ABSTRACT

Title of dissertation: MATHEMATICAL MODELING OF DRUG RESISTANCE AND CANCER STEM CELLS DYNAMICS

Cristian Tomasetti, Doctor of Philosophy, 2010

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In this dissertation we consider the dynamics of drug resistance in cancer and the related issue of the dynamics of cancer stem cells. Our focus is only on resistance which is caused by random genetic point mutations. A very simple system of ordinary differential equations allows us to obtain results that are comparable to those found in the literature with one important difference. We show that the amount of resistance that is generated before the beginning of the treatment, and which is present at some given time afterward, always depends on the turnover rate, no matter how many drugs are used. Previous work in the literature indicated no dependence on the turnover rate in the single drug case while a strong dependence in the multi-drug case.

We develop a new methodology in order to derive an estimate of the probability of developing resistance to drugs by the time a tumor is diagnosed and the expected number of drug-resistant cells found at detection if resistance is present at detection. Our modeling methodology may be seen as more general than previous approaches,

in the sense that at least for the wild-type population we make assumptions only on their averaged behavior (no Markov property for example). Importantly, the heterogeneity of the cancer population is taken into account. Moreover, in the case of chronic myeloid leukemia (CML), which is a cancer of the white blood cells, we are able to infer the preferred mode of division of the hematopoietic cancer stem cells, predicting a large shift from asymmetric division to symmetric renewal. We extend our results by relaxing the assumption on the average growth of the tumor, thus going beyond the standard exponential case, and showing that our results may be a good approximation also for much more general forms of tumor growth models.

Finally, after reviewing the basic modeling assumptions and main results found in the mathematical modeling literature on chronic myeloid leukemia (CML), we formulate a new hypothesis on the effects that the drug Imatinib has on leukemic stem cells. Specifically, we hypothesize that Imatinib is able to stop leukemic stem cells from dividing, putting them in a quiescent state. We elucidate the reasons why this hypothesis can explain the available data on CML better than all other previous assumptions made in the existing literature. Based on this hypothesis, we obtain new insights on the dynamics of the development of drug resistance in CML.

MATHEMATICAL MODELING OF DRUG RESISTANCE AND CANCER STEM CELLS DYNAMICS

by

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Dedication

This work is dedicated to those who most sacrificed their lives to allow me to be what I am and to know what I know.

To my parents,

Renzo and Caterina Tomasetti.

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List of Abbreviations

ODEs	ordinary differential equations
PDEs	partial differential equations
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
CSCs	cancer stem cells
LSCs	leukemic stem cells
CML	chronic myeloid leukemia
CP	chronic phase
AP	acute phase
BC	blast crisis
P_R	probability of drug resistance at detection
Eqn	equation

Chapter 1

Introduction

In this dissertation we consider the dynamics of drug resistance in cancer and the related issue of the dynamics of cancer stem cells. In this chapter we introduce and briefly discuss relevant biological aspects needed for the mathematical modeling of these topics.

1.1 Cancer as an evolutionary process and the cancer stem cell hypothesis

Cancer is thought to be monoclonal, that is, to originate from a single cell that has undergone harmful mutations [41, 85, 57]. These mutations cause the cell to ignore normal growth controls, thus initiating proliferation [8]. Surprisingly, the consequent clonal expansion will give rise to a tumor population with a large heterogeneity. For example, cancer cells within a primary tumor will typically differ in size, morphology, division rate, death rate, and resistance to a given drug [44, 8]. Only some of this diversity is caused by epigenetic plasticity; there is indeed evidence of genetic differences within a tumor cell population [91].

There are two fundamental concepts currently used in order to explain the cause for the observed tumor heterogeneity: the clonal evolution model and the cancer stem cell hypothesis. As of today, there is supporting evidence for both

models and it appears that a combination of the two ideas may provide the best explanation for tumor heterogeneity [91, 8].

The *clonal evolution model* assumes that a cancer cell has the potential to become any of the various types of cells present in a tumor. Such a process may require multiple divisions. The basic mechanism would be given by the well-known genetic instability of cancer cells [41]. Cancer cells acquire different combinations of mutations. This will give to different cancer cells different characteristics. Furthermore, some cells will acquire a selective growth advantage over other cancer cells. The key element of the clonal evolution model is thus given by the occurrence of various mutations. An important role is played by the genetic drift, i.e., the change in the frequency of a gene variant in the tumor population due to random sampling and, even more importantly, by natural selection, where a gene variant may become more or less common in the tumor population due to their reproductive and survival abilities [85, 8]. There is evidence of competition, predation, and mutualism within and around a tumor cell population [71].

On the other hand, the *cancer stem cell hypothesis* is based on experimental evidence that many tissues are maintained by a small group of slowly replicating cells, i. e., by a group of cells which are generally quiescent and only occasionally dividing [97, 78, 13]. Such relatively rare cells, known as "stem cells", are defined by their unique ability of both self-renewal and differentiation into more mature, specialized cells forming that tissue. This implies a hierarchy among cells, where only the subset of stem cells has the ability to self-renew while all other cells can only differentiate into more mature cells or die [78, 87, 118, 69, 119]. Moreover, the subset

of fully mature, differentiated cells does not have the ability to continue dividing. Stem cells are also very long lived, while mature, fully differentiated cells have a variable life span, which, depending on the tissue of origin, can range from a few days to several months, see [32]. Artur Pappenheim was the first to formulate the concept of stem cells for the hematopoietic system [90] and, years later, "hematopoietic stem cells have been isolated from humans, and have been shown to be responsible for the generation and regeneration of the blood-forming and immune systems" [97]. The hematopoietic system (blood cell) system is depicted in Figure 1.1. Lung, breast, prostate, and brain are additional instances of tissues for which the existence of stem cells have been shown [8]. The cancer stem cell hypothesis thus assumes a hierarchical structure in a tumor cell population. Because of this hierarchy, in a tumor, CSCs would then be the real "engine" behind cancer progression [97, 8]. Where exactly cancer stem cells (CSCs) originate from is still unclear though: from a healthy stem cell, which undergoes some harmful mutation, or from progenitor cells that acquire self-renewal capacity or both [13]? There exists some evidence that, at least in the case of CML, leukemic non-stem cells can acquire the ability to undergo self-renewal [13, 93, 40]. Furthermore, it has been often assumed that the slowly replicating CSCs would constitute only a very small fraction of the tumor cell population [8, 13, 93, 97]. However there is recent experimental evidence that CSCs may constitute a much larger proportion of a tumor [54, 94, 120, 114].

Aside the possible surgical removal of a tumor, chemotherapy is today one of the most common treatments against cancer (radiotherapy and immunotherapy being the other major ones). One of the main reasons for the failure of chemotherapy

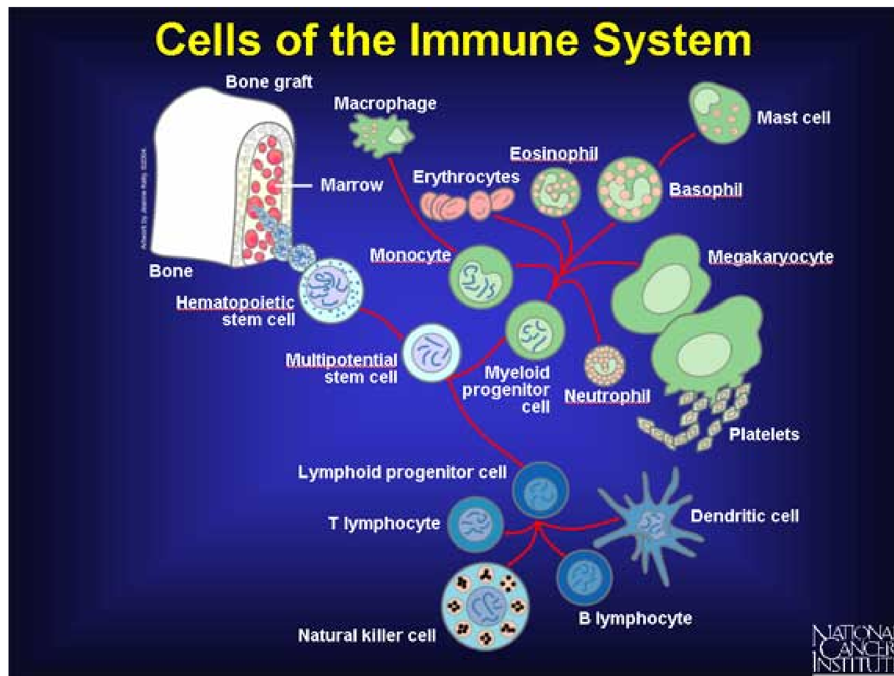

Figure 1.1: The hematopoietic system. (Jeanne Kelly; commons.wikimedia.org).

is the development of drug resistance. In order to better address the issue of cell resistance to anticancer drugs, we will briefly review what chemotherapy is, how it has evolved in time, and how it works.

1.2 Chemotherapy

A chemotherapeutic drug is, broadly speaking, a chemical compound with the ability to reduce the tumor load. At the end of the first millennium, Avicenna, an Arab physician who pioneered experimental medicine and randomized clinical trials, used arsenic compounds against cancer. While this procedure has been continuosly used for centuries, the effects were not very encouraging. Approximately a century ago, Paul Ehrlich coined the term chemotherapy to indicate a compound able to act

as the "magic bullet" against infections [92].

The first modern chemotherapy drug used against cancer was discovered in 1942, when the US government asked two Yale assistant professors, L. S. Goodman and A. Gilman, to study mustard gas, which had been developed and used as a chemical warfare agent in World War I. The two researchers found that nitrogen mustard, an alkylating agent derived from mustard gas, caused a dramatic regression of lymphoma in the patient under study. This discovery led in the late 1940s and early 1950s to the investigation and subsequent use in clinical practice of various compounds: A. Haddow used urethane in CML patients, J. Burchenal used methotrexate to treat leukemia in children, and S. Farber utilized aminopterin in acute childhood leukemia [92, 32].

Since then, many more compounds have been found that are able of producing a significant regression of a tumor load. We can broadly divide the standard chemotherapeutic agents as follows [106]:

1. *alkylating agents (and variations):* by attaching alkyl groups to the guanine base of DNA double-helix strands, they chemically modify a cell's DNA and thus impair its function. Indeed by crosslinking guanine bases in DNA they make the DNA strands unable to uncoil and separate. DNA replication is impaired and therefore the cell can no longer divide.

2. *antimetabolites:* chemicals that are similar in structure to metabolites (molecules that are part of the normal cell metabolism) prevent these substances from being incorporated into the DNA during the S phase of the cell cycle (synthesis

phase, when DNA replication occurs), consequently stopping normal cell development and division by damaging the DNA strand. In fact antimetabolites masquerade as purines or pyrimidines, molecules which are building blocks of DNA.

3. *plant alkaloids:* by inhibiting the assembly of microtubules they block cell division, due to the fundamental role played by microtubules in a cell.

4. *topoisomerase inhibitors:* by inhibiting topoisomerases, which are essential enzymes that maintain the topology of DNA, they cause damages to the transcription and replication of DNA (interfering with proper DNA supercoiling).

Importantly, these drugs do not differentiate between cancer and normal cells. However, given that cancer cells divide more often than normal cells, the inhibition of cell division is expected to harm cancer cells more than healthy cells, since the aforementioned drugs interfere with DNA replication or cell division. Unfortunately, a general phenomenon for all such chemotherapeutic agents is that, aside from the toxicity of the therapy, successive treatments will have decreasing therapeutic benefits due to the development of resistance. Thus, while chemotherapy has proven to be effective in the treatment of few types of cancer, such as lymphomas, germ cell (sexual) and pediatric malignancies, in the majority of cases the results are modest [116].

In the last decade new and less toxic drugs have been developed. These drugs are known as "targeted therapies". Instead of attacking both cancer and normal cells without discrimination, the new drugs are specifically designed to target a particular

molecular attribute (or mutation) that characterizes a given type of cancer. Imatinib mesylate, for example, works by inhibiting the bcr-abl enzyme activity, which is specific to CML (see [102, 19] and Chapter 6 of this dissertation for a more detailed description). Because of the potential of these new therapies, we are witnessing the rise of a new discipline, molecular oncology, whose focus is to understand the genetic and biochemical mechanisms involved in cancer [116].

Nevertheless, even for these targeted therapies, the development of drug resistance is still a fundamental problem. For example, mutations in the bcr-abl domain will cause almost invariably resistance to imatinib for patients in advanced stages of CML [68].

1.3 The biology of drug resistance

As already mentioned, drug resistance strongly limits the success of the therapy and reduces survival rates. This is a fundamental problem since cancer that becomes resistant to all available drugs may leave the patient with no therapeutic alternatives. There are various classifications of drug resistance, which are briefly summarized below. For a more comprehensive overview we refer to the book by Teicher [108] and to the references therein.

Drug resistance may be *permanent* or *temporary* (in which case it can reverse over time). Resistance is either *relative* or *absolute*, where *relative resistance* refers to a resistance that depends on the dosage. Typically, in relative resistance, the higher the dosage is, the smaller is the resistance that develops. On the other hand,

in *absolute resistance* no matter what the dose is, a resistant cell will not be affected by the drug. In general, contemporary literature seems to consider drug resistance to be mainly relative rather than absolute (see, e.g., [24, 37, 79, 92]).

Drug resistance appears to be both a *spontaneous* phenomenon caused by random genetic mutations, as well as an *induced* one (which means that patients that take a particular drug increase the chances of developing resistance to it). Indeed, there is clear experimental evidence that at least in the case of some drugs, known as "mutagenic drugs", the drug itself can induce resistance to itself (e.g., see [100, 101, 105]). Such resistance is referred to as "drug-induced resistance".

Patient's sensitivity to chemotherapy depends on many factors. We may divide them into broadly two categories [108, 34]:

1. *physiological resistance*, which depends on host factors, like, for example, the size, location, and growth rate of the cancer, the blood supply, the immune system status, the tumor microenvironment, the tumor pH, or the patient's intolerance to the effects of a drug.

2. *biological resistance*, given by kinetic resistance or genetic and epigenetic alterations in the cancer cells.

In the following we will focus only on the second kind. "Kinetic resistance" refers to the reduction in effectiveness of a drug which is caused by the cell division cycle. Such resistance is generally only temporary. Many drugs (such as methotrexate, vincristine, and citosine arabinoside, to name a few) are mainly effective during only one specific phase of the cell cycle, e.g., during the S phase, when the DNA is

synthesized. Thus, in the case of a short exposure to the drug, the cell will not be affected if during that time it is in a different phase. Even more importantly, the cell will be substantially invulnerable if it is out of the cell division cycle, i.e., in a "resting state" or in the G_0 state. This means that the number of cells that are affected by the drug is lower for cell populations that have low proliferation rates.

Resistance to drugs may instead develop as a consequence of genetic events such as mutations, rather than developing due to kinetic reasons. This category includes both "point mutations" and "chromosomal mutations", also known as "gene amplifications". *Point mutations* are random genetic changes that occur during cell division. These mutations cause the replacement of a single base nucleotide or pair with another nucleotide or pair in the DNA or RNA. This is a random event with a very small (yet nonzero) probability that modifies the cellular phenotype, making any of its daughter cells resistant to the drug. Other examples of genetic mutations are frameshift and missense mutations. *Gene amplification* is the consequence of an overproduction of a particular gene or genes. This means that a limited portion of the genome is reproduced to a much greater extent than the replication of DNA composing the remainder of the genome. Such a defect amplifies the phenotype that the gene confers on the cell, which, in turn, induces resistance by essentially providing the cells with more copies of a particular gene than the drug is able to cope with. While gene amplification (and consequently the resistance induced by it) may be a temporary phenomenon, point mutations are permanent.

The cause for genetic mutations is not completely clear. Is it a mainly random phenomenon, or rather a drug-induced, directed one, perhaps both? Such a

fundamental question has been the focus of the Nobel Prize winning work of Luria and Delbrück [65]. Using fluctuation analysis, Luria and Delbrück showed that drug resistance in *in vitro* bacterial cultures seems to have an important random component. Many further *in vitro* experiments with tumor cell lines confirmed this result. *In vivo* experiments have instead provided somewhat contradictory results [103, 32].

1.4 Structure of the dissertation

In this dissertation we consider the dynamics of drug resistance in cancer and the related issue of the dynamics of cancer stem cells. We would like to note that a fundamental consequence of the cancer stem cell hypothesis is that, in a tumor, long-lived resistance to chemotherapy will only be caused by those cells that have the ability of self-renewal, that is the cancer stem cells. Thus, if we assume such hierarchy in the cancer cell population, for the problem of drug resistance we may focus only on them. While there is a growing body of evidence on the existence and role of CSCs, to date there is little data on the correctness of this assumption and its clinical implications for therapeutic strategies [107, 13]. The rest of the dissertation is organized as follows.

In Chapter 2 we review the existing mathematical modeling literature on cancer stem cells and drug resistance. Starting with chapter 3, we introduce new research material.

In Chapter 3 we consider the question of the dependence - or not - of the dynamics of drug resistance on the so called "turnover rate", a variable which is an

indicator of the relative frequency of cell division with respect to cell death. We show how a very simple system of ordinary differential equations (ODEs) allows us to obtain results comparable to those found in the literature with one important difference. Indeed we show that the amount of resistance that is generated before the beginning of the treatment, and which is present at some given time afterward, always depends on the turnover rate, no matter how many drugs are used. Previous work in the literature indicated no dependence on the turnover rate in the single drug case while a strong dependence in the multi-drug case.

In Chapter 4 we develop a new methodology in order to derive an estimate of the probability of developing resistance to drugs by the time a tumor is diagnosed. Importantly, the heterogeneity of the cancer population is taken into account. Moreover, in the case of Chronic Myelogenous Leukemia (CML), we are able to infer the preferred mode of division of the hematopoietic cancer stem cells, predicting a large shift from asymmetric division to symmetric renewal.

In Chapter 5 we extend the results of Chapter 4 by relaxing the assumptions on the average growth of the tumor, thus going beyond the standard exponential case.

In Chapter 6 we briefly review the basic modeling assumptions and main results found in the mathematical modeling literature on CML, and formulate a new hypothesis on the effects that the drug Imatinib has on leukemic stem cells. Based on this hypothesis, we then use our mathematical results to obtain new insights on the dynamics of the development of drug resistance in CML.

Concluding remarks are provided in Chapter 7.

Chapter 2

Previous Mathematical Models

In this chapter we review the existing literature on the mathematical modeling of the cancer stem cell hypothesis and of drug resistance. We would like to comment on some of the works and refer interested readers to the references therein. We describe the main ideas of these models, the mathematical techniques involved and their main results.

