CAPITAL UNIVERSITY OF SCIENCE AND TECHNOLOGY, ISLAMABAD

Insilico Profiling of Bortezomib Resistant Biomarkers in Waldenstrom Macroglobulinemia and Pharmacokinetic-Pharmacodynamic Analysis

by

Anum Munir

A thesis submitted in partial fulfillment for the degree of Master of Science

in the

Faculty of Health and Life Sciences
Department of Biosciences

2019

First of all, I dedicate this research thesis to ***ALLAH Almighty****, the Most Gracious, the Most Merciful above the Throne established, always ready to help us and forgive us, the Creator of the Earth, the Heavens, and the universe, so of course not restricted in anything, the knower of all things, seen and unseen, causes life and death, and when He decides upon something, all He has to say is BE!*

And

Dedicate to ***Prophet Muhammad (PBUH)****, the most Poius of people,the one who refrains from arguing even if he is right, the gloriously adorned in the robe of divine revelation, the ocean of mercy, the king of the Night Journey, the prayer-leader of all the prophets, seal of the Prophets and the Kaba of Love,*

And

Dedicated to my ***Parents and Brothers****, who pray for me and always pave the way to success for me, the only true lovers, truly selfless, unconditional and forgiving, they're the only group that support me at every stage of my life*

And

Dedicated to my ***Teachers****, who are a persistent source of inspiration and encouragement for me, who transfered their knowledge to me and made me better human being*

CERTIFICATE OF APPROVAL

Insilico Profiling of Bortezomib Resistant Biomarkers in Waldenstrom Macroglobulinemia and Pharmacokinetic-Pharmacodynamic Analysis

by

Anum Munir

MBI171001

THESIS EXAMINING COMMITTEE

S. No.	Examiner	Name	Organization
(a)	External Examiner	Dr. Asma Gul	IIU, Islamabad
(b)	Internal Examiner	Dr. Marriam Bakhtiar	CUST, Islamabad
(c)	Supervisor	Dr. Sahar Fazal	CUST, Islamabad

Dr. Sahar Fazal
Thesis Supervisor
April, 2019

Dr. Sahar Fazal
Head
Dept of Biosciences
April, 2019

Dr. Muhammad Abdul Qadir
Dean
Faculty of Health and Life Sciences
April, 2019

Author's Declaration

I, **Anum Munir** hereby state that my MS thesis titled **"Insilico Profiling of Bortezomib Resistant Biomarkers in Waldenstrom Macroglobulinemia and Pharmacokinetic-Pharmacodynamic Analysis"** is my own work and has not been submitted previously by me for taking any degree from Capital University of Science and Technology, Islamabad or anywhere else in the country/abroad. This thesis is an account of research undertaken between July 2018 to January 2019 at The Department of Bioscience, Faculty of Health and Life Sciences, Capital University of Science and Technology, Islamabad.

At any time if my statement is found to be incorrect even after my graduation, the University has the right to withdraw my MS Degree.

(Anum Munir)

Registration No: MBI171001

Plagiarism Undertaking

I solemnly declare that research work presented in this thesis titled "**Insilico Profiling of Bortezomib Resistant Biomarkers in Waldenstrom Macroglobulinemia and Pharmacokinetic-Pharmacodynamic Analysis**" is solely my research work with no significant contribution from any other person. Small contribution/help wherever taken has been dully acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and Capital University of Science and Technology towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS Degree, the University reserves the right to withdraw/revoke my MS degree and that HEC and the University have the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized work.

(Anum Munir)

Registration No: MBI171001

Acknowledgements

In the name of Allah, the beneficent, the merciful. Praise be to the Lord of the Universe who has created us and made us into tribes and nations, that we may know each other, not that we may despise each other. I have been able to accomplish this research work and come up with final dissertation work which is necessary for the award of the degree of Masters in Bioinformatics.

Foremost, I would like to express my sincere gratitude to my Supervisor Dr. Sahar Fazal, and Co-Supervisor Dr. Aamer Iqbal Bhatti for the continuous support during my research, for their patience, motivation, enthusiasm, and immense knowledge. Their guidance helped me in all the time of research and writing of this thesis. I could not have imagined having better advisors and mentors for my MS. The doors to their offices were always open whenever I ran into a trouble spot or had a question about my research or writing. They steered me in the right direction whenever they thought I needed it.

I would also like to say heartiest thanks to my teachers Dr. Muhammad Abdul Qadir, Dr. Shaukat Iqbal Malik, Dr. Marriam Bakhtiar, Dr. Erum Dilshad, Dr. Aamer Nadeem, Dr. Nayyer Masood, and Dr. Arshia Ameen Butt for sharing their seemingly infinite knowledge and extraordinary kindness throughout my studies. They made me think, allowed me to dream, all of them also helped me in doing a lot of other Research and I came to know about so many new things I am really thankful to them.

Secondly, I would also like to say thanks to my parents for giving birth to me at the first place and supporting me through their prayers, spiritually, and financially throughout my life, my brothers who helped me a lot in finalizing this project within the limited time frame. Further, I am grateful to my friends Ms. Hajra Qayyum, Ms. Sana Masood, Ms. Sahar Aslam, Sana Elahi, and Ms. Anila Sajjad for encouraging me and for being always there for me to pray and support whenever I need them.

I would also like to say thanks to my hostel room mate Ayesha Shakeel from department of Management Sciences CUST and friend Afia Qummar from department of Computing CUST for helping me throughout my stay and in times of need. Last but not least, I would like to thank my students for praying whenever I needed their prayers, the faculty members, and colleagues of my College GPGCM for helping me throughout my studies, and for providing full support.

Thanks to All

Abstract

Waldenstrom's macroglobulinemia (WM) or lymphoplasmacytic lymphoma is a kind of non-Hodgkin lymphoma. The malignant cells make a lot of abnormal macroglobulin protein. It starts in B cells and develops essentially in the bone marrow that makes the distinctive types of platelets. This can prompt low dimensions of blood cells which makes it difficult for the body to fight disease. WM is characterized by hypersecretion of immunoglobulin-M and infiltration of neoplastic B-cells into the bone marrow and lymphoid tissues. Chemoimmunotherapy is commonly used for clinical management of WM, however, novel targeted agents such as the BTK-inhibitor, Ibrutinib and the proteasome inhibitor Bortezomib have shown significant improvement in patients with relapsed/refractory WM. Despite their activity though, drug resistance and relapse are common and there is limited insight into the mechanisms responsible for resistance to these targeted agents. The aim of the current study is to identify genes associated with Bortezomib-resistance in WM cells and their response to Pharmacokinetics-Pharmacodynamics modeling. Using Bortezomib-resistant and wild type isogenic WM cell lines, 25 genetic Biomarkers of acquired resistance to Bortezomib are identified through analysis of directed genes interaction networks and proposed for clinical studies. Among them, 12 are known. Resistant Biomarkers associated pathways are retrieved through the Reactome database and cross-checked through KEGG and other databases, the identified biomarkers are annotated and mapped to the pathways to determine their role in WM. Furthermore, the Pharmacokinetics-Pharmacodynamics (PK/PD) modeling is also done in the study to determine the effect of selected therapies on the tumor and response of Biomarkers against them. The PK profiles are generated using clinical trials data to determine the effects of the body on drug concentration, after PK profiles, the pharmacodynamics analysis is performed to determine the effects of drugs on the body. It is observed that the Bortezomib and Ixazomib reduced the tumor weight initially but as soon as the dose is stopped tumor again started to increase, Carfilzomib and Oprozomib showed better results, Rituximab more efficiently reduce the weight of tumor. Finally, the simulations of

the models are biologically validated through comparison with clinical pharmacokinetics results. In order to determine the response of Biomarkers against specific drugs, Biomarkers response model is designed by using gene expression data of WM, and drugs doses are administered. The dynamic levels of a Biomarkers observed for each dose are key marker uncovering the information and procedures of the medication activity. In conclusion, the properties and effects of drugs on tumor growth can be portrayed by this type of modeling studies and incorporated portion Biomarkers-response models can be developed with high integrity of-fit and incredible predictive capacity. This methodology shed new light on the itemized procedures and system of PK/PD modeling and may offer a significant reference for a proper dosing regimen in further clinical applications.

Contents

List of Figures

List of Tables

Abbreviations

AUC	Area Under Curve
BDR	Borteomib Dexamithasone Ritusimab
BendaR	Bendamustine-Rituximab
BTK	Bruton tyrosine Kinase
CaRD	Carfilzomib with Rituximab and Dexamithasone
CHOP	Cyclophasphamide
CHOP-R	Cyclophasphamide with Rituximab
Clint	Drug clearance
CL	Clearance
FDA	Food and Drug Administration
GO	Gene Ontology
HER	HUman epidermal growth factor receptor
HER	HUman epidermal growth factor receptor
KEGG	Kyoto encyclopedia of genes and genomes
MGUS	Monoclonal gammopathy of unknown significance
MDR	Multi drug resistance
MM	Multiple Myeloma
NF-KB	Nuclear Factor Kappa B
NP	Nucleoside Phosphorylase
PGK1	Phosphoglycerate Kinase-1
PSA	Prostate specific antigen
PD	Pharmacodynamics
PK	Pharmacokinetics
PK/PD	Pharmacokinetics-Pharmacodynamics

PPIN	Protein-protein interaction networks
SWM	Smoldring WM
TLR	Toll like receptor
TNF	Tumor Necrosis Factor
WHIM	Warts Hypogammaglobulinemia Infections Myelokathexis
WHO	World Health Organization
WM	Waldenstrom's Macroglobulinemia

Chapter 1

Introduction

1.1 Cancer

Cancer is the second driving reason for mortalities around the world. Generally, the prevalence of malignancy has really increased; just in the United States alone, around 1,665,540 individuals experienced tumor, and 585,720 of them died because of this malignancy by 2014 [1, 2]. Therefore, cancer is a serious issue influencing the health of every human. Tragically, it is a variable disease at the tissue level and this assortment is a noteworthy test for its particular determination, trailed by the viability of treatment [3, 4]. In men, the most elevated rates of cancer are the prostate, rectum and colon, bronchus, lungs, and urinary bladder. In ladies, malignancy pervasiveness is most astounding in the bronchus, breast, lung, colon, uterus, rectum, uterine corpus, ovarian, and thyroid, individually [5]. For children, the most noteworthy rate of malignancy are tumors identified with the lymph nodes, brain, and blood cancers [6, 7].

The tumor begins with a progression of progressive mutations in the genes with the goal that these mutations change cell functions. Generally, Cancer disturbs the activities of cells and results in the dysfunction of important genes. This disruption is viable in the cell cycle and prompts irregular multiplication [8, 9].

Proto-oncogenes are responsible for cell division and development under an ordinary condition, however, progress toward becoming oncogenes during hereditary mutations, which are most unsafe for cell presence[10]. In the previous three decades, researchers have revealed a significant volume of data about genes and proteins and their roles in tumor development. The actual role of transformed genes in malignant cells is the most vital disclosures. Recently, ecological variables identified with hereditary changes have been reported With the assistance of various molecular strategies [1].

Waldenstrom macroglobulinemia (WM) is an uncommon kind of malignancy that starts in the white blood cells. It is viewed as a kind of non-Hodgkin's lymphoma. It's occasionally called lymphoplasmacytic lymphoma. The most persistent feature of the bone marrow or lymph nodes of patients with WM is the appearance of pleomorphic B cells at various phases of development, for example, small lymphocytes, lymphoplasmacytoid cells, and plasma cells [11].

1.2 Molecular Basis and Treatment Options

There is a convincing proof that the hereditary changes are associated with the tumorigenesis. Hereditary changes that prompt genetic disorders and oncogene generation incorporate point mutations, gene amplification, chromosomal translocation, deletions, and insertions. Molecular investigations of cancer cells gather information that demonstrates several hereditary injuries of different combinations of oncogenes and tumor silencer genes in cancer, proposing participation of both kinds of genes. Hereditary damage of oncogenes ordinarily brings about gain of the function mutations while that of tumor silencer genes loss-of-function [12].

Cancer can be treated by chemotherapy, hormonal therapy, surgery, radiation therapy, targeted therapy, and synthetic lethality. The decision of treatment relies on the area and grade of the tumor and the phase of the illness, and in addition the general condition of the patient [13]. The poor diagnosis, visualization, and treatments of the ailment could be, for the most part, improper because of the

variety of severities, locations, durations, affectability and resistance against drugs, comprehension of pathogenesis, and cell origin and differentiation. With expanding proof that the networks and interactions amongst genes and proteins assume to play a critical role in the examination of molecular mechanisms of cancer, it is fundamental and vital to present another idea of systems clinical medicine, to integrate clinical science, system biology, omics-based innovation, computational biology and bioinformatics to enhance analysis, treatments and prognosis of cancer [14].

1.3 Drug Resistance in Cancer

Drug resistance is a common issue that occurs when an illness becomes tolerant of pharmaceutical medicines. This idea was first considered when microorganisms became resistant to specific anti-infection agents, yet from that point onwards comparable mechanisms have been found to occur in different sicknesses, including cancer. A few strategies for drug resistance are specific to the disease, while others, for example, drug efflux, which is seen in microorganisms and human cancers that are resistant to several drugs, are developmentally monitored. However, different kinds of cancers that are at first vulnerable to chemotherapy, after some time they can create resistance through these and different components, for example, DNA transformations and metabolic changes that cause medication degradation and inhibition [15].

There are several issues in the cancer treatment, for example, resistance against cytotoxic operators and harmful chemotherapy. Currently, 90 percent of disappointments in the chemotherapy occurs due to the intrusion and metastasis of cancer identified with drug resistance. In chemotherapy, due to the administration of a specific medication, an extensive number of patient tumor cells shows resistance to the medication. In this way, the drug resistance acts as a significant issue in the field of the tumor [16]. The mechanisms of resistance in a large number of cancer are unknown due to involvement of several pathways and genes.

Multidrug resistance (MDR) in the tumor chemotherapy has been taken attention because of the ability of cancer cells to resist against an extensive variety of hostile anti-cancer drugs. MDR might be created by the expanded drug release outside the cells. So the drug absorption is decreased in these cells [17]. Similarly, the Proteasome inhibitors are used to cure WM but the patients did not respond to treatment because of resistance mechanisms.

1.4 Bioinformatics in the Treatment of Cancer

Bioinformatics is a multidisciplinary field that turns out from the combination of different sciences and areas like software engineering, biology, chemistry, statistics, arithmetic, and considerably more [18–20]. It is one of these sciences which have an immense impression in the medical field, attracts people and enhances their interests in the medical industry[21, 22]. Because of expansive and quick strides in the medical field research, a ton of endeavors are reached out keeping in mind the end goal to figure out how to identify, analyze and treat such dangerous ailments. Additionally, the rise of the Human Genome project revelation in 2003 had put more weight on Bioinformatics to be connected in the tumor treatment.

Bioinformatics is presently being connected in the cancer research and treatment [23], and obviously, specialists and scientists have executed fast and extended measure of research on the tools of bioinformatics that are viewed as fundamental for the tumor treatments [24]. Bioinformatics is one of the different approaches to concentrate bioinformatics strategies in cancer, as per the specificity of disease metabolism, correspondence, signaling, and proliferation. The specificity, applicability, and integration of approaches, computational devices, programming, and databases which can be utilized to investigate the molecular components of disease, recognize and approve novel biomarkers, network-based biomarkers, and personalized pharmaceuticals in cancer [14].

In this study, we have discussed a method of the identification of Network-based resistant biomarkers for Bortezomib in Waldenstrom macroglobulinemia (WM)

and pharmacokinetic - pharmacodynamics (PK-PD) Modeling and simulations of Bortezomib and other Drugs used in WM.

1.5 Bioinformatics Approaches for Biomarkers Identification

Bioinformatics is playing a more vital role in the recognizable proof and approval of biomarkers, particular to clinical phenotypes identified with early findings and estimations to screen the advancement and progression of the disease, its reaction to treatment, and indicators for the change of patient's life quality [25]. Of genes, protein, peptide, synthetic or physic-based factors in the tumor, biomarkers are researched from a single to various markers, from the static network system to dynamic one and from the expression to function. Network-based biomarkers as another sort of biomarkers with protein-protein interactions are identified with the coordination of learning on protein interactions, annotations, and signaling pathway [26, 27]. Tumor biomarkers ought to have the characters of dynamics, networks, communications, and specificities to sickness analysis, prognosis, and treatment [28]. To determine the response of particular biomarker, A Pharmacokinetic-pharmacodynamics (PK/PD) model could be used. The models ascertain the drug concentration that accomplishes the desired response of biomarkers in a patient, not just PK changes in patients, yet in addition for variety in the pharmacological reaction.

1.6 Pharmacokinetic-Pharmacodynamic Modeling and Simulations

Pharmacokinetic (PK) and pharmacodynamic (PD) data arise from the scientific preface of present-day pharmacotherapy. Pharmacokinetics depicts the drug concentration-time courses in body fluids because of the administration of specific

drug dosage, where as pharmacodynamics is the prediction of observed effects because of a specific concentration of a drug. The basis for PK/PD-modeling is to connect pharmacokinetics and pharmacodynamics so as to build up and assess dose-concentration-response relationships and in this way depict and foresee the effect-time courses of a drug dose [29]. Generally, in light of the basic physiological process, PK/PD modeling ought to be favored at feasible times. The extended utilization of PK/PD modeling is thought to be very helpful for drug advancement and in addition, connected pharmacotherapy will doubtlessly enhance the present condition of therapeutics.

1.7 Purpose of the Study

The purpose of this study is to identify those biomarkers that show resistance against Bortezomib. These biomarkers should set up the right conclusion with high affectability and specificity, can likewise be utilized to predict whether a given patient may get benefit by a given treatment? These not just guide the determination of patient subsets for particular medicines, however, will distinguish new restorative targets. The purpose of this research study is also to perform PK/PD modeling to predict the Biomarkers response to Bortezomib. The biomarkers which will be predicted in this research will be used for the early identification of cancers, design of individual PK/PD modeling based therapies, and to distinguish underlying processes involved in the WM.

1.8 Gap Analysis

Cancer drug resistance keeps on being a major challenge in medicinal oncology. Clinically, resistance can emerge because of cancer treatment. Unfortunately, Tumor heterogeneity may likewise cause resistance, making the issue significantly more difficult. Therefore the biomarkers should be identified which builds up a certain level of resistance against drugs.

1.9 Problem Statement

The problem statement of this study is to identify those genes which show several levels of resistance against Bortezomib and pharmacokinetic-pharmacodynamic modeling and simulations of other drugs with Bortezomib to predict the response of biomarkers. The problem statement of this dissertation is composed of two parts.

- Network-based resistant biomarkers of Waldenstrom's macroglobulinemia as hub nodes in networks and have high prediction values for resistance against bortezomib
- The mechanistic pharmacokinetic-pharmaco-dynamic modeling and simulations of bortezomib and other drugs used as the treatment option.

1.10 Proposed Solution

Illustration of quantitative understanding of the resistance mechanisms that are most fundamentally engaged with development and progression of tumor and behavior of the modified cells and genes for deciding the site at which oncologist ought to intercede is of prime significance from the therapeutic perspective and analyzing their role against the treatment choices. Therefore, the possible proposed solution to solve resistance mechanisms is the identification of biomarkers that can be utilized to present a personalized treatment approach, where just patients with a high probability of treatment advantage will get the treatment. The PK/PD modeling and simulation of drugs by the utilization of scientific models; i.e. PK/PD models to depict the relationship between the concentration and effect, and the relationship between dose and concentration. The PK/PD modeling to determine the response of particular biomarker against specific drug therapy, either solitary or in combination. These approaches will help to identify appropriate treatment regimens for the disease as well as their effects on the abnormal behavior of biomarkers expressions.

1.11 Aims and Objectives

This thesis aims at delineating the idea of resistant biomarkers identification from gene and or protein interaction networks, developed through the gene expression profiles of established WM cell lines BCWM.1, MWCL-1 and RPCI-WM1, including their Bortezomib-resistant subclones BCWM.1/BR, MWCL-1/BR and RPCI-WM1/BR. The thesis also illustrates the idea of PK/PD modeling and simulation using the drugs involved in the treatment of WM. The aims and objectives of this work are following

1.11.1 Aims and Objectives of the Resistant Biomarkers Identification

1. Identification of resistant biomarkers that can be utilized to access change in Bortezomib reaction to a treatment
2. Advancement of new robotic speculations for the progression of disease or reaction to treatment interventions prompting new therapies or to a superior utilization of existing treatments
3. Completing clinical trials of existing and recently created drugs with putative advantageous effects for resistant biomarkers which tolerate or do not show a response to a bortezomib treatment
4. Breaking down the health-economic effect of biomarker-guided customized treatment

1.11.2 Aims and Objectives of PK/PD Modeling

1. Ideal pharmacodynamic sampling for conceivable future examinations.
2. Estimation of drugs effects and concentrations on the tumor.
3. Measurement of changes in plasma fixations and blood.

4. Alteration of dosages in light of estimations that could be advantageous to clinical results.

5. Comparison of a hypothetically simulated dosage individualized regimen to the standard dosage regimen.

1.12 Scope

This study has a wide range of scope due to its multidimensional nature, it involves both the bioinformatics and system biology. The bioinformatics area of this study covers the identification of resistant biomarkers of WM, whereas system biology involves the PK/PD modeling and simulations.

The identification of resistant biomarkers serves to help distinguish the most proper population for a specific treatment. Also, the identification of resistant biomarkers gives a dynamic and intense way to deal with understanding the range of WM progression and the reaction of these biomarkers to bortezomib treatment.

The PK/PD modeling acts as a guide for the drug disclosure researchers toward ideal design and conduction of PK/PD studies in the examination stage. PK/PD systems can be actualized in early research periods of drug disclosure tasks to empower a fruitful change to drug advancement. Successful PK/PD study design, investigation, and translation can enable researchers to clarify the connection amongst PK and PD, comprehend the mechanism of drug activity, and recognize PK properties for advance change and ideal compound design. Also, PK/PD modeling can help expand the interpretation of the potency of the compound in vitro to the in vivo setting, diminish the quantity of in vivo animal studies, and enhance interpretation of discoveries from preclinical species into the clinical setting.

The attempt to determine the effect of drugs on the expression level of Biomarkers will also help to design the proper drugs against them and change in the expression level will also help to determine proper dosage regimens.

1.13 Our Contribution

In this thesis, First, we focus on the identification of biomarkers that show resistance against the bortezomib treatment, for this reason, we specifically utilized separate gene sets or modules of every cell line and assembled their interaction networks. From the created networks we distinguished hubs nodes as corresponding biomarkers of resistance. Besides this, we have done the PK/PD modeling of bortezomib as a monotherapy option for WM. we likewise have done the PK/PD modeling of different medications which are similar to bortezomib, lying in a similar class, and are utilized as a part of the treatment of WM. In addition, we performed the PK/PD modeling of bortezomib in a combination with the aforementioned drugs as a combination therapy treatment option for Waldenstrom's macroglobulinemia

1.14 Organization of the Document

The remaining portion of the thesis is arranged as. The Chapter 2, explains the specialized background on ongoing related computational techniques utilized for biomarker recognition in the field of bioinformatics, the PK/PD modeling and simulations, and its uses in drug disclosure and improvement methodology in the field of systems biology. In this chapter, we also explained the issue of considering intuitive impacts in biomarker distinguishing proof and confinements of PK/PD methodology.

Chapter 3 comprises of two sections; section 1 center around the identification of resistant biomarkers, here we explained our new approach for the biomarkers identification and strategy used to validate the identified biomarkers. Part 2 focus on the methods and data used, for the development of mechanistic PK/PD model and the use of the developed model for PK/PD modeling of bortezomib and other drugs, end of the part two consists of details about the strategy applied for the validation of PK/PD model.

Chapter 4 consists of the distinguished biomarkers, by considering their effects on the treatment, and their contribution to critical pathways identified with Waldenstrom's macroglobulinemia. The last segment of this chapter includes the designed mechanistic PK/PD model, its segments and the detailed results of PK/PD modeling.

Lastly, in Chapter 5 we summarized the thesis and give conceivable suggestions for investigating in the computational biomarker identification and PK/PD modeling and simulation areas.

Chapter 2

Literature Review

2.1 Waldenstrom's Macroglobulinemia

The name Waldenstrm macroglobulinemia was coined by Jan Waldenstrm, a Swedish doctor, in 1944 [30, 31]. The World Health Organization (WHO) classifies it as a low-grade non-Hodgkin lymphoma subtype. WM is characterized as B-cell lymphoplasmacytic lymphoma, described by the presence of monoclonal immunoglobulin M protein in the serum and penetration of lymphoplasmacytic cells in bone marrow [32, 33]. Its occurrence is 0.38 cases per 100,000 people every year, with an increase in age, it raises to 2.85 in patients over 80 years [34]. Among patients of all ages, the males are more predominantly affected, the disease has a higher rate of incidence in American than other nations [35]. There is an increased occurrence of both strong tumors and hematologic malignancies among patients [36, 37]. WM is classified as an immuno-secretory issue with a basic lymphoplasmacytic lymphoma as indicated by the WHO [38].

The middle period of patients with WM at determination is 64 years. In spite of other hematological malignancies, the clinical course of WM is generally sluggish. It does not require quick treatment [39]. The median survival rate of patients with WM is 5 years and 10 percent of these makeup to 15 years after analysis, showing the variability for WM patients. A few reviews have been embraced

to recognize clinical parameters affecting prognosis [40–42]. Most investigations demonstrate age, beta 2-microglobulin levels, and hemoglobin ratio as solid indicators of survival. Recently, the discovery of trademark cytogenetic anomalies of the threatening clone has added prognostic information with respect to a few hematological malignancies. At present, for WM there is no cytogenetic variation from the norm that shows a relationship with the outcome of the disease [43, 44].

WM is presently taken as a well defined clinical substance characterized by the presence of an IgM monoclonal gammopathy, bone marrow invasion by small lymphocytes that show plasma cell separation and immunophenotype such as, surface IgM, CD5, CD10, CD19, CD20, CD23 and elimination of other lymphoproliferative issue, including lymphoma and chronic lymphocytic leukemia [45, 46]. Smoldering WM (SWM) is an inadequately depicted asymptomatic issue with an imminent danger of advancing to symptomatic WM which requires treatment. It is characterized by the existence of serum IgM 3 g/dL as well as 10 percent bone marrow lymphoplasmacytic invasion, however, no confirmation of organ or tissue damage such as characteristic frailty, hepatosplenomegaly, worse condition indications, hyperviscosity, or lymphadenopathy that can be credited to the proliferative disorderliness of plasma cell [47].

2.1.1 Genetic Mutations

WM patients can give a wide range of symptoms that effect symptomatic treatment. CXCR4 and MYD88 WHIM-like somatic mutations are available in greater than 90 percent, and 30-35 percent of WM patients, separately, yet are uncommon in other IgM emitting B-cell tumors. Over a portion of people with IgM secreting monoclonal gammopathy of obscure noteworthiness, the MYD88 L265P mutations, proposing its part as a rapid oncogenic manipulator [48–50]. CXCR4 somatic mutations are developed in the germline of Warts, Hypogammaglobulinemia, Infections, Myelokathexis (WHIM) disorder, and almost dependably are available in WM patients with MYD88 L265P. Both the nonsense and frameshift CXCR4 transformations happen in WM patients [51–53].

MYD88 and CXCR4 changes may affect the ratio of disease, treatment result, or potential survival. [54–56]. Insufficient tumor weight and serum IgM levels are related to wild type MYD88 (MYD88WT) sickness, while MYD88 L265P patients with CXCR4 WHIM mutations have more burden of the illness [57–59]. High serum IgM levels incorporating the hyperviscosity emergency have been seen in patients with CXCR4 WHIM/FS tumor. In one investigation, the low survival rate was observed in patients with the MYD88WT tumor yet was unaffected by CXCR4 mutation.

WM is normally not acquired, and most influenced individuals have no history of cancer in their family. The condition emerges from somatic mutations during the lifetime, which is not inherited. A few families appear to have an inclination to the condition. Around 20 percent of individuals with Waldenstrm macroglobulinemia have a relative with the condition or another disease consisting of abnormal B cells.

2.1.2 Clinical Features

WM shares numerous pathological and clinical features with other B-cell lymphomas and multiple myeloma (MM), which frequently makes the diagnosis of this entity difficult [54]. IgM Monoclonal Gammopathy of Unknown Significance (MGUS) is an antecedent state for WM. Around 2 percent of IgM MGUS patients advance to B-cell cancer every year, with the majority of these people progressing to WM [60–62]. The most common clinical features are thrombocytopenia, splenomegaly, lymphadenopathy, bleeding due to hyperviscosity, anemia, and peripheral neuropathy. lymphadenopathy and Splenomegaly are exceptional at the beginning to about 15-20 percent, however, at later stages, additional medullary sickness is more typical up to 60 percent of patients [63–65]. Morbidities related to WM might be shown by tumor cell invasion, as well as by the physiochemical and immunological properties of the monoclonal IgM protein secreted by WM cells [66–68]. The detailed clinical features are shown in Table 2.1 obtained through [69].

TABLE 2.1: Morbidities intervened due to the IgM monoclonal protein in WM patients, obtained through study of Treon et al [69].

IgM Monoclonal Protein properties	Condition	Clinical Features
Pentameric protein structure	Hyperviscosity	Headaches, epistaxis, obscured vision, retinal hemorrhages, leg pain, intracranial hemorrhage.
Protein gets precipitate while cooling	Type 1 Cryoglobulinemia	cold urticaria, Raynaud-like, ulcers, acrocyanosis, purpura
Glycoprotein (MAG), Ganglioside M1 (GM1), Auto-antibody activity to Myelin Associated Sulfatide moieties on peripheral nerve sheaths	Peripheral neuropathies	Sensori motor neuropathies, bilateral foot drop, ataxic gait,
Auto-antibody actions to IgG	Type 2 Cryoglobulinemia	Purpura, sensorimotor neuropathies, arthralgias, renal failure
Action of Auto-antibody to RBC antigens	Cold agglutinins	Hemolytic anemia, acrocyanosis, Raynauds phenomenom, ivedo reticularis.
Deposition in tissues as amorphous aggregates	Organ Dysfunction	Skin: bullous skin disease, Schnitzlers syndrome, papules, GI: diarrhea,bleeding Mal-absorption, Kidney: renal failure, proteinuria
Deposition in tissues as amyloid fibrils	Organ Dysfunction	weight loss, Fatigue, hepatomegaly, edema, macroglossia, involved organs: heart, liver, peripheral sensory and autonomic nerves, kidney

2.1.3 Symptoms

Few studies reported that Familial inclination towards the disease is well built in WM [70–72]. Familial patients of WM are young, have more pressure on the disease, and express poor response to non-proteasome inhibitor-based therapy [73].

People with symptomatic Waldenstrm macroglobulinemia can encounter general symptoms, for example, fever, weight reduction, and night sweats. A few different signs and symptoms of the condition are identified with the abundant IgM proteins, which can thicken blood and impair its flow, causing a hyperviscosity disorder. Hyperviscosity associated features incorporate nose or mouth bleeding, obscuring or loss of vision, cerebral pain, trouble in movement (ataxia), and dizziness. In some affected people, the IgM proteins cluster together in the hands and feet, where the temperature of a body is cooler than the center of the body. Such proteins are known as cryoglobulins, and their clumps result in as cryoglobulinemia. Cryoglobulinemia can prompt torment in the hands and feet or series of Raynaud phenomenon, in which the fingers and toes turn white or blue in response to cool temperatures [74].

The IgM protein can develop in organs, for example, kidneys and the heart, causing amyloidosis, which can prompt serious issues of kidneys and heart. A few people with Waldenstrm macroglobulinemia build up weakness and a loss of sensation in the appendages resulting in fringe neuropathy. Specialists are unsure why this character happens in patients, despite the fact that they theorize that the IgM protein appends to the protective covering of nerve cells (myelin) and breaks it. The harmed nerves cannot convey signals, prompting neuropathy [75].

Different features of Waldenstrm macroglobulinemia are because of lymphoplasmacytic cells clusters in various tissues. For instance, aggregation of these cells can prompt splenomegaly, hepatomegaly, or lymphadenopathy. The lymphoplasmacytic cells interfere with the development of normal red blood cells in the bone marrow, causing a deficiency of typical blood cells known as pancytopenia. Extreme tiredness because of lessening anemia is basic in affected people. Individuals

with Waldenstrm macroglobulinemia have an expanded danger of developing other cancers of different tissues and blood [76].

2.1.4 Deregulated Molecular Pathways

P13k/Akt and MTOR Pathways: The PI3k/Akt pathway regulates the survival of the cell, expanding proliferation of cell while repressing apoptosis [77, 78]. Furthermore, Akt action causes an increase in cell adhesion and migration [79–81]. Numerous studies have discovered over-expression of Akt in WM [79, 82]. This concurs with discoveries of PI3K pathway proteins over-expression. Constitutive initiation of Akt pathway brings about cell resistance by restraining apoptosis, maintaining cell cycle pathways, and supporting cell survival and proliferation [82]. Both IL-6 and IGF-1 activates the Akt pathways and give strong targets to therapy [79]. Recently, it was recommended that PTEN contrarily manages this pathway In WM [83], however, no mutations of PTEN has been observed, the expression of PTEN gene and protein are observed to be decreased, and it is proposed that low levels of PTEN prompt steady activation of the PI3K/Akt pathway. Additionally, PTEN adversely regulates the mTOR which, has increased activation as that of Akt, because of phosphorylation in WM.

The original mTOR inhibitors rapamycin and it's analog everolimus utilize a mechanism of allosteric inhibition to block the output of mTORC1 [84]. Stage II clinical trials utilizing everolimus as a solitary treatment option for WM patients demonstrated its high action, with a response rate of 70 percent and a medium toxicity value [85]. Interestingly, second mTOR inhibitors focus on the ATP binding site to hinder kinase action of both mTORC1 and mTORC2 (Figure 1) [84]. Another promising inhibitor is NVPBEZ235 which targets both Akt and mTOR pathways, effectively reverse the activation of both Akt and mTOR pathways and dually represses these overexpressed pathways [82]. These studies give urging information to the utilization of personalized treatment procedures to target overexpressed pathways in WM. Targeting the mTOR prompts huge clinical activities in WM patients up to 45 percent incomplete remission TORC1 inhibitor treatment [86].

Though, patients did not have a total reduction in disease, which demonstrates a resistance of TORC1 exists in WM [87]. Imperatively, these pathways additionally give numerous targets to particular treatments, as shown in Figure 2.1 obtained through [77].

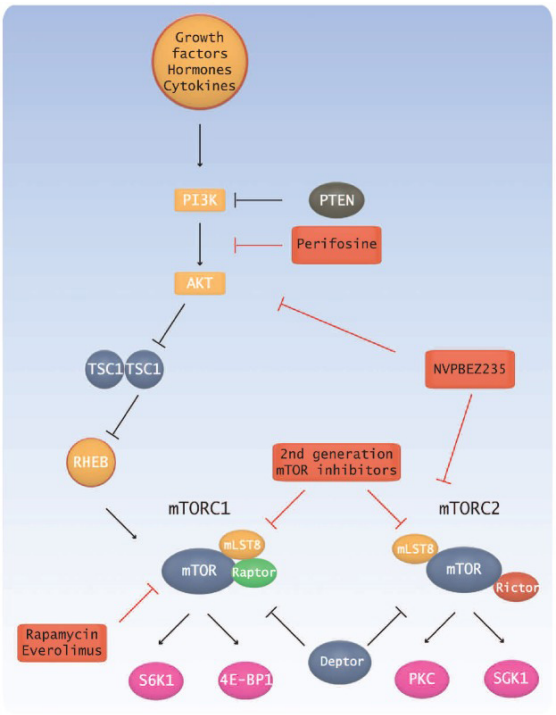


FIGURE 2.1: PI3K/Akt/mTOR pathway obtained through Braggio et al., [77]. The pathway gives various targets to particular therapies. Drugs targeting the pathway are represented by red boxes.

JAK/STAT Pathway: The Akt/PI3K pathway is over-expressed due to the elevation of STAT5 proteins [88]. The JAK/Stat pathway is an important one cytokine-initiated cascade that utilizes a few STAT proteins to accomplish biological and physiological functions, including hematopoiesis [89]. Loss of these receptors and second level messengers can be deadly and has been demonstrated to be extremely disrupting lymphoid and erythropoiesis movement. When constitutively dynamic, cells show an expansion in anti-apoptotic genes, tumor evasion, and cell cycle progression. Each of these features is noted in WM. Both the JAK1 and STAT3, initiated by IL-6, demonstrate over-expression in WM, in spite of no known mutations in their individual genes [90]. The persistent investigation of

these dysfunctional pathways in WM will help to give targets for treatments, as shown in Figure 2.2 retrieved from [90].

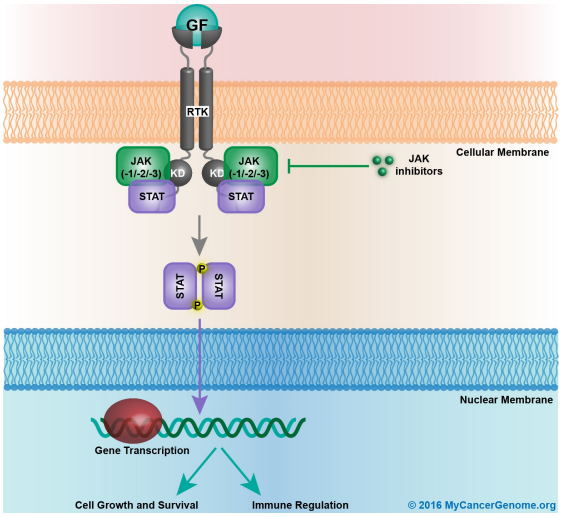


FIGURE 2.2: JAK/STAT pathway activation due to cytokines induction by Lucy et al., [90].

As the understandings with respect to the role of Jak/Stat signaling in cancer has expanded, various small inhibitors compounds of Jak and Stat proteins are being created. The adequacy of these molecules in WM isn't known so far, yet the administration of these inhibitors to patients with other hematologic disease has indicated extraordinary assurity [91]. Beyond their probable clinical use in WM, these inhibitors are likewise valuable apparatuses for looking at the connection between cytokine-activated Jak/Stat signaling and immunoglobulin emission in hematopoietic cancers. In the Waldenstrm's cell line, BCWM.1, utilization of the Jak inhibitors, JakI, totally block the IL-6 stimulated secretion of IgM [92]. This information gives starting proof embroiling IL-6-driven Jak/Stat signaling in the unnecessary emission of Ig and give more ways to look at the role of signaling transduction of Jak/Stat in the secretion of IgM proteins in the WM.

Furthermore, because of the significance of the Jak/Stat pathway in interceding cell development and endurance in response to the stimuli of cytokine, Jak-and Stat-particular inhibitors have moreover shown noteworthy cytotoxic and antiproliferative properties in numerous kinds of malignancies [93]. Lower Ig secretion results in decreased tumor weight, these inhibitor molecules in the treatment of

tumors secreting Igs, may end up being better than that of other drugs which focus on either the Ig production or secretion. As different cytokines are associated with controlling IgM production during hematopoiesis, there are chances that various cytokines in the tumor microenvironment are necessary for keeping up the elevated amounts of serum IgM protein normal to WM, and their personal effects on tumor development [94].

Distinguishing proof of these cytokines and the activities of the particular Jak and Stat proteins through which they intervene their belongings is a coherent initial phase in seeing how the Jak/Stat pathway adds to the pathological process of WM?. Clarifying the Jak/Stat role in signaling activity in WM will take into consideration the improvement and choice of suitable targets on medicines for the clinical assessment taken place so far [95].

TLR Signaling Pathway: The finding of mutations in MYD88 is of importance, demonstrating its activity as interleukin 1 receptor (IL-1R) adapter and Toll-like receptor (TLR) signaling [96]. All TLRs aside from TLR3 utilize MYD88 to facilitate the process of their signaling. After the stimulation of TLR or IL-1R, MYD88 is enrolled to the actuated receptor-complex as a homodimer, which then makes a complex with IRAK4 and initiates IRAK1 and IRAK2 [97–99]. Tumor necrosis factor receptor-related factor 6 (TNF-6) is then initiated by IRAK1, prompting the activation of nuclear factor kB (NF-kB) through the phosphorylation of IkBa [100]. Use of inhibitors of MYD88 pathway prompted a decrease in the phosphorylation of IRAK1 and IkBa and in addition the survival of WM cells expressing MYD88 L265P. NF-kB signaling is critical for WM development and survival [101]. In recent years, Yang and colleagues [102] demonstrated that Bruton tyrosine kinase (BTK) was also activated by MYD88 L265P. Active BTK co-immunoprecipitated with MYD88 that could be canceled by utilization of the BTK kinase inhibitor and overexpression of MYD88 L265P. In addition, knockdown of MYD88 by lentiviral transfection or utilization of an MYD88 homodimer inhibitor in WM cells with MYD88 L265P mutation revoked BTK activation. The process obtained through [103] is shown in Figure 2.3.

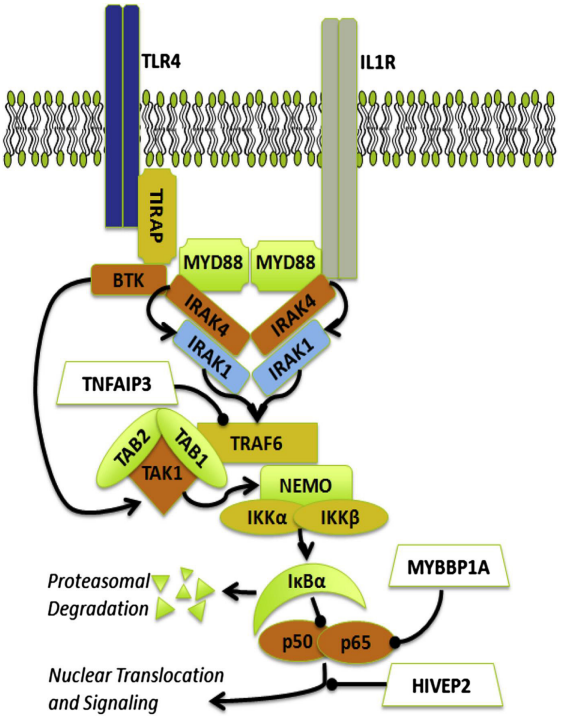


FIGURE 2.3: TLR signaling pathway dependant on MYD88. It also triggers NF-kB signaling through activation of IRAKs and BTK. Monoallelic losses in TNFAIP3,HIVEP2, and MYBBP1A might intensify NF-kB signaling in several patients of WM by Steven et al., [103].

