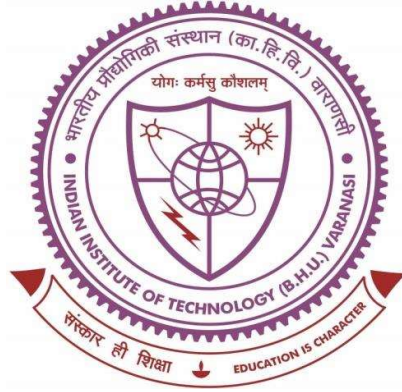


Computational Systems Biology based analysis of Tumour Regression



**Thesis submitted in partial fulfilment
for the Award of Degree**

Doctor of Philosophy

by

Bindu Kumari

**SCHOOL OF BIOMEDICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI – 221005, (U.P.)
INDIA**

ROLL NO: 17021508

2023



SCHOOL OF BIOMEDICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY
B.H.U., Varanasi – 221005
India

CERTIFICATE

It is certified that the work contained in the thesis titled “**Computational Systems Biology based analysis of Tumour Regression**” by “**Bindu Kumari**” has been carried out under my supervision and this work has not been submitted elsewhere for a degree.

It is further certified that the student has fulfilled all the requirements of Comprehensive Examination, Candidacy and SOTA for the award of Ph.D. Degree.

Prasun Roy
027/7/23

(Prof. Prasun Kumar Roy)
Supervisor

Neeraj Sharma

(Prof. Neeraj Sharma)
Co-supervisor



DECLARATION BY THE CANDIDATE

I, Bindu Kumari, certified that the work embodied in this Ph.D. thesis is my own bonafide work and carried out by me under the supervision of Prof. Prasun Kumar Roy and Prof. Neeraj Sharma from 27th December 2017 to 25th July 2023 at the School of Biomedical Engineering, Indian Institute of Technology (BHU), Varanasi. The matter embodied in this thesis has not been submitted for the award of any other degree/diploma.

I declare that I have faithfully acknowledged and given credits to the research workers wherever their work has been cited in my work in this thesis. I further declare that I have not wilfully copied any other's work, paragraphs, text, data, results, etc., reported in journals, books, magazines, reports dissertations, theses, etc., or available at websites and have not included them in this thesis and have not cited as my own work.

Date: 27/07/2023

Place: Varanasi

Bindu Kumari
Signature of the Student
(Bindu Kumari)

CERTIFICATE BY THE SUPERVISOR

It is certified that the above statement made by the student is correct to the best of our knowledge.

Prasun Roy 27/7/23
(Prof. Prasun Kumar Roy)
Supervisor

Neeraj Sharma
(Prof. Neeraj Sharma)
Co-Supervisor

Sy Kumar Kan
Coordinator 27/07/23

School of Biomedical Engineering
Indian Institute of Technology
Varanasi-221005, India

समन्वयक/CO-ORDINATOR

जैव चिकित्सा अभियांत्रिकी स्कूल
SCHOOL OF BIOMEDICAL ENGG.
भारतीय प्रौद्योगिकी संस्थान (का.डि.वि.)
INDIAN INSTITUTE OF TECHNOLOGY (B.H.U.)
वाराणसी 221005/VARANASI-221005



SCHOOL OF BIOMEDICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY
B.H.U., Varanasi – 221005
India

COPYRIGHT TRANSFER CERTIFICATE

Title of the Thesis: Computational Systems Biology based analysis of Tumour Regression.

Submitted by: Bindu Kumari

Copyright Transfer

The undersigned hereby assigns to the Indian Institute of Technology (BHU), Varanasi, India, all rights under copyright that may exist in and for the above thesis submitted for the award of the Doctor of Philosophy.

Date: 27/07/2023

Place: Varanasi, India.

Bindu Kumari
Signature of the Student
(Bindu Kumari)

Note: However, the author may reproduce or authorize others to reproduce material extracted verbatim from the thesis or derivative of the thesis for the author's personal use, provided that the source and the Institute's copyright notice are indicated.

ACKNOWLEDGEMENTS

It is my greatest pleasure to have this opportunity to express my sincere appreciation to everyone who helped me during my Ph.D. program at the Indian Institute of Technology (Banaras Hindu University), Varanasi. First, I want to thank my supervisor **Prof. Prasun Kumar Roy**, and Co-supervisor **Prof. Neeraj Sharma** for their valuable guidance, encouragement and paternal behaviour throughout the work. Their inspiring and excellent guidance is the key reason that I could successfully finish each of the research objectives. Without their guidance as great mentor, this work would not have been possible.