2.1 Models of the cancer stem cell hypothesis

While the first mathematical models of cancer were produced in the 1950s by Nordling [81], Armitage and Doll [2], and Fisher [22], it was in 1963 that Till, McCulloch and Siminovitch [109] proposed the first mathematical model of a cell population growth where the stem cell hypothesis was included. In their model it is assumed that a stem cell can either renew symmetrically, producing two daughter stem cells, or differentiate symmetrically, producing two differentiated (not stem cells) daughters. In this way the two division modes are simply equivalent to either a stem cell birth or death, allowing the modeling of the cell population growth by a birth and death process. Moreover the authors ran experiments on the growth of spleen colony-forming cells, considered to be stem cells. After collecting the data, they fit the stochastic model to the data, reaching the conclusion that the distri-

bution of the number of spleen stem cells present in a growing colony of spleen cells, after about ten days from the formation of the colony, was well approximated by a gamma distribution. It is important however to note that the model assumptions leave out from the dynamics the fundamental case of an asymmetric division in which a stem cell differentiates into one stem cell and one daughter cell (see Chapter 4). The model by Till, McCulloch and Siminovitch [109] was later used to describe human cancer growth (see for example [66]).

Today there is a growing literature on mathematical models which incorporate the cancer stem cell hypothesis among their assumptions and which are specific to a given type of cancer. Examples include [73, 98, 23] for chronic myeloid leukemia, which we will describe in detail in Chapter 6, [75, 51, 17, 52] for colorectal cancer, and [21] for breast cancer. We would like now to briefly review the mathematical models on colorectal and breast cancer mentioned above.

Michor *et al.* [75] study the colorectal tumorigenesis and whether chromosomal instability (CIN), present in more than 80% of colorectal cancers and caused by chromosomal mutations in certain genes, is an early occurring event driving tumorigenesis or rather a consequence of it. The cell population in the colonic crypt is assumed to be hierarchical. A colonic stem cell is at the base of the crypt and divides always asymmetrically producing one differentiated cell at the time, which enter in a line that makes it slowly migrate to the top of the crypt where it will commit apoptosis. Using numerical methods the authors estimate that even the presence of very few genes subject to chromosomal mutations, will ensure that chromosomal instability will emerge early in tumorigenesis.

Johnston *et al.* [51] present a three compartment model for the colonic crypt to account for stem cells, transit-amplifying, and fully differentiated cells. Both stem cells and transit-amplifying cells are assumed to have the ability to self-renew and differentiate. The dynamics of the tumor cell population is described by using a system of three coupled ordinary differential equations with saturation terms. The authors show that depending on the parameters, there are a series of stable equilibria corresponding to benign stages of a tumor. Exponential growth will occur only beyond certain parameters' values. Furthermore such instability may be driven by uncontrolled growth either in the stem cell compartment or in the transit-amplifying cells. In [52] Johnston *et al.* consider in detail both cases and show that in both cases it is possible to obtain any proportion of CSCs in a tumor under quite reasonable assumptions. This conclusion is in contrast to the generally accepted view that CSCs constitute only a small proportion of a tumor. We will see that this is also one of our conclusions in Chapter 4 [114].

Also D'Onofrio *et al.* [17] assume three compartments in the intestinal crypt: stem cells, semi-differentiated cells and fully differentiated cells. Both stem and semi-differentiated cells are assumed to be able to self-renew and differentiate, while fully differentiated cells no longer divide. A system of three difference equations is used. Interestingly, nonlinearity is introduced into the model in one of two ways: by considering random fluctuations in the parameters (where the parameters are now stochastic processes) or by assuming that the respective probabilities to self-renew, differentiate and commit apoptosis depend on the numbers of cells in each compartment. The model is characterized by bifurcation between an increase in

cell numbers leading to a stable equilibrium or to explosive exponential growth. In addition, the authors show that tumor progression may be a consequence of alterations in cell turnover or fluctuations in cell numbers, due to a normal event like tissue damage.

Enderling *et al.* [21] formulate a mathematical model to describe the process of mutations and stepwise development of breast cancer. They assume that the loss of two tumor suppressor genes is sufficient to give rise to a cancer, where for each gene two alleles are potential target for the first mutation in a gene compared to only one for the second mutation. A system of ordinary and partial differential equations is used where the effects of diffusion, random motility, and haptotaxis are included. Using numerical simulations, it is shown that only the presence of a large number of breast stem cells and tumor suppressor genes in the breast tissue can explain the high incidence of breast cancer in women. Furthermore, the mathematical model suggests that the most likely scenario is that the tumor arises from an area where a mutation occurred in stem cells at an early age.

Finally, in Dingli *et al.* [15] a microenvironment composed by a small number of stem cells (for example in the colonic crypt) is considered in order to study the insurgence of a tumor among stem cells due to random mutations. All three modes of stem cell divisions are considered while the number of stem cells is assumed to remain constant over time. Thus, a Moran process is used to model the dynamics of the stem cell population subject to mutations. Stochastic computer simulations show that in order for CSCs to take over the normal stem cell population it is generally required that the CSCs either have a fitness advantage or a higher probability to

renew symmetrically when compared with healthy stem cells.

2.2 Mathematical models of drug resistance

Drug resistance has been extensively studied in the mathematical literature. As already mentioned in Chapter 1, the modeling of resistance due to random point mutations was motivated by the experimental findings of Luria and Delbrück [65] in 1943 on the development of resistance to antibiotics in bacteria due to mutations. Using fluctuation analysis, the authors concluded that the development of resistance is primarily a random phenomenon rather than a drug-induced, directed one. The mathematical model the authors formulated in order to answer that fundamental question was also important for the estimation of mutation rates. In the model, Luria and Delbrück assume that the process starts with one normal cell and no mutants. The occurrence of mutations is modeled by a random process, specifically a Poisson process with an intensity function. Importantly, both normal and mutant cells are assumed to grow deterministically in an exponential fashion. The probability of no mutations is then calculated as well as the mean and the variance of the distribution of the number of mutants [65]. Given these estimates it was possible to implement methods for the estimation of mutations rates from the data. This Nobel Prize winning work has been followed by a large literature on the study of the distribution of the number of mutants in a population which grows exponentially, known as the Luria and Delbrück distribution [121].

The first model of resistance to chemotherapy due to point mutations in cancer

is the celebrated model by Goldie and Coldman and its extensions (see [10, 11, 29, 33, 30, 31, 32]).

In Goldie *et al.* [29] the growth of the drug sensitive cancer cell population is approximated by using a deterministic exponential curve. At each division there is a small positive probability that a cancer drug sensitive cell may give rise to one drug resistant cancer cell daughter because of a random point mutation. Such a mutant will generate a clone that grows according to a birth process. The number of mutations occurring in the drug sensitive population up to a given time is instead approximated by a Poisson distribution. It is also assumed that back mutations cannot occur. The number of mutants present in the cancer cell population is then given by a filtered Poisson process. The probability of having no resistant cells present in a tumor is then calculated, where the nonexistence of resistant cells is assumed to be the condition for being cured. We note that in the model by Goldie and Coldman, the drug-sensitive population is modeled deterministically as in Luria and Delbrück [65], but the drug resistant population is modeled stochastically rather than deterministically. The main results of Goldie *et al.* [29] are that the probability of having no drug resistance present in a tumor is inversely related to the tumor size and that more frequent dosage repetitions are more successful in minimizing the risk of drug resistance development than less frequent doses administered for a longer period of time.

In Coldman *et al.* [33, 30] the authors extend the model to multi-drug resistance. It is assumed that multiple drug resistance occurs in single steps where now a cancer cell may be sensitive to all drugs, resistant to only one of the drugs, to two

given drugs and so forth. The main conclusion of their study is that the best strategy is to use all available drugs simultaneously. Furthermore, it is shown that, if the simultaneous administration of all drugs is not possible, the sequential alternation of all drugs is optimal when these drugs are equally effective.

Interestingly, Goldie and Coldman extended their mathematical model to consider the development of drug resistance when the cancer stem cell hypothesis is considered [31, 32]. Unfortunately, they assumed that a stem cell can either renew symmetrically, producing two daughter stem cells, or differentiate symmetrically, producing two differentiated (not stem cells) daughters. In this way the two division modes are simply equivalent to either a stem cell birth or death, leaving out from the stem cell dynamics the fundamental case of an asymmetric division, where a stem cell and a differentiated daughter cell are produced. The model then reduces to the usual birth and death process of a growing population.

A more recent study on point mutations is by Komarova *et al.* [60, 58, 61, 59]. For example, in [60, 58], a model which is based on stochastic birth and death processes on a combinatorial mutation network is used to describe the development of resistance to multi-drug treatments. Thus, probabilistic methods and a hyperbolic PDE are used to show how the pre-treatment phase is more significant in the development of drug resistance than the treatment phase. This is a very natural, intuitive result given that the treatment will, in general, drastically reduce the cancer population and consequently also reduce the number of possible cell divisions and mutations. Moreover, the main result obtained in [58, 60] is the following: in the case of a single drug treatment, the probability to have resistant mutants generated

before the beginning of the treatment and present, including their progeny, at some given time afterward, does not depend on the cancer turnover rate. A consequence of such result is that also the probability of treatment success does not depend on such a rate. For the case of a multi-drug treatment, instead, in [58] it is shown that the probability of having resistant mutants strongly depends on the turnover rate, and, therefore, the probability of treatment success also will strongly depend on this rate. One of the goals of Chapter 3 is to understand the reason for such a difference between the single and multi-drug cases. In Komarova *et al.* [60, 59] the same methodology is used to analyze the development of resistance in CML. In [60] it is suggested that a combination of three drugs with different specificities might overcome the problem of resistance. In Komarova *et al.* [59] it is observed that combining more than two current drugs will not provide any further therapeutic advantage, due to the problem of cross-resistance.

Another recent work on point mutations is by Iwasa *et al.*, [50], in which continuous-time branching processes are used to calculate the probability of resistance at the time of detection of the cancer, as well as the expected number of mutants found at detection if resistance developed. These estimates are found both for the case where drug sensitive and drug resistant cells have the same birth and death rates as well as for the cases where the drug resistant cells have a fitness advantage or disadvantage with respect to the wild-type cancer cells. Similarly to the results of [29], the authors of [50] show that the probability of resistance is an increasing function of the detection size and the mutation rate. In a later paper [39] the model is extended to obtain the same estimates for the case of a two-drug treat-

ment. We refer to Chapter 4 for further details.

We will not consider in the next chapters the modeling of drug resistance due to gene amplification, kinetic resistance or drug induced resistance. We would like to comment on some of the works on these types of resistance and refer interested readers to the references therein.

Modeling of resistance due to gene amplification can be found, e.g., in [42, 43, 56]. Drug resistance in these works is studied using stochastic branching models. Kinetic resistance has been mathematically studied in [4] and [89]. The models in these papers are based on ODEs. An alternative approach on kinetic resistance, using age-structured models, can be found in [9, 14, 25, 26, 117]. Finally, for mathematical models and experimental findings on drug-induced resistance, we refer, e.g., to [36, 105, 88].

Chapter 3

The Dependence of Drug Resistance on the Turnover Rate

3.1 Introduction

In this chapter we introduce a very simple model for absolute drug resistance, which we assume is caused only by random genetic point mutations. For this particular setup, most existing mathematical models are based on stochastic methods. In contrast, our approach is based on a compartmental system of ODEs, whose variables are the normal cancer cell population (that is susceptible to the drug), and the population of cancer cells that are resistant to the drug due to point mutations.

The purpose of this model is to show that elementary ODE-based techniques can be successfully used to obtain comparable results to those that were previously derived using the more complex mathematical machinery of stochastic methods.

The structure of the chapter is as follows: In Section 3.2, we outline the basic modeling assumptions and develop our mathematical model of drug resistance. We start by considering the single drug case, and proceed with the more general case of two or more drugs. The main results that we obtain from the model are presented in Section 3.3. We show that the amount of resistance generated before the beginning of the treatment, and present at some given time afterward, always depends on the turnover rate, no matter how many drugs are used simultaneously. We also use our model to compare the amount of resistance that originates before and after the

treatment starts. Section 3.4 focuses on studying the differences between our results and other works in the field. Concluding remarks are provided in Section 3.5.

Once again we would like to stress that the main contribution of this chapter is in providing an elementary way to derive comparable results to those that were previously obtained using substantially more complex mathematical machinery. The content of this chapter has been published in [112, 113].

3.2 An elementary model for drug resistance

In this section we develop a simple mathematical model for absolute drug resistance in the presence of random genetic point mutations. Our approach is to model the process using a linear system of ODEs. In its essence, our model enjoys similarities with the well known model of Goldie and Coldman [30] (see also [25]). The main difference between our approach and [30], is that we do not make any use of probability theory. Instead, we rely on a purely deterministic system. The advantages and disadvantages of using such an approach will be examined below.

3.2.1 The single-drug case

We start with the case of resistance to a single drug. Accordingly, we follow two populations: The first group is composed of wild-type cancer cells (cells that are sensitive to the drug). We denote the number of wild-type cancer cells at time t, by $N(t)$. The second group are cells that have undergone a mutation. These cells are resistant to the drug. The number of mutated cells at time t is denoted by $R(t)$.

We assume that cancer grows exponentially according to the Skipper–Schabel–Wilcox model, also known as the log kill model (see [104]). We also assume that the drug therapy starts at t^*. Our model can then be written as:

$$\begin{cases} N'(t) = (L - D)N(t), \\ R'(t) = (L - D)R(t) + uN(t). \end{cases} \qquad t \leq t^*. \tag{3.1}$$

$$\begin{cases} N'(t) = (L - D - H)N(t), \\ R'(t) = (L - D)R(t) + uN(t). \end{cases} \qquad t > t^*. \tag{3.2}$$

The system (3.1) describes the pre-treatment phase, while the system (3.2) follows the dynamics after the treatment starts. The difference between both systems is the introduction of H, the drug-induced death rate, a term that appears only after the treatment starts, i.e., in (3.2). In both systems, L, D, and u denote the birth, death, and mutation rates, respectively. We assume that $0 \leq D < L$ and $0 < u \ll 1$. The system (3.1) is written assuming that mutations occur as a result of a wild-type cell differentiating into one wild-type and one mutant cell. This is a standard assumption, see, e.g., [58].

The initial conditions for the pre-treatment system (3.1) are given as constants $N(0) = N_0 \neq 0$ and $R(0) = 0$. The initial conditions for the system (3.2) are $N(t^*)$ and $R(t^*)$, which are the solutions of (3.1) at $t = t^*$.

Remarks:

1. In this model we assume that both the wild-type and the resistant (mutated) cells have the same birth and death rates. This assumption is made in order

to simplify the initial presentation, and can be easily modified to model situations where the resistant cancer cells R have a relative fitness advantage or disadvantage with respect to the wild-type cancer cells N. Indeed it is increasingly recognized that resistance to chemotherapy comes at a fitness cost, see for example [27]. If we consider the more general case where different birth and death rates are assumed for the wild-type and the mutated cells, in the single-drug case the model becomes:

$$\begin{cases} N'(t) = (L - D)N(t), \\ R'(t) = (\tilde{L} - \tilde{D})R(t) + uN(t). \end{cases} \qquad t \le t^*. \tag{3.3}$$

$$\begin{cases} N'(t) = (L - D - H)N(t), \\ R'(t) = (\tilde{L} - \tilde{D})R(t) + uN(t). \end{cases} \qquad t > t^*. \tag{3.4}$$

We assume that $(\tilde{L} - \tilde{D}) \neq (L - D)$, given that otherwise we are back to system (3.1).

2. Another modification could be to replace the exponential growth of cancer by a different model such as the gompertzian growth, which was empirically shown to provide a better fit (see the Norton-Simon hypothesis in [83], [82], [84]).

3. We assume that mutations happen only in one direction, i.e., wild-type cells mutate and become resistant but not vice versa. This seems to be a reasonable assumption in the case in which the focus is on point mutation resistance and not on resistance caused by gene amplification. Indeed, the probability of

reversal of a point mutation is much smaller than the probability of the point mutation itself, and can therefore be neglected (see [18, 73, 50, 58]).

4. The time of the beginning of the treatment, t^*, can be related to the size of the tumor at that time. If we assume that the total number of cancer cells at time t^* is M, we can use the exponential growth of cancer and the relatively small mutation rate u to estimate t^* as

$$t^* \approx \frac{1}{L-D} \ln \frac{M}{N_0}. \tag{3.5}$$

3.2.2 The 2-drug case

We now consider the case of a treatment in which two drugs are being simultaneously used. We denote by $R_1(t)$ and $R_2(t)$, the mutant cancer cell populations that mutated by time t so that they are resistant only to the first or to the second drug, respectively. We reserve the notation $R(t)$ for the population of cells that are resistant to both drugs at time t. With these notations, the model for drug

resistance with two drugs can be written as:

$$\begin{cases} N'(t) = (L-D)N(t), \\ R_1'(t) = (L-D)R_1(t) + uN(t), \\ R_2'(t) = (L-D)R_2(t) + uN(t), \\ R'(t) = (L-D)R(t) + uR_1(t) + uR_2(t). \end{cases} \quad t \le t^*. \tag{3.6}$$

$$\begin{cases} N'(t) = (L-D-H)N(t), \\ R_1'(t) = (L-D-H)R_1(t) + uN(t), \\ R_2'(t) = (L-D-H)R_2(t) + uN(t), \\ R'(t) = (L-D)R(t) + uR_1(t) + uR_2(t). \end{cases} \quad t > t^*. \tag{3.7}$$

Similarly to the single-drug case, also in the 2-drug case we distinguish between the pre-treatment dynamics (described by (3.6)) and the dynamics after the treatment started, which is given by (3.7).

Remarks:

1. Note that we assume that if a cell is already resistant to one drug it does not make it any less vulnerable to the combination of drugs (i.e., H remains unchanged). Such an assumption can be justified given that for cells that are resistant only to one drug, the second drug is still effective. This assumption can be easily modified to account for matters of physical or chemical nature that can influence the level of resistance that a cell (that is already resistant to one drug) may develop to the second drug.

2. We assume that the probability of a point mutation, and consequently the mutation rate u, is the same for any non fully resistant state in which a cell may be, an assumption that can also be easily modified.