2.1.5 Pathophysiology of Waldenstrom's Macroglobulinemia

Hereditary investigation of the hypervariable region of antibodies from patients having WM show that it creates from a post-germinal focus cell that has experienced physical hypermutation, perhaps affected by antigen choice. In this manner, WM emerges from the cells which express IgMs that changes after suspension of substantial transformation, however without starting switch occasions. Some un-mutated monoclonal IgM can be found and may emerge through a T-cell-autonomous system [104] The IgM monoclonal protein result in the elevation of blood viscosity by making cluster with each other, they binds their carbohydrate components with the water through covalent bonding and started to interact with the blood cells, thus, causing WM. The origin of WM is shown in Figure 2.4 obtained through [105].

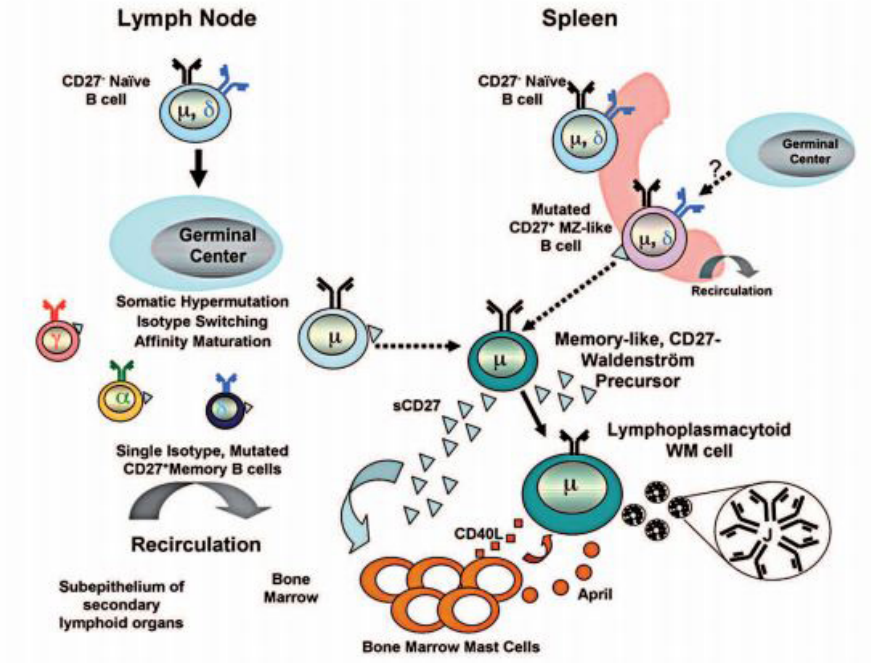


FIGURE 2.4: The origin of WM, explained by stone et al [105]

WM could emerge from a developed memory-like B cell of either peripheral zone or germinal focus which does not experience downstream switching but rather can obtain a lymphoplasmacytoid phenotype and capacity of IgM secretion

2.1.5.1 Hyperviscosity Syndrome

Side effects because of hyperviscosity happen in about thirty percent of patients having WM and might be the showing indication. The side effects and signs in patients with hyperviscosity disorder incorporate bleeding from mucosa and skin, visual aggravated retinopathy, and a number of neurological issues, and rare cardiovascular issues [106]. The fundoscopic exam demonstrates sausage in the retinal veins. Extraordinary ophthalmologic studies can identify lesser degrees of viscosity [107]. Serum viscosity is ordinarily 1.4 to 1.8 times as that of water at 37-degree centigrade. Hyperviscosity disorder is impossible except if the serum thickness is over four centipoises (cp). However, the consistency level associates intimately with signs and indications in a similar patient, to some degree because of the scope of natural viscosity estimations of various Waldenstrm IgM proteins

[108]. The treatment for hyperviscosity disorder is plasmapheresis with or without attending chemotherapy. Plasmapheresis will normally invert the signs of the disorder and is especially compelling in macroglobulinemia on the grounds that eighty percent of IgM is intravascular. It is essential to perceive hyperviscosity disorder and treat it instantly

2.1.5.2 Cryoglobulins

Cryoglobulins are immunoglobulins that form a gel at temperatures under 37-degree centigrade and re-breakdown at 37 degrees centigrade [109]. The stage change is temperature-reliant and reversible. Single cryoglobulins in patients having WM are because of the temperature-delicate insolubility of IgM and are typically dependant on a specific concentration. Most blended cryoglobulins are antigen-antibody complexes [110]. The cryoprecipitate is caused by the immune complex. Cryoglobulins are critical in the pathogenesis of symptoms in patients. The signs and symptoms may comprise of arthralgias, purpura, acrocyanosis, cutaneous vasculitis, coldly affected indications, visual aggravations, and mucosal bleeding. Once in a while, cerebral thrombosis, hepatosplenomegaly, lymphadenopathy, and renal malady, especially proliferative glomerulonephritis might also be present [111].

2.1.6 Treatment Options for WM

Since WM remains a serious disease, the objectives of treatment are to reduce symptoms and risk of end-organ harm. Treatment is generally necessary for symptomatic patients and those with extreme cytopenias. Whenever possible, clinical preliminaries ought to be considered for patients with recently analyzed WM. Active drugs incorporating monoclonal antibodies (ofatumumab and rituximab), nucleoside analogs (fludarabine and cladribine), alkylators (chlorambucil, bendamustine, and cyclophosphamide), proteasome inhibitors (carfilzomib and bortezomib), signal inhibitors (ibrutinib and everolimus), and immunomodulatory

drugs (lenalidomide, thalidomide, and pomalidomide)[112]. Table 2.2 demonstrates an outline of chosen clinical trials in WM, obtained through [32].

TABLE 2.2: Clinical trials of drugs, conducted for the WM, obtained through the Oza et al.,[32]

Regimen	Disease/Treatment status	Overall Response Rate	Complete Response Rate
Rituximab	Untreated and Treated	52.2%	0%
Bendamustine	Relapsed	90%	60%
Flludrabine	Untreated	95.3 %	4.7%
Bortezomib	Untreated and Treated	85%	0%
Thalamomide	Untreated and Treated	64%	4%

Combined therapies, especially with rituximab, generally have been accessed for the treatment of WM. several features, including the cytopenias, the requirement for faster illness control, autologous transplant treatment candidacy, and age, ought to be considered in clinical settling on the decision of first-line therapy. For patients who are the competitor for autologous transplant treatment, subjection to constant nucleoside analog or chlorambucil treatment, ought to be constrained, given the possibility for the stem cell damage. The utilization of nucleoside analogs may likewise build hazards for histologic change to diffused extensive B-cell lymphoma, myelodysplasia, and intense myelogenous leukemia [112].

2.1.6.1 Monoclonal Antibodies

Rituximab is a basic monoclonal antibody, its target is CD20; an antigen which commonly expressed on lymphoplasmacytic cells of WM patients. The utilization of rituximab at authoritative measurement prompts fractional or better reactions in around 27 to 35 percent of patients [113]. Individuals who received minor

doses of rituximab are benefited by enhanced platelet counts, hemoglobin, and lessening of splenomegaly as well as lymphadenopathy [114]. The median time of failure of the treatment extends from 8 - 271 months. Studies assessing an extensive rituximab plan comprising of 4 weeks course at 375 mg/m2 per week, with an additional 4-week course after 3 months, have exhibited higher significant Response rates (RR) of 44 to 48 percent, with time to the progressions estimation surpassing 29 months [115, 116].

Ofatumumab is a completely refined CD20-coordinated monoclonal antibody targeting the small loop of CD20, an epitope that is not the same as rituximab. A general reaction rate of 59 percent was seen in a progression of 37 symptomatic WM patients after the administration of Ofatumumab [117].

2.1.6.2 Alkylating Agents

Orally used alkylating drugs, when used as single and in a combined treatment with steroids, are being broadly assessed in the forthright treatment against WM. The best involvement with oral alkylator treatment has been with chlorambucil, which is given on both a constant day by day dosage and also an irregular dose schedule. Patients who receive chlorambucil daily get 0.1 mg per kg dose per day, though the patients who receive chlorambucil irregularly get 0.3 mg per kg, seven days, for six weeks [118]. Extra factors to be considered in chlorambucil treatment for patients having WM, incorporate the need for more quick control of ailment show slow reaction to this medication and thought for preventing the stem cells in patients who are the possibility for autologous transplant treatment. Chlorambucil should, accordingly, be given to patients who are nontransplant competitors with more indolent sickness [119].

The combination of doxorubicin, prednisone, vincristine, or cyclophosphamide, - (CHOP) with rituximab (CHOP-R) was examined in irregular examination by the German Low-Grade Lymphoma Study Group including sixty-nine patients, a large portion was suffering from WM. The expansion of rituximab to CHOP brought about a response rate up to 94 percent and an average time of progression is 22

months in contrast with patients who received only CHOP. Basic features were comparable for age, bone marrow association, earlier treatments, platelet number, hematocrit, and serum b2-microglobulin, despite the fact that high level of serum IgM levels were observed in patients who received CHOP-R treatment [120]. Examination of unfavorable occasions for these dosage designs demonstrated more chances for neutropenic fever and, in addition, treatment-related neuropathy in patients getting CHOP-R These examinations propose that for the treatment of WM, the utilization of doxorubicin and vincristine might be reduced to limit complexities related to the specific treatment. Along these lines, more extraordinary cyclophosphamide-based dosage, such as CHOP-R, ought to be minimized [121].

Bendamustine is newly approved by food and drug administration (FDA) for the treatment of lethargic non-Hodgkin lymphoma. Bendamustine has high structural likenesses to both purine analogs and alkylating agents [122]. The utilization of bendamustine along with rituximab was investigated by Rummel and associates [123] in the treatment of WM. As a major aspect of a random report, patients got six cycles of combined bendamustine-rituximab (Benda-R) or CHOP-R. An aggregate of five hundred and forty-six patients were enlisted in this examination including inactive non-Hodgkin lymphoma patients along with forty patients suffering from WM.

Patients in the Benda-R got bendamustine at 90 mg per m2 for 2 days and rituximab at 375 mg per m2 for a single day with the recurrence of a month for each cycle. The response rate was 96 percent for Benda-R and 94 percent for CHOP-R treated patients. Having a median perception time of 2 years and 2 months, 20 out of 23 Benda-R versus 9 out of 17 CHOPR treated WM patients stay free of disease. Benda-R was related to a lower rate of 3 or 4-grade neutropenia, irresistible intricacies, and alopecia [124]. However, treatment was very much endured in this investigation, delayed myelosuppression happened in patients who got earlier nucleoside analog treatment. Cytoreduction, including extramedullary sickness, is especially better with bendamustine-based treatment and can be assumed in patients with splenomegaly, adenopathy or symptomatic extramedullary illness. Therefore, large number of survived patients were observed after treatment.

2.1.6.3 Nucleoside Analogs

Both the cladribine and fludarabine have been assessed in untreated and also already under treatment patients of WM. Cladribine managed as a solitary medication by intravenous infusion, for two hours every day implantation or by subcutaneous bolus infusions for approximately 7 days has brought about response rate range from 38 - 54 percent [125–128]. The general response rate with day by day infusional fludarabine treatment regulated for the most part, on 5-days plan in patients of WM has gone from 30 - 40 percent. Myelosuppression normally happened after the delayed presentation to both of the nucleoside analogs, as well as lymphopenia with the supported exhaustion of both CD41 and CD81 T lymphocytes present in WM patients after the one-year of the inception of treatment. Treatment-related fatality rate because of myelosuppression or potentially sharp diseases owing to immunosuppression happened in up to five percent of every patient who received the therapy in some cases with either of the nucleoside analogs [129–131].

The efficacy of nucleoside analogs is subjected to examination for a few years. Thomas and colleagues [132] have announced their encounters in reaping stem cells in twenty-one patients with symptomatic WM in whom the collection of autologous peripheral blood stem cells was done. The study succeeded in 99 percent of patients who got non-nucleoside analog based treatment versus 33 percent of patients who got a nucleoside analog. The long haul safety of nucleoside analogs in WM was also inspected by Leleu and colleagues [133] in an extensive study of WM patients. A 7 times increment in a change to a fast-growing lymphoma and a 3 times increment in the improvement of intense myelogenous leukemia/myelodysplasia were seen among patients who got a nucleoside analog versus different treatments to cure the WM.

Recovery from Acute myeloid leukemia were observed in half of the patients uptill five years after treatment, with normal survival rate of 10 percent, but in some cases, the patients started to experience symptoms soon after closing treatment of nucleoside analogs.

2.1.6.4 Proteasome Inhibitors

Bortezomib has been broadly researched in WM. In several investigations of the WM, twenty-seven patients got up to eight cycles of bortezomib at 1.3 mg per m2 on 1, 4, 8, and 11 days. [134] 1 patient was suffering from refractory/relapsed illness. After treatment, average serum IgM levels reduced from 4660 mg per dL to 2092 mg per dL with a P ¡ 0.0001. The overall response rate was 85 percent, with 13 patients accomplishing a minor response and significant response, separately. All the responses were immediate and happened in the middle of 1.4 months. The average time to the movement for all reacting patients in this examination was 7.9 months, and the most widely recognized grade III or IV toxicities happened more noteworthy than half of the patients, 22 percent tactile neuropathies, 14.8 percent neutropenia, 18.5 percent leukopenia, 11 percent unsteadiness, and 7.4 percent thrombocytopenia. Significantly, tangible neuropathies settled or on the other hand enhanced in almost all patients after discontinuance of the treatment. As a feature of a National Cancer Institute of Canada study, Chen and partners [135] treated twenty-seven patients including both the previously treated and untreated.

Patients in another examination got bortezomib, utilizing the standard dosage procedure, they exhibited dynamic infection. The overall response rate in this examination was seventy-eight percent, with significant reactions seen in forty-four percent of patients. Tactile neuropathy happened in 20 patients, after 2 to 4 cycles of treatment. Among the patients building up a neuropathy, fourteen patients settled and 1 illustrated a change in tumor grade after 13 months. Nonetheless, these encounters with bortezomib monotherapy in WM, Dimopoulos, and partners [136] found the significant response rates in 6 of 10 percent already 60 percent treated WM patients. The combination of bortezomib, rituximab, and dexamethasone (BDR) has been examined as an essential treatment in WM patients. An overall response rate of 96 percent was seen with BDR [137]. The rate of grade three neuropathy was 30 percent in this investigation, which was utilized two times per week plan for bortezomib at 1.3 mg/m2 [138]. However, Bortezomib treatment showed promising results in other types of lymphoma.

Bortezomib often produces serious treatment associated fringe neuropathy in WM, the scientists examined the utilization of carfilzomib, proteasome-inhibitor to prevent from neuropathy, in mixture with rituximab and dexamethasone (CaRD) in symptomatic WM patients [139].

2.1.6.5 Immunomodulatory Agents

Thalidomide has been inspected in WM as monotherapy and also in the mixture with clarithromycin or potentially dexamethasone. Dimopoulos and colleagues [140] found a noteworthy response rate in 25 percent of patients who got a single dose of thalidomide. Low dosages of thalidomide with oral dexamethasone 40 mg dose once per week and 250 mg clarithromycin orally two times every day have been inspected, with 83 percent of patients exhibiting a noteworthy reaction [141]. Pomalidomide was also examined in a dosage regimen stage 1 with rituximab. [142] Patients indicated intolerance to the dose over 1mg, and rituximab increase prompted symptomatic hyperviscosity and new plasmapheresis in 3 of 7 patients. The general response rate was 43 percent.

2.2 Drug Resistance in Waldenstrom Macroglobulinemia

Treatment choices for WM incorporate alkylating agents, nucleoside analogs and rituximab as a single agent or in combination. However, these methodologies result in low clearance rates and short span of treatment survival in many occurrences. Besides, no particular drug has turned out to be better than others and, also, no treatment has been explicitly endorsed for WM. Thusly, new ways to deal with the treatment of WM are required. With an end goal to accomplish this, researchers have sought after targeted treatments, for example, bortezomib to cure WM, but this inhibitor also unable to cure WM properly because of Resistance due to certain unknown mechanisms [143].

2.3 Biomarkers Identification

Hulka and colleagues [144] first time defines the Biological markers also known as biomarkers as a biochemical, cell, or molecular changes that can be measured in biological media, for example, human fluids, cells, and tissues." Recently, the definition has been widened to incorporate biological characteristics that can be estimated and assessed as a pointer of biological processes, pharmacological reactions, or pathogenic procedures to a therapy intervention [145].

Generally, biomarkers incorporate the technologies and several tools that can help in understanding the cause, symptom, analysis, prediction, progression, or outcome of disease treatment. Biomarkers of all types have been utilized by the doctors, epidemiologists, and researchers to understand several human infections. The use of biomarkers in the diagnosis of a cardiovascular ailment, immunological and hereditary disorders, cancer, and infections are well-known [146]

There has been expanding proof that several kinds of cancers are exceedingly heterogeneous as in related somatic genes or different particles may vary in various patients which brings about various cancer subtypes with changing behavior, including different reactions to drugs, diverse rates of survival time, and distinctive recurrence rates [147–152].

The major critical issues in cancer informatics are tumor grading, where the objective is to discover the subtypes of tumor in a diverse populace of samples, discovering applicable subtypes, one other significant issue is to distinguish the biomarkers (genes or proteins) identified with each subtype with the end goal of customized treatment and prognosis. As a large number of gene or protein based Biomarkers have been identified for several types of cancer including liver, breast, thyroid, colorectal, ovarian, pancreatic cancer, melanoma, and several types of Leukemia. Therefore, instead of supervised learning for already proposed biomarkers identification methods, in this dissertation, the point is to locate the resistant biomarkers of WM against bortezomib profiling that better discriminate the outcome of drug response in an unsupervised manner where the causes of resistance are unknown.

2.3.1 Types of Biomarkers

Perera and Weinstein classified biomarkers into different types on the basis of a series of events from exposure to disease. There are two significant types of biomarkers: Anticident biomarkers, which are utilized to predict the risks, and biomarkers of illness, which are utilized in screening, determination, and to study the progression of a disease. In this study, we have been focused on the biomarkers of illness, which are causing the resistance to Bortezomib treatment in WM.

2.3.2 Biomarkers of Illness

Biomarkers allow pre-determination of disease or take into consideration the condition, important to be resolved at an earlier stage of the disease. They give important information for diagnosing infections. Biomarkers are utilized as a pointer of a biological factor that shows either a sub-clinical appearance, a surrogate indication of the disorder, or stage of the disease. Biomarkers utilized for screening or analysis usually show signs of the infection [153]. The significant uses of this class of biomarkers include:

1. Identification of patients having more chances to be affected or who are in the pre-clinical stages of the disease.
2. Lessening of the disease heterogeneity in epidemiological studies or in clinical trials.
3. The impression of the common history of disease incorporating the induction phases, dormancy, and detection.
4. Target for clinical trials.

2.3.3 Drug Resistant Biomarkers

Drug-resistance and Metastasis are real obstacles in the treatment of several cancers. More consideration is required in unraveling this area of cancer, keeping

in mind the end goal to produces more compelling treatments. In an examination, Goswami [154] has found the biomarkers that are resistant to chemotherapeutic medications. Distinguishing proof of molecular biomarkers for both the drug-resistant and invasive cells in the primary tumor opens another vista in the diagnosis of cancer. These resistant biomarkers also distinguish new pathways for helpful mediation which can be valuable to traditional therapies, meddling with the progression of tumor at few points. Biomarkers can anticipate the intrusive and drug-resistant tumor cells in light of an aberration in their expression level after treatment to specific medication [154]. So also, Li et al [155] have distinguished drug-resistant biomarkers in Leukemia K562/ADM cells, by utilizing nanoparticles as a new procedure to hinder multi-drug resistance in targeted tumor cells and as a delicate technique for the determination of specific tumors at early stages. In oncology, the resistant biomarkers are divided into predictive and prognostic biomarkers. Prognostic biomarkers give knowledge about the overall outcome of patient's tumor such as prostate-specific antigen (PSA), whereas predictive biomarkers can be utilized to calculate the reaction of a specific patient to a particular therapy, for example, the expression level of human epidermal growth factor receptor 2 (HER2) as an indicator of reaction to trastuzumab treatment [156].

2.3.4 Techniques for Biomarkers Identification

Biomarkers can give information on pathogenic procedures and pharmacological reactions for a helpful intervention. The recognizable proof of biomarkers for clinical analysis is one of a few fascinating topics in medicinal research. Despite the fact that estimations relating to biomarkers have been generally applied in the clinical procedures, distinguishing important biomarkers for clinical diagnostics in light of data-rich natural information is challenging [157]. To distinguish biomarkers, distinctive methodologies for selection of specific features required for a biomarker, for example, support vector machine recursive feature elimination [158], random forests[159], and genetic algorithms [160] have been broadly applied

[161]. These strategies select specific features in light of expression level among distinctive classes rather than feature relationship changes.

A feature is critical in the sense that it has an amazing effect on others. Since molecules are related to each other and show interaction among themselves, investigating changes in the interaction to get a detailed understanding of the mechanism of disease has gained expanding consideration [162, 163]. Consequently, breaking down the biological information from a network point of view could be a superior procedure for finding key biomarkers and encouraging the investigation of diseased phenotypes [164].

Distinctive network development and investigation strategies have been proposed to extract information from the disease data. Pearson correlation coefficient which measures related feature associations has been generally used to build the networks [165], and the center points (hubs) are held as key elements. Krumsiek et al [166] utilized the partial correlation coefficient to develop networks containing biological information. In metabolomics, a proportion could be assigned as the pathway response in which one metabolite is changed over into another metabolite by means of single or different response pathways.

Hence, Netzer et al. [167] developed a network in light of the matched biomarker identifier estimations of the metabolite proportions. PinnacleZ [168] connected common data to ascertain the discriminative capacity of the network. Diagram based iterative gathering investigation [169] positioned the features in the network and distinguishes the enlightening sub-networks in light of the p-value ascertained utilizing the positions of the specific features. Other proficient strategies exist, including a two-advance module cover [170] and condition-particular networks [171]. Palaniappan et al. [172] use strategies for network analysis to recognize novel biomarkers of the progression of colorectal malignancy on each stage.

In the present study, we utilized a computational method that defines potential resistant biomarkers of Bortezomib based on centrality analysis. It explores the hub nodes in the networks as key biomarkers of resistance.

2.3.5 Network-based Biomarkers

At the present time, a considerable measure of molecular networks has been constructed, including protein-protein interactions networks (PPINs), gene regulatory networks, metabolic networks, miRNA-gene interaction, etc. These molecular networks can be depicted as diagrams, where the nodes, refer to the molecules while the edges show the interactions [173]. Since these networks can depict different sorts of useful interactions among molecules, they are broadly utilized for recognizing biomarkers. In general, a network can be portrayed as a graph G(V, E), where V is the arrangement of nodes and E represents edges showing useful interactions [174]. In a network, aside from its own particular expression values, the significance of every node can be depicted by its topological properties. For instance, the nodes with high degrees are usually viewed as essential and have critical effects on different nodes in the system, where the degree of a node is the number of different nodes it associates within the network. In complex network systems, the nodes with most noteworthy degrees are called hub nodes [175].

Besides the degree, alternate types of centralities of a node are additionally broadly used to determine its significance. Among various kinds of centralities, betweenness is the most generally utilized, which is characterized as the number of the shortest paths from the node of interest to other connecting nodes in the network. Ozgur et al. positioned all genes in prostate cancer as for their topological attributes and found that betweenness yielded the most astounding exactness in ranking the genes associated with prostate cancer [176]. The identification of biomarkers is vital for developing precision pharmaceutical. Nonetheless, it is a troublesome task to create biomarkers with high exactness and power considering the complexity and variability of biological systems among patients [177, 178]. The molecular networks that depict the useful interactions among genes give a worldwide perspective of the complex natural systems. By studying these networks, the information about molecular mechanisms can be obtained. Numerous computational methodologies have been used to recognize biomarkers that can be

utilized. However, the drug-resistant biomarkers can also be determined by using the same methodologies of developing interaction networks [179, 180].

2.3.6 Application of Biomarkers

In clinical settings, the endpoints are the most tried attributes to accomplish the aftereffect of medical intervention and should be supported over surrogate endpoints. In some clinical settings the endpoints, for instance, survival requires long recognition time and patients number in clinical preliminaries to be accessed. This is impossible in a number of areas and renders the clinical advancement of new medications inefficient. There is a great need for such endpoint endeavors to increase the drug discovery process [181]. In spite of the fact that it is attractive that a biomarker, in the long run, turns into a surrogate endpoint, a biomarker that can't replace might be of remarkable motivation in drug advancement and change process in the patient care. A quantifiable difference in a biomarker in light of a pharmacological procedure may fill in as a proof-of-idea (POI) in early times of drug improvement.

This is significant knowledge when, for instance, a decision should be taken for a few hopeful drugs. Usually in pre-clinical testing of animals if there is an existence of biomarker in species other than humans. However, most of the medications follow up on receptors or physiological structures independent of disease, exist in patients, In volunteers, POI studies can give vital information. For instance, another anti-hypertensive drug that does not lessen the blood pressure of patients will most likely not be viable in hypertensive patients. Coordinating assessable biomarker data into a PK/PD model encourages basic decision making in regards to the dosage choice and preliminary plan or end of a project because of poor biomarker response. In clinical practice, doctors have been effectively utilizing biomarkers to manage individual treatment, for example, a globally standardized proportion for the oral treatment by anticoagulants, insulin treatment for blood-glucose for quite a long time, for decades, numerous of them don't act as a

biomarker [182]. The capability of biomarker-guided treatment is amazing both for existing drugs as well as drugs which will be produced later on.

2.3.7 Tools and Software used for the Network-based Biomarkers Analysis and Discovery

Network analysis has been done to identify several pathways and biomarkers associated with the diseases. Network-based biomarkers have not just effectively been used for the identification of metastatic versus non-metastatic tumors, yet in addition, exhibited higher reproducibility of networks as compared to individual biomarker distinguished by traditional methodologies. The regular method to translate and contextualize these biomarkers is with Kyoto Encyclopedia of Genes and Genomes (KEGG) [183], network-based enrichment Gene Ontology [184], and other comparative methodologies. This sort of examination focus on the functional relationship of markers. The network-based examination is an advanced technique of system biology, used to interpret large omic datasets [185]. By considering the cross-talk of different pathways, network modeling permits a more extensive investigation of a perplexing framework than the pathway-driven methodology. It has been demonstrated that system topological properties can be used for organizing candidate genes and anticipating novel competitor biomarkers [186], and modularity analysis could separate significant sub-networks identified with the considered infection.

Accordingly, the network-based examination has assumed to be an expanding job in current biomarker disclosure and drug discovery. A lot of tools and software are available for network analysis. A few programs center around the graphical representation of the network, while others include computational abilities, for example, clusters identification and modularity, helping in the elucidation of the biological functions hidden the unpredictable networks. The most popular among them are shown in Table 2.3.

TABLE 2.3: List of Tools and Software used for Network-based Biomarker Identification

Tool	Working	Limitations	Reference
Cytoscape	Visualize biological pathways and molecular networks, Build PPIN, GRN, retreive biological pathways etc.	Does not provide 3D representation, does not analyze complex networks	[187]
visANT	performs visual mining of biological networks, takes data from Genebank, SwissProt, and KEGG	Can't run on windows based systems, unable to integrate data from external sources	[188]
PINA	Studies interaction of proteins at network level, analyze intractomes and pathways	Can not build networks for all organism, only use six model organisms	[189]
atBioNet	Performs network analysis of genes and or proteins, integrates data of 7 PPI databases	only performs specific network analysis of humans, not all species	[190]
Gaphi	Visualize and analyze networks by modularity and centrality, able to develop more complex networks and pathways, differentiate network components in different colors through modularity	Unable to show large networks clearly due to small interface	[191]
BioPlat	Predicts novel cancer biomarkers by network mining, analyze gene expression data	Requires extra plugins, so take more memory	[192]
Mist	Integrates the data from gene mining and built protein interaction networks	Does not generate directions in the network	[190]
GeneMania	Provides networks of physical and genetic interactions	It is online	[185]

2.4 Pharmacokinetic-pharmacodynamic Modeling and Simulations

Dost presented the ideas of Pharmacokinetics in his 1953's book *Der Blutspiegel - Kinetik der Konzentrationsablufe in der Kreislaufflssigkeit* [193] after that, Nelson provided the explanation of four kinetic components, absorption, distribution, metabolism, and elimination in an global science journal in 1961 [194], the significance of pharmacokinetics was perceived in the territory of clinical pharmacological medicine. Nelson made a broad reference Toerell's work (1937) first depicted the action of xenobiotics in the body of human with scientific conditions [195]. These ideas are the premise of present-day pharmacokinetic (PK) data analysis based on the designed models.

Pharmacodynamic (PD) models are regularly based on the law of mass action or Langmuir's law of adsorption-desorption, later on, Ariens [196] and Stephenson [197] reformulated them for the instance the binding of antagonist with receptor. Central models of pharmacological reaction in connection to drug fixations were studied by Holford and Sheiner underscoring the significance of quantitative predictions of the effects of several drugs on human [198]. One of those fundamental PD models is the Emax, where a hyperbolic work relates a proportion of drug induction, such as blood fixation, dosage, and area under the curve (AUC), to some proportion of pharmacological reaction.

Most generally utilized numerical models to portray the time course of drug concentration in the plasma or blood are the mamillary compartmental models, where, the body of human is designed to an arrangement of associated compartments with the input and output in the form of the drug from a central compartment. These models could be depicted as frameworks of differential equations or poly-exponential terms. Numerous alterations to this basic model, for example, saturable elimination or delayed absorption have been portrayed, exhibiting its adaptability [199]. The variation of beneficial pharmacodynamic models is quite complex, difficult to design and implement, simulate and understand, hence

gigantic, running from straightforward linear models to complex robotic models depicting the gene or receptor intervened effects of corticosteroids [199].

In general PK/PD models, the drug amount in the dose compartment enters through the central compartment having a rate constant ka. It is distributed into the compartment and elimination of the drug from the central compartment is usually represented by Clearance rate constant or elimination rate constant. The differential equations are then built explaining the processes of absorption, distribution, and elimination.

2.4.1 PK-PD Modeling Techniques

A basic area of model-based examination of data is the model parameters evaluation by nonlinear regression. A wide range of calculations and estimation methods have been produced for the reason to *fit* some numerical functions to a given data of perceptions [199]. The most generally utilized strategy is the maximum likelihood estimation, given by Fisher in the middle of 1912 and 1922 [200]. Several optimization algorithms such as Gauss-Newton, Levenberg-Marquardt are also connected with models to discover the work by iteratively changing the estimation of the model parameters. Throughout the years NONMEM has turned into a most generally utilized programming tool for PK/PD modeling.

As compared to non-model methods, the models-based methods utilized for the modeling of data can give extra understanding into the drug behavior in an individual or in a populace, their quality lies in their capacity to make predictions of unstudied circumstances and consider the possible situations. Imperative inquiries like: How might a patient's reaction to a change in treatment if there is a decrease in renal capacity? or could extra advantage be gained by giving a specific drug twice a day in several cycles, instead of once per day? through the simulations results. In recent decades, the approaches of modeling and simulation have been progressively used in clinical and preclinical research and therapies and has inevitably changed into a complete discipline

It progressively used in clinical and preclinical research and therapies and has inevitably changed into a complete discipline Pharmacometrics - the investigation of quantitative pharmacology [201]Pharmacometrics - the investigation of quantitative pharmacology [201].

Drug design and discovery are ruled by the utilization of thermodynamic parameters, for example, Ki or IC50 values to choose and improve the lead candidates. These measurements are utilized to anticipate in vivo drug pharmacodynamics (PD) in view of the presumption that there is an equilibrium in drug and its target [202]. However, the concentration of drug at its target site is not constant because of dynamics, for example, absorption, distribution, metabolism, and elimination, and in this manner dug and target are probably not going to be at balance [203–206]. Instead, the overall description of drugtarget binding requires the consideration of drugtarget energy which can be utilized to better foresee changes in drug activity which are dependant on time[207–211]. This has prompted the improvement of mechanistic PK/PD models that incorporate the on and off rates for the drug-target binding, In the present work, we have used the mechanistic PK/PD model to quantitatively correlate the response rates of bortezomib with different drugs.

2.4.2 PK/PD Modeling Applications in Drug Development

Modeling and simulations are basic devices in this area and affirming idea and have a large number of applications. PK/PD models created on informations from experimental investigations allow the examination of maximum valuable dosages. Information from huge investigations can be utilized to distinguish several characteristics of patients affecting the dose-concentration-response relationship. The models are then used to In future clinical preliminaries can be design by reproducing outlines of diverse trials in these models with a specific end goal to distinguish the one with the most astounding likelihood of achievement [212]. Characterizing ideal testing times for concentration and response estimations between such trials

supplements this technique [213]. Fundamentally, the data picked up with modeling and simulations allows to make decisions on *whether* and *how* to proceed with the improvement of another drug, with less space for abstract experimentation and thinking. Modeling strategies are useful at any phase of the drug development procedure [214].

Concentration-time profiles in people can be anticipated with physiologically-based PK models, combining in vitro data and preclinical studies on animals. PK/PD models are utilized for the estimation of the primary dose of high-hazard drugs in people such as monoclonal antibodies [215]. Estimations of response such as biomarkers in clinical trials phase 1 studies in volunteers give the primary knowledge into the concentration-response relationship of medication in people and are hence refined patients study data. The capacity of models to be updated with recently arriving data, the spread of learning from one stage to the next, is an alluring feature for this approach. Mandema et al., assessed the potential advantages of lipid-lowering drug gemcitabine over the contender ezetimibe, utilizing simulations from a PK/PD model created with accessible data from the literature [216]. the benefits of modeling and simulations have also been confirmed by both the academia and pharmaceutical industries [217] and from the administrative perspective [218]. A noteworthy administration counseling organization issued a business proposal on PK/PD modeling and simulations entitled Pharma 2020: Virtual R and D [219], supporting the significance of this approach for the proficient development of creative drugs later on.

2.4.3 Applications in Personalized Treatment

Several drugs are available in the market showing that a similar dose ought to be similarly successful for each person, from the youth to aged one. Their utilization in such non-typical patient populaces is basically not prescribed because of the absence of detailed knowledge, anticipating these patients to profit by the drug or leaving the choice on the suitable dose to the doctor, without direction [220]. This is due to lack of learning of the dose-response relationship, yet more imperatively in

light of the fact that conventional drug development programs have been centered excessively around finding a dosing regimen that is basic and simple to use for doctors and patients [221]. Dosage individualization depends on characteristics of patients like body weight, age, or function of an organ such as clearance of creatinine from the body [222].

Population PK/PD modeling can be utilized to distinguish such attributes. Dosage changes in view of the event of symptoms, the absence of an effect, a biomarker estimation, or drug concentration are often done in clinical practice, despite the fact that it may not be expressed unequivocally in the medication's mark. These individualization dosage techniques could enormously profit by PK/PD models, directing dosage modification instead of abandoning it to experimentation. With a PK model, individual PK parameters such as the drug clearance of a patient can be determined by utilizing Bayesian estimation. The Bayesian technique consolidates information of the PK attributes of a drug in a populace and individual patient data in the form of body weight, plasma fixations and so on. With a specific end goal to estimate individual PK parameters. Knowing the individual drug clearance (CLint) rate, for instance, allows estimating the dosage regimen to get a predefined target concentration [223]. A PK/PD model could be used to ascertain the drug concentration that accomplishes the desired response of biomarkers in a patient, not just accounting for PK changes between patients, yet in addition for variety in the pharmacological reaction.

In the light of expanding interest for tailored therapies and personalized medicines, PK/PD-guided concentration individualization may get attention and many other techniques like this will be formed in near future [224].

2.4.4 Tools and Software used for PK/PD Modeling and Simulations

Evaluations of the PK/PD attributes are an indispensable part of the development of therapeutic agents and proteins. As compared to the small drug molecules,

remedial proteins have numerous particular PK/PD characteristics that require the utilization of altered or separate methodologies for identifying their PK/PD results. A number of PK/PD tools are currently being used to support the process of therapeutics development. Table 2.4 shows the most popular tools used for PK/PD modeling

TABLE 2.4: List of Tools and Software used for PK/PD modeling and Simulations

Tool	Working	Limitations	Reference
Phoenix 7.0 PK/PD	calculates pharmacokinetics, pharmacodynamics, and toxicokinetics by using PK/PD data, generates high resolution graphics,	Run on Linux	[225]
Simbiology Matlab	Performs PK/PD analysis and simulate biological pathways, analyze systems dynamics,	Takes more than 16 GB of memory and Matlab programing language	[226]
PFIM	Evaluates population design of drugs by using non linear mixed model	Only use fixed parameters	[227]
PolyPK	Performs poly PK analysis by using metabolomic approach and statistical analysis	requires R language and run on Linux	[228]
gPKPDSim	Preclinical and translational PK/PD modeling	run on default parameter values	[229]

DoseSim	Access the efficacy and safety of chemicals and construct dosage schedule	lack of user-friendly interface	[230]
PFIM	Performs PK/PD modeling on population data	Not suitable for single character data	[230]

Chapter 3

Material and Methods

The detailed flowchart of the applied methodology is shown below.