I would like to express my appreciation to my research progress evaluation committee (RPEC) members, **Dr. Sanjeev K. Mahto** and **Prof. Neeraj Sharma**, School of Biomedical Engineering, IIT (BHU) and **Dr. G.P. Modi**, Department of Pharmaceutical Engineering, for their kind cooperation during the course of this work. I offer my special thanks to **Prof. Sanjeev Kumar Mahto**, Coordinator of the School of Biomedical Engineering for providing all the necessary facilities of the School.

I would like to thank all the respected faculty members of the School of Biomedical Engineering, IIT(BHU): **Dr. Sanjeev K. Mahto**, **Prof. Neeraj Sharma**, **Dr. Shiru Sharma**, **Dr. Sanjay Kumar Rai**, **Dr. Marshal**, **Dr. Pradip Paik**, **Dr. Jac Fredo**, **Dr. Deepesh Kumar**, **Dr. Sudip Mukherjee**, **Dr. Brijesh Kumar** and **Dr. Gowri Balachander** for their kind support and valuable suggestions. Thanks to our corroborators **Dr. Chandrashekhar Sakode**, Indian Institute of Information Technology, Nagpur and **Dr. L. Raghavendran**, Jawaharlal Nehru University, New Delhi for their help.

I would also like to express my gratitude to my wonderful lab-mates **Mr. Pratik Purohit**, **Ms. Anindita Bhattacharjee**, **Mr. Brajesh Baghel**, **Mr. Mahtim Singh** and **Ms. Ranjitha Ruttala** for their stimulating discussions and creating a cheerful environment in the lab. I would also like to thank my batch-mates and friends **Dr. Alok**

Prakash, Dr. Ajay Sahi, Dr. Romel Bhattacharjee, Dr. Uvanesh Kasiviswanathan, Dr. Hemlata Shakya, Ms. Kirti Wasnik, Ms. Prachi Sirvastava, Mr. Vivek Kumar, Ms. Aarushi Mishra, Mr. Ashish Shrivastava and Mr. Bhaskar Roy and all my juniors for their extended support. I thank all my beloved friends across the globe for sharing my happy and sorrow moments at all times.

I would like to sincerely thank all the technical and non-technical staff members of the department for their kind help whenever I required. Especially our lab in charge **Mr. Bhuwaneshwari Saran** for immense support and help. I would also like to thank **MHRD (now Ministry of Education)** for financially supporting the thesis work and providing fellowship.

I can never forget the invaluable contribution of both my parents **Mr. Babu Lal & Mrs. Meena**, and parents in laws **Mr. Ramesh Gupta** and **Mrs. Meera Gupta**, both my brothers **Mr. Manish Kumar** and **Mr. Dilip Kumar** and sister **Indu**, my brother in law **Mr. Naman Jain** and sister in law **Mrs. Tanvi Jain** whose boundless love, constant inspiration, emotional support and blessings has provided me encouragement at every step of life. My parents' everlasting shower of blessing kept me moving easily with all hazards vanishing miraculously. I am extremely grateful to my husband **Mr. Manas Gupta** for always being my pillar of strength and support without which I could not have completed my thesis. My word power fails to express my feeling of gratitude to my loving family members for their encouragement and moral support during my work.

Lastly, I would like to thank the Almighty, for having made everything possible by giving me strength and courage to do this work.

Bindu Kumari

(Bindu Kumari)

Date: 25/07/2023

Place: Varanasi

Dedicated
To
My Beloved Parents,
My Loving Husband and
my In-laws

Table of Contents

LIST OF FIGURES	xv
LIST OF TABLES	xxi
LIST OF ABBREVIATIONS	xxiii
PREFACE.....	xxv
Chapter 1: Introduction	2
1. Introduction.....	2
1.1 Reservations with respect to conventional therapy	2
1.2 Spontaneous cancer regression	3
1.3 Quantitative systems biology formulation	5
1.4 Mechanism of complete tumour regression negative using biasing	6
1.5 Melanoma cancer: as a case study of spontaneous regression process.....	9
1.6 Melanoma signalling pathway.....	11
1.6.1 MAP Kinase-ERK dependent pathway.....	12
1.6.2 PI3Kinase-AKT dependent pathway	13
1.7 Research objective	13
1.8 Scope of the thesis	14
Chapter 2: Computational systems biology approach for Permanent tumour elimination and normal tissue protection using negative biasing.....	18
2. Outline.....	18
2.1 Introduction.....	19
2.2 Materials and methods	22
2.2.1 Formulating the computational framework of spontaneous tumour regression: 22	
2.2.2 Systems analysis of tumour extinction:.....	24
2.2.3 Equivalence between endogenous and exogenous tumour regression	26
2.2.4 Formulation of extinction of tumour cells.....	29
2.2.5 Protection of normal host tissue	34
2.2.6 Bounds of parameters in endogenous and exogenous tumour regression	34
2.2.6.1 Bounds of DNA damage.....	34
2.2.6.2 Bounds of antitumour factors	36
2.2.7 Path of complete tumour regression process	39

