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Hybrid Modeling of Cancer Drug Resistance Mechanisms

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Abstract

Cancer is a multi-scale disease and its overwhelming complexity depends upon the multiple interwind events occurring at both molecular and cellular levels, making it very difficult for therapeutic advancements in cancer research. The resistance to cancer drugs is a significant challenge faced by scientists nowadays. The roots of the problem reside not only at the molecular level, due to multiple type of mutations in a single tumor, but also at the cellular level of drug interactions with the tumor. Tumor heterogeneity is the term used by oncologists for the involvement of multiple mutations in the development of a tumor at the sub-cellular level. The mechanisms for tumor heterogeneity are rigorously being explored as a reason for drug resistance in cancer patients. It is important to observe cell interactions not only at intra-tumoral level, but it is also essential to study the drug and tumor cell interactions at cellular level to have a complete picture of the mechanisms underlying drug resistance.

The multi-scale nature of cancer drug resistance problem require modeling approaches that can capture all the multiple sub-cellular and cellular interaction factors with respect to different scales for time and space. Hybrid modeling offers a way to integrate both discrete and continuous dynamics to overcome this challenge. This research work is focused on the development of hybrid models to understand the drug resistance behaviors in colorectal and lung cancers. The common thing about the two types of cancer is that they both have different mutations at epidermal growth factor receptors (EGFRs) and they are normally treated with anti-EGFR drugs, to which they develop resistances with the passage of time. The acquiring of resistance is the sign of relapse in both kind of tumors.

The most challenging task in colorectal cancer research nowadays is to understand the development of acquired resistance to anti-EGFR drugs. The key reason for this problem is the KRAS mutations appearance after the treatment with monoclonal antibodies (moAb). A hybrid model is proposed for the analysis of KRAS mutations behavior in colorectal cancer with respect to moAb treatments. The colorectal tumor hybrid model is represented as a single state automata, which shows tumor progression and evolution by means of mathematical equations for tumor sub-populations, immune system components and drugs for the treatment. The drug introduction is managed as a discrete step in this model. To evaluate the drug performance on a tumor, equations for two types of tumors cells are developed, i.e KRAS mutated and KRAS wild-type. Both tumor cell populations were treated with a combination of moAb and chemotherapy drugs. It is observed that even a minimal initial concentration of KRAS mutated cells before the treatment has the

ability to make the tumor refractory to the treatment. Moreover, a small population of KRAS mutated cells has a strong influence on a large number of wild-type cells by making them resistant to chemotherapy. Patient's immune responses are specifically taken into considerations and it is found that, in case of KRAS mutations, the immune strength does not affect medication efficacy. Finally, cetuximab (moAb) and irinotecan (chemotherapy) drugs are analyzed as first-line treatment of colorectal cancer with few KRAS mutated cells. Results show that this combined treatment could be only effective for patients with high immune strengths and it should not be recommended as first-line therapy for patients with moderate immune strengths or weak immune systems because of a potential risk of relapse, with KRAS mutant cells acquired resistance involved with them.

Lung cancer is more complicated then colorectal cancer because of acquiring of multiple resistances to anti-EGFR drugs. The appearance of EGFR T790M and KRAS mutations makes tumor resistant to a gefitinib and AZD9291 drugs, respectively. The hybrid model for lung cancer consists of two non-resistant and resistant states of tumor. The non-resistant state is treated with geftinib drug until resistance to this drug makes tumor regrowth leading towards the resistant state. The resistant state is treated with AZD9291 drug for recovery. In this model the complete resistant state due to KRAS mutations is ignored because of the unavailability of parameter information and patient data. Each tumor state is evaluated by mathematical differential equations for tumor growth and progression. The tumor model consists of four tumor sub-population equations depending upon the type of mutations. The drug administration in this model is also managed as a discrete step for exact scheduling and dosages. The parameter values for the model are obtained by experiments performed in the laboratory. The experimental data is only available for the tumor progression along with the gefitinib drug. The model is then fine tuned for obtaining the exact tumor growth patterns as observed in clinic, only for the gefitinib drug. The growth rate for EGFR T790M tumor sub-population is changed to obtain the same tumor progression patterns as observed in real patients. The growth rate of mutations largely depends upon the immune system strength and by manipulating the growth rates for different tumor populations, it is possible to capture the factor of immune strength of the patient. The fine tuned model is then used to analyze the effect of AZD9291 drug on gefitinib resistant state of the tumor. It is observed that AZD9291 could be the best candidate for the treatment of the EGFR T790M tumor sub-population.

Hybrid modeling helps to understand the tumor drug resistance along with tumor progression due to multiple mutations, in a more realistic way and it also provides a way for personalized therapy by managing the drug administration in a strict pattern that avoid the growth of resistant sub-populations as well as target other populations at the same time. The only key to avoid relapse in cancer is the personalized therapy and the proposed hybrid models promises to do that.

Dedication

Dedicated to my beloved husband, Usman Rauf, for all his love and support. I may not be able to thank him enough for the sacrifices he made for me during this period of work.

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Chapter 1

Introduction

Over the decades, many improvements have been made to elucidate the molecular mechanisms for cancer development and prevention but still cancer cure is a crucial question for researchers. One of the major problems faced in cancer treatment is its resistance to chemotherapeutics. In order to discover the mechanisms of disease progression, it is inevitable to first understand the complex processes of living body. Over the years various mathematical and computational methods have been devised to decode the digital information of human genome for studying the behavior of human body in both normal and disease state and also for assessing the effect and dynamics of drug inside the body. In order to quantify and asses the interactions of molecular elements in biological systems, new scientific discipline of system biology has emerged [4, 5]. Computer scientists joined biologists in the quest of determining the reactive nature of biological systems and as a result many computational techniques are introduced to address the behavior of components of biological systems, their communication and coordination with each other and also with the environment to produce cascading biochemical reactions [6]. Hornberg and his colleagues declared cancer as system biology disease [7]. So, in order to study the overwhelmingly complex cancer disease, computational modeling is essential.

Commonly used approaches for the study of biological systems arise from mathematics, in particular ordinary differential equations (ODE). ODE can be used to quantitatively analyze biological systems and also for understanding therapeutic efficacy inside these systems by in silico study of drug dynamics. However, this deterministic modeling approach becomes difficult to apply when the complexity of the modeled biological system increases. Further researches indicate that biological systems are not only deterministic but they have inherently embedded stochasticity [8]. In many cases when there is a situation of chemical instability and there is interaction of only few entities evolving dynamically, the best recommendable solution for modeling is to use stochastic models. While for stable systems which involve a large number of components deterministic modeling is recommendable. Moreover, many processes governed by nature inherently work in a hybrid way comprising of both deterministic and continuous events. The classic example in molecular biology is the switching on and off of certain genes according to environmental conditions [9]. This kind of behavior can be modeled as sets of ODE, and the discrete events in temperature

rise or fall change the dynamic behavior from one set of ODE to another[10].

Modeling of biological systems is one of the most developing area in computer science nowadays. The efforts in modeling and simulation of biological processes are mainly focused on single biological levels or scales, e.g., genomic/proteomic, cellular, tissue, organ, whole body, behavioral, and population. There is a need to seamlessly integrate the models from microscales to macroscales. Such multiscale models are necessary to produce quantitative and predictive models of complex biological behaviors such as *cancer*. At the same time, developing the abstractions to integrate between scales will lead to a much deeper understanding of the universal or generic features of biological phenomena.

Complexity of cancer resides on the multiscale nature of the disease. In the era of the human genome project, scientists thought that genetic mutation information could be the answer to the quest of cancer therapy. However, as the time passed it has become clear that genetics is not the only governing factor for cancer initiation. The involvement of genetic and biochemical pathways, signal transduction networks and cellular interactions made it more and more difficult to understand the mechanism of cancer progression. Zooming into the biological system for cancer evaluation proves the involvement of multiple molecular systems and similarly when we zoom out to get the overall picture there is the participation of many cellular processes to the pathogenicity of the disease. Hence, cancer is termed as system biology disease because in order to fully understand cancer disease all of the scales of biological information ranging from molecular to cellular and then cellular to organism must be taken into consideration [11].

Hybrid modeling is a key for the multiscale modeling of biological systems by integrating discrete and continuous processes going inside the organism. The dynamic and complex nature of the biological systems can be captured by a hybrid modeling approach. In cancer therapy it is long awaited by the physicians to not only see the impact of drug on the overall tumor growth at cellular level but also peek through at the molecular scale for the mutational burden carried in each tumor and response of these mutations to the drug at the same time. Hybrid modeling seems promising to combine both continuous and discrete events in cancer therapy, by taking drug administration as a discrete event and tumor growth as a continuous process. The mathematical modeling helps to observe the continuous processes of tumor growth with respect to mutation populations. Hence, hybrid modeling technique help us to develop a model to capture cancer progression and therapy at a multiscale viewpoint to help elucidate personalized treatment procedures for this disease.

1.1 Motivation

Drug resistance is major impediment in cancer research. There are two types of cancer drug resistances: intrinsic or acquired. The presence of resistant cells before the start of any therapy indicates the occurrence of intrinsic resistance, while the activation of resistance after the start of therapy is known as acquired resistance to the drugs [12, 13, 14, 15]. There is another recently recognized explanation for the appearance of resistance to cancer

drugs and that is termed as *tumor heterogeneity*. It explains that tumor is comprised of multiple mutated cell populations including initially unrecognized small number of resistant cells, which then grows in number unnoticed. In the meanwhile, target specific drug kills non-resistant cells without having any effect on resistant cells until the state of complete resistance is acquired [16, 17, 18]. Genetic variations have high tendency to influence the efficacy and ineffectiveness of a drug inside human body. Small therapeutic index for cancer treatment drugs increases the urge to study pharmacogenetic associations for cancer therapy. Nowadays cancer therapy is a personalized treatment because of difference in mutation burden of every patient For example, in case of colorectal cancer the cetuximab drug kills EGFR mutated tumor cells but KRAS mutated cells show no response to the drug so different proportions of both mutations in colorectal tumors pose different behaviors for same drug.

Various biological models have been devised to help physicians in the treatment of different diseases but growing mortality rate due to cancer, show dire need for computer scientists and biologists to work together in understanding the pathological behavior of disease. Hybrid modeling is an approach recently been employed by computer scientists for better understanding biological systems. Due to the dynamic and complex nature of biological systems it is suitable to model them by using various deterministic and stochastic approaches together. This research aims to provide a hybrid modeling approach for the analysis of drug resistance mechanisms in cancer. To date there is no cancer hybrid model available which analyze the drug resistance mechanisms in tumors along with assessing the immune strength role on tumor growth. The aim of this research is to incorporate the cellular processes of tumor growth, drug dynamics and immune response to study the drug resistance by the tumor at molecular level. Two clinical case studies of lung and colorectal tumors regarding drug resistance in cancer therapy are analyzed by utilizing a hybrid modeling approach.

1.2 Hybrid modeling framework for the personalized therapy of cancer

A generalized hybrid modeling framework for the solution of emerging issues in cancer therapy has been proposed (Fig:1.1). This framework is adopted for exploring the reasons for drug resistance mechanisms in colorectal and lung cancer in this thesis.

Hybrid models have two components: continuous and discrete. Modeling cancer by using hybrid modeling approach makes it possible to study various aspects of the disease including tumor progression, influence of immune system and the therapeutic procedure, altogether. The tumor progression and immune system interactions with tumor is a continuous process, while the drug administration is a discrete step. So, the hybrid model for cancer can take two kinds of input parameters, the continuous parameters regarding tumor progression and immune system strength, and the discrete parameter of therapeutic strategy. The tumor cells and immune system evolve in a continuous manner so they

are represented as ordinary differential equations. The therapeutic strategy works as drug control unit and it manages drug administration on discrete time steps.

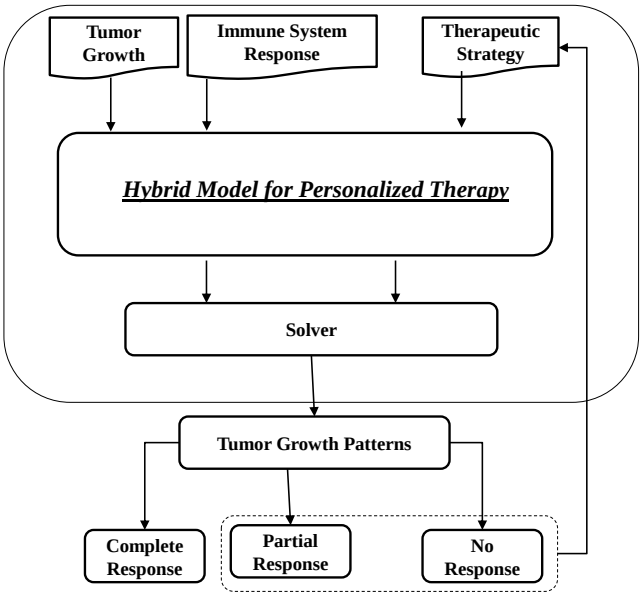

Figure 1.1: Hybrid Modeling Framework for Personalized Therapy of Cancer

The hybrid model is then developed by utilizing both continuous and discrete input information. The tumor cells and immune system cells evolve as continuous functions, while drugs are administered at certain discrete levels. In order to analyze the impact of therapeutic control as discrete step on the continuously evolving tumor and immune system cells, the hybrid modeling is the answer. The hybrid model development phase is discussed in detail in chapter 3 and 4. Various solvers can be used for implementing the hybrid model e.g. Mathematica, Matlab, Octave etc. In this research work Octave version 3.6.1 is used for the implementation of hybrid models. The implementation phase is discussed in detail in chapter 5. The output of the solver is obtained in the form of graphical illustrations of tumor growth patterns with respect to the therapy. The results obtained can be categorized into three ways: one, in which tumor shows complete response to the therapy by rapid or gradual decline in number of tumor cells until the complete removal of tumor cells. Second, in which tumor shows partial response to the therapy with some decline in number but not the complete removal of the cells. The tumor maintains a specific amount of cells throughout therapeutic procedure. Third, no response to the therapy as sign of complete

failure of treatment. The output with partial response or no response results are then analyzed by different therapies or with different drug administration schedules to obtain the required results.

The proposed framework can provide a way towards the personalized therapy of cancer by solving various other kind of cancer issues related to tumor heterogeneity and cancer stem cells by hybrid modeling.

1.3 Contributions

Nonlinear hybrid models are constructed to describe the drug resistance mechanisms in colorectal and lung cancers. The progression of the tumor during the treatment and appearance of resistance is thoroughly studied to obtain the following results:

First, the models are validated with clinical data obtained from both wet lab experiments and already published data from literature. The wet lab experimental data is used for the understanding of the lung cancer case study while for colorectal cancer previously published data is used. The overall tumor progression rate is determined by taking into account the individual mutation population for each tumor. Effect of drug on each population also governs the growth pattern of the tumor. Immune strength is also taken into account as a factor of patient variability in treatment procedures and also helps to reproduce the accurate patient populations for the analysis.

Second, the models handle various critical aspects such as tumor proliferation rate with respect to treatment, tumor dynamics with respect to each mutation population and tumor growth with respect to different drug administration schedules. The impact of drug on overall tumor along with the individual mutation population and the ratios of resistant VS non-resistant populations provides the global picture of resistance mechanism taking place at both molecular and cellular levels.

Third, the results are obtained to deeply understand the resistant mutations and their behaviors with respect to specific drugs. Patient specific treatment schedules and possible responses for the drugs are highlighted.

Fourth, the role of immune system is also taken into account to obtain unique patient specific simulation results. Possibility of immunotherapy as future treatment options is also discussed briefly according to model results.

Fifth, a computational framework is proposed for identifying patient-specific drug administration schedules with respect to mutation status for postponing the potential cancer relapse. Personalized model parameters are obtained for each patient by fitting clinical data and impact of other drugs is obtained by using the same parameters to obtain realistic results.

1.4 Structure of the Thesis

Chapter 2: Provides quick review of biological modeling approaches. The formalisms developed to model biological systems including differential equations, boolean networks, bayesian networks, Petri nets, process algebras and hybrid systems are briefly reviewed to have overall knowledge about formal modeling in biological systems.

Hybrid models in biology and specifically for cancer are also extensively discussed. Cancer hybrid models are distributed into four classes according to their biological action plan e.g, tumor growth and metastasis, drug-tumor interactions, tumor-immune system interaction and tumor-drug resistance mechanisms.

Two types of hybrid modeling formalisms: hybrid automata and hybrid Petri nets are defined in both formal and informal ways along with examples. The similarities and comparison of these formalisms are explained and finally, a Justification has been made for choosing hybrid automata over hybrid Petri nets for modeling of drug resistance in cancer.

Chapter 3: A hybrid model designed for the study of drug resistance mechanism in colorectal cancer is described in detail. In the hybrid model, the tumor and drug populations are modeled as mathematical non-linear ordinary differential equations and the therapeutic drugs introduction to the system is modeled as discrete step. Hybrid automata with clocks are used to model this phenomenon, in which drug scheduling and administration is monitored using clocks. The mathematical model not only comprise of tumor equations but it also takes into account of all components of immune system involved in tumor growth. The effect of monoclonal antibody (moAb)therapy and chemotherapy in combination is evaluated on KRAS mutated or wild-type tumor populations.

Chapter 4: Application of hybrid modeling in lung cancer case study is explained in detail. The lung tumor is modeled as a two state hybrid automaton as non-resistant or resistant tumor states. Lung tumor undergoes various drug resistance mechanisms related to each type of mutation and drug. The gefitinib and AZD9291 drug resistances are evaluated in lung tumor comprising of four tumor sub-populations. The relation of immune system and tumor growth factors for each sub-population is evaluated. The efficacy of AZD9291 drug is calculated by using clinically evaluated parameters for lung tumors.

Chapter 5: Implementation of proposed hybrid models is explained in detail.

Chapter 6: Conclusion and possible ideas for the extension of proposed models as future work are briefly described.

1.5 Published material

The part of thesis related to mathematical model of colorectal cancer has been partially published in [19],

Sameen, Sheema, et al. A Mathematical Model for Assessing KRAS Mutation Effect on Monoclonal Antibody Treatment of Colorectal Cancer. Software Engineering and Formal Methods. Springer International Publishing, 2014. 243-258.

The complete mathematical model for colorectal cancer has been published in the Journal of Theoretical Biology [20].

Sameen, Sheema, et al. "Mathematical modeling of drug resistance due to KRAS mutation in colorectal cancer." Journal of theoretical biology 389 (2016): 263-273.

The paper related to the part of thesis about lung cancer model is in preparation phase for the publication.

Chapter 2

Background

2.1 Modeling Formalisms in Biology

Computational modeling and analysis methods are playing a vital role in understanding the overwhelmingly complex dynamics of biological systems. In last few years huge progress is observed in the development of various mathematical and computational models varying from qualitative to quantitative behaviors of biological systems. The modeling formalisms are chosen according to the biological problem or on the modeling requirements.

Biological models can be basically divided into three broader categories: discrete models,continuum models and hybrid models.

Discrete models can represent individual cells in space and time. Each cell update its internal state according to a predefined set of biological rules. This approach is very helpful in studying cell-cell interactions and cell proliferation. Both lattice-based and lattice free methods are used by the discrete models. The computational complexity of such models increases with the increase in number of cells in study and hence limits the size of the tumor to be modeled.

Continuum models are used to study continuous behavior by utilizing differential equations. This gives global picture of the biological system instead of focusing on individual discrete events of cells. Such kind of models are ideal for studying the tumor growth and progression with respect to the genetic and cellular characteristics. Although larger spatial and temporal scales are easier model by continuum approach but it often fails to capture details of cell-level behavior.

Hybrid models integrate both discrete and continuous modeling approaches. They perfectly combine the advantages from both modeling practices and limits their drawbacks. The discrete part is modeled computationally while continuous part is captured mathematically. Hence provide the extensive multi-scale view for the biological processes. Hybrid models are powerful in a way that by integrating discrete and continuous modeling techniques they are able to incorporate the subcellular, cellular and tissue scales of the living systems. Hybrid modelling is a key to investigate biological processes at multi-scale level[21].

Many formalisms have been used to model biological systems [22]. The two main categories for formal modeling approaches depends upon the property of biological system to be studied. The biological system can either be studied by taking into consideration of qualitative aspect or by quantitative properties of a system. Fisher and Henzinger categorized the biological models into computational versus mathematical [23] and Bonzanni et al. phrase it as operational versus denotational models [24]. Denotational or mathematical models calculate mathematical relationships between quantities and change in their behavior with time. Operational models are generally qualitative in nature and provide high abstraction level.

Different formalisms are proposed to study qualitative and quantitative aspects of biological systems. The qualitative aspect reflect on the state dependent properties e.g. reachability of states. On the contrary, the quantitative aspects are related to time and probability dependent properties e.g. time or probability required to reach a certain state. The tumor dynamics is mostly studied by quantitative means in order to calculate the time for tumor progression or relapse of the disease. Quantitative aspects are often represented as stochastic formal methods. Biological processes are intrinsically stochastic, this property of biological systems makes stochastic modeling a fundamental approach of research [25]. Most famous stochastic formulation is provided by Gillispie, in which the chemical kinetics of biochemical reactions is based upon the interactions among the molecules [26].

The main formalisms developed to model biological systems including, differential equations, boolean networks, bayesian networks, Petri Nets, process algebras and hybrid Systems are briefly discussed below:

Differential equations

Mathematical modeling is considered to have a significant role in the study of biological systems. They are essential for providing insight for precise theoretical arguments about the factors affecting system under study and they can also be utilized for making predictions about future evolution of the system under observation. Differential equations are mostly used for dynamical systems modeling. They measure the rate of change of continuous variables. Ordinary differential equations (ODEs) have been used extensively in systems biology for modelling biochemical pathways, regulatory networks,gene expression processes and many more. The first model for metabolic dynamics in Saccharomyces cerevisiae was made using differential equation [27]. The Tyson et al. described the dynamics of regulatory and signalling pathways in detail by non-linear differential equations [28]. The metabolic processes of carbon metabolism of Escherichia coli is modeled using ordinary differential equations by Chassagnole [29]. The idea of modeling gene expression by mathematical equations is given by Chen et al.[30].

The use of mathematical models has also some limitations: for example, such models are often based on differential equations describing how the concentration of a substance in a solution changes over time. Differential equations are continuous and deterministic. This makes them suitable to model solutions with substances in high concentrations, and to analyse such models efficiently by means of numerical integration. However, differential

equations are not suitable to model systems with components which occur in small numbers and whose behaviour could be determined by choices between alternatives associated with probabilities. Another limitation of differential equation modelling is that they require a lot of experimental data and insight into reactions in order to build realistic models.

Boolean networks

Kauffman introduced boolean networks to model gene regulatory networks [31]. A boolean network consists of a discrete set of variables. Each variables is assigned with a function that takes inputs from variables and produces output which determines the state of the variable. In a genetic network, boolean variables that represent active and inactive states. The state of each gene is calculated by a function at each time step and a global state is achieved by combining states of all of the genes. Rene Thomas has introduced a kinetic logic to model biological regulatory networks based upon the boolean network approach. Kinetic logic is a multivalued logic, which allows us to approximate the sigmoid nature of biological entities more closely [32].

Boolean modeling for system biology has a very big limitation that, it only deals with two levels, i.e., 0 and 1. Whereas biological systems are very complex as they contain a lot of biological entities which demands more than two levels for capturing their dynamics more accurately. For large biological networks it is almost impossible to explore all possible states.