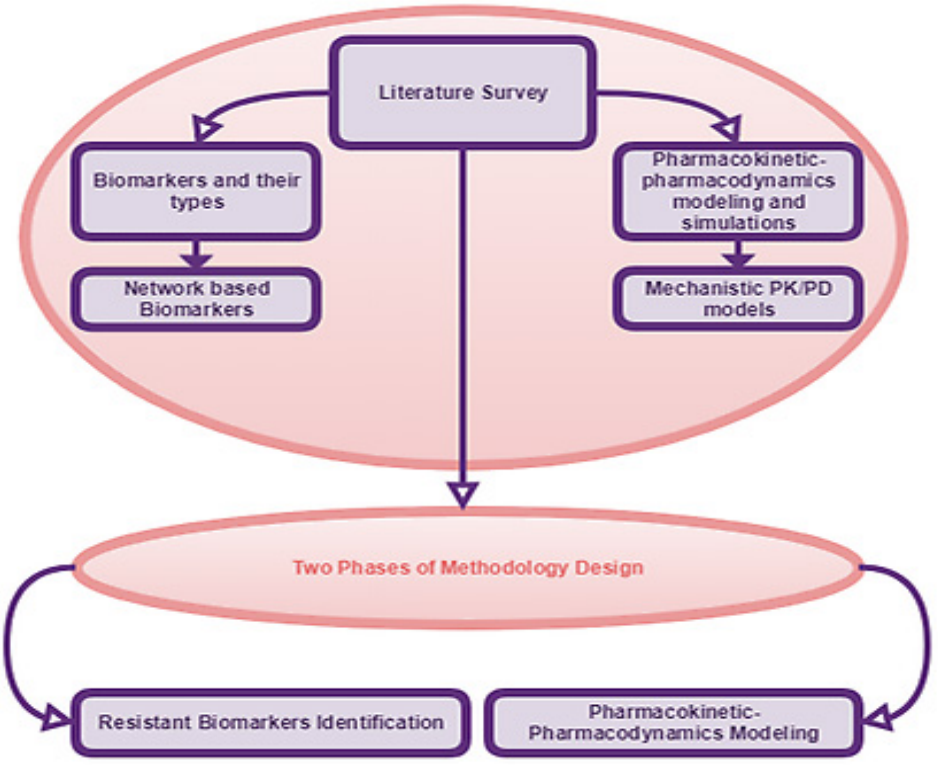


FIGURE 3.1: Detailed flowcharts literature survey for the identification of resistant biomarkers and PK/PD modeling

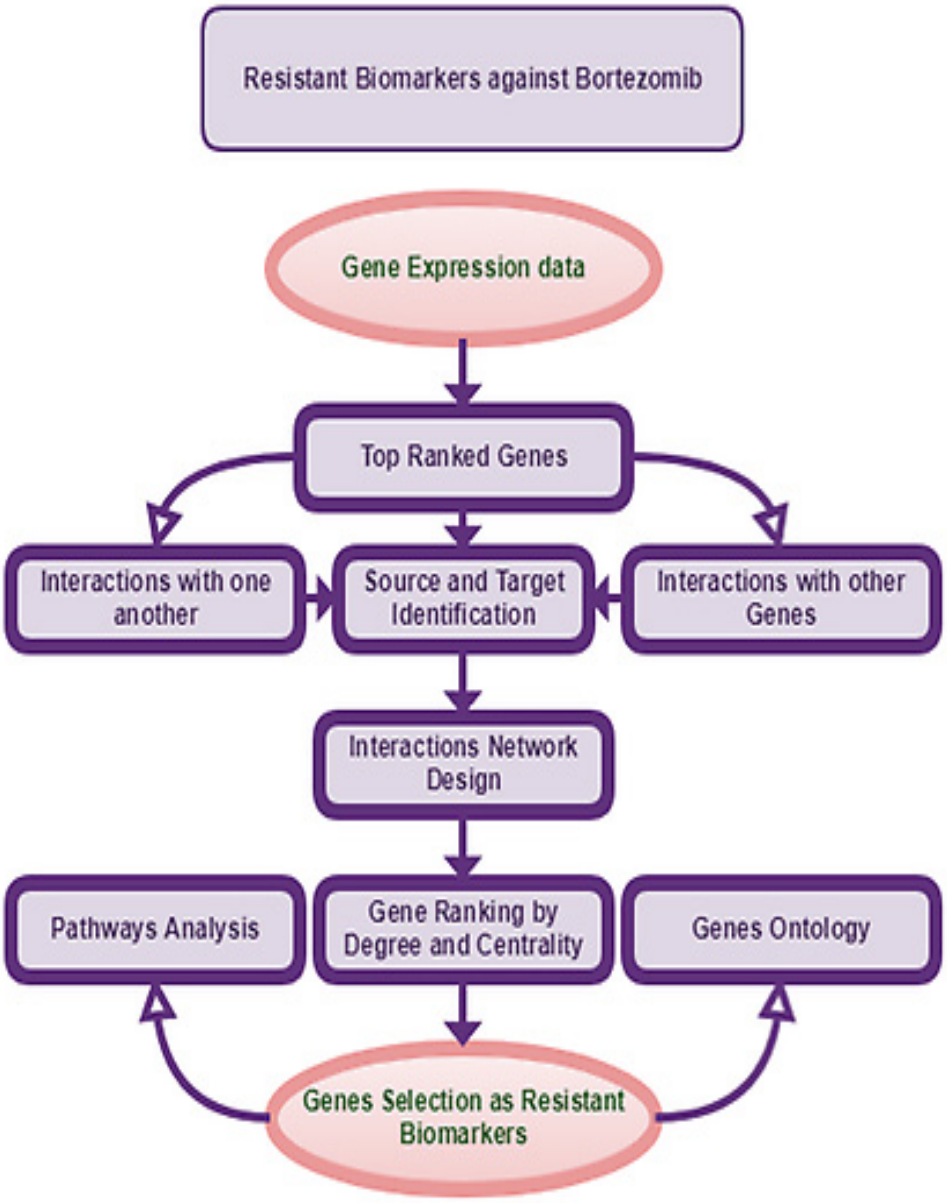


FIGURE 3.2: Detailed flowcharts of methodology applied for the identification of resistant biomarkers

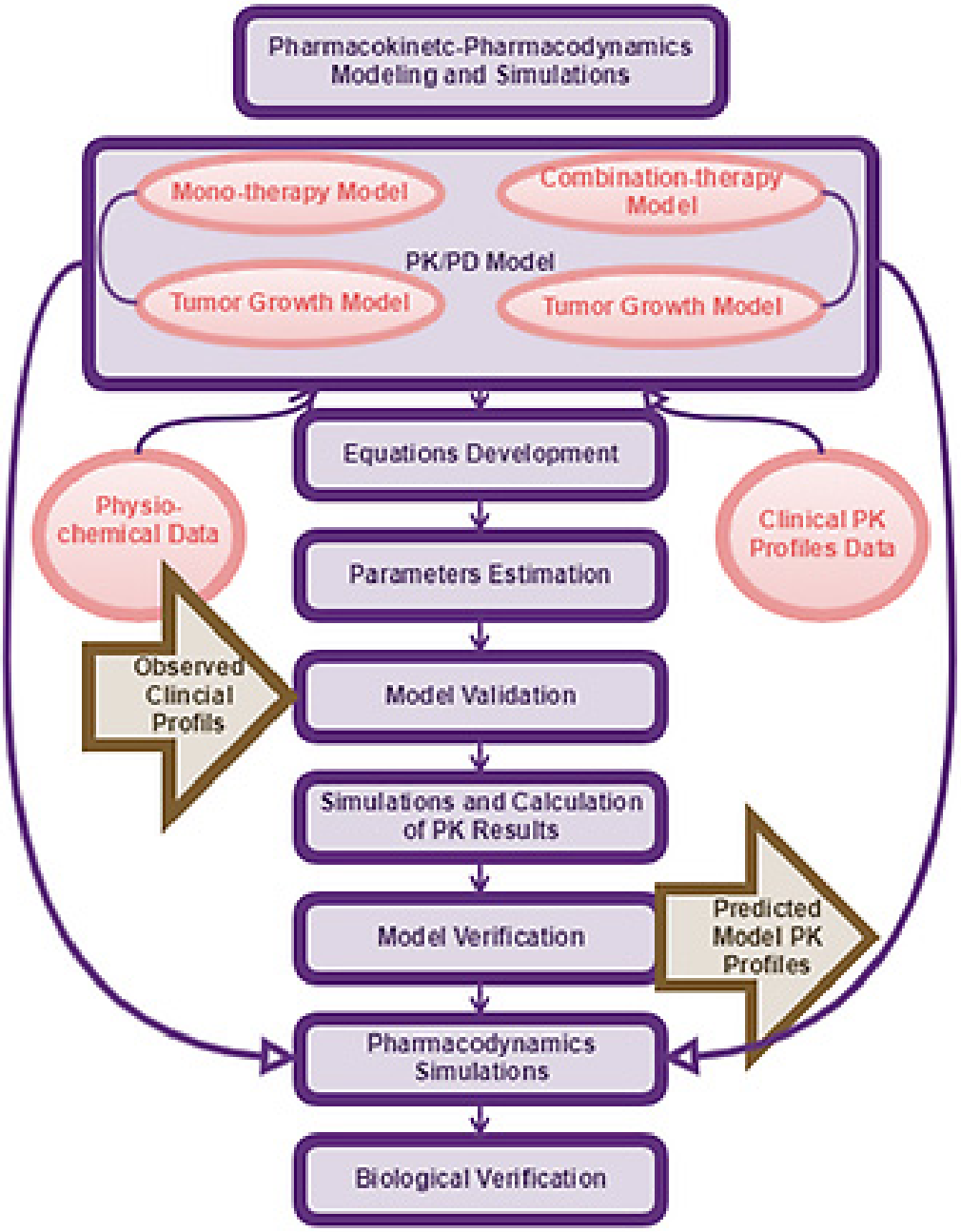


FIGURE 3.3: Detailed flowcharts of methodology applied for PK/PD modeling

3.1 Gene Expression Dataset

Gene expression profiling dataset of the clinical trials of bortezomib against WM, having established WM cell lines BCWM.1, MWCL-1 and RPCI-WM1, including their bortezomib-resistant subclones BCWM.1/BR, MWCL-1/BR and RPCI-WM1/BR were obtained through the Mayo Clinic Florida. Mayo Clinic Jacksonville is the hospital located in Jacksonville, Florida. It is one of three grounds alongside Phoenix/Scottsdale, Arizona, and Rochester, Minnesota. Groups of researchers and doctors direct research with the objective of enhancing persistent consideration. Their focus is to discover new and better approaches to anticipate, analyze, predict and ideally treat complex brain conditions, Cancers, and other illnesses. Analysts work cooperatively in research facilities, on clinical trials, and on epidemiological investigations to perform important discoveries for disease treatment.

3.2 Ranking of Genes According to Resistance

Framing the level of resistance as the unit of investigation, to acquire the resistant driver genes, we split the gene expression datasets of cell lines into three portions. Each portion comprised of gene expression values ranging from 0-0.9 of one resistant cell line and normal cell line alongside genes names list. To identify novel driver genes, we calculated the absolute of difference between the gene expression values of each normal cell line and its corresponding resistant cell line to find out the genes which have changed their expression under prolonged exposure to Bortezomib and named them as resistant driver genes. In a similar manner, we obtained the resistant driver genes of the remaining portions of the dataset. It is argued that the potential drug-resistant genes will undergo significant change in their responses when subjected to an extended treatment of Bortezomib. To eliminate non-specific genes from the analysis, we screened each portion of the dataset and selected only those genes whose absolute difference values were greater than

or equal to the arbitrarily selected threshold value which happens to be 0.9 in this case.

3.3 Novel Driver Genes Identification and Interaction Analysis

To find the co-expression and interaction of the resistant driver genes with each other and other genes of the expression dataset, we submitted the resistant driver genes lists of the first portion of the dataset into the FunCoup tool. FunCoup (http://FunCoup.sbc.su.se) is a database of functional couplings, or practical relationship, among the genes and their products. Recognizing these functions is vital in the comprehension of the larger amount of functions performed by complex processes of the cell. FunCoup recognizes four classes of genes interactions: genes role in a similar signaling network, cooperation in the equivalent metabolic process, co-enrollment in a complex of proteins and physical association [231]. We found different sorts of interactions of the particular resistant driver genes: 'physical', 'protein-protein interactions' and 'genetic interactions. All the genes and or proteins which demonstrate the certain level of interactions with the driver genes were selected and considered as novel resistant driver genes. In a similar manner, we obtained the novel resistant driver genes of the remaining portions of the dataset. These novel resistant driver genes were used to build Networks for each portion of the dataset.

3.4 Source and Targets Identification

The resistant driver genes set distinguished above were submitted to GeneMania tool to identify the source and target nodes, which yielded directed networks. GeneMANIA (https://genemania.org/) is an adaptable, easy to use the web interface for producing predictions about functions of the genes, analyzing the genes

set and organizing genes for practical measures. Given an inquiry list, GeneMANIA expands the genes list with practically comparative genes that it recognizes utilizing accessible genomics and proteomic information [232]. In a similar manner, we obtained the source and target genes of the remaining portions of the dataset.

3.5 Network analysis and Identification of Potential Biomarkers

The development and analysis of the networks were supported by Gephi [233]. The degree distribution of each network was figured out and the integrity of fit with a power-law distribution was resolved utilizing the coefficient of assurance (R2). A high R2 of few genes demonstrate them as the central hub genes. Modification of the abilities of these genes because of change, translocation or copy number variation could bring about injurious genes harming cell development. To investigate the structure of each network, we performed modularity analysis, Power-law degree distribution test, and centrality analysis. Centrality analysis recognized the central hubs in each system by different measurements. Four measurements of centrality were used to rank the genes, viz. the between-ness centrality [234], closeness centrality [235], Harmonic centrality [235], and Eigen-centrality [236]. The between-ness centrality was calculated by the formula shown in equation 1

$$C(v) = \sum \sigma \frac{sp^{(v)}}{\sigma_{sp}} \tag{3.1}$$

where C(v) is used for the centrality, sp is the total number of shortest paths from node s to node p and sp (v) is the number of those paths that pass through v. The closeness centrality and harmonic centrality was calculated by the formula shown in equation 2

$$C(v) = \frac{n}{\sum d(x, y)} \tag{3.2}$$

where d(x, y) is the distance between vertices x and y and N is the total number of nodes. The Eigen centrality was calculated by the formula shown in equation 3

$$C_{eg}(V) = \frac{1}{\lambda \sum a_{vt} x_t} \tag{3.3}$$

where avt is the matrix, and xt are constants. The modularity was calculated by the formula shown in equation 4

$$M = \sum [\frac{i}{E} - \frac{d}{2E}^{2}] \tag{3.4}$$

where E is the number of edges in the network, i represent the number of strongly connected edges, and d is the degree of a node. These analyses were selected for their estimation of the integral properties of hub gene significance. The genes having higher values from each measure were picked to yield an accord set of central genes for each stage. These are the "hub" genes or potential resistant biomarkers recognized in our work. A gene basic to each set is a driver and a hub.

3.6 Genes Enrichment Ontology

The experimentally validated biomarkers, identified through networks are submitted to the Gene Ontology tool for identification of their molecular function. Gene Ontology is the structure of the model of science. The GO characterizes ideas/-classes used to portray gene functions, and connections between these ideas. It characterizes functions along three viewpoints: molecular function, cellular component, and biological process. The Gene Ontology analysis Consortium is available at (http://www.geneontology.org/page/go-enrichment-analysis)

Gene ontology consortium retrieves the activities of genes performed at molecular level, cellular level, and also, calculates the biological processes performed by genes to achieve the maximum number of activities a gene can perform, it also characterize the genes according to eukaryotic or prokaryotic specie.

3.7 Identification of Pathways Associated with Resistant Biomarkers

The associated pathways for each resistant biomarker were identified through EnrichNet tool available at (http://www.enrichnet.org/). The benefit of EnrichNet is the analysis of genes set with known inclusion in human ailments, identification of new pathway affiliations and sub-interaction networks between their protein products [237]. The identified pathways were further cross-validated by the KEGG Mapper (https://www.genome.jp/kegg/tool/map_module2.html) is the latest version of KEGG pathways where the set of genes or proteins can be given and it returns their associated pathways and modules.

3.8 Implementation of PK/PD Model

The PK/PD model in the form of monotherapy and combination therapy PK/PD was designed in the Simbiology toolbox of Matlab, the tumor growth model was integrated with the mono and combination therapy PK/PD models collectively, the tumor growth model used for the integration was suggested by Simoni et al., [238]. This model consists of several attributes that shift with the passage of time, also the model describes the growth rate of the tumor without the administration of drug and change in the tumor growth rate after the administration of several drugs doses.

Four variants were set in the combination therapy PK/PD model in the form of PK estimates of drug 1, PK estimates of drug 2, cell line 1, and cell line 2. As our gene expression data consists of both normal and resistant cell lines and each biomarkers network consists of genes from one normal cell line along with its Bortezomib resistant sub-clone cell line, therefore, the cell line 1 and cell line 2 variants were created in the model. here in this study, PK/PD modeling was done to access the effect of drugs on biomarkers individually as well as in the

combination. For the purpose of Combination therapy, PK/PD modeling the PK estimates of drug 1 and 2 variants were created.

3.9 Parameters Estimation and Differential Equations Development

To determine the underlying processes of every component in the model, several parameters are required to decide the reaction kinetics, reaction kinetics mechanisms, and the conduct of the model components [239]. The parameters of the model were obtained through the study of Li et al.,[240]. The parameters of drugs were obtained through literature. Five same class drugs - Rituximab, Carfilzomib, Bortezomib, Oprozomib, and Ixazomib- were used for the PK/PD modeling against biomarkers response.

The parameters for the drugs were retrieved through clinical PK profiles. The important parameters considered for this study were molecular weight, EC50, and dose [241–244]. All the parameters were estimated by ODE Solver(23) toolbox of Matlab. For the estimation of parameters, the PK data of drugs were uploaded to the PK compartment of the model and 'data fit' function was performed through non-linear least squares numerical function. Several ordinary differential equations for the PK model, monotherapy PK/PD model, combination therapy PK/PD model and tumor growth model were developed collectively.

3.10 Model Validation and Verification

Model validation is taken as a major aspect of the model design and development process [245]. The validation procedure is attempted with the end goal to guarantee that the model created is adequately precise for the current reason [246]. Therefore, for this purpose, the clinical trials data in the form of a number of patients, dosage regimens, and a number of cycles for the Rituximab, Carfilzomib,

Bortezomib, and Oprozomib were retrieved through literature [247–251]. The data were imported into the models consecutively and the model simulations were performed. The simulations of models were compared with the clinical trials data of drugs to validate our PK/PD model.

3.11 Pharmacokinetics-Pharmacodynamics Modeling

The pharmacokinetics modeling is the use of pharmacokinetic standards to the safe and successful restorative administration of medications in an individual patient. An effect of the drug is frequently identified with its concentration at the site of action, so it is valuable to screen this concentration [252]. Similarly, Pharmacodynamic modeling depends on a quantitative reconciliation of pharmacokinetics, pharmacological systems, and pathophysiological procedures for understanding the time-course of medication effects and their intensity on the body. Use of such models to the examination of important exploratory information takes into account the evaluation and prediction of drugbody interactions for both restorative and unfavorable medication responses [253].

The PK/PD modeling was done for both monotherapies and combination therapies. Their concentration in the central compartment and their effects on the growth of tumor were determined to identify proper dosage regimens against the resistant biomarkers of WM. The tumor growth dynamics were modeled because subject variability is the most important characteristic of cancer. The change in tumor growth was determined for each drug

3.12 Biological Verification

Computational models are just reflections of the natural biological phenomenon and in this manner should be approved before they are utilized for real life or

natural applications. To guarantee the dependability and vigor of a model the approval methodology ought to be depicted in a reproducible way, the purpose behind which standard documentation or general rules could be conceivably helpful. There is no particular method for validating the models in biological or in different fields. The procedure is the equivalent all over sciences, several numerical measures are used to predict the accuracy of model [254]. Keeping this thing in mind, the simulations results of PK/PD models were biologically verified by their comparison with the clinical PK/PD results of selected drugs available in literature [247–251].

3.13 Modeling of Biomarkers Expression Level in Response to Particular Drugs

For modeling the response of biomarkers against specific medicines, the biomarkers interactions model was developed and drugs doses were induced into the model to predict the change in expression level of each biomarker collectively. All the Parameters of biomarkers were identified through the obtained gene expression data and estimated through the ODE solver nonlift- lease square method.

Chapter 4

Results and Discussions

4.1 Identification of Driver Resistant Genes

The WM cell lines dataset consisted of total 34683 genes and proteins, among which many genes and or proteins are predicted and not validated experimentally. However, among the cell lines dataset, Six-cell lines represent Bortezomib drug response values. Among these established WM cell lines three were normal: BCWM.1, MWCL-1 and RPCI-WM1 and three were bortezomib-resistant subclones: BCWM.1/BR, MWCL-1/BR and RPCI-WM1/BR. Considering the issue of resistance against Bortezomib, we tried to identify the resistant driver genes from cell line data to find the potential biomarkers for WM. Only those genes were selected as resistant driver genes whose absolute values were lying in the range from 0.90-0.99. A disease driver gene is characterized as one whose mutations increases the net cell development under the particular micro-environmental conditions that exist in the cell in vivo. The aggregate number of driver genes is obscure [255], Keeping this phenomenon in mind, we gave the name resistant driver genes to our identified genes. Table 4.1 demonstrates the number of driver genes identified at first progression of our screening methodology.

TABLE 4.1: The resistant driver genes identified in each portion of the dataset

Cell Lines: BCWM.1/BR and BCWM.1	Cell Lines: RPCI-WM1/BR and RPCI-WM1	Cell Lines: MWCL-1/BR and MWCL-1
PELI2	HS.572121	YAP1
ACP5	GALNTL4	ZNF256
LOC646084	HS.514454	LOC643612
ERCC-00076	LOC389105	LRRC31
CCDC48	LONRF1	HS.563400
PGLYRP1	JMJD2A	DPYS
LOC647190	MT1M	LRRN3
LOC653257	HS.336593	PALLD
SH2D7	CUEDC1	AGMAT
SMOC2	HS.540022	SYNJ2
MIR513A2	MAOB	LOC647089
LOC100132923	LOC652542	LOC646064
CATSPERB	XDH	KRT39
SLC46A2	LOC283050	HIST1H2AJ
HS.576915	TPSG1	RNF144B
FXYD6	SNORA51	INSM2
LOC648147	LOC647881	MGC16291
SEPN1	COL17A1	CRYBA4
HS.537149	LOC646892	MORN4
	LOC284441	HS.554274
	ZNF541	LOC643699
	OPN1SW	IAPP
	HIST1H2AH	LOC440956
	CPS1	OR5J2

	RRAD	TNNI3K
	LOC652764	TMEM119
	UNC13C	PAG1
	LOC645359	ENG
		MTTP
		IL1A
		PRAM1
		LOC100134353
		NUP62CL
		ST18
		PBXIP1
		TMSB4Y
		MIR197
		HS.581341
		LOC284757
		LRRK2
		LOC339352
		LOC644171
		LOC645585
		LOC541469
		LOC391771
		C8ORF47
		LOC650651

4.1.1 Identification of Novel Resistant Driver Genes

The resistant driver genes identified were then checked for their interactions with other genes of the cell lines to identify the novel driver genes. The novel driver genes in this research work were those which demonstrate a maximum number

of interactions with other genes of the cell lines. By adopting the methodology of finding interactions of resistant driver genes with other genes, we found the maximum number of novel resistant driver genes. The novel resistant driver genes identified for the first portion of data were lying in the range of 0.004 to 0.5, the novel resistant driver genes identified for the second portion of data were lying in the range of 0.0002 to 0.298, and the for the third portion of the data was in the range of 0.0002 to 0.619. The final sets of novel driver genes for each cell line utilized as a part of the consequent Gene Mania search are given in Table 4.2

TABLE 4.2: The novel resistant driver genes identified through Gene Mania for each portion of the dataset

Cell Lines: BCWM.1/BR and BCWM.1	Cell Lines: RPCI-WM1/BR and RPCI-WM1	Cell Lines: MWCL-1/BR and MWCL-1
ACP5	JMJD2A	DDAH1
C6orf25	ACOX1	PTEN
CAMP	DAO	MTTP
CATSPERB	OPN1SW	SLIT3
CCDC48	ERO1B	RASGRF2
CEACAM8	MAOB	HIST1H4H
DEFA4	CYB5R3	COL16A1
ERCC-00076	IVD	LRRN3
FXYD2	MAOA	AQP3
FXYD3	XDH	SATB1
FXYD4	CPS1	PALLD
FXYD5	ACAD8	HIST1H2BJ
FXYD6	GSR	AGMAT
FXYD7	GALNTL4	ST18
FYDX1	MT1M	SYNJ2

FXYD7	GALNTL4	ST18
FYDX1	MT1M	SYNJ2
FXYD6	SNORA51	INSM2
HS.537149	ZNF541	CDK6
SEPN1	COL17A1	CRYBA4
HS.576915	AIFM1	IL1A
LOC100132923	TPSG1	RNASE1
LOC646084	COL17A1	IAPP
LOC647190	POR	UTY
LOC653257	GFER	EIF1AY
MIR513A2	LONRF1	HIST1H2BO
MPO	HIST1H2AH	HIST1H2BB
NRXN3	ACOX1	PTEN
PELI1	HS.572121	HIST1H2AJ
PELI2	HS.514454	RPS4Y1
PELI3	LOC389105	YAP1
PGLYRP1	HS.336593	ZNF256
PGLYRP2	HS.540022	LRRC31
PGLYRP3	LOC652542	DPYS
RAPGEF1	LOC283050	PAG1
SEPN1	SNORA51	OR5J2
SH2D7	LOC647881	RNF144B
SLC46A2	HS.540022	ENG
SMOC2	LOC652542	INSM2
SPOCK2	LOC283050	CRYBA4
	SNORA51	TMEM119
	LOC647881	TMSB4Y
	LOC646892	C8orf47
	RRAD	LRRK2

	LOC652764	LOC643612
	UNC13C	HS.563400
	LOC645359	LOC647089
		LOC646064
		KRT39
		MGC16291
		MORN4
		HS.554274
		LOC643699
		LOC440956
		OR5J2
		PRAM1
		LOC100134353
		NUP62CL
		MIR197
		HS.581341
		LOC284757
		PBXIP1
		LOC339352
		LOC644171
		LOC645585
		LOC541469
		LOC391771
		LOC650651
		HIST1H1E
		HIST1H2AJ
		HIST1H2AE
		HIST1H2BE
		HIST1H2AM
		HIST1H1E

4.2 Identification of Resistant Biomarkers from Genes Interaction Network

The novel driver genes were used to design directed networks of interactions. We examined the degree distribution of each directed network and found that the node distribution of all the three networks adjusted better to a power law distribution than a direct model, A power-law fit suggests the nearness of a couple of exceedingly connected nodes (i.e. hubs) in the networks. Generally, Hub could incline susceptibility to the disease. Transformations in these hub genes could prompt functional changes in the related protein which could produce changes in its interaction with different proteins. This could prompt a failure in the network and cause illness [256]. In this specific situation, a power law behavior suggests that changes in the hub genes could expand vulnerability to the signs of cancer [257] and encourage the spread of the agitation in the network. In this way, distinguishing proof of the hub genes could also pinpoint the key genes whose failure would underscore the development of cancer or bring about resistance to specific medication. The network of the novel resistant driver genes obtained for the cell lines BCWM.1 and BWCM.1/BR is shown in Figure 4.1.

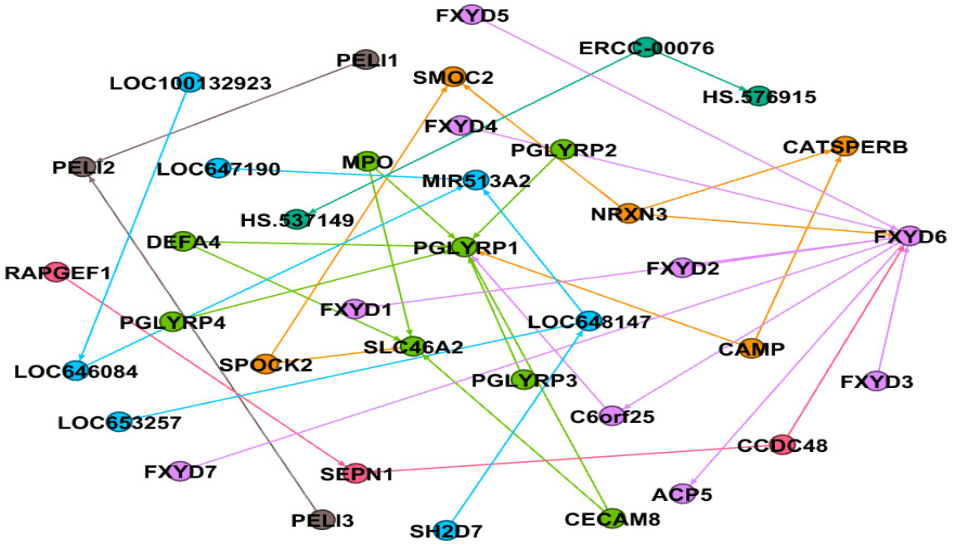


FIGURE 4.1: The network of novel driver genes of the cell lines BCWM.1 and BCWM.1/BR

Only five hub driver genes or the potential biomarkers were identified and displayed a higher degree, and centrality values in figure 4.1: FXYD6, C6orf25, LOC646084, MIR-513A2, and LOC648147. Among these biomarkers, only FXYD6 and C6orf25 are previously validated by experiments and are reported in several databases and literature, remaining ones are predicted. These genes represented themselves as strongly resistant against the Bortezomib and can be used as potential biomarkers for WM. The same method was applied to design the directed networks of the other cell lines. The network of the novel driver genes of the cell line RPCI-WM1/BR and RPCI-WM1 is shown in figure 4.2.

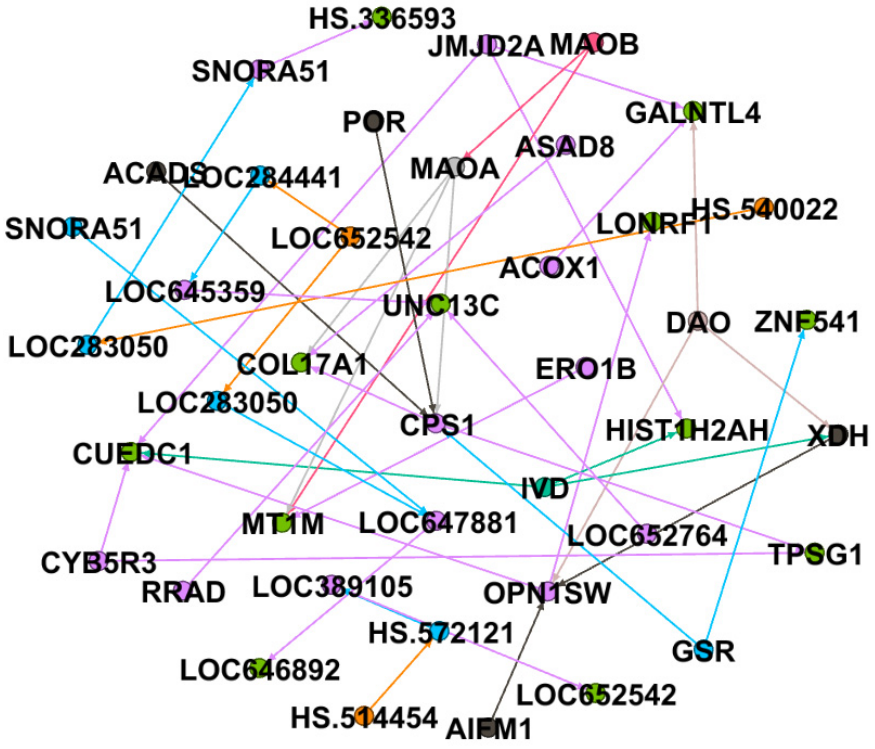


FIGURE 4.2: The network of the novel driver genes of the cell line RPCI-WM1/BR and RPCI-WM1

The potential biomarkers identified in the network of cell lines MCF6A and MCF3A were OPN1SW, MAOA, XDH, CPS1, HS.572121, LOC389105, LOC283050, SNORA51, LOC-283050, LOC647881, LOC284441, and LOC645359. Among these biomarkers, only OPN1SW, MAOA, XDH, CPS1, and SNORA51, are already reported experimentally validated genes and remaining ones are predicted. The network

of the novel resistant driver genes obtained for the cell lines MWCL-1/BR and MWCL-1 is shown in figure 4.3.

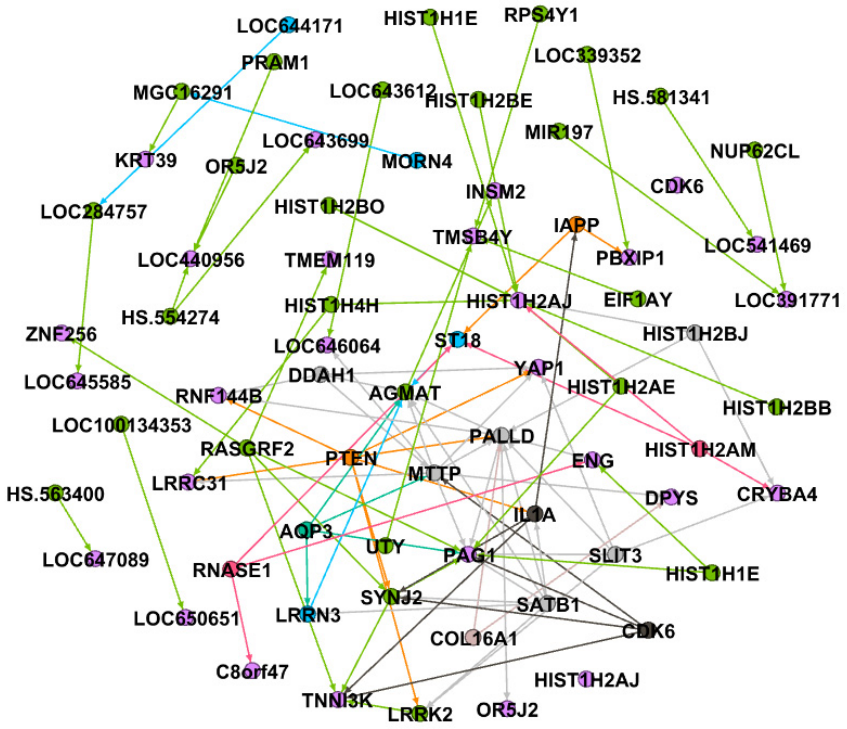


FIGURE 4.3: The network of novel driver genes of the cell lines MWCL-1/BR and MWCL-1

The potential biomarkers identified in the network of cell lines shown in figure 4.3 were MTTP, PALLD, AGMAT, SYNJ2, IL1A, IAPP, LRRK2, and LOC284757, all the genes are already reported experimentally validated except the LOC284757, and can be used as potential biomarkers against bortezomib resistance in WM.

4.3 Degree Distribution and Centrality Analysis of Predicted Resistant Biomarkers

Formally a centrality is a capacity C which gives each vertex v of a network, a numeric esteem C(v). As we were interested in the ranking the vertices of a

given network [256], we have utilized four kinds of centrality measures, closeness centrality, betweenness centrality, harmonic centrality, and the Eigen centrality, the power-law degree distribution and centrality measures of three networks are displayed in table 4.3 - 4.5.

TABLE 4.3: The centrality measure values and power-law degree distribution analysis of the first network

Label	In-Degree	Out Degree	Total Degree	Closeness Centrality	Harmonic Centrality	Betweenness Centrality	Modularity
FXYD6	8	3	11	0.8	0.875	31	1
C6orf25	1	1	2	1	1	9	1
LOC-646084	1	1	2	0.67	0.75	2	6
MIR-513A2	2	1	3	1	1	4	6
LOC-648147	1	2	3	0.75	0.83	3	6

TABLE 4.4: The centrality measure values and power-law degree distribution analysis of the Second network

Label	In-Degree	Out Degree	Total Degree	Closeness Centrality	Harmonic Centrality	Betweenness Centrality	Modularity
OPN1-SW	3	2	5	1	1	7	1
MAOA	1	3	4	0.8	0.875	3	1
CPS1	4	2	6	1	1	8	1

HS.-572121	1	1	2	0.66	0.75	2	3
LOC-389105	1	1	2	1	1	2	3
LOC-283050	1	1	2	0.66	0.75	2	4
SNOR-A51	11	1	2	1	1	2	4
LOC-647881	2	1	3	1	1	3	7
LOC-284441	1	1	2	0.66	0.75	2	7
LOC-645359	1	1	2	1	1	2	73

TABLE 4.5: The centrality measure values and power-law degree distribution analysis of the Third network

Label	In-Degree	Out Degree	Total Degree	Closeness Centrality	Harmonic Centrality	Betweenness Centrality	Modularity
MTTP	3	7	10	0.73	0.81	25	4
PALLD	3	5	11	0.85	0.91	35.5	4
AGMAT	7	1	8	1	1	16	4
SYNJ2	5	2	7	1	1	2.16	3
IL1A	2	3	5	0.5	0.63	6.66	3
IAAP	1	2	3	0.57	0.7	8	14
LRKK2	3	1	4	1	1	1.66	1
LOC-284757	1	1	2	1	1	1	10

The degree of a node in a network is the number of its interactions with different nodes and the degree distribution is the likelihood distribution of these degrees over the entire system of the network, The higher the degree of a node more are its

interactions with other nodes and it is more relatedly considered as hub or potential biomarker [258]. Centrality examination is the positioning of network components used to distinguish intriguing nodes of a network is one of these techniques [259]. From table 4.3, it is clearly observed that the minimum weighted degree is 2 and maximum is 12, means none of the biomarkers have the degree value 0. Similarly, the minimum centrality value starts at 0.5 and end at 1. It is especially valuable to recognize key players in natural biological procedures. For instance, it has been demonstrated that very associated vertices in protein interaction network are frequently practically essential and the deletion of such vertices are identified with lethality [257].

4.4 Gene Ontology of Resistant Biomarkers

Total of 25 genes and or proteins were identified as biomarkers or hub driver genes from the networks. Drug resistance is an outstanding concept that occurs when illnesses become tolerant to pharmaceutical medicines. Numerous anticancer medications require metabolic actuation, and in this manner, tumor cells can create resistance through diminished medication enactment. The drug resistance is additionally accomplished by the changes in the signal transduction process that intervene drug activation [260]. To overcome the issues of drug resistance, the Gene Ontology (GO) of the distinguished biomarkers was done, just 12 biomarkers represented the GO profiles, which are tentatively approved, remaining 13 didn't demonstrate any GO profile. The gene ontology of the resistant biomarkers is shown in table 4.6

TABLE 4.6: The Gene Ontology of the potential biomarkers identified from the networks

Mapped IDs	Gene Names	Function
PALLD	Palladin	actin binding

LRRK2	Leucine-rich repeat serine/threonine-protein kinase 2	MAP kinase kinase activity, ion channel binding, tubulin binding, Rho GTPase binding, GTPase activator activity
SYNJ2	Synaptojanin-2	PDZ domain binding, SH3 domain binding
MTTP	Microsomal triglyceride transfer protein large subunit	apolipoprotein binding, lipid transporter activity, lipid binding
MAOA	Amine oxidase [flavin-containing] A	serotonin binding, flavin adenine dinucleotide binding
IL1A	Interleukin-1 alpha	interleukin-1 receptor binding, copper ion binding, cytokine activity
FXYD6	FXYD domain-containing ion transport regulator 6	enzyme modulator ion channel activity
CPS1	Carbamoyl-phosphate synthase [ammonia], mitochondrial	endopeptidase activity, metal ion binding
OPN1SW	Short-wave-sensitive opsin 1	receptor activity, G-protein coupled receptor activity
IAPP	Islet amyloid polypeptide	hormone activity, receptor binding

XDH	Xanthine dehydrogenase/oxidase	2 iron, 2 sulfur cluster binding, flavin adenine dinucleotide binding, iron ion binding
AGMAT	Agmatinase, mitochondrial	metal ion binding

Gene Ontology is a noteworthy bioinformatics activity to bring together the portrayal of gene and gene item traits overall species [261] "Ontologies" comprise a portrayal of things that are perceptible or straightforwardly noticeable and the relationships between those things.

LRRK2 Gene: This gene is an individual from the leucine-rich repeat kinase family and encodes a protein with a region of ankyrin repeat, the kinase domain, a leucine-rich repeat domain, a DFG-motif, a GTPase domain, a WD40 domain a RAS domain, and an MLK-like domain. The protein is available to a great extent in the cytoplasm yet in addition partners with the mitochondrial external film. Changes in this gene have been related to Parkinson ailment [262].

PALLD Gene: This gene encodes a cytoskeletal protein that is required for arranging the actin cytoskeleton. The protein is a segment of actin-containing microfilaments, and it is engaged with the control of cell shape and grip. Polymorphisms in this gene are related to a weakness to pancreatic cancer type 1, and furthermore with a hazard for myocardial necrosis [263].

MTTP Gene: MTP encodes the huge subunit of the heterodimeric microsomal triglyceride exchange protein. Protein disulfide isomerase (PDI) finishes the heterodimeric microsomal triglyceride exchange protein, which has appeared to assume a focal part in the assembly of lipoprotein. Transformations in MTP can cause abetalipoproteinemia [264].

MAOA Gene: This gene is one of two neighboring gene relatives that encode mitochondrial catalysts which catalyze the oxidative deamination of amines, for example, dopamine, norepinephrine, and serotonin. Transformation of this gene

outcome in Brunner disorder. This gene has likewise been related to an assortment of another mental issue, including reserved conduct. On the other hand, grafted transcript variations encoding numerous isoforms have been observed [265].

SYNJ2 Gene: The gene is an individual from the inositol polyphosphate 5-phosphatase family. The encoded protein connects with the Ras-related C3 botulinum poison substrate 1, which causes translocation of the encoded protein to the plasma membrane where it restrains clathrin-intervened endocytosis [266].

IL1A Gene: The protein encoded by this gene is an individual from the interleukin 1 cytokine family. This cytokine is a pleiotropic cytokine associated with different immune reactions, inflammatory procedures, and hematopoiesis. This cytokine is delivered by monocytes and macrophages as a proprotein, which is proteolytically handled and discharged in light of cell damage, and along these lines instigates apoptosis. This gene and eight other interleukin 1 family genes made a cytokine gene group on chromosome 2. It has been recommended that the polymorphism of these genes is related to rheumatoid joint pain and Alzheimer's sickness [267].

FXYD6 Gene: This gene encodes an individual from the FXYD group of transmembrane proteins. This specific protein encodes phosphohippolin, which likely influences the movement of Na, K-ATPase. Different on the other hand joined transcript variations encoding a similar protein have been depicted. Related pseudogenes have been distinguished on chromosomes 10 and X [268].

CPS1 Gene: The mitochondrial enzyme encoded by this gene catalyzes the production of carbamoyl phosphate from salts and bicarbonate. This response is the principal submitted venture of the urea cycle, which is vital in the excretion of an overabundance of urea from cells. Three transcript variations encoding diverse isoforms have been found for this gene [269].

XDH Gene: Xanthine dehydrogenase is a member of molybdenum-containing hydroxylases engaged with the oxidative digestion of purines. The encoded protein has been distinguished as a working protein in view of its abilities to perform

robotically particular functions. Xanthine dehydrogenase can be changed over to xanthine oxidase by reversible sulfhydryl oxidation or by irreversible proteolytic adjustment [270].

OPN1SW: This gene is a member of the G-protein coupled receptor 1 family, opsin subfamily. It encodes the blue cone pigment gene which is one of three types of cone photoreceptors accountable for normal color visualization. Imperfections in this gene are the reason for Tritan color blindness. Pretentious persons lack blue and yellow sensory mechanisms while recollecting those for red and green [271].

AGMAT Gene: AGMAT gene encodes an enzyme agmanitase and belongs to the family of hydrolases and extremely expressed in the kidney and liver. Similarly, it also originates in skeletal muscle, skin, testis, fetal liver, brain, and the gastrointestinal tract [272].

IAPP Gene: This gene encodes an individual from the calcitonin group of peptide hormones. This hormone is discharged from pancreatic beta cells to manage blood glucose levels. Human patients with type 1 and 2 diabetes show lessened levels of the encoded hormone in blood and pancreas. This protein additionally shows bactericidal, antimicrobial activities [273].

4.5 Pathways Associated with the Predicted Biomarkers

Each predicted biomarker was then checked for its role in several pathways leading to the WM. Majority of the predicted resistance biomarkers have shown their roles in the metabolic pathways, signaling pathways, and biosynthesis pathways. AGMAT, SYNJ2, CPS1, MAOA, and XDH have shown their major roles in the arginine and proline metabolic pathways, IL1A is involved in the majority of signaling pathways including NF-kappa B, AKT signaling, TGF-beta signaling etc. the identified biomarkers were annotated and mapped to the associated pathways to determine their role in WM. The pathways were identified for only already

reported experimentally verified gene, the details of the pathways are shown in Table 4.7.