2.2.8	Computational modelling of Endogenous or Exogenous regression	41
2.2.9	Model corroboration by collateral experimental findings: Complete elimination of malignant cell population by first-order kinetics.....	43
2.3	Results.....	44
2.3.1	Mathematical modelling and computational simulation for tumour regression model	44
2.3.2	Tumour system behavior	44
2.3.2.1	Tumour regression under conventional therapy (without Negative Bias): Tumour Relapse	45
2.3.2.2	Tumour regression by negative bias formulation: Permanent tumour elimination	46
2.3.2.3	Behaviour of cancer stem cells, natural killer cells and circulating lymphocytes	49
2.3.2.4	Adaptability and robustness of tumour elimination process	50
2.3.2.5	General characteristics of tumour regression process	50
2.3.2.6	Basis of the tri-phasic activation.....	52
2.3.3	Model corroboration by collateral experimental findings: Complete tumour eradication by First-order kinetics	54
2.3.3.1	Exponential decline without negative bias:	57
2.3.3.2	Exponential decline with negative bias:	58
2.4	Discussion.....	60
2.4.1	Normal tissue protection	60
2.4.2	Robustness of cancer stem cell abolition with negative bias	61
2.4.3	Double pulsed lymphocyte activation for final tumour eradication.....	62
2.4.4	Therapeutic implications of the natural process of spontaneous regression of tumour	63
2.5	Conclusions	64
Chapter 3: Experimental validation of the profile of antitumour entities that enable permanent tumour elimination.....		68
3.	Outline.....	68
3.1	Introduction	69
3.2	Materials and methods	72
3.2.1.	Validation of our computational model using experimental biological system	72
3.2.2	Pre-clinical investigation	73
3.2.3	Normalization and statistical analysis.....	74
3.2.4	Time dependent biological function analysis using pathway study	74
3.3.	Results	75
3.3.1	First-order kinetics: Path for complete tumour regression	75

3.3.2 Overall profile of Permanent Tumour Remission process	77
3.3.3 Experimental validation.....	78
3.3.4. Malignant melanoma elimination	79
3.3.4.1. Melanoma microarray data preprocessing	79
3.3.4.2. Identification of signaling pathways.....	79
3.3.4.3. Identification of genes	82
3.3.5 Negative bias related genes: CASP7, GZMB	84
3.3.6 Malignant histiocytoma elimination	85
3.4. Discussion	86
3.4.1 Practical implementation: Enforcement of tumour extinction—computational feedback approach.....	88
3.4.2 Clinical translation: Towards complete tumour elimination by negative biasing 89	
3.5. Conclusions.....	90
Chapter 4: A Mechanistic Analysis of Spontaneous Cancer Remission Phenomenon Identification of Genomic Basis and Effector Biomolecules for Therapeutic Applicability: Melanoma as a case study.....	94
4. Outline	94
4.1 Introduction.....	95
4.2 Materials and Methods	98
4.2.1 Formulation of Genomic and Pharmacological Analysis	98
4.2.2 Microarray investigation of preclinical study	98
4.2.3 Identification of differentially expressed genes (DEGs) of tumour regression	99
4.2.4 Biological Signaling Pathway analysis.....	99
4.2.5 Gene Ontology and Pathway Enrichment Analysis	99
4.2.6 Protein-Protein Interaction Network Construction and Hub-Gene Identification..	100
4.2.7 Identification of Candidate Drugs from Hub Gene Interaction	100
4.2.8 Molecular Processes Study and Drug-Ligand Interaction.....	100
4.2.9 Corroboration on Human Subjects: Normal Controls and Melanoma Tumour Patients.....	101
4.3 Results	101
4.3.1 Formulation of Genomic and Pharmacological Analysis	101
4.3.2 Microarray Investigation and Analysis	101
4.3.3 IPA Signaling Pathway Analysis	103
4.3.4 Identification of Genes (DEGs) enabling first-order kinetics of tumour regression	107
4.3.5 Protein-Protein Networking analysis.....	116
4.3.6 Functional Enrichment analysis.....	120