3.2.3 The n-drug case

Extending the 2-drug model (3.6)–(3.7) to the setup of n drugs is straightforward. The resulting general n-drug case (with $n \geq 1$) is as follows. The system for the pre-treatment phase ($t \leq t^*$) is:

$$
\begin{aligned}
&N'(t) = (L-D)N(t),\\
&R_1'(t) = (L-D)R_1(t) + uN(t),\\
&\quad\vdots\\
&R_n'(t) = (L-D)R_n(t) + uN(t),\\
&R_{1,2}'(t) = (L-D)R_{1,2}(t) + uR_1(t) + uR_2(t),\\
&\quad\vdots\\
&R_{1,n}'(t) = (L-D)R_{1,n}(t) + uR_1(t) + uR_n(t),\\
&R_{2,3}'(t) = (L-D)R_{2,3}(t) + uR_2(t) + uR_3(t),\\
&\quad\vdots\\
&R_{2,n}'(t) = (L-D)R_{2,n}(t) + uR_2(t) + uR_n(t),\\
&\quad\vdots\\
&R_{1,\dots,n-1}'(t) = (L-D)R_{1,\dots,n-1}(t) + u\sum_{i=1}^{n-1} R_{(1,\dots,n-1)\setminus i}(t),\\
&R_{2,\dots,n}'(t) = (L-D)R_{2,\dots,n}(t) + u\sum_{i=2}^{n} R_{(2,\dots,n)\setminus i}(t),\\
&\quad\vdots\\
&R_{1,\dots,n-2,n}'(t) = (L-D)R_{1,\dots,n-2,n}(t) + u\sum_{i=1, i\neq(n-1)}^{n} R_{(1,\dots,n-2,n)\setminus i}(t),\\
&R'(t) = (L-D)R(t) + u\sum_{i=1}^{n} R_{(1,\dots,n)\setminus i}(t).
\end{aligned}
\tag{3.8}
$$

The system that describes the dynamics during the treatment $(t > t^*)$ is:

$$
\begin{aligned}
&N'(t) = (L - D - H)N(t),\\
&R_1'(t) = (L - D - H)R_1(t) + uN(t),\\
&\quad\vdots\\
&R_n'(t) = (L - D - H)R_n(t) + uN(t),\\
&R_{1,2}'(t) = (L - D - H)R_{1,2}(t) + uR_1(t) + uR_2(t),\\
&\quad\vdots\\
&R_{1,n}'(t) = (L - D - H)R_{1,n}(t) + uR_1(t) + uR_n(t),\\
&R_{2,3}'(t) = (L - D - H)R_{2,3}(t) + uR_2(t) + uR_3(t),\\
&\quad\vdots\\
&R_{2,n}'(t) = (L - D - H)R_{2,n}(t) + uR_2(t) + uR_n(t),\\
&\quad\vdots\\
&R_{1,\dots,n-1}'(t) = (L - D - H)R_{1,\dots,n-1}(t) + u\textstyle\sum_{i=1}^{n-1} R_{(1,\dots,n-1)\backslash i}(t),\\
&R_{2,\dots,n}'(t) = (L - D - H)R_{2,\dots,n}(t) + u\textstyle\sum_{i=2}^{n} R_{(2,\dots,n)\backslash i}(t),\\
&\quad\vdots\\
&R_{1,\dots,n-2,n}'(t) = (L - D - H)R_{1,\dots,n-2,n}(t) + u\textstyle\sum_{i=1,i\neq(n-1)}^{n} R_{(1,\dots,n-2,n)\backslash i}(t),\\
&R'(t) = (L - D)R(t) + u\textstyle\sum_{i=1}^{n} R_{(1,\dots,n)\backslash i}(t).
\end{aligned}
\tag{3.9}
$$

In these equations we used the obvious extension of the notation. For example, $R_{(1,\dots,n)\backslash i}$ denotes the mutant cancer cell population that is resistant to all drugs with the exception of drug $\#i$.

3.3 Analysis and results

In this section we discuss the main results that can be obtained by analyzing the models for drug resistance that were introduced in Section 3.2.

3.3.1 Dependence on the turnover rate D/L

Our first result is that the amount of resistance present at the time when the treatment starts, t^*, always depends on the turnover rate D/L no matter how many drugs are simultaneously used. This result is easily obtained by considering the solutions of $R(t^*)$ in the single-drug systems (3.1), the two-drug system (3.6), and the n-drug system (3.8). We briefly outline the derivation of the solutions (3.10) and (3.12). By writing the ODE system (3.1) in matrix form, we obtain the following matrix:

$$\begin{pmatrix} L-D & 0 \\ u & L-D \end{pmatrix} = (L-D)I + \begin{pmatrix} 0 & 0 \\ u & 0 \end{pmatrix},$$

where I is the identity matrix. The last matrix is nilpotent of order 2, thus the fundamental matrix is given by

$$e^{(L-D)t} \begin{pmatrix} 1 & 0 \\ ut & 1 \end{pmatrix}.$$

From this it follows that for the single drug case the solution is (using (3.5) to evaluate t^*):

$$R(t^*) = N_0 u t^* e^{(L-D)t^*} \approx \frac{Mu \ln(M/N_0)}{L(1-D/L)}, \tag{3.10}$$

where M is the total number of cancer cells when the therapy begins. In the more general case where different birth and death rates are assumed for the wild-type and

the mutated cells the solution of (3.3) for $R(t)$ is given by:

$$R(t^*) = N_0 \frac{u}{(\tilde{L}-\tilde{D})(L-D)}[e^{(\tilde{L}-\tilde{D})t^*} - e^{(L-D)t^*}] \approx u \left[\frac{M - N_0 \, (M/N_0)^{\frac{\tilde{L}-\tilde{D}}{L-D}}}{(\tilde{L}-\tilde{D})(L-D)} \right]. \tag{3.11}$$

Similarly, by writing the ODE system (3.6) in matrix form, we obtain the following matrix:

$$\begin{pmatrix} L-D & 0 & 0 & 0 \\ u & L-D & 0 & 0 \\ u & 0 & L-D & 0 \\ 0 & u & u & L-D \end{pmatrix} = (L-D)I + \begin{pmatrix} 0 & 0 & 0 & 0 \\ u & 0 & 0 & 0 \\ u & 0 & 0 & 0 \\ 0 & u & u & 0 \end{pmatrix}.$$

The last matrix is nilpotent of order 3, thus the fundamental matrix is given by

$$e^{(L-D)t} \begin{pmatrix} 1 & 0 & 0 & 0 \\ ut & 1 & 0 & 0 \\ ut & 0 & 1 & 0 \\ (ut)^2 & ut & ut & 1 \end{pmatrix},$$

from which it follows that the solution for the two-drug therapy is the following:

$$R(t^*) = N_0 (ut^*)^2 e^{(L-D)t^*} \approx M \left[\frac{u \ln(M/N_0)}{L(1-D/L)} \right]^2. \tag{3.12}$$

The extension to the general n-drug case is obvious:

$$R(t^*) = N_0 (ut^*)^n e^{(L-D)t^*} \approx M \left[\frac{u \ln(M/N_0)}{L(1-D/L)} \right]^n. \tag{3.13}$$

The various expressions for $R(t^*)$, (3.10)–(3.13), contain the turnover ratio D/L. We can thus conclude that the slower the growth of the cancer is (i.e., the closer the turnover rate D/L is to 1) the larger is the pre-treatment drug resistance.

Conversely, the faster the tumor grows (i.e., the closer the turnover rate is to zero) the smaller is the resistance that develops prior to the beginning of the treatment. This result is independent of the number of drugs.

3.3.2 How much resistance originates before the treatment?

Assume that mutations could be terminated after time t^* so that the only drug resistance that is present after t^* would be the "progeny" of the resistance generated before therapy started. We refer to such resistance as the "pre-treatment resistance at time t" and denote it by $R^p(t)$. We would like to compare $R^p(t)$ with the resistance that is generated exclusively by mutations that occur during treatment, which we denote by $R^d(t)$, and refer to as the "during-treatment resistance at time t". Mathematically we can stop the mutations by setting $u = 0$.

Our second result is that for any $t > t^*$, the pre-treatment resistance is greater than the during-treatment resistance, i.e., $R^p(t) \geq R^d(t)$. This result holds under the assumptions that $(L - D) < H$ and $M/N_0 \geq C$, where the constant C depends on the number of drugs. For example we will see that in the one-drug case, $C = e$, and in the two-drug case $C = \exp(1 + \sqrt(3))$.

Indeed, in the single drug case, the solution of the system (3.2), subject to the initial conditions N(0) = M and R(0) = 0, is given by

$$R^d(t) = M\frac{u}{H}[e^{(L-D)t} - e^{(L-D-H)t}]. \tag{3.14}$$

Here, to simplify the notations, time is measured from the beginning of the treatment, i.e., $t = 0$ refers to what we previously considered to be $t = t^*$.

On the other hand, $R^p(t)$ is the solution of (3.1) at time t^* that is then multiplied by an exponential term $e^{(L-D)t}$ that accounts for the growth of this resistance during treatment,

$$R^p(t) = \frac{Mu \ln(M/N_0)}{L-D} e^{(L-D)t}. \tag{3.15}$$

Thus, clearly $R^p(t) \geq R^d(t)$ for any $t \geq 0$. Moreover, for sufficiently large t, we have

$$\frac{R^p(t)}{R^d(t)} \approx \frac{H}{L(1-D/L)} \ln(M/N_0) = Ht^*, \tag{3.16}$$

which nicely illustrate the key players in determining the proportion between the two populations. We would like to stress that in the case of mutagenic drugs, where the mutation rates are much higher during treatment, the result may be easily reversed, i.e., the resistance generated after the beginning of the treatment may exceed the pre-treatment resistance.

For two drugs, the solution of the system (3.7), subject to the initial conditions

$$\begin{cases} N(0) = M, \\ R_1(0) = \dfrac{Mu \ln(M/N_0)}{L-D}, \\ R_2(0) = \dfrac{Mu \ln(M/N_0)}{L-D}, \\ R(0) = 0, \end{cases}$$

is given by

$$\begin{aligned} R^d(t) &= 2M\frac{u^2}{H^2}\left[e^{(L-D)t} - e^{(L-D-H)t} - tHe^{(L-D-H)t}\right] \\ &\quad + \frac{2Mu^2 \ln(M/N_0)}{H(L-D)}\left[e^{(L-D)t} - e^{(L-D-H)t}\right]. \end{aligned} \tag{3.17}$$

Similarly to the single drug case, $R^p(t)$ is the solution of (3.6) multiplied by the exponential term $e^{(L-D)t}$ to account for the growth of such resistance during treat-

ment,

$$R^p(t) = M\left[\frac{u\ \ln(M/N_0)}{L-D}\right]^2 e^{(L-D)t}. \tag{3.18}$$

It is now easy to verify that $R^p(t) \geq R^d(t)$ for any $t \geq 0$, unless t is large and

$$1-\sqrt{3} < \frac{H\ \ln(M/N_0)}{L-D} < 1+\sqrt{3},$$

which is impossible given the assumptions.

Remarks:

1. A similar result can be obtained in the n-drugs case. $R^p(t)$ is the solution of (3.8) multiplied by the exponential term $e^{(L-D)t}$ to account for the growth of such resistance during treatment. Thus we find that

$$R^p(t) = M\left[\frac{u\ \ln(M/N_0)}{L-D}\right]^n e^{(L-D)t}. \tag{3.19}$$

Instead, for $R^d(t)$ we find that for a sufficiently large t,

$$\begin{aligned} R^d(t) \quad \approx \quad & \binom{n}{n} n!M\left(\frac{u}{H}\right)^n e^{(L-D)t} \\ & + \binom{n}{n-1}(n-1)!M\left(\frac{u}{H}\right)^{n-1}\left[\frac{u\ \ln(M/N_0)}{L-D}\right]^1 e^{(L-D)t} \\ & + \binom{n}{n-2}(n-2)!M\left(\frac{u}{H}\right)^{n-2}\left[\frac{u\ \ln(M/N_0)}{L-D}\right]^2 e^{(L-D)t} + \ldots \\ & + \binom{n}{1}(n-1)!M\left(\frac{u}{H}\right)^1\left[\frac{u\ \ln(M/N_0)}{L-D}\right]^{n-1} e^{(L-D)t}. \end{aligned}$$

2. Equations (3.15), (3.18) and (3.19) clearly show how the amount of resistance generated before the beginning of the treatment and present, including its progeny, at any given time afterward depends on the turnover rate.

3. We note that in the two-drug case, the interval in which the pre-treatment resistance is smaller than the during-treatment resistance, i.e., $R^p(t) < R^d(t)$, is slightly larger than in the single drug case.

4. If we allow the drug-induced death rate to take different values, for example, $H/2$ for the populations R_1 and R_2 and H for the population N, (which models a situation in which only one of the two drugs can kill these cells), then the interval for which $R^p(t) < R^d(t)$ can be made larger. For the particular choice of these values, the interval becomes

$$2 - \sqrt{2} < \frac{H \ln(M/N_0)}{L - D} < 2 + \sqrt{2}.$$

3.4 Discussion

The discussion in this section is devoted to comparing the results obtained with our model and the results obtained in [50, 60, 58]. One of our motivations is indeed to understand the main result obtained in [58, 60], which is the following. In the case of a single drug treatment, the probability to have resistant mutants generated before the beginning of the treatment and present, including their progeny, at some given time afterward, does not depend on the cancer turnover rate. A consequence of such result is that also the probability of treatment success will not depend on such rate. For the case of a multi-drug treatment, instead, in [58] it is shown how there appears to be a strong dependence of the probability to have resistant mutants on the turnover rate, and therefore also the probability of treatment success will strongly depend on such rate. Our goal is to understand the reason for such a

difference between the single and multi-drug cases. This is accomplished by using our different, simpler approach.

The problem studied in all papers is similar, i.e., drug resistance caused by random mutations, though the mathematical techniques being used are different. Here we use a deterministic system of ODEs as opposed to the various stochastic methods used in [50, 60, 58]. The specific goals of each paper are somewhat different. In [50] the probability to have developed resistance by the time of clinical detection of the cancer is calculated. Instead, in [60, 58] the focus is on the probability to have resistance as $t \to \infty$. Finally, in our paper we calculate the "average" amount of resistance developed at any given time before and after the beginning of the treatment. We would like to note that while in this work we deal with numbers of cells, in [50, 60, 58] the quantities of interest are probabilities.

Comparing our results with some of the main results of [50, 60, 58], the outcome is as follows:

1. Our results agree with [50], in the sense that both works clearly show the dependence of resistance on the turnover rate at the time of detection (see equation (3.15) and table 1 of [50, page 2563], also found below as equation (3.21)). Furthermore, our results agree with [50] also on the effect that the mutation rate and the detection size have on resistance.

2. Most of our results agree with the results of [58, 60] which are summarized on page 352 of [58]. For example, in [58] it is shown that the pre-treatment phase is more significant in the development of resistance than the treatment phase.

This is a very natural, intuitive result given that the treatment will in general drastically reduce the cancer population, and therefore also the number of possible cell divisions and mutations. With our model such result was easily obtained. However there is one important difference between our results and those found in [58]. In our work, we show that no matter how many drugs are used in the treatment, the amount of fully resistant mutants (generated before the beginning of the treatment and present, including their progeny, at some given time afterward) depends on the turnover rate. See (3.10)–(3.13) and Remark 2 in Section 3.3. In contrast, one of the main results of [58, 60] was that this dependence holds only in the multi-drug setup, and not for the single drug case (see [58, page 360]). The reason for this difference is due to the fact that [58] studies the probability to have such resistance in the limit, as $t \to \infty$. It is only at $t = \infty$ that the results of [58] show a lack of dependence of the resistance on the turnover rate (see page 365, equation (49) and the following discussion in [58]). This result does not hold at any finite time. This result can be further understood by the following argument. Using mixed techniques of ordinary differential equations and branching processes it is possible to calculate - as we will see in chapter 4 - the probability to have resistant mutants generated before the beginning of the treatment and present, including their progeny, at some given time afterward. This probability is given by the following formula

$$P_R(t) = 1 - \exp\left(-Mu\frac{L}{D\exp(-(L-D)t)}\ln\frac{1}{1-\frac{D\exp(-(L-D)t)}{L}}\right). \quad (3.20)$$

Here the time t is measured from the start of the treatment. Once again it is clear that this probability given by (3.20) does depend on the cancer turnover rate for any finite time t . The asymptotic analysis found in [58] loses this information, indeed it is only asymptotically that such dependence will disappear. Clearly, the strength of such dependence will depend on the actual values of the parameters.

Furthermore, the conclusion in [58] that in the single drug case, the probability of treatment success does not depend on the turnover rate (see [58, page 352]), is related to the definition of a successful treatment as a complete extinction of the tumor as time becomes infinite. Different definitions of a successful treatment (such as allowing tumors not to exceed a certain size or simply considering finite times) will lead to a dependence on the turnover rate also in the single drug case.

3. The elementary mathematical tools that we used in formulating our model, enable us to analytically study the n-drug case. Of course, with an increasing number of drugs, any deterministic model that considers averaged quantities has a limited validity. For two drugs or more, the results obtained using the approach in [60, 58] rely on numerical simulations.

4. Due to the simplicity of our model is it easy to consider the dynamics of resistance also in the more realistic case of treatment regimes where the drugs are administered intermittently. In such a scenario, one interesting issue is to study the connection between the frequency of the administration of the

drug (e.g. when the total dosage is fixed) and the development of resistance. It is also straightforward to compare different therapy regimes. Such a study would be particularly interesting if we assume a different model for the cancer growth. This setup is beyond the scope of this paper and it is left for future research.

5. Since our approach is deterministic, it has a definite disadvantage when compared with stochastic models as it does not provide the time-dynamics of the probability distribution of the generated resistance. At the same time, it is important to note that the stochastic approach may yield results of a limited value. Indeed, according to [50], the probability that resistance develops by the time of detection in the one-drug case is given by

$$P = 1 - \exp\left(-\frac{MuL}{D}\ln\frac{L}{L-D}\right). \tag{3.21}$$

Here, M is the detection size. Hence, for values of $M = 10^9$ (which, at present, is approximately the lower limit of clinical detection, see [92, page 31]) and $u \geq 10^{-8}$ [63, 73, 111], the probability that resistance develops by the time a tumor is detected is always greater than .9999. This is the case since $(L/D)\ln(L/(L-D))$ is always greater than 1 if $D < L$, where the last inequality is supported by experimental data [110]. Hence, in this case the actual calculation of the probability distribution of the generated resistance, while showing the explicit dependence on the parameters, may not provide useful information if the product of M and u is greater than 1.

6. While from a mathematical point of view, it is a common practice to compute

asymptotics as $t \to \infty$, it is more desirable in the problem of drug resistance (and its related concept of treatment success) to study the dynamics for finite time, a time that is at most of the order of several years.

Importantly, we would like to note that also the results found in Michor *et al.* [73] (see also [99]), which are supported by clinical data, indicate the dependance of drug resistance on the cancer turnover rate.