TABLE 4.7: The Pathways associated with predicted resistant biomarkers

Gene Names	Associated Pathways	Databases
OPNS1W	G-alpha signaling in GPCR pathway	Reactome
	GPCR ligand binding pathway	
MAOA	Metabolism pathways	Reactome, KEGG, INOH
XDH	Metabolism pathways	Reactome, KEGG, INOH
CPS1	Metabolism pathways	Reactome, KEGG, INOH
MTTP	Metabolism pathways	Reactome, KEGG
	Transport of small molecules	
AGMAT	Metabolism pathways	Reactome, KEGG
SYNJ2	Metabolism pathways	Reactome, KEGG
IL1A	IL1A Pathway	NetPath
	Cytokine Signaling in Immune system pathway	Reactome
	Immune System pathway	
	MAPK signaling pathway	KEGG

	Cytokine-cytokine receptor interaction pathway	
	GPCR signaling pathway	INOH
	IL-1 signaling pathway	
	JAK STAT pathway and regulation pathway	
	Nfk-b signaling pathway	PID Biocarta

4.5.1 G-alpha signaling in the GPCR Pathway

Transducin (Gt) is a heterotrimeric G protein encoded by GNAT genes. Two types of G proteins are the alpha-1 chain and alpha-2 chain. OPSINS after stimulation, can bind to G (t) alpha subunits and act as GEFs. in this manner, the GDP is replaced with GTP. Hence, activated G (t) alpha proteins separate from the complex. Their activation results in the phototransduction cascade. Cyclic GMP Phosphodiesterase is activated which decreases cGMP levels. Lower cGMP levels would then be able to prompt the blockage of cGMP-directed Na+ and Ca2+ particle channels and a hyperpolarized film potential. The signaling component for G alpha (I) inhibits the cAMP pathway through hindrance of adenylate cyclase. Diminished generation of cAMP from ATP results in diminished action of cAMP-dependant protein kinases. Different elements of G alpha (I) incorporates activation of the protein tyrosine kinase Src [274]. Regulator of G-protein Signaling proteins can manage the activity of G alpha (I). As the OPSINS acts as GEFs to activate the G-alpha which in turn activates the Src Kinase. The Src tyrosine kinase regulates adhesion and chemotaxis in WM and produces resistance against therapeutic agents in WM patients [275]. The Src tyrosine kinase also shows overexpression in WM cells as compared to cancers of B cells, and the Src inhibitor AZD0530 when use for treatment led to significant inhibition of adhesion, migration, and cytoskeletal signaling. The pathway is shown in Figure 4.4

4.5.2 Metabolism Pathway

In human peripheral blood monocytes, Interleukin-4 and 3 essentially up-regulate the proteins associated with inflammation including the outer-membrane protein monoamine oxidase-A (MAOA) in mitochondria. MAOA catalyzes the oxidative deamination of biogenic and dietary amines to regulate homeostasis. MAOA requires FAD as a cofactor, specially oxidizes biogenic amines, for example, 5-hydroxytryptamine (5HT), dopamine, noradrenaline, and adrenaline. 5HT is deaminated to 5-hydroxy indol-acetaldehyde -(5HIALD). Peripheral monocytes of WM patients show a differential expression of genes to their up-regulation [276]. The MAOA associated pathway is shown in figure 4.5

Cytosolic xanthine dehydrogenase (XDH) catalyzes the response of hypoxanthine with water and oxygen to shape xanthine and hydrogen peroxide. The dynamic type of the protein is a dimer. XDH also catalyzes the response of xanthine with water to generate urate. Cytosolic nucleoside phosphorylase (NP) trimer catalyzes the reversible response of inosine or deoxyinosine with orthophosphate to produce hypoxanthine and ribose 1-phosphate or deoxyribose 1-phosphate. The activation of NP with either nucleotide in vitro or in vivo, constraining the degree of this response. NP inadequacy in vivo is related to deformities in purine nucleotide and prompts immunodeficiency resulting in several types of Lymphoma. The changes in the activities of NP most likely reflect changes in the lymphocyte subpopulations and don't appear to have an etiological role in the pathogenesis of the disturbed response of immune system [277]. Therefore, the mutations in NP hiders the production of XDH leading to several subtypes of Lymphoma. The XDH associated pathway is shown in figure 4.6

This reaction occurs in the mitochondrial network and is interceded by the carbamoyl-phosphate synthase (CPS) forming CPS1 dimer. Mitochondrial N acetylglutamate synthetase (NAGS) catalyzes the response of glutamate and acetyl-CoA to produce N-acetyl-glutamate and CoA. Arginine activates the NAGS and produces N-acetylglutamatein in the response thusly it is required to initiate CPS1. NAGS

transformations in people are related with hyperammonemia whereas CPS1 is responsible for urea synthesis in the liver, any change in CPS1 leads to chronic liver disease, which in turns manifest into WM [278]. The CPS1 associated pathway is shown in figure 4.7

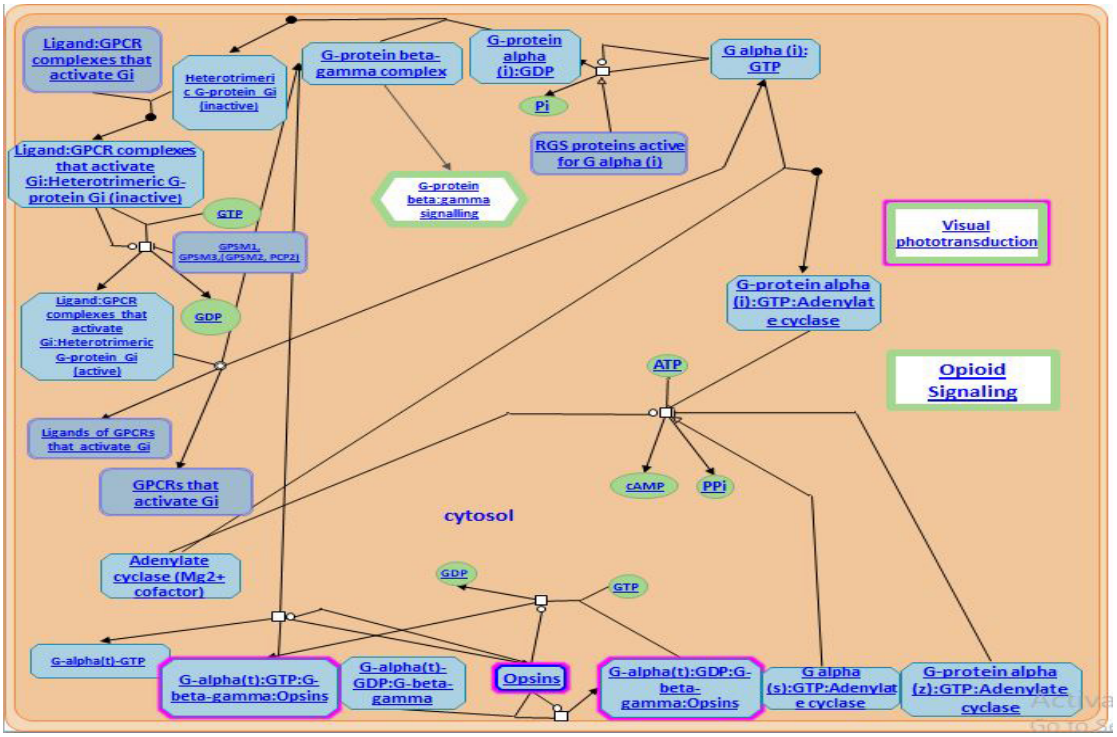


FIGURE 4.4: The role of OPSINS in G-alpha signaling in GPCR signaling pathway

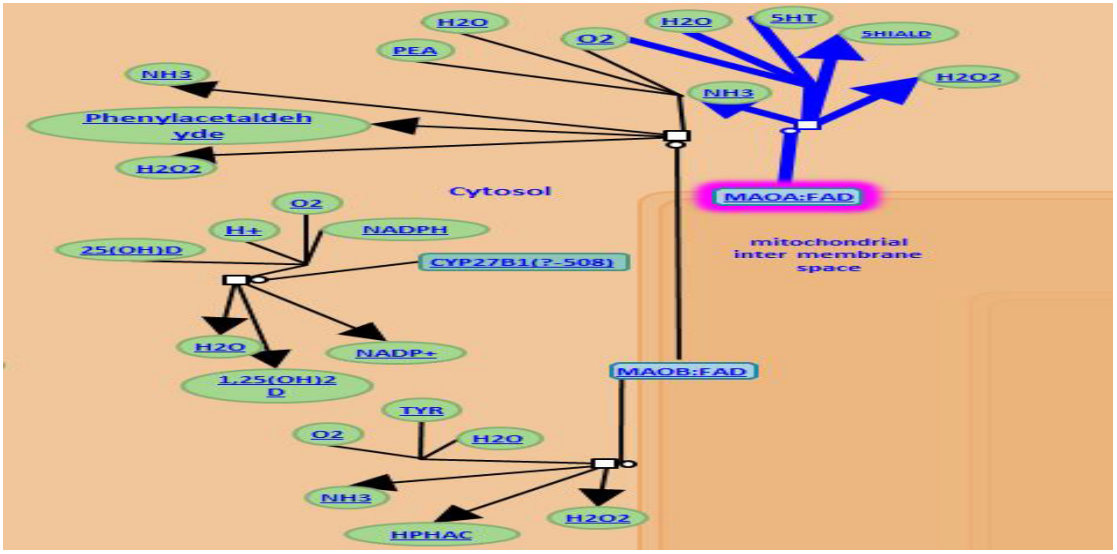


FIGURE 4.5: The role of MAOA in Metabolism pathway

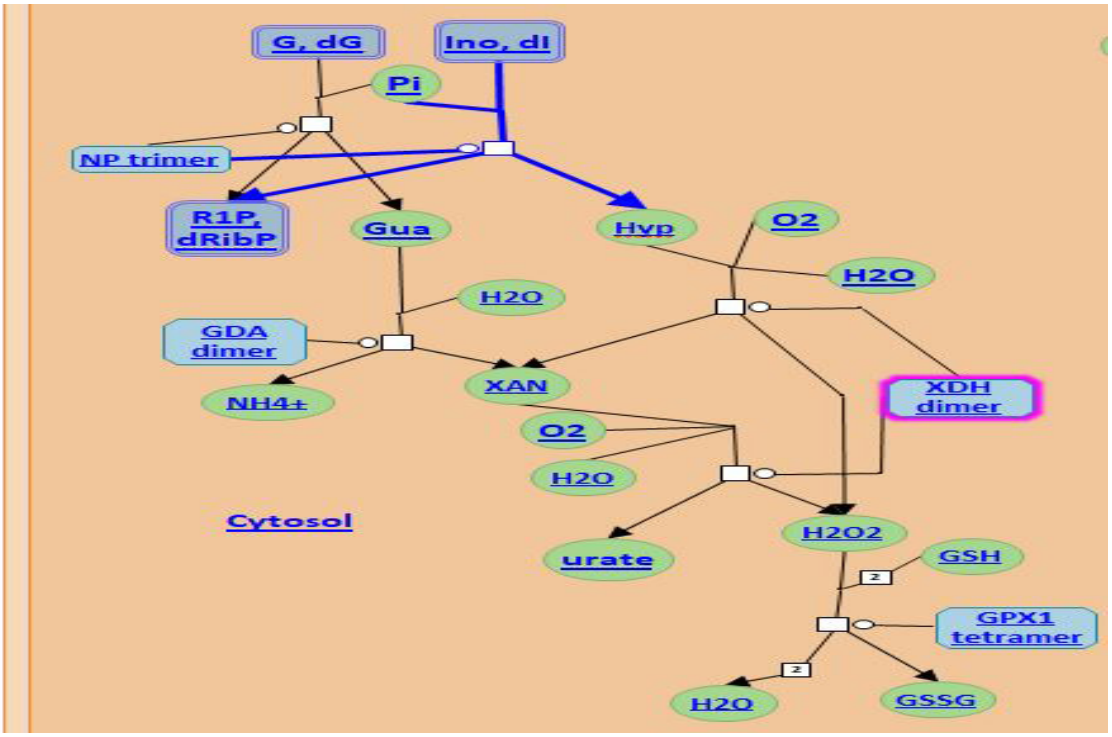


FIGURE 4.6: The role of XDH in Metabolism pathway

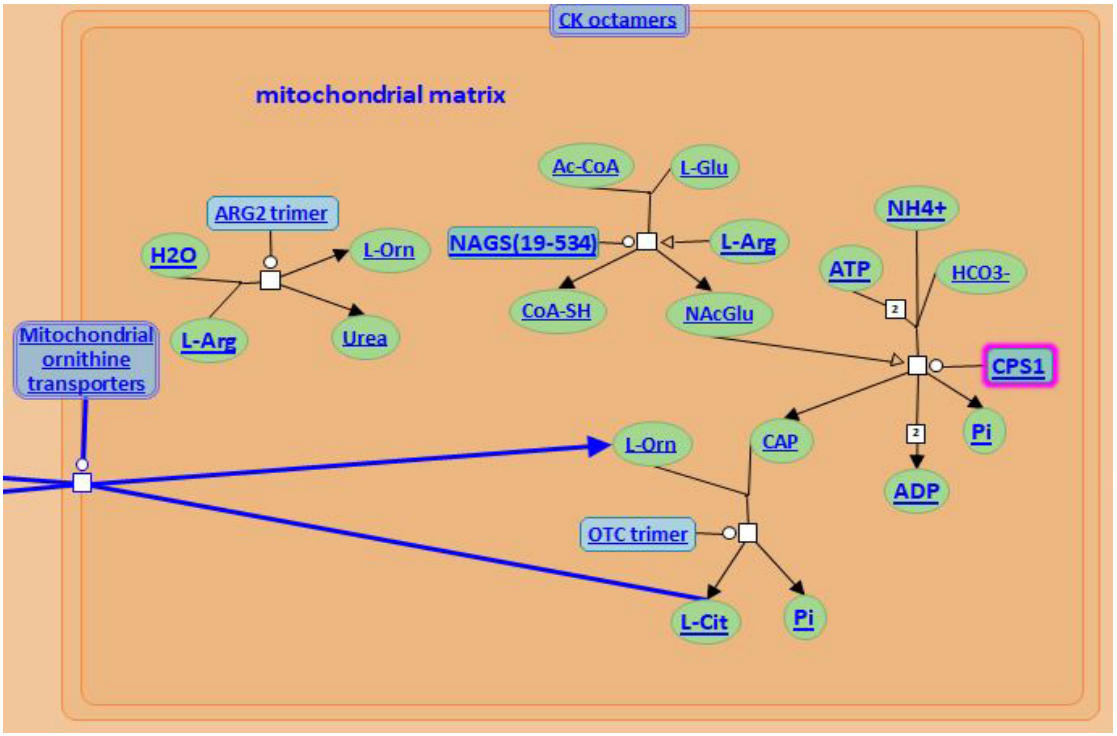


FIGURE 4.7: The role of CPS1 in Metabolism pathway

Phospholipid (PL) and triacylglycerol (TG) are linked in the translation of the

apo B-48 polypeptide. MTTP (microsomal triacylglycerol exchange protein) mediates the whole process as an MTTP: PDI (protein disulfide isomerase) heterodimer. MTTP binds the small amount of PL and TG and effectively exchanges the bound lipid between membranes. MTTP: PDI specifically associates with the apoB-48 polypeptide and is thought to exchange lipid from the membranes of endoplasmic reticulum to incipient apoB-48. While a portion of the MTTP functions stays misty, the patients who need MTTP can't create chylomicrons. Chylomicron metabolism is understudied in the number of malignancies, in spite of its immediate contribution to the patient nutrition status. the nonappearance of chylomicron has been found in Hodgkin and non-Hodgkin lymphoma and WM patients [279]. The MTTP pathway is shown in figure 4.8

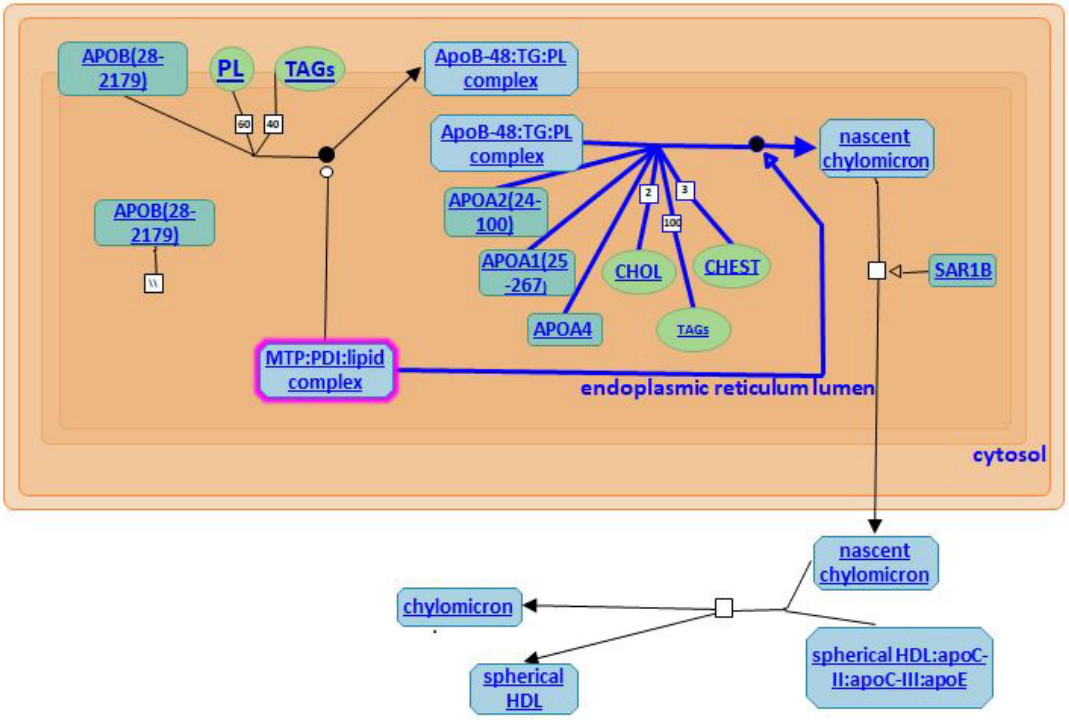


FIGURE 4.8: The role of MTTP in Metabolism pathway

Agmatinase (AGMAT) is considered as a member of the arginase superfamily because it hydrolyzes a guanidino group within agmatine and constitutes signature amino acid residues that act as ligand binding sites for the potential Mn(++) cofactor. Polyamines are a group of molecules (i.e. putrescine, spermine, spermidine) obtained from ornithine as indicated by a decarboxylation process. Recently,

it has been exhibited that arginine can be used by a similar pathway prompting agmatine development. Polyamines are basic for the development, the upkeep and the capacity of typical cells. The multifaceted nature of their metabolism and the way that polyamines homeostasis is firmly managed help the possibility that polyamines are basic to cell survival. Different variations from the norm in the control of polyamines digestion may be involved in a few pathological procedures including several malignancies. Similarly, the excess of arginine has been observed in patients of non-Hodgkin lymphoma and WM [280]. The AGMAT associated pathway is shown in figure 4.9

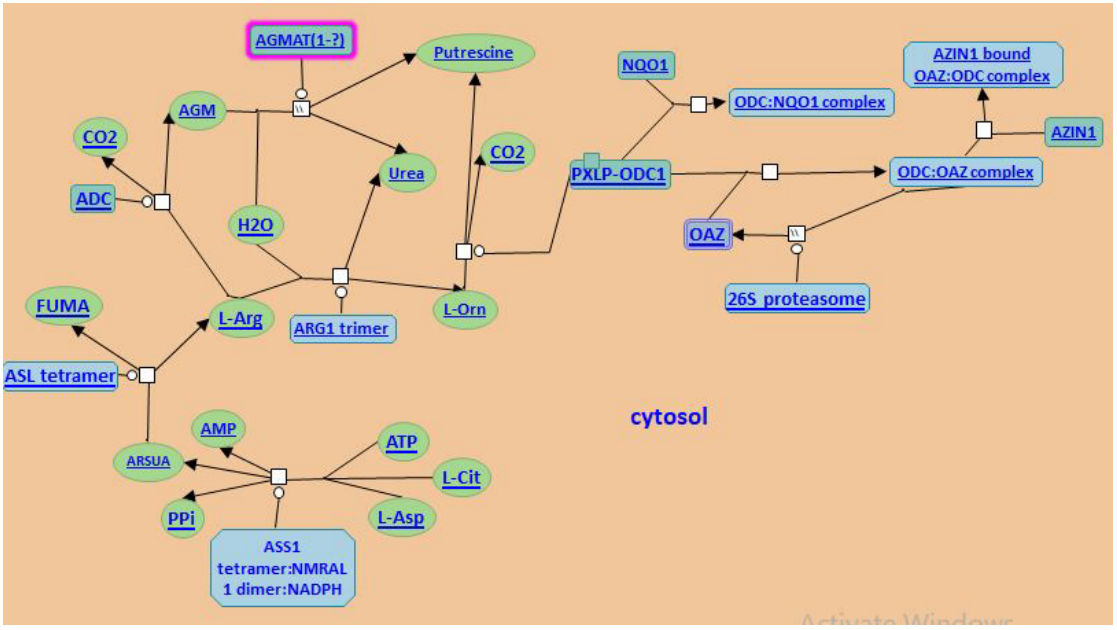


FIGURE 4.9: The role of AGMAT in Metabolism pathway

At the plasma membrane, Synaptojanin-1 (SYNJ1) and -2 (SYNJ2), inositol polyphosphate 5-phosphatase K (INPP5K) also known as SKIP, phosphatidylinositol 4,5 bis-phosphate 5-phosphatase A (INPP5J) also called PIPP dephosphorylates the phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) to form phosphatidylinositol 4-phosphate (PI4P). Both the SYNJ1 and SYNJ2 have a central 5-phosphatase domain, N-terminal Sac1-like domain, and a C-terminal proline-rich segment, This reaction is of extreme importance because of its regulation by small GTPases of the RHO and ARF families. Mutations in these families hinder the

dephosphorylation reaction, thus play a role in the development of several lymphoma types, including WM [281]. The SYNJ2 associated pathways are shown in figure 4.10 and 4.11

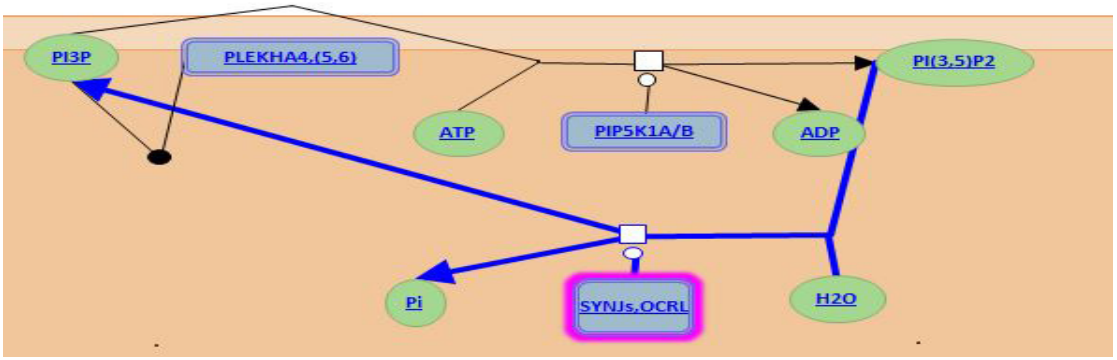


FIGURE 4.10: The role of SYNJs in Metabolism pathway

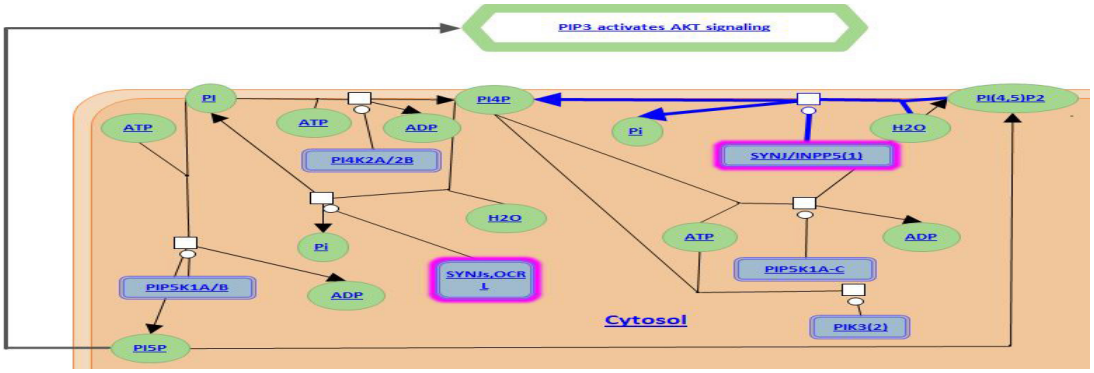


FIGURE 4.11: The role of SYNJ2 in Metabolism pathway

4.5.3 Cytokine Signaling and Nfk-b Signaling Pathway

The interleukin-1 (IL1) gene promotor contains AP-1 binding sites which are possessed by the AP-1 (FOS: JUN) complex, bringing about the activation of its transcription. IL10 regulates cytokines expression, myeloid cell surface atoms, and soluble mediators to initiate and sustain inflammatory and immune responses. The impacts of IL10 on cytokine generation and capacity of human macrophages are commonly like those on monocytes. IL10 inhibits the production of IL1A, IL1B, IL6, IL12, IL18, CSF2 (GM-CSF), CSF3 (G-CSF), CSF1 (M-CSF), TNF, LIF, PAF and itself by initiated monocytes/macrophages. The impact of IL10 on IL-1 and TNF generation is especially imperative as these cytokines synergically

affect the process of inflammation, enhancing their effect by instigating secondary mediators, for example, chemokines, prostaglandins, and PAF [282]. IL10 additionally restrains enacted monocyte generation of inducible chemokines that are engaged with irritation, to be specific CCL2 (MCP1), Ccl12 (MCP-5, in mice), CCL3, CCL3L1 (Mip-1alpha), CCL4 (Mip-1beta), CCL20 (Mip-3alpha), CCL19 (Mip-3beta), CCL5 (Rantes), CCL22 (MDC), CXCL8 (IL-8), CXCL10 (IP-10), CXCL2 (MIP-2) and CXCL1 (KC, Gro-alpha) [283]. These are associated with the enlistment of monocytes, dendritic cells, neutrophils, and T cells, and influence both Th1 and Th2 reactions. CXCL1 is initiated by IFN gamma and attracts the Th1 cells; IL-4 induced CCL2 which in turns attract Th2 cells.

IL-10 also upregulates the expression of IL-1A through hindrance of mRNA degradation. In human blood, monocytes Interleukin-4 and Interleukin-13 fundamentally downregulate the IL1, IL-6, IL-8, IL-18, CCL2, and TNF. IL13 hinders monocyte and macrophage creation of IL1-A, IL6, IL8, TNF, and IL12 through a system that incompletely suppresses Nuclear factor NF-kappa-B, regularly seen in WM patients. IL-1 and IL-6 genes and the most fundamentally related pathway for them is the mitogen-activated protein kinase (MAPK) pathway, proposing that these genes assume to play an important role in WM by motioning through the MAPK pathway [284]. The IL1A associated pathway is shown in figure 4.12

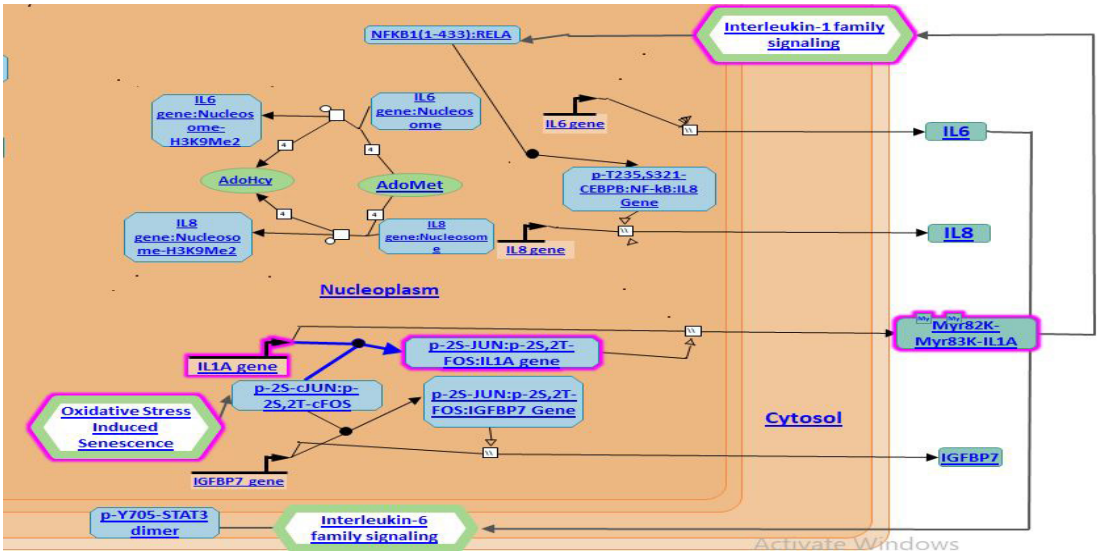


FIGURE 4.12: The role of IL1A in Cytokines signaling pathway

4.6 Collection of Drugs data used in WM Treatment for PK/PD Modeling

All the drugs used for the treatment of lymphoma subtypes and WM were accessed through a literature survey, their classes, pharmacological data, dosage, pharmacodynamics, and ADMET properties were accessed through the Drug Bank database. The details of the information are given in table 4.8

TABLE 4.8: The detailed information of drugs used in the treatment of WM

Drug name	Class	Dosage	Pharmacological data	Pharmacodynamics	ADMET properties
Bortezomib	Carboxylic acids and derivatives	1 3.5 mg	Available	N-A	Available
Carfilzomib	Carboxylic acids and derivatives	10 mg	Available	Predicted	Predicted
Ixazomib	Benzene and substituted derivatives	2- 4mg	Available	Predicted	NA
Oprozomib	Carboxylic acids and derivatives	20mg	N-A	N-A	N-A
Rituximab	Carboxylic acids and derivatives	10 -1400 mg	Available	Predicted	Available
Bendamustine	Benzimidazoles	25 100 mg	Available	Predicted	Available

Chlorambucil	Organo-nitrogen compounds	2 mg	Available	N-A	Available
Cladribine	Purine nucleosides	1 10 mg	Available	Available	Available
Cyclophosphamide	Organo-nitrogen compounds	2 -500 mg	Available	Available	Available
Doxorubicin	Anthracyclines	2 -150 mg	Available	Available	Available
Vincristine	Vinca alkaloids	1 5 mg	Available	Available	Available
Prednisone	Steroids and derivatives	1 50 mg	Available	Available	Available
Fludarabine	Purine nucleosides	10 50 mg	Available	Available	Available
dexamethasone	Steroids and derivatives	1 10 mg	Available	Available	Available
Thalidomide	Isoindoles and derivatives	50 200 mg	Available	Available	Available

As this research study focuses on the Bortezomib resistance profiling in WM, the Bortezomib is a proteasome inhibitor and belongs to carboxylic acids and their derivatives, therefore only those drugs, lying in the same class and proteasome inhibitors were selected along with bortezomib for PK/PD analysis. From Table 4.8 it is clearly seen that only Rituximab, Oprozomib, and Carfilzomib have similar class as that of Bortezomib, the Ixasomib have different class but it is proteasome

inhibitor, therefore, it was also selected. However, all other drugs (table 4.8) are being used in the WM treatment but the majority of the drugs have different classes, therefore, we excluded them from our study

4.7 Mono Therapy and Combination Therapy PK/PD models

Model-design based research is remodeling the manner in which researchers and scientists work and enables them to design the clinical laboratory tasks to the work area of the computer screens [285]. To access the PK and PD simulations for the aforementioned selected drugs two types of models were built monotherapy and combination therapy. The purpose of designing both models was to access their PK/PD profiles individually as well as in combination.

The monotherapy model consists of the single central compartment, consisting of single drug dose and its elimination route from the compartment, whereas combination therapy model consists of two central compartments named as central 1 and central 2. Both the models are given in figure 4.13

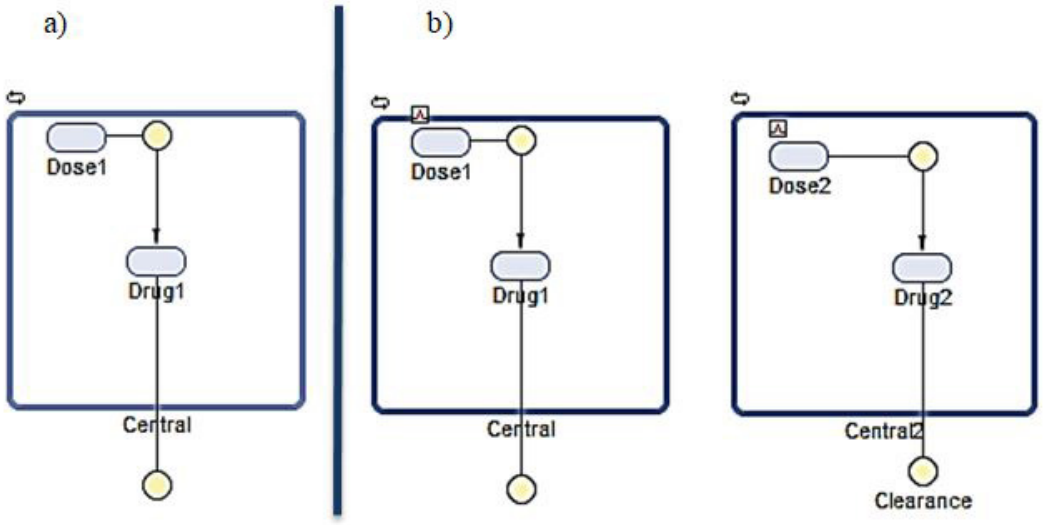


FIGURE 4.13: The PK/PD models, a) mono therapy PK/PD model, b) Combination therapy PK/PD model

Figure 4.13 shows that the drug enters into the central compartment, produces its effect on the compartment and then eliminate from the compartment. The circles linking the arrows with one another represent the reactions. Both the models can be explained in terms of differential equations are as follows

$$\frac{d(Dose)}{dt} = -kaCentral \times Dose \tag{4.1}$$

$$\frac{d(Drug)}{dt} = \frac{1}{Central} \times (kaCentral \times Dose - (keCentral \times Drug) \times Central) \tag{4.2}$$

where the Ka represents the absorption transition of dose in the central compartment, Ke denotes drug elimination from the central compartment. Plasma pharmacokinetics were modeled utilizing the monotherapy model. Nonlinear least squares weighting function was used as 1/y2 observed For Bortezomib, Carfilzomib, Oprozomib, Ixazomib, and Rituximab. Pharmacokinetic parameters were assessed from mean levels of drug concentrations. Since PK data for the aforementioned drugs were acquired through the clinical trials[247–251], the pharmacokinetic simulations were performed utilizing a naive pooled approach.

4.7.1 Tumor Growth Model

To determine the effects of each selected drug on the biomarkers as well as on tumor weight, the tumor growth model suggested by Simoni et al., [238] was understood properly and designed in the Simbiology toolbox of Matlab. The model was then integrated with both mono and combination therapy models to access the effects of drugs on tumor individually, as well as their effects in combination. Several concepts were undertaken while designing the model including proliferation rate of tumor, and change in its weight due to several treatment measures, the effects of combination therapy on tumor growth were also considered. The Tumor growth model is shown in figure 4.14

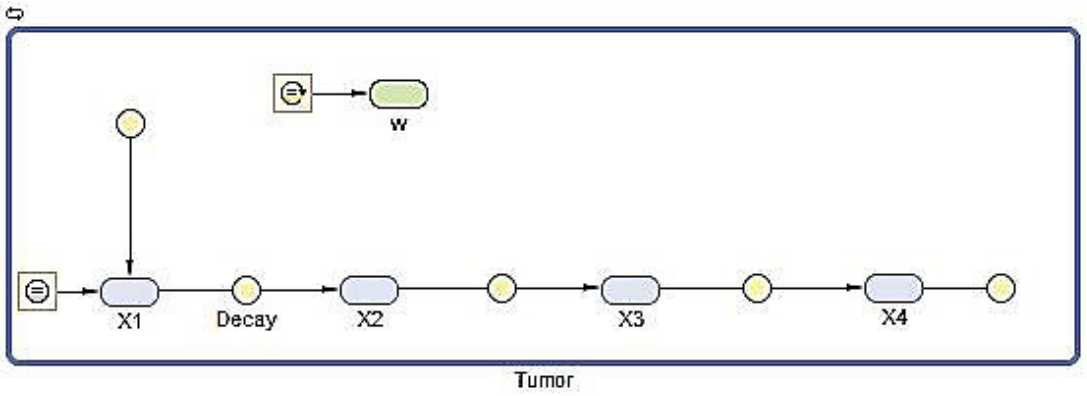


FIGURE 4.14: Tumor growth model

The tumor growth model comprises weight denoted by W and several growth rate attributes from X1 - X4 that changes with the passage of time when the drug dosage is induced into the model. The decay reaction in the model tells the decrease in weight of the tumor, after drug induction.

The model (fig. 4.14) demonstrates the two unique stages: an underlying exponential development in the weight pursued by linear growth before the treatment. The tumor development changes from exponential to linear development at a threshold tumor mass (wth). This expects all cells are proliferating (X1) when tumor occurs. At the point when the medication is induced into the model, a few cells got damaged by it and returned to the non proliferating (X2, X3, X4) and further turned out to be dead cells through a mortality chain. The differential equations representing the tumor growth model are:

$$\frac{d(X1)}{dt} = L1 \times L0 \times \frac{X1^2/(L1 + L0 \times X1)}{w} - phil2 \times K1 \times X1 \times Drug1 + K2 \times X1 \times Drug2 \tag{4.3}$$

$$\frac{d(X2)}{dt} = phil2 \times K1 \times X1 \times Drug1 + K2 \times X1 \times Drug2 - K1 \times X2 \tag{4.4}$$

$$\frac{d(X3)}{dt} = K1 \times X2 - K1 \times X3 \tag{4.5}$$

$$\frac{d(X4)}{dt} = K1 \times X3 - K1 \times X4 \tag{4.6}$$

where the L1 and L0 are fixed parameters according to the tumor growth before treatment, K1 and K2 are rate constants for non proliferating cells, the phil2 is used to show the effects of interaction between two drugs, On the off chance that if there is no interaction between the two medications, phil2 would equal to 1.0, which demonstrates the additive effect of two drugs combination. On the other hand, phil2 is greater than or less than 1, shows that there is a synergistic or an opposing effect between the two drugs. However, in the tumor integrated PK/PD model, All the parameters were set according to the monotherapy and combination model. This integrated model was further used in this study to analyze drug pharmacodynamics.

4.8 Parameters Selection and Estimation for the Models

All the parameters required for the drugs and models were estimated through a nonmixed effect model with isqnonlin (nonlinear least square model) on the pooled data. for this purpose, the clinical trials data of selected drugs were exported into the model one by one and nonlinear least square function was applied to calculate the combined model parameters for all the drugs. Parameters of the model are given in Table 4.9

The parameters displayed in the table 4.9 were further used to perform PK/PD simulation of the model to determine different dosage regimens for the selected drugs.

TABLE 4.9: The detailed information of drugs Parameters used in the PK/PD model

Parameter name	Value	Unit
Cl-Central	1	mL kg $^{-1}$. d^{-1}
ka-Central	0	ug. mL^{-1}
ke-Central	1	mL. kg^{-1}
w0	0.085	cm^3
K1	0.469	d^{-1} cm^3
phil2	1.98	NA
K2-2	0.00732	d^{-1}
K2-1	0.01542	d^{-1}
L1	0.334	d^{-1}
L0	0.334	d^{-1}
ka-Central2	1	ug. mL^{-1}
Cl-Central2	1	ug. mL^{-1}
ke-Central2	1	ug. mL^{-1}
Central	1	L
Central2	1	L
w	3.085	cm^3
X1	0.085	cm^3
X2 to X4	1	cm^3

4.9 PK Modeling and Simulation of Drugs

The reason for PK is to research what the body does to a drug and PK modeling quantitatively or numerically shows the procedures of drug ADME. The pharmacokinetic conduct of a drug is a basic determinant of both its adequacy and safety. To determine the PK profiles of drugs, their pharmacokinetics modeling was done

on the clinical trials data, their concentration in the central compartment, drug absorbance and clearance were determined, the PK profiles of the drugs are shown from figure 4.15 to 4.19 and Table 4.10

TABLE 4.10: The PK profiles generated for the selected drugs used in the treatment of WM

Drug name	Dose	Cl-Central	Ka-Central
Bortezomib	3.5 mg	2.10549862 mL kg^{-1}. d^{-1}	3.71848844 ug. mL^{-1}
Carfilzomib	10 mg	6.9535123 mL kg^{-1}. d^{-1}	7.20526531 ug. mL^{-1}
Ixazomib	4 mg	2.41730632 mL kg^{-1}. d^{-1}	4.221432 ug. mL^{-1}
Oprozomib	20 mg	10.012763 mL kg^{-1}. d^{-1}	16.5001832 ug. mL^{-1}
Rituximab	30 mg	16.85307726 mL kg^{-1}. d^{-1}	30.135141 ug. mL^{-1}

Clearance (CL) is amongst the most essential PK parameters and is characterized as the volume of body liquid (e.g., plasma) from which a drug is eliminated by bio-transformation as well as discharge, per unit of time. To a great extent, CL explains the fate of a drug compound in the body. The prediction of human CL is basic in medication disclosure [286]. It is clearly observed that all the drugs have a higher clearance rate and represented a good agreement between the Simulations results and Clinical Trials. Ka refers to the absorption rate of the drug in the central compartment, dependent upon drug dose. The higher the dose of a drug higher should be its absorption rate, it is a characteristic of effective drug compounds.

The figure 4.15 represents the Time-course of Bortezomib plasma concentrations in the central compartment following 3.5 $mg.m^{-2}$ Bortezomib IV administration for 50 hours on a daily basis, the x-axis represents the time in hours whereas y-axis shows concentration in $mg.m^{-2}$. The circles represent the observed values obtained

through the data from [247], and solid lines show predicted PK profiles based on scaling approach.

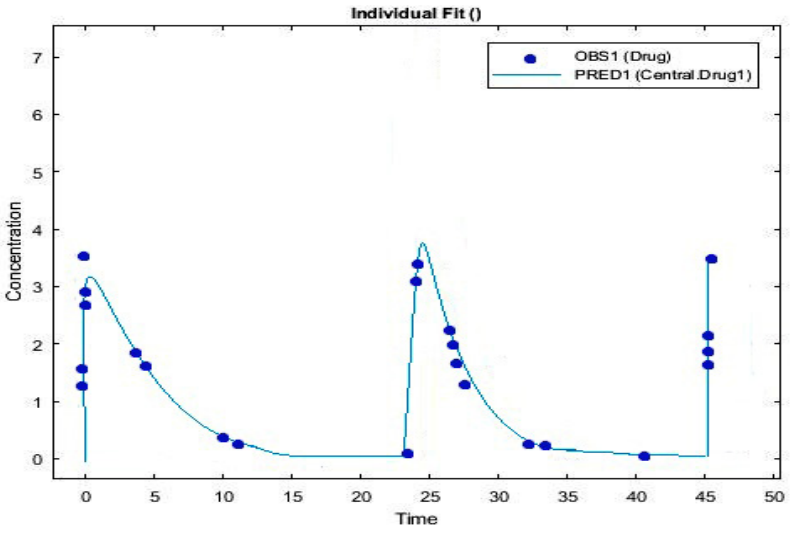


FIGURE 4.15: The PK modeling of Bortezomib in central compartment

According to the model predictions (fig(4.15)), in the first peak, 3.5 mg.m^{-2} dose of bortezomib was observed concentration in the clinical pharmacokinetics data, 3.2 mg.m^{-2} was predicted through the model with a difference of 0.2, in the second peak the model predictions is 3.7, again with a difference of 0.2, but the third peak is as exact as observed profiles.