4.3.7 Protein-Protein interaction analysis and Sub-network analysis.....	122
4.3.8 Identification of Hub Genes:	125
4.3.9. Identification of Candidate Drugs	127
4.3.10 Molecular docking for TOP2A	130
4.3.11 Corroboration from Human Subjects and Clinical Patients	134
4.4 Discussion.....	136
4.5 Conclusion.....	140
Chapter 5: Spontaneous cancer regression as reversion of spontaneous cancer progression: Molecular targets and pharmacological implications	142
5. Outline.....	142
5.1 Introduction	143
5.2 Methods and Materials:.....	146
5.2.1 Data Collection and Investigation:	146
5.2.2 Identification of differentially expressed genes (DEGs) of spontaneous melanoma regression data:	146
5.2.3 Gene Ontology Analysis:	147
5.2.4 Identification of Melanoma regression genes:	147
5.2.5 Identification of target proteins.....	148
5.2.6 Drug Identification	151
5.2.7 Validation of Drug using Molecular docking.....	151
5.2.7.1 Molecular docking with BRAF and NRAS:	151
5.2.7.2 Molecular dynamic simulation:.....	153
5.3 Result	154
5.3.1 Microarray data analysis and DEGs identification	155
5.3.2 Gene enrichment analysis of DEGs	156
5.3.3 Identification of melanoma tumour regression driving genes	158
5.3.4 Downstream signaling pathway of Melanoma and its target protein	161
5.3.5 Identification of Drugs	162
5.3.6 Molecular Docking with BRAF and NRAS	162
5.3.7 Molecular dynamics simulation investigation	167
5.4 Discussion.....	171
5.5 Conclusion.....	175
Chapter 6: Conclusion	178
6.1 Conclusion.....	178
6.2 Future scope of work	182
REFERENCES.....	185

APPENDIX-I	199
APPENDIX-II	209
APPENDIX – III (A)	229
APPENDIX – III (B)	247

LIST OF FIGURES

- Figure 1.1** Permanent Tumour Elimination Process by First order kinetics. 9
- Figure 2.1** Complete tumour elimination process by principle of negative biasing. (a): In conventional therapy, the elimination of the tumour cell population $M(t)$ follows an exponentially-decreasing trajectory, with tumour cells always persisting asymptotically under the curve, thereby leading to tumour relapse after therapy duration has ended. (b): The Negative bias shift process enables the residual tumour cell population to become zero at a finite time t_F . This curve $M(t)$ decreases exponentially by approaching the negative bias ($-M^*$) value, so that, at time point F , it hits the horizontal x-axis, where tumour cell population is zero. Thus, at F the tumour cell population becomes extinct and there is no further tumour cell to replicate, i.e., complete and permanent tumour regression occurs, eliminating the malignant lesion..... 23
- Figure 2.2** Multimodal equivalence between 1) Therapy-induced elimination of tumour, i.e., Exogenous tumour regression, and 2) Host tissue-induced elimination to tumour, i.e., Endogenous tumour regression. Endogenous regression of tumour is due to internally-generated factors, while Exogenous regression is due to externally-generated factors. Each of the three causative entities of Exogenous regression (therapy-initiated regression) and Endogenous regression (tissue-initiated regression) have similar factors, as detailed in the three boxed entities on the left side..... 25
- Figure 2.3** Estimation of DNA interference in endogenous spontaneous regression in terms of equivalent alkylation units. (the motifs are drawn in ChemDraw, the DNA helix is from Open Source, the Trex enzyme is from RCSB-PDB database). 28
- Figure 2.4** Computational systems analysis of activation of multimodal entities (Interleukin-2, Cytotoxic T-cells, and DNA damage) which enables tumour eradication, endogenously or exogenously..... 29
- Figure 2.5** Interaction between the malignant lesion and the entities in its environment during endogenous or exogenous regression of the lesion (i.e. host-induced or therapy-induced regression). 30
- Figure 2.6** Flow chart for computational modelling of Endogenous or Exogenous regression of malignant lesion (i.e., Spontaneous tumour regression or Treatment-induced tumour regression respectively)..... 43
- Figure 2.7** Conventional treatment protocol using chemotherapy and immunotherapy. This uses DNA blocking alkylator drug dacarbazine, and immunotherapy (Cytotoxic T-lymphocyte and Interleukin-2). The protocol fails to eliminate malignant melanoma tumour cells, and after treatment duration there is relapse of the malignancy. 46