3.5 Conclusion

In this chapter we developed a simple compartmental ODE model for absolute drug resistance, which is assumed to be caused only by random genetic point mutations. Our model was derived and analyzed for an arbitrary number of drugs. The simplicity of our model is the main strength of our approach. Alternative approaches to the problem that are based on stochastic methods, become very complex with an increasing number of drugs. Of course, with an increasing number of drugs, any deterministic model that considers averaged quantities has a limited validity.

One of our goals was to understand the reasons behind the difference in the results of Komarova [58] for the single and multi-drug cases. We have shown that the amount of resistance that is generated before the beginning of the treatment, and which is present at some given time afterward, always depends on the turnover rate, no matter how many drugs are used. The dependence on the turnover rate in the single drug case is simply weaker that the dependence in the multi-drug case. This result contradicts some results in the literature, as discussed in Section 3.4.

Additional results that were obtained with our model, validate the corresponding results in the literature. In particular, we demonstrate that, with the reasonable biological assumptions $L - D - H < 0$ and $M/N0 > C$ (see [58]), the resistance whose origin is before treatment is greater than the resistance created after the therapy starts, at any given point in time. Note that a violation of the first assumption would imply that the therapy is not able to reduce the size of the tumor, even without any resistance being present.

In conclusion, we consider the modeling via a system of ODEs to be a simpler and at least in some sense a better way to approach the problem of acquired drug resistance, especially if the focus of the analysis is on the dynamics following the time of the tumor's detection. Due to the simplicity of our approach, it is possible to easily improve the validity of our model by considering, e.g., other types of models for cancer growth.

Chapter 4

The Role of Symmetric and Asymmetric Division of Stem Cells

4.1 Introduction

In this chapter, we will focus only on resistance due to genetic mutations and, due to the results of Luria and Delbrück, we will assume that such resistance is caused by a random mutation mechanism. Furthermore, we will consider the case of absolute resistance, i.e., resistance that is not relative to the dosage of the drug administered. A cancer cell is either resistant to the drug or not, once the dosage of the drug is prescribed.

In a recent work [50], Iwasa *e*t al. have shown that the probability that resistance to a drug develops by the detection time is given by

$$P = 1 - \exp\left(-\frac{MuL}{D}\ln\frac{L}{L-D}\right). \tag{4.1}$$

Here, M is the total number of cancer cells found at detection; u the probability of mutation per cell division; and L and D are the birth and death rates, respectively. This result was obtained using Markov chains and continuous time branching processes. Furthermore, in the case where $Mu \ll 1$, the expected number of resistant cancer cells found at detection, conditional on the presence of resistance, is

$$\bar{Y} \approx \frac{\ln M}{(L/D - 1)\ln\left(L/(L-D)\right)}. \tag{4.2}$$

It is important to emphasize that the study in [50] is based on assuming that

cancer cells are a homogeneous population, which is why M is taken to be the total number of cells. Yet, recent experimental evidence suggests that tumors should not be thought of as homogeneous. Indeed, it appears that tissues are maintained by a small subset of slowly replicating cells. These so-called "stem cells" have the capacity of both self-renewal and differentiation into more mature cells. Stem cells are very long lived, while mature, fully differentiated cells have a variable life span, which, depending on the tissue of origin, can typically range from a few days to several months.

From the point of view of drug resistance, the heterogeneity hypothesis implies that only the cells that have the capacity for self-renewal can propagate drug resistance. Therefore, these cells should be taken into account in any model of drug resistance in cancer. In fact, these are the only cells that should be taken into account.

The rest of the chapter is devoted to deriving, presenting, and applying the results we obtained while calculating the probability of developing resistance to drugs by the time a tumor is diagnosed, this time taking into account the heterogeneity of the population.

To demonstrate the significance of our result, we focus on chronic myeloid leukemia (CML) for which a recent study has been published on a six-year follow up of patients that receive imatinib as the first line of treatment [45]. The clinical study provides us with a concrete estimate of the percentage of patients that shift from the chronic phase to the acute phase (or enter into a blast crisis). We use this estimate as an upper bound on the number of patients that have developed resistance to the drug

by the time CML was diagnosed. By integrating the mathematical estimates with clinical and experimental data, we are able to infer the preferred mode of division of the hematopoietic cancer stem cells, predicting a large shift from asymmetric division to symmetric renewal. Such a shift is required in order to explain the clinical data. The content of this chapter has been published in [114].

4.2 Model derivation

We derive the mathematical model using ordinary differential equations for the wild-type cancer population and branching processes for the mutant cells. In Iwasa et al. [50] both the wild-type and the mutant cancer populations are modeled as stochastic processes. Our deterministic approach in modeling the total number of drug-sensitive cancer stem cells (CSCs) is equivalent to considering their averaged behavior. For completeness we derive in Section 4.2.6 the corresponding model in which both populations are represented by branching processes. The standard theory for these multi-type branching processes can be found in [3, 56] and especially in the book by Mode [76]. As we will show, however, this approach does not allow to obtain completely satisfactory results. Thus the methodology we used in the paper may be seen as a more satisfactory approach with respect to the standard multi-type branching processes approach. Moreover our methodology appears to be a simplified version of the stochastic approach taken by [50, 20] and in this sense it has the advantage of easily allowing modifications. For example, an exponential cancer growth may be a good assumption only when the cancer population is small.

To replace it by any other growth model, while straightforward with the ODEs approach, would be a somewhat more complex undertaking when dealing directly with stochastic methods. Furthermore, we would like to note that arguably our modeling methodology may be seen as more general, in the sense that at least for the wild-type population we only make assumptions on their averaged behavior (no Markov property for example). Finally, we note that if the cells are assumed to be a homogeneous population then our results, obtained through a partially deterministic methodology, match exactly the results of Durrett et al. obtained by the completely stochastic approach [20].

4.2.1 Ordinary differential equations

We assume that a stem cell may divide in the following three ways (shown in Figure 4.1):

1. **asymmetric division:** a stem cell divides into one progenitor cell and one stem cell;

2. **symmetric differentiation:** a stem cell divides into two progenitor cells;

3. **symmetric renewal:** a stem cell divides into two stem cells.

All three types of division have been observed experimentally (see [78, 87, 118, 69, 119]). We can therefore assign probabilities to the three division paths of stem cells as follows: we denote by a the probability of an asymmetric division, by b the probability of a symmetric differentiation, and by $c = 1 - a - b$ the probability of a faithful symmetric renewal. Clearly, $0 \leqslant a, b, c \leqslant 1$ and $a + b + c = 1$.

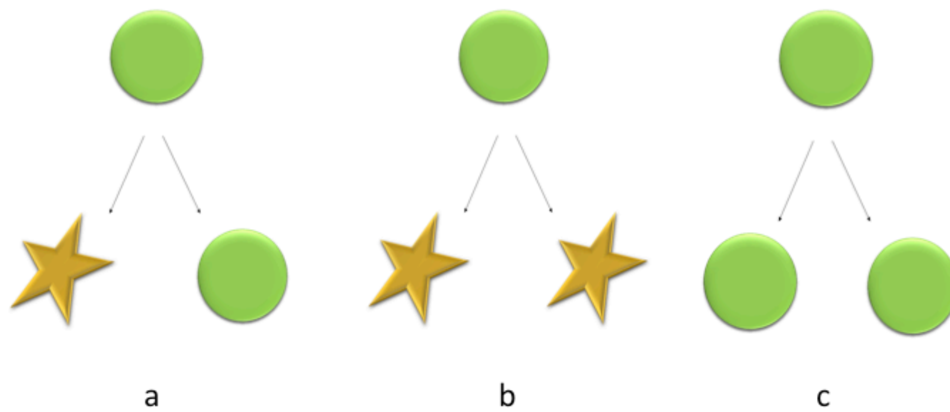

Figure 4.1: The different ways in which a stem cell may divide: (1) asymmetric division in which a stem cell divides into one progenitor cell and one stem cell; (2) symmetric differentiation in which a stem cell divides into two progenitor cells; and (3) symmetric renewal in which a stem cell divides into two stem cells.

Since we are interested in the dynamics of drug resistance in cancer, we will focus from now on on cancer stem cells. In addition we focus on the case of a single drug therapy. We denote the total number of wild-type, i.e., drug-sensitive cancer stem cells at time t by $S(t)$. This population is assumed to grow exponentially. The second group of cells, the mutated cancer stem cells that developed resistance to the drug are denoted by $R(t)$. The dynamics of the averaged behavior of the two cell populations can be described using the following system:

$$
\begin{aligned}
S'(t) &= \left[L(1-u)(1-a-b) - \left(D + bL + \frac{uaL}{2}\right)\right] S(t), \\
R'(t) &= \left[L(1-a-b) - (D+bL)\right] R(t) + uL\left(1 - \frac{a}{2} - b\right) S(t).
\end{aligned}
\tag{4.3}
$$

We assume that at time $t = 0$, we start with a single wild-type cancer stem cell, and no mutated stem cells. Consequently, the initial conditions for (4.3) are given by $S(0) = 1$ and $R(0) = 0$. As previously stated, u is the mutation probability per cell division, while L and D are the birth and death rates, respectively. To be precise, from now on, we will refer to L as the division rate, since a division may not result in the birth of a stem cell. In addition, since the mutation probability is usually extremely small, the system (4.3) is written under the assumption that mutations may occur in the division process only to one of the two daughter cells. This is a standard assumption, see e.g. [60, 50].

In the first equation in (4.3), the size of the wild-type cell population can increase only as a result of a symmetric renewal, were no mutation occurs. The probability of such an event is $(1-u)(1-a-b)$. Multiplying this probability by the division rate L provides the birth rate of $S(t)$. On the other hand, a decrease in the wild-type population will occur in the following cases: a cell death (D), a symmetric differentiation into two progenitors (bL), and if there is an asymmetric division (aL) in which the stem cell daughter is the mutant. The stem cell daughter will be the mutant with probability $u/2$, since u is the probability of mutations, and in this scenario only one of the two daughter cells is a stem cell.

For the second equation in (4.3), the size of the mutant population can once again increase only due to a symmetric renewal. Only that this time, we assume that mutated cells can no longer mutate back to the wild-type state (due the rarity of this event for point mutations). A decrease in the mutant population will be the result of cell death and of a symmetric differentiation into two progenitors. The last term of the equation are the wild-type cells that mutated. They either come from a symmetric renewal: $uL(1-a-b)$, or from an asymmetric division in which a mutation hits the daughter stem cell: $uLa/2$. The sum of these two terms is $uL(1-a/2-b)$.

Given that u is very small, it can be eliminated from the first equation. However, it cannot be removed from the second equation since $S(t)$ is much larger than $R(t)$. The system (4.3) can be thus reduced to the following:

$$
\begin{aligned}
S'(t) &= [L(1-a-2b)-D]S(t), \\
R'(t) &= [L(1-a-2b)-D]R(t) + uL\left(1-\frac{a}{2}-b\right)S(t).
\end{aligned}
\tag{4.4}
$$

We would like to make the following remarks.

1. Since we are modeling a cancer stem cell population that is assumed to grow from one cell to a large number, it must be that $[L(1-a-2b)-D] > 0$, which implies that $(1-a-2b) > 0$ and therefore $(1-\frac{a}{2}-b) > 0$.

2. Since the mutant population is always considered to be very small, we are actually not going to use the equation for $R(t)$ from (4.4). This equation will be replaced with a stochastic approach. It is still instructive to keep this equation as part of the system (4.4) since it can be used to understand the meaning of the final formulas.

3. In this model we assume that both the wild-type and the resistant stem cells have the same division and death rates and the same division events probabilities a, b. This assumption is made in order to simplify the presentation, and can be easily modified to model situations where the mutant cancer cells $R(t)$ have a relative fitness advantage or disadvantage with respect to the wild-type cancer cells $S(t)$ (as done in [50]).

4. Due to the simplicity of our approach, the assumption regarding the exponential growth of cancer can be easily replaced by any other growth model. Such a

modification is more complex when dealing directly with stochastic methods.

5. We assume that mutations happen only in one direction, i.e., wild-type cells mutate and become resistant but not vice versa, and thus $(1-u)$ is not multiplying L in the second equation. This seems to be a reasonable assumption in the case in which the focus is on point mutation resistance and not on resistance that is caused by gene amplification. Indeed, the probability of reversal of a point mutation is much smaller than the probability of the point mutation itself, and can therefore be neglected.

6. By modeling the cancer stem cell population growth in a deterministic way instead of as a random process, we lose the sensitivity to events that can happen for small populations, such as a population going extinct. Our focus in this study is on modeling the case where the wild-type cancer stem cell population reaches a detection size, M, and on its impact on drug resistance. Such a deterministic approach cannot provide the probability distribution of how long it takes for the tumor to reach detection size. However, our study provides the average behavior of such time. Interestingly, the formulas that we obtain using our mixed approach, are in exact agreement with the results that were derived with the full-blown stochastic approach (in the simple case where only symmetric renewal is considered, the only case that was previously studied with stochastic methods [50]).

4.2.2 The expected number of mutations when the size of the wild-type population is x

At this stage we can use the system (4.4) to estimate the expected number of mutations, m_x, that occur once the wild-type population is of size x.

Consider the first equation of system (4.4). As first step, we are interested in finding the expected lengths of time for which the total number of wild-type cancer stem cells $S(t)$ is equal to $1, 2, \ldots, M-1$. Here, M is the number of wild-type cancer stem cells at detection time. Note that, given the very small probability of a mutation, M is also a good approximation of the total number of cancer stem cells found at detection, i.e. both wild-type and mutated ones. Since the solution of (4.4) for $S(t)$ is given by

$$S(t) = \exp[(L(1-a-2b)-D)t], \tag{4.5}$$

then the "average" time at which the wild-type cancer stem cell population reaches size x, defined as $t_{(x)}$, is given by

$$t_{(x)} = \frac{\ln(x)}{L(1-a-2b)-D}. \tag{4.6}$$

Thus the "average" length of time for which the population will consist of exactly x stem cells is

$$t_{(x+1)} - t_{(x)} = \frac{\ln(x+1)-\ln(x)}{L(1-a-2b)-D} = \frac{\ln(1+1/x)}{L(1-a-2b)-D}. \tag{4.7}$$

Hence, the "expected number" of mutations occurring when there are exactly x wild-type cancer stem cells, m_x, is given by

$$m_x = xuL\left(1-\frac{a}{2}-b\right)\frac{\ln(1+1/x)}{L(1-a-2b)-D}. \tag{4.8}$$

This expression is obtained by multiplying the number of wild-type stem cells present at that time, x, by the mutation rate $uL(1 - \frac{a}{2} - b)$, and by the length of time for which the total stem cell population equals x.

Finally, note that $\varphi := x \ln(1+1/x) \sim 1$, (e.g., $\varphi \approx .9$ for $x = 4$ and $\varphi \approx .98$ for $x = 20$). Hence, since we are ultimately interested in large values of x, equation (4.8) can be reduced to

$$m_x = \frac{uL\left(1 - \frac{a}{2} - b\right)}{L(1 - a - 2b) - D}. \tag{4.9}$$

Note that if the cancer stem cell population is growing, $L(1 - a - 2b) - D$ must be greater than zero and hence the expression in (4.9) is well defined.

4.2.3 Branching processes

We are ultimately interested in calculating the probability of developing drug resistance by the time a tumor is detected. At this stage of the analysis, it is proper to take advantage of stochastic methods.

Assume that the tumor population grows exponentially starting from one wild-type stem cell. Let r_x be the actual number of mutations produced when there are $x = 1, 2, \ldots, M - 1$ wild-type cancer stem cells. Assume that all the random variables, r_x, follow a Poisson distribution with mean m_x, given by equation (4.9), and that they are independent. Note that the Poisson distribution seems to be a good choice given the very small probability of a mutation u.

Consider the clone initiated by a mutant cancer stem cell which originated when there were x wild-type cancer stem cells. Assume that the population size of

such clone follows a continuous-time branching process where in each time step of length Δt, a stem cell divides with probability $L\Delta t$ and dies with probability $D\Delta t$.

Let $g_x(\xi)$ be the generating function of such clone for which the original mutation happened when $S(t) = x$. To calculate this generating function we proceed as follows.

To simplify the notation we let $\tilde{L} = L(1 - a - b)$ and $\tilde{D} = Lb + D$. Then by the Kolmogorov backward equation we have

$$\begin{aligned} E\left[\xi^{R(t+\Delta t)} \mid R(0) = 1\right] &= \tilde{L}\Delta t E\left[\xi^{R(t)} \mid R(0) = 1\right]^2 \\ &+ \tilde{D}\Delta t E\left[\xi^{R(t)} \mid R(0) = 0\right] \\ &+ (1 - (\tilde{L} + \tilde{D})\Delta t) E\left[\xi^{R(t)} \mid R(0) = 1\right], \end{aligned} \tag{4.10}$$

since $E[\xi^{R(t)} \mid R(0) = 2] = E[\xi^{R(t)} \mid R(0) = 1]^2$, by independence. The three terms on the right hand side of (4.10) describe the three possible events occurring in the interval of time Δt: the clone-originating mutated cancer cell divides with probability $\tilde{L}\Delta t$ producing two resistant daughter stem cells, it dies or differentiates symmetrically with probability $\tilde{D}\Delta t$ extinguishing the clone, or nothing happens with probability $1 - (\tilde{L} + \tilde{D})$ and we still have the one mutated stem cell. The time $t = 0$ in (4.10) is the time of occurrence of the original mutation that generates the clone, i.e., when the wild-type population is of size x. The time t in (4.10) measures time starting from this $t = 0$.