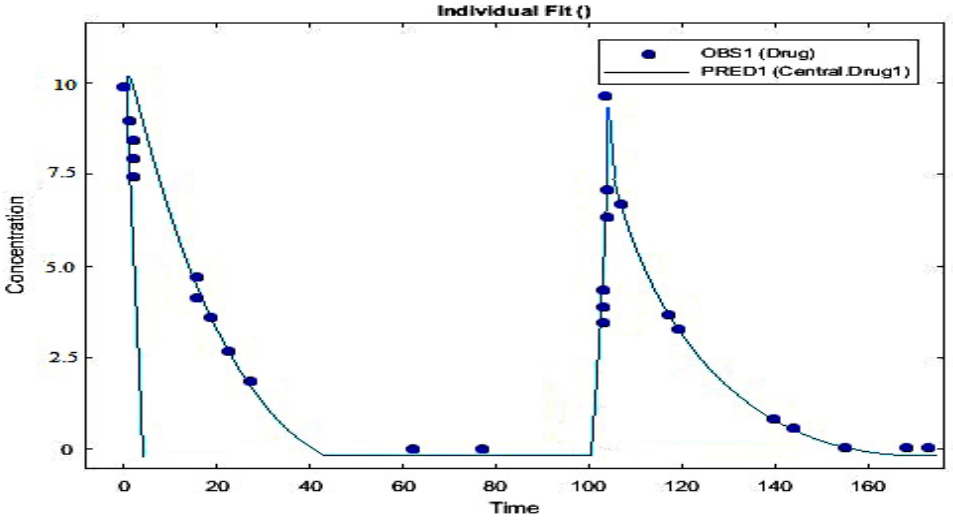


FIGURE 4.16: The PK modeling of Carfilzomib in central compartment

In figure 4.16 the x-axis represents the time in hours whereas y-axis shows concentration in mg.m^{-2}. The above figure represents the Time-course of Carfilzomib plasma concentrations in the central compartment following 10 mg.m^{-2} dose administration for 160 hours on the planned schedule, the circles represent the observed values obtained through [248], and solid lines show predicted PK profiles of Carfilzomib, based on scaling approach.

The model predictions (fig 4.16) shows that, 10 mg.m^{-2} dose of carfilzomib concentration was actual in the observed data, the model predicted 10.1 mg.m^{-2} with a difference of 0.1, in second peak the model predicted concentration of about 9.9 mg.m^{-2} again with a difference of 0.1

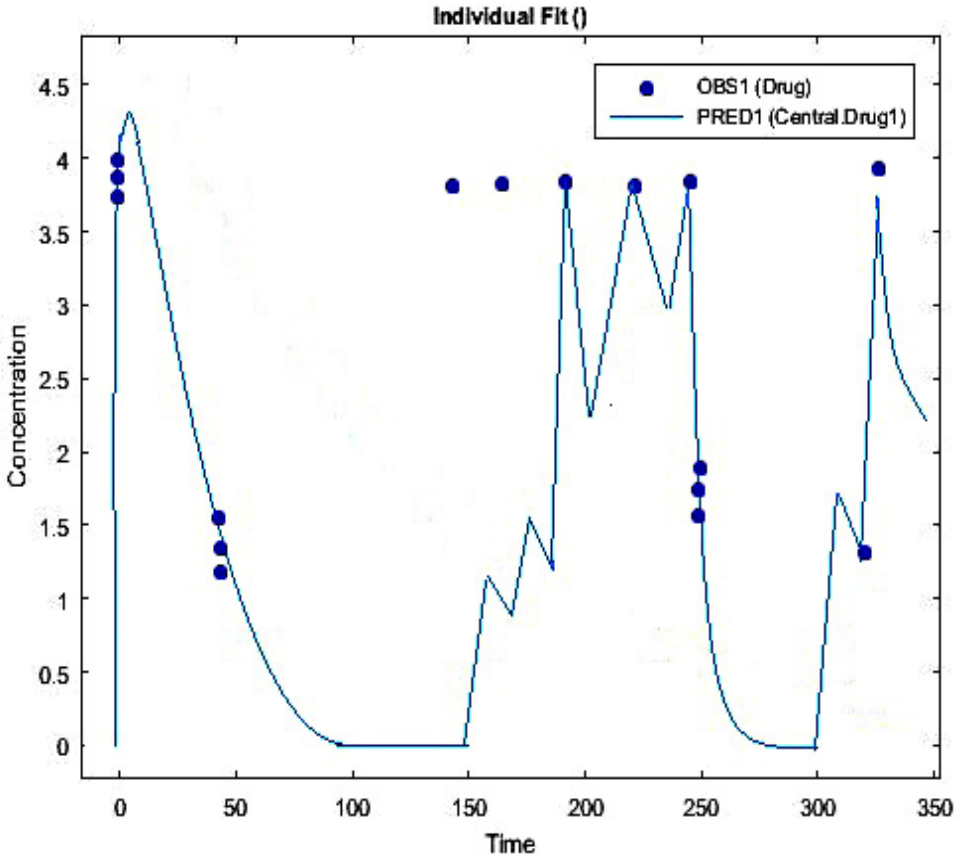


FIGURE 4.17: The PK modeling of Ixazomib in central compartment

In figure 4.17 the x-axis represents the time in hours whereas y-axis shows concentration in mg.m^{-2}. The above figure represents the Time-course of Ixazomib

plasma concentrations in the central compartment following 4 $mg.m^{-2}$ dose administration for 360 hours on a planned schedule of 3 intervals, the circles represent the observed values obtained through [251], and solid lines show predicted PK profiles of Ixazomib, the model predictions show that 4 $mg.m^{-2}$ dose of Ixazomib concentration was actual in the observed data, the model predicted 4.2 $mg.m^{-2}$ with a difference of 0.2, in second peak the model predicted concentration of 4 $mg.m^{-2}$ perfectly as observed and in the third peak it gives 3.8 $mg.m^{-2}$ concentration again with a difference of 0.2.

The PK modeling of Ixazomib also shows that it is cytotoxic in nature with a larger half-life than normal, so it can not be given to patients with mild symptoms.

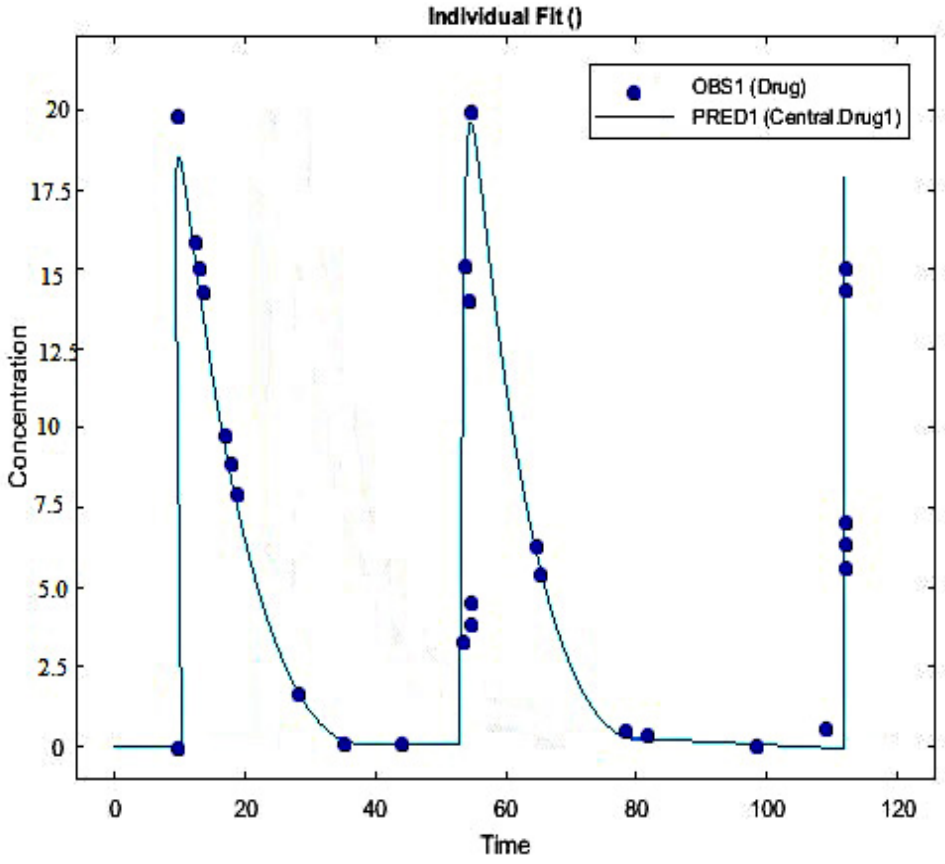


FIGURE 4.18: The PK modeling of Oprozomib in central compartment

Figure 4.18 represents the Time-course of Oprozomib plasma concentrations in the central compartment following 20 $mg.m^{-2}$ dose administration for 120 hours, according to planned dosage schedule. The x-axis represents the time in hours

whereas y-axis shows concentration in $mg.m^{-2}$, the circles represent the observed values obtained through the reference [249], and solid lines show predicted Oprozomib PK profiles.

This figure 4.18 also shows the 0.2 difference between model prediction and observed clinical PK data, 20 $mg.m^{-2}$ are observed in clinical data, where as in model the concentration rate is 18 $mg.m^{-2}$.

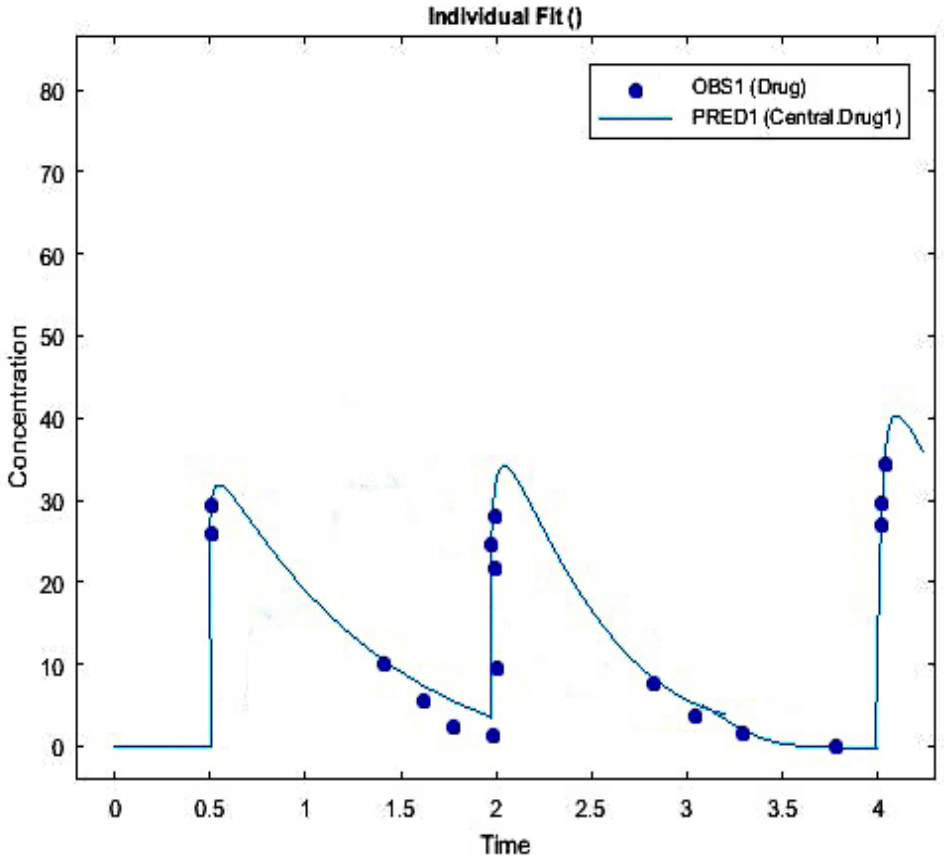


FIGURE 4.19: The PK modeling of Rituximab in central compartment

Figure 4.19 represents the Time-course of Rituximab concentrations in the central compartment following 30 $mg.m^{-2}$ dose administration for 4 days, on a daily basis. The x-axis represents the time in days whereas y-axis shows concentration in $mg.m^{-2}$, the circles represent the observed values obtained through the clinical trials [250], and solid lines show predicted PK profiles of Rituximab 30 mg per milliliter dose given to patient intravenously for 4 days.

In figure 4.19 again the difference in all peaks is near to 0.2, the observed concentration rate was 30 mg/m2, the model predicted 30.2 to 32 mg/m2 concentration at each peak. It is clearly observed from figure 4.15 - 4.18 that our model predictions are quite similar to those of clinical data obtained through [247–251]. However, only a difference of 0 to 2 occurs in the model prediction and actually observed profile, with a standard model error of 0.2, representing minimal and acceptable error rate in the model predictions.

4.10 PD Modeling and Simulations of Drugs

PD is the investigation of what a drug does to the body. PK/PD modeling characterizes a scientific connection among PD impact and drug exposure to the body and decides how much drug dose is required, and to what extent, to acquire the proper effect [287]. To perform PD modeling of drugs, the doses of compounds were enter into the model, simulations time was set to 30 days, PK estimates of drugs were set as variant and graphs were generated to determine the effects of individual drugs on the tumor weight (mono-therapy), as well as their effects in combination with Bortezomib (combination-therapy). The results of monotherapy PD modeling are given in figures 4.20 - 4.24

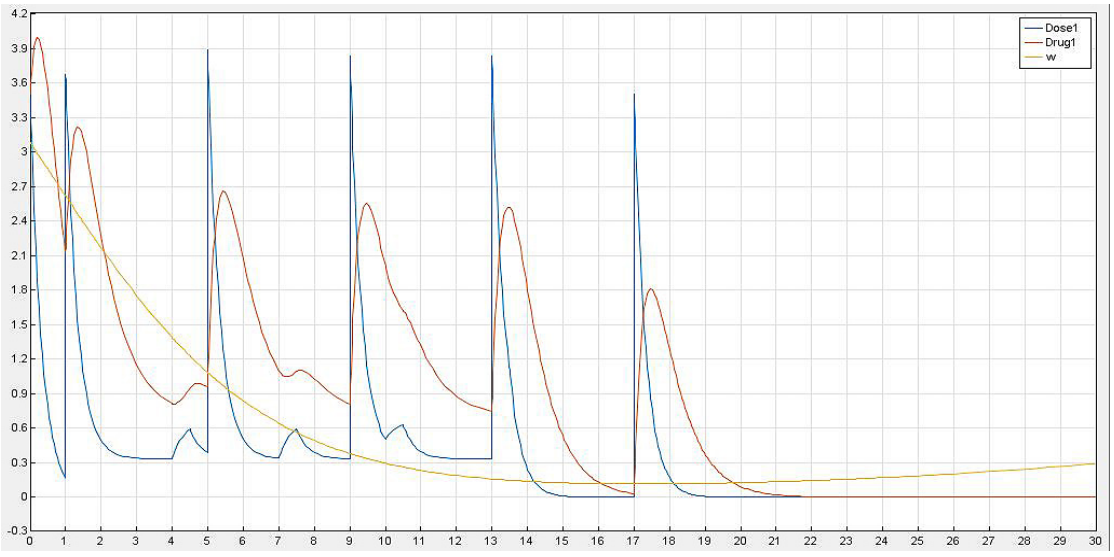


FIGURE 4.20: The PD modeling to determine the effects of Bortezomib concentration on tumor weight

3.5 $mg.m^{-2}$ dose of Bortezomib was introduced into the monotherapy PK/PD model on the first day as a treatment therapy with a repeat rate of every 4th day for a 28-day cycle. In figure 4.20 the X-axis represents the days whereas Y-axis shows concentration values.

It was observed that at initial the weight of tumor was 3 gram, as the treatment started the tumor weight decreased and reach to 0.2 g, but as soon as the doses stopped after the 17th day of treatment, the tumor again stated to increase in weight and reached to 0.3 gram on the 30th day. The absorption rate of the dose is shown by blue color, it is clearly seen that the absorption rate of Bortezomib is quite high on each dose to about 3.5 to 3.7 mL kg^{-1} d^{-1}, which shows its efficacy. The drug clearance is shown by red color solid lines, lying in the range of 3.6 to 2.1 ug. mL^{-1}. After the stop of drug dose on the 18th day, the drug is completely cleared from the body after 21 days.

Like for various other anticancer drugs, the surface area of the body did not seem to affect clearance of drugs, proposing that they could be tested given at a settled portion [288]. Generally, the huge effect of body measure on PK/PD, anticancer medications ought to be given at a fixed dosage since it is more advantageous and may even be more secure. Keeping this statement in mind the fixed dosage of 3.5 $mg.m^{-2}$ was given to model.

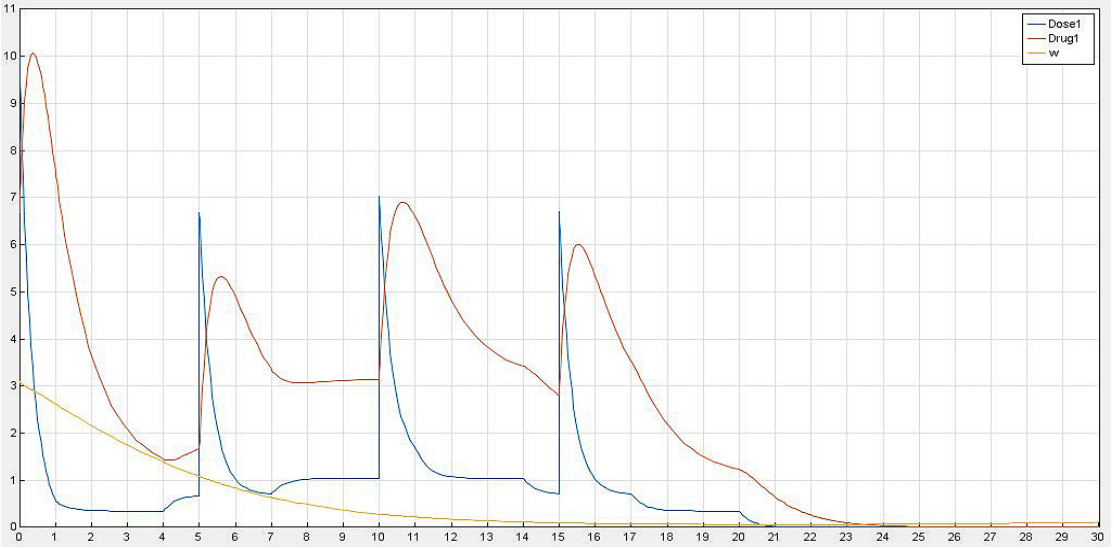


FIGURE 4.21: The PD modeling to determine the effects of Carfilzomib concentration on tumor weight

In figure 4.21 the X-axis represents the days whereas Y-axis shows concentration values. 10 $mg.m^{-2}$ dose of carfilzomib was introduced into the monotherapy PK/PD model on the first day as a treatment therapy with a repeat rate of every 5th day for a 28-day cycle. It was observed that at initial the weight of the tumor was 3 gram, as the treatment started the tumor weight started to decrease and diminished on the 17th day after the last dose.

The absorption rate of the dose is shown by blue color, it is clearly seen that the absorption rate of Carfilzomib is lying in the range of 6.7 to 7.0 mL kg^{-1}. d^{-1}. The drug clearance is shown by red color solid lines, lying in the range of 5 to 6.4 ug. mL^{-1}. After the stop of drug dose on the 15th day, the drug is completely cleared from the body after 21 days.

It was also observed that after the administration of Carfilzomib first dose, its concentration declined quickly with time, and most of the drug was disposed of from the compartment before the administration of the second dose. Carfilzomib accumulation was not seen among doses, and also, its exposure was not changed upon continue dosing.

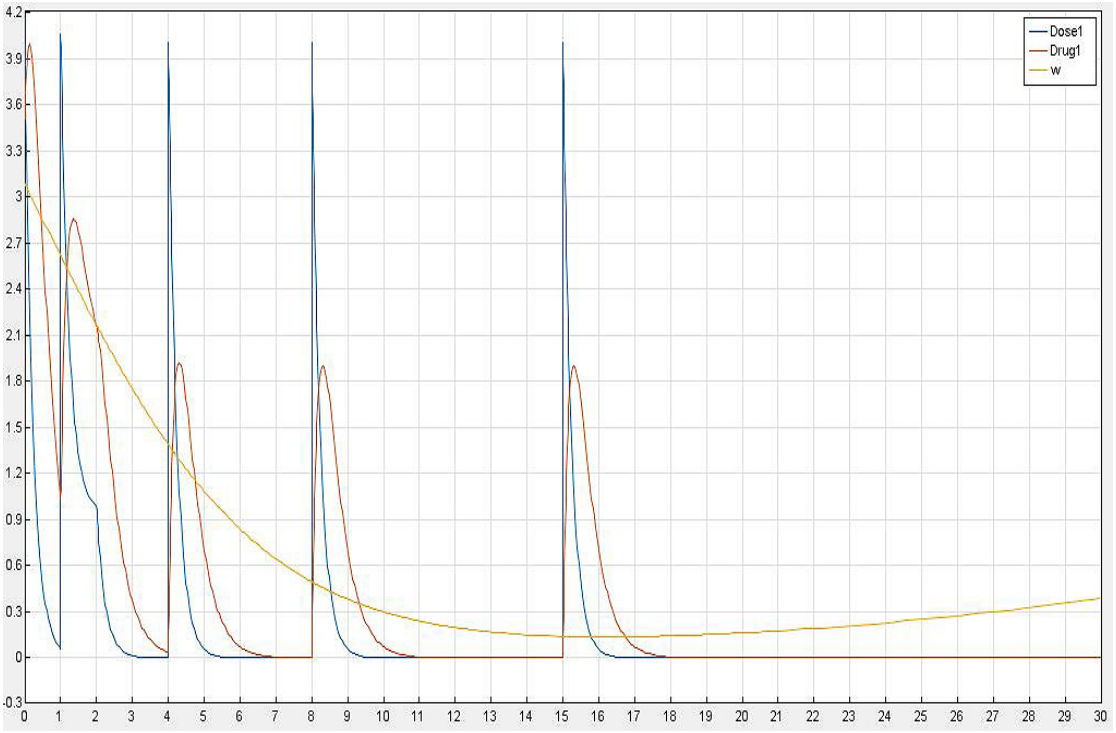


FIGURE 4.22: The PD modeling to determine the effects of Izxazomib concentration on tumor weight

In figure 4.22 the X-axis represents the days whereas Y-axis shows concentration values. 4 $mg.m^{-2}$ dose of Ixazomib was introduced into the monotherapy PK/PD model on the first day as a treatment therapy with a repeat rate of 8th, 9th, and 15th day for a 28-day cycle. It was observed that the Ixazomib absorbed quickly into the central area with an absorption rate of 3.9 to 4 $mL.kg^{-1}$. d^{-1}, and cleared from a compartment at a rate of 1.8 to 2.7 ug. mL^{-1}. At initial, the weight of tumor was 3 gram, as the treatment started the tumor weight started to decrease and reach to 0.2 g, but as soon as the doses stopped after the 15th day of treatment, the tumor again stated to increase in weight and reached to 0.4 gram on the 30th day. The absorption rate of the dose is shown in blue color. The drug clearance is shown by red color solid lines. After administration of the first dose of Ixazomib, it was observed that it quickly absorbs in the compartment within the median time, but does not produce fruitful effects on the tumor size.

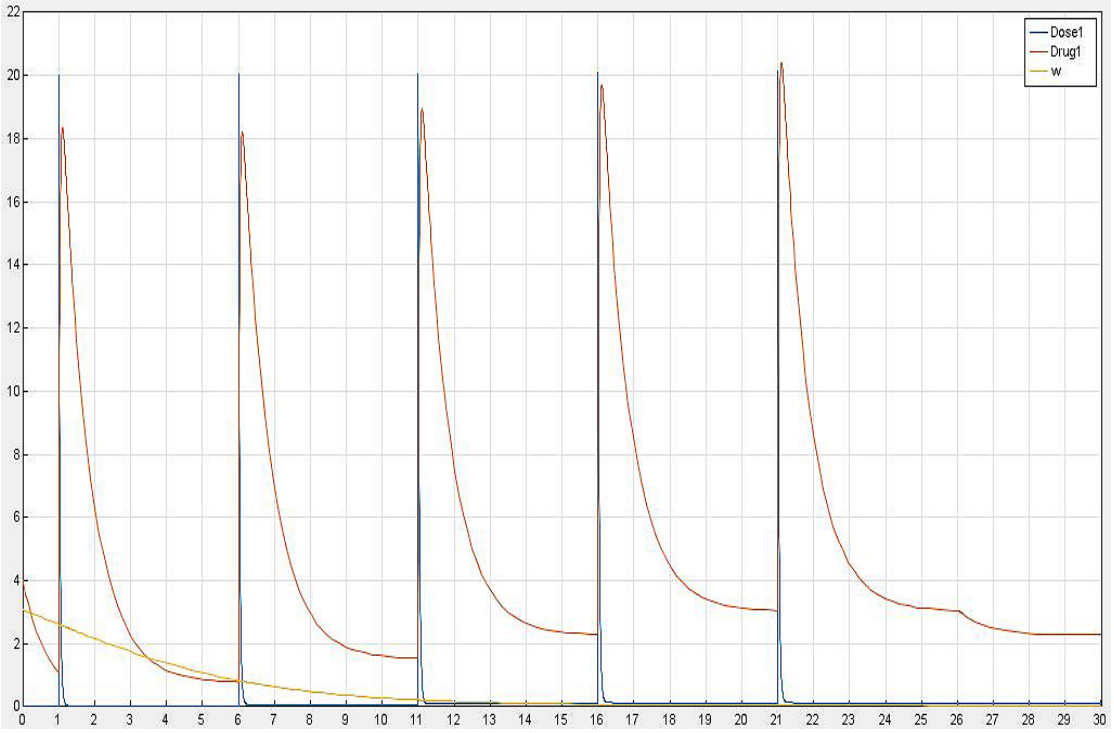


FIGURE 4.23: The PD modeling to determine the effects of Oprozomib concentration on tumor weight

In figure 4.23 the X-axis represents the days whereas Y-axis shows concentration values. 20 $mg.m^{-2}$ the dose of Oprozomib was introduced into the monotherapy PK/PD model on the first day as a treatment therapy with a repeat rate of every

5th day for a 25-day cycle. It was observed that at initial the weight of the tumor was 3 gram, as the treatment started the tumor weight started to decrease and diminished on the 12th day. The absorption rate of Oprozomib is lying in the range of14 to 16.5 mL.kg $^{-1}$. d^{-1}. The drug clearance is, lying in the range of 6.5 to 10 ug. mL^{-1}.

The dose-dependent decrease in the tumor was observed in the case of Oprozomib, change in the dose range also changed the tumor inhibition rate. In the divided dose treatment plan, the maximum decrease in tumor weight was found after simulations, bringing about 90 percent hindrance in Tumor size.

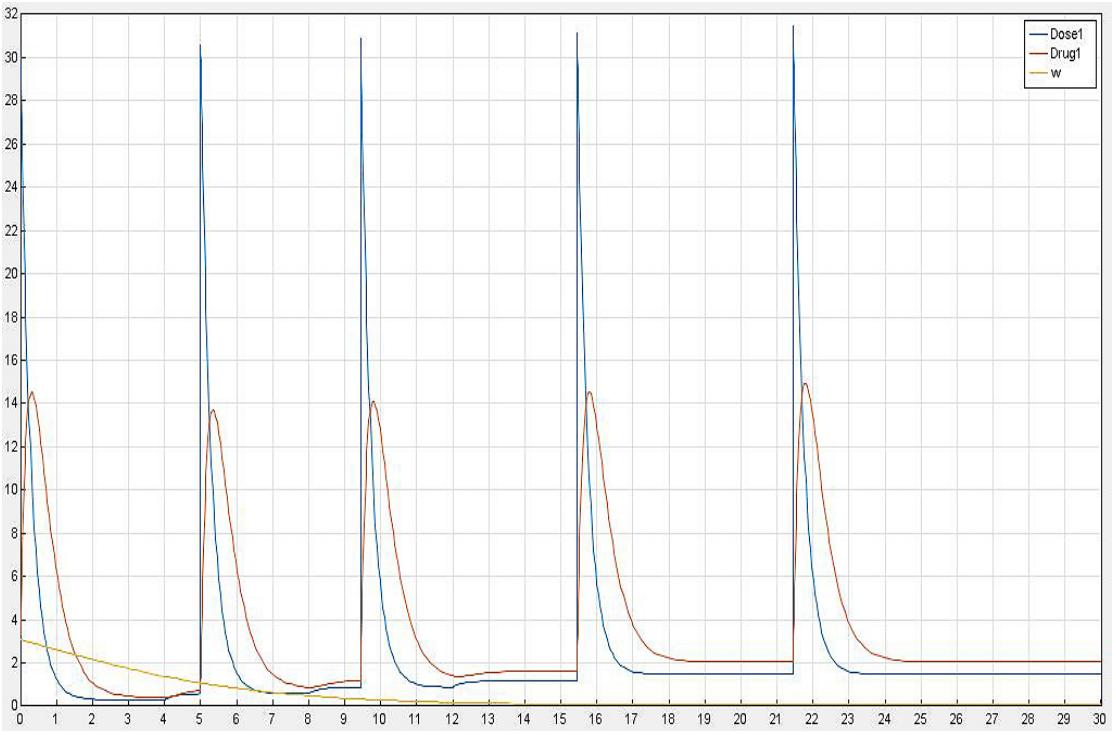


FIGURE 4.24: The PD modeling to determine the effects of Rituximab concentration on tumor weight

In figure 4.24 the X-axis represents the days whereas Y-axis shows concentration values. 30 mg.m^{-2} dose of Rituximab was introduced into the monotherapy PK/PD model on the zero-day as a treatment therapy with a repeat rate of every 5th day for a 24-day cycle. It was observed that at initial the weight of the tumor was 3 gram, as the treatment started the tumor weight started to decrease and diminished on the 10th day after the last dose. The absorption rate of the dose is

quite high lying in the range of 30 to 31 mL.kg $^{-1}$. d^{-1}. The drug clearance is lying in the range of14 -16 ug. mL^{-1}. After the stop of drug dose on 20th da,y the drug is completely cleared from the body after 22 days. Quite good Rituximab PK is shown by monotherapy PK/PD model comparing to target-interceded behavior of drug, the clearance of Rituximab is again quite similar to its clinical response.

As our study focuses on Bortezomib resistance profiling and its treatment efficacy, therefore the PD modeling of drugs was simulated in combination with Bortezomib one by one. The results of combination therapy PD modeling are shown in figure 4.25 - 4.28

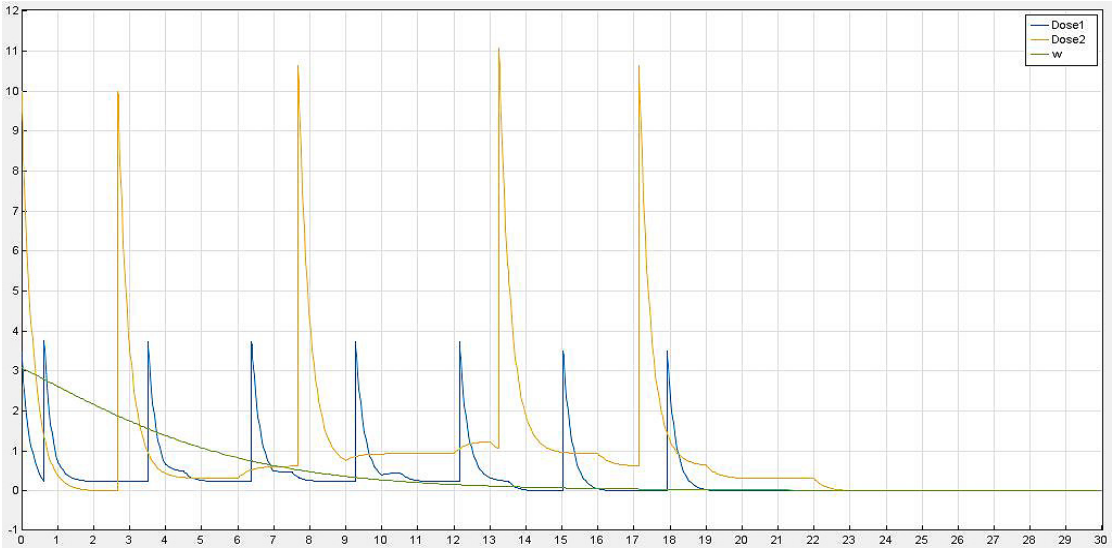


FIGURE 4.25: The PD modeling to determine the effects of Bortezomib in combination with Carfilzomib on tumor weight

In figure 4.25 of the combination therapy, PK/PD model, the X-axis represents the days whereas Y-axis shows concentration values. The dose criteria were set to 3.5 mg.m^{-2} dose of Bortezomib on the start day as a treatment therapy with a repeat rate of every 2.8 days for a 28-day cycle. and 10 mg.m^{-2} Carfilzomib dose started from the first day with a rate of mixed dosing interval up to 17 days, using such combination, it was observed that the tumor weight reached to 0 g from 3 g on 12 days of the treatment cycle and never raised again. The blue color lines show dose 1 (Bortezomib) and yellow solid lines represent dose 2(Carfilzomib), the tumor is represented by the green line.

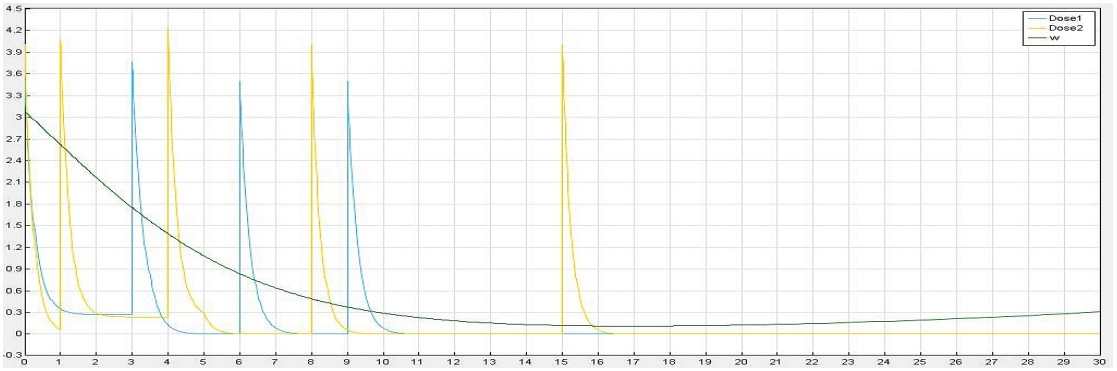


FIGURE 4.26: The PD modeling to determine the effects of Bortezomib in combination with Ixazomib on tumor weight

In figure 4.26 the X-axis represents the days whereas Y-axis shows concentration values. The dose criteria was set to 3.5 mg.m^{-2} dose of Bortezomib on the start day as a treatment therapy with a repeat rate of 3rd, 6th, and 9th day and 4 mg.m^{-2} of Ixazomib on day 1, 8th, and 15th for a 28-day cycle. Using such combination, it was observed that the tumor weight reached to 0.2 g on the 15th day of the treatment cycle, as the treatment stopped, the tumor again started to increase and reached to 0.3 on the 30th day since the start of treatment. The blue color lines show dose 1 (Bortezomib) and yellow solid lines represent dose 2(Ixazomib), the tumor is represented by the green line.

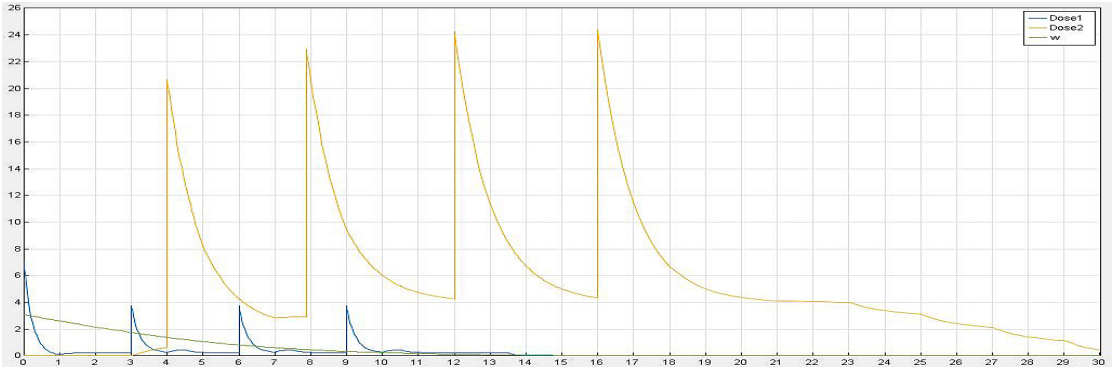


FIGURE 4.27: The PD modeling to determine the effects of Bortezomib in combination with Oprozomib on tumor weight

In figure 4.27 the X-axis represents the days whereas Y-axis shows concentration values. The dose criteria was set to 3.5 mg.m^{-2} dose of Bortezomib on the start of treatment therapy with a repeat rate of every 3 days for a 28-day cycle. and

20 mg.m^{-2} Oprozomib dose started from day 4 with a rate of every 4th day up to 17 days, using such combination, it was observed that the tumor weight reached to 0 g from 3 g on 12 days of the treatment cycle and never raised again. The blue color lines show dose 1 (Bortezomib) and yellow solid lines represent dose 2(Oprozomib), the tumor is represented by the green line.

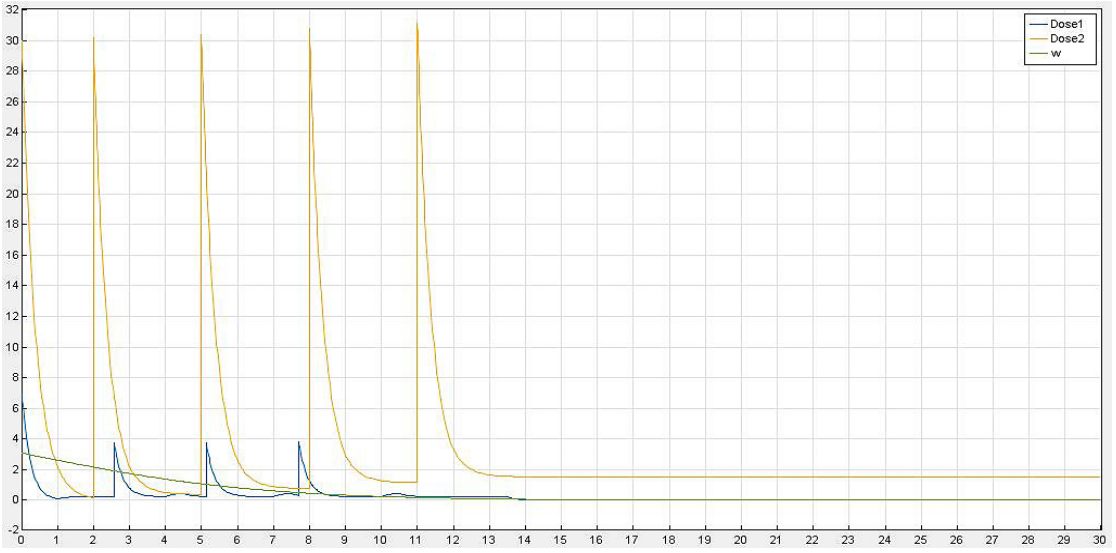


FIGURE 4.28: The PD modeling to determine the effects of Bortezomib in combination with Rituximab on tumor weight

In figure 4.28 the X-axis represents the days whereas Y-axis shows concentration values. The dose criteria was set to 3.5 mg.m^{-2} dose of Bortezomib was on the start day as a treatment therapy with a repeat rate of every 3rd day for a 28-day cycle. and 30 mg.m^{-2} Rituximab dose started from the 2nd day with a rate of every 5th day up to 17 days, using such combination, it was observed that the tumor weight reached to 0 g from 3 g on 10 days of the treatment cycle and never raised again. The blue color lines show dose 1 (Bortezomib) and yellow solid lines represent dose 2(Rituximab), the tumor is represented by the green line.

From figure 4.25 - 4.28 it is clearly observed that the combination of Bortezomib with Ixazomib does not produce satisfactory results as compared to other combinations. The combination of Bortezomib with Rituximab shows the most significant decrease in the tumor weight in only 10 days after the start of treatment.

