PROJECT THESIS

PROGNOSTIC SIGNIFICANCE AND CLINICAL APPLICATION OF MNS16A (VNTR) POLYMORPHISM IN NORTH INDIAN LUNG CANCER PATIENTS

Submitted in partial fulfillment of the requirements for the award of a Degree of

Master of Technology

In

Biotechnology

Under the Supervision of:

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DECLARATION

I as a result of this declare that the work done in the seminar report entitled, "**Prognostic significance and clinical application of MNS16A (VNTR) polymorphism in North Indian lung cancer patients"** submitted towards partial fulfilment of the requirement for the award of **Master of Technology degree** in Biotechnology in the **Department of Biotechnology** at **Thapar Institute of Engineering and Technology, Patiala,** is an authentic record of work carried out by me under the supervision and guidance of Dr. Siddharth Sharma, Professor at the Department of Biotechnology in Thapar Institute of Engineering and Technology, Patiala.

I further declare that the contents of this report accurately represent my independent work and research and have not been submitted, wholly or in part, to any other university or institute for the award of any degree or diploma.

ISHMEET KAUR

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This is to certify that the above declaration made by the student concerned is correct to the best of my knowledge and belief.

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CERTIFICATE

This is to certify that the dissertation entitled, "**Prognostic significance and clinical application of MNS16A (VNTR) polymorphism in North Indian lung cancer patients",** submitted by **Ishmeet Kaur** in partial fulfilment of the requirements for the award of M.Tech in Biotechnology at Thapar Institute of Engineering and Technology, Patiala is an authentic work carried out by her under the supervision and guidance.

To the best of my knowledge, the matter embodied in the dissertation has not been submitted to any other university/institute for the award of any Degree or Diploma.

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ABSTRACT

Background: Telomerase is critical in maintaining telomere integrity in normal and cancer cells, with hTERT being a key factor in telomere biology. The activity of hTERT is directly connected to cellular ageing and the development of various health issues, including cancer. The MNS16A polymorphism in the hTERT gene has been identified as an essential factor influencing telomerase activity and linked to cancer susceptibility, further highlighting the complexity of telomere-related genetics in cancer research. Although prior studies have examined this association, the results have varied significantly across populations.

Purpose: We aimed to investigate the potential prognostic role of the MNS16A polymorphism in the hTERT gene among lung cancer patients in the North Indian Population.

Experimental Design: In this study, we genotyped 401 lung cancer samples, all undergoing platinum-based chemotherapy, for the MNS16A polymorphism using PCR to explore its association with lung cancer risk. Overall survival of lung cancer patients was evaluated using the univariate Kaplan-Meier method, while Cox regression analysis was used to calculate the adjusted hazard ratio. Stratified analyses were conducted to assess risks for subgroups based on various clinicopathological parameters, clinical outcomes, and toxicity, with odds ratios (ORs) and 95% confidence intervals (CIs) being calculated. A p-value of less than 0.05 was considered statistically significant in all analyses.

CHAPTER 1

INTRODUCTION

1) INTRODUCTION

Lung cancer, or bronchogenic carcinoma, arises from abnormal cell growth in the lung tissue or bronchial tubes. This condition is typically triggered by genetic mutations induced by factors like smoking or exposure to harmful substances. These genetic alterations disrupt the normal function of airway cells, leading to uncontrolled cell division and tumour formation. Unchecked lung tumour growth can impair their function, causing breathing difficulties and other respiratory symptoms. As the disease progresses, lung tumours can potentially metastasize, spreading to distant organs like the liver, brain, or bones. This metastatic spread poses a significant challenge in lung cancer treatment and often leads to more severe health complications. As per the statistics report of Globocan 2018, lung cancer is the most common cancer, causing a significant number of deaths. Among males, lung cancer leads to both incidence and mortality, followed by other types like prostate and colorectal cancer. In females, breast cancer tops the list in both cases, with lung cancer ranking high as well (Bray *et al*., 2018). In India, it constitutes 5.9% of all cancers and 8.1% of cancer-related deaths, with 80% of patients being smokers (Mohan *et al*., 2020). Unlike many high-income countries like the United States and Canada, where smoking rates have decreased, India has persistent high smoking rates and increasing numbers of smokers (Islami *et al*., 2015). Data from 28 population-based and 58 hospital-based cancer registries during 2012-2016 identified 22,645 lung cancer cases. Predictions anticipate a seven-fold rise to over 1.61 lakh cases by 2025, with 30,000+ cases in females and 81,000+ males (Kumar *et al*., 2022). This trend and delayed diagnoses are concerning, as highlighted in the Indian Journal of Medical Research.