Let $g(\xi, t) = E[\xi^{R(t)} \mid R(0) = 1]$. As $\Delta t \to 0$ we get

$$\frac{\partial}{\partial t} g = \tilde{L} g^2 + \tilde{D} - (\tilde{L} + \tilde{D}) g. \tag{4.11}$$

Solving (4.11) with the initial condition $g(\xi, 0) = \xi$ gives

$$g(\xi, t) = \frac{(\xi - 1)(\tilde{D}/\tilde{L})e^{(\tilde{L}-\tilde{D})t} - (\xi - \tilde{D}/\tilde{L})}{(\xi - 1)e^{(\tilde{L}-\tilde{D})t} - (\xi - \tilde{D}/\tilde{L})}. \tag{4.12}$$

Because $M \approx x \exp((\tilde{L} - \tilde{D})t_{M-x})$, where t_{M-x} is the time it takes for the cancer stem cells to go from x to M, we obtain the generating function

$$g_x(\xi) = g(\xi, x) \approx \frac{(\xi - 1)(\tilde{D}/\tilde{L})(M/x) - (\xi - \tilde{D}/\tilde{L})}{(\xi - 1)(M/x) - (\xi - \tilde{D}/\tilde{L})}. \tag{4.13}$$

We now denote by T the total number of drug resistant cancer stem cells that are present when the cancer is detected, i.e., when the total stem cell population is M. We let $G_T(\xi)$ be its generating function. To calculate $G_T(\xi)$, we let K_x be the total amount of resistant cells found at detection whose originating mutation occurred when there were x sensitive cancer stem cells. Then

$$\begin{aligned}
G_T(\xi) &= E[\xi^T] = E[\xi^{(K_1 + \ldots + K_{M-1})}] \\
&= E[\xi^{K_1}] \cdots E[\xi^{K_{M-1}}] \\
&= E[E[\xi^{K_1} \mid r_1]] \cdots E[E[\xi^{K_{M-1}} \mid r_{M-1}]] \\
&= \prod_{x=1}^{M-1} \sum_{r_x=0}^{\infty} \left(\frac{m_x^{r_x}}{r_x!} e^{-m_x} g_x(\xi)^{r_x} \right) \qquad (4.14) \\
&= \prod_{x=1}^{M-1} \exp((m_x g_x(\xi) - m_x)) \\
&= \exp\left(- \sum_{x=1}^{M-1} m_x (1 - g_x(\xi)) \right).
\end{aligned}$$

Note that, on line four, we used the fact that $g_x(\xi)^{r_x}$ is the generating function of all the r_x clones for which the original mutations happened when $S(t) = x$, that is the product of r_x generating functions $g_x(\xi)$.

4.2.4 The probability of having resistant cancer stem cells at the time of detection

By (4.14),(4.9),(4.13) we have that the probability of having resistant cancer stem cells at the time of detection is

$$\begin{aligned} P_R = 1 - G_T(0) &= 1 - \exp\left(-\sum_{x=1}^{M-1} m_x(1-g_x(0))\right) \\ &= 1 - \exp\left(-\frac{u(1-\frac{a}{2}-b)}{(1-a-2b)-D/L}\sum_{x=1}^{M-1}\frac{1-C}{1-C(x/M)}\right) \end{aligned} \tag{4.15}$$

where $C = \frac{D+Lb}{L(1-a-b)}$. By replacing the summation with an integral, and with a change of variable, we get

$$P_R \approx 1 - \exp\left(-\frac{uM(1-\frac{a}{2}-b)}{(1-a-b)}\int_0^1 \frac{1}{1-\frac{D+Lb}{L(1-a-b)}y}dy\right), \tag{4.16}$$

from which we obtain the desired expressions:

$$P_R = 1 - \exp\left(-uM\left(\frac{1-\frac{a}{2}-b}{1-a-b}\right)\right), \tag{4.17}$$

if $D = 0$. Otherwise, when $D \neq 0$, we obtain

$$P_R = 1 - \exp\left(-uM\left(\frac{1-\frac{a}{2}-b}{1-a-b}\right)\frac{1}{C}\ln\left(\frac{1}{1-C}\right)\right), \tag{4.18}$$

where $C = \frac{D+Lb}{L(1-a-b)}$.

4.2.5 The expected value of resistant cells found at detection if resistance occurred

Given (4.18), we calculate the expected value of the number of resistant cells that are found at detection, assuming that resistance has indeed developed by the

time of detection. This conditional expectation of resistant cells is given by

$$
\begin{aligned}
E(T \mid resistance) &\approx \frac{1}{P_R}\left(\frac{u(1-\frac{a}{2}-b)}{(1-a-2b)-D/L}\right)\sum_{x=1}^{M-1}\frac{M}{x} \\
&\approx \frac{M}{P_R}\left(\frac{u\left(1-\frac{a}{2}-b\right)}{(1-a-2b)-D/L}\right) ln(M).
\end{aligned} \tag{4.19}
$$

Equation (4.19) is obtained noting that $E(T \mid resistance) = E(T)/P_R = G'_T(1)/P_R$. Here, P_R is given by (4.18) and G_T is the generating function of the random variable T, which is the total number of resistant cancer stem cells that are present when the cancer is detected.

4.2.6 Alternative derivation: Mode's theory

We consider here the model where both populations are represented by branching processes and show an alternative way to derive equation (4.17). The standard theory for these multi-type branching processes can be found in the book by Mode [76] as well as partially in [3, 56].

Let $p^{(1)}(j,k)$ the probability for one wild-type cancer cell to give birth to j wild-type cancer cells and k drug-resistant cancer cells. Let $p^{(2)}(j,k)$ be the same probability for a drug-resistant mother cell.

Then the only values for which $p^{(i)}(j,k)$ is different from zero are the following: $p^{(1)}(2,0) = (1-u)(1-a-b)$, $p^{(1)}(1,1) = u(1-a-b)$, $p^{(1)}(1,0) = (1-u/2)a$, $p^{(1)}(0,1) = (ua)/2$, $p^{(1)}(0,0) = b$; $p^{(2)}(0,2)(1-a-b)$, $p^{(2)}(0,1) = a$, $p^{(2)}(0,0) = b$. Let $D = 0$.

Let $f^{(1)}(s_1, s_2)$ and $f^{(2)}(s_1, s_2)$ be the probability generating functions of these

distributions. Thus

$$\begin{cases} f^{(1)}(s_1, s_2) = (1-u)(1-a-b)s_1^2 + u(1-a-b)s_1 s_2 + \\ \qquad +(1-u/2)as_1 + \dfrac{ua}{2}s_2 + b, \\ f^{(2)}(s_1, s_2) = (1-a-b)s_2^2 + as_2 + b. \end{cases} \tag{4.20}$$

Let $Z_1(t)$ and $Z_2(t)$ be the number of cancer cells at time t, which are wild-type and drug-resistant respectively. Note that these are both random variables now. Let $F^{(1)}(s_1, s_2; t)$ be the probability generating function of the total number of cancer cells (both types) present at time t, for the process that was started at time 0 by one wild-type cancer cell, that is

$$\sum_{j,k} P(Z_1(t) = j, Z_2(t) = k | Z_1(0) = 1, Z_2(0) = 0) s_1^j s_2^k. \tag{4.21}$$

Similarly, let $F^{(2)}(s_1, s_2; t)$ be the probability generating function of the total number of cancer cells (both types) present at time t, for the process that was started at time 0 by one drug-resistant cancer cell. Note that since we do not consider backward mutations, $F^{(2)}(s_1, s_2; t)$ is given by

$$\sum_{0,k} P(Z_1(t) = 0, Z_2(t) = k | Z_1(0) = 0, Z_2(0) = 1) s_2^k, \tag{4.22}$$

that is, it is not a function of s_1.

By formula (2) in [3, page 225] we have that the two probability generating functions satisfy the following system:

$$\begin{cases} F^{(1)}(s_1, s_2; t) = e^{-Lt}s_1 + \\ \qquad + \int_0^t f^{(1)}[F^{(1)}(s_1, s_2; t-y), F^{(2)}(s_2; t-y)]Le^{-Ly}dy, \\ F^{(2)}(s_2; t) = e^{-Lt}s_2 + \int_0^t f^{(2)}[0, F^{(2)}(s_2; t-y)]Le^{-Ly}dy. \end{cases} \tag{4.23}$$

These equations can be differentiated side-by-side to yield the following system (see [56, pages 70 and 88]):

$$\begin{cases} \dfrac{dF^{(1)}}{dt} = -LF^{(1)} + Lf^{(1)}(F^{(1)}, F^{(2)}), \\ \dfrac{dF^{(2)}}{dt} = -LF^{(2)} + Lf^{(2)}(F^{(1)}, F^{(2)}). \end{cases} \tag{4.24}$$

Thus, substituting (4.20) into (4.24) we obtain the following system:

$$\begin{cases} \dfrac{dF^{(1)}}{dt} = L(1-u)(1-a-b)(F^{(1)})^2 - L(1-a+\dfrac{au}{2})F^{(1)} + \\ \qquad +Lu(1-a-b)F^{(1)}F^{(2)} + L\dfrac{ua}{2}F^{(2)} + Lb, \\ \dfrac{dF^{(2)}}{dt} = L(1-a-b)(F^{(2)})^2 - L(1-a)F^{(2)} + Lb. \end{cases} \tag{4.25}$$

Note that we have already solved the second equation in (4.25), it is equation (4.12),

with $\tilde{D} = Lb$,

$$F(s_2, t) = \frac{(s_2 - 1)(\tilde{D}/\tilde{L})e^{(\tilde{L}-\tilde{D})t} - (s_2 - \tilde{D}/\tilde{L})}{(s_2 - 1)e^{(\tilde{L}-\tilde{D})t} - (s_2 - \tilde{D}/\tilde{L})}. \tag{4.26}$$

Now we can substitute (4.26) in the first equation of system (4.25). However, note that the resulting equation for $F^{(1)}$ does not have a known closed-form solution. Thus, perturbation methods are needed to obtain an approximate solution, as done in [39], and even in that case the approximation does not appear to be satisfactory, as acknowledged by the same authors in [39], since it does not provide a good fit for the values obtained by simulating the process numerically. Finally note that, once we obtain $F^{(1)}$, the probability to have resistance developed by the time the tumor is detected is given by $P_R = 1 - F^{(1)}(1, 0; T)$, where T is the time of detection of the tumor.

4.3 Results

We have considered the different ways in which a stem cell may divide (shown in Figure 4.1): (1) asymmetric division in which a stem cell divides into one progenitor cell and one stem cell (with probability a); (2) symmetric differentiation in which a stem cell divides into two progenitor cells (with probability b); and (3) symmetric renewal in which a stem cell divides into two stem cells (with probability $c = 1 - a - b$).

All three possibilities have been experimentally observed (see [78, 118, 69, 119]). Generally, these three modes of division coexist. Growth curves for different

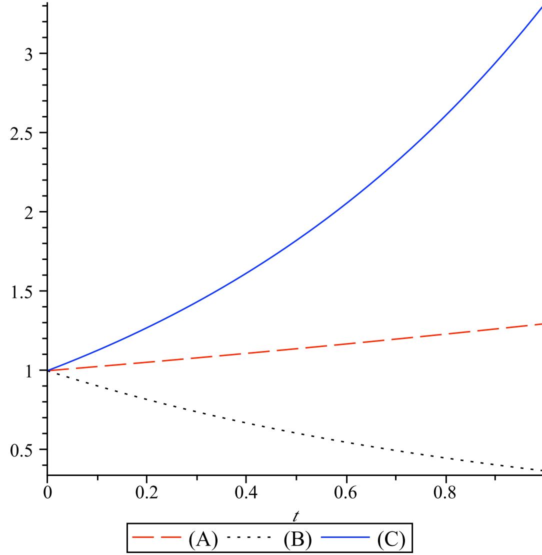

Figure 4.2: Growth curves for the stem cell population. (A) Predominantly asymmetric division: $a = 0.75$ and $b = 0.01$. (B) Predominantly symmetric differentiation: $a = 0.2$ and $b = 0.6$. (C) Predominantly symmetric renewal: $a = 0.2$ and $b = 0.05$. In all plots $D = 0.2$ and $L = 2$.

choices of parameters are illustrated in Figure 4.2. We recall that the division rate is denoted by L, the death rate by D, and the mutation probability per cell division by u. When a tumor is detected, the number of cancer stem cells is assumed to be M. Note that this M is different than the M that was used in (4.1) where it denoted the total number of cancer cells. An example of the time course of the growth of the drug resistant population versus the sensitive one is shown in Figure 4.3.

First, we have computed the expected number of mutations occurring when there are exactly x wild-type (drug-sensitive) cancer stem cells in the population. This expected number of mutations, which we denote by m_x is given by

$$m_x = \frac{u\left(1 - \frac{a}{2} - b\right)}{(1 - a - 2b) - D/L}.$$

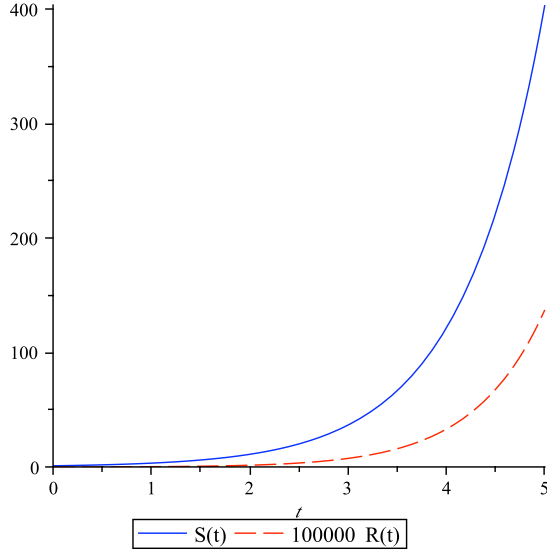

Figure 4.3: An example of the growth of drug-resistant cancer stem cells $R(t)$ versus the sensitive ones $S(t)$. The parameters are $a = 0.2$, $b = 0.05$, $D = 0.2$, $L = 2$, and $u = 4 \cdot 10^{-7}$.

Note that m_x is independent of x, a result which is not surprising since when the population is large there are many more cells which may divide but also substantially less time for them to do so before the population increases to $x+1$. A similar result was obtained using Markov chains in [50].

Our next result has been the calculation of the probability, P_R, that at the time of detection, there are cancer stem cells that developed resistance to the drug. If the death rate D is assumed to be zero, then this probability is given by (4.17)

$$P_R = 1 - \exp\left(-uM\left(\frac{1-\frac{a}{2}-b}{1-a-b}\right)\right).$$

Otherwise, when $D \neq 0$, we obtain (4.18)

$$P_R = 1 - \exp\left(-uM\left(\frac{1-\frac{a}{2}-b}{1-a-b}\right)\frac{1}{C}\ln\left(\frac{1}{1-C}\right)\right),$$

where $C = \frac{D+Lb}{L(1-a-b)}$. We note that equation (4.18) is an extension of equation (4.1) (which can be recovered by setting $a = b = 0$, and setting M as the total population size).

Given (4.18), we have also calculated the expected value of the number of resistant cells that are found at detection, assuming that resistance has indeed developed by the time of detection. This conditional expectation of resistant cells is given by (4.19)

$$E(T \mid resistance) \approx \frac{M}{P_R} \left(\frac{u\left(1 - \frac{a}{2} - b\right)}{(1 - a - 2b) - D/L} \right) ln(M). \tag{4.27}$$

Having obtained these results, we would like to demonstrate their significance by considering a concrete problem. Our goal is to study the division pattern of hematopoietic leukemic stem cells in chronic myeloid leukemia (CML). This can be accomplished by estimating the probabilities a, b, and c in CML.

It is estimated that in CML, the leukemic stem cell population at early detection is of the order of $M \approx 2.5{\cdot}10^5$ cells [47, 73]. It is also estimated that the probability of a random point-mutation conferring resistance to imatinib is $u \approx 4 \cdot 10^{-7}$ [73]. The turnover rate D/L is estimated to be in the range $0.1 - 0.5$ [110]. The model parameters are summarized in Table 4.1.

A recent clinical study [45] follows CML patients that have been treated with imatinib over a period of 6 years. It is shown that no more than 15% of the patients stop responding to the drug at some point during this time period. Interestingly, after 5 years, patients seem to stop relapsing, at least during the time-frame of the study. Since it has been recognized that imatinib has a positive effect on eliminating

Table 4.1: Model parameters

Parameter	Description	Estimate
M	Cancer stem cell population at detection	$2.5 \cdot 10^5$
u	Mutation rate	$4 \cdot 10^{-7}$
D/L	Turnover rate	$0.1 - 0.5$

differentiated leukemic cells, the cases of observed relapses must be traced back to the subpopulation of leukemic stem cells that developed resistance to imatinib. This implies that the probability that cancer stem cells mutated and developed resistance to imatinib by the time of detection cannot exceed 15%, a figure that can be used as an upper bound on the probability of developing resistance by the time the disease is detected, P_R.

Using these estimated parameters, we use equation (4.18) to fit the parameters a and b; c is then given by $1 - a - b$. In Figure 4.4 we plot the range of a and b for which $P_R < 0.15$. In this case we set the turnover rate $D/L = 0.1$. Clearly, the figure indicates that in order for $P_R < 0.15$, a and b must be relatively small. For example, if $a = 0.87$ and $b = 0.01$ then $P_R = 0.71$. However, if $a = 0.2$ and $b = 0.05$ then $P_R = 0.12$, which is in the desirable range. Similar results are obtained for other values of the turnover rate. The larger D/L is, the smaller the admissible region is.

This result is not surprising for the probability of a symmetric differentiation, b, which is typically estimated to be very small. However, the estimated value of

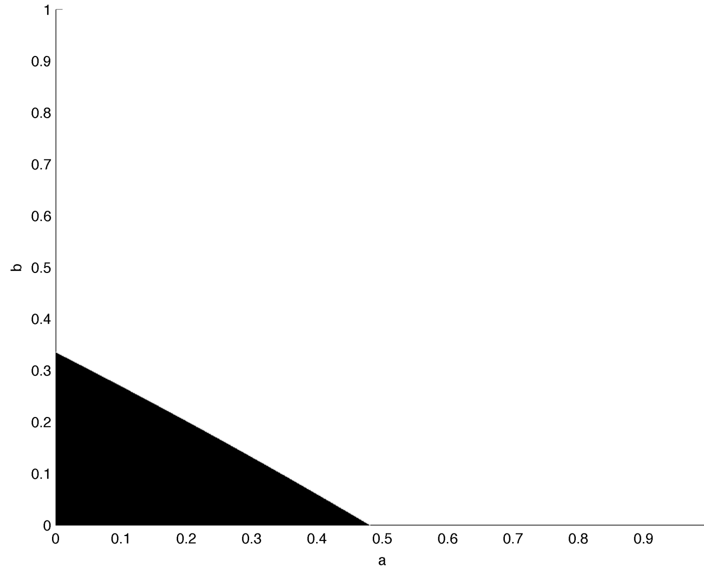

Figure 4.4: The range of a and b for which $P_R < 0.15$. The turnover rate is $D/L = 0.1$.

the asymmetric division probability a is interesting, since it has been observed that for healthy hematopoietic stem cells, a should be close to 1, and generally above 0.9 [5, 28].

These estimates suggest that leukemic hematopoietic stem cells should have a much lower than normal tendency to divide asymmetrically (i.e., a low a), hence making a substantial shift toward an increased symmetric renewal.