Despite of their strong limitation for biological modeling boolean models are still being used for exploring some interesting processes in biology [33]. Some recent examples for boolean modeling of biological systems include, boolean model for yeast apoptosis helps to understand the apoptotic processes in yeast and humans [34], a cardiac gene regulatory network is identified using a boolean model [35], another model predicts mutant phenotypes of fission yeast [36].

Bayesian networks

Bayesian networks (BNs) are also known as belief networks [37]. They are probabilistic graphs in which the nodes represent variables and the conditional dependencies on the variables are represented by edges between variables. The probabilistic dependencies are often calculated by statistical and computational methods. There are learning methods to infer both structure and probability parameters which makes Bayesian networks suitable for biological applications. They have been used for modeling gene regulatory [38, 39] and signaling networks [40]. The drawback of Bayesian networks is that they do not model feedback loop, which makes them inappropriate for biological systems. The extension in the form of dynamic Bayesian networks is proposed to overcome this problem [41].

Petri nets

Petri nets are bipartite graphs having two types of nodes, places and transitions. These nodes are connected by directed arcs. Petri nets are non-deterministic and good in design to

model concurrent events. The capturing of concurrent events and the intuitive graphical notations are considered main advantages of Petri nets. Biochemical networks can be suitably modeled using Petri nets by representing gene or proteins as *places* in Petri net and biochemical reactions can be represented as *transitions* and a transition is fired to execute a reaction.

Reddy with his co-workers was the first one to report the application of Petri nets in the biological modeling of metabolic pathways [42]. A lot of contributions in system biology are based on Petri nets including the modeling of reaction-diffusion systems [43], multi-scale modeling of ion channels, the modeling of bacterial regulatory networks [44], analysis of metabolic systems [45], signal transduction pathway analysis [46]. Recent tools designed for the analysis of biochemical pathways and networks are STEPP [47] and Snoopy [48]. The main disadvantage of Petri nets is that they become very large to handle with relatively big systems and also it is not possible to treat separately every single components of a model.

Process algebras

Process algebras is an umbrella term for formal languages that model concurrent systems. They usually consist of a set of process primitives, operators for sequential and parallel composition of processes, and communication channels.

One of the first process algebra is the Calculus of Communicating Systems (CCS) developed by Milner [49]. The CCS is extended into the $\pi - calculus$. The π-calculus [50] is a process algebra originally developed for describing concurrent processes, that are able to communicate by using channels. The key feature of the calculus is its ability to represent *mobility*, i.e. the movement of an agent to a different location in a virtual space of processes.

Bio-PEPA [51, 52, 53] has been developed for modeling biochemical networks, it allows to express stoichiometric coefficients and general kinetic laws in reactions. The abstraction of the behavior of complex systems with missing low-level details becomes easier when general kinetic laws are known. It also allows the use of different compartments of different sizes.

Brane Calculi [54] is proposed by Cardelli for the modeling of interactions of biological membranes. Another calculus inspired by the π-calculus and Mobile Ambients give rise a new calculus which deals with the compartment is known as BioAmbients [55]. Beta Binders [56] models biological membranes, by enclosing π-calculus processes inside membranes. In that way the communication with the external environment is possible through specific interfaces. Another interesting formalism for protein-protein interactions is $\kappa - calculus$ [57]. BlenX [58] is proposed to simulate the evolution of biological networks.

Other formalisms

Recent advances in formalisms for biological entities arise by the idea of membrane computing. Paun was the first one to propose that idea as membrane systems [59]. Membrane

systems (or P systems) is a computational formalism designed to represent biochemical reactions inside membranes as evolution rules from multisets of objects present on each membrane [60]. Various extensions of P systems have been introduced to model various aspects of biological systems, they include: metabolic P systems derived for modeling the evolution of biochemical processes deterministically [61], ecological P systems extension is based upon the probabilistic approach of P systems to describe the behavior of meta-populations [62, 63], multi-environment P systems applied to the modelling of ecosystems [64, 65], tissue like P system [66, 67] and lattice population P systems [68, 69].

Similarly, the Calculus of Looping Sequences (CLS) proposed by Barbuti et al. is a formalism focused on the evolution of a biological system, which is represented by means of rewrite rules [70, 71, 72]. This rewrite rule based approach is ideal for modeling complex biochemical reactions.

CLS allows an easy description of microbiological systems and their evolution. It allows modeling membranes, which are first-class elements of the calculus and, thus, can evolve and take part in reactions. Some extensions of the calculus have been developed. Stochastic CLS [73] allows the description of quantitative aspects of the modeled systems, such as the frequency of chemical reactions. CLS with links (LCLS) allows for the description of protein interaction at the domain level [74].

The P systems and Calculus of Looping Sequences are suitable for modeling the complex behaviors of tumor growth, progression and its behavior on the use of chemotherapeutics.

Deterministic-stochastic hybrid modeling

To date a variety of hybrid models have been developed in system biology. One of the first kind of hybrid models were based upon the idea of mixing stochastic and deterministic approaches to obtain more efficient simulations of systems. For example, the Tau leap method has been proposed by Gillespie [75, 76]. Similarly, Haseltine and Rawlings [77] proposed efficient hybrid simulations by separating systems into the slow and fast reactions. In another approach by Rao and Arkin [78] some reactions are simulated using Gillespie algorithm explicitly while the others by random variables distributed according to the probability density functions at quasi-stationary state. Salis and Kaznessis [79] came up with a method in which they described parameters to define number of reactions occurring in a time step. A Stochastic Concurrent Constraint Programming (SCCP) algebra proposed by Bortolussi and Policriti [80] provide semantics by combining discrete and continuous steps hybrid automata. A hybrid technique is established for ODE with the Runge-Kutta numerical approximation method [81]. Stochastic calculus technique, hybrid method developed by integrating ODEs and stochastic simulations [82, 83]. The HYPE process algebra is also introduced in to hybrid modeling [84]. Finally in 2012, hybrid simulation method is introduced for systems described in Calculus of Wrapped Compartments (CWC)[85].CWC was developed to model biological systems, which is simplification of CLS. That approach was very good to analyze various biological processes but it was not much efficient according to complexity of large biological systems.

2.2 Cancer hybrid models

Cancer modeling is being utilized from decades to understand all the molecular processes taking place in the formation of a tumor. Until now, many key processes involved in cancer are modeled by different approaches. These include modeling of tumor growth and progression [86, 87], tumor angiogenesis [88, 89], tumor metastasis [90, 91, 92], intracellular pathways involved in tumor initiation [93, 94], drug dynamics [95, 96] and many more [97]. All of these models can be categorized as discrete or continuous models [98], which means that all of these models have limitations related to their respective modeling approaches.

The cancer hybrid models can be categorized into four main groups according to biological processes; Tumor growth and metastasis hybrid models, drug-tumor interaction hybrid models, tumor-immune system interaction hybrid models and tumor-drug resistance hybrid models.

2.2.1 Tumor growth and metastasis hybrid models

Recently, cancer modeling is more focused towards hybrid modeling approaches. Rejniak and Anderson has categorized hybrid models of tumor growth into on-lattice models and off-lattice models [99]. On-lattice models mainly use cellular automata. Gerlee and Anderson et al., produced hybrid cellular automata models to study the solid tumor growth and also to configure the impact of environment on tumor growth [100, 101]. These on-lattice models has gained much consideration among scientists because of their ability to observe tumor growth and invasion by hybrid discrete-continuum method. It suggests that tumor morphology variation depends upon tumor micro-environment conditions. But the on-lattice models has certain limitations because of the use of rule-based cellular automata on square lattice. In the work done by Gerlee and Anderson the cell motility factor is not captured well because of that limitation. Off-lattice models can easily overcome such type of limitations as they give more realistic representation of cell spatial locations because they are not required to uniformly spaced on a fixed grid. Hence, an off-lattice model of cell motility with detailed forces describing cell-cell and cell-ECM interactions can provide more detailed information of tumor growth and progression[102]. These hybrid modelling formalisms only provide the representation of tumor growth mechanisms because they are specifically spatio-temporal in nature but they do not give any explanation about the molecular or genetic burden underlying in tumor growth. Neither they explain the cancer drugs behavior with respect to tumor growth dynamics.

Agent based modelling is also a discrete-based hybrid modeling approach. It not only allows to study the cell populations but it also gives insight within each individual cell [103]. This kind of modelling is suitable for the cancer metastasis as it provides more phenotypic information and helps to generate 3D representations.

2.2.2 Drug-tumor hybrid models

Drug dynamics is extensively studied for the impact on tumor growth. However, studying both drug and tumors dynamics together as hybrid systems is not a very old idea. Wang and his colleagues recently published a very interesting work which combines drug pharmacokinetic-pharmacodynamic (PK-PD) with the agent based model (ABM)[104]. Both of these are widely applied in cancer research as PK-PD explain about the drug efficacy and resistance in cancer while ABM are extensively studied for predicting tumor growth and progression. This seems a promising hybrid model for explaining the drug dynamics and tumor response in both time and scale but it does not give any clue about the effect of mutation on the tumor progression. There are some other models published in previous years for explaining the effect of chemotherapeutic drugs on solid tumors, e.g, Powathil et al., studied the effect of cell-cycle heterogeneity within each cell of tumor and response of tumor to the chemotherapy. The cell cycle heterogeneity among tumor cells has large impact on the response to the drugs. This is again studied by cellular automata giving more phenotypic information [105].

2.2.3 Tumor-immune system hybrid models

Human immune strength plays a decisive role in the success of all cancer therapies. Taking consideration of immune responses in tumor modelling is being done from last decade but initially the immune response was thought to be limited to only cytotoxic T lymphocytes. With the passage of time other components of immune system are also being considered gradually for complete immune strength assessment.

Tumor-immune system interactions are studied by Mallet and De Pillis by presenting hybrid cellular automata with partial differential equation model. This model explicates the role of immune system in tumor suppression or progression [106]. Kim and Lee investigated the interactions of cytotoxic T lymphocyte cells with tumor to predict the impact of immunotherapy as future cancer treatment. The tumor-immune interactions are observed using an agent-based model (ABM) for tumor and dynamics in the lymph node by using a system of delay differential equations (DDEs). Model revealed conditions for rapid tumor destruction by immunotherapy [107]. A model of the immune response to chronic myelogenous leukemia in post-transplantation dynamics using time delays is proposed by Niculescu and her colleagues [108]. This model considers that immune strength only relies upon cytotoxic T lymphocyte. But there are many other immune cell populations including natural killer cells, helper T cells, lymphocytes etc. that are needed to be taken into account. Nanda et al., developed a discrete-continuous model for the B cell chronic lymphocytic leukemia (B-CLL). B-CLL tumors have extreme heterogeneity depending upon the immune strengths of the patients, which makes each patient respond differently to the same therapy. This model includes almost all essential immune system components of the body involved in cancer progression e.g. natural killer cells, cytotoxic T lymphocytes and helper T cells. Influence of immune systems on each patient is thoroughly examined and its impact on disease progression is explored to have better personalized treatments

in future[109]. Another model proposed by De Pillis et al., studies the effect of regulatory T-cells on renal cell carcinoma treated with immunotherapy [110].

2.2.4 Tumor-drug resistance hybrid models

Drug resistance is a key hurdle in cancer therapy since it leads to a relapse of the disease after some time in which the therapy was successfully administered. There is no single reason for drug resistance hence it is encouraged to find the solution in pharmacogenetics.

There are very few hybrid models which allow for analyzing drug-resistance phenomena. Almost all these models include drug resistance as a measure of heterogeneity among cells in tumor tissue. The heterogenous nature of tumor mass is currently being studied as a reason for drug resistance in tumor therapeutic procedures. Each cell in a tumor is considered to be unique because of its biochemical interaction and own internal cellular pathways contributing towards major changes at cellular levels. These individual characteristics of each cell makes tumor a heterogenous mass of cells. Tumor heterogeneity is considered to be a reason for chemotherapy resistance in cancers. This is first time studied by hybrid modelling in a research by Chaplain and Powathil in year 2015 [111].

2.3 Hybrid modeling

All of the classes of hybrid models described in previous section deals with a specific aspect of cancer. Small therapeutic index for cancer treatment drugs increases the urge to study all of these aspects of cancer research to find a way towards personalized medicine. In this work, it is tried to capture all of the tumor, drug and immune system interactions for the solution of drug resistance problem. It is possible to address all these characteristics by incorporating hybrid modelling techniques. Hybrid system is described as the system comprised of discrete and continuous events dynamically. The interplay between discrete and continuous behaviors of several processes allows a system to flow and jump simultaneously. Biological processes mainly work as hybrid models as the discrete and continuous functions occur together at the same time. The complete understanding of biological phenomena is dependent upon retrieving the global picture of the molecular processes that takes place together in cell and nowadays this task has become easier by the development of various hybrid modeling formalisms and their applicability in the field of system biology. There can be various formalisms to deal with hybrid modeling but the formalisms that are frequently explored in the field of system biology include hybrid automata and hybrid Petri nets.

In this section we will recall the definition of hybrid automata and hybrid Petri nets. We will start with timed automata that will be useful to define hybrid automata.

2.3.1 Timed Automata

Informal Definition

A timed automaton [112] is essentially a finite automaton with real-valued variables representing the logical clocks in the system. The clocks are initialized with zero at the start of the execution of the system and then their value increments along with the passing time. Clock constraints are guards over clocks and a transition occurs when the clock value satisfies the guard on the edge. Alur et al., has discussed in detail the theory of time automata.

Formal Definition

Formally, a timed automaton $\mathcal{T}$ is a tuple,

$$\mathcal{T} = (\mathrm{V}, \sum, V_0 \text{ ,C,E, Inv})$$

Where:

- V is a set of states
- $V_0 \in \mathrm{V}$ is the set of initial states
- $\sum$ is a finite alphabet of actions
- C is a finite set of clocks, and
- $\mathrm{E} \subseteq \mathrm{V} \times \mathcal{B}(C) \times \sum \times 2^C \times \mathrm{V}$ is a set of edges, in which $\mathcal{B}(C)$ is the constraint over clocks.
- Inv: $\mathrm{V} \rightarrow \mathcal{B}(C)$ assigns clock constraint to states

An edge $\mathrm{V} \xrightarrow{g,a,S} V'$, represents a transition from V to V' on input symbol a. S is a set of clocks to be reset with the transition while the g is a guard over C. A timed automata is labeled with finite set of clock variables, called clocks. Clocks are different from usual variables, as their access is limited: clocks may only be inspected, and reset to zero. After every transition clocks are reset to zero after which they start increasing their value implicitly as time progresses. All clocks proceed at rate one, i.e., after the elapse of d time units, all clocks advance by d. Clocks can intuitively be considered as stopwatches that can be started and checked independently of one another. Conditions on the values of the clocks are used as enabling conditions (i.e., guards) of actions: only if the condition is fulfilled the action is enabled and capable of being taken; otherwise, the action is disabled. Conditions which depend on clock values are called clock constraints. In timed automata all clocks change at the same rate.

2.3.2 Hybrid Automata

Informal Definition

A very informal and commonly used definition of hybrid systems says that a hybrid system is a system with combined discrete and continuous behaviors.

A hybrid automaton can also be defined as a finite-state automaton connected with the collection of dynamical systems with continuously evolving variables. The discrete events are edges of the automaton that allows the automaton to switch from one dynamical state to another. The continuous evolution is modeled by differential equations. In each mode of automaton the evolution laws for continuous variables may change but the set of continuous variables remains the same.

A hybrid automaton can be also described as an extension of timed automata. In timed automata the clocks change continuously with the passing time and they all behave in same way but in hybrid automata it is possible to define how each variable changes with time by means of ordinary differential equations. In other words, a timed automata is defined as a hybrid automata when each $c \in C$ changes according to $\frac{dc}{dt} = 1$.

Formal Definition

Formally, a hybrid automaton $\mathcal{H}$ is a tuple,

$$\mathcal{H} = (\mathrm{V},\ \mathrm{E},\ \mathbf{X},\ \mathit{Init},\ \mathit{Inv},\ \mathit{Flow},\ \mathit{Jump})$$

where:

- G = (V,E) is a control graph. This is a finite labelled graph consisting of a set of vertices V and edges E which represent control modes and control switches of a discrete event respectively.
- X is a finite set of real valued variables represented as $\{x_1, x_2, \ldots, x_n\}$. The set of $\dot{X}$ variables $\{\dot{x_1}, \dot{x_2}, \ldots, \dot{x_n}\}$ represent variables evolving at continuous state, while, X' $=\{\ x'_1,\ x'_2, \ldots,\ x'_n\ \}$ represent variables at the conclusion of each discrete state.
- *Init*, *Inv* and *Flow* are the conditions associated with continuous evolution of the variables at each state. In other words, *Init*, *Inv* are conditions that govern the continuous flow of differential equations associated with each vertex $v \in V$.
  - *Init* : X $\rightarrow R$ describes initial conditions whose free variables are from X and which states the possible valuations for those variables at the start of each state v.
  - *Inv* is a formula associated with each $v \in V$, whose free variables are from X and which calculates the possible valuations for those variables during the continuous evolution of variables in a v.
  - Flow conditions are described as differential equations, whose free variables are from $X \cup \dot{X}$.

- *Jump* is an edge labelling function that assigns to each control switch $e \in E$. It defines the transitions values for each variable. Each jump condition $jump(e)$ has free variables from $X \cup X'$

Hybrid automata generalize time automata by varying clock variables with different rates or in a dynamic way. In the hybrid automata edges are labeled with (g, α, D), Where g $\subseteq CC(C)$ is a set of constraint over clocks, $\alpha \subseteq$ Act is an action to be performed if guards are satisfied and $D \subseteq C$ a set of clocks. It means that a automaton can move from x to x', where x and $x' \in X$, when clock constraint g holds. Besides, when moving from state x to x', any clock in D will be reset to zero and action α is performed.

Examples

The hybrid modeling is a well established field of research as it has many applications in various fields of studies, including biology. The working of hybrid models is explained by two examples, one is a very simple classic example of a thermostat stated by the father of hybrid automata, Thomas A. Henzinger in his paper [1]. Another example is the most recent application of hybrid modeling in cancer therapeutic research.

Thermostat: A thermostat controls the environmental temperature at desired level. In hybrid model of a thermostat the temperature is represented by variable x. There are two control modes for heater, off and on. The flow conditions for each mode are:

- Control mode Off: $\dot{x} = -0.1x$
- Control mode On: $\dot{x} = 5 - 0.1x$

Initially the heater is on and the temperature is 20 degrees, hence, the initial temperature is x=20. The jump condition is $x \leq 19$, Which means that the heater may go on as soon as the temperature falls below 19 degrees and go off as soon as temperature is greater than 22. The invariant condition which tells the condition for a heater to stay within Off state is $x \geq 18$. It controls that a heater in a way that, the heater must start at the latest when temperature falls to 18 degrees. Similarly, the invariant condition for the 'On' state is $x \leq 22$. The graphical representation of thermostat automata is given in Fig. 2.1.

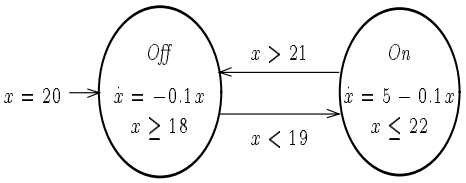

Figure 2.1: Thermostat Automaton by Heinzinger [1]

Hybrid model for hormone therapy of prostate cancer: Liu et al. proposed a hybrid automata model for prostate cancer cell dynamics in response to therapy [2]. The tumor progression is monitored during the therapeutic treatment of intermittent androgen suppression. The progression and survival of prostate cancer depends upon the levels of androgens. The prostate tumor consist of two sub-populations of cells, the hormone sensitive cells and castration resistant cells. Drop in androgen level considerably reduces the rate of tumor proliferation of hormone sensitive cells but at the same time it also increases the conversion rate of hormone sensitive cells to castration resistant cells. Intermittent androgen suppression (IAS) was proposed to limit the duration of androgen-poor conditions and avoid emergence of castration resistant cells. This IAS therapy switches between on-treatment and off-treatment modes by monitoring the serum level of a tumor marker called prostate-specific antigen (PSA). When PSA level decreases to some lower threshold limit the androgen suppression is stopped and when PSA level increases to the upper threshold level the androgen suppression is resumed. Hence the proposed hybrid model deals with two states of therapy, on-treatment mode and off-treatment mode. This hybrid model provide framework for identification of patient specific treatment schedules for avoiding cancer relapse. It takes into account the population of HSCs, the population of CRCs, as well as the serum androgen concentration, represented as x(t), y(t), and z(t), respectively. In addition, it also includes the serum prostate-specific antigen (PSA) level v(t), which is a commonly used biomarker for assessing the total population of prostate cancer cells. In the off treatment mode of model the androgen concentration is maintained at the normal level z0 by homeostasis, while, in the On-treatment mode the androgen is cleared at a rate $\frac{1}{\tau}$. The basal androgen production rate is also introduced at the rate of μ_z. The effect of both mode of treatments are carefully observed with respect to growth of tumor sub-populations. The proposed hybrid automata by Liu et al. is represented in figure 2.2.

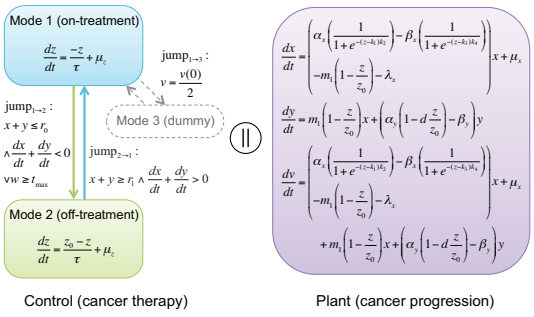

Figure 2.2: A simplified hybrid automata example for prostate cancer hormone therapy by Liu et al. [2]

2.3.3 Hybrid Petri nets

Formal Definition

A Hybrid Petri net $\mathcal{Q}$ is a tuple,

$$\mathcal{Q} = (\text{P, T, h, pre, post, } M_0)$$

where:

- Places are represented by $P = \{P_1, P_2, \ldots, P_n\}$
- Transitions are represented by $T = \{T_1, T_2, \ldots, T_n\}$
- h: $P \cup T \rightarrow \{D, C\}$ indicates the discrete or continuous status for each place or transition. A non-negative integer is always associated with a discrete place ($h(P_i) = D$) , it is called as the number of token. A non-negative real number is always associated with a continuous place ($h(P_i) = C$), it is called as the mark
- $Pre(Pi, Tj)(Post(Pi, Tj))$ is a function that defines an arc from a place P_i to a transition T_j, where an arc has a non-negative integer (non-negative real number)as weight if $h(Pi) = D(h(Pi) = C)$.

  Pre and Post functions have the following criterion: if P_i and T_j are a place and a transition such that P_i is discrete and T_j is continuous then $Pre(P_i, T_j) = Post(P_i, T_j)$ must be verified;

- M_0 is called as initial marking of Petri net as it maps the set of places to the set of non-negative integers or the set of non-negative real numbers.

Example: Gene operon

An operon is a functioning unit of genomic DNA containing a cluster of genes under the control of a single promoter. Matsuno et al., described the hybrid Petri net for an operon containing only two genes. The figure [3] shows the Petri net for operon of two genes transcription and then translation process.