4.11 Biological Verification of Modeling and Simulations Results

The model simulations were biologically validated by the clinical pk results of the drugs, given in literature [250, 251, 289–291]. For this purpose the same dosing schedule was given to the model and simulations were performed, it was observed that for each drug dosing regimen same simulation graphs were generated as present in the [250, 251, 289–291] which confirms the accuracy of PK/PD model. The comparisons results are shown in figures 4.29 - 4.33

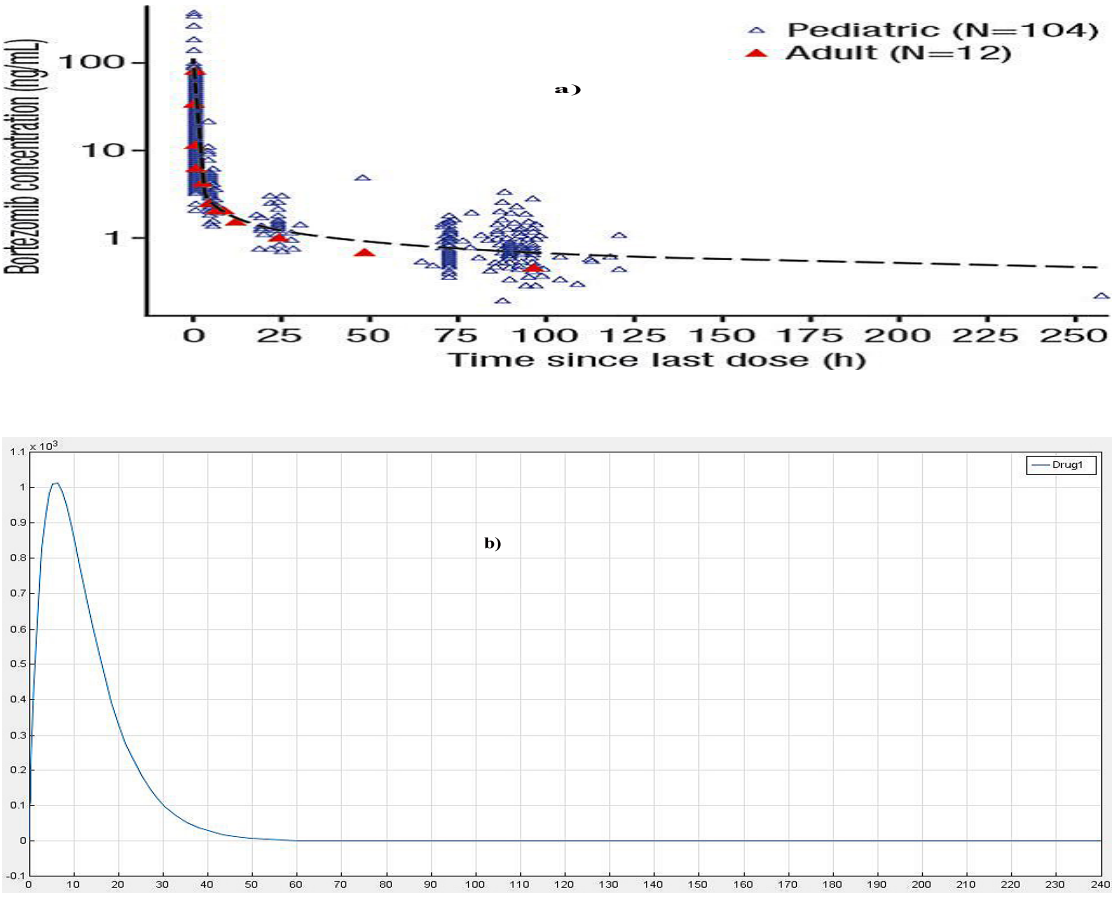
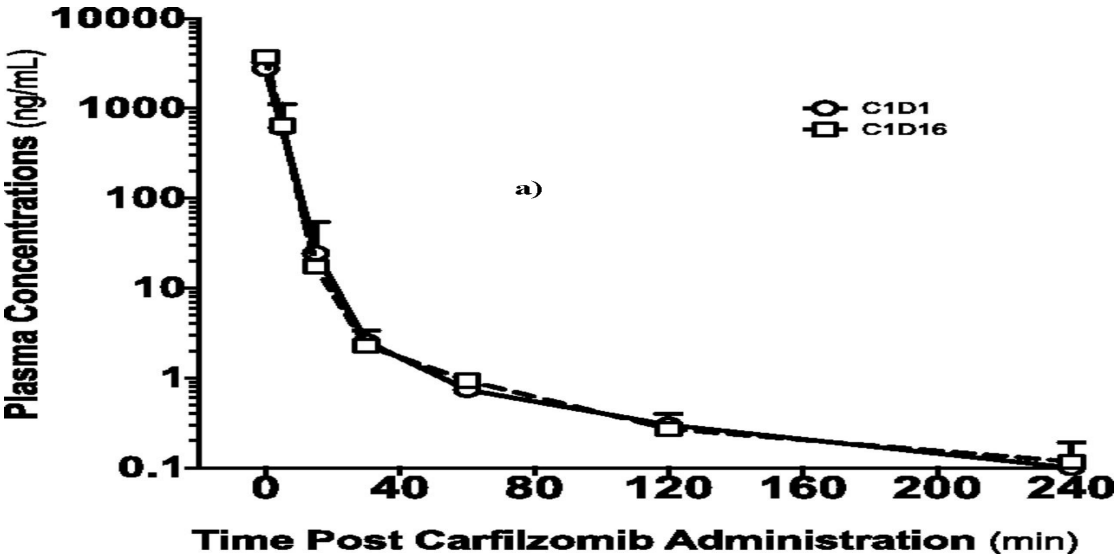


FIGURE 4.29: Comparison of the Clinical PK/PD results of Bortezomib, obtained through [289] with simulations of the model: a) Clinical Results, b) Model simulations

In the figure 4.29, a) shows the clinical PK/PD results of Bortezomib, 3.5 mg.m^{-2} was administered into the WM patients. The dose concentration was reached to its maximum on 100 ng/mL soon after administration and started to decrease and

reached to 2 ng/mL after 25 hours, after 250 hours it was completely cleared from the body. Same model simulations were produced for the Bortezomib. According to the model simulations b) the concentration of Bortezomib was 0.001 mg/mL (100 ng = 0.01 mg) and reached to the 0.2 x 10^{-3} after 25 hours, same as that of Clinical results.



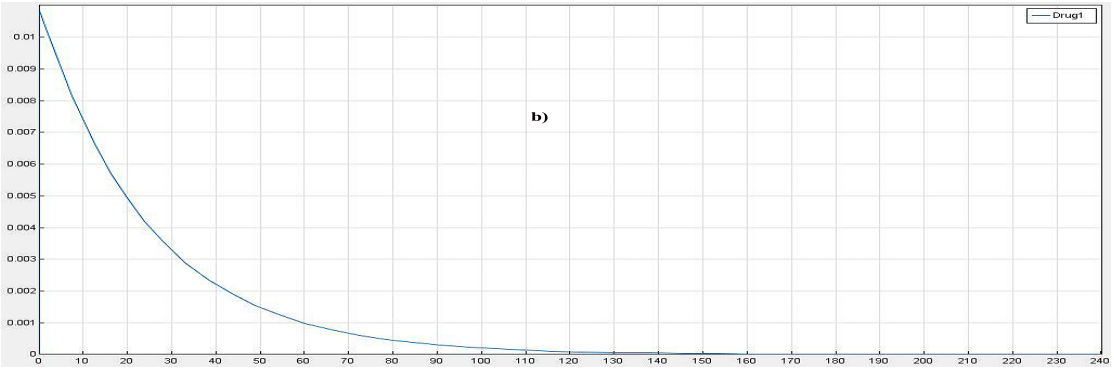


FIGURE 4.30: Comparison of the Clinical PK/PD results of Carfilzomib, obtained through [290] with simulations of the model: a) Clinical Results, b) Model simulations

In figure 4.30, a) shows the clinical PK/PD results of Carfilzomib, 10 mg.m^{-2} was administered into the WM patients. The dose concentration was reached to its maximum on 10000 ng/mL soon after administration and started to decrease and reached near to 0.1 ng/mL after 120 minutes. Same model simulations were produced for the Carfilzomib.

According to the model simulations b) the concentration of Carfilzomib was 0.01 mg/mL (10000 ng = 0.01 mg) and reached to 0.1 after 120 minutes.

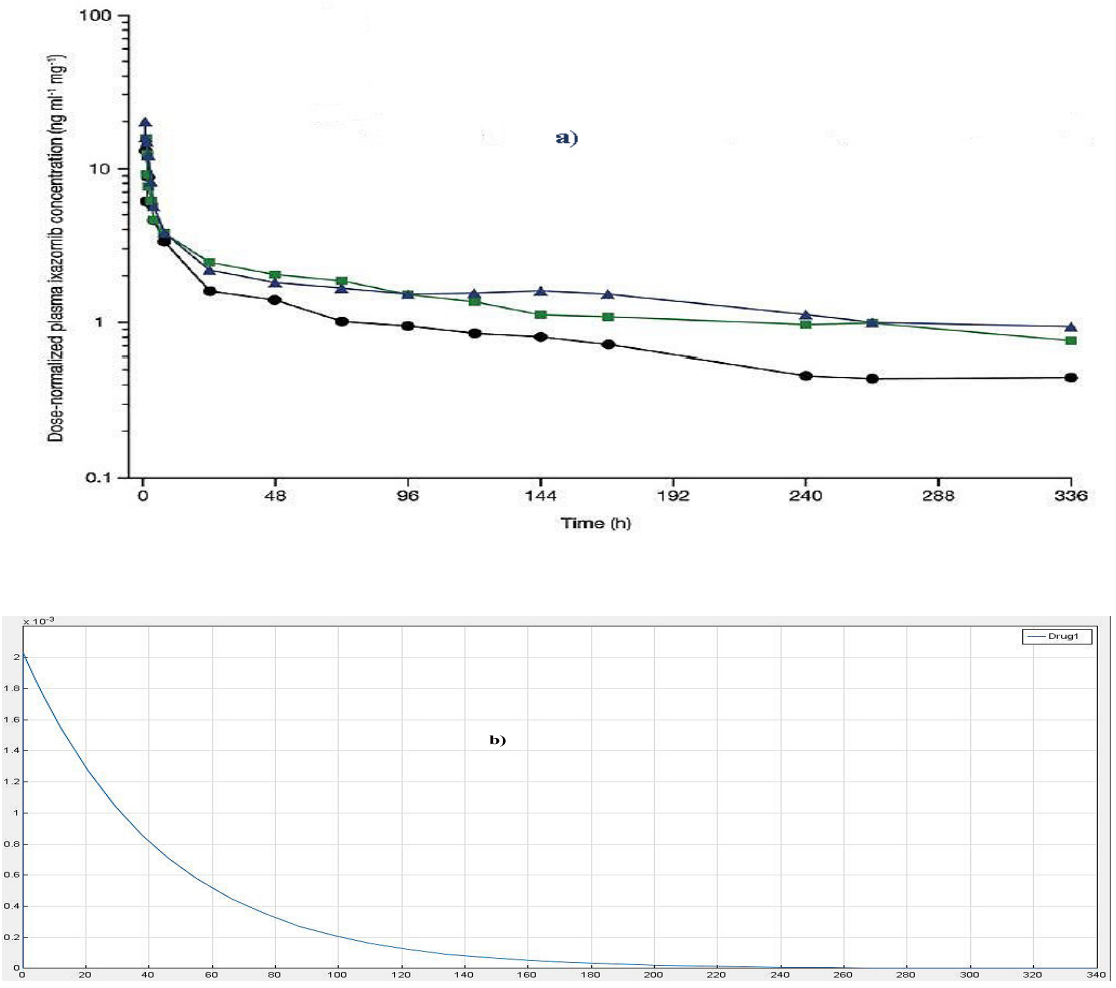


FIGURE 4.31: Comparison of the Clinical PK/PD results of Ixazomib, obtained through [285] with simulations of the model: a) Clinical Results, b) Model simulations

In figure 4.31, a) shows the clinical PK/PD results of Ixazomib, 20 mg.m^{-2} was administered into the patients. The dose concentration was reached to its maximum on 20 ng/mL soon after administration and started to decrease and reached to 1 ng/mL after 144 hours.

Same model simulations were produced for the Ixazomib. According to the model simulations the concentration of Ixazomib was 2 x 10^{-2} mg/mL (20 ng = 2 x 10^{-2}) and reached to the 0.1 x 10^{-2} after 144 hours.

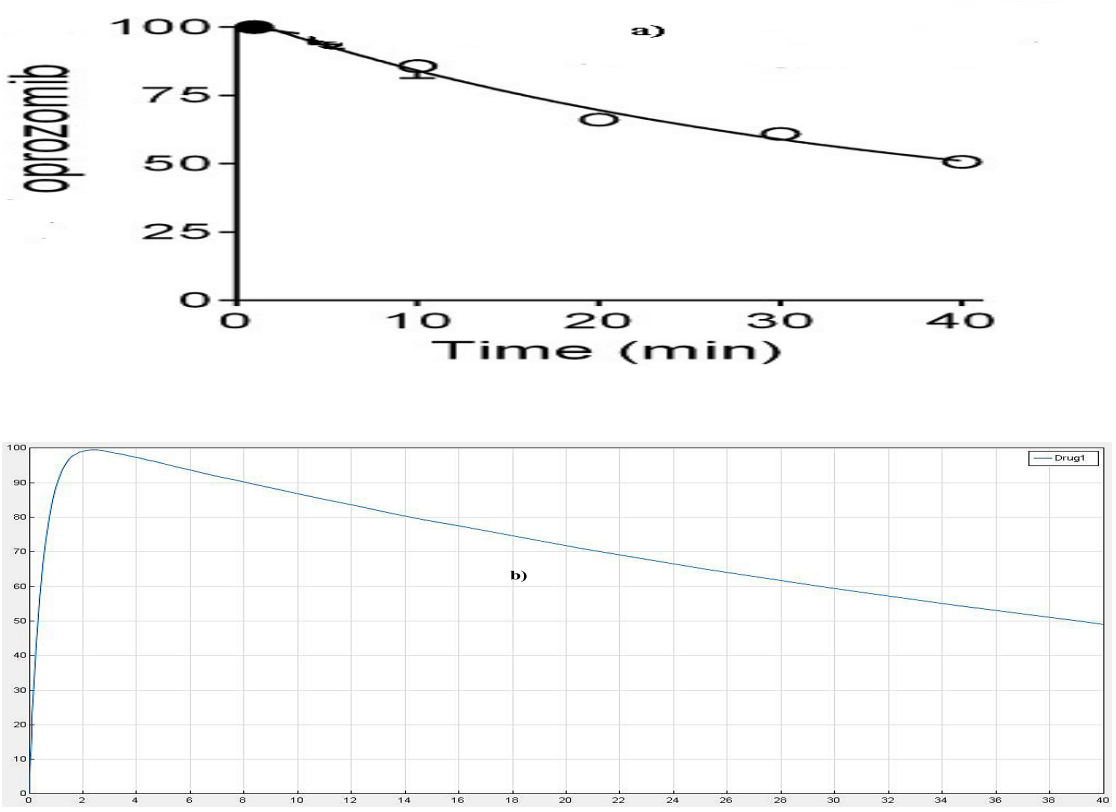
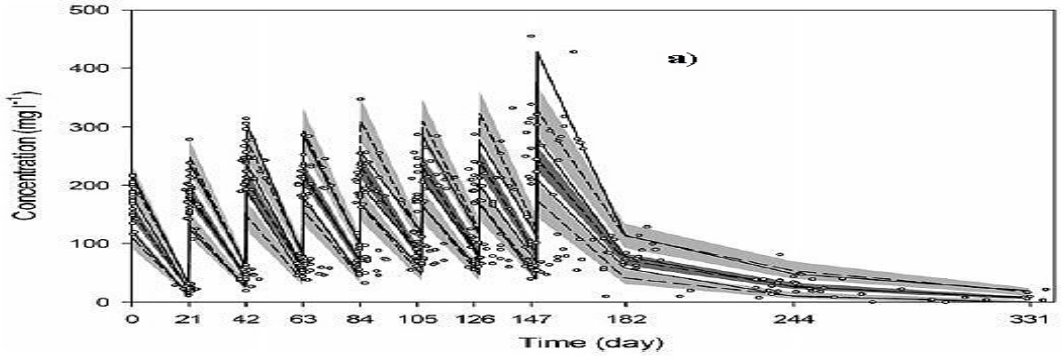


FIGURE 4.32: Comparison of the Clinical PK/PD results of Oprozomib, obtained through [291] with simulations of the model: a) Clinical Results, b) Model simulations

In figure 4.32, a) shows the clinical PK/PD results of Oprozomib, 20 mg.m^{-2} was administered into the patients. The dose concentration was at its maximum on 100 mg/mL soon after administration and started to decrease and reached to 50 mg/mL after 40 minutes of administration. According to the model simulations, the concentration of Oprozomib was 100 mg/mL and reached to the 50 mg/mL after 40 minutes of administration.



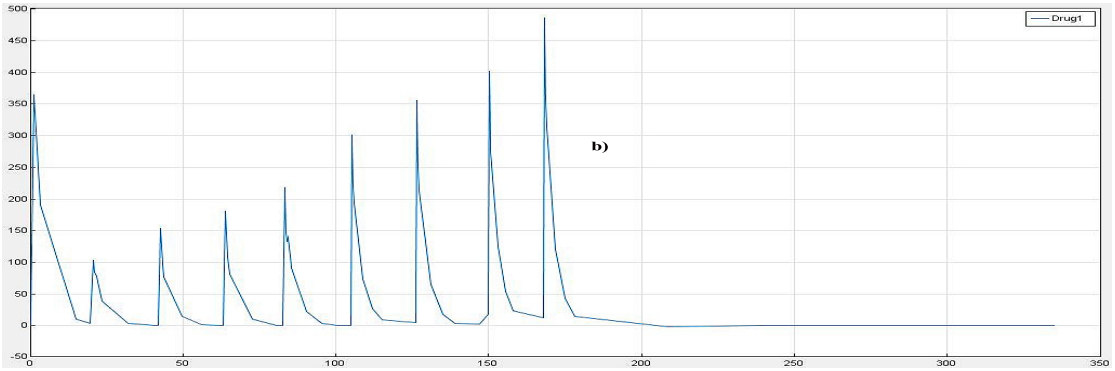


FIGURE 4.33: Comparison of the Clinical PK/PD results of Rituximab, obtained through [284] with simulations of the model: a) Clinical Results, b) Model simulations

In figure 4.33, a) shows the clinical PK/PD results of Rituximab 30 mg.m^{-2} dose was administered into the patients with an interval of 21 days for 331 days cycle. The dose concentration was started from 100 mg/mL, continues to increase and reached to 400 mg/mL on 147th-day of dosing cycle. The concentration started to decrease and again reached to 100 mg/mL on the 182nd day and eliminated from the body after 130 days of dosing cycle. Same model simulations were produced for the Rituximab.

4.12 Biomarkers Response Against Drugs

The Biomarkers that can characterize the patients with various visualizations after chemotherapy stays limited and require further investigation. The motivation behind this study is to additionally distinguish response of Biomarkers for evaluating chemotherapy viability by means of computational Bioinformatics examination of gene expression profiles of WM patients. In order to determine the effect of drugs on the expression level of Biomarkers, the Biomarkers interaction model was developed on the basis of interactions networks, in the Simbiology Matlab, the parameters of Biomarkers were set same as to the normalized values of gene expression cell lines data and the simulations were produced. Responses were generated only for those genes which are experimentally validated. The Biomarkers response model and their responses to drugs are given in figure 4.34

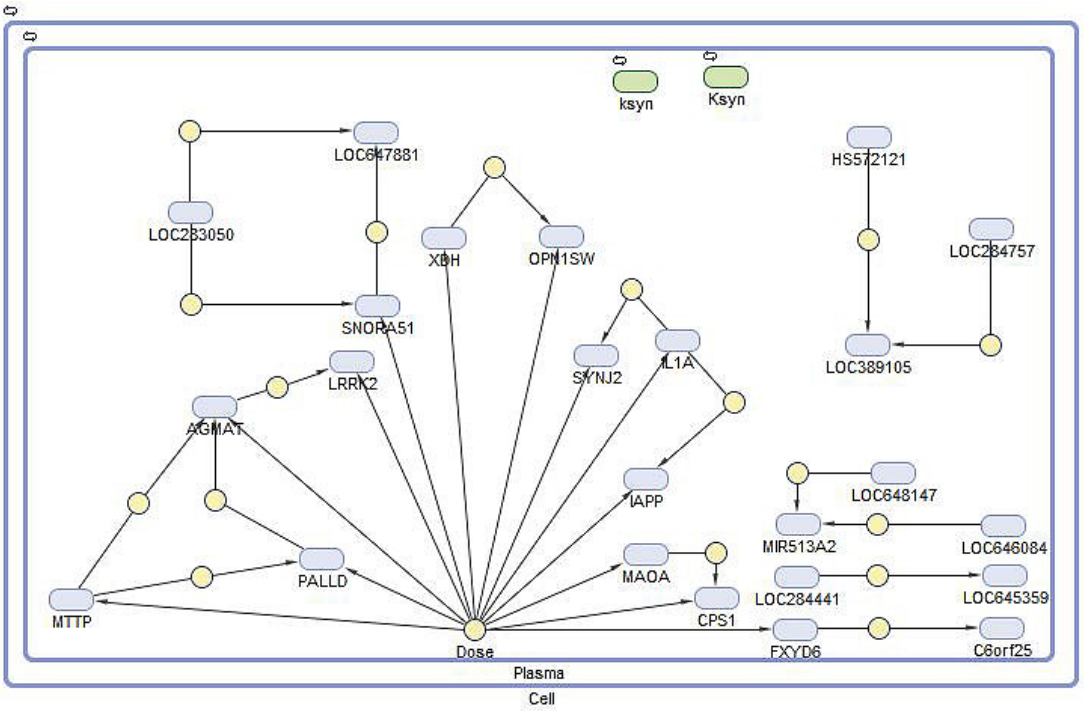


FIGURE 4.34: Doses interaction and biomarkers response model

In figure 4.34, the arrows show interactions (identified through gene interaction networks) the dose reaction was formed and linked to only those genes which are experimentally validated. The differential equations produced for the model are following

EV genes = (IL1A + SNORA51 + LRRK2 + MTTP + PALLD + MAOA + FXYD6 + CPS1 + IAPP + SYNJ2 + XDH + OPN1SW)

$$\frac{d(IL1A)}{dt} = Ksyn \times MAOA - ksyn \times IL1A + DrugDose \times (EVgenes) \quad (4.7)$$

$$\frac{d(IAPP)}{dt} = Ksyn \times IL1A + DrugDose \times (EVgenes) \quad (4.8)$$

$$\frac{d(SYNJ2)}{dt} = Ksyn \times IL1A + DrugDose \times (EVgenes) \quad (4.9)$$

$$\frac{d(XDH)}{dt} = Ksyn \times XDH + DrugDose \times (EVgenes) \tag{4.10}$$

$$\frac{d(OPN1SW)}{dt} = Ksyn \times XDH + DrugDose \times (EVgenes) \tag{4.11}$$

$$\frac{d(MAOA)}{dt} = Ksyn \times MAOA + DrugDose \times (EVgenes) \tag{4.12}$$

$$\frac{d(CPS1)}{dt} = Ksyn \times MAOA + DrugDose \times (EVgenes) \tag{4.13}$$

$$\frac{d(FXYD6)}{dt} = Ksyn \times FXYD6 + DrugDose \times (EVgenes) \tag{4.14}$$

$$\frac{d(SNORA51)}{dt} = Ksyn \times LOC647181 - SNORA51 + DrugDose \times (EVgenes) \tag{4.15}$$

$$\frac{d(MTTP)}{dt} = -Ksyn \times MTTP + DrugDose \times (EVgenes) \tag{4.16}$$

$$\frac{d(PALLD)}{dt} = Ksyn \times PALLD + Ksyn \times MTTP + DrugDose \times (EVgenes) \tag{4.17}$$

$$\frac{d(AGMAT)}{dt} = Ksyn \times AGMAT + Ksyn \times PALLD + DrugDose \times (EVgenes) \tag{4.18}$$

$$\frac{d(LRRK2)}{dt} = Ksyn \times AGMAT + DrugDose \times (EVgenes) \tag{4.19}$$

Ksyn parameter was used in the model as synthesis rate of proteins, several units for the parameters were assumed, the Parameters and units used in the model are given in table 4.11

TABLE 4.11: Parameters values used in the Biomarkers interaction and Response model

Biomarker	Value	Unit
Ksyn	1	Liter
DrugDose	35 - 30	$mg.m^{-2}$
IL1A	0.91559	molecule
IAPP	0.92857	molecule
SYNJ2	0.95975	molecule
XDH	0.92468	molecule
OPN1SW	0.90909	molecule
LOC648147	0.90519	molecule
MIR513A2	0.91818	molecule
LOC646084	0.95974	molecule
MAOA	0.68311	molecule
CPS1	0.9039	molecule
LOC284441	0.91559	molecule
LOC645359	0.9	molecule
FXYD6	0.28182	molecule
LOC389105	0.95325	molecule
C6orf25	0.93435	molecule
LOC284757	0.90649	molecule
SNORA51	0.91948	molecule
LOC283050	0.92468	molecule

LOC647881	0.91816	molecule
MTTP	0.92078	molecule
PALLD	0.96364	molecule
AGMAT	0.96104	molecule
LRRK2	0.90629	molecule
HS572121	0.96494	molecule

Before adding any dose into the model it was observed that PALLD, AGMAT, FXYD6, IL1A, XDH, and MAOA genes were down regulating where-as others were overexpressed shown in Figure 4.35. The Dose reaction was designed into the model with variable values, for each selected drug the value of dose reaction was changed according to the drug dosage regimens and simulations were produced to determine the response of biomarkers against specific drug dosages, the simulation results are shown in figures 4.35 - 4.39.

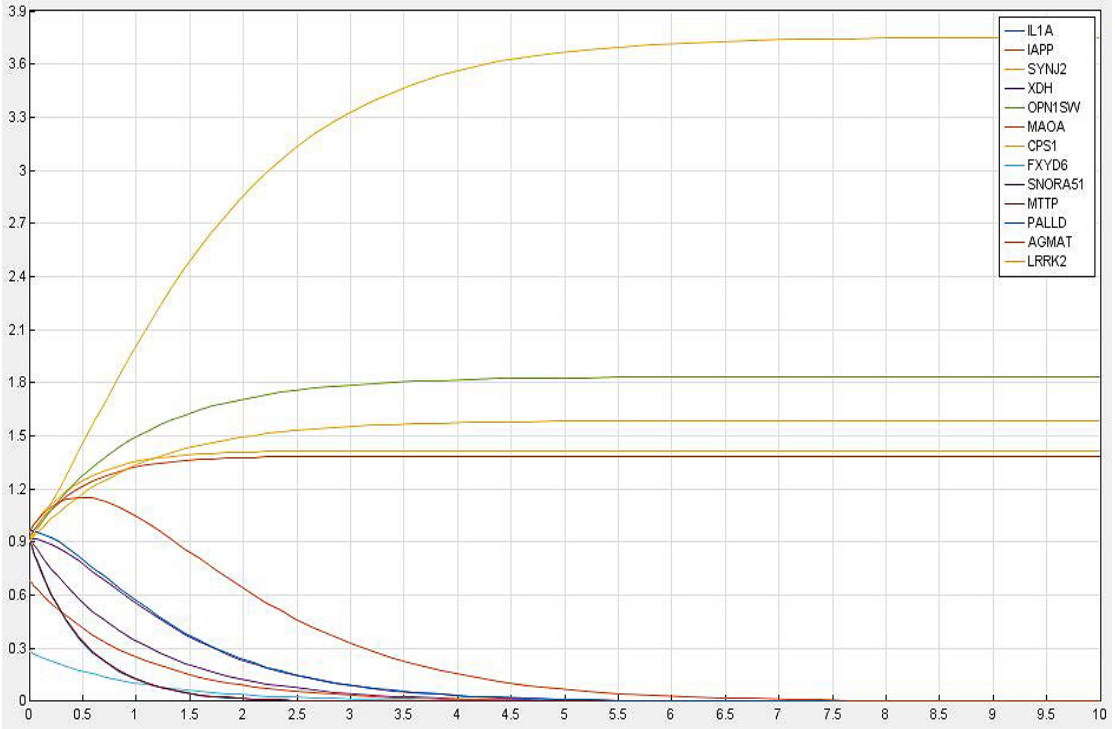


FIGURE 4.35: Expression level of Biomarkers before any dose induction

In figure 4.35, the X-axis represents time in hours and Y-axis shows expression levels of Biomarkers in mole/hour, the expression levels start from 0.9 and reached to a maximum of 3.6 for a biomarker. Similarly, the down expression starts from 0.9 and reached to 0.

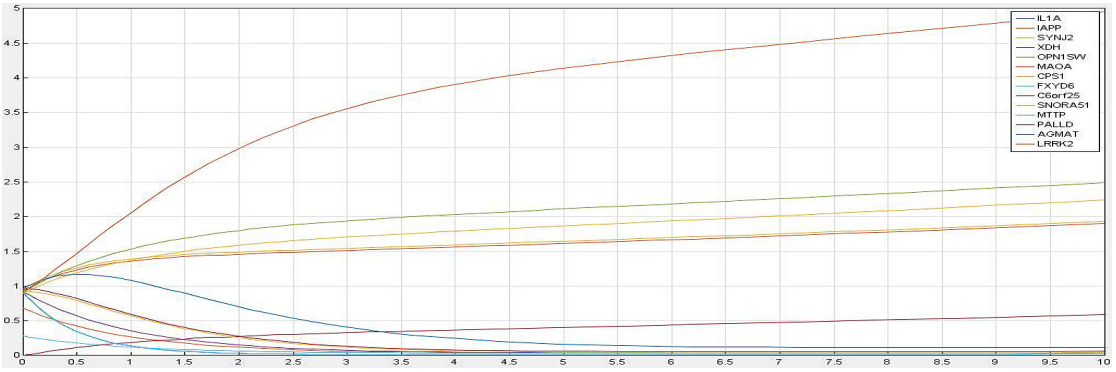


FIGURE 4.36: The Biomarkers response against Bortezomib 3.5 mg

In figure 4.36, it was observed that when 3.5 $mg.m^{-2}$ was added into the model, Decrease in the expression level of PALLD, AGMAT, FXYD6, IL1A, XDH, and MAOA was seen, whereas other biomarkers showed overexpression with a slight change in the expression levels.

It was observed that after the dose, the expression of LRRK2 got increase from 3.6 to 5, OPN1SW from 1.8 - 2.0, the expression levels of CPS1, SYNJ2, and IAPP also increased from previous expressions but PALLD, AGMAT, FXYD6, IL1A, XDH, and MAOA expressions did not show much change in their expressions.

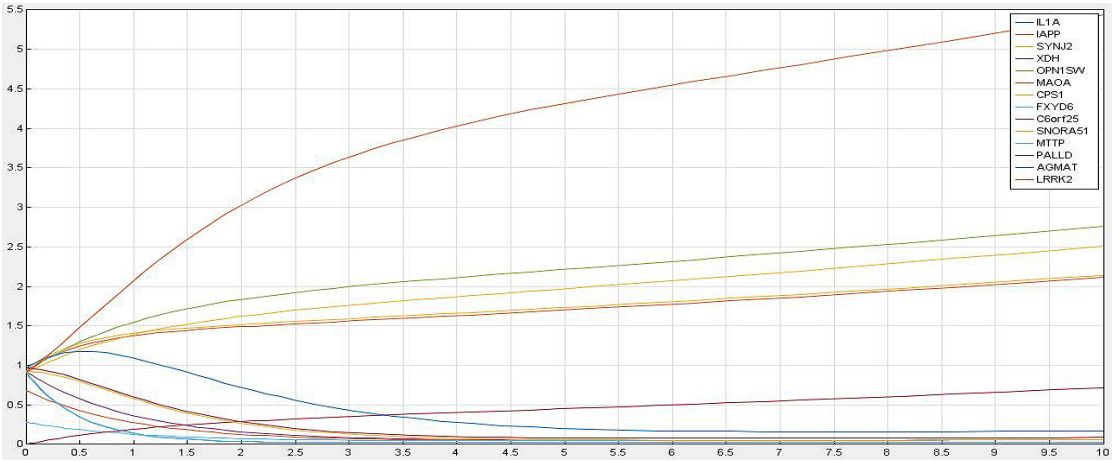


FIGURE 4.37: The Biomarkers response against Ixazomib 4 mg

Same simulation results were produced for Ixazomib 4 mg.m^{-2} dose in figure 4.37 as that of Bortezomib with a slight variation in the expression levels.

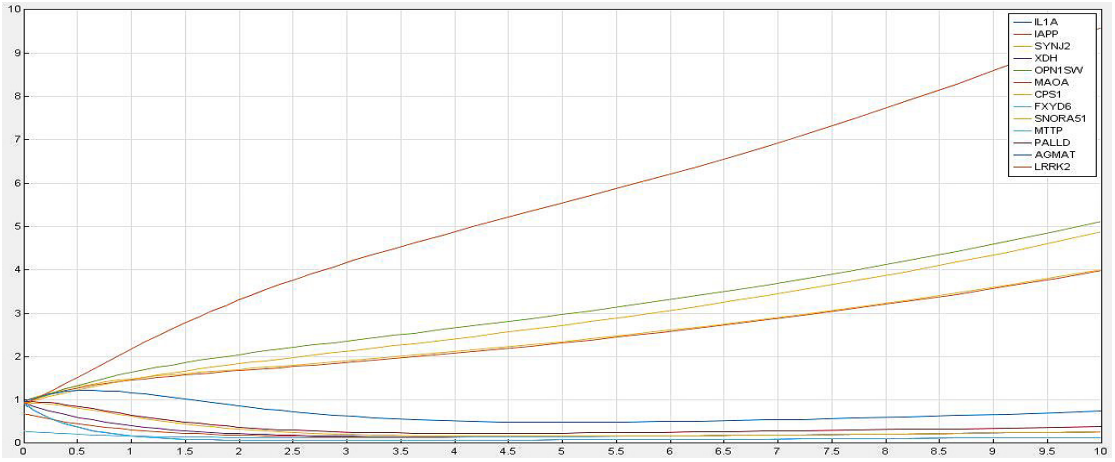


FIGURE 4.38: The Biomarkers response against Carfilzomib 10 mg

In figure 4.38, Again same simulation results were produced for Carfilzomib 10 mg.m^{-2} dose as that of Bortezomib and Ixazomib with a slight variation in the expression levels.

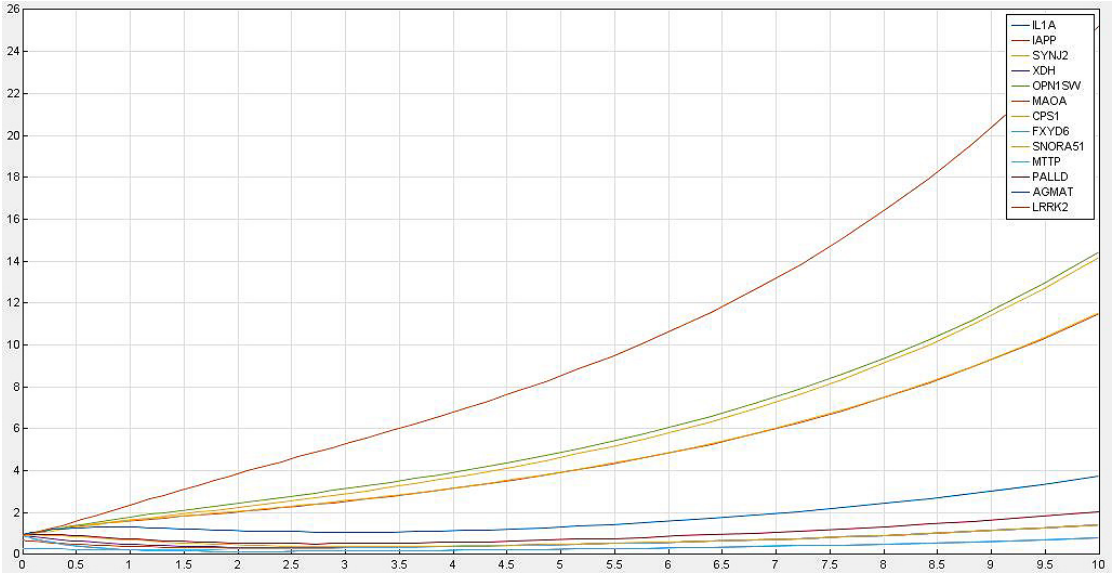


FIGURE 4.39: The Biomarkers response against Oprozomib 20 mg

The figure 4.39 shows that when 20 mg.m^{-2} of Oprozomib was given to the model, all the Biomarkers show a change in their expression level, PALLD, AGMAT,

FXYD6, IL1A, XDH, and MAOA showed an increase in their expression from 0, and other Biomarkers showed much elevation in their expression level.

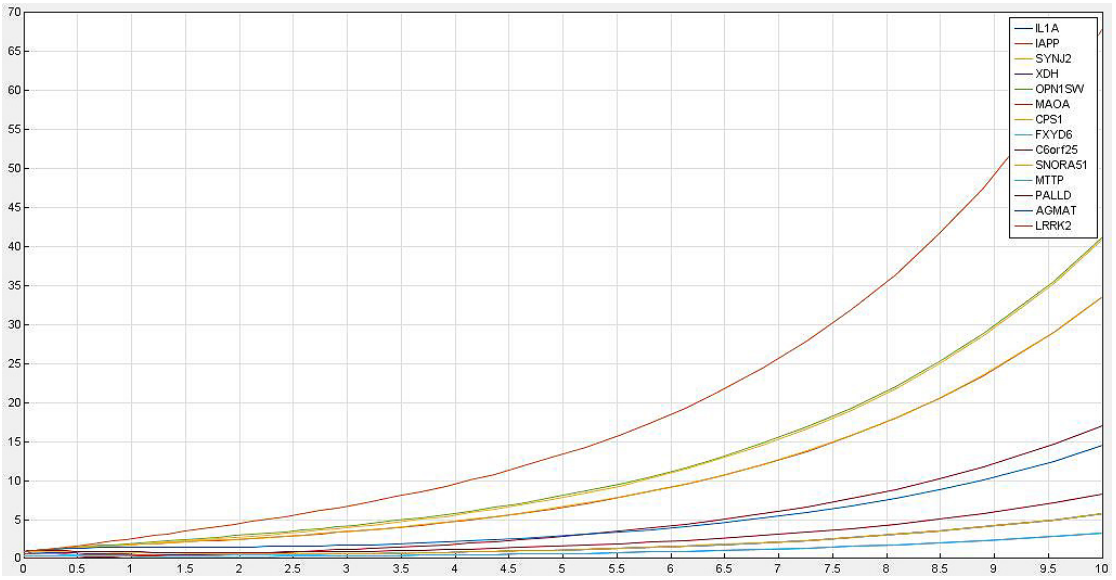


FIGURE 4.40: The Biomarkers response against Rituximab mg

Same results were produced for Rituximab in figure 4.40, when its 30 mg.m^{-2} of Oprozomib was given to the model, all the Biomarkers show change in their expression level, PALLD, AGMAT, FXYD6, IL1A, XDH, and MAOA showed an increase in their expression from 0, and other biomarkers showed much elevation in their expression level. When doses were given in combination with Bortezomib collectively it was seen that all the biomarkers showed an increase in their expression levels than the level without induction of doses.

A few investigations have shown a progression of Biomarkers related to chemotherapy reaction. For instance, Sun et al [291] revealed that phosphoglycerate kinase-1 (PGK1) is upregulated in breast cancer tissues at both mRNA and protein levels, as compared to normal tissues. Additionally, patients with elevated amounts of PGK1 expression show shorter in general survival regardless of whether the paclitaxel chemotherapy routine is planned, demonstrating PGK1 might be an autonomous prognostic biomarker for chemoresistance to paclitaxel [292]. Ataseven et al explained that patients with high expression of protein tyrosine kinase 7 displayed a fundamentally poorer 3-year sickness free survival increment. Notwithstanding, while accepting taxane-based chemotherapy, they indicated essentially

preferred disease-free survival over those receiving no chemotherapy, recommending protein tyrosine kinase 7 might be a prognostic biomarker related with the affectability to taxane [293]

Chapter 5

Conclusion and Future Recommendation

Genomic, proteomic, and other omic-based methodologies are currently being utilized in biomedical research to encourage the understanding of mechanisms involved in diseases and identification of molecular targets and biomarkers for remedial and diagnostic advancement. The Omics innovations and bioinformatics devices for investigating Omics information are quickly progressing, numerous endeavors have been made to find novel biomarkers for early illness in oncology, disease-specific biomarkers, and resistant biomarkers to chemotherapies. However, the absence of proficient computational techniques blocks the disclosure of such biomarkers for better understanding and the board of treatment results. In this study, the resistant biomarkers against Bortezomib profiling in Waldenstrom's macroglobulinemia were studied. Waldenstrom macroglobulinemia is a kind of non-Hodgkin lymphoma. The malignant cells make a lot of proteins (called a macroglobulin). Another name for WM is lymphoplasmacytic lymphoma.

To distinguish the biomarkers associated with resistance to bortezomib in WM cells, we identified novel driver genes. Each of these genes is a potential novel analytic resistant biomarker against bortezomib, utilized for the WM. The modularity analysis, power-law degree distribution and centrality analysis reaffirmed

these key genes, by recognizing the bigger networks of which they are apart. More than a single gene, it could be the networks of genes that are basic to the malignancy development and subsequently, these networks could fill in as indicative biomarkers for bortezomib resistance and in addition, act as a target to treat. The Gene Ontology investigation has revealed insight into certain novel functions of these biomarkers. This study has been powerful in uncovering novel biomarkers and mechanisms that are driving the cancers. A total of 25 markers associated with bortezomib-resistance are recognized in this study, utilizing the gene interaction network analysis. The consequences of this research work give various solid directions to advance examination of the biology of WM cells. For each identified biomarker, their associated pathways were determined and about thirteen different pathways, linked to the WM in some manner were found. IL1A showed a maximum number of associated pathways including Cytokine signaling and Nfk-b pathways. PK/PD modeling and simulation can be utilized as a 'connected science' instrument to give replies on the viability and safety of medications quicker and at a lower cost. Keeping this thing in mind, the information about the drugs used for WM treatment were collected, and only 5 drugs were selected for PK/PD modeling, the monotherapy and combination therapy PK/PD models were developed and simulations were performed collectively, it was observed that among the selected drugs, Carfilzomib and Rituximab displayed strong effect on tumor weight and behaved better as compared to other drugs, similarly, in combination with bortezomib again both Carfilzomib and Rituximab demonstrated improved effects. In order to determine the effects of these drugs on biomarkers another biomarker interaction model was developed and the effects of drugs on their expression level were compared, again the Carfilzomib, Oprozomib and Rituximab have shown improved effects on the expression level of biomarkers.