4.4 Discussion

The main result of Iwasa *et al.* [50] is given by equation (4.1). How should this equation be understood given actual estimates of the parameters? We recall that in (4.1), the parameter M is the total number of cells in the tumor. At present, an

approximation of the lower limit of clinical detection of solid tumors is $M \approx 10^9$, see, e.g., [92, page 31]. For the mutation rate, a common estimate is on the order of $u \geqslant 10^{-8}$ (see [63, 111]). Hence, according to the results of Iwasa *et al.*, since $(L/D)\ln(L/(L-D)) > 1$ if $D < L$, the probability that resistance develops by the time a tumor is detected must be always greater than .9999.

Another quantity of interest is the expected number of resistant cancer cells found at detection $\bar{Y}$, assuming that resistance developed. An estimation of $\bar{Y}$ is given in [50] by equation (4.2). Unfortunately equation (4.2) is valid only when $Mu \ll 1$, which is not the case when $M = 10^9$ and $u = 10^{-8}$. Hence, we estimate $\bar{Y}$ for $M = 10^6$ and $u = 10^{-8}$, which according to (4.2) is $\bar{Y} > 13$. Consequently, for $M = 10^9$ we could expect thousands of mutated, drug-resistant cancer cells. Such a result implies that resistance should always be present in large numbers at the detection time. Therefore, clones that are generated by such resistant cells are invulnerable to the treatment. They will expand, and no patient will be able to survive the disease in the long run. This result is in contradiction with clinical data. It turns out that, e.g., among CML patients that are treated with imatinib in the chronic phase, only in less than 15% of the patients the drug is unable to keep the disease under control which results in a relapse [45, 73]. The apparent contradiction between the mathematical analysis and the experimental data suggests that something may be missing from the modeling assumptions of Iwasa *et al.*. Alternatively, the experimental estimates of the mutation rates might have to be adjusted.

This dichotomy has been addressed in recent works, e.g. [73, 98], in which the

modeling assumptions include a heterogeneous tumor population, comprising from stem cells and other cells that are at various stages of maturation. For example, Michor *et al.* focus only on the stem cell compartment and accordingly use a much smaller value as an estimate for M [73]. Hence, they effectively think of M as the number of cancer stem cells found at cancer detection, and not as the total number of cells in the tumor. As already mentioned in the Results Section, it is estimated that in the case of CML in the early chronic phase, the stem cell population at detection is approximately $2.5 \cdot 10^5$ cells and the mutation probability u is approximately $4 \cdot 10^{-7}$. With such values in mind, Michor *et al.* obtain an estimate (using formula (4.1) with $D/L = 0.5$) for the probability of resistance mutations present at the time of diagnosis. This probability is calculated in [73] to be approximately 13%, an estimate that is in agreement with the data [73, Table 1]. Unfortunately, the methodology of [73] is based on applying the results of [50] to small cell populations in order to consider only stem cells. The problem with this approach is that equation (4.1) was derived assuming a homogeneous tumor population. It is not valid for describing the dynamics of stem cells, and should not be applied directly to heterogeneous populations as done in [73].

In contrast, the model that we study in this work is based on the "stem cell hypothesis". It incorporates the different ways a stem cell may divide and studies the dynamics that emerges due to these division paths. We would like to emphasize once again that from the point of view of drug resistance, the heterogeneity in the tumor cell population implies that it is only the stem-like long-lived cells, those cells that have the ability of self-renewal, that propagate the drug resistance. Any

model of drug resistance in cancer must therefore take into account the cancer stem cells. Cancer cells that do not have self-renewal capabilities, cannot propagate the resistance in the long run, and should be disregarded.

In order to address the heterogeneity of the tumor cell population we chose to simplify the mathematical tools. In modeling the wild-type cancer stem cell population we replace the Markov chains that were used in [50] by ordinary differential equations. Our approach amounts to using a deterministic model which considers the averaged behavior of such a population. Intriguingly, notwithstanding such simplification, our approach provides identical results to those found in Iwasa *et al.* if we also assume a homogeneous cell population. This can be immediately obtained by setting $a = 0$, and $b = 0$ in equations (4.17)–(4.18). Thus, in the context of the specific questions we are studying, nothing is lost by using a partially deterministic approach. On the contrary, our simplified approach enables us to extend the results of [50].

The prediction of a large shift from asymmetric division to symmetric renewal of hematopoietic stem cells in CML is an important examples of what the mathematical modeling of drug resistance can tell us about cancer. Our estimates were based on a resistance probability of approximately 15%. If instead, we use a less conservative estimate that the probability of resistance is less than 10% (as could be interpreted from the data in [45]) then the conclusion of our study is that cancer stem cells must generally renew symmetrically.

In order to discuss the confidence level at which the conclusions were obtain, we make the following observations on the sensitivity to the parameter estimates

and on certain extensions of the model.

It is important to comment on the sensitivity of the result on the shift from asymmetric division to symmetric renewal to the parameter estimates. It is easy to check that reasonable variations in the turnover rate do not affect our conclusion. With respect to the dependence on the estimates of M and u, it seems unlikely that the estimate for M, the cancer stem cell population size present at the time of detection, would be much smaller than what is estimated in the literature [47]. Rather, it appears reasonable to assume that for many patients, M will actually be larger (depending on the detection time). In this case, there must be an even more substantial shift toward symmetric renewal. The same is true for the mutation probability u. If u is larger than the estimated value then there will have to be an even stronger shift to symmetric renewal in order to explain the clinical data.

Our calculations did not include the possibility of recruitment of progenitor cells (and their offsprings) into the cancer stem cell compartment. It is possible that progenitor cells whose normal function has been perturbed will acquire stem-like self-renewal properties [62]. However, we note that if such a recruitment takes place, this would only strengthen our result. The total amount of drug resistance that is found at detection and is due to the original compartment of cancer stem cells would have to be smaller than our original estimates due to the recruitment of progenitors. In this case, the probability of asymmetric division would be even smaller than our current estimates. Our model also did not take into account gene amplification, a cellular process which amplifies the phenotype that the gene confers on the cell, preventing the absorption of the drug by the cell. However, a similar

reasoning to the case of recruitment of progenitor cells, shows that the inclusion of this kind of resistance would again only strengthen our conclusions.

Our results do not contradict the somewhat sparse experimental data. In the contrary, there is some experimental evidence to support the hypothesis that cancer stem cells change their mode of division. For example, it has been observed that when the mechanism regulating asymmetric divisions is disrupted, *Drosophila* neuroblasts begin dividing symmetrically and form tumors [64]. It is also known that some gene products can both induce symmetric cell divisions and function as oncogenes in mammalian cells [64, 95]. Thus there seems to be a link between symmetric divisions and cancer progression.

It could be of great interest in our model could be tested experimentally, at least in vitro, on cancer cell lines. Ideally, a direct experimental method would require to isolate cancer stem cells and healthy stem cells and compare the growth of both stem cell populations.

From a methodological perspective, the mathematical analysis allows us to use indirect information about the mechanisms that control the dynamics of the disease evolution to reach specific conclusions that go beyond the present reach of experiments. While we have applied our mathematical results to the available clinical data on CML, the approach is not limited to this specific disease, and we expect similar conclusions about the division of cancer stem cells in other types of cancer as well. Specifically, our model should apply to any cancer for which stem cells our known to be the driving force of the progression of the disease and for which point mutations are a source of drug resistance.

Chapter 5

An Extension of the Stem Cell Model

5.1 Introduction

There have been many attempts in the literature to model and simulate tumor growth. Clearly a specific growth curve will depend on a very large number of factors, for example, the type of tumor, its location, and patient-specific characteristics. Due to its simplicity, an exponential growth has been the most commonly used curve in cancer modeling, and we also have made use of it in the previous chapters. For the initial phase of a tumor growth, the exponential curve appears to be a reasonable approximation.

However, because of limits in space and nutrients, tumors generally appear to slow down their growth after having attained a certain size. Exponential growth models do not reflect this critical saturation. Thus, exponential cancer growth may be a good assumption only when the cancer population is small. To account for the more advanced phases of the tumor, the logistic and Gompertz growth models have been successfully introduced, as in the celebrated work on the modeling of human breast cancer by Moolgavkar [77], where the logistic curve was used.

For a recent example we refer to Nakasu *et al.* [80], where various growth curves are compared by the authors in the case of meningiomas in order to determine which one provides the best fit to the data. Nonlinear regression analyses were performed

against power, exponential, logistic, and Gompertzian curves. Their conclusion was that Gompertzian and logistic growth curves gave the best fit. Interestingly they found that atypical meningiomas continue to grow quasi-exponentially.

Surprisingly, more realistic models of tumor growth have not been fully integrated into existing mathematical models of drug resistance. In the literature on drug resistance, there are some works in which the possibility of considering non-exponential tumor growth types is mentioned, for example in [58]. However, to the best of our knowledge, for the case of non-exponential tumor growth there are no estimates of the probability, P_R, that, by the time a tumor reaches detection size, drug resistant stem cells are already present. One possible reason is given by the fact that to replace the exponential curve with any other growth model is mathematically a more complex undertaking, especially when dealing with stochastic methods. Moreover the Markovian assumptions, that are usually inherent to the stochastic models, require exponentially distributed times, thus making it very natural to use the corresponding exponential growth.

In this chapter we extend our previous results by replacing the exponential cancer growth model by more realistic models. The simplicity of our methodology allows us to obtain an estimate for P_R for the case of logistic tumor growth. Furthermore we are able to draw some conclusions for more general growth models. In this sense, this chapter represents an extension of the stem cell model found in the previous chapter and a generalization of its results. For example, the exponential growth model can be seen as a logistic growth model where the carrying capacity is infinite.

5.2 The logistic case: model derivation

As in Chapter 4, we adopt a mixed technique and derive the mathematical model using ordinary differential equations (ODEs) for the wild-type cancer population and branching processes for the mutant cells.

5.2.1 Ordinary differential equations

Recall that cell division rate is denoted by L, the death rate by D, and the mutation probability per cell division by u. As in the previous chapter, a denotes the probability of an asymmetric division, b the probability of a symmetric differentiation, and $c = 1 - a - b$ the probability of a faithful symmetric renewal. When a tumor is detected, the number of cancer stem cells is assumed to be M. We denote the total number of wild-type, i.e., drug-sensitive cancer stem cells at time t by $S(t)$. The second group of cells, the mutated cancer stem cells that developed resistance to the drug, are denoted by $R(t)$.

Importantly, these populations are now assumed to grow logistically. The dynamics of the averaged behavior of the two cell populations can then be described using the following system:

$$S'(t) = \left[L(1-u)(1-a-b) - \left(D + bL + \frac{uaL}{2}\right)\right]\left(1 - \frac{S(t)}{K}\right)S(t),$$

$$R'(t) = \left[\left[L(1-a-b) - (D+bL)\right]R(t) + uL\left(1 - \frac{a}{2} - b\right)S(t)\right]\left(1 - \frac{S(t)}{K}\right). \tag{5.1}$$

We note that the only difference with (4.3) is the inclusion of the term $\left(1 - \frac{S(t)}{K}\right)$, which accounts for the carrying capacity K of the logistic growth, where $S(t)$ must be less than K for all t, and also $M < K$. The initial conditions for (5.1) are given by $S(0) = 1$ and $R(0) = 0$.

Given that the mutation rate u is very small, it can be eliminated from the first equation. However, it cannot be removed from the second equation since $S(t)$ is much larger than $R(t)$. The system (5.1) can be thus reduced to the following:

$$\begin{aligned} S'(t) &= [L(1-a-2b) - D]\left(1 - \frac{S(t)}{K}\right) S(t), \\ R'(t) &= \left[[L(1-a-2b) - D]R(t) + uL\left(1 - \frac{a}{2} - b\right) S(t)\right]\left(1 - \frac{S(t)}{K}\right). \end{aligned} \tag{5.2}$$

with $S(0) = 1$ and $R(0) = 0$. Again, the only difference with (4.4) is the inclusion of the term $\left(1 - \frac{S(t)}{K}\right)$.

5.2.2 The expected number of mutations when the size of the wild-type population is x

Using system (5.2) we estimate the expected number of mutations, m_x, that occur once the wild-type population is of size x. The solution of (5.2) for $S(t)$ is given by

$$S(t) = \frac{K}{1 + (K-1)\exp[(L(1-a-2b) - D)t]}. \tag{5.3}$$

This means that the "average" time at which the wild-type cancer stem cell population reaches size x, defined as $t_{(x)}$, where $S(t_{(x)}) = x$, is given by

$$t_{(x)} = \frac{\ln\left(\frac{(K-1)x}{K-x}\right)}{L(1-a-2b)-D}. \tag{5.4}$$

Thus the "average" length of time for which the population will consist of exactly x stem cells is

$$t_{(x+1)} - t_{(x)} = \frac{\ln\left(\frac{(K-1)(x+1)}{K-(x+1)}\right) - \ln\left(\frac{(K-1)x}{K-x}\right)}{L(1-a-2b)-D} = \frac{\ln\left(\frac{(K-x)(x+1)}{[K-(x+1)]x}\right)}{L(1-a-2b)-D}. \tag{5.5}$$

Hence, the "expected number" of mutations occurring when there are exactly x wild-type cancer stem cells, m_x, is given by

$$m_x = xuL\left(1-\frac{a}{2}-b\right)\left(1-\frac{x}{K}\right)\frac{\ln\left(\frac{(K-x)(x+1)}{[K-(x+1)]x}\right)}{L(1-a-2b)-D}. \tag{5.6}$$

This expression is obtained by multiplying the number of wild-type stem cells present at that time, x, by the mutation rate $uL(1-\frac{a}{2}-b)\left(1-\frac{x}{K}\right)$, and by the length of time for which the total stem cell population equals x.

Finally, note that since $x\left(1-\frac{x}{K}\right)\ln\left(\frac{(K-x)(x+1)}{[K-(x+1)]x}\right) \approx 1$, equation (5.6) can be reduced to

$$m_x = \frac{uL\left(1-\frac{a}{2}-b\right)}{L(1-a-2b)-D}. \tag{5.7}$$

Interestingly, this is exactly the same expression found for the exponential growth case, equation (4.9). There is an intuitive way to understand why the expected number of mutations occurring when there are exactly x wild-type cancer stem cells turns out to be the same expression when assuming an exponential model or a logistic model for the cancer growth: the closer the cancer stem cell population is

to the carrying capacity K the smaller the division rate but also the larger the time spent by the tumor in the state $S(t) = x$. The two effects turn out to balance each other.

5.2.3 Branching processes

Assume now that the tumor population grows logistically starting from one wild-type stem cell. Let r_x be the actual number of mutations produced when there are $x = 1, 2, \ldots, M-1$ wild-type cancer stem cells. Assume that all random variables, r_x, follow a Poisson distribution with mean m_x, given by equation (5.7), and that they are independent.

Consider the clone initiated by a mutant cancer stem cell which originated when there were x wild-type cancer stem cells.

Assume that the population size of such clone follows a continuous-time branching process where in each time step of length Δt, a cancer stem cell divides with probability $L\left(1 - \frac{S(t)}{K}\right)\Delta t$ and dies with probability $D\left(1 - \frac{S(t)}{K}\right)\Delta t$. This means that births and deaths occur according to an exponential distribution with parameters $\tilde{L}(t) = L(1-a-b)\left(1 - \frac{S(t)}{K}\right)$ and $\tilde{D}(t) = (Lb + D)\left(1 - \frac{S(t)}{K}\right)$ respectively. Note that the difference with the exponential growth case is again the presence of the extra factor $\left(1 - \frac{S(t)}{K}\right)$. By (5.3),

$$1 - \frac{S(t)}{K} = 1 - \frac{S(0)}{S(0) + (K - S(0))\exp(-rt)}, \tag{5.8}$$

where $r := (L(1-a-2b) - D)$. Thus, for a clone of mutants generated when the wild-type population was equal to $S(t) = x$, we have that births and deaths in such

a clone occur according to an exponential distribution with parameters

$$\tilde{L}(t) = L(1-a-b)\left(1 - \frac{x}{x + (K-x)\exp(-rt)}\right) = L(1-a-b)\frac{K-x}{(K-x) + x\exp(rt)}, \tag{5.9}$$

and

$$\tilde{D}(t) = (Lb + D)\left(1 - \frac{x}{x + (K-x)\exp(-rt)}\right) = (Lb + D)\frac{K-x}{(K-x) + x\exp(rt)} \tag{5.10}$$

respectively. Here time is measured starting from the generation of the clone.

Let $g_x(\xi)$ be the generating function of such a clone for which the original mutation happened when $S(t) = x$. Then, by the Kolmogorov backward equation, we have as in (4.10) for the exponential case,

$$\begin{aligned} E\left[\xi^{R(t+\Delta t)} \mid R(0) = 1\right] &= \tilde{L}(t)\Delta t E\left[\xi^{R(t)} \mid R(0) = 1\right]^2 \\ &+ \tilde{D}(t)\Delta t E\left[\xi^{R(t)} \mid R(0) = 0\right] \\ &+ (1 - (\tilde{L}(t) + \tilde{D}(t))\Delta t) E\left[\xi^{R(t)} \mid R(0) = 1\right], \end{aligned} \tag{5.11}$$

since $E[\xi^{R(t)} \mid R(0) = 2] = E[\xi^{R(t)} \mid R(0) = 1]^2$, by independence. The time $t = 0$ in (5.11) is the time of occurrence of the original mutation that generates the clone, i.e., when the wild-type population is of size x. The time t in (5.11) measures time starting from this $t = 0$.