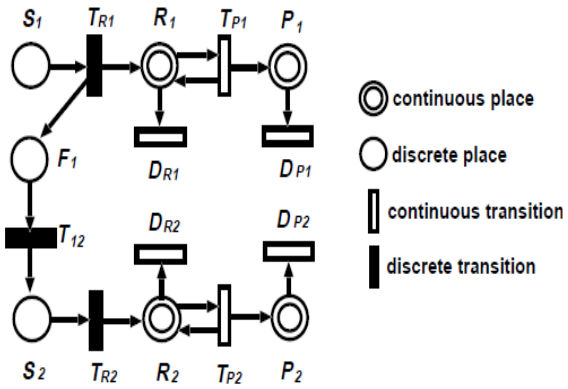

Figure 2.3: 2-Gene operon hybrid Petri net model by Matsuno et al. [3]

The figure explains the transcription and translation of two genes, gene 1 and gene 2 in an operon in which:

- Discrete places are: S1, F1, and S2.
- Continuous places are: R1, P1, R2, and P2.
- Discrete transitions are: TR1, T12, and TR2.
- Continuous transitions are: TP1, DR1, DP1, TP2, DR2, and DP2

In the initial marking, the discrete place S1 has a token (this reflects that RNA polymerase binds to the promoter of the operon), whereas the marks of other places are zero. A variable dT_j is assigned to manage the delay time of T_j to each discrete transition $T_j(h(T_j) = D)$ and for the continuous transition $T_j(h(T_j) = C)$, another variable vT_j is assigned for the speed of T_j.

The time required for the transcription of RNA polymerase of the gene1 is the delay time, which is represented as d_{TR1}, associated with the discrete transition TR1. The place S1 gets a token and then the transition TR1 is fired after time d_{TR1}. After the transcription of gene1 the mark of continuous place R1 which represent the concentration of mRNA of gene1 is increased by Post(R1,TR1). The degradation rate of concentrations of mRNA of gene1 is given by $Pre(R1, DR1)v_{DR1}$. The speed v_{TP1} of the continuous transition TP1 is the speed of translation of gene1. The place R1 is simultaneously an input and an output of the transition TP1, because it is required for translation but should not be consumed.

The increasing rate of the concentration of protein of the gene1 is given by $Post(P1, TP1)$ v_{TP1}. The degradation rate of concentration of protein of gene1 is given by $Pre(P1, DP1)$ v_{DP1} . The delay time d_{T12} of the transition T12 represents the time needed for RNA polymerase moving between the end of gene1 and the beginning of gene2. At the moment when the place S2 gets a token, RNA polymerase begins the transcription of gene2. The same process is repeated for gene 2.

Hybrid Petri nets (HPN) to hybrid automata (HA)

The hybrid Petri nets and hybrid automata are the formalisms which can be considered as substitute for each other. They both have significant similarities with each other and because of this resemblance in representation they both can be interchanged by the choice of modeler. A hybrid Petri net can be easily converted into hybrid automata. In order to explain this relationship, the above explained example of gene operon HPN is converted into HA. In order to develop hybrid automata from the gene operon HPN, all the discrete places in HPN are represented as states or locations of HA. Similarly, the discrete transitions are represented as jump statements for the transition from one state to other. All other continuous processes in HPN including continuous places and transitions are first converted in to continuous equations, which are represented by real valued variables.

Continuous processes in HPN are converted to continuous equations in HA. The translation process of a gene is represented as a continuous process in above mentioned HPN. There are three reactions going on in the process of translation of single gene. The actual reactions which are taking place in this process can be described as:

$$\mathrm{R}_1 \xrightarrow{V_{DR1}} \epsilon \tag{2.1}$$

$$\mathrm{R}_1 \xrightarrow{V_{TP1}} \mathrm{R}_1\mathrm{P}_1 \tag{2.2}$$

$$\mathrm{P}_1 \xrightarrow{V_{DP1}} \epsilon \tag{2.3}$$

These reactions simply describe the chemical process going on in gene translation process. The R1 is either degraded by V_{DR1} or it produces product R1P1 by the rate of V_{TP1}. The product P1 can also be self degraded as represented in equation 3.

Similar reactions are taking place in gene 2.

$$\mathrm{R}_2 \xrightarrow{V_{DR2}} \epsilon \tag{2.4}$$

$$\mathrm{R}_2 \xrightarrow{V_{TP2}} \mathrm{R}_2\mathrm{P}_2 \tag{2.5}$$

$$\mathrm{P}_2 \xrightarrow{V_{DP2}} \epsilon \tag{2.6}$$

These reactions can be represented as in the form of differential equations. Two equations can be developed for each gene e.g. for gene1 the equations are R1 and P1 e.g.

$$\frac{dR_1}{dt} = -R_1\, V_{DR1} \tag{2.7}$$

$$\frac{dP_1}{dt} = R_1\, V_{DR1} - P_1 V_{DP1} \tag{2.8}$$

Similarly for second gene, the equations will be:

$$\frac{dR_2}{dt} = -R_2\, V_{DR2} \tag{2.9}$$

$$\frac{dP_2}{dt} = R_2 V_{DR2} - P_2 V_{DP2} \tag{2.10}$$

The initial state of the hybrid automaton is the state where RNA polymerase binds to the promoter of the operon and the gene translation begins after the delay time of d_{TR1}, which is the time required for the transcription of gene 1. So, the transition to the state one starts when $t >= d_{TR1}$. The states of the resulting hybrid automata are two, first state in which only gene one is activated and the second state in which gene 1 and gene 2 both are activated, named as State 1 and State 2. In the state 1 the continuous variables of gene 1 are executed and in the state 2 the continuous variables of both of the genes are executed. The transition between both states takes place when $t >= d_{T12} + d_{TR2}$. The resulting hybrid automaton is shown in figure 2.4.

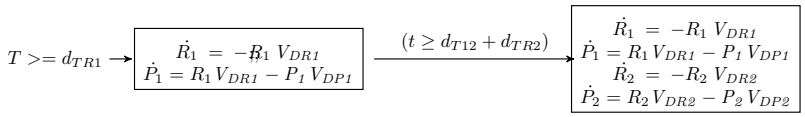

Figure 2.4: 2-Gene operon hybrid Automata model

Hybrid Petri nets (HPN) vs hybrid automata (HA)

The concept of hybrid Petri nets is not new as it was first introduced by Bail et al. in 1991 [113]. The basic idea is the same as to integrate and evaluate the discrete and continuous processes altogether in a single model. Various biochemical processes has been studied utilizing HPN e.g. Troncale et al.[114], proposed a HPN model for the modeling of Haematopoiesis, which is a complex phenomenon for the generation of all types of mature blood cells. The mature blood cell population arises from haematopoitic stem cells (HSC). The haematopoitic stem cell can either goes to a self renewal phase or they can mature into blood cells, this fate of HSC is defined by interleukin-6 (IL-6), cytokines. The sub-cellular phase in which HSC convert to mature blood cells is described as discrete switch of states. While the processes which govern the switch between these states are continuous in nature and IL-6 activation at molecular state during a continuous process triggers the switch from one state to another. The analysis of discrete and continuous phases of haematopoiesis offers deep understanding of the phenomena. Continuous processes in this work are not described as differential equations which are normally considered as standard representation of continuous processes in biochemical pathways but instead by exploiting the features of

continuous Petri nets, by continuous firing of transitions at a rate v(t). The resulting model is made up of classic Petri nets, initially described for discrete events, and continuous Petri nets.

Keeping in account the above example for the application of hybrid Petri nets in biology, it persuades system biologists to work with this type of formalism with advantage of capturing concurrency and parallelism inherent in biological processes. But the continuous processes in biological processes are not so easy to be handled by continuous Petri nets, as the number of variables to deal with becomes enormously high in various processes. The cancer progression is an example of such processes and that is why it is easier to model cancer related processes by differential equations. David Gilbert and Monika Heiner [115] were the first one to realize the necessity of incorporating differential equations into the Petri nets to model biochemical processes. In this paper they have first developed a discrete Petri net model and then converted it into a continuous Petri net in step wise manner. The resulting model is developed in a way that the continuous model preserves the structure of the discrete model developed at initial step. They explained there continuous Petri net model as structured description of ODEs. Although this approach seems to give a solution to the problem of incorporating differential equations but they have proved their work by a very small example. It is clearly understood that the proposed extension in the Petri net formalism can work but for the simple and small case studies. More complex the example becomes the more difficult it will be to handle the resulting Petri net model and the graphical illustration. For big examples of Petri nets will become complicated for the modeler.

Petri nets are sometimes defined as umbrella formalism for qualitative, stochastic and continuous models. To easily move between these paradigms the models can be converted into each other e.g. from discrete Petri net to continuous Petri nets, as in above example. Snoopy is a tool to handle all of such processes together [116]. This tool is very efficient in modeling and integrates stochastic and continuous Petri nets functionality to develop a generalized hybrid Petri net. These hybrid Petri nets are specifically developed for models in which stochastic and continuous behaviors require an inter-play. The application of this generalized hybrid Petri net,is useful to model the biochemical reactions which are composed by two types of reactions, slow and fast reactions for stochastic and continuous transitions, simultaneously [117]. Overall, the work on hybrid Petri nets by utilizing differential equations is not only recent but very less explored for the complex biochemical reactions with enormous number of continuous variables. So, for modeling complex cancer problems it is required to explore some other formalism for the ease and accuracy.

Hybrid automaton is a largely explored formalism for all types of hybrid processes including biological phenomena. The hybrid automata have an edge over hybrid Petri nets by having greater power of analysis. The reduced analysis power of Petri nets as compared to hybrid automata is often tried to overcome by coupling it with hybrid automata [118]. The hybrid automata are widely considered for modeling cellular processes or biochemical processes [119, 120]. The cancer development is a complicated biochemical process and the hybrid automata has showed promising solution to model almost every aspect of cancer progression and differentiation. Lots of cancer hybrid automata models have been

developed to solve different issues regarding identification, observation and solution of the problem. The basic cancer progression models are developed by differential equations and automata can easily incorporate differential equations of even very complex nature. Few examples of such cancer models are briefly described below:

Patel et al.[121] proposed a hybrid cancer model for the evaluation of tumor growth and the increased anaerobic tumor metabolism. The glucose concentration around the tumor or uptake by the tumor is represented in the form of differential equations. Similarly, Gerlee et al.[122], has proposed a cancer model for estimating the impact of cellular micro-environment on the tumor growth. The impact of tissue oxygen concentration is specifically observed on the clonal expansion on the tumor. Ribba et al.[97], has also emphasized on the importance of hybrid cancer model by providing a framework for the improvement of cancer therapy. The cancer drugs are administered on discrete steps and their effect is observed on cancer cells.

The above examples convince for the use of hybrid automata in exploration of cancer research questions. As the all previous mathematical models are originally developed in differential equations that is why it is easier to develop new models based on previous ones, so that new arising issues in cancer research can be addressed efficiently. The hybrid automata are good way to model such biological questions.

In this work the formalism used for modelling is the hybrid automata because of two main reasons. Main reason is the complex biological nature of the problems aimed to be studied and analyzed in this research. Cancer is said to be very complicated biological process with complex intertwined biochemical processes. Although, the hybrid Petri nets for such type of problems can be developed but the resulting model of such processes shows very complex graphical illustration which makes it very difficult for the modeler to handle and analyze. Second, important reason is that, this work is related to clinical practices performed for cancer treatment. It directly aids the physicians in therapeutic process and the physicians are more familiarized with automata as compared to Petri net models because of their generalized and easy way of representation.

Chapter 3

Hybrid Modeling of Colorectal Cancer Drug Resistance Mechanism

3.1 Introduction

The World Health Organization (WHO) declared colorectal cancer (CRC) as the second most common cause of cancer mortality in Europe [123]. The epidermal growth factor, commonly known as EGFR, has been the focus of main attention in the therapy of colorectal cancer as almost all of the colorectal tumors have mutations in EGFR gene. The specifically designed drugs for targeted therapy in colorectal tumors are called as anti-EGFR drugs because they target only EGFR mutations. Monoclonal antibody (moAb) drug has been introduced as the most promising treatment to fight against colorectal cancer disease by directly targeting the EGFR. This kind of therapy is more favored then chemotherapy because of its targeted nature. The chemotherapy has a big drawback of inability to differentiate between tumor and normal cells. This limitation is overcome by the introduction of targeted therapy such as anti-EGFR therapy in case of colorectal cancer. Targeted anti-EGFR treatment promises to increase the therapeutic index of disease along with the maximization of the benefits of drugs and reducing of toxicity. The efficacy of moAb treatment in colorectal tumors is challenged by KRAS mutations. The development of acquired resistance to the moAb drug, due to KRAS mutations, makes the problem very complex in terms of personalized treatment.

In current work, the role of KRAS mutations is critically analyzed in case of moAb targeting EGFR in metastatic colorectal cancers. In order to do so,a system of non-linear ordinary differential equations (ODEs)is developed to model the impact of KRAS mutations on the moAb and chemotherapy combination treatment of colorectal cancer. The behavior of moAb and chemotherapy is studied with respect to patient immune responses and moAb drug is also explored as a potential candidate for first-line therapy of CRC, in combination with chemotherapeutic drug.

The considered treatment pattern of colorectal tumors includes the moAb along with the chemotherapy. The drugs are administered according to different schedules on discrete

time steps e.g. moAb is administered bi-weekly while the chemotherapy is given weekly. The therapeutic procedure is modeled by a hybrid modeling approach in which the effect of drugs on tumor is analyzed as a continuous function, while, drugs are administered as a discretely.

The proposed hybrid model for colorectal cancer comprise of two units, the mathematical model for evaluating the role of mutations on tumor growth due to therapy and the drug scheduling unit which delivers drug on discrete intervals of time.

EGFR signaling pathway

The *epidermal growth factor receptor* (HER) is a network composed by four components: EGFR also known as HER1 or erbB1, erbB2 also known as HER2, erbB3 also called as HER3, and erb4 or HER4. ErbB receptors are glycoproteins composed of an extracellular ligand-binding domain, a transmembrane region, and an intracellular protein tyrosine kinase domain with a regulatory carboxyl terminal segment. This whole pathway is responsible for generating signals crucial for the survival of cell. The mutation in any component of this network leads towards carcinogenesis.

EGFR is basically a receptor on cell membrane, when its ligand binds to it a signaling pathway is activated. The ligand for EGFR is EGF, which is an epidermal growth factor. EGF-EGFR makes a dimer and upon the formation of dimer the intrinsic protein tyrosine kinase autophosphorylation is triggered. This autophosphorylation further triggers cascades of signaling processes. One of the important EGFR downstream pathway is the $RAS - RAF$ mitogen activated protein kinase (MAPK) pathway. The second important network activated due to EGFR signaling is the phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Both of EGFR triggered signalling networks are crucial for various key important cellular activities including DNA repair, cell survival and proliferation, differentiation, motility, adhesion etc.

Due to the critical role of EGFR in cellular processes, it is most widely studied for the involvement in tumor growth and progression, angiogenesis, invasion and metastasis, and inhibition of apoptosis. Approximately 90% of colorectal tumors have EGFR mutations. So, the EGFR targeted therapies are introduced to treat colorectal tumors [124].

Monoclonal antibodies targeting EGFR in CRC

In the case of CRC, two antibodies are approved for treatment of metastatic disease, cetuximab and panitumumab [124]. The highly recommended, most effective and advanced anti-EGFR drug is the cetuximab. cetuximab is IgG1 monoclonal antibody directed to the EGFR. It has higher binding affinity when it attaches with extracellular domain of the human EGFR. This removes the dimers on EGFR and blocks the downstream signaling pathway. Cetuximab antitumor activity includes, cell cycle inhibition, favoring of apoptosis, anti-angiogenesis and the production of immunological response by enhancing antibody-dependent cellular cytotoxicity (ADCC). This high performance makes cetuximab antitumor efficacy greater than other drugs for colorectal tumors. The treatment of

cetuximab in combination with either chemotherapy or radiotherapy is more effective for eradicating metastatic tumors.

Panitumumab is second recently approved drug for colorectal tumor therapy. It is human IgG2 monoclonal antibody directed to the EGFR. It binds with high affinity to EGFR and blocks binding of epidermal growth factor and transcription growth factor α. It inhibits tumor cell activation and induces apoptosis of tumor cells.

Colorectal cancer therapy and KRAS mutations

Monoclonal antibodies are a major breakthrough in CRC therapeutic research because of their anti-EGFR activity [125, 126]. The Food and Drug Administration (FDA) approved moAb drugs for colorectal cancer including cetuximab and panitumumab. These drugs produce promising results when administered in combination with chemotherapeutic drugs [127, 128]. They kill tumor cells in three ways: by directly blocking the EGFR pathway, by enhancing the activity of chemotherapeutic drugs and by enabling antibody-dependent cellular cytotoxicity (ADCC) from natural killer cells.

The most relevant hypothesis concerning the CRC progression during the moAb therapy is the selection of the treatment of KRAS mutated cells. In particular, it is retained that there is a small fraction of KRAS mutated cells into the majority of wild-type CRC cells, that will be selected by the moAb therapy while they will not be killed from the treatment and they will survive, given treatment acquired resistance [129, 130]. It has been frequently reported that patients having KRAS mutations show no significant response to moAb treatment [131, 132]. KRAS mutations are found in approximately 35%-45% of CRCs [133, 134, 135]. For this reason KRAS mutational status is considered as predictive marker for determining the efficacy of anti-EGFR therapies, and KRAS screening tests are prescribed by physicians before the start of treatments [136]. Only patients having wild type KRAS are eligible for moAb therapy [137]. Interestingly, some patients who have initially only KRAS wild type cells before treatment, still remain irresponsive to the medication because of the emergence of KRAS mutations.

Previous models

Various colorectal cancer mathematical models have been developed for basic tumor cell populations, cell proliferation and for the more complex pharmacodynamic and pharmacokinetics in colorectal cancer treatment [138]. These include models of colon crypts [139, 140, 141, 142, 143] and models of chemotherapy for colorectal cancer [144, 145]. Recently, DePillis et al. proposed a model which includes both chemotherapy and immunotherapy along with considerations of patient specific immunity parameters. This is a comprehensive model which includes tumor cell and immune cell populations, chemotherapy and monoclonal antibody treatment. Results show the effect of drugs on chemorefractory tumors [146].

The hypothesis of drug resistance of KRAS mutations in colorectal cancer is quite recent. Diaz Jr. et al. recently published a paper in which they proved that pre-existed small

number of KRAS mutated cells are responsible for developing resistance to panitumumab [147]. Another very recent paper by Stites describes a mathematical model which evaluates how different KRAS mutated polymorphisms show different sensitivity to the EGFR inhibitors [148].

3.2 Hybrid modeling of colorectal cancer

The hybrid model for colorectal cancer is developed to monitor tumor growth with respect to KRAS mutational status during and after the moAb therapy. In the model, tumor is evaluated on the basis of KRAS wild-type or KRAS mutated status. The mutated KRAS tumor subpopulation is resistant to the moAb and chemotherapy but wild-type KRAS population responds to both of the therapies. It is essential to keep in mind that all of the tumor population is EGFR mutated as well and further categorization of EGFR mutated tumor is done on the basis of KRAS mutated or wild-type tumor cells. Tumor grows in continuous fashion in the presence of the therapies and the drugs are introduced at different intervals depending upon the standard drug schedules. The hybrid automata is developed in order to study the dynamic behavior of tumors, the effect of drugs on tumor growth and appearance of resistance to the drugs in the presence of KRAS mutated cells.

Hybrid automaton for CRC therapy

The colorectal tumor hybrid automata is represented a single state S, which represent a tumor state.

The state S is governed by a variety of continuous variables. There are three major types of continuous variables in tumor state S. The tumor variables, the variables for therapies, and the clocks.

- The continuous variables for tumor include; T_w and T_m describing two type of tumor populations, the tumor cells with witld-type KRAS T_w and tumor population with mutated KRAS T_m. The therapy is only effective on wild-type KRAS cells, so, drugs M and A are only active on wild-type tumor cell population.

- The continuous variables for therapy are M and A representing each drug given to the colorectal patients. The M represent the chemotherapy drug, while A represent moAb drug. The concentration of both of the drugs are maintained into the system by specific decay rates calculated in the respective equations.

- Two clocks C_1 and C_2 are added into the hybrid automata each clock is associate with each drug. They monitor the scheduling or timing of administration of each drug.

The detailed mathematical model with all tumor, immune system and therapy equations is provided in section 3.3. The equations of tumor mentioned in the hybrid model representation are incomplete and just provided only to give an idea of tumor progression

and effects of two drugs on KRAS wild-type tumor. The other continuous variables describing the representation of immune system components in tumor state are not mentioned here just to make model simple to understand.

The FDA approved drug schedule for M , which is a chemotherapy drug (irinotecan) in our model, is the weekly introduction to a system with $dose = \kappa$. This means that M is introduced after every 7 days. Similarly, The FDA approved drug schedule for A , which is a moAb drug (cetuximab)in our model, is bi-weekly introduction to a system with $dose = \xi$. This means that A is introduced after every 14 days.

The *Inv*, invariant conditions for the state S are $(C_1 < 7)$ or $(C_2 < 14)$

The *Init* or initial conditions for M and A are κ and ξ respectively. The clocks are are also initialized by zero, $C_1 := 0$ and $C_1 := 0$.

The *jump* conditions are associated with clocks, $(C_1 = 7)$ or $(C_2 = 14)$ The transition within the state S takes place when any of the two *jump* conditions $(C_1 = 7)$ or $(C_2 = 14)$ is satisfied.

The edges in the hybrid automata are labeled as $((C_1 = 7), (M = M+\kappa), (C_1 := 0))$ and $((C_2 = 7), (A = A+\xi), (C_2 := 0))$. In which $(C_1 = 7)$ and $(C_2 = 14)$ are guard conditions, $(M = M + \kappa)$ and $(A = A + \xi)$ are the Act or actions performed when the guards are satisfied and then both of clocks are reset to zero again by $(C_1 := 0)$ and $(C_2 := 0)$.

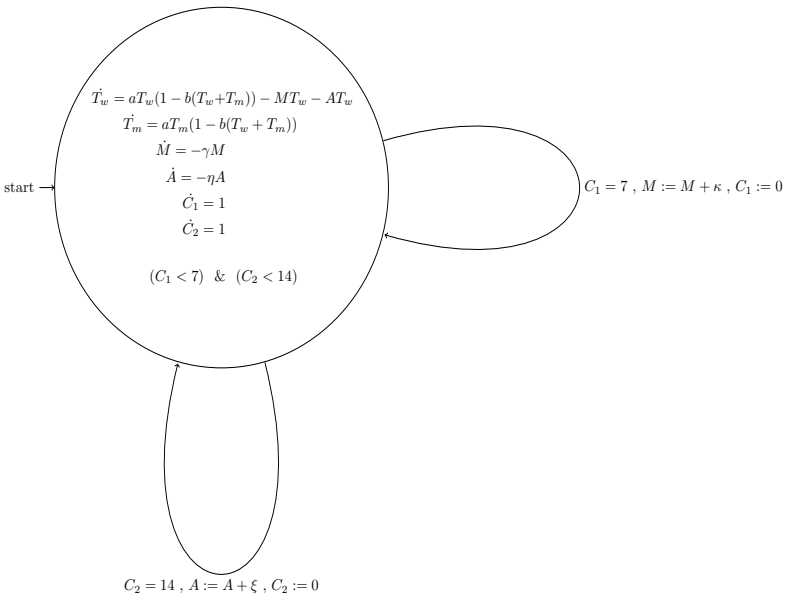

Figure 3.1: Colorectal Tumor Hybrid Automata

The resulted hybrid automata gives the drug introduction for M and A at discrete steps. The modeling of treatment pharmacodynamics as flow condition is shown in the

figure. 3.2. Where, the M is introduced after every 7 days while, M is introduced after every 14 days.