Figure 2.8 Complete elimination of melanoma tumour under negative bias. (a) Consistent decline of tumour cell population with time: Complete elimination of tumour at 46 days by following negative biasing behavior of fig. 1(b) scheme. (b) Bimodal temporal profile of cytotoxic T-cell required for eliminating Tumour cells. (c) Unimodal profile of level of DNA damage required for eliminating Tumour cells (DNA damage is estimated in terms of equivalent amount of alkylator substance dacarbazine that produces similar amount of DNA damage, see text). (d) Concentration profile of Interleukin-2 required for tumour elimination (the curve displays a stationary level). (e) Temporal profile of circulating lymphocyte level required for tumour elimination (the curve levels off at a saturating value). (f) Temporal profile of natural killer cell level required for tumour elimination (the curve levels off at a saturating value). (g) Interleukin-2 input rate that would enable tumour elimination. (h) DNA blockade input rate that would enable tumour elimination (estimated in equivalent amount of rate of input of alkylator substance dacarbazine). (i) Tumour-infiltrating lymphocyte input rate that would enable tumour elimination..... 48

Figure 2.9 Complete elimination of cancer stem cells. The tumour will need more time for the cancer stem cells to be extinct (in 59 days) with chemotherapy sensitivity at 1% of usual cancer cells..... 49

Figure 2.10 Decrease of tumour cells population with different initial conditions. Extinction of the tumour occurs regardless of the initial conditions. 50

Figure 2.11 For complete regression of tumours with different initial conditions, the time-wise alteration of the tumour-affecting entities involved does follow the common pattern template: (a) Bimodal intensity of Cytotoxic T-cell, (b) Unimodal intensity of DNA blockade factor, (c) Uniform stationary intensity of Interleukin-2, (d) Saturating intensity of Natural killer cells and Circulating lymphocytes..... 52

Figure 2.12 Basis of the tri-phasic activation process in tumour regression as illustrated by the three temporal transitions..... 53

Figure 3.1 Permanent elimination of malignant melanoma tumour under first-order kinetics..... 75

Figure 3.2 To enable permanent regression of malignant tumour with different initial conditions, the antitumour-entities are found to have the following common time-wise varying patterns: (a) Unimodal temporal intensity of DNA blockade factor, (b) Bimodal temporal intensity of Cytotoxic T-cell, (c) Uniform stationary temporal intensity of Interleukin-2, (d) Saturating temporal intensity of Natural killer cells and Circulating lymphocytes. 77

Figure 3.3 Volcano plots for differentially expressed genes. ANOVA analysis results showing DEGs plot: blue color signifies the downregulated genes, red color signifies the upregulated genes, and black color signifies the non-significant genes..... 78

Figure 3.4 Comprehensive experimental validation of the prediction of the computational model. Behaviour of the various formulated entities needed for malignant tumour regression (melanoma) are shown, the entities being antitumour T-cells, DNA damage, Circulating lymphocytes, and Natural killer cells. (a)–(d) Activation of the biological readouts obtained using microarray data of the respective tissues at the various time-points, the vertical y -axis is in LLS units, i.e., log level of significance (i.e., $-\log(p\text{-value})$). Regarding the four curves, it is evident that their activations are respectively of bimodal (panel (a)), unimodal (panel (b)), and saturation patterns (panel (c), (d)), which are also predicted by our computational model developed theoretically for tumour regression (patterns of the computationally predicted theoretical curves are in Figures 3.1)..... 80

Figure 3.5 Permanent regression of malignant tumour: Empirical confirmation of the activation functions of the computational model, the tumour being malignant histiocytoma. (a) Interleukin-2 level as tumour regression ensues across time: the activation level is practically stationary. (b) Intensity of DNA damage, as gauged by level of $\text{TNF-}\alpha$ that induces DNA strand breaks. Note that here the activation function is unimodal..... 85

Figure 4.1 Graphical summary of comparison between two different time points: the first time point is when the tumour is progressing (left panel), the second time point is when it is regressing (right panel). The networks show a ready overview of the major biological entities such as canonical pathways, upstream regulators, diseases, and biological functions; here orange color nodes show the predicated activated entities, while blue color nodes show the predicated inhibited entities (the intensity of color is based on magnitude of z -score value in the analysis). In the regression phase (right panel), one can note that the inhibited region (blue) is in the upper right portion) and is mainly concerned with the first-order kinetics based decline of malignant cell population, namely by DNA interference and inhibition of tumour cells replication. The lower red colored region (right panel) show activation of the other components producing tumour regression: Interleukin-2 and Lymphocyte activation.....103