Let $g(\xi, t) = E[\xi^{R(t)} \mid R(0) = 1]$. As $\Delta t \to 0$ we get

$$\frac{\partial}{\partial t} g = \tilde{L}(t) g^2 + \tilde{D}(t) - [\tilde{L}(t) + \tilde{D}(t)] g, \tag{5.12}$$

again a Riccati equation as for the exponential growth case (see equation 4.11), but

this time with variable coefficients rather than constant,

$$\frac{\partial}{\partial t}g = \frac{K-x}{(K-x)+x\exp(rt)}[L(1-a-b)g^2-(L(1-a-b)+(Lb+D))g+(Lb+D)]. \tag{5.13}$$

Solving (5.13) with the initial condition $g(\xi,0)=\xi$ gives

$$g(\xi,t) = 1+\frac{1}{\left[\frac{1}{(\xi-1)[r(1+\frac{K-x}{x})]}+\frac{L(1-a-b)}{r^2}\left(\frac{1}{1+\frac{x\exp(rt)}{K-x}}-\frac{1}{1+\frac{x}{K-x}}\right)\right]\left[r\left(1+\frac{K-x}{x\exp(rt)}\right)\right]}. \tag{5.14}$$

Since $M \approx \frac{xK}{x+(K-x)\exp(-rt_{M-x})}$, where t_{M-x} is the time it takes for the cancer stem cells to go from x to M, by letting $\exp(rt_{M-x}) = \frac{M(K-x)}{x(K-M)}$ into (5.14) we obtain the generating function

$$g_x(\xi) = g(\xi,x) \approx 1+\left[\frac{x}{(\xi-1)M}+\frac{L(1-a-b)}{r}\left(\frac{x-M}{M}\right)\right]^{-1}. \tag{5.15}$$

We denote by T the total number of drug resistant cancer stem cells that are present when the cancer is detected, i.e., when the total stem cell population is M. Finally we let $G_T(\xi)$ be its generating function and recall that, by (4.14),

$$G_T(\xi) = E[\xi^T] = \exp\left(-\sum_{x=1}^{M-1} m_x(1-g_x(\xi))\right). \tag{5.16}$$

5.2.4 The probability of having resistant cancer stem cells at the time of detection

By (5.7),(5.15),(5.16) we have that the probability of having resistant cancer stem cells at the time of detection is

$$\begin{aligned} P_R = 1 - G_T(0) &= 1 - \exp\left(-\sum_{x=1}^{M-1} m_x(1-g_x(0))\right) \\ &= 1 - \exp\left(-\frac{u(1-\frac{a}{2}-b)}{(1-a-2b)-D/L}\sum_{x=1}^{M-1}\frac{M}{x+\frac{L(1-a-b)}{L(1-a-b)-(Lb+D)}(M-x)}\right). \end{aligned} \tag{5.17}$$

By replacing the summation with an integral, and with a change of variable, we get

$$P_R \approx 1 - \exp\left(-\frac{uM(1-\frac{a}{2}-b)}{(1-a-2b)-D/L}\int_0^1 \frac{1}{y+\frac{L(1-a-b)}{L(1-a-b)-(Lb+D)}(1-y)}dy\right), \tag{5.18}$$

from which we obtain

$$P_R = 1 - \exp\left(-uM\frac{(1-\frac{a}{2}-b)}{b+D/L}\ln\left(\frac{1-a-b}{(1-a-2b)-D/L}\right)\right). \tag{5.19}$$

It is immediate to see that such expression is equivalent to

$$P_R = 1 - \exp\left(-uM\left(\frac{1-\frac{a}{2}-b}{1-a-b}\right)\frac{1}{C}\ln\left(\frac{1}{1-C}\right)\right). \tag{5.20}$$

where $C = \frac{D+Lb}{L(1-a-b)}$. It is important to note that equation (5.20) is precisely equation (4.18), obtained for the exponential growth case. Finally, given (5.19), it follows that the expected value of the number of resistant cells that are found at detection, assuming that resistance has indeed developed by the time of detection is given by

$$E(T \mid resistance) \approx \frac{M}{P_R}\left(\frac{u\left(1-\frac{a}{2}-b\right)}{(1-a-2b)-D/L}\right) ln(M), \tag{5.21}$$

as in the exponential growth case.

In summary, we have calculated the probability, P_R, that by the time a tumor reaches detection size, there are drug resistant stem cells in a logistically growing cancer population. We have found that this probability, given by equation (5.19), is the same as for the exponential growth case. This result may appear somewhat surprising. However, it can be explained by making the following observation. We note that the closer the cancer stem cell population is to the carrying capacity, K, the smaller the time-dependent division probability $L\left(1-\frac{S(t)}{K}\right)\Delta t$ (which in the exponential growth case is constant) but also more time is spent by the cancer stem cells in the state $S(t)=x$, with the two effects balancing each other.

The simplicity of our methodology allowed us to obtain an estimate for P_R and $E(T \mid \mathit{resistance})$ for the case of a logistic tumor growth. We would like to note that arguably our modeling methodology may be seen as more general, in the sense that at least for the wild-type population we only made assumptions on their averaged behavior. We only assumed that, on average, the cancer stem cell population grows logistically. We did not assume, for example, that the time to division is exponentially distributed or that the Markov property holds.

5.3 On other types of tumor growth

While a logistic term has been used successfully to model tumor growth, there are many other possible types of growth that can be considered. Thus, it is natural to ask whether our result, on the probability to find resistance by the time of tumor detection, is independent of the averaged way in which the cancer stem cells grow.

Would we get the same estimate for the probability P_R if the average growth of the tumor population were to be for example a Gompertz law or a power law rather than exponential or logistic?

While we do not have a complete answer, we would like to make the following observation about a possible generalization of our results. Consider the following system:

$$\begin{aligned} S'(t) &= [L(1-a-2b)-D]f(t)S(t), \\ R'(t) &= \left[[L(1-a-2b)-D]R(t) + uL\left(1-\frac{a}{2}-b\right)S(t)\right]f(t). \end{aligned} \tag{5.22}$$

with $S(0) = 1$ and $R(0) = 0$. Here $f(t)$ is any strictly positive real function of t. Naturally we may have $f(t) = g(S(t))$ for some strictly positive real function g.

The only difference with (5.2) is that now we allow for a much more general form of tumor growth. The system (5.22) includes both the exponential and the logistic growth cases considered previously, as well as many other types of growth like, for example, the Gompertz law.

From the first equation of system (5.22) we have that

$$\Delta S(t) = S(t+\Delta t) - S(t) \approx [L(1-a-2b)-D]f(t)S(t)\Delta t. \tag{5.23}$$

Thus if we let $\Delta S(t) = 1$, that is, starting at time t, S goes from x to $x+1$ in time Δt, then

$$\Delta t = \frac{\Delta S}{[L(1-a-2b)-D]f(t)S(t)} = \frac{1}{[L(1-a-2b)-D]f(t)S(t)}. \tag{5.24}$$

Hence, the "expected number" of mutations occurring when there are exactly x wild-type cancer stem cells, m_x, is given by

$$m_x = uL\left(1 - \frac{a}{2} - b\right) f(t)\, x\, \frac{1}{[L(1-a-2b)-D]f(t)x} = \frac{uL\left(1-\frac{a}{2}-b\right)}{L(1-a-2b)-D}. \tag{5.25}$$

Note that equation (5.25) is identical to the corresponding results that were derived in the exponential growth model (see equation (4.9)) and in the case of a logistic growth model (see equation (5.7)). Note that we could obtain the same result in the even more general case where $S'(t) = [L(1-a-2b)-D]f(t)$.

For simplicity, let $D = 0$ and $b = 0$. In this case, every time a mutation occurs the resulting clone will never become extinct. This means that the probability, P_R, that by the time a tumor reaches detection size there are drug resistant stem cells in the cancer population will be given by

$$P_R = 1 - \exp\left(-uM\left(\frac{1-\frac{a}{2}-b}{1-a-b}\right)\right), \tag{5.26}$$

which is the same expression found for the exponential and logistic cases, given by equation (4.17). Thus, at least for the case where $D = 0$ and $b = 0$, formula (5.26) applies to very general tumor growth models and the probability to find resistance by the time of tumor detection is independent of the way the cancer stem cell population grows on average. While we are not able to give a complete answer for the case where $D \neq 0$ and $b \neq 0$, the experimental evidence indicates that $b \approx 0$ (see [5, 28]) and D is very small compared to L. In fact, some researchers consider stem cells as eternal [1, 41], which corresponds to $D = 0$. In conclusion, our results

seem to be a good approximation for a rather general model of cancer growth.

Chapter 6

On Resistance to Imatinib in Chronic Myeloid Leukemia

6.1 Introduction

Chronic myeloid leukemia (CML) is a cancer of the white blood cells. It causes unregulated proliferation of immature blood-forming myeloid cells in the bone marrow, leading to accumulation of mature granulocytes (neutrophils, eosinophils, and basophils) and their precursors in the bone marrow and in the blood. The hematopoietic (blood cells) system is depicted in Figure 1.1.

CML is characterized by a chromosomal translocation between chromosome 9 and 22 known as the "Philadelphia chromosome". As a result, the fusion gene BCR-ABL is created, which encodes the bcr-abl fusion protein, a tyrosine kinase. This enzyme is able to transfer a phosphate group from ATP (Adenosine-5'-triphosphate) to a protein, by attaching it to the amino acid tyrosine. This phosphorylation controls the properties and function of the protein receiving the phosphate group. Specifically, it has been shown that the bcr-abl tyrosine kinase contributes via tyrosine phosphorylation to growth factor independence, increased proliferation, genetic instability and suppression of apoptosis in leukemic cells (see [70, 96, 45] and the references therein). The methods used to detect the "Philadelphia chromosome" are bone marrow cytogenetics analysis, which studies directly the chromosome, and reverse transcriptase polymerase chain reaction (RT-PCR), which enables detection

of residual leukemic cells at the level of one cell in $10^5 - 10^6$ normal cells by measuring the levels of bcr-abl transcripts in the blood of the patient and obtaining an estimate of the fraction of terminally differentiated leukemic cells (see Branford *et al.* [6]).

There are three distinct clinical phases in CML. Most people are diagnosed with CML during the so called "chronic phase" (CP), as a result of a routine blood test showing an elevated white blood cell count, which leads to further investigations. Patients in this phase are either asymptomatic or with mild symptoms of fatigue, fever, easy bleeding or abdominal pain. In this phase the concentration of myeloblasts in the blood is above normal limits. The myeloblast is a unipotent stem cell, which differentiates into one of the mature granulocytes. As the disease progresses, patients enter an "accelerated phase" (AP), when the percentage of myeloblasts in the blood or bone marrow reaches 10 - 19%, followed by a almost invariably fatal "blast crisis" (BC), in which the hematopoietic differentiation has become arrested and many immature myeloblasts fill much of the bone marrow (at least 20%) and are found in high concentrations in the blood [70, 115].

As of today, allogeneic stem-cell transplantation is the only known cure able to eradicate the disease. However, the success of transplantion is limited, because the procedure is viable in approximately 25% of the patients and relapses occur in 20 - 30% of cases [48, 86].

The recently introduced drug Imatinib (also known as STI571, Gleevec, and Glivec) is a potent inhibitor of the bcr-abl tyrosine kinase. After 18 months of treatment, a complete cytogenetic response (CCyR) is achieved in 87% of previ-

ously untreated patients, thus dramatically improving patients' survival [86]. CCyR is defined as the complete absence of detectable bcr-abl transcripts in the blood, that is, when 0% of detectable blood cells are Ph+ (Philadelphia chromosome positive). Because of its ability to directly target the bcr-abl protein, Imatinib mesylate belongs to the group of the so called "targeted therapies". These drugs differentiate themselves from the more traditional chemotherapeutic treatments by targeting molecules specifically involved in tumor growth rather than attacking all (healthy or not) rapidly dividing cells. Specifically, Imatinib works by blocking the ATP binding site of bcr-abl, thus inhibiting the enzyme activity, e.g. [102, 19]. This mechanism is depicted in Figure 6.1. This fact explains why point mutations in the bcr-abl domain can cause resistance to Imatinib [68].

The structure of the chapter is as follows. In Section 6.2, we review the most recent data available on CML patients treated with Imatinib and make a series of key observations. In Section 6.3, we briefly review the basic modeling assumptions and main results found in the mathematical modeling literature on CML and the related development of drug-resistance. In Section 6.4, we use our observations to formulate a new hypothesis, which is able to explain, for the first time, the most recent data on CML. In Section 6.5, we provide new insights on the diagnostic and therapeutic strategies against CML.

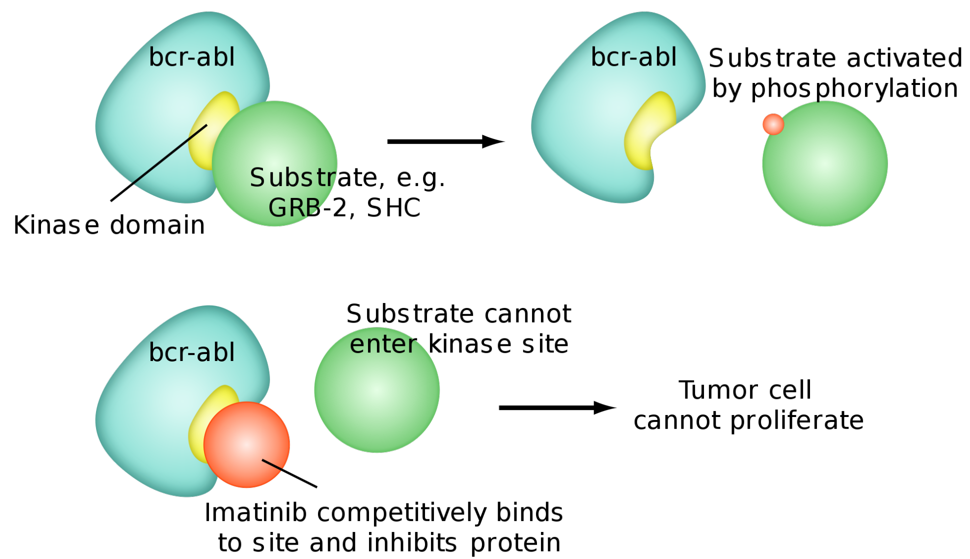

Figure 6.1: The effect of Imatinib on the bcr-abl tyrosine kinase. (Sodium; commons.wikimedia.org under GNU Free Documentation License).

6.2 The data and some key observations

In this section we consider the most recent data found in the literature on CML patients treated with Imatinib. In particular we refer to the data coming from the largest international clinical trial, known as the IRIS (International Randomized Study of Interferon vs STI571) trial (see for example Hochhaus *et al.* [45]), where 1106 previously untreated CML patients in chronic phase were randomized, between June 2000 and January 2001, to receive treatment with Imatinib (553 patients) or interferon-α (IFN) plus cytarabine (553 patients). IFN plus cytarabine represented the standard treatment for CML patients in chronic phase at the time of the beginning of this trial (see [86]). Furthermore we also consider some patient-specific data published in Branford *et al.* [7] and Michor *et al.* [73].

We make the following observations derived from the data on Imatinib.

1. At 18 months into the trial, Imatinib provided complete cytogenetic responses for 76.2% of the patients versus 14.5% obtained with interferon-α (IFN) plus cytarabine (see [38, 86]). Thus, as a result of the data from the first years of this IRIS trial, Imatinib has become the standard first-line therapy for patients with newly diagnosed CML in chronic phase [86]. Of 553 patients originally on the IFN-α plus cytarabine treatment, 359 crossed over to Imatinib, 181 patients discontinued treatment, and only 13 patients remained on IFN-α plus cytarabine treatment (see [38]).

2. At 48 months after starting Imatinib, the estimated percentages of freedom from progression to accelerated phase or blast crisis was 91%. After 54 months

under treatment, 81% of patients had a complete cytogenetic remission [38]. At 72 months, both of these percentages were basically unchanged [45].

3. For CML patients in CP, within few months after the start of treatment, the vast majority of leukemic cells had generally died out (see [73]).

4. In the presence of point mutations or gene amplification, there are drug-resistant cells that survive and generally will cause a relapse and progression of the disease to AP and BC (see [7, 73]).

5. It appears that drug-resistant cells are not the only problem. Indeed if Imatinib is removed, even after up to three years into the treatment, often the disease relapses almost immediately and quickly reach levels at or beyond the pre-treatment baseline. Thus Imatinib does not seem to be able to completely eradicate the disease [73].

6. As long as a CML patient in CP does not discontinue the treatment and as long as there is not the development of resistance, the disease is generally kept under control in the long run [45] .

7. Importantly, relapses due to drug resistance seem to stop occurring at around 5 years into the treatment [45]. Note how in Figure 6.2, the "curve" for relapses has a peak around two years and then approaches zero around 5 years. This is somewhat similar to the case of relapses in bone marrow transplants.

8. Finally, we would like to note that a consequence of the stem cell hypothesis is that long-lived resistance will be only caused by those cells that have the

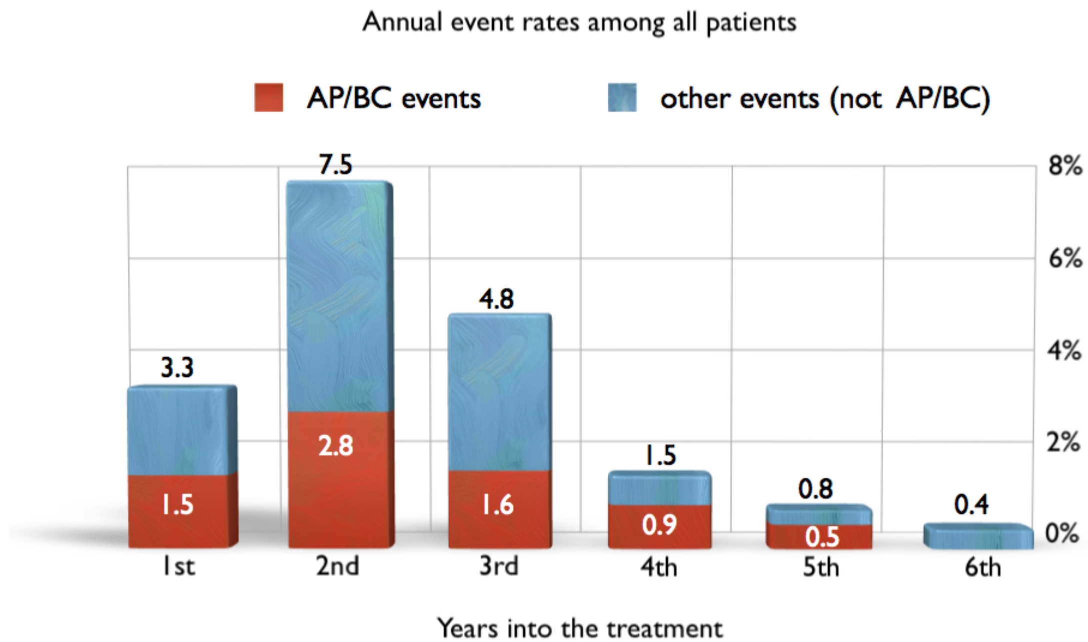

Figure 6.2: The annual event rates for patients under treatment with Imatinib in the IRIS study. The red bars represent the progression of the disease to an Acute Phase or Blast Crisis (AP/BC). The blue bars include all other events (Based on Figure 2 in Hochhaus *et al.* [45]).

ability of self-renewal, that is the stem-like cells. Thus, for the problem of drug resistance, we may focus only on them [114].

6.3 Current mathematical models of CML which include the stem cell hypothesis

There is a growing literature on mathematical models of CML which include the stem cell hypothesis among their assumptions. Of particular relevance are the two *Nature* papers by Michor *et al.* [73] and Roeder *et al.* [98], as well as the work by Kim *et al.* [55], Komarova *et al.* [61], Dingli *et al.* [16], Michor *et al.* [74, 72] and Foo *et al.* [23]. In this section we briefly review some of the main assumptions and conclusions of [73, 98, 23].