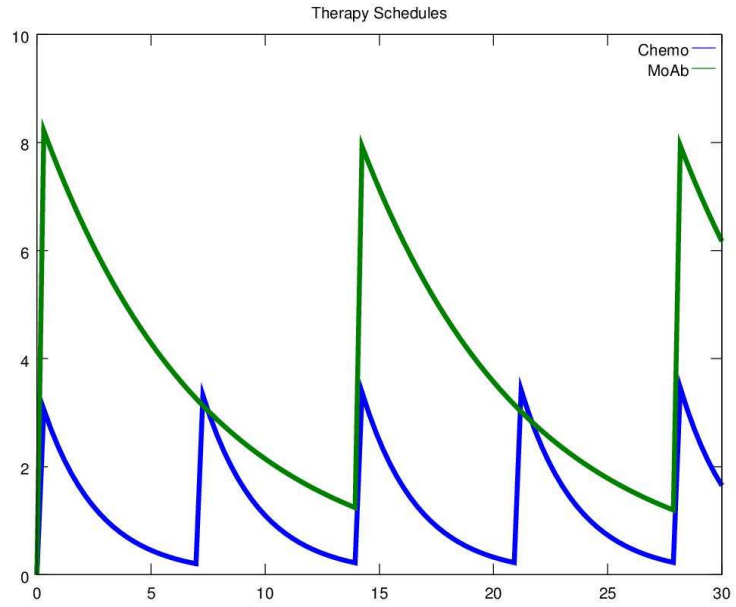

Figure 3.2: Colorectal Tumor Therapy Control

3.3 Mathematical model

The proposed mathematical model for colorectal cancer is an extensive model which not only captures the tumor growth with drug dynamics but it also models the drug resistance and the probability of the relapse of cancer. Current model is an advanced model for colorectal cancer and it is based upon previously proposed model of colorectal tumor. Although, there are various mathematical models for examining the tumor progression mechanism in colorectal tumors, some models also take into account the therapies but an interesting and extensive recently proposed model is by DePillis [146]. Current model is mostly based upon the model by DePillis. In this section, firstly, the mathematical model by DePillis is briefly described and then extension to this model is proposed after that.

3.3.1 DePillis' model for colorectal cancer

DePillis et al. [146] has recently proposed a mathematical model for colorectal tumors and the effect of monoclonal antibody treatments on tumor growth. They have explore the possible dosing schedules for moAb drugs to obtain maximum promising results for colorectal cancer therapy. A system of nonlinear ordinary differential equations (ODE) was proposed to model colorectal cancer growth and treatment. The model by DePillis et al., is a very

extensive tumor model because it includes not only the tumor cell population and the effect of drug on tumor but it also takes into account the host immune response. The consideration of immune strength of a patient in colorectal tumor model is very significant because moAb therapy is actually an immunotherapy and the response of other immune system components provide good insight into the actual tumor progression process. The modeled immune system components are; natural killer cells (NK), CD8+ T-Cells, lymphocytes and interleukins. The model also includes two type of treatment equations; chemotherapy and moAb therapy by two moAb drugs; cetuximab and panitumumab.

There are basically three main treatment action pathways followed by the moAb drugs which result in tumor growth rate reduction, complement activation, and possibly other mechanisms for tumor death. The model captures all these interaction pathways for the activity of moAb therapy:

- Interactions between moAb drugs, natural killer cells, and tumor cells. These interactions result in invoking the antibody-dependent cellular cytotoxicity (ADCC) from NK cells thus increases the NK cells cytotoxicity.
- Interactions between moAbs, chemotherapy and tumor cells. The moAb therapy along with chemotherapy is proven to be associated in the improvement of chemotoxicity. The effectiveness of chemotherapy increase when it is administered alon with the moAb drugs.
- Interactions between moAbs and tumor cells. The moAb drugs are very effective in directly killing of egfr mutated colorectal tumor cells.

The proposed model tracks the following populations and quantities:

- Cell Populations
  - T(w) the KRAS wild-type tumor cell sub-population;
  - T(m) the KRAS mutated tumor cell sub-population;
  - N(t) the concentration of NK cells per liter of blood (cells/L);
  - L(t) the concentration of cytotoxic T lymphocytes (CD8 +) per liter of blood (cells/L);
  - C(t) the concentration of lymphocytes per liter of blood, not including NK cells and active CD8+T cells (cells/L).
- Medications and Cytokines
  - M(t) the concentration of chemotherapy per liter of blood (mg/L);
  - I(t) the concentration of interleukin per liter of blood (IU/liter);
  - A(t) the concentration of monoclonal antibodies per liter of blood (mg/liter); The specific explored treatments are the chemotherapeutic drug irinotecan , and moAbs cetuximab or panatumumab.

The model incorporates patient-specific parameters to account for individual variations in immune system strength and in medication efficacy against the tumor. The simulations are performed on groups of virtual patients using a range of biologically reasonable patient-specific parameter values. The parameter for the models are derived from available clinical trial data. The model was evaluated for multiple dosing schedules and the new most effective treatment schedules are proposed for significant reduction in tumor size. It was also found that tumor growth is most dependent upon the drug specific pharmacokinetic and pharmacodynamic parameters.

3.3.2 Extending dePillis' model

The purpose of this mathematical model is to monitor tumor growth with respect to KRAS mutational status during and after the moAb therapy. The model is an extension of the model developed by DePillis et al. [146] by representing tumor cell populations using two equations, Eq(3.1) for tumor cells with wild type KRAS and Eq(3.2) for mutant KRAS tumor cells. All the other equations for natural killer cells (NK), cytotoxic T lymphocytes (CTL), lymphocytes excluding NK cells and CTLs and medications are as in the original model by DePillis et al. [146]. The model is implemented using the octave programming environment [149]. For detailed information about the parameter values of the model see the Appendix. The model includes equations for:

1. wild type tumor cell (T_w) and mutant tumor cell (T_m) populations;
2. patient immune system including, Natural killer cells (N), CD8+ T-Cells (L), Lymphocytes (C) and Interleukins (I);
3. chemotherapy (M) and monoclonal antibody (A) treatment;
4. patient immune strength (D).

3.4 Proposed ODE model

The four groups of equations are described below in detail

3.4.1 Equations for tumor cells

Equation for KRAS wild-type tumor cells

Tumor cells with KRAS wild-type nature go through natural clonal expansion process to form a tumor mass. The only two factors that interrupt the logistic growth of tumor cells are immune system and therapy. This fact is modeled in Eq. (3.1).

$$\begin{aligned} \frac{dT_w}{dt} &= aT_w(1-b(T_w+T_m)) - (c+\xi\frac{A}{h_1+A})NT_w \\ -DT_w &- (K_t+K_{at}A)\frac{T_w}{\alpha T_m+T_w}(1-e^{-\delta TM})T_w - \psi AT_w \end{aligned} \tag{3.1}$$

Logistic tumor growth is modeled by term $aT_w(1 - b(T_w + T_m))$. The innate immune system of the body fights tumor cells with the help of natural killer cells (term $-cNTw$) and CD8+ T cells (term $-DT_w$). Two other ways by which tumor cells experience death are chemotherapy (term $Kt\frac{T_w}{\alpha T_m+T_w}(1 - e^{-\delta TM})T_w$) and monoclonal antibody treatment. The triple action of monoclonal antibody, which is valid only for KRAS wild-type tumor cells, includes terms for:

- direct killing ($-\psi AT_w$);
- killing by enhancement of chemotherapy ($K_{at}A\frac{Tw}{\alpha T_m+T_w}(1 - e^{-\delta TM})T_w$);
- killing by assisting natural killer cells ($-\xi\frac{A}{h_1+A}NT_w$).

Equation for KRAS mutant tumor cells

KRAS mutant cells behave differently from the KRAS wild-types by disturbing the triple action behavior of monoclonal antibody treatment. The monoclonal antibody is not able to directly kill KRAS mutant tumor cells and also fails to create chemosensitization in KRAS mutants. This fact is modeled in Eq. (3.2).

$$\begin{aligned}\frac{dT_m}{dt} = aT_m(1 - b(T_w + T_m)) - (c + \xi\frac{A}{h_1 + A})NT_m \\ -DT_m - (K_t\frac{T_w}{\alpha T_m + T_w})(1 - e^{-\delta TM})T_m\end{aligned} \tag{3.2}$$

Thus Eq(3.2) is obtained from Eq(3.1) by removing the two terms for moAb induced tumor death in KRAS wild-type tumor cell equation and moAb-induced tumor death by enhancing activity of chemotherapy.

Both of the equations for tumor contain the terms which describe the interaction of moAb therapy with natural killer cells or chemotherapy and their effect on tumor growth. The enhancement of natural killer cells activity induced by moAb therapy is the same for both mutated and wild-type cells. This is represented in both equations by the $-\xi\frac{A}{h_1+A}NT_w$ term. Chemotherapy has reduced effectiveness against tumor cells during monoclonal antibody treatment because of mutant cells. This is represented in the model by $-Kt\frac{T_w}{\alpha(T_m)+T_w}$. The chemotherapy effectiveness decreases with the increase of the number of mutated cells. This term is introduced in both the equations of wild-type and mutant tumour cells for controlling the rate of chemotherapy induced tumor death. K_t is the maximum rate of chemotherapy induced tumor death in the absence of KRAS mutant cells. The above term makes the effectiveness of the chemotherapy dependent on the ratio of wild-type and total tumor cells. This ratio is controlled by the parameter α in such a way that, by increasing α, the rate of chemotherapy induced death is decreased with respect to the increase in the mutant population. Similarly, by increasing the initial number of KRAS mutated cells or by decreasing the initial number of KRAS wild-type cells, the rate of chemotherapy induced tumor death becomes much lower. Hence, the function clearly models the phenomenon of

chemotherapy ineffectiveness, in conjunction with monoclonal antibody treatment, in case of presence of KRAS mutant cells.

Determining accurate α is the key task for producing realistic results. α not only modulates the ratio of wild-type VS mutated cells but it also depicts the influence of mutated cells on wild-type cells in making them resistant to the chemotherapy as well. KRAS mutated cells has the ability to render the neighboring wild-type cells insensitive to the chemotherapy and the α determines the range of mutated cell microenvironment.

3.4.2 Equations for immune response

Natural killer cells, CD8+ T-Cells, other lymphocytes, and interleukins all play a vital role in creating immediate immune response with the initiation of tumor. Thus, in order to analyze the effect of immune system response and strength on the tumor proliferation the four equations are described. one for each component of immune system.

Natural killer cells

Natural Killer (NK) cells are a fundamental part of host first-line defense system. Their activity is modeled in Eq. (3.3).

$$\begin{aligned}\frac{dN}{dt} = eC - fN - (p + p_a\frac{A}{h_1 + A})N(T_w + T_m) + \frac{p_n NI}{g_n + I} \\ -K_n(1 - e^{-\delta NM})N\end{aligned} \tag{3.3}$$

They are produced from circulating lymphocytes (term eC) and their activity is stimulated by interleukins (term $\frac{p_n NI}{g_n+I}$). NK turnover is modeled by term fN. In case of tumor cells NK cells exhibit a special killing mechanism known as Antibody-dependent cell-mediated cytotoxicity (ADCC). In this process NK cells recognize tumor cells by special receptors that identify attached antibodies on the surface of tumor cells. After recognition, NK cells release some cytotoxic granules into the tumor cell which consequently cause death. The cytotoxic granules are actually tumor killing resources of NK cell; in case of exhaustion of these resources the NK cells die (term $(p + p_a\frac{A}{h_1+A})N(T_w + T_m)$). In addition, NK cells may die due to chemotherapy toxicity (term $-K_n(1 - e^{-\delta N_m})N$).

CD8+ T-cells

Cytotoxic lymphocytes are part of cell-mediated immunity. They kill target cells by releasing into them specialized granules that program them to undergo apoptosis. They are vital for killing tumor cells. Their activity is modeled in Eq. (3.4).

$$\begin{aligned}\frac{dL}{dt} = \frac{\theta m L}{\theta + I} + j\frac{T_w + T_m}{k + (T_w + T_m)}L - qL(T_w + T_m) + (r_1 N + r_2 C) \\ (T_w + T_m) - \frac{uL^2CI}{\kappa + I} - K_l(1 - e^{-\delta LM})L + \frac{p_i LI}{g_i + I}\end{aligned} \tag{3.4}$$

CD8+ T cell turnover is modeled by term $\frac{\theta mL}{\theta+I}$ and the breakdown of their surplus in presence of of IL-2 is modeled by term $\frac{uL^2CI}{\kappa+I}$. CD8+ T cells activity is stimulated by dead tumor cells, lysed by themselves (term $j\frac{T_w+T_m}{k+(T_w+T_m)}L$), NK cells (term $r_1N(T_w+T_m)$) or the general lymphocyte population (term $r_2C(T_w+T_m)$). Interleukins also perform stimulating effect on CD8+ T cells (term $\frac{p_iLI}{g_i+I}$). CD8+ T cell may die because of exhaustion of these tumor killing resources (term $qL(T_w+T_m)$) or due to chemotherapy toxicity (term $K_l(1-e^{-\delta LM})L$).

Lymphocytes

Lymphocyte count is the most important parameter to be considered while modeling tumors undergoing chemotherapy. Chemotherapy kills normal cells along with the tumor cells; hence, patients are constantly checked for their lymphocyte count during treatment. Reduction in lymphocyte count means weakening of immune system, which makes the body more vulnerable. Lymphocyte activity is modeled in Eq. (3.5).

$$\frac{dC}{dt} = \alpha - \beta C - K_c(1-e^{-\delta CM})C \tag{3.5}$$

Lymphocytes are synthesized in the bone marrow (term α) and their turnover is modeled by term βC. In addition, lymphocytes may be killed by chemotherapeutic drugs (term $K_c(1-e^{-\delta CM})C$).

Interleukins

Interleukin-2 is a major regulatory factor of immune responses. It belongs to a immune signaling group of cytokines. Interleukin-2 work as an immune response system by increasing the activity of cytotoxic T-cells. Their activity is modeled in Eq. (3.6).

$$\frac{dI}{dt} = -\mu I + \phi C + \frac{\omega LI}{\varsigma + I} \tag{3.6}$$

Interleukin-2 is produced in response to activated CD8+ T-cells (term $\frac{\omega LI}{\varsigma+I}$) or by naive CD8+T cells and CD4+T cells in the body (ϕC). Its turnover is modeled by term $-\mu I$.

3.4.3 Equations for treatments

In order to monitor treatments, separate equations are defined for chemotherapy (irinotecan) and monoclonal antibody (cetuximab) Eq. (3.7) and Eq. (3.8), respectively.

Chemotherapy/irinotecan

The activity of chemotherapy depends on the concentration of drug present in body at a specific time. This can be understood by the rate of excretion of drug from body, which is

modeled by term $-\gamma M$. Chemotherapy using irinotecan is modeled by Eq. (3.7)

$$\frac{dM}{dt} = -\gamma M \tag{3.7}$$

Monoclonal Antibody/cetuximab

Monoclonal antibodies bind to the epidermal growth factor receptors (EGFRs) present on the surface of tumor cells. As an average cell contains thousands of EGFRs, many molecules of moAb drug are consumed in a single tumor cell. The loss of moAb molecules due to their binding with the tumor (term $\lambda(T_w + T_m)\frac{A}{h_2+A}$) is an important factor to be considered while modeling moAb drug treatment to tumor. The rate of excretion of drug from body is modeled by term $-\eta A$.

$$\frac{dA}{dt} = -\eta A - \lambda(T_w + T_m)\frac{A}{h_2 + A} \tag{3.8}$$

3.4.4 Patient immune strength formula

Immune strength, i.e. the effectiveness of CD8+ T-cells, is calculated using Eq. (3.9). The formula uses the lymphocyte count L and total tumor mass $T_w + T_m$ along with other parameters to compute immune strength.

$$D = d\frac{(L/(T_w + T_m))^l}{s + (L/(T_w + T_m))^l} \tag{3.9}$$

Immune strength D is calculated by considering the following parameters:

d = immune strength coefficient;
l = immune-system strength scaling coefficient;
s = value of ratio $(L/(T_w + T_m))^l$ necessary for half maximal CD8+ T-cell effectiveness against tumor.
(It tells how quickly CD8+ T-cells respond to the presence of tumor.)

In our simulation the parameters are varied to generate three types of immune strength values: strong, moderate and weak.

3.4.5 Initial conditions and drug dosages

The initial conditions for the model are taken from DePillis model except the number of KRAS mutated cells. The initial number of KRAS mutated cells, which can cause resistance to the treatment, is not available in the literature. Thus, a small number for

KRAS mutated cells is assumed , say 35, because even such a small number of mutated cells is able to cause resistance. The initial conditions for the model are as follows.

$$\begin{aligned} Tw &= 4.65928 \times 10^9 \\ Tm &= 35 \\ N &= 9 \times 10^7 \\ L &= 1.8 \times 10^5 \\ C &= 9 \times 10^8 \\ M &= 0 \\ I &= 1173 \\ A &= 0 \end{aligned}$$

The parameter values in our model are also taken from DePilis except the rate of chemotherapy induced tumor death, which is reduced to the minimum level because of KRAS mutations. As DePillis, it is assumed that patients are already gone through first-line chemotherapy and are refractory to the treatment. Therefore, the initial tumor is assumed to have a very large number of cells: 4.65928×10^9. If tumor size becomes less than 2^7 cells during the treatment, it is assumed that the tumor is showing complete response to the therapy. Similarly, tumors which remain larger then 2^7 but do not continue to grow during the treatment are considered to have partial response.

Treatment comprised individual or combination of monoclonal antibody and chemotherapeutic drug, cetuximab and irniotecan, respectively. The drugs are administered according to standard FDA approved dosages and timings. For irinotecan, a $125mg/m^2$ dose is given over 90 minutes once a week, for 4 weeks. For cetuximab, a loading dose of $400mg/m^2$ is administered for two hours, followed by a $250mg/m^2$ dose over 60 minutes given every week for one month.

The implementation details of resulted hybrid automata are provided in chapter 5.

3.5 Results

3.5.1 Monoclonal antibody effect on chemotherapy

As described before, the effectiveness of chemotherapy against tumor cells gradually reduce during monoclonal antibody therapy due to KRAS mutated cells. This gradual ineffectiveness with the increase of mutated cells is modulated by term α in the model. All of the possible values for α are explored, ranging from 10 to 10^9. Initially, very low range of α is explore but in that case, obtained results contradict with the reported experimental data. The α value is then raised up to $\alpha = 10^6$ or $\alpha = 10^7$ to get the actual results (Figures 3.3 and 3.4). The reason for this much high α is that a single KRAS mutated cell has the tendency to influence thousands of wild-type cells.

The results are also analyzed by varying α values along with the varying initial number of mutated cells. Lower the α greater is the impact of chemotherapy but with the increase

in number of mutated cell this effect is not much significant. But with lower α and small number of mutated cells the drug has profound effect, which is not an actual phenomenon. In reality, chemotherapy also tend to become ineffective with the passage of time.

In the simulations the value $\alpha = 10^7$ is used because this shows a gradual decrease in the efficiency of the chemotherapy as compared to a too rapid reduction experimented with the smaller value $\alpha = 10^6$.

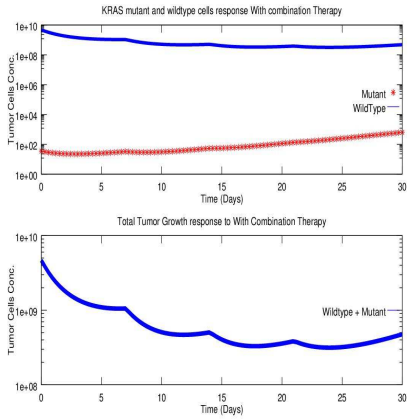

Figure 3.3: α value: 10^6 shows rapid decrease in wild-type and increase in mutant KRAS cells (Red: mutant; Blue: wildtype)

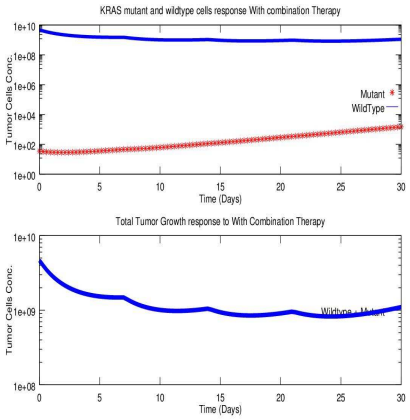

Figure 3.4: α: 10^7 shows gradual decrease in wild-type and increase in mutant KRAS cells (Red: mutant; Blue: wildtype)

3.5.2 Treatment trial simulations for KRAS mutated colorectal cancer tumors

Our model has been evaluated for standard treatments by chemotherapy and monoclonal antibodies for tumors with KRAS mutations. The KRAS mutated tumors are treated according to standard dosage of drugs and are evaluated for both monotherapy and combination therapy.

Cetuximab and irinotecan monotherapy

In accordance with the literature, in our model cetuximab monotherapy has no impact on colorectal tumors because of the number of elevated KRAS mutated tumor cells (Figure 3.6). Similarly, irinotecan monotherapy has no impact on the tumor because of the chemorefractory status of tumor. Here, no increase in KRAS mutated cells is noticed (Figure 3.5). Results show that, although both drugs fail as monotherapies, failure of cetuximab is specifically caused by an increase in the number of KRAS mutated cells.

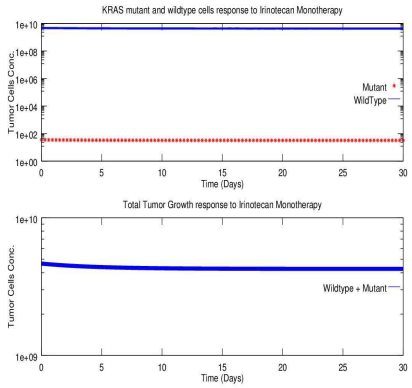

Figure 3.5: Irinotecan monotherapy(Red: mutant; Blue: wildtype)

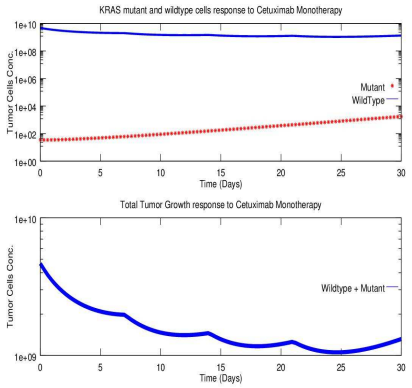

Figure 3.6: Cetuximab monotherapy(Red: mutant; Blue: wildtype)

Cetuximab and irinotecan combination therapy

For patients presenting metastatic colorectal cancer, cetuximab and irinotecan are recommended in combination. The model is used to test the combination of the two drugs. This allowed to understand the impact of combined therapy on KRAS mutated tumor cells (Figure 3.7). KRAS mutated cells grow with the passage of time and KRAS wild type cells start to reduce. However, as the initial number of KRAS mutated cells is very small, their increase is not clearly visible in the figure. Anyway, even this very low level of KRAS mutated cells is still able to gradually reduce the activity of drugs (Figure 3.7). The combination therapy is only effective for KRAS wild-type tumours (Figure 3.8).