In Future this work can be further used in the In vitro to validate the identified biomarkers as being resistant against Bortezomib, thus, these biomarkers could anticipate the degree of disease. This method could also be extended able to the analysis of the numerous types of cancer to yield novel helpful biomarkers. The predictions of the model in this dissertation will have various essential potential

clinical implications. The PK/PD model can be further used to study the PK/PD of other drug compounds, the modeling work presented in this study can be instrumental in illuminating the structure of further investigations, and can add to clinical research being performed in an increasingly successful and effective way. However, this study still has some limitations as it is In-silico and needs In-vitro confirmation.

Bibliography

[1] S. H. Hassanpour and M. Dehghani, "Review of cancer from perspective of molecular," *Journal of Cancer Research and Practice*, vol. 4, no. 4, pp. 127–129, 2017.

[2] R. Siegel, D. Naishadham, and A. Jemal, "Cancer statistics, 2013," *CA: a cancer journal for clinicians*, vol. 63, no. 1, pp. 11–30, 2013.

[3] C. E. Meacham and S. J. Morrison, "Tumour heterogeneity and cancer cell plasticity," *Nature*, vol. 501, no. 7467, pp. 328–332, 2013.

[4] R. Fisher, L. Pusztai, and C. Swanton, "Cancer heterogeneity: implications for targeted therapeutics," *British journal of cancer*, vol. 108, no. 3, pp. 479–484, 2013.

[5] R. L. Siegel, K. D. Miller, and A. Jemal, "Cancer statistics, 2016," *CA: a cancer journal for clinicians*, vol. 66, no. 1, pp. 7–30, 2016.

[6] P. Correa, "Human gastric carcinogenesis: a multistep and multifactorial processfirst american cancer society award lecture on cancer epidemiology and prevention," *Cancer research*, vol. 52, no. 24, pp. 6735–6740, 1992.

[7] K. Y. Yoo and H. R. Shin, "Cancer epidemiology and prevention," *Korean Journal of Epidemiology*, vol. 25, no. 1, pp. 1–15, 2003.

[8] M. Seto, K. Honma, and M. Nakagawa, "Diversity of genome profiles in malignant lymphoma," *Cancer science*, vol. 101, no. 3, pp. 573–578, 2010.

[9] J. C. Cigudosa, N. Z. Parsa, D. C. Louie, D. A. Filippa, S. C. Jhanwar, B. Johansson, F. Mitelman, and R. Chaganti, "Cytogenetic analysis of 363

consecutively ascertained diffuse large b-cell lymphomas," *Genes, Chromosomes and Cancer*, vol. 25, no. 2, pp. 123–133, 1999.

[10] E. Shtivelman, B. Lifshitz, R. P. Gale, and E. Canaani, "Fused transcript of abl and bcr genes in chronic myelogenous leukaemia," *Nature*, vol. 315, no. 6020, pp. 550–554, 1985.

[11] A. Vijay and M. A. Gertz, "Waldenström macroglobulinemia," *Blood*, vol. 109, no. 12, pp. 5096–5103, 2007.

[12] T. Yamamoto, "Molecular basis of cancer: oncogenes and tumor suppressor genes," *Microbiology and immunology*, vol. 37, no. 1, pp. 11–22, 1993.

[13] R. A. Lockhart, "Cancer in myth and dream," *Psychotherapeutic Treatment of Cancer Patients/ed. by JG Goldberg. Transaction Publishers*, vol. 71, no. 3, pp. 11–22, 1990.

[14] M. Hashemi, N. Behrangi, H. Borna, A. Akbarzadeh *et al.*, "Evaluating new targets of natural anticancer molecules through bioinformatics tools," *J Proteomics Bioinform*, vol. 5, no. 1, pp. 50–53, 2012.

[15] G. Housman, S. Byler, S. Heerboth, K. Lapinska, M. Longacre, N. Snyder, and S. Sarkar, "Drug resistance in cancer: an overview," *Cancers*, vol. 6, no. 3, pp. 1769–1792, 2014.

[16] B. Mansoori, A. Mohammadi, S. Davudian, S. Shirjang, and B. Baradaran, "The different mechanisms of cancer drug resistance: A brief review," *Advanced pharmaceutical bulletin*, vol. 7, no. 3, pp. 339–344, 2017.

[17] H. Zahreddine and K. Borden, "Mechanisms and insights into drug resistance in cancer," *Frontiers in pharmacology*, vol. 4, pp. 28–34, 2013.

[18] M. Pop and S. L. Salzberg, "Bioinformatics challenges of new sequencing technology," *Trends in Genetics*, vol. 24, no. 3, pp. 142–149, 2008.

[19] J. Zadeh, "An undergraduate program in bioinformatics," *IEEE Potentials*, vol. 25, no. 3, pp. 43–46, 2006.

[20] M. Fulekar and J. Sharma, "Bioinformatics applied in bioremediation," *Innovative Romanian Food Biotechnology*, vol. 3, no. 1, pp. 28–33, 2008.

[21] N. M. Luscombe, D. Greenbaum, and M. Gerstein, "What is bioinformatics? a proposed definition and overview of the field," *Methods of information in medicine*, vol. 40, no. 04, pp. 346–358, 2001.

[22] D. Poe, N. Venkatraman, C. Hansen, and G. Singh, "Component-based approach for educating students in bioinformatics," *IEEE Transactions on Education*, vol. 52, no. 1, pp. 1–9, 2009.

[23] G. H. Fernald, E. Capriotti, R. Daneshjou, K. J. Karczewski, and R. B. Altman, "Bioinformatics challenges for personalized medicine," *Bioinformatics*, vol. 27, no. 13, pp. 1741–1748, 2011.

[24] M. Al-Rajab and J. Lu, "Bioinformatics: an overview for cancer research," *Journal of Drug research*, vol. 12, no. 3, pp. 777–780, 2012.

[25] H. Chen, Z. Zhu, Y. Zhu, J. Wang, Y. Mei, and Y. Cheng, "Pathway mapping and development of disease-specific biomarkers: protein-based network biomarkers," *Journal of cellular and molecular medicine*, vol. 19, no. 2, pp. 297–314, 2015.

[26] D. Wu, D. Zhu, M. Xu, R. Rong, Q. Tang, X. Wang, and T. Zhu, "Analysis of transcriptional factors and regulation networks in patients with acute renal allograft rejection," *Journal of proteome research*, vol. 10, no. 1, pp. 175–181, 2010.

[27] H. Chen, Z. Song, M. Qian, C. Bai, and X. Wang, "Selection of disease-specific biomarkers by integrating inflammatory mediators with clinical informatics in aecopd patients: a preliminary study," *Journal of cellular and molecular medicine*, vol. 16, no. 6, pp. 1286–1297, 2012.

[28] Y. Cun and H. Fröhlich, "Prognostic gene signatures for patient stratification in breast cancer-accuracy, stability and interpretability of gene selection approaches using prior knowledge on protein-protein interactions," *BMC bioinformatics*, vol. 13, no. 1, pp. 69–76, 2012.

[29] B. Meibohm and H. Derendorf, "Basic concepts of pharmacokinetic/pharmacodynamic (pk/pd) modelling," *International journal of clinical pharmacology and therapeutics*, vol. 35, no. 10, pp. 401–413, 1997.

[30] J. Paludo and S. M. Ansell, "Waldenström macroglobulinemia: biology, genetics, and therapy," *Blood and Lymphatic Cancer: Targets and Therapy*, vol. 6, no. 1, pp. 49–58, 2016.

[31] J. Waldenström, "Incipient myelomatosis or essential hyperglobulinemia with fibrinogenopeniaa new syndrome?" *Acta Medica Scandinavica*, vol. 117, no. 3-4, pp. 216–247, 1944.

[32] A. Oza and S. Rajkumar, "Waldenstrom macroglobulinemia: prognosis and management," *Blood cancer journal*, vol. 5, no. 3, pp. e394–e401, 2015.

[33] M. A. Gertz, "Waldenström macroglobulinemia: 2013 update on diagnosis, risk stratification, and management," *American journal of hematology*, vol. 88, no. 8, pp. 703–711, 2013.

[34] J. Monge, E. Braggio, and S. M. Ansell, "Genetic factors and pathogenesis of waldenströms macroglobulinemia," *Current oncology reports*, vol. 15, no. 5, pp. 450–456, 2013.

[35] H. Wang, Y. Chen, F. Li, K. Delasalle, J. Wang, R. Alexanian, L. Kwak, L. Rustveld, X. L. Du, and M. Wang, "Temporal and geographic variations of waldenstrom macroglobulinemia incidence: a large population-based study," *Cancer*, vol. 118, no. 15, pp. 3793–3800, 2012.

[36] M. Varettoni, A. Tedeschi, L. Arcaini, C. Pascutto, E. Vismara, E. Orlandi, F. Ricci, A. Corso, A. Greco, S. Mangiacavalli *et al.*, "Risk of second cancers

in waldenström macroglobulinemia," *Annals of oncology*, vol. 23, no. 2, pp. 411–415, 2011.

[37] R. Ojha and R. Thertulien, "Second malignancies among waldenstrom macroglobulinemia patients: small samples and sparse data," *Annals of oncology*, vol. 23, no. 2, pp. 542–543, 2011.

[38] N. L. Harris, E. S. Jaffe, J. Diebold, G. Flandrin, H. K. Muller-Hermelink, J. Vardiman, T. A. Lister, and C. D. Bloomfield, "The world health organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the clinical advisory committee meeting, airlie house, virginia, november, 1997," *Annals of Oncology*, vol. 10, no. 12, pp. 1419–1432, 1999.

[39] L. S. Hodge, A. J. Novak, D. M. Grote, E. Braggio, R. P. Ketterling, M. K. Manske, T. L. P. Troska, S. C. Ziesmer, R. Fonseca, T. E. Witzig *et al.*, "Establishment and characterization of a novel waldenström macroglobulinemia cell line, mwcl-1," *Blood*, vol. 117, no. 19, pp. e190–e197, 2011.

[40] T. Facon, M. Brouillard, A. Duhamel, P. Morel, M. Simon, J. P. Jouet, F. Bauters, and P. Fenaux, "Prognostic factors in waldenström's macroglobulinemia: a report of 167 cases." *Journal of Clinical Oncology*, vol. 11, no. 8, pp. 1553–1558, 1993.

[41] P. Morel, M. Monconduit, D. Jacomy, P. Lenain, B. Grosbois, C. Bateli, T. Facon, I. Dervite, F. Bauters, A. Najman *et al.*, "Prognostic factors in waldenström macroglobulinemia: a report on 232 patients with the description of a new scoring system and its validation on 253 other patients," *Blood*, vol. 96, no. 3, pp. 852–858, 2000.

[42] R. García-Sanz, S. Montoto, A. Torrequebrada, A. G. De Coca, J. Petit, A. Sureda, J. A. Rodríguez-García, P. Massó, A. Pérez-Aliaga, M. D. Monteagudo *et al.*, "Waldenström macroglobulinaemia: presenting features and outcome in a series with 217 cases," *British journal of haematology*, vol. 115, no. 3, pp. 575–582, 2001.

[43] P. Morel, A. Duhamel, P. Gobbi, M. A. Dimopoulos, M. V. Dhodapkar, J. McCoy, J. Crowley, E. M. Ocio, R. Garcia-Sanz, S. P. Treon *et al.*, "International prognostic scoring system for waldenström macroglobulinemia," *Blood*, vol. 113, no. 18, pp. 4163–4170, 2009.

[44] I. M. Ghobrial, R. Fonseca, M. A. Gertz, M. F. Plevak, D. R. Larson, T. M. Therneau, R. C. Wolf, R. J. Hoffmann, J. A. Lust, T. E. Witzig *et al.*, "Prognostic model for disease-specific and overall mortality in newly diagnosed symptomatic patients with waldenstrom macroglobulinaemia," *British journal of haematology*, vol. 133, no. 2, pp. 158–164, 2006.

[45] R. Kyle and S. V. Rajkumar, "Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma," *Leukemia*, vol. 23, no. 1, pp. 3–9, 2009.

[46] S. Konoplev, L. J. Medeiros, C. E. Bueso-Ramos, J. L. Jorgensen, and P. Lin, "Immunophenotypic profile of lymphoplasmacytic lymphoma/waldenström macroglobulinemia," *American Journal of Clinical Pathology*, vol. 124, no. 3, pp. 414–420, 2005.

[47] R. A. Kyle, J. T. Benson, D. R. Larson, T. M. Therneau, A. Dispenzieri, S. Kumar, L. J. Melton, and S. V. Rajkumar, "Progression in smoldering waldenstrom macroglobulinemia: long-term results," *Blood*, vol. 119, no. 19, pp. 4462–4466, 2012.

[48] S. P. Treon, L. Xu, G. Yang, Y. Zhou, X. Liu, Y. Cao, P. Sheehy, R. J. Manning, C. J. Patterson, C. Tripsas *et al.*, "Myd88 l265p somatic mutation in waldenström's macroglobulinemia," *New England Journal of Medicine*, vol. 367, no. 9, pp. 826–833, 2012.

[49] S. P. Treon and Z. R. Hunter, "A new era for waldenstrom macroglobulinemia: Myd88 l265p," *Blood*, vol. 121, no. 22, pp. 4434–4436, 2013.

[50] M. Varettoni, L. Arcaini, S. Zibellini, E. Boveri, S. Rattotti, R. Riboni, A. Corso, E. Orlandi, M. Bonfichi, M. Gotti *et al.*, "Prevalence and clinical significance of the myd88 (l265p) somatic mutation in waldenström's

macroglobulinemia and related lymphoid neoplasms," *Blood*, vol. 32, no. 5, pp. 314–317, 2013.

[51] C. Jiménez, E. Sebastián, M. Chillón, P. Giraldo, J. M. Hernandez, F. Escalante, T. Gonzalez-Lopez, C. Aguilera, A. G. de Coca, I. Murillo *et al.*, "Myd88 l265p is a marker highly characteristic of, but not restricted to, waldenströms macroglobulinemia," *Leukemia*, vol. 27, no. 8, pp. 1722–1732, 2013.

[52] S. P. Treon and Z. R. Hunter, "A new era for waldenstrom macroglobulinemia: Myd88 l265p," *Blood*, vol. 121, no. 22, pp. 4434–4436, 2013.

[53] S. Ansell, L. Hodge, F. Secreto, M. Manske, E. Braggio, T. Price-Troska, S. Ziesmer, Y. Li, S. Johnson, S. Hart *et al.*, "Activation of tak1 by myd88 l265p drives malignant b-cell growth in non-hodgkin lymphoma," *Blood cancer journal*, vol. 4, no. 2, pp. e183–e189, 2014.

[54] Z. R. Hunter, L. Xu, G. Yang, Y. Zhou, X. Liu, Y. Cao, R. J. Manning, C. Tripsas, C. J. Patterson, P. Sheehy *et al.*, "The genomic landscape of waldenström macroglobulinemia is characterized by highly recurring myd88 and whim-like cxcr4 mutations, and small somatic deletions associated with b-cell lymphomagenesis," *Blood*, vol. 123, no. 11, pp. 1637–1646, 2014.

[55] A. M. Roccaro, A. Sacco, C. Jimenez, P. Maiso, M. Moschetta, Y. Mishima, Y. Aljawai, I. Sahin, M. Kuhne, P. Cardarelli *et al.*, "C1013g/cxcr4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma," *Blood*, vol. 123, no. 26, pp. 4120–4131, 2014.

[56] J. Schmidt, B. Federmann, N. Schindler, J. Steinhilber, I. Bonzheim, F. Fend, and L. Quintanilla-Martinez, "Myd 88 l265p and cxcr 4 mutations in lymphoplasmacytic lymphoma identify cases with high disease activity," *British journal of haematology*, vol. 169, no. 6, pp. 795–803, 2015.

[57] S. Poulain, C. Roumier, A. Venet-Caillault, M. Figeac, C. Herbaux, G. Marot, D. Emmanuelle, E. Bertrand, S. Geffroy, F. Lepretre *et al.*, "Genomic landscape of cxcr4 mutations in waldenstrom's macroglobulinemia," *Clinical Cancer Research*, vol. 12, no. 1, pp. 0641–0646, 2015.

[58] S. P. Treon, Y. Cao, L. Xu, G. Yang, X. Liu, and Z. R. Hunter, "Somatic mutations in myd88 and cxcr4 are determinants of clinical presentation and overall survival in waldenström macroglobulinemia," *Blood*, vol. 123, no. 18, pp. 2791–2796, 2014.

[59] S. P. Treon, C. K. Tripsas, K. Meid, D. Warren, G. Varma, R. Green, K. V. Argyropoulos, G. Yang, Y. Cao, L. Xu *et al.*, "Ibrutinib in previously treated waldenströms macroglobulinemia," *New England Journal of Medicine*, vol. 372, no. 15, pp. 1430–1440, 2015.

[60] R. A. Kyle, T. M. Therneau, S. V. Rajkumar, E. D. Remstein, J. R. Offord, D. R. Larson, M. F. Plevak, and L. J. Melton, "Long-term follow-up of igm monoclonal gammopathy of undetermined significance," *Blood*, vol. 102, no. 10, pp. 3759–3764, 2003.

[61] S.-M. Bang, S. R. Park, S. H. Park, E. K. Cho, S.-S. Yoon, D. B. Shin, J. H. Lee, S. Park, B. K. Kim, and N. K. Kim, "Clinical features of waldenstrom macroglobulinemia in korea," *The Korean journal of internal medicine*, vol. 19, no. 3, pp. 137–146, 2004.

[62] L. Baldini, M. Goldaniga, A. Guffanti, C. Broglia, S. Cortelazzo, A. Rossi, E. Morra, M. Colombi, V. Callea, E. Pogliani *et al.*, "Immunoglobulin m monoclonal gammopathies of undetermined significance and indolent waldenstroms macroglobulinemia recognize the same determinants of evolution into symptomatic lymphoid disorders: proposal for a common prognostic scoring system," *J Clin Oncol*, vol. 23, no. 21, pp. 4662–4668, 2005.

[63] I. M. Ghobrial, M. Gertz, B. LaPlant, J. Camoriano, S. Hayman, M. Lacy, S. Chuma, B. Harris, R. Leduc, M. Rourke *et al.*, "Phase ii trial of the oral

mammalian target of rapamycin inhibitor everolimus in relapsed or refractory waldenström macroglobulinemia," *Journal of Clinical Oncology*, vol. 28, no. 8, pp. 1408–1412, 2010.

[64] A. Tedeschi, G. Benevolo, M. Varettoni, M. L. Battista, P. L. Zinzani, C. Visco, V. Meneghini, P. Pioltelli, S. Sacchi, F. Ricci *et al.*, "Fludarabine plus cyclophosphamide and rituximab in waldenstrom macroglobulinemia: an effective but myelosuppressive regimen to be offered to patients with advanced disease," *Cancer*, vol. 118, no. 2, pp. 434–443, 2012.

[65] R. A. Kyle, "Monoclonal gammopathy of undetermined significance: natural history in 241 cases," *The American journal of medicine*, vol. 64, no. 5, pp. 814–826, 1978.

[66] J.-L. Preud'homme and M. Seligmann, "Immunoglobulins on the surface of lymphoid cells in waldenström's macroglobulinemia," *The Journal of clinical investigation*, vol. 51, no. 3, pp. 701–705, 1972.

[67] A. Marmont and G. Merlini, "Monoclonal autoimmunity in hematology." *Haematologica*, vol. 76, no. 6, pp. 449–459, 1991.

[68] S. Treon, Z. Hunter, A. Aggarwal, E. Ewen, S. Masota, C. Lee, D. D. Santos, E. Hatjiharissi, L. Xu, X. Leleu *et al.*, "Characterization of familial waldenström's macroglobulinemia," *Annals of Oncology*, vol. 17, no. 3, pp. 488–494, 2005.

[69] S. P. Treon, "How i treat waldenström macroglobulinemia," *Blood*, vol. 114, no. 12, pp. 2375–2385, 2009.

[70] M. L. McMaster, G. Csako, T. R. Giambarresi, L. Vasquez, M. Berg, S. Saddlemire, B. Hulley, and M. A. Tucker, "Long-term evaluation of three multiple-case waldenström macroglobulinemia families," *Clinical Cancer Research*, vol. 13, no. 17, pp. 5063–5069, 2007.

[71] S. Y. Kristinsson, M. Björkholm, L. R. Goldin, M. L. McMaster, I. Turesson, and O. Landgren, "Risk of lymphoproliferative disorders among first-degree

relatives of lymphoplasmacytic lymphoma/waldenström macroglobulinemia patients: a population-based study in sweden," *Blood*, vol. 112, no. 8, pp. 3052–3056, 2008.

[72] S. P. Treon, C. Tripsas, C. Hanzis, L. Ioakimidis, C. J. Patterson, R. J. Manning, P. Sheehy, B. Turnbull, and Z. R. Hunter, "Familial disease predisposition impacts treatment outcome in patients with waldenström macroglobulinemia," *Clinical Lymphoma Myeloma and Leukemia*, vol. 12, no. 6, pp. 433–437, 2012.

[73] T. Izumi, R. Sasaki, S. Tsunoda, M. Akutsu, H. Okamoto, and Y. Miura, "B cell malignancy and hepatitis c virus infection." *Leukemia (08876924)*, vol. 11, no. 4, pp. 25–32, 1997.

[74] P. Kapoor, J. Paludo, N. Vallumsetla, and P. R. Greipp, "Waldenström macroglobulinemia: What a hematologist needs to know," *Blood reviews*, vol. 29, no. 5, pp. 301–319, 2015.

[75] R. A. Kyle, T. M. Therneau, S. V. Rajkumar, J. R. Offord, D. R. Larson, M. F. Plevak, and L. J. Melton III, "A long-term study of prognosis in monoclonal gammopathy of undetermined significance," *New England Journal of Medicine*, vol. 346, no. 8, pp. 564–569, 2002.

[76] R. A. Kyle, J. T. Benson, D. R. Larson, T. M. Therneau, A. Dispenzieri, S. Kumar, L. J. Melton, and S. V. Rajkumar, "Progression in smoldering waldenstrom's macroglobulinemia: long-term results," *Blood*, vol. 119, no. 19, pp. 4462–4466, 2012.

[77] E. Braggio, C. Philipsborn, A. Novak, L. Hodge, S. Ansell, and R. Fonseca, "Molecular pathogenesis of waldenström macroglobulinemia," *Haematologica*, vol. 97, no. 9, pp. 1281–1290, 2012.

[78] I. Vivanco and C. L. Sawyers, "The phosphatidylinositol 3-kinase–akt pathway in human cancer," *Nature Reviews Cancer*, vol. 2, no. 7, pp. 489–494, 2002.

[79] X. Leleu, X. Jia, J. Runnels, H. T. Ngo, A.-S. Moreau, M. Farag, J. A. Spencer, C. M. Pitsillides, E. Hatjiharissi, A. Roccaro *et al.*, "The akt pathway regulates survival and homing in waldenstrom macroglobulinemia," *Blood*, vol. 110, no. 13, pp. 4417–4426, 2007.

[80] M. Yoeli-Lerner and A. Toker, "Akt/pkb signaling in cancer: a function in cell motility and invasion," *Cell cycle*, vol. 5, no. 6, pp. 603–605, 2006.

[81] S. Brader and S. A. Eccles, "Phosphoinositide 3-kinase signalling pathways in tumor progression, invasion and angiogenesis," *Tumori Journal*, vol. 90, no. 1, pp. 2–8, 2004.

[82] A. Sacco, A. Roccaro, and I. M. Ghobrial, "Role of dual pi3/akt and mtor inhibition in waldenstrom's macroglobulinemia," *Oncotarget*, vol. 1, no. 7, pp. 578–581, 2010.

[83] P. Tassone, P. Neri, J. L. Kutok, O. Tournilhac, D. D. Santos, E. Hatjiharissi, V. Munshi, S. Venuta, K. C. Anderson, S. P. Treon *et al.*, "A scid-hu in vivo model of human waldenström macroglobulinemia," *Blood*, vol. 106, no. 4, pp. 1341–1345, 2005.

[84] D. A. Guertin and D. M. Sabatini, "The pharmacology of mtor inhibition," *Sci. Signal.*, vol. 2, no. 67, pp. pe24–pe24, 2009.

[85] I. M. Ghobrial, M. Gertz, B. LaPlant, J. Camoriano, S. Hayman, M. Lacy, S. Chuma, B. Harris, R. Leduc, M. Rourke *et al.*, "Phase ii trial of the oral mammalian target of rapamycin inhibitor everolimus in relapsed or refractory waldenström macroglobulinemia," *Journal of Clinical Oncology*, vol. 28, no. 8, pp. 1408–1419, 2010.

[86] I. M. Ghobrial, T. E. Witzig, M. Gertz, B. LaPlant, S. Hayman, J. Camoriano, M. Lacy, P. L. Bergsagel, S. Chuma, D. DeAngelo *et al.*, "Long-term results of the phase ii trial of the oral mtor inhibitor everolimus (rad001) in relapsed or refractory waldenstrom macroglobulinemia," *American journal of hematology*, vol. 89, no. 3, pp. 237–242, 2014.

[87] A. M. Roccaro, A. Sacco, E. N. Husu, C. Pitsillides, S. Vesole, A. K. Azab, F. Azab, M. Melhem, H. T. Ngo, P. Quang *et al.*, "Dual targeting of the pi3k/akt/mtor pathway as an antitumor strategy in waldenstrom macroglobulinemia," *Blood*, vol. 115, no. 3, pp. 559–569, 2010.

[88] N. Harir, C. Pecquet, M. Kerenyi, K. Sonneck, B. Kovacic, R. Nyga, M. Brevet, I. Dhennin, V. Gouilleux-Gruart, H. Beug *et al.*, "Constitutive activation of stat5 promotes its cytoplasmic localization and association with pi3-kinase in myeloid leukemias," *Blood*, vol. 109, no. 4, pp. 1678–1686, 2007.

[89] A. C. Ward, I. Touw, and A. Yoshimura, "The jak-stat pathway in normal and perturbed hematopoiesis," *Blood*, vol. 95, no. 1, pp. 19–29, 2000.

[90] L. S. Hodge and S. M. Ansell, "Jak/stat pathway in waldenström's macroglobulinemia," *Clinical Lymphoma Myeloma and Leukemia*, vol. 11, no. 1, pp. 112–114, 2011.

[91] E. Atallah and S. Verstovsek, "Prospect of jak2 inhibitor therapy in myeloproliferative neoplasms," *Expert review of anticancer therapy*, vol. 9, no. 5, pp. 663–670, 2009.

[92] E. Hatjiharissi, H. Ngo, A. A. Leontovich, X. Leleu, M. Timm, M. Melhem, D. George, G. Lu, J. Ghobrial, Y. Alsayed *et al.*, "Proteomic analysis of waldenstrom macroglobulinemia," *Cancer research*, vol. 67, no. 8, pp. 3777–3784, 2007.

[93] L. S. Hodge, S. C. Ziesmer, Z.-Z. Yang, F. J. Secreto, A. J. Novak, and S. M. Ansell, "Constitutive activation of stat5a and stat5b regulates igm secretion in waldenström's macroglobulinemia," *Blood*, vol. 123, no. 7, pp. 1055–1058, 2014.

[94] K. A. Z. Siddiquee and J. Turkson, "Stat3 as a target for inducing apoptosis in solid and hematological tumors," *Cell research*, vol. 18, no. 2, pp. 254–260, 2008.

[95] S. Malin, S. McManus, C. Cobaleda, M. Novatchkova, A. Delogu, P. Bouillet, A. Strasser, and M. Busslinger, "Role of stat5 in controlling cell survival and immunoglobulin gene recombination during pro-b cell development," *Nature immunology*, vol. 11, no. 2, pp. 171–176, 2010.

[96] T. M. Watters, E. F. Kenny, and L. A. O'neill, "Structure, function and regulation of the toll/il-1 receptor adaptor proteins," *Immunology and cell biology*, vol. 85, no. 6, pp. 411–419, 2007.

[97] L. Cohen, W. J. Henzel, and P. A. Baeuerle, "Ikap is a scaffold protein of the iκb kinase complex," *Nature*, vol. 395, no. 6699, pp. 292–298, 1998.

[98] M. Loiarro, G. Gallo, N. Fanto, R. De Santis, P. Carminati, V. Ruggiero, and C. Sette, "Identification of critical residues of the myd88 death domain involved in the recruitment of downstream kinases," *Journal of Biological Chemistry*, vol. 284, no. 41, pp. 28 093–28 103, 2009.

[99] S.-C. Lin, Y.-C. Lo, and H. Wu, "Helical assembly in the myd88–irak4–irak2 complex in tlr/il-1r signalling," *Nature*, vol. 465, no. 7300, pp. 885–895, 2010.

[100] T. Kawagoe, S. Sato, K. Matsushita, H. Kato, K. Matsui, Y. Kumagai, T. Saitoh, T. Kawai, O. Takeuchi, and S. Akira, "Sequential control of toll-like receptor–dependent responses by irak1 and irak2," *Nature immunology*, vol. 9, no. 6, pp. 684–690, 2008.

[101] X. Leleu, J. Eeckhoute, X. Jia, A. M. Roccaro, A.-S. Moreau, M. Farag, A. Sacco, H. T. Ngo, J. Runnels, M. R. Melhem *et al.*, "Targeting nf-κb in waldenstrom macroglobulinemia," *Blood*, vol. 111, no. 10, pp. 5068–5077, 2008.

[102] G. Yang, Y. Zhou, X. Liu, L. Xu, Y. Cao, R. J. Manning, C. J. Patterson, S. J. Buhrlage, N. Gray, Y.-T. Tai *et al.*, "A mutation in myd88 (l265p) supports the survival of lymphoplasmacytic cells by activation of bruton's tyrosine kinase in waldenstrom's macroglobulinemia," *Blood*, vol. 122, no. 7, pp. 1222–1232, 2013.

[103] S. P. Treon, Z. R. Hunter, J. J. Castillo, and G. Merlini, "Waldenström macroglobulinemia," *Hematology/Oncology Clinics*, vol. 28, no. 5, pp. 945–970, 2014.

[104] W. J. Chng, R. F. Schop, T. Price-Troska, I. Ghobrial, N. Kay, D. F. Jelinek, M. A. Gertz, A. Dispenzieri, M. Lacy, R. A. Kyle *et al.*, "Gene-expression profiling of waldenström macroglobulinemia reveals a phenotype more similar to chronic lymphocytic leukemia than multiple myeloma," *Blood*, vol. 108, no. 8, pp. 2755–2763, 2006.

[105] M. J. Stone, G. Merlini, and V. Pascual, "Autoantibody activity in waldenström's macroglobulinemia," *Clinical lymphoma*, vol. 5, no. 4, pp. 225–229, 2005.

[106] M. J. Stone, "Pathogenesis and morbidity of autoantibody syndromes in waldenstrom's macroglobulinemia," *Clinical Lymphoma Myeloma and Leukemia*, vol. 11, no. 1, pp. 157–159, 2011.

[107] M. N. Menke, G. T. Feke, J. W. McMeel, and S. P. Treon, "Ophthalmologic techniques to assess the severity of hyperviscosity syndrome and the effect of plasmapheresis in patients with waldenström's macroglobulinemia," *Clinical Lymphoma and Myeloma*, vol. 9, no. 1, pp. 100–103, 2009.

[108] M. J. Stone, "Waldenström's macroglobulinemia: hyperviscosity syndrome and cryoglobulinemia," *Clinical Lymphoma and Myeloma*, vol. 9, no. 1, pp. 97–99, 2009.

[109] S. Sugai, R. Pillarisetty, and N. Talal, "Monoclonal macroglobulinemia in nzb/nzw f1 mice," *Journal of Experimental Medicine*, vol. 138, no. 4, pp. 989–1002, 1973.

[110] M. J. Stone and J. E. Fedak, "Studies on monoclonal antibodies: Ii. immune complex (igm-igg) cryoglobulinemia: the mechanism of cryoprecipitation," *The Journal of Immunology*, vol. 113, no. 4, pp. 1377–1385, 1974.

[111] M. J. Stone and V. Pascual, "Pathophysiology of waldenströms macroglobulinemia," *haematologica*, vol. 95, no. 3, pp. 359–367, 2010.

[112] K. C. Anderson, M. Alsina, W. Bensinger, J. S. Biermann, A. D. Cohen, S. Devine, B. Djulbegovic, E. A. Faber, C. Gasparetto, F. Hernandez-Ilizaliturri *et al.*, "Waldenströms macroglobulinemia/lymphoplasmacytic lymphoma, version 2.2013," *Journal of the National Comprehensive Cancer Network*, vol. 10, no. 10, pp. 1211–1219, 2012.

[113] M. A. Gertz, M. Rue, E. Blood, L. S. Kaminer, D. H. Vesole, and P. R. Greipp, "Multicenter phase 2 trial of rituximab for waldenström macroglobulinemia (wm): an eastern cooperative oncology group study (e3a98)," *Leukemia & lymphoma*, vol. 45, no. 10, pp. 2047–2055, 2004.

[114] S. P. Treon, D. B. Agus, B. Link, G. Rodrigues, A. Molina, M. Q. Lacy, D. C. Fisher, C. Emmanouilides, A. I. Richards, B. Clark *et al.*, "Cd20-directed antibody-mediated immunotherapy induces responses and facilitates hematologic recovery in patients with waldenstroms macroglobulinemia," *Journal of Immunotherapy*, vol. 24, no. 3, pp. 272–279, 2001.

[115] M. A. Dimopoulos, C. Zervas, A. Zomas, C. Kiamouris, N. A. Viniou, V. Grigoraki, C. Karkantaris, C. Mitsouli, D. Gika, J. Christakis *et al.*, "Treatment of waldenstroms macroglobulinemia with rituximab," *Journal of Clinical Oncology*, vol. 20, no. 9, pp. 2327–2333, 2002.

[116] S. Treon, C. Emmanouilides, E. Kimby, A. Kelliher, F. Preffer, A. Branagan, K. Anderson, and S. Frankel, "Extended rituximab therapy in waldenström's macroglobulinemia," *Annals of Oncology*, vol. 16, no. 1, pp. 132–138, 2005.

[117] M. A. Dimopoulos, E. Kastritis, R. G. Owen, R. A. Kyle, O. Landgren, E. Morra, X. Leleu, R. García-Sanz, N. Munshi, K. C. Anderson *et al.*, "Treatment recommendations for patients with waldenström macroglobulinemia (wm) and related disorders: Iwwm-7 consensus," *Blood*, vol. 124, no. 9, pp. 1404–1411, 2014.

[118] R. A. Kyle, P. R. Greipp, M. A. Gertz, T. E. Witzig, J. A. Lust, M. Q. Lacy, and T. M. Therneau, "Waldenström's macroglobulinaemia: a prospective study comparing daily with intermittent oral chlorambucil," *British journal of haematology*, vol. 108, no. 4, pp. 737–742, 2000.

[119] V. Leblond, S. Johnson, S. Chevret, A. Copplestone, S. Rule, O. Tournilhac, J. F. Seymour, R. D. Patmore, D. Wright, P. Morel *et al.*, "Results of a randomized trial of chlorambucil versus fludarabine for patients with untreated waldenström macroglobulinemia, marginal zone lymphoma, or lymphoplasmacytic lymphoma," *Journal of Clinical Oncology*, vol. 31, no. 3, pp. 301–307, 2012.

[120] C. Buske, E. Hoster, M. Dreyling, H. Eimermacher, H. Wandt, B. Metzner, R. Fuchs, J. Bittenbring, B. Woermann, K. Hohloch *et al.*, "The addition of rituximab to front-line therapy with chop (r-chop) results in a higher response rate and longer time to treatment failure in patients with lymphoplasmacytic lymphoma: results of a randomized trial of the german low-grade lymphoma study group (glsg)," *Leukemia*, vol. 23, no. 1, pp. 153–159, 2009.

[121] M. A. Dimopoulos, A. Anagnostopoulos, M.-C. Kyrtsonis, K. Zervas, C. Tsatalas, G. Kokkinis, P. Repoussis, A. Symeonidis, S. Delimpasi, E. Katodritou *et al.*, "Primary treatment of waldenstrom macroglobulinemia with dexamethasone, rituximab, and cyclophosphamide," *Journal of Clinical Oncology*, vol. 25, no. 22, pp. 3344–3349, 2007.

[122] S. P. Treon, Z. Hunter, and A. R. Branagan, "Chop plus rituximab therapy in waldenström's macroglobulinemia," *Clinical lymphoma*, vol. 5, no. 4, pp. 273–277, 2005.

[123] B. D. Cheson and M. J. Rummel, "Bendamustine: rebirth of an old drug," *Journal of Clinical Oncology*, vol. 27, no. 9, pp. 1492–1501, 2009.

[124] M. J. Rummel, N. Niederle, G. Maschmeyer, G. A. Banat, U. von Grünhagen, C. Losem, D. Kofahl-Krause, G. Heil, M. Welslau, C. Balser *et al.*, "Bendamustine plus rituximab versus chop plus rituximab as first-line

treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial," *The Lancet*, vol. 381, no. 9873, pp. 1203–1210, 2013.

[125] S. P. Treon, C. Hanzis, C. Tripsas, L. Ioakimidis, C. J. Patterson, R. J. Manning, and P. Sheehy, "Bendamustine therapy in patients with relapsed or refractory waldenström's macroglobulinemia," *Clinical Lymphoma Myeloma and Leukemia*, vol. 11, no. 1, pp. 133–135, 2011.

[126] M. A. Dimopoulos, H. Kantarjian, D. Weber, S. O'Brien, E. Estey, K. Delasalle, E. Rose, F. Cabanillas, M. Keating, and R. Alexanian, "Primary therapy of waldenström's macroglobulinemia with 2-chlorodeoxyadenosine." *Journal of Clinical Oncology*, vol. 12, no. 12, pp. 2694–2698, 1994.

[127] E. S. Liu, C. Burian, W. E. Miller, and A. Saven, "Bolus administration of cladribine in the treatment of waldenström macroglobulinaemia," *British journal of haematology*, vol. 103, no. 3, pp. 690–695, 1998.

[128] A. Hellmann, K. Lewandowski, J. Zaucha, M. Bieniaszewska, K. Hałaburda, and T. Robak, "Effect of a 2-hour infusion of 2-chlorodeoxyadenosine in the treatment of refractory or previously untreated waldenström's macroglobulinemia," *European journal of haematology*, vol. 63, no. 1, pp. 35–41, 1999.

[129] D. Betticher, S.-F. Hsu Schmitz, D. Ratschiller, A. Von Rohr, T. Egger, P. Pugin, M. Stalder, U. Hess, M. Fey, and T. Cerny, "Cladribine (2-cda) given as subcutaneous bolus injections is active in pretreated waldenström's macroglobulinaemia," *British journal of haematology*, vol. 99, no. 2, pp. 358–363, 1997.

[130] M. A. Dimopoulos, S. O'Brien, H. Kantarjian, S. Pierce, K. Delasalle, B. Barlogie, R. Alexanian, and M. J. Keating, "Fludarabine therapy in waldenström's macroglobulinemia," *The American journal of medicine*, vol. 95, no. 1, pp. 49–52, 1993.

[131] J. M. Foran, A. Z. Rohatiner, B. Coiffier, T. Barbui, S. A. Johnson, W. Hiddemann, J. A. Radford, A. J. Norton, S. M. Tollerfield, M. P. Wilson *et al.*,

"Multicenter phase ii study of fludarabine phosphate for patients with newly diagnosed lymphoplasmacytoid lymphoma, waldenstrom's macroglobulinemia, and mantle-cell lymphoma," *Journal of Clinical Oncology*, vol. 17, no. 2, pp. 546–546, 1999.

[132] S. P. Treon, C. J. Patterson, E. Kimby, and M. J. Stone, "Advances in the biology and treatment of waldenström's macroglobulinemia: a report from the 5th international workshop on waldenström's macroglobulinemia, stockholm, sweden," *Clinical Lymphoma and Myeloma*, vol. 9, no. 1, pp. 10–15, 2009.

[133] X. Leleu, J. Soumerai, A. Roccaro, E. Hatjiharissi, Z. R. Hunter, R. Manning, B. T. Ciccarelli, A. Sacco, L. Ioakimidis, S. Adamia *et al.*, "Increased incidence of transformation and myelodysplasia/acute leukemia in patients with waldenstrom macroglobulinemia treated with nucleoside analogs," *J Clin Oncol*, vol. 27, no. 2, pp. 250–255, 2009.

[134] S. P. Treon, Z. R. Hunter, J. Matous, R. M. Joyce, B. Mannion, R. Advani, D. Cook, J. Songer, J. Hill, B. R. Kaden *et al.*, "Multicenter clinical trial of bortezomib in relapsed/refractory waldenstrom's macroglobulinemia: results of wmctg trial 03-248," *Clinical cancer research*, vol. 13, no. 11, pp. 3320–3325, 2007.

[135] C. I. Chen, C. T. Kouroukis, D. White, M. Voralia, E. Stadtmauer, A. K. Stewart, J. J. Wright, J. Powers, W. Walsh, and E. Eisenhauer, "Bortezomib is active in patients with untreated or relapsed waldenstrom's macroglobulinemia: a phase ii study of the national cancer institute of canada clinical trials group," *Journal of clinical oncology*, vol. 25, no. 12, pp. 1570–1575, 2007.

[136] M. A. Dimopoulos, A. Anagnostopoulos, M. C. Kyrtsonis, E. Castritis, A. Bitsaktsis, and G. A. Pangalis, "Treatment of relapsed or refractory waldenstrom's macroglobulinemia with bortezomib," *Haematologica*, vol. 90, no. 12, pp. 1655–1658, 2005.

[137] S. P. Treon, L. Ioakimidis, J. D. Soumerai, C. J. Patterson, P. Sheehy, M. Nelson, M. Willen, J. Matous, J. Mattern *et al.*, "Primary therapy of waldenström macroglobulinemia with bortezomib, dexamethasone, and rituximab: Wmctg clinical trial 05-180," *Journal of Clinical Oncology*, vol. 27, no. 23, pp. 3830–3834, 2009.