6.3.1 Michor *et al.*

Michor *et al.* [73] uses the stem cell hypothesis in analyzing the dynamics of chronic myeloid leukemia (CML). In their hierarchical model the leukemic population is divided into four sub-populations: stem cells (the only cells with an indefinite potential for self-renewal), which give rise to progenitors, which produce differentiated cells, which in turn produce terminally differentiated cells. Thus a four-compartmental ODE system is used, where the coefficients are estimated using a 169-patient data set. Clearly the dynamics of this system are somewhat different from those obtained by modeling the tumor as homogeneous. More precisely, there are three sets of equations in this model: a set of equations for healthy cells,

a set of equations for leukemic cells, and a set of equations for leukemic mutated drug-resistant cells. Each set of equations has four equations in order to account for the dynamics of stem cells, progenitor cells, differentiated cells, and terminally differentiated cells. Also, it is not assumed in the model that the drug kills any cells. The action of the drug in this model is accounted for with a lower (than normal) proliferation rate for the leukemic cells and is done through the terms for the proliferation rate constants of the leukemic progenitor, differentiated and fully differentiated cells respectively.

A fundamental assumption of the model is that Imatinib is not able to affect the leukemic stem cell compartment. The reasons behind this assumption are the experimental evidence that, even if the drug has been administered for as long as three years, there is a relapse of bcr-abl transcripts within three months after discontinuation (see [73, 12]), and the fact that leukemic stem cells appear to be insensitive to the drug (see [35, 67]). Thus, in this model, the cells that are resistant to the drug are both the leukemic stem cells (LSCs) and the entire hierarchy of mutated drug-resistant cells. Additionally, the model has no upper limits on the proliferation of the LSCs.

Therefore one of the main assumptions of the paper is that Imatinib is not able to deplete the leukemic stem cell compartment. The probability of developing resistance to Imatinib by the time of detection is also calculated using formula (4.1), and estimated at 13%, which fit well with the data (see [73, see table 1]).

Remarks:

1. The model predicts that, over time, leukemia always wins, since the LSC compartment can do nothing else other than to keep growing. This is due to the assumptions of the model that LSCs are not affected by the drug and their growth is not constrained by saturation. Thus, overall, patients should not be able to survive.

2. Importantly, because of what mentioned in Remark 1, drug resistance should propagate throughout the uninterrupted production of LSCs and of mutated cells. Thus, it should be possible to see patients who relapse at any point in time after the start of treatment.

3. The issues raised in Remarks 1 and 2 are clearly in contradiction with the up-to-date data (see [45]), where actually we see the majority of the patients survive and the disease is kept under control with Imatinib. Furthermore, in the data reported in [45], there is no evidence of relapses after the fifth year, that is, after a peak at around two years into the treatment, the rate of relapse appears to go to zero at around 5 years.

4. Finally note that the probability of developing resistance to Imatinib by the time of detection was calculated using formula (4.1), which is derived from a model where the cancer population is assumed to be homogeneous and not a hierarchical one (see [50]). However this is in contradiction with the fact that the model by Michor *et al.* assumes the stem cell hypothesis. It is indeed clear that the dynamics should be different.

In summary: the model by Michor *et al.* [73] would predict that over time,

the disease would progress for all patients, as there is no mechanism to stop the propagation of LSCs and of drug resistance. This is clearly not the case.

6.3.2 Roeder *et al.*

In a recent paper, Roeder *et al.* [98] have proposed a model for the dynamics of the relapse in chronic myeloid leukemia (CML) where the role of quiescent G_0 stem cells and their ability to escape Imatinib-induced apoptosis was included. Roeder *et al.* assume that the stem cells are divided into two compartments: active and quiescent. With certain probabilities, stem cells can pass between the compartments. Only stem cells that are in their active state can proliferate. The transition between the compartments is based on some internal counter - named “affinity” - which increases over time (though upper bounded) and is related to the transition probabilities. There is actually no data to support the existence of an “affinity” parameter, even though the experimental data do support a model in which stem cells can be active or dormant. What causes them to shift between the states is unknown. In this model, the drug does affect LSCs, an important difference between this model and [73]. However, it acts only on the active cells, which is a reasonable assumption. In such a model, over time, drug resistance will develop in the active LSC compartment. Since LSCs shift between compartment, the drug resistance can then propagate from the active cells into the dormant compartment, and stay there indefinitely. In such a way, even if all active LSCs were eliminated, LSCs that are resistant to the drug may still return to the system by shifting out of the dormant

state. However, if wild-type LSCs became active during treatment they would be eliminated, thus there should not be progression of the disease for patients under treatment who did not develop drug resistance before the start of the treatment; rather the disease should be eradicated in these patients. The prediction of Roeder's model is that Imatinib has the potential to induce a complete eradication of the disease in the long run, except for those patients who developed drug resistance. There are several remarks we would like to make about this model.

Remarks:

1. The model predicts that Imatinib has the potential to induce a complete cure from the disease, in the long run, for patients under treatment who did not develop drug resistance before the treatment starts. The logic is as follows. The active LSCs would be eliminated by the drug. The quiescent LSCs instead would need to become active in order to proliferate. Thus, we could expect these reactivated LSCs to be typically eliminated by the drug before the mutation hits, given that mutations are rare events. Usually, then, patients should be able to reach complete remission from the disease. However the data implies that this may not be the case. Indeed it appears that as soon as the therapy is interrupted, often (but not always) there is a quick relapse of the disease (see [73, 12]).

2. Another major concern about this model is that if a temporary remission is attained due to the residue of LSCs being dormant, once the drug is removed there is no clear mechanism by which the disease will relapse immediately, i.e.,

within few weeks, which is instead what has been observed in patients [73, 12]. Furthermore the relapse will often cause an increase in the number of leukemic cells well beyond the pre-treatment baseline, again something difficult to explain with this model, given that cycling LSCs are assumed to be eliminated by Imatinib. Thus, it might be hard to explain with this model and its transition probabilities, a rapid transition of stem cells between states. Usually, stem cells are being thought of as having relatively stable and slow dynamics. It could be that LSCs have somewhat different characteristics, but to the best of our knowledge, this is unknown.

3. The entire concept of "affinity", which is an integral part of the works of Roeder and his co-authors, is not based on any actual biological knowledge. In particular, it is hard to understand why cells that have been dormant longer are more likely to become active. Is there really an internal clock involved, or is the activation of stem cells related to signals that they received, regardless of the time that they became dormant? The latter is probably a more likely biological scenario.

6.3.3 Foo *et al.*

In this work [23], the authors incorporate, as in Roeder *et al.* [98], the idea of splitting the stem cells into two compartments: cycling and quiescent. These states are temporary: quiescent stem cells can start cycling and vice versa. In addition, they consider the possibility of LSCs being susceptible to the drug. Two possible

scenarios are considered: the case where all LSCs are completely insensitive to the drug and the case where only the quiescent LSCs are drug resistant (not because of a mutation but just due to kinetic resistance), while the cycling LSCs are depleted by the drug Imatinib. Finally, they assume that there exists an equilibrium, a steady state for the system, reached by the time of detection, which guarantees that the total amounts of normal and leukemic stem cells remain constant until the treatment initiates.

Remarks:

1. The main concern with this model is that it does not explain the data. Indeed the model makes either one of two opposite predictions: if only quiescent LSCs are resistant to Imatinib then a full cure will ultimately be obtained (given that quiescent states are temporary) or Imatinib will ultimately fail if all LSCs are resistant to it (since the LSC compartment will keep growing and furthermore more drug resistance will be created).

2. Another problem with this model is not the idea of a level of equilibrium, which might exist, but the fact that it is assumed that this steady state is reached before the therapy starts. In reality, an equilibrium, if any, should be reached much later than at detection time. Indeed, at detection the disease is usually in chronic phase and not in acute phase or blast crisis, where the leukemic cell load is much higher. Thus, if we did not take into account the treatment, after the time of CML detection there would still be a lot more time for the leukemic cell population to grow in numbers before reaching a level of

saturation or any possible balance with the healthy hematopoietic population.

6.4 A new hypothesis

In this section, we formulate a new hypothesis driven by the data and the observations made in Section 6.2.

From observations 4 and 5 of Section 6.2 it follows that Imatinib does not seem to eradicate the disease. However, under treatment, LSCs do not appear to be growing in number (see observations 1-3, 6-7). Thus, we hypothesize that the drug shuts down the division process of all leukemic cells, i.e., it inhibits proliferation caused by the BCR-ABL rearrangement. Interestingly, there is a general consensus in the medical literature that this is the effect of Imatinib on leukemic non-stem cells (see [46, 35, 96, 70, 45] and the references therein). Thus an observed decline of all types of leukemic cells, with the exception of the stem cells, is due to Imatinib blocking the cells from dividing. The leukemic cells remain quiescent until they reach the end of their lifespan. Given that, with the exception of the stem cells, all blood cells are relatively short-lived (one day to several months, depending on the type of cell [32]), all such cells will slowly die out leaving only, as a residue, the very long-lived leukemic stem cells. We assume that Imatinib does affect stem cells exactly in the same way as it acts on all other leukemic cells. The different life-spans of different types of cells is what leads to different outcomes. This does not contradict the results of the experiments mentioned earlier [35, 67], where it appears that Imatinib is unable to deplete the LSCs: indeed Imatinib shifts the

LSCs to a quiescent state. As soon as the treatment is discontinued, the LSCs generally resume their cycling activity, which causes the observed relapse.

From observation 3 of Section 6.2 we note that relapses seem to occur over a period of five years; however it has been estimated that it takes approximately 5-7 years for CML to reach detection size [49, 53, 73]. Thus if random point mutations causing drug resistance could happen during treatment (due to the continuing division of LSCs), then we should see various cases of patients having relapses well after five years from the start of the therapy. This does not appear to be the case [45]. This observation should be viewed as another argument that the drug is able to shut down the cycling mechanism among the leukemic stem cells putting them in a quiescent state. If this were not the case, there would still be production of drug resistant stem cells, due to the random mutation mechanism which is always acting on each cell division. Note that this is another example of what drug resistance can tell us about cancer, in addition to our results on the division pattern of the LSCs we obtained in Chapter 4.

Now, if we assume that Imatinib blocks all leukemic cells from dividing, then we come to the conclusion that there are two possible scenarios: either a patient has already drug resistant LSCs present at detection, which should cause a relapse in the future, or he/she does not, in which case a relapse is not expected, at least not due to this type of drug resistance. Indeed once the treatment starts, no more mutations are produced because LSCs do not divide anymore due to the presence of Imatinib. Thus the creation (or lack) of resistant mutants in the leukemic stem cell compartment before the therapy starts will generally determine the long-term outcome for a

patient continuously under treatment with Imatinib. It is natural, however, to expect some patient-level variability in the effectiveness of Imatinib in blocking LSCs from dividing, due to the factors like dosing schedules, drug pharmacokinetics, and patient-specific characteristics.

We would like to mention that such an assumption leaves the door open to the possibility of a complete eradication of the disease. Indeed, unless the LSCs are eternal, sooner or later they should die out. Furthermore the immune system may have an important role during therapy in eliminating this quiescent LSC residue [55].

6.5 Insights for the diagnostic and therapeutic strategies in CML

We have elucidated the reasons why our new hypothesis is capable of explaining the current data on CML better than all other previous assumptions made in the existing literature. Based on this hypothesis, we now use the mathematical results of Chapter 4 to obtain new insights on the dynamics of the development of drug resistance in CML and to provide an important recommendation for the standard medical practice, when dealing with CML.

Recall that the dynamics of the averaged behavior of the wild-type cancer stem cell populations, denoted by $S(t)$, can be described using the following equation (see system 4.4):

$$S'(t) = [L(1 - a - 2b) - D]S(t). \tag{6.1}$$

Note that we are assuming that the stem cell population is growing exponentially. However, this is a realistic assumption for CML that is detected at an early

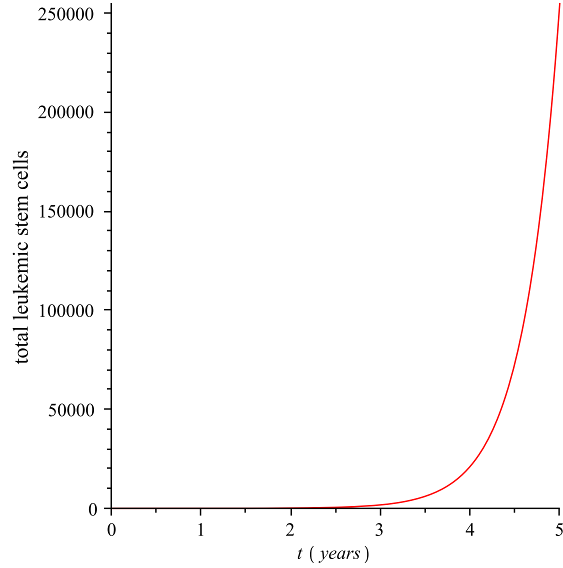

Figure 6.3: Growth curve for the leukemic stem cell population, where the exponential rate constant is $[L(1-a-2b)-D] \approx 2.49$. Time t is measured in years.

stage. Indeed, as previously mentioned, if there were to be a saturation level, it should be reached much after the detection time.

It is estimated that the number of leukemic stem cells found in the case of an early detection of CML is on the order of $M \approx 2.5 \cdot 10^5$ cells [47, 73]. It has also been estimated that it takes approximately 5-7 years for CML to reach detection size [49, 53, 73]. Thus if we set, for example, $S(5) = 2.5 \cdot 10^5$, where time is measured in years, we obtain from equation (6.1) that $[L(1-a-2b)-D] \approx 2.49$. The exponential growth of such leukemic stem cell population up to detection size is depicted in Figure 6.3.

It is interesting to note that according to this curve, if drug resistance occurs in the five years before detection, it will occur in the last year with a probability of

approximately 0.92, in the last six months with a probability of 0.7, and in the last three and a half months with a probability of 0.5. This is due to the fact that, for example, after four years the number of leukemic stem cells is approximately equal to $21,000$, which is only about 8% of the total population found at the end of the fifth year (detection time).

This calculation has fundamental consequences for the standard of practice in the diagnostic and therapeutic strategies of CML. Indeed, with the hypothesis that Imatinib blocks all leukemic cells from dividing, then, as we already mentioned, either a patient has already developed drug resistance in the LSC compartment at detection, which will generally cause a future relapse, or he/she does not, in which case a relapse is not expected, at least not due to this type of drug resistance. Thus having drug-resistant mutants in the leukemic stem cell compartment before the therapy starts will determine the expected long-term outcome. This argument translates into a clear need to start the treatment as soon as possible, in order to stop the occurrence of further LSC divisions, which have the risk of creating drug-resistant LSCs. Moreover, it is critical to detect CML before the occurrence of drug resistance. As demonstrated, if a random point mutation causing the insurgence of resistance hits a leukemic stem cell, it is most likely to occur in the last several months before detection. It is therefore essential to detect CML as early as possible. Detecting CML even only a few weeks earlier could potentially make a substantial difference on the expected final outcome of the disease.

Interestingly, we would like to note that this approach is not, in general, followed by the standard medical practice, when dealing with CML. To the contrary,

it is often assumed that the time of detection of CML has no connection with possible future relapses. The development of resistance is not assumed to be related to a late detection or a delay in the start of the treatment. This is the opposite of what we conclude by studying the dynamics of the development of CML under Imatinib and the related problem of drug resistance. In conclusion, we suggest that our hypothesis provides an important indication for the diagnostic and therapeutic strategies against CML.

Chapter 7

Conclusion

In this dissertation we have considered the dynamics of drug resistance in cancer and the related issue of the dynamics of cancer stem cells. After introducing the relevant biological aspects needed for the mathematical modeling of these topics in Chapter 1, we reviewed the existing mathematical modeling literature on cancer stem cells and drug resistance in Chapter 2.

In Chapter 3 we considered the question of the dependence - or not - of the dynamics of drug resistance on the so called "turnover rate", a variable which is an indicator of the relative frequency of cell division with respect to cell death. Here our focus is on resistance which is caused only by random genetic point mutations. A very simple system of ODEs allows us to obtain results comparable to those found in the literature with one important difference. Indeed we have shown that the amount of resistance that is generated before the beginning of the treatment, and which is present at some given time afterward, always depends on the turnover rate, no matter how many drugs are used.

In Chapter 4 we developed a new methodology in order to derive an estimate of the probability of developing resistance to drugs by the time a tumor is diagnosed. Importantly, the heterogeneity of the cancer population is taken into account. Moreover, in the case of CML, we were able to infer the preferred mode of division of the

hematopoietic cancer stem cells, predicting a large shift from asymmetric division to symmetric renewal. From a methodological perspective, the mathematical analysis has allowed us to use indirect information about the mechanisms that control the dynamics of the disease evolution to reach specific conclusions that go beyond the present reach of experiments. While we have applied our mathematical results to the available clinical data on CML, the approach is not limited to this specific disease, and we expect similar conclusions about the division of cancer stem cells in other types of cancer as well. Specifically, our model should apply to any cancer for which stem cells our known to be the driving force of the progression of the disease and for which point mutations are a source of drug resistance. It could be of great interest if our results were to be tested experimentally, at least *in vitro*, on cancer cell lines. Ideally, a direct experimental method would require isolating cancer stem cells and healthy stem cells and compare the growth of both stem cell populations.

In Chapter 5 we extended the results of Chapter 4 by relaxing the assumptions on the average growth of the tumor, thus going beyond the standard exponential case. We calculated the probability that by the time a tumor reaches detection size, there are drug resistant stem cells in a cancer population growing logistically. We found that this probability is the same as for the exponential growth case. We would like to note that arguably our modeling methodology may be seen as more general, in the sense that, at least for the wild-type population, we only made assumptions on their averaged behavior. Moreover we showed that our results may be a good approximation also for a much more general form of tumor growth.

Finally, in Chapter 6 we reviewed the basic modeling assumptions and main

results found in the mathematical modeling literature on CML, and have formulated a new hypothesis on the effects that the drug Imatinib has on leukemic stem cells. Specifically, we have hypothesized that Imatinib is able to stop LSCs from dividing, putting them in a quiescent state. We have elucidated the reasons why this hypothesis is capable of explaining the current data on CML better than all other previous assumptions made in the existing literature. Based on this hypothesis, we then used the mathematical results of Chapter 4 to obtain new insights on the dynamics of the development of drug resistance in CML. We concluded with converting the mathematical results to clinical recommendations regarding the diagnostics and treatment of CML.

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