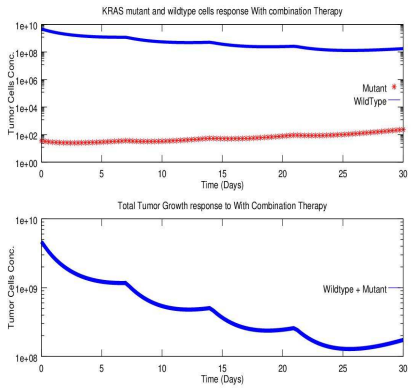

Figure 3.7: Cetuximab and irinotecan as combination therapy with KRAS mutant (Red: Mutant; Blue: Wildtype)

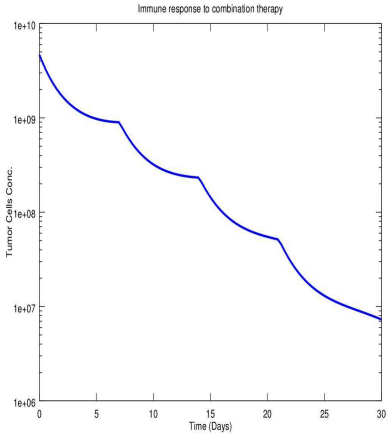

Figure 3.8: Cetuximab and irinotecan as combination therapy without KRAS mutant (Red: Mutant; Blue: Wildtype)

3.5.3 Patient responses to the therapy

The model is simulated for patients with different immune strengths. Generally, it is believed that a strong immune system both helps the medication and facilitates quick recovery, while patients with weak immunity do not respond well to the medicine. The the interaction between patient immune strength and treatment is analyzed in case of mutation development during and after medication. The hypothetical immune strength values are calculated for generating weak, moderate and strong immune responses. These values are generated by the formula for immune strength (Eq. (**??**)) by changing the values of its parameters.

Table 3.1: Cetuximab and irinotecan combination therapy

	With KRAS Mutation	Without KRAS mutation
Strong Immunity	NR/PR (Fig. 3.9)	CR (Fig. 3.10)
Moderate Immunity	NR (Fig. 3.11)	PR (Fig. 3.12)
Weak Immunity	NR (Fig. 3.13)	NR (Fig. 3.14)

Results are summarized in Table 3.1. Patients without KRAS mutations have complete response (CR), partial response (PR) and no response (NR) for strong, moderate and weak immunity, respectively. With KRAS mutations the immune strength has no significant impact on the treatment. KRAS mutated tumours normally show no response to the treatment but sometimes there is a partial response in presence of a high immune strength. For moderate and weak immunity there is no response at all.

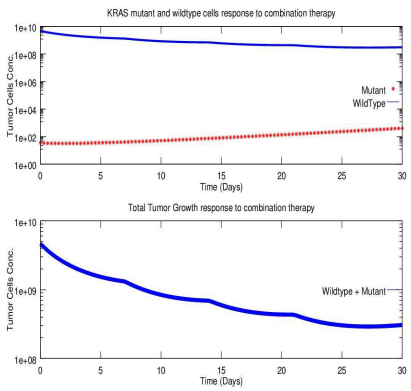

Figure 3.9: Strong immunity response with KRAS mutation

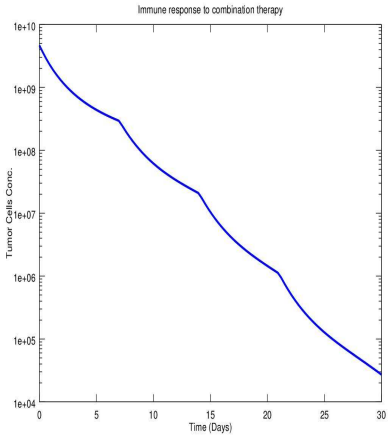

Figure 3.10: Strong immunity response without KRAS mutation

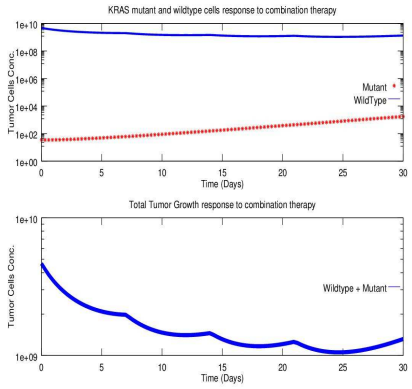

Figure 3.11: Moderate immunity response to KRAS mutation

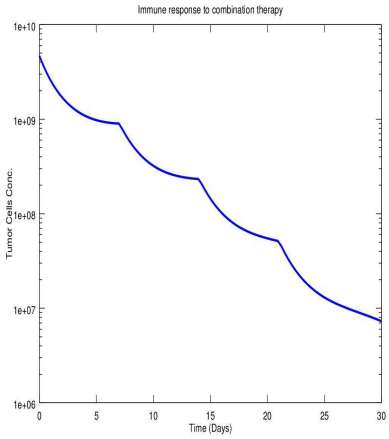

Figure 3.12: Moderate immunity response without KRAS mutation

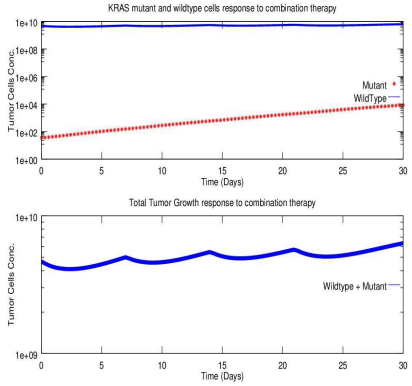

Figure 3.13: Weak immunity response with KRAS mutation

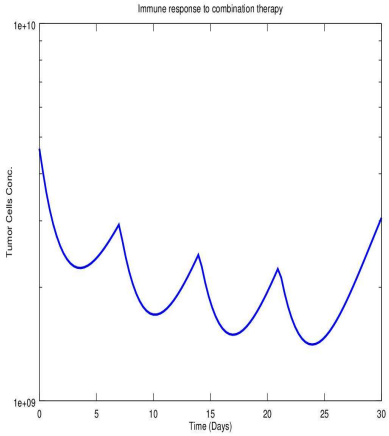

Figure 3.14: Weak immunity response without KRAS mutation

3.5.4 Cetuximab and irinotecan as first-line therapy

In this section the possibility of using cetuximab and irinotecan as first-line therapy is explored. Initial conditions are the same as shown in Section 3.4.5.

Patients having weak immunity do not show any significant response to the cetuximab and irinotecan as first-line therapy (Fig. 3.15). Tumor size reduces significantly in patients with moderate immunity, but the number of KRAS mutated cells show a relevant increase (Figure 3.16). The response to the therapy is only observed in patients with strong immunity and very low number of initial KRAS mutated cells (Fig. 3.17).

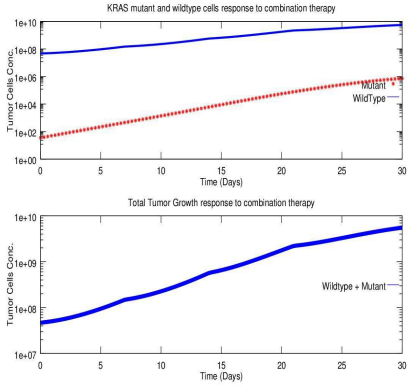

Figure 3.15: Cetuximab and irinotecan as first-line therapy: weak immune response (Red: Mutant; Blue: Wildtype)

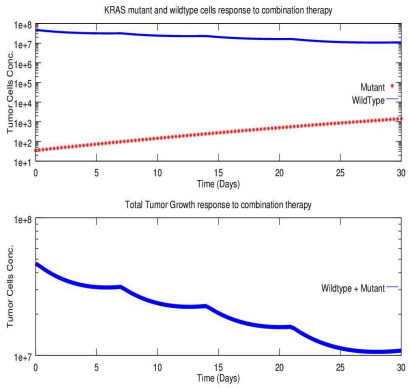

Figure 3.16: Cetuximab and irinotecan as first-line therapy: moderate immune response (Red: Mutant; blue: Wildtype)

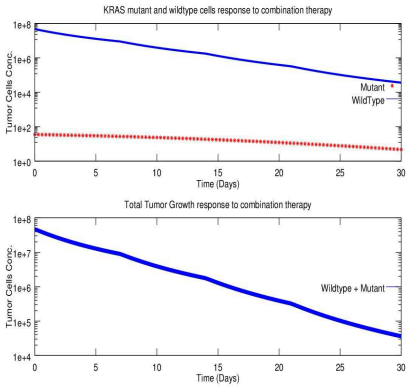

Figure 3.17: Cetuximab and irinotecan as first-line therapy: strong immune response (Red:Mutant; Blue:Wildtype)

3.6 Discussion

Emergence of KRAS mutated status is an alarming situation for colorectal cancer patients being treated with anti-EGFRs. Presence of KRAS mutations in a tumor treated with monoclonal antibodies is a sign of becoming refractory to treatments. In order to understand the phenomenon of developing resistance to the anti-EGFRs a mathematical model is developed with separate equations for KRAS mutant and wild-type cells.

KRAS mutations are considered as driver of resistance to anti-EGFR therapy in colorectal cancer. Subset of KRAS wild-type cells in colorectal tumor initially responds very

effectively to the anti-EGFR drugs but the presence of traces of KRAS mutations prior to treatment ultimately results in the development of acquired resistance to the drug. Hence, the treatment of colorectal tumor with anti-EGFRs is only recommended when the KRAS mutation status is zero. The anti-EGFR treatments to colorectal tumors containing minute quantity of KRAS mutated cells consequently develop resistance to the therapy. The pre-existing subclones of mutated cells multiply very rapidly during the treatment and on the other hand KRAS wild-type cells reduce significantly in their number due to efficient targeting procedure of monoclonal antibody drugs. Eventually, tumor mass repopulate with the mutated cells and makes tumor much more refractory to other treatments as well. So, the initial drop in the number of wild-type cells does not contribute much in shrinking the overall size of the tumor after the treatment. Diaz et al. was the first to discover the presence of small number of KRAS mutated cells in circulating tumor DNA at very early stage of drug treatment. This indicates that ostensibly looking KRAS wild-type tumors hide inside some fatal KRAS mutated cells as well. The mathematical model by Diaz et al. suggests that circulating DNA analysis for KRAS mutation detection can act as a marker for early detection of relapse of disease [147]. Misale et al., also confirmed the Diaz et al. results by in vitro analysis and declared that the reason for development of resistance is the pre-existence of clones of KRAS mutated cells [150].

A major problem in colorectal cancer is to identify the behavior of monoclonal antibody therapy in presence of KRAS mutations and the impact of the mutations on other therapies. More specifically, exploring the sensitivity of monoclonal antibody drugs to the chemotherapy and natural killer cells activity in the presence of mutations is another key issue in understanding drug efficacy [151]. It is speculated in our model that in case of natural killer cells, cetuximab has equal enhancing effect on both KRAS mutant and wild-type cells. In other words, KRAS mutational status has no significant impact on the antibody-dependent cellular cytotoxicity (ADCC) mediated by the drug [152].

The anti-EGFR drugs along with chemotherapy give promising results for colorectal tumors with wild-type KRAS. Monoclonal antibody is not only a perfect EGFR blocker but it also boosts the activity of chemotherapeutic drug molecules and results in enhancement of overall antitumor activity [153, 154, 155, 156, 157, 158]. Hence, the monoclonal antibody therapy in combination with chemotherapy is proved to be effective in avoiding relapses and increasing the progression free survival period in colorectal cancer patients. The presence of KRAS mutation in colorectal tumor leaves this combination therapy with no profound effects on tumor size this means that the chemosensitization operation of moAb drugs does not imply on KRAS mutated tumor cells [159]. The wild-type KRAS tumors have longer progression free survival as compared to mutated KRAS tumors when treated with combination therapy [160, 161, 162, 163, 164]. This reduced survival period due to mutated KRAS cells is also confirmed in our experiments. The ineffectiveness of both cetuximab and irinotecan drugs increases with increase in number of mutated cells as these drugs only influence the wild-type cells. Cetuximab has been frequently reported to increase chemotherapeutic activity upon combination with irinotecan drug in tumor cells [165, 156]. Studies show that KRAS mutant cells do not allow cetuximab to produce such type of chemosensitization [166, 167]. Chemotherapy is effective only at very early stage

of treatment when KRAS mutated cells are significantly lower in numbers but when the number of mutated cells in the tumor start to increase the sensitivity of chemotherapy reduces gradually. In the initial phase of treatment with combination therapy the drugs seem to reduce tumor a little but soon it goes back to the much bigger and drug refractory state. Time tumor takes to go back in to maximum or more resistant state is the time between the relapse which is significantly lower in case of KRAS mutations.

Our results repeatedly confirmed that small number of mutated cells can have an influence on whole tumor for making it refractory to the therapies. The major point to ponder is that how a very small proportion of mutated cells make the drug insensitive even to the wild-type cells. The moAb drug effectiveness on the wild-type cells is well explained but understanding the change in behavior of wild-type cells because of growing number of mutated cells during treatment is a challenging task for the researchers. BL Parsons and MB Myers explained this myth of KRAS wild-type cells behavior by blaming KRAS mutant cells responsible for every unexplained resistance mechanism of the tumor. The mutated cells undergo negative selection process in poly clonal tumors. Parson and Myers suggested KRAS mutations as transdriver mutations, these are the mutations which are able to speed up the tumor progression process even if they are in small proportion[168, 169].

The possible reasons for strong influence of small number of mutated cells on wild-type cells for producing phenomenal resistance against anti-EGFR drugs lies in tumor heterogeneity and in the theory of cancer stem cells. Tumor heterogeneity is the reason for failure of chemotherapy induced tumor cell death, not only for mutated cells but also for wild-type cells when treated along with the moAb drug[170, 171, 172]. In order to model this phenomenon the rate of chemotherapy was regulated for induced tumor death. It is assumed that the effect of chemotherapy decrease with the increase in KRAS mutated cells. Therefore, one cannot take any benefit from the chemosensitization activity of moAb drugs in case of KRAS mutations. The chemotherapy may work effectively only at the beginning of the treatment but then, with the increase of KRAS mutant population, it starts to loose its strength. Tumor heterogeneity explains the dispersal of mutated cells in the tumor mass. These distributed resistant cells have strong impact on their microenvironment[173]. They perform like small radiators emitting some harmful radiations which affect a range of surrounding cells, leaving them resistant to the therapies irrespective of their original wild-type status. Hobor et al., reported that wild-type KRAS cells has the ability to grow during the cetuximab treatment when KRAS mutated cells are present in the tumor. Resistant mutated cells maintain micro-environment inside tumor by influencing their neighboring cells rendering drug sensitive wild-type cells to resistant cells. The KRAS mutated cells secrete increased amount of ligands that has the ability to protect wild-type cells from EGFR blockade by cetuximab drug. The secretion of TGF α and amphiregulin by moAb resistant KRAS mutated cells sustain the EGFR signaling in wild-type cells too [174].

Tumor heterogeneity is also the major reason for the failure of initial KRAS mutation screening test. The incorrect assignment of wild-type status to the tumor is because of widely dispersed mutated cells inside the tumor. The test is only applied to a small chunk of tumor and there is a chance that the block selected for test may not contain KRAS mutation and hence give wrong result [175, 171]

Tumor heterogeneity role in therapeutic resistance is irrefutable but the root cause of heterogeneity development is the cancer stem cells (CSCs) [176, 177]. CSCs are best described as the cells having selective advantage of proliferation over the other cells in the tumor. They occur as minority in tumor and tend to have self renewing property that drive tumorigenesis [178, 179, 180, 181]. CSCs are rare cells with potential of being naturally resistant to chemotherapy. The area of CSC research is still underdeveloped that is why there is no concrete description about the process of chemotherapeutic drug resistance due to CSCs. If KRAS mutations are considered as CSC then failure of chemotherapy in our results is justified. KRAS mutated cells are recently been explored as potential CSCs in colorectal tumors. Evidences support the hypotheses of KRAS mutated cells as CSCs, which gives us enough explanation about the acquired resistance in colorectal tumor due to KRAS mutations [182, 183].

Patient immune responses play a vital role in oncotherapeutic processes and this role varies from positive to negative with strong to weak immune strength respectively. The immune strength becomes unimportant for KRAS mutated patients because the initially strong immunity turns into a weak one due to the development of secondary KRAS mutations during the treatment [184]. Even with the highest immune strength, the response to the drugs is only partial (sometimes). In our simulations tumor size was set to its maximum and it is considered refractory to the chemotherapy given as first-line to the patients. The reason for adopting these criteria is because cetuximab is generally given as third- or fourth-line treatment to the patients as final rescue [185, 186]. Hence it is proved that there is no correlation between immune strength and combination treatment for KRAS mutated patients.

The cetuximab and irinotecan combination therapy is proved to be very effective as first-line therapy for colorectal cancer but this is true only for KRAS wild-type patients [187, 135]. Although KRAS screening tests are always performed before starting monoclonal antibody treatments, there is a risk of minimal quantities of KRAS mutated cells that are not detected by common sequencing processes of laboratories. In this case critical questions arise about the patient response to cetuximab and irinotecan as first-line therapy. Our results show complete response only in patients with strong immunity. High immune strength means little number of KRAS mutations, so there is a chance that the drug kills wild-type cells quickly and chemotherapy also gets the chance to kill mutant cells. The first-line therapy seems to work also for moderately immune persons but, at the same time, increases the KRAS mutation level, which is a sign of recurrence of disease. Patient responses are also dependent upon the initial KRAS mutant cell concentrations. If the initial mutant level is very low then a complete response can be obtained. However, in case of greater level of initial KRAS mutants, the response is only partial with decrease in tumor size and significant increase in KRAS mutant levels, which doubles the chances of relapse. The relapse after cetuximab as first-line therapy will be more lethal because of acquired resistance to the drugs due to increased KRAS mutant populations.

In conclusion, the cetuximab and irinotecan combination therapy shows the rapid increase in levels of KRAS mutations and the partial or no response on the tumor size is an indications of the development of resistance to the drugs. Patients with stronger immunity

can be highly recommended cetuximab and irinotecan as first-line therapy but there is no instrument to accurately judge immunity of a person. Thus there is a potential risk associated with standard dosage cycles of drugs. The failure of the treatment will ultimately lead towards tumor progression with much higher rates. Moreover, the increased number of KRAS mutations makes the problem even more complex by creating resistance against the drugs. The co-occurrence of EGFR and KRAS mutations in a colorectal cancer patient is indeed the worst case scenario.

Chapter 4

Hybrid Modeling of Lung Cancer Drug Resistance Mechanisms

4.1 Introduction

Lung cancer is the most frequent cause of worldwide cancer mortality, accounting for more than 1 million deaths per year. Despite therapeutic advances, the overall 5-year survival remains only 15%. This public health problem is most significant in men. Lung cancer is of several types but almost 80% of all lung cancer cases are non small cell lung cancer (NSCLC).

4.1.1 Lung cancer mutations

Non-small-cell lung cancer (NSCLC) is caused by disturbances in epidermal growth factor receptor EGFR signalling pathway. Multiple mutations occurring in this pathway leads towards lung cancer. Hence, EGFR signalling is a key network involved in lung cancer initiation.

EGFR signaling is triggered by the binding of growth factors, such as epidermal growth factor EGF, resulting in the dimerization of EGFR molecules. Autophosphorylation of the receptors through their intrinsic tyrosine kinase domains leads to the activation of various downstream signalling pathways emitting proliferative and cell-survival signals [188, 189, 4, 5, 6]. EGFR signaling plays a key role in promoting the growth and survival of various types of solid tumors, including non small cell lung cancer (NSCLC) [190, 191, 192, 193]. This ensure the EGFR role as an important therapeutic target.

EGFR is a transmembrane receptor that is expressed on the cell surface in approximately 80 to 85% of patients with NSCLC. The most commonly found EGFR mutations in patients with NSCLC are deletions in exon 19 (Ex19del in 45% of patients) and a mutation in exon 21 (L858R in 40 %). Both mutations are commonly known as activating EGFR mutations and result in activation of the tyrosine kinase domain and are associated with sensitivity to the small-molecule tyrosine kinase inhibitors (TKIs: eg. erlotinib, gefitinib). Initial studies with the EGFR tyrosine kinase inhibitors (TKIs) demonstrated

biological and clinical activity in only a relatively limited subset of lung cancers [194]. Further investigation demonstrated that the highest response rates to these TKIs were seen in patients with somatic mutations within the EGFR-TK domain, particularly exon 19 deletion, exon 21 L858R, and exon 18 G719X [195]. These mutations occur within EGFR exons, which encodes a portion of the EGFR kinase domain. Approximately 90 percent of these mutations are exon 19 deletions or exon 21 L858R point mutations [196]. These mutations increase the kinase activity of EGFR, leading to hyperactivation of downstream pro-survival signaling pathways.

Even though lung cancer patients harboring a mutation in the epidermal growth factor receptor (EGFR) gene exhibit an initial dramatic response to EGFR tyrosine kinase inhibitors (EGFR-TKIs), acquired resistance is almost inevitable after a progression-free period of approximately 10 months. A secondary point mutation that substitutes methionine for threonine at amino acid position 790 (T790M), is a molecular mechanism that produces a drug-resistant variant of the targeted kinase. The T790M mutation is present in about half of the lung cancer patients with acquired resistance, and reported to act by increasing the affinity of the receptor to adenosine triphosphate, relative to its affinity to TKIs [197]. Nevertheless, several lines of evidence indicate that the T790M mutation confers growth advantage to cancer cells. The T790M mutation is association with resistance to TKI therapy and has been reported in approximately 50 percent of patients with disease progression [198, 199, 200].

Mutations in epidermal growth factor receptor (EGFR)and KRAS mutually are exclusive in patients with NSCLC, and the presence of each mutation can influence response to targeted therapy. Therefore, these mutations are regularly tested in NSCLC patients in order to tailor therapy according to genetic profile of a patient [201, 202, 203].

4.1.2 Non-small cell lung cancer (NSCLC) therapy

NSCLC patients were previously treated only with chemotherapy but this treatment has adverse side effects despite of prolonging the survival time of patients[144]. The use of cytotoxic chemotherapy is associated with a response rate of 20 to 35 percent and a median survival time of 10 to 12 months among patients with advanced non small cell lung cancer[145, 204].

Recently, introduction of tyrosine kinase inhibitors has hugely modified the treatment of NSCLC. Gefitinib is the most effective tyrosine kinase inhibitor for NSCLC patients. Gefitinib targets the ATP cleft within the tyrosine kinase epidermal growth factor receptor (EGFR),[205] which is overexpressed in 40 to 80 percent of non small cell lung cancers and many other epithelial cancers[206]. Gefitinib is the first agent designed with a known molecular target to receive FDA approval for the treatment of lung cancer, yet its activity is limited to a subgroup of patients with non small cell lung cancer. It is effective for the treatment of all activating EGFR mutations including L858R and Ex19del activating EGFR mutations. The efficacy of first-line gefitinib was superior to that of standard chemotherapy, with acceptable toxicity, in patients with advanced non small cell lung cancer harboring sensitive EGFR mutations [207, 208, 209].

The positive response of gefitinib is only limited to the activating EGFR mutations. In this work the included patients are with both type of activating mutations (L858R and Ex19del), as the activity and effectiveness of gefitinib is almost similar for both type of activating EGFR mutations. Dramatic responses of gefitinib are observed in NSCLC patients with activating EGFR mutations.

As gefitinib remains ineffective for EGFR T790M cells, a new drug is introduced for the treatment trials, AZD9291. 60 % of the patients have experienced tumor shrinkage in recent trial studies by Pasi and colleagues [210]. This third-generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) known as AZD9291 showed promising clinical activity and progression-free survival (PFS). In the simulations, we have virtually treated geftinib resistant patients with AZD9291 drug and predicted positive results in almost all of the patients. The positive response observed by AZD9291 drug in geftinib resistant tumors due to EGFR T790M is not long lasting. Scientists are exploring the role of KRAS mutations in making tumor resistant to this drug.

Almost all of the NSCLC patients contain EGFR T790M from the beginning but initially their number is significantly smaller than activating EGFR mutations but with the passage of time these cells continue to multiply in number hence they render tumor resistant to the therapy effective for activating EGFR mutations.