[138] I. M. Ghobrial, W. Xie, S. Padmanabhan, A. Badros, M. Rourke, R. Leduc, S. Chuma, J. Kunsman, D. Warren, T. Poon *et al.*, "Phase ii trial of weekly bortezomib in combination with rituximab in untreated patients with waldenström macroglobulinemia," *American journal of hematology*, vol. 85, no. 9, pp. 670–674, 2010.

[139] S. P. Treon, C. K. Tripsas, K. Meid, S. Kanan, P. Sheehy, S. Chuma, L. Xu, Y. Cao, G. Yang, X. Liu *et al.*, "Carfilzomib, rituximab and dexamethasone (card) is active and offers a neuropathy-sparing approach for proteasome-inhibitor based therapy in waldenstrom's macroglobulinemia," *Blood*, vol. 85, no. 10, pp. 697–712, 2014.

[140] M. A. Dimopoulos, A. Zomas, N. A. Viniou, V. Grigoraki, E. Galani, C. Matsouka, O. Economou, N. Anagnostopoulos, and P. Panayiotidis, "Treatment of waldenstroms macroglobulinemia with thalidomide," *Journal of Clinical Oncology*, vol. 19, no. 16, pp. 3596–3601, 2001.

[141] M. A. Dimopoulos and A. Anagnostopoulos, "Treatment of waldenstroms macroglobulinemia," *Current treatment options in oncology*, vol. 8, no. 2, pp. 144–153, 2007.

[142] S. Treon, C. Tripsas, D. Warren, C. Patterson, P. Sheehy, G. Yang, and Z. Hunter, "phase i study of pomalidomide, dexamethasone and rituximab (pdr) in patients with relapsed or refractory waldenstrom's macroglobulinemia (wm): 536.: 536," *Hematological Oncology*, vol. 31, no. 1, pp. 267–271, 2013.

[143] J. P. Laubach, C. S. Mitsiades, A. M. Roccaro, I. M. Ghobrial, K. C. Anderson, and P. G. Richardson, "Clinical challenges associated with bortezomib therapy in multiple myeloma and waldenströms macroglobulinemia," *Leukemia & lymphoma*, vol. 50, no. 5, pp. 694–702, 2009.

[144] S. A. Bingham, "Biomarkers in nutritional epidemiology," *Public health nutrition*, vol. 5, no. 6a, pp. 821–827, 2002.

[145] S. Naylor, "Overview of biomarkers in disease, drug discovery and development," *Drug Discovery*, vol. 47, no. 1, pp. 21–27, 2005.

[146] F. P. Perera and I. B. Weinstein, "Molecular epidemiology: recent advances and future directions," *Carcinogenesis*, vol. 21, no. 3, pp. 517–524, 2000.

[147] J. N. Weinstein, E. A. Collisson, G. B. Mills, K. R. M. Shaw, B. A. Ozenberger, K. Ellrott, I. Shmulevich, C. Sander, J. M. Stuart, C. G. A. R. Network *et al.*, "The cancer genome atlas pan-cancer analysis project," *Nature genetics*, vol. 45, no. 10, pp. 1113–1119, 2013.

[148] M. Hofree, J. P. Shen, H. Carter, A. Gross, and T. Ideker, "Network-based stratification of tumor mutations," *Nature methods*, vol. 10, no. 11, pp. 1108–1114, 2013.

[149] P. A. Konstantinopoulos, D. Spentzos, and S. A. Cannistra, "Gene-expression profiling in epithelial ovarian cancer," *Nature Reviews Clinical Oncology*, vol. 5, no. 10, pp. 577–581, 2008.

[150] P. A. Konstantinopoulos, D. Spentzos, B. Y. Karlan, T. Taniguchi, E. Fountzilas, N. Francoeur, D. A. Levine, and S. A. Cannistra, "Gene expression profile of brcaness correlates with responsiveness to chemotherapy in patients with epithelial ovarian cancer," *Journal of clinical oncology*, vol. 28, no. 22, pp. 3555–3559, 2010.

[151] J. S. Reis-Filho and L. Pusztai, "Gene expression profiling in breast cancer: classification, prognostication, and prediction," *The Lancet*, vol. 378, no. 9805, pp. 1812–1823, 2011.

[152] F. J. Esteva, A. A. Sahin, M. Cristofanilli, K. Coombes, S.-J. Lee, J. Baker, M. Cronin, M. Walker, D. Watson, S. Shak *et al.*, "Prognostic role of a multigene reverse transcriptase-pcr assay in patients with node-negative breast cancer not receiving adjuvant systemic therapy," *Clinical cancer research*, vol. 11, no. 9, pp. 3315–3319, 2005.

[153] R. Mayeux, "Biomarkers: potential uses and limitations," *NeuroRx*, vol. 1, no. 2, pp. 182–188, 2004.

[154] C. Holohan, S. Van Schaeybroeck, D. B. Longley, and P. G. Johnston, "Cancer drug resistance: an evolving paradigm," *Nature Reviews Cancer*, vol. 13, no. 10, pp. 714–723, 2013.

[155] J. Li, X. Wang, C. Wang, B. Chen, Y. Dai, R. Zhang, M. Song, G. Lv, and D. Fu, "The enhancement effect of gold nanoparticles in drug delivery and as biomarkers of drug-resistant cancer cells," *ChemMedChem: Chemistry Enabling Drug Discovery*, vol. 2, no. 3, pp. 374–378, 2007.

[156] C. Lathia, D. Amakye, W. Dai, C. Girman, S. Madani, J. Mayne, P. MacCarthy, P. Pertel, L. Seman, A. Stoch *et al.*, "The value, qualification, and regulatory use of surrogate end points in drug development," *Clinical Pharmacology & Therapeutics*, vol. 86, no. 1, pp. 32–43, 2009.

[157] E. Saccenti, H. C. Hoefsloot, A. K. Smilde, J. A. Westerhuis, and M. M. Hendriks, "Reflections on univariate and multivariate analysis of metabolomics data," *Metabolomics*, vol. 10, no. 3, pp. 361–374, 2014.

[158] I. Guyon, J. Weston, S. Barnhill, and V. Vapnik, "Gene selection for cancer classification using support vector machines," *Machine learning*, vol. 46, no. 1-3, pp. 389–422, 2002.

[159] L. Breiman, "Random forests," *Machine learning*, vol. 45, no. 1, pp. 5–32, 2001.

[160] D. E. Goldberg and J. H. Holland, "Genetic algorithms and machine learning," *Machine learning*, vol. 3, no. 2, pp. 95–99, 1988.

[161] E. Tapia, P. Bulacio, and L. Angelone, "Sparse and stable gene selection with consensus svm-rfe," *Pattern recognition letters*, vol. 33, no. 2, pp. 164–172, 2012.

[162] F. Long, J.-H. Su, B. Liang, L.-L. Su, and S.-J. Jiang, "Identification of gene biomarkers for distinguishing small-cell lung cancer from non-small-cell lung cancer using a network-based approach," *BioMed research international*, vol. 2015, no. 1, pp. 13–19, 2015.

[163] L. Feng, R. Tong, X. Liu, K. Zhang, G. Wang, L. Zhang, N. An, and S. Cheng, "A network-based method for identifying prognostic gene modules in lung squamous carcinoma," *Oncotarget*, vol. 7, no. 14, pp. 18 006–188 017, 2016.

[164] X. Huang, X. Lin, J. Zeng, L. Wang, P. Yin, L. Zhou, C. Hu, and W. Yao, "A computational method of defining potential biomarkers based on differential sub-networks," *Scientific Reports*, vol. 7, no. 1, pp. 14 339–14 348, 2017.

[165] X. Zhang, H. Yang, B. Gong, C. Jiang, and L. Yang, "Combined gene expression and protein interaction analysis of dynamic modularity in glioma prognosis," *Journal of neuro-oncology*, vol. 107, no. 2, pp. 281–288, 2012.

[166] J. Krumsiek, K. Suhre, T. Illig, J. Adamski, and F. J. Theis, "Gaussian graphical modeling reconstructs pathway reactions from high-throughput metabolomics data," *BMC systems biology*, vol. 5, no. 1, pp. 21–27, 2011.

[167] M. Netzer, K. G. Kugler, L. A. Müller, K. M. Weinberger, A. Graber, C. Baumgartner, and M. Dehmer, "A network-based feature selection approach to identify metabolic signatures in disease," *Journal of theoretical biology*, vol. 310, no. 1, pp. 216–222, 2012.

[168] M. Netzer, K. M. Weinberger, M. Handler, M. Seger, X. Fang, K. G. Kugler, A. Graber, and C. Baumgartner, "Profiling the human response to physical exercise: a computational strategy for the identification and kinetic analysis of metabolic biomarkers," *Journal of clinical bioinformatics*, vol. 1, no. 1, pp. 34–43, 2011.

[169] H.-Y. Chuang, E. Lee, Y.-T. Liu, D. Lee, and T. Ideker, "Network-based classification of breast cancer metastasis," *Molecular systems biology*, vol. 3, no. 1, pp. 140–143, 2007.

[170] T. Aittokallio and B. Schwikowski, "Graph-based methods for analysing networks in cell biology," *Briefings in bioinformatics*, vol. 7, no. 3, pp. 243–255, 2006.

[171] J. G. Mezey, J. M. Cheverud, and G. P. Wagner, "Is the genotype-phenotype map modular?: a statistical approach using mouse quantitative trait loci data," *Genetics*, vol. 156, no. 1, pp. 305–311, 2000.

[172] H. Ma, E. E. Schadt, L. M. Kaplan, and H. Zhao, "Cosine: Condition-specific sub-network identification using a global optimization method," *Bioinformatics*, vol. 27, no. 9, pp. 1290–1298, 2011.

[173] A. Palaniappan, K. Ramar, and S. Ramalingam, "Computational identification of novel stage-specific biomarkers in colorectal cancer progression," *PloS one*, vol. 11, no. 5, pp. e0 156 665–e0 156 669, 2016.

[174] G. Zhu, X.-M. Zhao, and J. Wu, "A survey on biomarker identification based on molecular networks," *Quantitative Biology*, vol. 4, no. 4, pp. 310–319, 2016.

[175] J. A. Santiago and J. A. Potashkin, "Network-based metaanalysis identifies hnf4a and ptbp1 as longitudinally dynamic biomarkers for parkinsons disease," *Proceedings of the National Academy of Sciences*, vol. 112, no. 7, pp. 2257–2262, 2015.

[176] Y. Li, J. Xu, H. Chen, J. Bai, S. Li, Z. Zhao, T. Shao, T. Jiang, H. Ren, C. Kang *et al.*, "Comprehensive analysis of the functional microrna–mrna regulatory network identifies mirna signatures associated with glioma malignant progression," *Nucleic acids research*, vol. 41, no. 22, pp. e203–e203, 2013.

[177] A. Özgür, T. Vu, G. Erkan, and D. R. Radev, "Identifying gene-disease associations using centrality on a literature mined gene-interaction network," *Bioinformatics*, vol. 24, no. 13, pp. i277–i285, 2008.

[178] A. De Gramont, S. Watson, L. M. Ellis, J. Rodón, J. Tabernero, A. De Gramont, and S. R. Hamilton, "Pragmatic issues in biomarker evaluation for targeted therapies in cancer," *Nature reviews Clinical oncology*, vol. 12, no. 4, pp. 197–203, 2015.

[179] G. Qin and X.-M. Zhao, "A survey on computational approaches to identifying disease biomarkers based on molecular networks," *Journal of theoretical biology*, vol. 362, no. 1, pp. 9–16, 2014.

[180] R. Liu, X. Wang, K. Aihara, and L. Chen, "Early diagnosis of complex diseases by molecular biomarkers, network biomarkers, and dynamical network biomarkers," *Medicinal research reviews*, vol. 34, no. 3, pp. 455–478, 2014.

[181] B. Munos, "Lessons from 60 years of pharmaceutical innovation," *Nature reviews Drug discovery*, vol. 8, no. 12, pp. 959–963, 2009.

[182] P. J. Williams and E. I. Ette, "Pharmacometrics: impacting drug development and pharmacotherapy," *Pharmacometrics: The science of quantitative pharmacology*, vol. 16, no. 1, pp. 1–21, 2007.

[183] M. Kanehisa and S. Goto, "Kegg: kyoto encyclopedia of genes and genomes," *Nucleic acids research*, vol. 28, no. 1, pp. 27–30, 2000.

[184] M. Ashburner, C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig *et al.*, "Gene ontology: tool for the unification of biology," *Nature genetics*, vol. 25, no. 1, pp. 25–32, 2000.

[185] A.-L. Barabási, N. Gulbahce, and J. Loscalzo, "Network medicine: a network-based approach to human disease," *Nature reviews genetics*, vol. 12, no. 1, pp. 56–62, 2011.

[186] A. Lachmann and A. Ma'ayan, "Lists2networks: integrated analysis of gene/protein lists," *BMC bioinformatics*, vol. 11, no. 1, pp. 87–64, 2010.

[187] G. Su, J. H. Morris, B. Demchak, and G. D. Bader, "Biological network exploration with cytoscape 3," *Current protocols in bioinformatics*, vol. 47, no. 1, pp. 8–13, 2014.

[188] Z. Hu, J. Mellor, J. Wu, and C. DeLisi, "Visant: an online visualization and analysis tool for biological interaction data," *BMC bioinformatics*, vol. 5, no. 1, pp. 17–23, 2004.

[189] M. J. Cowley, M. Pinese, K. S. Kassahn, N. Waddell, J. V. Pearson, S. M. Grimmond, A. V. Biankin, S. Hautaniemi, and J. Wu, "Pina v2. 0: mining interactome modules," *Nucleic acids research*, vol. 40, no. D1, pp. D862–D865, 2011.

[190] Y. Ding, M. Chen, Z. Liu, D. Ding, Y. Ye, M. Zhang, R. Kelly, L. Guo, Z. Su, S. C. Harris *et al.*, "atbionet–an integrated network analysis tool for genomics and biomarker discovery," *BMC genomics*, vol. 13, no. 1, pp. 325–327, 2012.

[191] M. Bastian, S. Heymann, M. Jacomy *et al.*, "Gephi: an open source software for exploring and manipulating networks." *Icwsm*, vol. 8, no. 2009, pp. 361–362, 2009.

[192] M. D. Butti, H. Chanfreau, D. Martinez, D. García, E. Lacunza, and M. C. Abba, "Bioplat: a software for human cancer biomarker discovery," *Bioinformatics*, vol. 30, no. 12, pp. 1782–1784, 2014.

[193] S. L. Shafer and J. R. Varvel, "Pharmacokinetics, pharmacodynamics, and rational opioid selection," *Anesthesiology*, vol. 74, no. 1, pp. 53–63, 1991.

[194] E. Nelson, "Kinetics of drug absorption, distribution, metabolism, and excretion," *Journal of pharmaceutical sciences*, vol. 50, no. 3, pp. 181–192, 1961.

[195] T. Teorell, "Kinetics of distribution of substances administered to the body, i: the extravascular modes of administration," *Archives internationales de pharmacodynamie et de therapie*, vol. 57, no. 1, pp. 205–225, 1937.

[196] E. J. Ariëns *et al.*, "Affinity and intrinsic activity in the theory of competitive inhibition. 1. problems and theory." *Archives internationales de pharmacodynamie et de thérapie*, vol. 99, no. 1, pp. 32–49, 1954.

[197] R. Stephenson, "A modification of receptor theory," *British journal of pharmacology and chemotherapy*, vol. 11, no. 4, pp. 379–393, 1956.

[198] N. H. Holford and L. B. Sheiner, "Kinetics of pharmacologic response," *Pharmacology & therapeutics*, vol. 16, no. 2, pp. 143–166, 1982.

[199] E. I. Nielsen and L. E. Friberg, "Pharmacokinetic-pharmacodynamic modeling of antibacterial drugs," *Pharmacological reviews*, vol. 65, no. 3, pp. 1053–1090, 2013.

[200] D. E. Mager, E. Wyska, and W. J. Jusko, "Diversity of mechanism-based pharmacodynamic models," *Drug Metabolism and Disposition*, vol. 31, no. 5, pp. 510–518, 2003.

[201] J. Aldrich *et al.*, "Ra fisher and the making of maximum likelihood 1912-1922," *Statistical science*, vol. 12, no. 3, pp. 162–176, 1997.

[202] F. Daryaee, Z. Zhang, K. R. Gogarty, Y. Li, J. Merino, S. L. Fisher, and P. J. Tonge, "A quantitative mechanistic pk/pd model directly connects btk target engagement and in vivo efficacy," *Chemical science*, vol. 8, no. 5, pp. 3434–3443, 2017.

[203] H. Lu and P. J. Tonge, "Drug–target residence time: critical information for lead optimization," *Current opinion in chemical biology*, vol. 14, no. 4, pp. 467–474, 2010.

[204] R. A. Copeland, D. L. Pompliano, and T. D. Meek, "Drug–target residence time and its implications for lead optimization," *Nature reviews Drug discovery*, vol. 5, no. 9, pp. 730–739, 2006.

[205] D. C. Swinney, "Applications of binding kinetics to drug discovery," *Pharmaceutical Medicine*, vol. 22, no. 1, pp. 23–34, 2008.

[206] R. A. Copeland, "The drug–target residence time model: a 10-year retrospective," *Nature Reviews Drug Discovery*, vol. 15, no. 2, pp. 87–96, 2016.

[207] D. C. Swinney, "Biochemical mechanisms of new molecular entities (nmes) approved by united states fda during 2001-2004: mechanisms leading to optimal efficacy and safety," *Current topics in medicinal chemistry*, vol. 6, no. 5, pp. 461–478, 2006.

[208] P. J. Tummino and R. A. Copeland, "Residence time of receptor- ligand complexes and its effect on biological function," *Biochemistry*, vol. 47, no. 20, pp. 5481–5492, 2008.

[209] D. C. Swinney, "The role of binding kinetics in therapeutically useful drug action." *Current opinion in drug discovery & development*, vol. 12, no. 1, pp. 31–39, 2009.

[210] R. Zhang and F. Monsma, "The importance of drug-target residence time." *Current opinion in drug discovery & development*, vol. 12, no. 4, pp. 488–496, 2009.

[211] R. A. Copeland, "Drug-target interactions: Stay tuned," *Nature chemical biology*, vol. 11, no. 7, p. 451, 2015.

[212] P. J. Williams and E. I. Ette, "Pharmacometrics: impacting drug development and pharmacotherapy," *Pharmacometrics: The science of quantitative pharmacology*, vol. 16, no. 1, pp. 1–21, 2007.

[213] N. H. Holford, H. C. Kimko, J. P. Monteleone, and C. C. Peck, "Simulation of clinical trials," *Annual review of pharmacology and toxicology*, vol. 40, no. 1, pp. 209–234, 2000.

[214] S. B. Duffull, F. Mentré, and L. Aarons, "Optimal design of a population pharmacodynamic experiment for ivabradine," *Pharmaceutical research*, vol. 18, no. 1, pp. 83–89, 2001.

[215] J. Y. Chien, S. Friedrich, M. A. Heathman, D. P. de Alwis, and V. Sinha, "Pharmacokinetics/pharmacodynamics and the stages of drug development: role of modeling and simulation," *The AAPS journal*, vol. 7, no. 3, pp. E544–E559, 2005.

[216] C. for Medicinal Products for Human Use *et al.*, "Guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products," *London, European Medicines Agency*, vol. 2, no. 3, pp. 1–12, 2007.

[217] J. W. Mandema, D. Hermann, W. Wang, T. Sheiner, M. Milad, R. Bakker-Arkema, and D. Hartman, "Model-based development of gemcabene, a new lipid-altering agent," *The AAPS journal*, vol. 7, no. 3, pp. E513–E522, 2005.

[218] R. Lalonde, K. Kowalski, M. Hutmacher, W. Ewy, D. Nichols, P. Milligan, B. Corrigan, P. Lockwood, S. Marshall, L. Benincosa *et al.*, "Model-based drug development," *Clinical Pharmacology & Therapeutics*, vol. 82, no. 1, pp. 21–32, 2007.

[219] V. A. Bhattaram, B. P. Booth, R. P. Ramchandani, B. N. Beasley, Y. Wang, V. Tandon, J. Z. Duan, R. K. Baweja, P. J. Marroum, R. S. Uppoor *et al.*, "Impact of pharmacometrics on drug approval and labeling decisions: a survey of 42 new drug applications," *The AAPS journal*, vol. 7, no. 3, pp. E503–E512, 2005.

[220] J. Cross, H. Lee, A. Westelinck, J. Nelson, C. Grudzinskas, and C. Peck, "Postmarketing drug dosage changes of 499 fda-approved new molecular entities, 1980–1999," *Pharmacoepidemiology and drug safety*, vol. 11, no. 6, pp. 439–446, 2002.

[221] D. R. Stanski, M. Rowland, and L. B. Sheiner, "Getting the dose right: report from the tenth european federation of pharmaceutical sciences (eufeps) conference on optimizing drug development," *Journal of pharmacokinetics and pharmacodynamics*, vol. 32, no. 2, pp. 199–211, 2005.

[222] M. Fernandez, "{published data only} mar fernandez de gatta md, victoria calvo m, ardanuy r, dominguez-gil a, lanao jm, moreno sr. evaluation of population pharmacokinetic models for amikacin dosage individualization in critically ill patients," *Journal of Pharmacy & Pharmacology*, vol. 61, no. 6, pp. 759–66, 2009.

[223] J. E. Wallin, L. E. Friberg, and M. O. Karlsson, "A tool for neutrophil guided dose adaptation in chemotherapy," *Computer methods and programs in biomedicine*, vol. 93, no. 3, pp. 283–291, 2009.

[224] G. Downing, "Biomarkers definitions working group. biomarkers and surrogate endpoints," *Clinical Pharmacology & Therapeutics*, vol. 69, no. 1, pp. 89–95, 2001.

[225] J. Sun, X. Xiao, R.-J. Huang, T. Yang, Y. Chen, X. Fang, T. Huang, Y.-F. Zhou, and Y.-H. Liu, "In vitro dynamic pharmacokinetic/pharamcodynamic (pk/pd) study and co pd of marbofloxacin against haemophilus parasuis," *BMC veterinary research*, vol. 11, no. 1, p. 293, 2015.

[226] D. Houcque *et al.*, "Introduction to matlab for engineering students," *Northwestern University*, vol. 3, no. 1, pp. 1–64, 2005.

[227] C. Dumont, G. Lestini, H. Le Nagard, F. Mentré, E. Comets, T. T. Nguyen *et al.*, "Pfim 4.0, an extended r program for design evaluation and optimization in nonlinear mixed-effect models," *Computer methods and programs in biomedicine*, vol. 156, pp. 217–229, 2018.

[228] M. Li, S. Wang, G. Xie, X. Ma, T. Chen, and W. Jia, "polypk: an r package for pharmacokinetic analysis of multi-component drugs using a metabolomics approach," *Bioinformatics*, vol. 34, no. 10, pp. 1792–1794, 2017.

[229] I. Hosseini, A. Gajjala, D. B. Yadav, S. Sukumaran, S. Ramanujan, R. Paxson, and K. Gadkar, "gpkpdsim: a simbiology®-based gui application for pkpd modeling in drug development," *Journal of pharmacokinetics and pharmacodynamics*, vol. 45, no. 2, pp. 259–275, 2018.

[230] B. Reisfeld, J. H. Ivy, M. A. Lyons, J. M. Wright, J. L. Rogers, and A. N. Mayeno, "Dosesim: a tool for pharmacokinetic/pharmacodynamic analysis and dose reconstruction," *Bioinformatics*, vol. 29, no. 3, pp. 400–401, 2012.

[231] T. Schmitt, C. Ogris, and E. L. Sonnhammer, "Funcoup 3.0: database of genome-wide functional coupling networks," *Nucleic acids research*, vol. 42, no. D1, pp. D380–D388, 2013.

[232] J. Montojo, K. Zuberi, H. Rodriguez, F. Kazi, G. Wright, S. L. Donaldson, Q. Morris, and G. D. Bader, "Genemania cytoscape plugin: fast gene function predictions on the desktop," *Bioinformatics*, vol. 26, no. 22, pp. 2927–2928, 2010.

[233] M. Bastian, S. Heymann, M. Jacomy *et al.*, "Gephi: an open source software for exploring and manipulating networks." *Icwsm*, vol. 8, no. 2009, pp. 361–362, 2009.

[234] M. E. Newman, "A measure of betweenness centrality based on random walks," *Social networks*, vol. 27, no. 1, pp. 39–54, 2005.

[235] P. Boldi and S. Vigna, "Axioms for centrality," *Internet Mathematics*, vol. 10, no. 3-4, pp. 222–262, 2014.

[236] J. M. Fletcher and T. Wennekers, "From structure to activity: Using centrality measures to predict neuronal activity," *International journal of neural systems*, vol. 28, no. 02, pp. 1 750 013–1 750 015, 2018.

[237] E. Glaab, A. Baudot, N. Krasnogor, R. Schneider, and A. Valencia, "Enrichnet: network-based gene set enrichment analysis," *Bioinformatics*, vol. 28, no. 18, pp. i451–i457, 2012.

[238] M. Simeoni, P. Magni, C. Cammia, G. De Nicolao, V. Croci, E. Pesenti, M. Germani, I. Poggesi, and M. Rocchetti, "Predictive pharmacokinetic-pharmacodynamic modeling of tumor growth kinetics in xenograft models after administration of anticancer agents," *Cancer research*, vol. 64, no. 3, pp. 1094–1101, 2004.

[239] A. Munir, S. Hussain, and E. Dilshad, "Silver nanoparticles conjugated with neurotrophin 3 upregulate myelin gene transcription pathway," *Journal of theoretical biology*, vol. 459, pp. 111–118, 2018.

[240] J.-y. Li, Y.-p. Ren, Y. Yuan, S.-m. Ji, S.-p. Zhou, L.-j. Wang, Z.-z. Mou, L. Li, W. Lu, and T.-y. Zhou, "Preclinical pk/pd model for combined administration of erlotinib and sunitinib in the treatment of a549 human nsclc xenograft mice," *Acta Pharmacologica Sinica*, vol. 37, no. 7, pp. 930–937, 2016.

[241] D. Leveque, M. C. M. Carvalho, and F. Maloisel, "Clinical pharmacokinetics of bortezomib," *In vivo*, vol. 21, no. 2, pp. 273–278, 2007.

[242] Z. Wang, J. Yang, C. Kirk, Y. Fang, M. Alsina, A. Badros, K. Papadopoulos, A. Wong, T. Woo, D. Bomba *et al.*, "Clinical pharmacokinetics, metabolism, and drug-drug interaction of carfilzomib," *Drug Metabolism and Disposition*, vol. 41, no. 1, pp. 230–237, 2013.

[243] J. R. Infante, D. S. Mendelson, H. A. Burris, J. C. Bendell, A. W. Tolcher, M. S. Gordon, H. H. Gillenwater, S. Arastu-Kapur, H. L. Wong, and K. P. Papadopoulos, "A first-in-human dose-escalation study of the oral proteasome inhibitor oprozomib in patients with advanced solid tumors," *Investigational new drugs*, vol. 34, no. 2, pp. 216–224, 2016.

[244] S. Rozman, I. Grabnar, S. Novaković, A. Mrhar, and B. Jezeršek Novaković, "Population pharmacokinetics of rituximab in patients with diffuse large b-cell lymphoma and association with clinical outcome," *British journal of clinical pharmacology*, vol. 83, no. 8, pp. 1782–1790, 2017.

[245] N. Tsioptsias, A. A. Tako, and S. Robinson, "Model validation and testing in simulation: a literature review," *Clinical Oncology*, vol. 12, no. 1, pp. 35–48, 2016.

[246] S. Robinson, "Modes of simulation practice: approaches to business and military simulation," *Simulation Modelling Practice and Theory*, vol. 10, no. 8, pp. 513–523, 2002.

[247] D. Leveque, M. C. M. Carvalho, and F. Maloisel, "Clinical pharmacokinetics of bortezomib," *In vivo*, vol. 21, no. 2, pp. 273–278, 2007.

[248] Z. Wang, J. Yang, C. Kirk, Y. Fang, M. Alsina, A. Badros, K. Papadopoulos, A. Wong, T. Woo, D. Bomba *et al.*, "Clinical pharmacokinetics, metabolism, and drug-drug interaction of carfilzomib," *Drug Metabolism and Disposition*, vol. 41, no. 1, pp. 230–237, 2013.

[249] J. R. Infante, D. S. Mendelson, H. A. Burris, J. C. Bendell, A. W. Tolcher, M. S. Gordon, H. H. Gillenwater, S. Arastu-Kapur, H. L. Wong, and K. P. Papadopoulos, "A first-in-human dose-escalation study of the oral proteasome inhibitor oprozomib in patients with advanced solid tumors," *Investigational new drugs*, vol. 34, no. 2, pp. 216–224, 2016.

[250] S. Rozman, I. Grabnar, S. Novaković, A. Mrhar, and B. Jezeršek Novaković, "Population pharmacokinetics of rituximab in patients with diffuse large b-cell lymphoma and association with clinical outcome," *British journal of clinical pharmacology*, vol. 83, no. 8, pp. 1782–1790, 2017.

[251] N. Gupta, M. J. Hanley, K. Venkatakrishnan, R. Perez, R. E. Norris, J. Nemunaitis, H. Yang, M. G. Qian, G. Falchook, R. Labotka *et al.*, "Pharmacokinetics of ixazomib, an oral proteasome inhibitor, in solid tumour patients with moderate or severe hepatic impairment," *British journal of clinical pharmacology*, vol. 82, no. 3, pp. 728–738, 2016.

[252] L. B. Sheiner, D. R. Stanski, S. Vozeh, R. D. Miller, and J. Ham, "Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine," *Clinical Pharmacology & Therapeutics*, vol. 25, no. 3, pp. 358–371, 1979.

[253] D. E. Mager, E. Wyska, and W. J. Jusko, "Diversity of mechanism-based pharmacodynamic models," *Drug Metabolism and Disposition*, vol. 31, no. 5, pp. 510–518, 2003.

[254] N. Rapin, O. Lund, M. Bernaschi, and F. Castiglione, "Computational immunology meets bioinformatics: the use of prediction tools for molecular

binding in the simulation of the immune system," *PLoS One*, vol. 5, no. 4, pp. e9862–e9863, 2010.

[255] T. Sjöblom, S. Jones, L. D. Wood, D. W. Parsons, J. Lin, T. D. Barber, D. Mandelker, R. J. Leary, J. Ptak, N. Silliman *et al.*, "The consensus coding sequences of human breast and colorectal cancers," *science*, vol. 314, no. 5797, pp. 268–274, 2006.

[256] B. Zhang and S. Horvath, "A general framework for weighted gene co-expression network analysis," *Statistical applications in genetics and molecular biology*, vol. 4, no. 1, pp. 123–129, 2005.

[257] H. Jeong, S. P. Mason, A.-L. Barabási, and Z. N. Oltvai, "Lethality and centrality in protein networks," *Nature*, vol. 411, no. 6833, p. 41, 2001.

[258] R. Albert and A.-L. Barabási, "Statistical mechanics of complex networks," *Reviews of modern physics*, vol. 74, no. 1, p. 47, 2002.

[259] D. Koschützki, H. Schwöbbermeyer, and F. Schreiber, "Ranking of network elements based on functional substructures," *Journal of theoretical biology*, vol. 248, no. 3, pp. 471–479, 2007.

[260] D. Hanahan and R. A. Weinberg, "Hallmarks of cancer: the next generation," *cell*, vol. 144, no. 5, pp. 646–674, 2011.

[261] M. A. Harris, J. I. Deegan, A. Ireland, J. Lomax, M. Ashburner, S. Tweedie, S. Carbon, S. Lewis, C. Mungall, J. Day-Richter *et al.*, "The gene ontology project in 2008," *Nucleic acids research*, vol. 36, no. SUPPL. 1, pp. 1–34, 2008.

[262] M. Sierra, I. Martínez-Rodríguez, P. Sánchez-Juan, I. González-Aramburu, M. Jiménez-Alonso, A. Sánchez-Rodríguez, J. Berciano, I. Banzo, and J. Infante, "Prospective clinical and dat-spect imaging in premotor lrrk2 g2019s-associated parkinson disease," *Neurology*, vol. 23, no. 1, pp. 10–1212, 2017.

[263] T. A. Brentnall, "Arousal of cancer-associated stromal fibroblasts: palladin-activated fibroblasts promote tumor invasion," *Cell adhesion & migration*, vol. 6, no. 6, pp. 488–494, 2012.

[264] D. Rubin, A. Schneider-Muntau, M. Klapper, I. Nitz, U. Helwig, U. R. Fölsch, J. Schrezenmeir, and F. Döring, "Functional analysis of promoter variants in the microsomal triglyceride transfer protein (mttp) gene," *Human mutation*, vol. 29, no. 1, pp. 123–129, 2008.

[265] M. Balestri, R. Calati, A. Serretti, A. M. Hartmann, B. Konte, M. Friedl, I. Giegling, and D. Rujescu, "Maoa and maob polymorphisms and personality traits in suicide attempters and healthy controls: a preliminary study," *Psychiatry research*, vol. 249, no. 1, pp. 212–217, 2017.

[266] Q. Du, X. Guo, X. Zhang, W. Zhou, Z. Liu, J. Wang, T. Zhang, Z. Mao, J. Luo, T. Jin *et al.*, "Synj2 variant rs9365723 is associated with colorectal cancer risk in chinese han population," *The International journal of biological markers*, vol. 31, no. 2, pp. 138–143, 2016.

[267] J. Li, Y. Feng, M. S. Sung, T. H. Lee, and S. W. Park, "Association of interleukin-1 gene clusters polymorphisms with primary open-angle glaucoma: a meta-analysis," *BMC ophthalmology*, vol. 17, no. 1, pp. 218–223, 2017.

[268] Q. Gao, X. Chen, H. Duan, Z. Wang, J. Feng, D. Yang, L. Song, N. Zhou, and X. Yan, "Fxyd6: a novel therapeutic target toward hepatocellular carcinoma," *Protein & cell*, vol. 5, no. 7, pp. 532–543, 2014.

[269] M. Çeliktaş, I. Tanaka, S. Chandra Tripathi, J. F. Fahrmann, C. Aguilar-Bonavides, P. Villalobos, O. Delgado, D. Dhillon, J. B. Dennison, E. J. Ostrin *et al.*, "Role of cps1 in cell growth, metabolism, and prognosis in lkb1-inactivated lung adenocarcinoma," *JNCI: Journal of the National Cancer Institute*, vol. 109, no. 3, pp. 1–9, 2017.

[270] R. D. Huff, A. C. Hsu, K. S. Nichol, B. Jones, D. A. Knight, P. A. Wark, P. M. Hansbro, and J. A. Hirota, "Regulation of xanthine dehydrogensase

gene expression and uric acid production in human airway epithelial cells," *PloS one*, vol. 12, no. 9, p. e0184260, 2017.

[271] R. C. Baraas, L. A. Hagen, E. W. Dees, and M. Neitz, "Substitution of isoleucine for threonine at position 190 of s-opsin causes s-cone-function abnormalities," *Vision research*, vol. 73, no. 4, pp. 1–9, 2012.

[272] W. C. Skarnes, B. Rosen, A. P. West, M. Koutsourakis, W. Bushell, V. Iyer, A. O. Mujica, M. Thomas, J. Harrow, T. Cox *et al.*, "A conditional knockout resource for the genome-wide study of mouse gene function," *Nature*, vol. 474, no. 7351, p. 337, 2011.

[273] Z.-X. Xu, Q. Zhang, G.-L. Ma, C.-H. Chen, Y.-M. He, L.-H. Xu, Y. Zhang, G.-R. Zhou, Z.-H. Li, H.-J. Yang *et al.*, "Influence of aluminium and egcg on fibrillation and aggregation of human islet amyloid polypeptide," *Journal of diabetes research*, vol. 2016, no. 1, pp. 1–29, 2016.

[274] H. T. Ngo, A. K. Azab, M. Farag, X. Jia, M. M. Melhem, J. Runnels, A. M. Roccaro, F. Azab, A. Sacco, X. Leleu *et al.*, "Src tyrosine kinase regulates adhesion and chemotaxis in waldenstrom macroglobulinemia," *Clinical Cancer Research*, vol. 43, no. 1, pp. 1078–1092, 2009.

[275] S. Janz, "Waldenström macroglobulinemia: clinical and immunological aspects, natural history, cell of origin, and emerging mouse models," *ISRN hematology*, vol. 2013, no. 1, pp. 123–134, 2013.

[276] W. J. Chng, R. F. Schop, T. Price-Troska, I. Ghobrial, N. Kay, D. F. Jelinek, M. A. Gertz, A. Dispenzieri, M. Lacy, R. A. Kyle *et al.*, "Gene-expression profiling of waldenström macroglobulinemia reveals a phenotype more similar to chronic lymphocytic leukemia than multiple myeloma," *Blood*, vol. 108, no. 8, pp. 2755–2763, 2006.

[277] Y. Sidi, P. Boer, I. Pick, J. Pinkhas, and O. Sperling, "Activity of adenosine deaminase and of purine nucleoside phosphorylase in peripheral lymphocytes from patients with acquired immunological disorders." *Biomedicine/[publiee pour l'AAICIG]*, vol. 33, no. 2, pp. 39–41, 1980.

[278] D. M. Jensen, M. Papadakis, and J. Payne, "Chronic liver disease manifesting as waldenström's macroglobulinemia." *Archives of internal medicine*, vol. 142, no. 13, pp. 2318–2319, 1982.

[279] R. P. Gonçalves, V. T. Hungria, C. S. Chiattone, D. B. Pozzi, and R. C. Maranhão, "Metabolism of chylomicron-like emulsions in patients with hodgkins and with non-hodgkins lymphoma," *Leukemia research*, vol. 27, no. 2, pp. 147–153, 2003.

[280] J. Storr and A. Burton, "The effects of arginine deficiency on lymphoma cells," *British journal of cancer*, vol. 30, no. 1, p. 50, 1974.

[281] F. Fueller and K. F. Kubatzky, "The small gtpase rhoh is an atypical regulator of haematopoietic cells," *Cell Communication and Signaling*, vol. 6, no. 1, p. 6, 2008.

[282] L. M. Pedersen, G. W. Jürgensen, and H. E. Johnsen, "Serum levels of inflammatory cytokines at diagnosis correlate to the bcl-6 and cd10 defined germinal centre (gc) phenotype and bcl-2 expression in patients with diffuse large b-cell lymphoma," *British journal of haematology*, vol. 128, no. 6, pp. 813–819, 2005.

[283] D. Giri, M. Ozen, and M. Ittmann, "Interleukin-6 is an autocrine growth factor in human prostate cancer," *The American journal of pathology*, vol. 159, no. 6, pp. 2159–2165, 2001.

[284] S. F. Elsawa and S. M. Ansell, "Cytokines in the microenvironment of waldenström's macroglobulinemia," *Clinical Lymphoma and Myeloma*, vol. 9, no. 1, pp. 43–45, 2009.

[285] A. Munir, S. Hussain, and E. Dilshad, "Silver nanoparticles conjugated with neurotrophin 3 upregulate myelin gene transcription pathway," *Journal of theoretical biology*, vol. 459, no. 1, pp. 111–118, 2018.

[286] R. S. Obach, J. G. Baxter, T. E. Liston, B. M. Silber, B. C. Jones, F. Macintyre, D. J. Rance, and P. Wastall, "The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data," *Journal of Pharmacology and Experimental Therapeutics*, vol. 283, no. 1, pp. 46–58, 1997.

[287] X.-Q. Yu and A. G. Wilson, "The role of pharmacokinetic and pharmacokinetic/pharmacodynamic modeling in drug discovery and development," *Future medicinal chemistry*, vol. 2, no. 6, pp. 923–928, 2010.

[288] C. N. Papandreou, D. D. Daliani, D. Nix, H. Yang, T. Madden, X. Wang, C. S. Pien, R. E. Millikan, S.-M. Tu, L. Pagliaro *et al.*, "Phase i trial of the proteasome inhibitor bortezomib in patients with advanced solid tumors with observations in androgen-independent prostate cancer," *Journal of clinical oncology*, vol. 22, no. 11, pp. 2108–2121, 2004.

[289] C. R. C. Tan, S. Abdul-Majeed, B. Cael, and S. K. Barta, "Clinical pharmacokinetics and pharmacodynamics of bortezomib," *Clinical pharmacokinetics*, vol. 12, no. 7, pp. 1–12, 2018.

[290] Z. Wang, J. Yang, C. Kirk, Y. Fang, M. Alsina, A. Badros, K. Papadopoulos, A. Wong, T. Woo, D. Bomba *et al.*, "Clinical pharmacokinetics, metabolism, and drug-drug interaction of carfilzomib," *Drug Metabolism and Disposition*, vol. 41, no. 1, pp. 230–237, 2013.

[291] Z. Wang, Y. Fang, J. Teague, H. Wong, C. Morisseau, B. D. Hammock, D. A. Rock, and Z. Wang, "In vitro metabolism of oprozomib, an oral proteasome inhibitor: role of epoxide hydrolases and cytochrome p450s," *Drug Metabolism and Disposition*, vol. 13, no. 8, pp. 109–117, 2017.

[292] S. Sun, X. Liang, X. Zhang, T. Liu, Q. Shi, Y. Song, Y. Jiang, H. Wu, X. Lu, and D. Pang, "Phosphoglycerate kinase-1 is a predictor of poor survival and a novel prognostic biomarker of chemoresistance to paclitaxel treatment in breast cancer," *British journal of cancer*, vol. 112, no. 8, pp. 1332–1339, 2015.

[293] B. Ataseven, A. Gunesch, W. Eiermann, R. E. Kates, B. Högel, P. Knyazev, A. Ullrich, and N. Harbeck, "Ptk7 as a potential prognostic and predictive marker of response to adjuvant chemotherapy in breast cancer patients, and resistance to anthracycline drugs," *OncoTargets and therapy*, vol. 7, no. 1, pp. 1723–1724, 2014.