CHAPTER 1

Motivation and Outline of the Thesis

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1.1. Motivation of the present work:

The ability to manage cancer is quickly being outstripped by the rising worldwide burden of the disease. In 2020, there were more than 19 million novel cases of cancer identified and 10 million cancer-related deaths [1]. According to Global Cancer Observatory, by 2040, there will be 16 million cancer-related deaths and approximately 30 million new cases of cancer yearly.

A new study in The Lancet examines the relationship between markers of metabolic, environmental, occupational, environmental, as well as behavioural risk factors associated with malignancies around the world, as outlined by researchers from Global Burden of Disease Study [2]. The authors found that these risk factors contributed to 4.45 million deaths & 105 million disability-adjusted life years (DALYs), accounting for 44.4% of cancer deaths and 42.0% of cancer DALYs, utilising estimates of the cancer incidence, mortality, as well as risk factor data from 204 countries. The leading causes of cancer deaths worldwide were tobacco use, alcohol use, high BMI, and unsafe sex. These risk factors were also among the top three risk factors in the low- and low-middle SDI countries. Additionally, between 2010 and 2019, they discovered an alarming 20.4% upsurge in cancer mortality that may have been avoided.

As with all studies that compile data from several international sources, methodological difficulties are inevitably present during the compilation of the data. However, it is impossible to determine how long a person has been exposed over the course of their lifetime to complex risk factors, such as dietary components and their correlating links with cancer, even with the best data available. This problem is made worse in a worldwide research by the wide variation in data availability and completeness between nations and regions. It should also be noted that, in addition to these difficulties, there is also one of the fundamental problems of the consistently flawed reliability of data, especially that derived from observational studies, which purports to link certain risk factors, including dietary variables, to certain types of cancer [3]. Since there is not real-time data available, residual confounding, and the inherent complexity of these risk variables, estimating lifetime exposure is necessary

because of the timing, dose, interactions with other exposures, lack of contemporaneous data, residual confounding, and intrinsic complexity of the risk variables. Causal inference is challenging due to this. In many regions of the world, where routinely gathered health data, including cancer registration, are lacking, even measuring cancer incidence itself is extremely difficult [4].

The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) team has considerable expertise in handling these complications and has included improbability estimates to partially address this. However, it is hard to predict the extent of mismeasurement, and unavoidably, not all estimations will be correct. The number of risk variables included in the GBD research is astonishing. However, several significant cancer risk factors, such as UV radiation and infectious agents, are not mentioned. The burden of cancers caused by infectious diseases is significant and rising worldwide [5]. Infections, such as Helicobacter pylori, human papillomavirus (HPV), as well as the viral hepatitis, were responsible for millions of newly diagnosed cancer cases in 2018, with this burden being unevenly spread throughout geographical areas [6].

Chronic diseases such hepatitis B & C, HIV, as well as HPV cause a larger percentage of cancer mortality in low SDI nations [6]. However, the significance of malignancies like prostate and breast cancer that are triggered by nutrition and hormones is growing [6]. In the decades that follow tobacco's introduction and when measures are taken to decrease the ensuing exposure, the incidence of malignancies linked to tobacco use develops a predictable pattern [7].

It is not a coincidence that behaviors linked to an increased chances of cases of cancer are organized in relation to poverty, especially within nations [8, 9]. People who live in poverty experience adverse impacts on their living environments, as well as adverse impacts on their lifestyle decisions [8]. As a result of concerted efforts both within and outside the health sector, cancer can be prevented. In addition to providing access to immunizations that protect against cancer-causing illnesses like hepatitis B and HPV, this measure includes particular policies targeted at lowering exposure to risk factors for cancer, such as smoking and alcohol consumption [9-11]. A study found that spending US$100 million in the prevention might avert US$100 billion in the treatment expenses, making cancer prevention a very cost-effective endeavor [12]. But larger policy measures, including those that prioritize education, gender equality, child health,

and economic distribution, play a significant role in developing settings that not only support health and well-being generally but also cancer prevention [8].

The best chance of lowering the future cancer burden is primary prevention, which involves eliminating or decreasing modifiable risk factors. The health and well-being of people will increase and reducing this load will also ease the financial strain on cancer services and also the larger health system, as well as the compounding impacts on people [4].

Due to low knowledge, limited access to cheap care, and poor prognoses, cancer has a significant negative impact on lower middle-income nations like **India** [13, 14]. Geographical differences exist in genetic factors, environmental exposures, and patterns of malignancies between areas due to variability in ancestries, socioeconomic and cultural characteristics, dietary habits, and lifestyles [15, 16]. To improve cancer epidemiology within nation, programmes that collect data on cancer in both urban as well as rural areas systematically and provide high-quality data are crucial. In order to gather and compile trustworthy cancer data from diverse regions of India [17], the National Cancer Registry Programme (NCRP), which the Indian Council of Medical Research (ICMR) launched in 1981, has built a network of cancer registries. The NCRP operates through hospital- and population-based cancer registries (HBCRs) in several Indian cities. 38 population-based cancer registries (PBCRs) are now operating under the NCRP and providing information on the many cancer types that are common in various regions of India as well as the geographic differences in cancer pathology. Additionally, 253 HBCRs are sharing information on the type of therapy used and the overall survival rate (*https://www.ncdirindia.org/*).