4.2 Lung cancer hybrid model

Lung cancer is a complex disease because of appearance of multiple mutations at the same time and the different behaviors of the mutations towards different therapeutic drugs leading towards multiple resistant states. Lung tumor at initial stage before application of any therapy is mostly comprised of activating EGFR mutations. This type of mutations are treated with an anti-EGFR drug named as gefitinib. After certain level of time gefitinib therapy starts to loose its strength and tumor shows progression of disease due to appearance resistance towards the therapy. The resistance to gefitinib is because of the growth of EGFR T790M mutated tumor cells. In other words, the tumor goes from non-resistant to resistant state when EGFR T790M population becomes greater than all other mutation populations. This kind of mutated cells are resistant towards gefitinib therapy, so, the gefitinib administration is stopped at the progression of tumor. In order to treat EGFR T790M mutated tumor cells another drug named as AZD9291 is prescribed. This drug treats such kind of mutations very effectively at a certain level of time until tumor again starts to progress due to triggering of resistance mechanism for AZD9291 drug as well. The factors of resistant towards AZD9291 are not exactly well understood but most of cancer scientists believe this to be because of KRAS mutations. At this stage, the tumor is at so much progressive state that no therapy can be able to completely eradicate it. So, chemotherapy or other drugs are prescribed at this stage just to prolong the patient life. The multiple resistant states evoke multiple resistance mechanisms to the drugs.

A hybrid model is developed to understand the evolution of tumor from non-resistant to resistant state. In this model only one resistant state is considered, which is the resistance

to gefitinib drug. Second resistant is ignored in this model just because of absence of sufficient information required for model parameters.

4.2.1 Hybrid automaton for drug resistant state of lung tumor

The lung cancer hybrid automata $\mathcal{L}$ is a tuple: $\mathcal{L}$ = (L, E, **X**, *Init*, *Inv*, *Flow*, *Jump*)

The illustration of lung hybrid automata shown in Figure 4.1

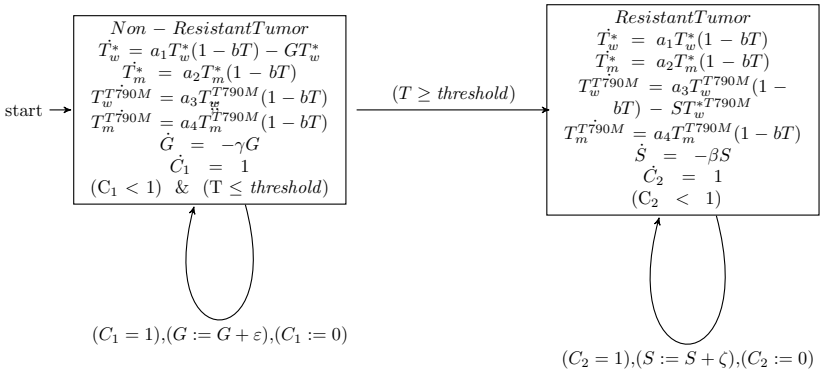

Figure 4.1: Lung Tumor Hybrid Automata

Control states *L*

The lung cancer hybrid automata $\mathcal{L}$ is comprised of two control states of tumor; drug sensitive state L_1 and a drug resistant state $L_2 \in L$. The non-resistant and resistant states of tumor depends upon the level of each mutation comprising a tumor. The lung tumor hybrid automata is comprised of two control states:

- Non-resistant tumor state L_1. This is the initial state of tumor in which tumor is a collection of multiple mutated cells. The major proportion of the tumor is composed of activating EGFR mutation, which is sensitive to gefitinib therapy. There very few cells of other mutated populations and *that's* why it is called as non-resistant tumor state.

- Resistant tumor state L_2. This is a state where tumor becomes resistant to gefitinib drug because of reduction of activating EGFR mutation and increase in EGFR T790M populations.This tumor is resistant to gefitinib drug but sensitive to another therapeutic drug known as AZD9291.

Continuous variables ($\boldsymbol{X}$) Each control state is comprised of following three type of continuous variables; tumor variables, the variables for therapies, and the clock variable.

1. The continuous variables for tumor include four tumor sub-populations given below.

   - Activating EGFR mutated tumor population with wild-type KRAS, represented by T_w^* in model.
   - Activating EGFR and KRAS mutated tumor population, represented by T_m^* in model.
   - Activating EGFR and EGFR T790M mutated tumor population with wildtype KRAS, represented by T_w^{T790M} in model.
   - Activating EGFR, EGFR T790M and KRAS mutated tumor population, represented by T_m^{T790M} in model.

   The *gefitinib* therapy or drug G is effective on T_w^* tumor sub-population, while, the AZD9291 therapy or drug S is effective on T_w^{T790M} tumor cells.

2. The continuous variables for therapy are S and G representing each drug given to the lung cancer patients. The G represent the *gefitinib* drug, while S represent AZD9291 drug. The concentration of both of the drugs are maintained into the system by specific decay rates calculated in the respective equations.

   The detailed mathematical model with all tumor therapy equations is provided in 4.3. The equations of tumor mentioned in hybrid model representation are incomplete and just provided only to give an idea of tumor progression and effects of two drugs on KRAS wild-type tumor.

3. Two clocks C_1 and C_2 are added into each state L_1 and L_2, respectively. Each clock is associate with each drug. They monitor the scheduling or timing of administration of each drug. Clock variables C_1 and C_2 are added to increment the value of each clock by 1. The clocks takes only two values, initialized by zero, they trigger the switch state for drug when clock is equal to 1 because the drug is administered on daily basis.

   Each clock monitors the time duration for the drugs. The clock C_1 handles the time schedule for G at state L_1 and the clock C_2 handles the time schedule for S at state L_2.

Control Switches ($\boldsymbol{E}$)

There are two types of control switches $e \in E$ in the model, where E is the set of edges:

- The first type of switch is from tumor state L_1 to L_2. This switch depends upon the tumor size. When the tumor reaches a maximum *threshold* value, the state of tumor changes from non-resistant L_1 to resistant L_2. The *threshold* value depends upon the

clinical information data for each patient. The clinical experiments are performed on regular intervals of time to measure the tumor size and when the tumor starts to progress again its size is measures and it is considered as threshold tumor size for that patient. As the growth pattern of tumor varies from patient to patient, the threshold value also varies. So, the jump condition $jump(e)$ from L_1 to L_2 is dependent upon the tumor size. When the jump condition ($T >= threshold$) is fulfilled, the transition from non-resistant to resistant tumor state takes place.

- Another type of control switch is the therapy control switch over each state. Both states L_1 and L_2 are associated with certain kind of therapy, e.g. the control state L_1, which is the non-resistant tumor state can only be treated by $gefitinib$ drug. The $gefitinib$ drug is administered on daily basis according to FDA protocols, in order to introduce drug at daily regular intervals a control switch is added which is associated with clocks. Similarly, the control state L_2, the resistant tumor state, is associated with another drug, AZD9291. This drug is also administered on daily basis. The discrete step is evoked for such kind of regular inputs of drugs to the state. The time of administration is modeled by adding clock here as well. The drugs represented as G and S are associated with state L_1 and L_2, respectively.

The *Init* or initial conditions for G and S are ε and ζ respectively. The clocks are also initialized by zero, $C_1 := 0$ and $C_2 := 0$. The initial tumor size T_0, varies from patient to patient. Clinical data is generated by wet lab experiments for the initial tumor size calculation. The *jump* conditions are associated with clocks, ($C_1 = 1$) or ($C_2 = 1$) and jump condition for transition from S_1 to S_2 is dependent on threshold value for tumor progression, which is $T >= Threshold$.

4.3 Mathematical Model for Lung Cancer

4.3.1 Equations for Tumor

The model includes four types of tumor equations:

1. Tumor cell populations having Activating EGFR mutation and wild-type KRAS;
2. Tumor cell populations having Activating EGFR mutation and mutated KRAS;
3. Tumor cell populations having Activating EGFR, EGFR T790M mutation and wild-type KRAS;
4. Tumor cell populations having Activating EGFR, EGFR T790M mutation and mutated KRAS.

All of the tumor sub-populations are essentially activating EGFR mutated as well but for the simplification purposes in the model only EGFR T790M mutations are mentioned everywhere.

Equation for tumor with Activating EGFR and wildtype KRAS (T_w^*)

Tumor cells with Activating EGFR mutation (T^*) and wildtype KRAS (T_w) are treated with gefitinib. The only two factors that interrupt the logistic growth of tumor cells are immune system and therapy. Gefitinib drug is very effective in case of activating EGFR mutations. This is modeled in Eq. (4.1)

$$\frac{dT_w^*}{dt} = a1T_w^*(1 - bT) - cIT_w^* - \psi GT_w^* - \phi ST_w^* \tag{4.1}$$

- Logistic tumor growth: $a1T_w^*(1 - bT)$, here the T refers to overall tumor size by adding all tumor sub-populations essential to maintain the overall tumor size under the carrying capacity.
- The immune response: cIT_w^*
- The effect of gefitinib drug: ψGT_w^*
- The effect of AZD9291 drug: ϕST_w^*

Equation for tumor with Activating EGFR and mutated KRAS (T_m^*)

Tumor cells with Activating EGFR mutation (T^*) and mutated KRAS (T_m) do not respond to gefitinib or AZD9291 therapy KRAS mutations. So these tumor cells grow without any difficulty during the treatment with Gefitnib or AZD9291.

$$\frac{dT_m^*}{dt} = a2T_m^*(1 - bT) - cIT_m^* \tag{4.2}$$

- Logistic tumor growth: $a2T_m^*(1 - bT)$
- The immune response: cIT_m^*

Equation for tumor with EGFR T790M and wild-type KRAS (T_w^{T790M})

Tumor cells with EGFR T790M mutation (T^{T790M}) and wild-type KRAS (T_w) are treated with AZD9291 as the gefitinib drug is ineffective for EGFR T790M cells.

$$\frac{dT_w^{T790M}}{dt} = a3T_w^{T790M}(1 - bT) - cIT_w^{T790M} - \phi ST_w^{T790M} \tag{4.3}$$

- Logistic tumor growth: $a3T_w^{T790M}(1 - bT)$
- The immune response: cIT_w^{T790M}
- The effect of AZD9291 drug: ϕST_w^{T790M}

Equation for tumor with EGFR T790M and mutated KRAS (T_m^{T790M})

Tumor cells with EGFR T790M mutation (T^{T790M}) and mutated KRAS (T_m) are resistant to AZD9291 treatment and $that's$ why no treatment is effective on the tumors cells containing both EGFR T790M and KRAS as in Eq. (4.4)

$$\frac{dT_m^{T790M}}{dt} = a4T_m^{T790M}(1 - bT) - cIT_m^{T790M} \tag{4.4}$$

- Logistic tumor growth: $a4T_m^{T790M}(1 - bT)$
- The immune response: cIT_m^{T790M}

4.3.2 Equations for therapy

In order to monitor treatments, separate equations are defined for geftinib and AZD9291 drugs, Eq. (4.5) and Eq. (4.6) respectively. In therapy equations the drug decay rates are represented by the γ and β values for gefitinib (4.5) and AZD9291 (4.6),respectively. These values are obtained by the formula $\frac{ln(2)}{t\frac{1}{2}}$, in which, term $t\frac{1}{2}$ refers to the half life of the drug. This formula, based on exponential decay of drug is taken from the DePillis et al. mathematical model paper for colorectal cancer [146].

Gefitinib

Gefitinib is administered orally on a daily basis. The daily dosage of 250mg gefitnib administration is maintained in the model as illustrated in the Eq. (4.5). The activity of drug depends on the concentration of drug present in body at a specific time. This can be understood by the rate of excretion of drug from body, which is modeled by term $-\gamma S$ in equation for gefitinib therapy.

$$\frac{dG}{dt} = -\gamma G \tag{4.5}$$

AZD9291

AZD9291 drug is also administered daily like gefitnib but the required dosage is 80mg. The drug excretion rate is modeled by term $-\beta$ in Eq. (4.6).

$$\frac{dSG}{dt} = -\beta SG \tag{4.6}$$

The implementation details of resulted hybrid automata are provided in chapter 5.

4.4 Materials and methods for clinical experiments

4.4.1 Experimental data

Several kinds of experimental data has been obtained from 7 non small cell lung cancer patients for model evaluation:

- Tumor size data is obtained by computed tomography scan measurement procedures. Tumor size is calculated before starting the treatment and then after regular intervals of 2-3 months to observe the effect of drug on tumor.
- Genetic profile data for each patient obtained by ddPCR of cell free tumor DNA (cftDNA) or data obtained after biopsy explaining the presence of activating EGFR, EGFR T790M or KRAS mutations.
- Drug administration schedule data for each patient. Describing the start and stop dates of drug according to progression time.

The experiments are performed clinically and in the laboratory of pharmacological unit of University of Pisa, Italy. The data for seven patients is obtained for analysis by performing various techniques and experiments. The important data acquired for modelling purposes is summarized in table 4.1.

4.4.2 Clinical experiments for tumor size measurement

Every 2-3 months a CT (computed tomography) scan is performed. Measure of tumor nodules (primary tumor and the relative metastases) are taken according to RECIST criteria [211, 212]. In this procedure some nodules are chosen according to the physician judgment and their maximum diameter is measured. The sum of each maximum diameter of the target nodules gives you an idea how the tumor is changing over time. For example, comparing the sum of the maximum diameter of a new CT scan with the previous, you will say that the tumor is in progression (PD) if the sum $> 20\%$, is in partial response (PR) if the sum is $< 30\%$, is in complete response (CR) if the tumor disappear and is stable (SD) if the sum $30\% < x < 20\%$. There is an exception if a novel tumor node (metastasis) compares the tumor is considered in progression (PD).

4.4.3 Tumor cell free DNA experiments for identification of mutations

Plasma collection and cftDNA extraction

Six ml of blood were collected in EDTA and centrifuged at 4°C for 10 min at 3000 rpm within two hours after blood drawing. Plasma samples were stored at $-80°C$ until analysis. cftDNA was extracted using a QIAmp Circulating nucleic acid Kit (*Qiagen*, Valencia, CA) from 1 to 3 ml of plasma following the protocol by manufacturer and the DNA was eluted in 100 μ l of buffer.

Analysis of cell free tumor DNA (cftDNA)

The cell free tumor DNA analysis was performed to investigate the contribution of mutations in tumors. The lung tumors are checked for the EGFR and KRAS mutations using this analysis. This kind of experiment is significant because it can reveal the presence of mutation even when it is little in amount and also it removes the need for biopsy for finding the mutations in a tumor. This is the reason that, this technique is more reliable and efficient then other type of simple polymerase chain reaction(PCR) methods. The investigational part of this study was the assessment of KRAS codon 12 and EGFR c.2369C>T (p.T790M) mutations in cftDNA. Other mutations potentially associated with EGFR-TKI resistance were not examined because of the limited amount of cftDNA available.

The analysis of cftDNA was performed by digital droplet PCR (ddPCR, *BioRad*, Hercules, CA, USA) and ddPCR Mutation Assay (*BioRad*). The analytic procedure was unable to discriminate the nature of the KRAS mutations detected because the analysis was performed with a ddPCR KRAS Multiplex assay. PCR reactions were assembled into individual wells of a single-use injection molded cartridge, according to the following protocol:

20 ng of template DNA (4µl), 1µl of 20X target primer/probe assay (FAM), 1µl of 20X wild type primer/probe assay (HEX), 10µl of 2X ddPCR Super Mix and 4 µl of DNAse/RNAse-free water up to a total volume of 20µl. Droplet generation oil (70µl) was then loaded and the cartridge was placed into the droplet generator. Using vacuum, sample and oil were mixed, generating mono-disperse droplets. Thereafter, 40 µl of packed droplets were transferred into a 96 well PCR plate for thermal cycling amplification. The protocol was standardized for all mutations to the following conditions: $95°C \times 10min, 94°C \times 30sand55°C \times 60s(35cycles), 98°C \times 10min, and4°C$ hold. The droplet reader (*BioRad*) was used for fluorescence signal quantification. The concordance between KRAS and $c.2369C > T$ (p.T790M) mutational status was assessed on pairwise cftDNA and tissue DNA of 8 patients who underwent re-biopsy for diagnostic purposes. DNA was extracted from formalin-fixed paraffin-embedded biopsies using the QIAmp DNA Mini Kit (*Qiagen*) and analyzed using conventional diagnostics as reported above. As a positive control for mutKRAS, the cftDNA from 30 patients with known KRAS mutated pancreatic cancer was used, while the DNA extracted from plasma of 43 healthy blood donors was employed as negative control for KRAS and p.T790M mutations. A 0.1 % and 0.2% cut-off was set for the detection of p.T790M and KRAS mutant alleles, respectively, as per manufacturers manual[213, 214].

4.5 Results

4.5.1 Wet lab experimental results

Data obtained from wet lab experiments provides three types of information: mutation status of a patient, gefitinib start and stop dates, tumor size at start of therapy and at

disease progression. All this information has been summarized in a table 4.1. The data has been collected from 7 NSCLC patients.

Each patient has activating EGFR mutation with mixed proportion of L858R and Ex19del activating EGFR mutation type. Gefitninb drug is very effective for both type of activating EGFR mutations. So, each patient is treated with gefitinib initially after the diagnosis of NSCLC. Tumor size is calculated at several intervals to check for the progressive disease. The only one patient among 7 shows complete response to the gefitinib therapy (patient ID=30) and one patient shows progressive disease at the second month after initialization of treatment. All others show partial response to the treatment as the gefitnib drug slowly eliminates the activating EGFR mutations but other mutations continue to increase in number.

Table 4.1: Lung cancer patient summarized experimental data; PD= Progressive disease, CR= Complete response, PR= Partial response, Gef= Gefitinib

Patient ID	EGFR Mutation	KRAS status	Initial tumor size	Tumor size at PD1	Tumor size at PD2	PD after Gef start(days)	Stop Gef (days)	Response
32	Ex19del	No	2.7×10^{11}	2.9×10^{10}	1.2×10^{12}	450	450	PR
24	Ex19del	yes	No data	NA	NA	803	1235	PR
30	Ex19del	No	22.4×10^{10}	NA	NA	NA	250	CR
20	L858R	yes	No data	NA	NA	600	682	PR
29	L858R	No	1.574×10^{11}	45.12×10^{11}	NA	550	NA	PR
26	L858R	yes	8.5×10^{10}	1.8×10^{11}	NA	74	74	PD
27	L858R	yes	1.15×10^{13}	4.5×10^{11}	7.5×10^{11}	144	144	PR

4.5.2 Calculating number of cells in tumor

In laboratory experiments tumor size is normally obtained in millimeters. For our simulation experiments we require to describe tumor size in number of cells in a tumor. It is hard to explain number of cells present in a tumor mass. Scientists believe that $1cm^3$ of tumor contains 1×10^9 cells [215, 216]. According to this hypothesis we have calculated number of tumor cells from tumor size in millimiter. May it is not so much accurate way to calculate number of cells but it surely provides an estimate for the analysis.

4.5.3 Initial conditions and parameter value for model

Initial values for tumor equations

Initial tumor size is obtained from the real tumor size of the patients calculated by laboratory experiment before the start of gefitinib treatment. The initial conditions for the four tumor populations is dependent upon that real patient data. As we know by literature that initially lung tumors are mostly comprised of activating EGFR mutations, so the initial condition for activating EGFR tumor population is almost the same as the original size of the tumor calculated in the laboratory. We assume that EGFR T790M cells are also present in the lung tumor but initially they are in very small numbers which varies from patient to patient. In this model the initial value for EGFR T790M cells is also not fixed for all patients but it is changed in every patient to obtain the same curves or trend of

tumor growth as observed in real patients but this value is never set to be greater than 200 cells. KRAS mutated tumor population initial values are dependent upon the detection of KRAS mutations in tumor by ddPCR laboratory test. If the laboratory test shows the presence of KRAS cells then the fixed initial values are assigned for all patients having KRAS positive status, otherwise the KRAS mutated populations are set to be zero. In case of KRAS positive test the activating EGFR and KRAS population is initiated with only 35 number of cells, while, EGFR T790M and KRAS population initial value is 15 cells.

Grow rates for tumor populations

The grow rates for activating EGFR mutations is derived from the literature (ref) but for all of the other mutated populations the values are assumed, as no experimental proof is available in previous research. In lung cancers the KRAS mutations are multiplied in very slow motion. They grow very slowly and gradually in numbers in an unnoticed way until they reach a significant number and makes tumor resistant to almost all of the drugs. The grow rates for both KRAS mutated populations is set to be very small. The grow rate of EGFR T790M population is varied in each patient to obtain the same tumor growth curve as observed in real patients. The grow rate for EGFR T790M for each patient is summarized in table 4.2. Greater the grow rate more quickly tumor reaches to its resistant state, similarly, smaller the grow rate greater time tumor takes to reach resistant state. The grow rate parameter in each tumor equation is named as a1, a2, a3 and a4 for activating, activating + KRAS, EGFR T790M and EGFR T790M + KRAS populations, respectively.

$$a1(ActivatingEGFR) = 0.5 \tag{4.7}$$

$$a2(ActivatingEGFR + KRAS) = 0.05 \tag{4.8}$$

$$a3(EGFRT790M) = variesineverypatient \tag{4.9}$$

$$a4(EGFRT790M + KRAS) = 0.05 \tag{4.10}$$

Table 4.2: EGFR T790M grow rate for tumor samples

Patient ID	EGFR T790M Grow Rate
32	0.01
24	0.009
30	0.05
20	0.01
29	0.1
26	0.3
27	0.0

Immune strength parameter

The immune strength represented by term $'cI'$ in all four equations of the tumor. This immune strength is actually a estimate of natural killer cells activity on tumor cells. It

is taken as a constant value parameter instead of measuring dynamics of immune cells response according to therapy. It is because the effect of immune strength on the NSCLC therapy is not so evident from the literature. In the term $'cI'$, the parameter $'c'$ represents the tumor cell kill rate by natural killer cell and $'I'$ represents the number of natural killer cells.

4.5.4 Validation of model by experimental results

The mathematical model is verified by producing same results as obtained through experimental techniques. This involved the fine tuning of only two parameters, the initial number of mutated cells and grow rates for each mutated populations. The overall initial tumor size was maintained same as calculated by experimental techniques. As the lung tumor is initially comprised of activating EGFR mutations. So, the initial number of activating EGFR mutations is set to be highest in the simulations. While, the initial percentage of EGFR T790M and KRAS mutated cells are set to be very low in order to obtain exact replica of real patient tumor. The grow rate of activating EGFR mutations is greater as compared to EGFR T790M so the initial number of both type of mutated cells play a significant role in overall grow rate of the tumor and this also explains that how quickly a tumor can reach to its resistant state.The gathered patient experimental data only gives the result of Gefitnib treatment on patients. We have validated the same effect of Gefitnib in our simulations and hence developed the exact patient profiles for our model experiments as real patients studied in laboratory experiments.

Initial size of the tumor is not the only parameter that govern the overall dynamics of tumor progression. The grow rate of EGFR T790M is also tailored to get the required growth pattern of the tumors to obtain the results like real patients. The grow rates for activating EGFR and KRAS mutations are set to be the same for all patients (0.5 for activating EGFR and 0.05 for KRAS). Different patient profiles generated by only varying the grow rate for EGFR T790M and initial number of mutated cells. To our surprise, this produces the virtual patient profiles approximately similar to the real patients.