Figure 4.2 The most significant gene network detected by IPA Pathway analysis at time point t_2 (tumour regression process has started and is underway): A total of 30 focus genes were identified and mapped to top IPA functional classes, such as cancer, cell cycle, and hematological disease (significance score = 45). The green nodes are the downregulated biological diseases and functions, while the red nodes are the upregulated molecules; and the intensity of the focus nodes is based on the data measurement value in our dataset. 105

Figure 4.3 Venn diagram: Identification of differentially expressed genes (DEGs) responsible for first-order decreasing curve of tumour extinction, while the tumour undergoes regression across the duration of regression, i.e. along the three time points t_2 , t_3 , t_3 . The common intersection zones indicated the genes that were effective at all the time points, throughout the regression process. Thereby, a total of **(a)** 176 upregulated and **(b)** 116 downregulated genes are obtained for further analysis.106

Figure 4.4 Gene ontology (GO) analysis and significant enrichment of “Cellular Component” responsible for exponential decreasing curve in melanoma regression model; (a, b) GO analysis for upregulated differentially expressed genes (DEGs); (c, d) GO analysis for downregulated DEGs.....	115
Figure 4.5 Gene Ontology analysis and significant enrichment of “Molecular Function” responsible for exponential decreasing curve in melanoma regression model; (a, b) GO analysis for upregulated DEGs; (c, d) GO analysis for downregulated DEGs.....	116
Figure 4.6 Gene Ontology analysis and significant enrichment of “Biological Process” responsible for exponential decreasing curve in melanoma regression model; (a, b) GO analysis for upregulated DEGs; (c) pi-chart representation of GO analysis.	117
Figure 4.7 Gene Ontology analysis and significant enrichment of “Biological process” responsible for exponential decreasing curve in melanoma regression model; (a, b) GO analysis for <i>downregulated</i> DEGs; (c) pi-chart representation of GO analysis.	118
Figure 4.8 Gene ontology analysis and significant enrichment of biological pathway responsible for exponential decreasing curve in the melanoma regression model for downregulated differentially expressed genes. The blue bars denote the percentage of genes, while the yellow line is the level of statistical significance of the genes [$-\log_{10}(\text{p-value})$]. Note that there is very significant downregulation of the genes associated with tumour cell multiplication, such as Mitosis function, DNA replication, Cell cycle checkpoint functionality, Kinase signaling, etc. Such inhibition of malignant cell reproduction produces rapid decline in tumour cell population.	119
Figure 4.9 Relationship of 164 Human Upregulated DEGs (differentially expressed genes) and their 428 linking edges during spontaneous tumour regression, as obtained by Protein-Protein Interaction analysis.....	121
Figure 4.10 Relationship of 115 Human Downregulated DEGs (differentially expressed genes) and their 1,797 linking edges during spontaneous tumour regression, as obtained by Protein-Protein Interaction analysis.	122
Figure 4.11 Sub-network of top 3 modules with seed node representation (in yellow color) from Protein-Protein Interaction (PPI) network: (a) Module-1, having KIF4A gene as a seed node; (b) Module-2 having RAC2 gene as a seed node; (c) Module13 having FCER1G gene as a seed node; (d) Biological pathway enrichment analysis of Module 1.	123
Figure 4.12 (a) Construction of Protein-Protein Interaction network of the top 10 hub genes, having 10 nodes and 45 edges; (b) The co-expression analysis of the 10 hub genes using the STRING platform, the color intensity of the triangle-matrix shows the level of confidence between two functionally connected proteins.	125
Figure 4.13 (a) An oncoprint summary across a set of melanoma tumours (skin cutaneous melanoma TCGA Pan-Cancer data) shows the genetic alteration connected with four main	

genes in a total of 442 patients; **(b)** Common drugs from three databases (DGIdb, Cytoscape, and IPA).....128

Figure 4.14 Computed structural comparison and binding features respectively of **(a)** Teniposide, **(b)** Etoposide, **(c)** Epirubicin, and **(d)** Doxorubicin against TOP2A receptor (PDB ID: 4FM9) (Visualization using UCSF Chimera and AutoDock).131

Figure 4.15 Expression level of four hub genes in human subjects and clinical patients. The four panels of the heat maps show the expression levels of the four hub genes [**(a)** TOP2A, **(b)** CDK1, **(c)** CCNA2, and **(d)** CHEK1] in melanoma tissue (skin cutaneous melanoma) vis-à-vis normal skin tissue, based on TCGA data analyzed using GEPIA platform. The red and grey box plots in each panel represents melanoma tissues and normal skin tissues respectively.133