Since the beginning of the first cancer registries in this area in 2003, the north-eastern (NE) States of India have continuously had the highest incidence rates of all locations of cancer [18]. Additionally, compared to other locations, this region has a greater prevalence of upper digestive tract malignancies, including oesophageal, stomach, and hypopharyngeal tumours. In addition, a combined study of the HBCR data collected from the North-East revealed lower survival rates, a lower rate of localised case discovery, and distinct cancer patterns when compared to other parts of India.

Understanding the unique cancer trends in this area, creating suitable programmes, and establishing research objectives are urgently needed. Although there have been a

number of publications attempting to address different aspects of the cancer incidence and mortality in the region, either with a focus on particular cancer types or reviews highlighting the cancer profile, a thorough analysis of all pertinent cancers, associated aetiological factors, and potential public health measures is lacking [19-23]. **Table 1.1, 1.2, 1.3,** and **1.4** show the recent statistics on cancer in the north-eastern states of India [18, 24-26].

Table 1.1. *Data quality indicators of North-Eastern population-based cancer registries for all sites (ICD-10: C00-C97) for the period of 2012-2016. Taken from [18].*

State	Registry	Per cent MV	Per cent DCO	Per cent O and U
Arunachal Pradesh	West Arunachal	94.1	0.1	2.6
	Papumpare district	95.5	0.0	2.3
	Pasighat	88.3	1.6	7.4
Assam	Cachar district	82.8	3.0	12.2
	Dibrugarh district	78.7	9.8	4.9
	Kamrup Urban	81.1	8.2	5.4
Manipur	Manipur State	93.2	0.6	4.2
	Imphal West district	94.2	0.5	4.4
Meghalaya	Meghalaya State	86.8	9.9	8.3
	East Khasi Hills district	89.7	7.0	6.3
Mizoram	Mizoram State	85.2	5.0	10.0
	Aizawl district	88.0	2.6	7.5
Nagaland	Nagaland	96.6	0.5	3.3
Sikkim	Sikkim State	88.1	4.8	8.3
Tripura	Tripura State	93.8	0.1	8.1

Per cent MV: the proportion of microscopically verified cases;

Per cent DCO: proportion of death certificate 'only' cases;

Per cent O and U: relative proportion of cancers that fell into 'other and unspecified sites (O and U)' group as per ICD-10 (including codes C26, C39, C48, C75, C76, C77, C78, C79, C80, C97).

Table 1.2. *Cancer-related health facilities in north-eastern States of India. Taken from [18].*

State	Population	Cancer-treating facilities	Radio-therapy facilities	Cancer patient welfare schemes	Palliative care centres
Arunachal Pradesh	1,383,727	1	1	0	0
Assam	31,205,576	6	6	9	8
Manipur	2,855,794	1	0	0	1
Meghalaya	2,966,889	7	1	0	1
Mizoram	1,097,206	5	1	3	2
Nagaland	1,978,502	6	1	0	1
Sikkim	610,577	1	0	0	1

Tripura	3,673,917	1	1	0	1

Table 1.3. *Ranking of north-eastern population-based cancer registries (PBCRs) with highest site-specific age-adjusted rate (AAR) in men and women for 2012-2016. Taken from [18].*

Rank 1		**Rank 2**	**Rank 3**		
Outside NE region		AAR	PBCR area	AAR	
	Oesophagus				
Patiala	11.5	54.6	Aizawl district	46.7	
Patiala	8.2	23.0	Kamrup urban	17.9	
	Stomach				
Chennai	10.5	40.3	Mizoram State	39.1	
Chennai	5.1	21.7	Mizoram state	18.8	
	Lung				
Kollam	23.1	32.1	Papumpare district	20.1	
Hyderabad district	6.0	27.6	Imphal west district	16.6	
	Liver				
Mumbai	6.6	21.5	Aizawl district	12.2	
Mumbai	3.3	8.0	Mizoram State	5.9	
	Gall bladder				
Delhi	5.4	5.6	Dibrugarh district	4.4	
Delhi	11.6	11.9	Papumpare district	10.7	
	Nasopharynx				
Thiruvanathapuram	0.6	9.3	Mizoram state	5.2	
Chennai	0.3	3.9	Aizawl district	2.8	
	Breast				
Hyderabad district	48.0	29.6	Kamrup urban	27.1	
	Cervix				
Bengaluru	17.7	27.4	Mizoram State	23.2	

Site	Rank 1		
	PBCR area	AAR	PBCR area
Men	East Khasi Hills district	75.4	Meghalaya State
Women	East Khasi Hills district	33.6	Meghalaya State
Men	Aizawl district	44.2	Papumpare district
Women	Papumpare district	27.1	Aizawl district
Men	Aizawl district	38.8	Mizoram State
Women	Aizawl district	37.9	Mizoram State
Men	Papumpare district	35.2	West Arunachal
Women	Papumpare district	14.4	West Arunachal
Men	Kamrup urban	7.9	Cachar district
Women	Kamrup urban	16.2	Cachar district
Men	Nagaland	14.4	Papumpare district
Women	Nagaland	6.5	Papumpare district
Women	Aizawl district	30.7	Papumpare district
Women	Papumpare district	27.7	Aizawl district