Patient ID 30

This patient has Ex19del activating EGFR mutation. There is also evidence of EGFR T790M presence from biopsy data but KRAS status is zero. We set the KRAS population equal to zero throughout in our simulation for this patient. Patient shows quick and significant response to the first-line therapy of gefitinib drug without any relapse. The fast and positive response to the gefitinib therapy is may be because of two reasons; very small number of initial EGFR T790M mutated cells (30 cells) and negative status of KRAS mutated cells (fig:4.2). The grow rate of EGFR T790M is set to be 0.05.

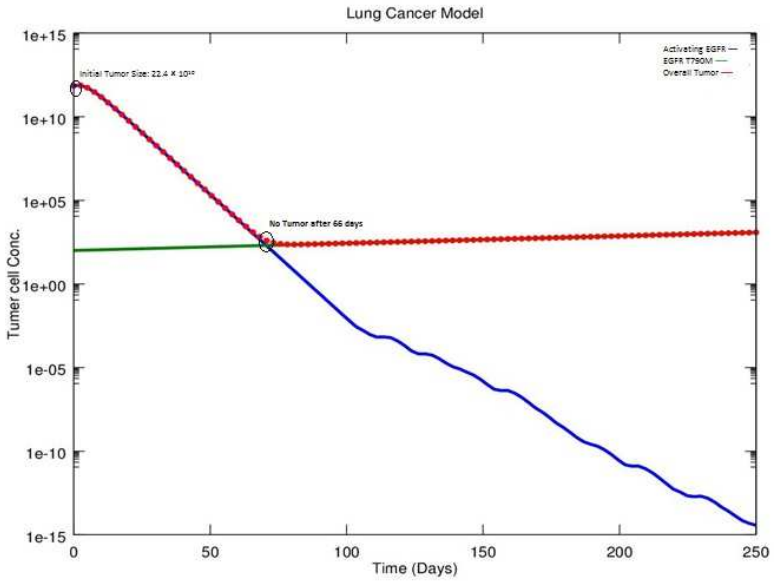

Figure 4.2: Complete Response to Gefitinib Therapy (Patient ID:30)
*The encircled points represent the real data points obtained by lab experiments

Patient ID 26

This patient shows progressive disease just after 2 months of gefitnib therapy start date. This means that initial number of EGFR T790M is much higher in tumor which makes tumor resistant to gefitinib at the beginning stage. The figure shows little decrease in tumor size in the start but this decrease is not so significant and tumor again grow back and shows progression of disease(fig:4.3). For simulation the initial number of EGFR T790M is set to be equal to 300, while the grow rate for this mutation is set to be 0.3, which is much higher but still lower than activating EGFR growth rate of 0.5.

Patient ID 29

This tumor shows partial response to the gefitinib treatment (fig:4.4) but significant complete response to the AZD9291 drug because of absence of KRAS mutations (fig:4.13).

Patient ID 27

This *patient's* tumor shows partial response to the gefitinib treatment (fig:4.5.

Patient ID 32

This tumor shows partial response to the gefitinib treatment (fig:4.6)

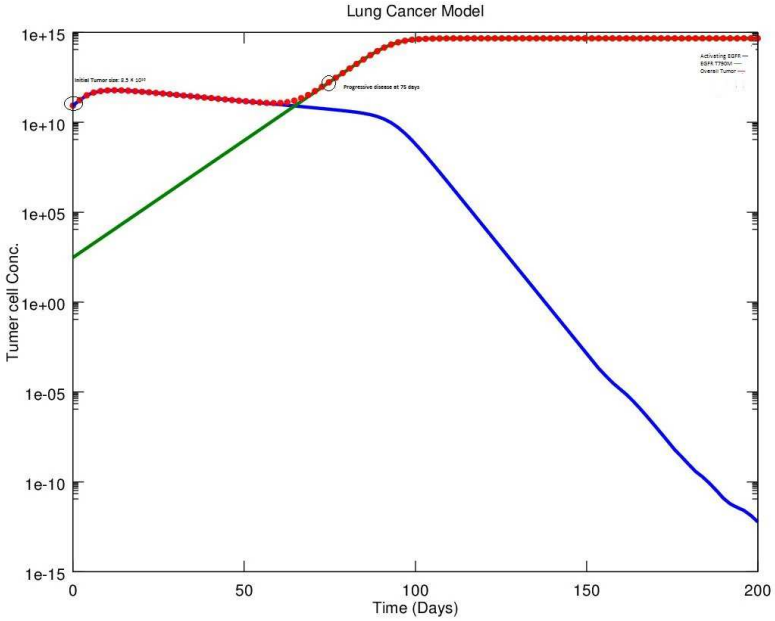

Figure 4.3: No response to gefitinib therapy (Patient ID:26)
*The encircled points represent the real data points obtained by lab experiments

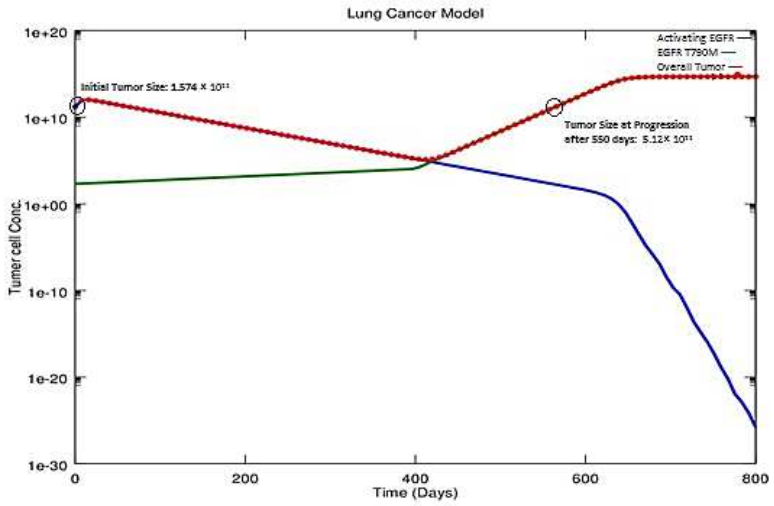

Figure 4.4: Partial response to gefitinib therapy (Patient ID:29)
*The encircled points represent the real data points obtained by lab experiments

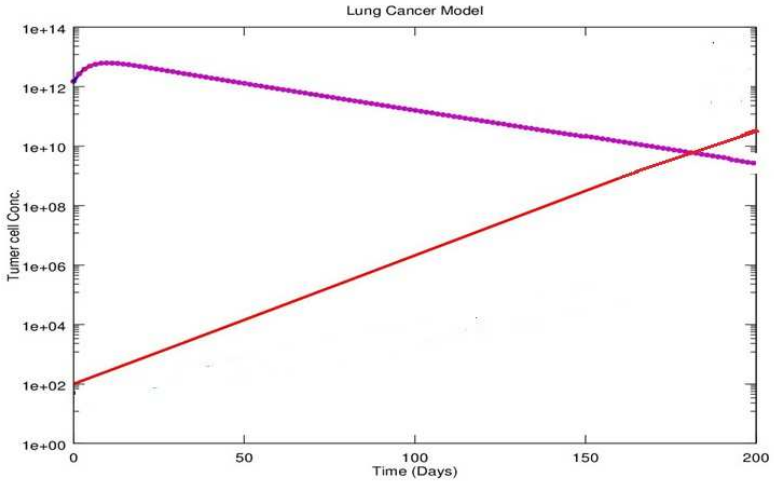

Figure 4.5: Partial response to gefitinib therapy (Patient ID:27)
*The encircled points represent the real data points obtained by lab experiments

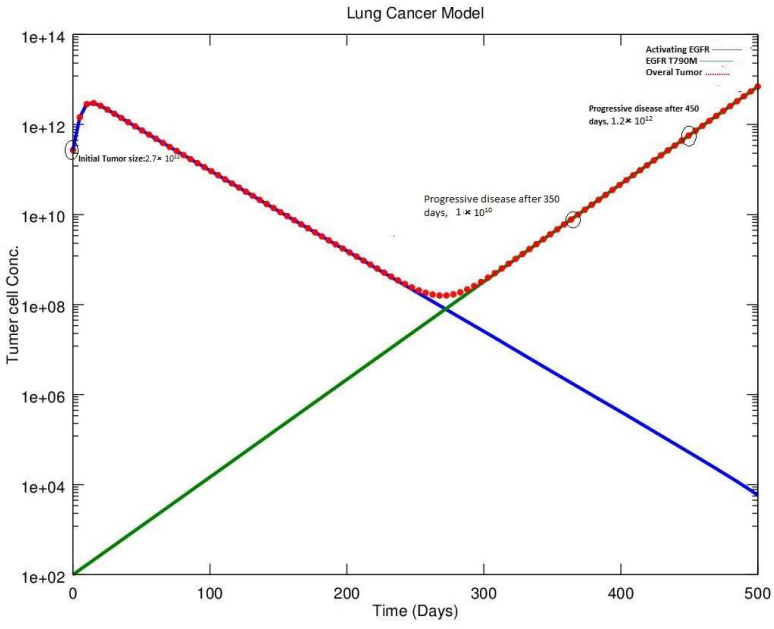

Figure 4.6: Partial response to gefitinib therapy (Patient ID:32)
*The encircled points represent the real data points obtained by lab experiments

Patient ID 20

No tumor size data is available for this patient. The tumor growth curves obtained by only considering the dates of progressive disease and drug start and stop time. This tumor also shows partial response to the gefitinib treatment (fig:4.7)

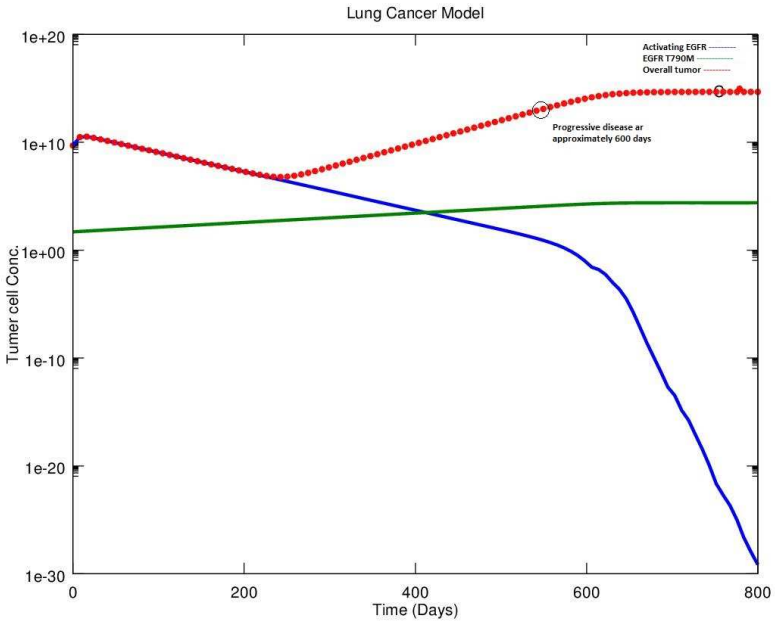

Figure 4.7: Partial response to gefitinib therapy (Patient ID:20)

Patient ID 24

This patient has maximum survival period in our selected group of patients even with the presence of KRAS mutations. In simulations there is only EGFR T790M grow rate who is responsible for this but in real case study the reason is not clear (fig:4.8)

4.5.5 AZD9291 drug effect on EGFR T790M tumor cells induced resistance to gefitinib

AZD9291 is a new drug undergoing experimentation by the scientists to be checked for its clinical usability as second line therapy for NSCLC tumors experiencing EGFR T790 mutations. This drug directly targets EGFR T790M and is very effective to overcome the gefitinib drug resistance crisis caused by EGFR T790M cells. After the validation of lung cancer mathematical model parameters, the model is used to monitor the effects of AZD9291 drug on patients previously treated with Gefitnib. The activity of Gefitnib drug on tumor cells is determined by obtaining the same tumor growth patterns from the real clinical data of the patients. So gefitinib induced death rate, referred as ψ in (4.1)is

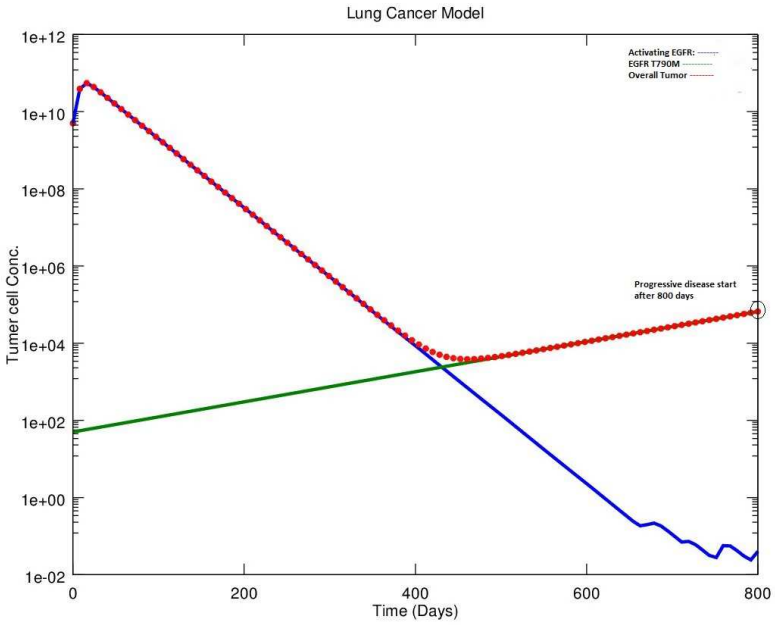

Figure 4.8: Partial response to gefitinib therapy (Patient ID:24)

set to be 0.005 for all patients because this value gives perfect curves as observed in real patients but we do not have any clinical data to assess the activity of AZD9291 drug on our patients. And also there is no sufficient information available in literature for assessing this parameter, so, in the simulations we have estimated the rate of AZD9291 induced cell death by varying the parameter ϕ in (4.3). Every simulation for testing AZD9291 is performed for three different ϕ values (0.05, 0.005 and 0.009)for evaluating the most suitable values for ϕ that provides best representation of drug activity for each patient. The best suitable value is chosen to be

The tumor growth reduction curves obtained with three different values for ϕ can be seen in Fig.4.9, 4.10 and 4.11, respectively. The rate of 0.05 kills all tumor cells very quickly just before 40 days, which is the most unrealistic assumption. The kill rate 0.009 seems to be little OK as compared to previous 0.05 value but it is still a quick elimination of tumor within 3 months which is not so acceptable behaviour. Hence, it is evident that 0.005 value of ϕ gives more realistic result with moderate kill rate of tumor cells as tumor reaches to only 10×7 size after 100 days. Approximately same growth patterns are observed in all of the patients with varying ϕ values and 0.005 value is turned out to be most acceptable value for our simulations

Patient having complete response from gefitinib

The patient having complete response from gefitinib (Patient ID:30) is excluded from this analysis.

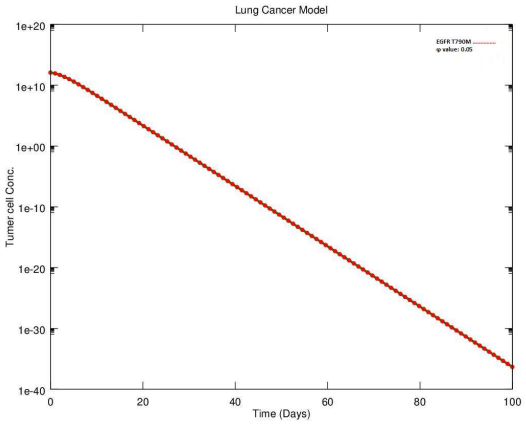

Figure 4.9: ϕ value 0.05

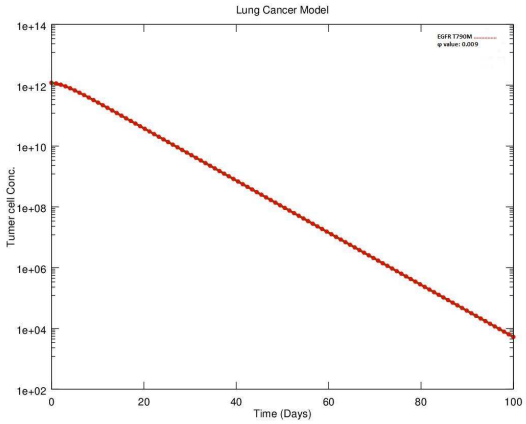

Figure 4.10: ϕ value 0.009

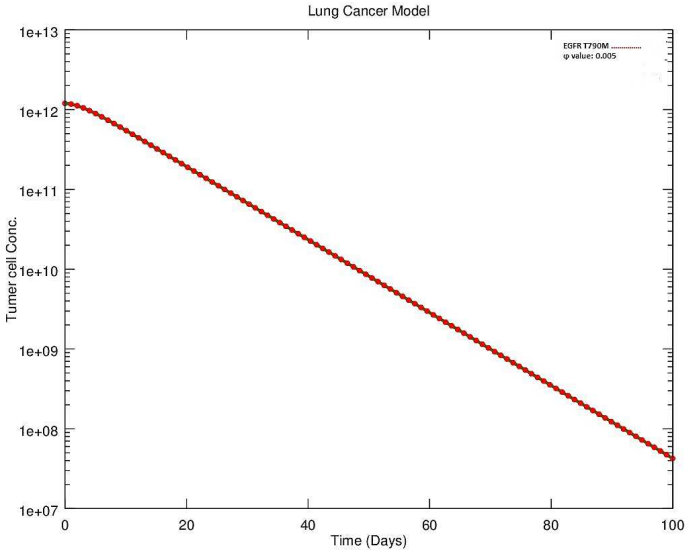

Figure 4.11: ϕ value 0.005

Patient with progressive disease with gefitinib

The treatment with AZD9291 drug is totally ineffective for progressive disease lung tumor(Patient ID:26). As the number of EGFR T790M cells and the grow rate of this mutation was set to be very high at the stop time of gefitinib drug, the treatment seems to fail according to our simulations. Although the appearance of KRAS mutations is also confirmed through laboratory tests but in simulations the KRAS status is not considered as the drug is ineffective even without KRAS mutations. The simulated treatment with AZD9291 drug initially seems to be effective for such type of tumor but the remarkable increase in KRAS cells shows alarming condition which means that this tumor shrinkage due to AZD9291 drug is not so long (fig:4.12).

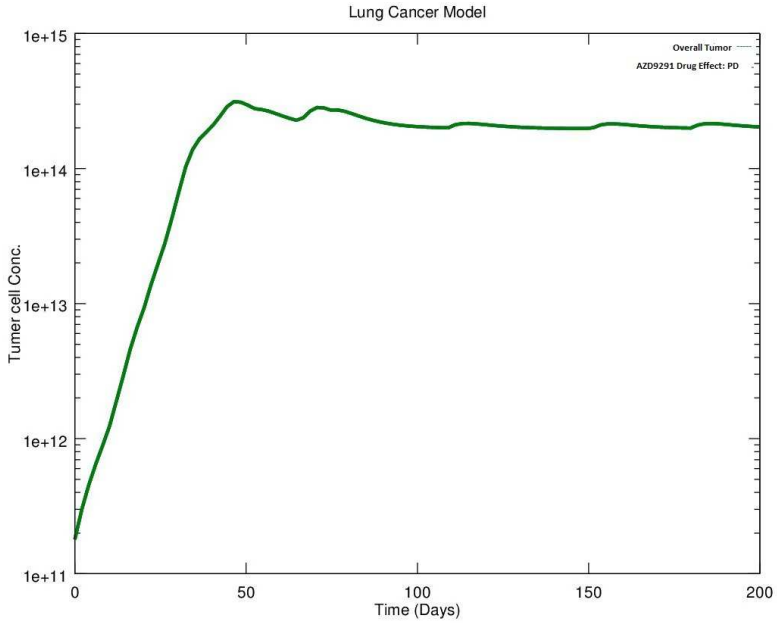

Figure 4.12: Partial response to second-line AZD9291 therapy (Patient ID:26SG)

Patients with partial response to gefitinib without KRAS mutation

The patients having partial responses in previous gefitinib treatment have notable responses to the AZD9291 drug provided with zero KRAS mutation status (Patient ID:29 and Patient ID:32).

Patients with partial response to gefitinib and KRAS mutation

Patients who obtained partial response from the gefitinib and also have presence of KRAS mutation confirmed by experimental ddPCR method also shows the reduction in tumor size with AZD9291 drug(fig:4.15). If we take into account the contribution of KRAS mutated

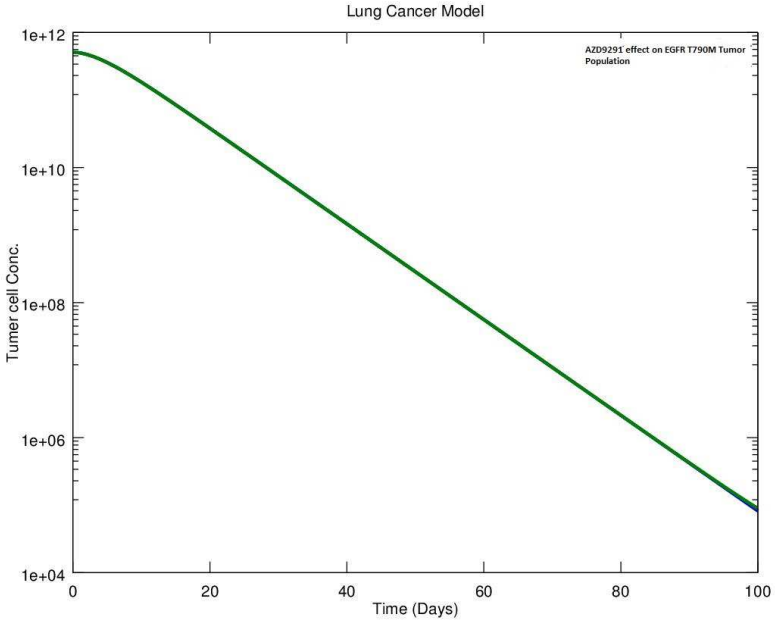

Figure 4.13: Partial response to gefitinib therapy (Patient ID:29)

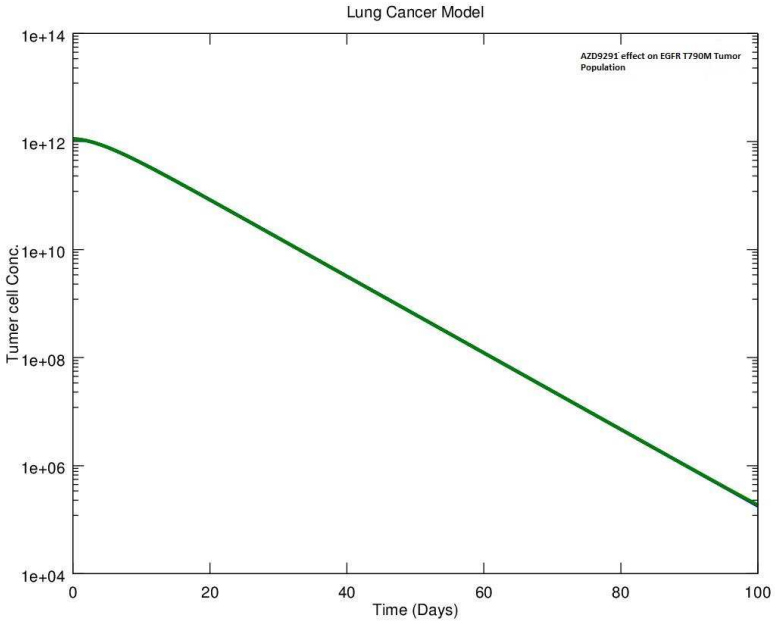

Figure 4.14: complete response to AZD9291 therapy (Patient ID:32)

cells to the overall tumor size. It shows that tumor is going towards the ultimate resistant position, the KRAS resistant state (fig:4.16).

