(B).

$$\begin{aligned} T_2^i(c_1) &= \frac{(b_i - B_i)^2}{(c_1 - B_i)^2} \\ B_i &= \frac{2.3\sqrt{K} - b_i}{\sqrt{K} - 1} \end{aligned} \tag{5.13}$$

The variable parameter for second family is b_i and the value that is set for three curves within the family is $b_1 = 5, b_2 = 6, b_3 = 7$. After the toxicity constrained has been established we find the area under the toxicity curve in order to minimize the probability of resistance. The optimal point should lie within this area. Let us focus our attention on the first toxicity constraint family. The rest follows the same procedure of optimization. Consider the curve with the following parameters $i = 1, K = 28, a_1 = 2.1, M = 10^9, u = 5.1^{-10}, r_2 = r_1, d_2 = d_1$. For this curve

$$\begin{aligned} B_1 &= \frac{2.1\sqrt{28} - 6}{\sqrt{28} - 1} = 1.1912 \\ T_1^1(c_1) &= \frac{(6 - 1.1912)^2}{(c_1 - 1.1912)^2} \\ &\underset{c_i}{\text{Minimize}}\ c_1 T_1^1(c_1) \\ &\implies c_1 = 3.15 \end{aligned}$$

If we put the value in figure 5.5(A) we get the corresponding T_{on} value as 6.05 days. Now we can obtain other optimal values once we get T_{on}. Now, $T_{off} = K - T_{on} = 21.95$ days. We can calculate all other parameters values using $c_1 = 3.15$. That conclude our optimal drug dosing regimen.

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