Table 1.4. *Hospital-Based Cancer Registry (2012-2014) in north-eastern States of India. Taken from [22].*

State	Within NE (%)	Outside NE (%)
Assam	93.4	6.6
Arunachal Pradesh	82.4	17.6

Meghalaya	80.9	19.1
Mizoram	41.8	58.2
Manipur	37.6	62.4
Sikkim	1.7	98.3
Tripura	63.5	36.5
Nagaland	21.3	78.7

This vibrant region (North-East India) appears to be being overtaken by cancer, which is pervasive. In the Northeast (NE), tobacco use is currently the main cause of cancer, and tobacco use is known to be the cause of 22% of cancer deaths globally. People must go outside of NE to receive treatment since the region has a significant cancer burden yet the infrastructure is insufficient. The development of an extensive cancer control programme is urgently required. Given the foregoing, the discussion section's multilevel, interdisciplinary, and multidimensional approach may prove useful in partially controlling the issue. To emphasize the many components for lowering the occurrence of cancer, extensive study on NE is required.

The protein 53 (p53) is better known as the " Guardian of the Genome" as it acts as the dominant tumor suppressor protein. And its main antagonist is the Murine Double Minute 2 (MDM2) protein, which binds to the p53 protein leading to disruption of all its functions, mainly the tumor suppressing function of p53. This acts as the major step in tumor progression, finally leading to metastasis in cancer patients.

Thus, it will be worthwhile in studying (i) the p53-MDM2 interactions between different sites of p53 and MDM2, (ii) the p53-Inhibitor/MDM2-Inhibitor complexes resulting in successful inhibition of p53-MDM2 interactions, and (iii) how the p53-MDM2 interaction inhibitors can be regulated using different approaches (eg. Photo-regulation).

1.2. Outline of the thesis:

Chapter 2 briefs about cancer, its epidemiology, causes of cancer, therapies of cancer available, structural characteristics of p53 molecule, cellular functions of p53 molecule, structural characteristics of MDM2 molecule, cellular functions of MDM2 molecules in

cancer, auto regulatory feedback loop of p53 andMDM2, and types of inhibitors of p53-MDM2 interaction. **Chapter** 2 also focuses on the Scope of this thesis.

Chapter 3 gives a description about the various computational techniques and the key principle of Molecular Dynamics (MD) simulation and other computational tools, including web servers and and softwares used in this thesis.

Chapter 4 describes the binding and unbinding mechanism of the complex formed by the Transactivation Domain 1 (TAD1) of p53 and the N-Terminal Domain (NTD) of MDM2 using different force fields. **Chapter 4** also describes the salient structural features, including the protein-protein interaction profile of the lowest energy structure of p53(TAD1)-MDM2(NTD) complex.

Chapter 5 describes the salient structural features, conformational dynamics, and protein-protein interaction profile of the complex formed by the DNA Binding Domain (DBD) of p53 and the central Acidic Domain (AD) of MDM2, as this site of interaction is crucial for proper ubiquitination of p53 molecules.

Chapter 6 describes the protein-protein interaction profile between the C-Terminal Domain (CTD/REG) Domain of p53 and the N-Terminal Lid present in the NTD of MDM2. **Chapter 6** also describes about the effect of the p53(CTD) on the structural characteristics and conformational dynamics of the MDM2(NTD) along with the N-Terminal Lid of MDM2 in the presence as well as absence of p53(CTD) (i.e. when p53(CTD) is bound as well as unbound to the N-Terminal Lid of MDM2.

Chapter 7 describes the molecular interaction between MDM2(NTD) and its inhibitor idasanutlin when Idasanutlin is bound to, as well as free from a Photoremovable Protecting Group (PPG).

Chapter 8 describes the binding and unbinding mechanism of the complex formed by the NTD of MDM2 and its active inhibitor Idasanutlin.

Chapter 9 describes the protein-ligand interaction profile, conformational dynamics, as well as stability of the complex between MDM2(NTD) and a novel inhibitor of p53-MDM2 interaction, known as XR-2.

Chapter 10 describes the protein-ligand interaction profile, conformational dynamics, as well as stability of the complex between p53(NTD) and a novel inhibitor of p53-MDM2 interaction, known as Epigallocatechin Gallate (EGCG).

Chapter 11 describes the protein-ligand interaction profile, conformational dynamics, as well as stability of the complex between p53(NTD) and a potent inhibitor of p53-MDM2 interaction, known as Reactivating p53 and Inducing Tumor Apoptosis (RITA). The aim of **Chapter 11** is to check whether RITA remains bound to p53(NTD) in a region other than the p53(TAD1), or it shifts to and then gets bound to p53(TAD1).

Chapter 12 presents a summary the significant findings and also the future prospects of this work.