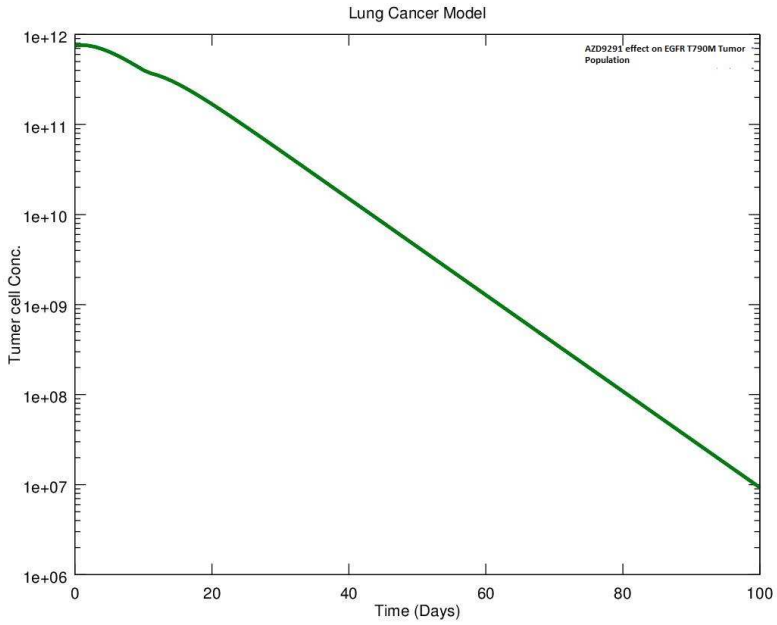

Figure 4.15: Partial response to AZD9291 therapy (Patient ID:27)

4.6 Discussion

Experimental results confirms gefitinib as a very effective TKI drug but only for activating EGFR mutations[217, 218, 219]. The main reported reason for the resistance to gefitinib drug is EGFR T790M. According to our results and previously published research, the appearance of EGFR T790M is inevitable in NSCLC patients. Data obtained from real patients has been utilized to validate our mathematical model. Parameter tuning for the model is done by following *patient's* clinical evaluation data and also by wet lab tumor size calculation results. Most important parameter is the grow rate estimation for each mutation. For our simulations we have only tailored the grow rate for EGFR T790M to obtain different patient profiles matching with real patient data. Although, there is no biological explanation for using differed grow rates of EGFR T790M for each patient but it was necessary to obtain same results as in real patients. It is noticed in our results that smaller the grow rate of EGFR T790M more time it takes to reach the resistant state. Further investigation is required to explore the governing factors for the change in grow rate of mutations from patient to patient. It is a possibility that immune system may have any impact on preferential growth of the mutations because immune system is reportedly involved in pathogenicity and progression of lung tumors [220]. The idea of immunoregulation as lung cancer therapy is recently being explored by researchers [221, 222]. The

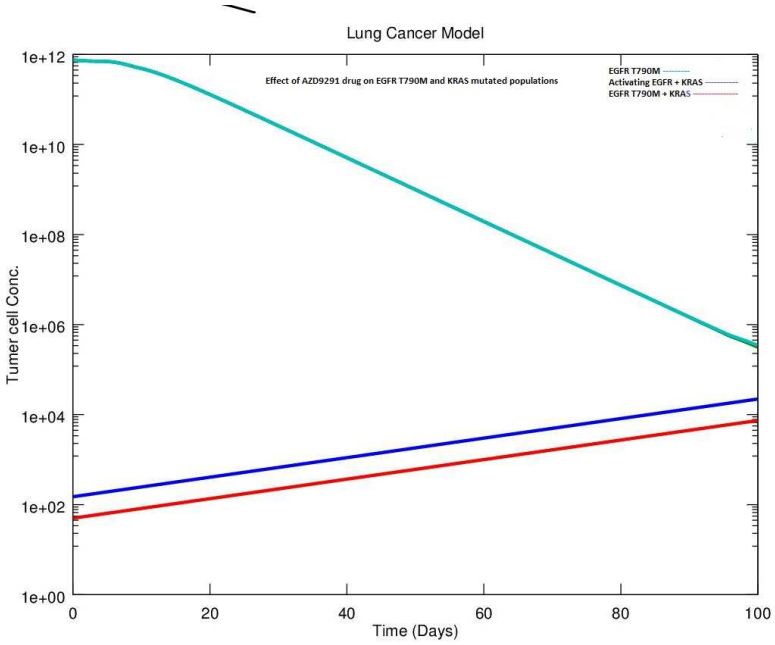

Figure 4.16: Partial response to AZD9291 therapy (Patient ID:27)

modulation of mutation grow rates by immune system can provide key idea towards the immunotherapy of lung cancer. The verified model parameters allows to further utilize this model to investigate the effect of other drugs using same patient specific parameters on NSCLC tumors. In this case the authentication and higher precision of simulated results is also very much guaranteed.

Recent studies introduce a new drug named AZD9291, with promising results for treatment of EGFR T790M. AZD9291 high activity has been confirmed in a very recent study conducted by Janne and colleagues [223]. In previous years various other researchers has also proved that irreversible tyrosine kinase inhibitor activity of AZD9291 has the ability to overcome the resistance issues due to EGFR T790M [224, 225]. AZD9291 drug ability of selective targeting of EGFR T790M has proved this drug to be most effective in near future [226, 227, 228, 229]. This drug has passed few trials for NSCLC patients but still it is not so commonly used by the physicians. The main reason for this is that this drug is still going through the trials. In current research we did simulations to study the impact of AZD9291 on the real patient data treated with gefitinib drug. We collected data for 8 NSCLC patients and produced exactly same results obtained in real patients treated with gefitinib. Then we continued our simulations to check that if the same patients are treated with AZD9291 drug then what will be the effect on tumor size. To our surprise, all of the patients having partial responses with gefitinib drug showed a significant response to AZD9291 drug. Except one patient (patient ID:26) who have progressive disease status after approximately 4 months of the start of treatment with gefitnib drug. Although this patient also shows a significant reduction in tumor size after starting therapy with

AZD9291 but at that time the KRAS mutated cells are much higher in numbers that it is evident that tumor again goes back to more resistant state then before, the KRAS resistant state.

KRAS mutations seem to have no significant impact on the tumor resistance to gefitinib drug but it may effect later during AZD9291 activity. Very recent study by Eberlein and his colleagues pointed out the activation of RAS signalling pathway during AZD9291 treatment and appearance of resistance for this drug [230]. The possible explanation for this behavior is that, if the tumor show slower response to these therapies and continue to stay in almost same state after initial reduction in size then KRAS mutated cells can get a chance to survive and multiply with the passage of time and become able to produce resistance against AZD9291 drug. Hence, AZD9291 drug which tends to be an ideal solution for EGFR T790M merely becomes a life incremental drug by only increasing the life span of patient because underlying KRAS mutations are so strong by that time that its too late for any other drug.

In order to avoid tumor to become KRAS resistant, we need to explore the possibility of treatment of EGFR T790M and KRAS mutations simultaneously. Currently, the practiced lab tests can only reveal about the presence of certain mutations. If we are able to quantify the number of mutated cells for all EGFR T790M and KRAS mutations then it would help in controlling the disease progression towards threshold mortality or death point by making drug administration choices smartly on quantities of mutated populations. At the time of appearance of gefitinib resistance the EGFR T790M is much more in quantity then KRAS so the AZD9291 drug could be the first priority for the treatment of major resistance mutation but before the start of AZD9291 treatment KRAS status must be checked specifically by ddPCR for more accuracy. If the patient is KRAS positive then administer AZD9291 drug very carefully and measure the tumor size on regular basis and if tumor shows little bit of progression then change the AZD9291 drug with chemotherapy or any other drug specifically for KRAS mutations.

In conclusion, the lung cancer patients with EGFR T790M can be recommended to be treated with AZD9291 but only if the patient shows partial response with gefitinib treatment. The behavior of KRAS mutations in lung cancer treatment with AZD9291 drug is still not clear so there is dire need to observe more closely the behavior of KRAS mutations for producing resistance towards AZD9291drug.

Chapter 5

Implementation of Hybrid Models

The hybrid models described in this thesis are implemented in Octave version 3.6.1 [149]. Octave is a free GNU program for doing numerical mathematics. It is a high-level language for numerical computations and it provides a way to find numerical solutions for linear and non-linear problems. The commands of octave are mostly compatible with Matlab. It also has graphics capabilities for data manipulation and visualization.

The hybrid models can be implemented in octave as it has several solvers for the solution of continuous processes e.g. FSOLVE, LSODE etc. The choice of solver depends upon the nature of problem. Non-linear ordinary differential equations can be solved by LSODE solver. The function LSODE uses the solver by Hindmarsh for ordinary differential equations [231] Hybrid models have two parts, continuous and discrete. The implementation of continuous part is easier in octave but incorporating discrete events during continuous process is tricky.

Solving the continuous part of a hybrid model

Solving ordinary differential equations in octave requires just a call to the function. The function LSODE can be used to solve ODEs of the form

$$\frac{dx}{dt} = f(x, t); \tag{5.1}$$

with $x(t_0) = x_0$, which is the initial state of the system.

The differential equations can be written as in the form of a vector called xdot. The coupled differential equations can be solved by representing each equation as xdot(1), xdot(2), xdot(3),...xdot(n).

LSODE takes three arguments: the function $f(x, t)$ containing differential equations as xdot, the initial condition for each xdot equation and the time vector t.

In order to understand how LSODE works, let us consider the following small example of differential equation

$$\frac{dx}{dt} = 10; \tag{5.2}$$

In octave this equation is represented as xdot vector, $xdot(1) = 10;$, and it is written in the function as:

```
function xdot = f(x,t)
 xdot(1)= 10;
endfunction
```

Then in the main file LSODE solver calls this function

```
x0 = [0];
t = linspace (0,20);
y =lsode (@ (x,t) f (x,t), x0, t);
A = (y(:,1));
plot (t,A)
```

This was the simple illustration for solving differential equations by LSODE.

Adding the discrete behavior

Implementing discrete transitions to continuous processes in octave is a bit tricky part. Simple discrete transitions can be implemented as simple if, else conditions inside the xdot function. For example in equation

$$\frac{dx}{dt} = 10 + Transition(x); \tag{5.3}$$

where the discrete function transition is

$$Transition(x) = \begin{cases} 20, & \text{if } x \geq 100 \\ 10, & \text{otherwise} \end{cases}$$

The discrete step of this type can be easily implemented in if,else conditions for xdot equations

```
function xdot = f(x,t)
if (x(1)<100)
    xdot(1) = 10;
else
   xdot(1) = 20;
endif
endfunction
```

But the problem arises when modeler try to change the value of $x(1)$ by some external function because LSODE do not allow to change the values of xdot equations. This can be considered as the limitation of LSODE. So, trying to add discrete events needs to be done by playing some tricks with the solver and this method can vary according to the modeling problem.

The implementation of proposed hybrid models for the colorectal and lung cancer case studies differs on the basis of discrete processes in both case studies.

Discrete step implementation in colorectal cancer case study

In the first case study of colorectal cancer the discrete event is the administration of two drugs simultaneously at different time steps, one is administered weekly and other bi-weekly. The discrete function is added in the equation for both of the drugs as:

$$\frac{dM}{dt} = -\gamma M + v_M(t) \tag{5.4}$$

$$\frac{dA}{dt} = -\eta A - \lambda(T_w + T_m)\frac{A}{h_2 + A} + v_A(t) \tag{5.5}$$

In both equations the $v_M(t)$ and $v_A(t)$ can be described as discrete drug administration functions, which is the dosage of drug. Adding drug dose is easier but decide when to administer this dose is difficult because LSODE computes its own time steps for the computations and adding discrete function at time steps defined by LSODE returns wrong results. So, in order to define at which time step a discrete step of drug administration is performed there is the need to define some external function. The external function which can manage to handle time steps in LSODE is the basic requirement for this. Octave provides a way for this by tailoring $'train - sigmoidal'$ function with discrete steps. $Sigmoid - train$ function simply evaluates a train of sigmoid functions at t. It takes range, rc and t. Range determines the number and duration of each sigmoid, *rc* is an array that defines the rising and falling time constants of each sigmoid and t is the time vector. This function can be manipulated to handle the time of discrete events to be performed. Octave code for using this function and passing the resulting values as external parameters to LSODE is given below. It simply describes the timing of the discrete events to be performed.

```
range= [0  0.05;7  7.05;14  14.05;21  21.05];
rc= [0  0;0  0;0  0;0  0];
sigmoid_train= @ (t) sigmoid_train (t, range, rc);
y = lsode (@ (x,t) xdot (x,t,sigmoid_train), x0, t);
```

Inside the xdot function of above drug equations the information from $sigmoid - train$ function is added as:

```
xdot(1)= -gamma x(1) + (sigmoid_train(t))*dose1;
xdot(2)= -eta x(2)-lambda*(Tw+Tm)*x(2)}/{h2+x(2)} +
         (sigmoid_train(t))*dose2;
```

Where the dose1 and dose 2 correspond to the administration of dose for drug 1 and drug 2, respectively.

Discrete step implementation in lung cancer case study

In lung cancer case study the discrete step is also an administration of two drugs but not simultaneously and is dependent upon the overall size of the tumor. The tumor is initially

treated with drug1 and when it reaches to certain threshold size then the drug is switched to drug2. There are again two equations (5.6)(5.7) for two type of drugs but switching between drug1 and drug2 on the basis of tumor size is the main discrete step. Another discrete step is same as in the previous model, the drug administration at discrete time steps and is done in the same way as described before. The equation (5.6) is for gefitinib drug, the drug administration at regular intervals is maintained with discrete function vG(t). The equation (5.7)is for the drug AZD9291, here again the drug administration is maintained by discrete function vSG(t). Both vG(t) and vSG(t)are implemented in the same way as previous model.

$$\frac{dG}{dt} = -\gamma G + vG(t) \tag{5.6}$$

$$\frac{dSG}{dt} = -\beta SG + vSG(t) \tag{5.7}$$

The switch between therapy is simply done by if, else condition, where T represents overall tumor size.

```
if(T<= threshold)
xdot(1)= -gamma x(1)
else
xdot(2)= -beta x(2)
endif
```

The proposed hybrid models for the colorectal and lung cancers can also be implemented in some other ways by using Mathematica, Matlab or by R language, but the octave is chosen for the implementation because of its free availability and having good numerical computation power. It is very easy to solve differential equations by octave and the described models containing many complex differential equations. The octave code for colorectal cancer and lung cancer case studies can be accessed on the links provided at [232]and [233], respectively.

Chapter 6

Conclusion and Future Work

The hybrid modeling for cancer drug resistance mechanisms can provide a breakthrough in personalized cancer therapy. The proposed hybrid models for colorectal and lung cancer very much enlightens about the drug resistance mechanisms specific to these cancers. The involvement of immune system is very critical in the treatment procedure of cancer and its role during the therapy is very much highlighted in the development of the models. Further extensions to these models may lead towards more efficient and accurate therapeutic procedures.

In case of colorectal cancer, by utilizing the proposed model, the level of KRAS mutations could be measured, that can be tolerated to avoid resistance to anti-EGFRs. This could provide information to stop the anti-EGFR treatment before reaching the threshold value for KRAS mutant cells. The treatment could be switched from anti-EGFR to anti-KRAS drugs. The clinical perspective about switching treatments is not known, but this could provide a better way to solve the secondary KRAS mutation problem in colorectal cancers.

The application of different monoclonal drugs and by varying administration schedules or dosages for same drugs in colorectal cancer, may also provide a solution for the precise personalized therapy of colorectal tumors. The further exploration of KRAS mutated cells fate as cancer stem cells and development of tumor heterogeneity is required. Tumor heterogeneity makes the problem of resistance against the drugs even worse, as a small number of mutated cells are able to make drugs ineffective even for a large number of wild-type cells. The future plan is to further investigate this interplay between wild-type and mutant cells caused by tumor heterogeneity.

As future work, a stochastic feature can be introduced for KRAS mutations in the current model in order to increase the accuracy of the model. The current model could also be upgraded to cellular hybrid automata to observe the behavior of small number of KRAS mutations in the presence of drug and the impact of minute quantity of these mutations on surrounding tumor populations. The cellular automata are well known for modeling the spatial information of tumors. In order to analyze the growth and effect of KRAS mutated population in colorectal cancer the spatial information for these cells is essential to consider in a model.

Similarly, in case of lung cancer, the strict regulations of treatment procedures is required to avoid the transition of tumor from non-resistant to resistant states. The simultaneous or combined treatment of gefitinib and AZD9291 drugs could be checked for controlling the resistant sub-populations in tolerable proportions.

In order to make the current lung hybrid model more easier for the management of drug introduction and scheduling in future, it is needed to upgrade this model by parallel composition of lung tumor hybrid automata with drug controller hybrid automata. The idea behind the parallel composition of tumor and drug automata is to make the personalized treatment of lung tumors easier. In the parallel compositions of the model tumor automata will be only comprised of tumor sub-populations and drug automata will control the introduction of both of the drugs. The tumor automaton will be composed/sunchronized with the drug controller via the help of some shared variables. The tumor progression with increase in multiple sub-populations in separate tumor automata and the parallel drug controller automaton will help the drug management with respect to the proportions of resistant sub-populations of the tumor. It will become easier to model the effect of any other drug for the lung tumor by having a drug controller automata independent of tumor growth automata. The lung tumor heterogeneity can also be observed and managed by introducing a descriptive automaton linked to tumor state, which can provide the description of dominant tumor sub-population as the tumor progresses with respect to the therapy. This tumor heterogeneity information along with tumor progression behavior seems promising to point out the more effective drug administration procedures for personalized treatments of lung cancer.

Appendix

Table 6.1: Tumor Equations Parameters

Parameter	Description	Value
a	Logistic tumor growth	$2.31 \times 10^{-1}(Day^{-1})$
b	Inverse of carrying capacity	$2.146 \times 10^{-10}(Cells^{-1})$
c	Rate of NK induced tumor death	$5.156 \times 10^{-14}(LCells^{-1}Day^{-1})$
ξ	Rate of NK induced tumor death through ADCC	$6.5 \times 10^{-10}(LCells^{-1}Day^{-1})$
h_1	Concentration of moAbs for half maximal increase in ADCC	$1.25 \times 10^{-6}(mgL^{-1})$
K_t	Rate of chemotherapy induced tumor death	$0 - 8.1 \times 10^{-1}(Day^{-1})$
K_{at}	Additional chemotherapy induced tumor death due to moAbs	$4 \times 10^{-4}(Lmg^{-1}Day^{-1})$
δ	Medicine efficacy coefficient	$2 \times 10^{-1}(Lmg^{-1})$
ψ	Rate of moAb induced tumor death	$2.28 \times 10^{-2}(Lmg^{-1}Day^{-1})$

Table 6.2: NK Cell Equation Parameters

Parameter	Description	Value
$\frac{e}{f}$	Ratio of NK cell synthesis rate with turnover rate	$\frac{1}{9}$
f	Rate of NK cell turnover	$1 \times 10^{-2}(Day^{-1})$
p	Rate of NK cell death due to tumor interaction	$5.156 \times 10^{-14}(LCells^{-1}Day^{-1})$
p_a	Rate of NK cell death due to tumor moAb complex interaction	$6.5 \times 10^{-10}(LCells^{-1}Day^{-1})$
p_n	Rate of IL 2 induced NK cell proliferation	$5.13 \times 10^{-2}(Day^{-1})$
g_n	Concentration of IL 2 for half maximal NK cell proliferation	$2.5036 \times 10^{5}(IUL^{-1}))$
K_n	Rate of NK depletion from chemotherapy toxicity	$9.048 \times 10^{-1}(Day^{-1})$
δN	Chemotherapy toxicity coefficient	$2 \times 10^{-1}(Lmg^{-1})$

Table 6.3: CD8+ T Cell Equation Parameters

Parameter	Description	Value
θ	$Concentration of IL-2 to halve CD8+ T-cell turnover$	$2.5036 \times 10^{-3}(IUL^{-1})$
m	Rate of activated $CD8+$ $T-cell$ turnover	$5 \times 10^{-3}(Day^{-1})$
j	Rate of $CD8+$ T cell lysed tumor cell debris activation of $CD8+$ T cells	$1.245 \times 10^{-4}(Day^{-1})$
k	Tumor size for half maximal $CD8+$ T lysed debris $CD8+$ T activation	$2.019 \times 10^{7}(Cells)$
q	Rate of $CD8+$ T cell death due to tumor interaction	$5.156 \times 10^{-17}(Cells^{-1}Day^{-1})$
r_1	Rate of NK lysed tumor cell debris activation of $CD8+T$ cells	$5.156 \times 10^{-12}(Cells^{-1}Day^{-1})$
r_2	Rate of $CD8+$ $T-cell$ production from circulating lymphocytes	$1 \times 10^{-15}(Cells^{-1}Day^{-1})$
u	$CD8+$ $T-cell$ self limitation feedback coefficient	$3.1718 \times 10^{-14}(L^2Cells^{-2}Day^{-1})$
κ	Concentration of $IL-2$ to halve magnitude of $CD8+$ $T-cell$ self regulation	$2.5036 \times 10^{3}(IUL^{-1})$
K_l	Rate of $CD8+$ $T-cell$ depletion from chemotoxicity	$4.524 \times 10^{-1}(Day^{-1})$
δL	Chemotherapy toxicity coefficient	$2 \times 10^{-1}(Lmg^{-1})$
p_i	Rate of $IL-2$ induced $CD8+$ $T-cell$ activation	$2.4036(Day^{-1})$
g_i	Concentration of $IL-2$ for half-maximal $CD8+$ $T-cell$ activation	$2.5036 \times 10^{3}(IUL^{-1})$

Table 6.4: Lymphocyte Equation Parameters

Parameter	Description	Value
$\frac{\alpha}{\beta}$	Ratio of rate of circulating lymphocyte production to turnover rate	$3 \times 10^9 (CellsL^{-1})$
β	Rate of lymphocyte turnover	$6.3 \times 10^{-3} (Day^{-1})$
K_c	Rate of lymphocyte depletion from chemotherapy toxicity	$5.7 \times 10^{-1} (Day^{-1})$
δC	Chemotherapy toxicity coefficient	$2 \times 10^{-1} (Lmg^{-1})$

Table 6.5: Interleukin Equation Parameters

Parameter	Description	Value
μ	Rate of excretion and elimination of $IL-2$	$11.7427(Day^{-1})$
ϕ	Rate of $IL-2$ production from $CD4+/naive\ CD8+T$ cells	$1.788 \times 10^{-7} (IUCells^{-1}Day^{-1})$
ω	Rate of $IL-2$ production from $CD8+T$ cells	$7.88 \times 10^{-2} (IUCells^{-1}Day^{-1})$
ς	Concentration of $IL-2$ for half-maximal $CD8+\ T-cell\ IL-2$ production	$2.5036 \times 10^3 (IUL^{-1})$

Table 6.6: Medication Equation Parameters

Parameter	Description	Value
γ	Rate of excretion and elimination of chemotherapy drug	$4.077 \times 10^{-1} (Day^{-1})$
η	Rate of moAb turnover and excretion	$1.386 \times 10^{-1} (Day^{-1})$
λ	Rate of moAb-tumor cell complex formation	$8.9 \times 10^{-14} (mgCells^{-1}L^{-1}Day^{-1})$
h_2	Concentration of moAbs for half-maximal EGFR binding	$4.45 \times 10^{-5} (mgL^{-1})$

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