Figure 4.16 Correspondence of Hub Genes with Survival Rates in Clinical Patients of Malignant Melanoma tumour. The Human Protein atlas was used to evaluate the overall survival rates of high and low expression of the genes: **(a)** TOP2A, **(b)** CDK1, **(c)** CCNA2, and **(d)** CHEK1 in patients with melanoma. Blue graphs: low expression of gene; Red graphs: high expression of gene.....133

Figure 5.1 The MAP/ERK and PI3K/AKT signaling pathway for melanoma tumour proliferation.148

Figure 5.2 The Protein-Protein interaction (PPI) network: (a) Upregulated differentially expressed genes (DEGs); (b) Downregulated differentially expressed genes (DEGs)....153

Figure 5.3 Gene Ontology (GO) term analysis of significantly enriched DEGs, classified in 3 groups (Cellular component, Biological process and Molecular function): (a) Upregulated DEGs; (b) Downregulated DEGs.....155

Figure 5.4 Significant Biological Pathway enrichment analysis of DEGs: (a) Biological pathway of upregulated DEGs; (b) Biological pathway of downregulated DEGs.....156

Figure 5.5 Venn Diagram representing the common genes between microarray data, NCBI database and Gene Card database.....157

Figure 5.6 Enrichment analysis of significant Biological Pathway analysis of melanoma driving genes.....159

Figure 5.7 3D representation of the target receptors docked with ligands, illustrating the interacting residues in 2D interaction diagram. (a) BRAF complex with Alpelisib and Obatoclax, (b) NRAS complex with Alpelisib and Obatoclax.....163

Figure 5.8 The 3D representation of Protein-Ligand complex: (a) Alpelisib with BRAF(5C9C); (b) Obatoclax with BRAF protein (5C9C); (c) Alpelisib with NRAS protein (6ZIZ); (d) Obatoclax with NRAS(6ZIZ).164

Figure 5.9 3D representation of the target receptors docked with Cetuximab, illustrating the interacting residues. (a) BRAF-Cetuximab docked complex, (b) NRAS-Cetuximab docked complex. Color scheme used for dashed line showing interactions as follows: hydrogen bond (black), salt-bridge (green), and pi-cation (dark green).	165
Figure 5.10 RMSD analysis of protein-ligand complexes throughout the simulation trajectories. The RMSD of the protein is plotted on the left Y-axis, and the RMSD of the ligand is plotted on the right Y-axis, against simulation time (ns) on the X-axis. (a) RMSD of BRAF protein along with three selected ligands (Alpelisib, Obatoclox, and Cetuximab). (b) RMSD of NRAS protein along with three selected ligands (Alpelisib, Obatoclox, and Cetuximab).....	166
Figure 5.11 RMSF analysis of protein C-alpha atoms for each residue throughout the simulation time, depicting the flexibility of the protein residues in the complexes. (a) RMSF plot of the BRAF complex, and (b) RMSF plot of the NRAS complex.	167
Figure 5.12 Intermolecular interactions between the protein and ligand were calculated during the simulation to measure the stability of the complexes. The interaction profiles of the following complexes are depicted in the figure: (a) BRAF-Alpelisib, (b) BRAF-Obatoclox, (c) BRAF-Cetuximab, (d) NRAS-Alpelisib, (e) NRAS-Obatoclox, and (f) NRAS-Cetuximab.	168
Figure 5.13 Radius of gyration plot of the protein-ligand complexes illustrating changes in protein compactness throughout the simulation. In this figure, the radius of gyration of the protein is depicted for each complex: (a) BRAF-Alpelisib complex, (b) BRAF-Obatoclox complex, (c) BRAF-Cetuximab complex, (d) NRAS-Alpelisib complex, (e) NRAS-Obatoclox complex, and (f) NRAS-Cetuximab complex.....	169
Figure 5.14 The cell cycle process under the genes that actuate the spontaneous regression of malignant melanoma tumour.	171
Figure A.1 Cellular component (CC) of enrichment analysis: (a) Upregulated genes; (b) Downregulated genes.	241
Figure A.2 Biological process (BP) of enrichment analysis: (a) Upregulated genes; (b) Downregulated genes.	242
Figure A.3 Molecular function (MF) of enrichment analysis: (a) Upregulated genes; (b) Downregulated genes	243
Figure A.4 Molecular Dynamics (MD) simulation trajectories analysis of both BRAF (PDB ID: 5C9C) and NRAS (PDB ID: 6ZIZ) with both the ligand (Alpelisib and Obatoclox). Panel (a, b, c, and d) illustrate the RMSF plot showing how the protein chain's alpha helixes (red color) and beta sheets (blue color) distributions, as well as local fluctuations, changes during the course of the simulation. Green bars represent the ligand interaction locations with the particular residues.....	244

LIST OF TABLES

Table 2.1 Values of the biological parameters of the tumour system	33
Table 2.2 Limits of upper and lower bounds	38
Table 2.3 Tumour cell population decline with time during the spontaneous regression process.....	56
Table 3.1 Biologically-based experimental corroboration of the computed activation functions of the antitumour entities which enable complete tumour elimination: The characteristic computed functions [Eqs (1)–(4), (6) of chapter 2] are in the first column, rows 2–6), and for each of these functions, their corresponding biological significance and relationships are provided in the other columns. The negative bias function is also included as row 7).....	82
Table 3.2 Genes inducing Negative bias with their activity values at different time points.	84
Table 4.1 List of Upregulated Genes; These are expressed at all the three time points across the duration of the spontaneous tumour regression process (t_2 , t_3 , & t_4), each time point being at three-weekly intervals.	107
Table 4.2 List of Downregulated Genes; These are expressed at all the three time points across the duration of spontaneous tumour regression process (t_2 , t_3 , t_4), each time point being at three-weekly intervals.	112
Table 4.3 Top 10 hub genes identification: CytoNCA analysis of PPI network	124
Table 4.4 Identification of candidate pharmacological molecules.....	126
Table 4.5 List of all the Nine Drugs with their binding energy and inhibitory constant (K _i) value, exhibiting inhibitory action on TOP2A gene (PDB ID: 4FM9).	129

Table 4.6 The binding energy, inhibitory constant (K _i) and interacting amino acid residues of the candidate drugs Teniposide, Etoposide, Epirubicin, and Doxorubicin against TOP2A receptor (PDB ID: 4FM9).....	130
Table 5.1 The top 10 hub genes with their fold change value for melanoma regression.	157
Table 5.2 The 17 genes common between microarray data, NCBI database and Gene Card database with their fold change values.	158
Table 5.3 Docking results of selected compounds with BRAF and NRAS receptors....	161

LIST OF ABBREVIATIONS

WHO	World Health Organization
NK cell	Natural Killer cell
DNA	Deoxyribonucleic acid
MAPK	Mitogen-activated protein kinase
PI3K	phosphoinositide 3-kinase
RAF	Rapidly Accelerated Fibrosarcoma
BRAF	Murine sarcoma viral oncogene homolog B
RAS	Rat sarcoma virus
NRAS	Neuroblastoma RAS viral oncogene homolog
KRAS	Kirsten rat sarcoma virus
HRAS	Harvey Rat sarcoma virus
ARAF	Serine/threonine-protein kinase A-Raf
CRAF	RAF proto-oncogene serine/threonine-protein kinase
ERK	Extracellular signal-regulated kinase
AKT3	AKT Serine/Threonine Kinase 3
PTEN	Phosphatase and tensin homolog deleted on chromosome ten
BAD	BCL2 associated agonist of cell death
BCL-2	B-cell leukemia/lymphoma 2 protein
MDM2	murine double minute 2
IL-2/15	Interleukin-2/ Interleukin-15
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol 3,4,5-triphosphate
CCND1	Cyclin D1
CDC2	Cell division control protein 2 homolog
CHEK2	Checkpoint kinase 2
IL2RG	Interleukin (IL)-2 receptor common gamma chain gene
TRGV5	T Cell Receptor Gamma Variable 5
CD28	Cluster of Differentiation 28
CASP7	Caspase 7
GZMB	Granzyme B

TOP2A	topoisomerase IIalpha gene
DEGs	Differentially Expressed Genes
FC	Fold Change
FDR	False Discovery Rate
IPA	Ingenuity Pathway Analysis
GO	Gene Ontology
NCBI	National Center for Biotechnology Information
CC	Cellular Component
BP	Biological Process
MF	Molecular Function
DAVID	Database for Annotation, Visualization, and Integrated Discovery
PDB	Protein Data Bank
Å	Angstrom
MD	Molecular dynamics
RMSD	Root Mean Square Deviation
RMSF	Root Mean Square Fluctuation
ANOVA	Analysis of variance
3D	3 Dimensional
SB	Salt Bridges