Telomeres, protective caps at the ends of chromosomes, are essential for maintaining genetic stability (Artandi *et al*., 2005). They consist of repeating TTAGGG sequences and are guarded by a shelterin complex, which shields them from DNA damage responses (de Lange *et al*., 2005). Over time, as cells divide, telomeres naturally shorten, eventually reaching a critical length that triggers cellular senescence, and the process is called the “mitotic clock for ageing” (Blasco *et al*., 2005). Telomerase or terminal transferase activity in cancer cells prevents this shortening, allowing them to maintain their ability to proliferate indefinitely as they act as RNA-dependent DNA polymerase. This enzyme adds TTAGGG sequences onto chromosome ends, thus enabling cells to proliferate continuously (Greider *et al*., 1985).

Telomerase consists of two essential components: a functional RNA part known as hTERC, which is encoded by the TERC gene located at the 3q26 region of the chromosome, and a

catalytic reverse transcriptase called hTERT, which is encoded by the TERT gene located at chromosome 5p13.33 (Zhang *et al*., 2012). The hTERC segment acts as a template for synthesizing telomeric DNA, pivotal in maintaining chromosome integrity and stability. On the other hand, the hTERT gene encodes the catalytic subunit responsible for extending telomeres, thus preserving cellular longevity and function (Feng *et al*., 1995). While telomerase is naturally active in germline and stem cells, its heightened activity in cancer cells plays a significant role in tumorigenesis, including lung cancer (Shay & Wright 2011). New findings indicate that variations in the 5p15.33 region of the hTERT gene could influence cancer risk (Baird 2010). Abnormal changes in the hTERT gene can increase telomerase activity, a common trait in cancer cells. This can happen through gene amplification or hTERT promoter methylation, which promotes cancer cell immortality. Mutations in the hTERT promoter are linked to various cancers, including uroepithelial, bladder, squamous cell carcinomas, and glioblastoma. These mutations further boost telomerase activity, fueling cancer cell growth and survival (Leao *et al*., 2018).

MNS16A refers to polymorphic tandem repeat minisatellites found within the TERT gene. It is positioned downstream of exon 16 of the hTERT gene and upstream in the potential promoter region of an antisense hTERT transcript containing two repeat elements that form either a 23bp core sequence or a 26bp core sequence with a CAT insertion. This core sequence includes a binding site for the GATA-1 transcription factor. Based on the size of the polymerase chain reaction (PCR) fragments, researchers identified two variable numbers of tandem repeats (VNTRs) alleles known as VNTR-302 and VNTR-243. Other less common alleles, VNTR-272 and VNTR-333, were also observed in cancer cell lines. For statistical analysis, Wang *et al*. classified VNTR-243 and VNTR-272 as short (S) alleles and VNTR-302 and VNTR-333 as long (L) alleles. The MNS16A polymorphism, which involves a variable number tandem repeat (VNTR), has been studied in different contexts such as nasopharyngeal carcinoma (Zhang *et al*., 2011), colorectal cancer (Hofer *et al*., 2011), prostate cancer, bladder cancer (Diler *et al*., 2020) and even in normal human ageing (Concetti *et al*., 2013). Given the conflicting findings from earlier studies regarding the link between hTERT genomic variations and cancer risk, there is a pressing need to validate this relationship across various populations with different genetic backgrounds. The attention drawn to the association of hTERT genomic variation MN16A with tumour biology underscores the importance of conducting thorough investigations in diverse populations to understand these complex interactions comprehensively.

CHAPTER 2

REVIEW OF LITERATURE

2) REVIEW OF LITERATURE

2.1) Cancer

Malignant tumors develop from unchecked cell growth and proliferation, which is the hallmark of the complex and diverse disease known as cancer. With current research concentrating on genetic pathways, interactions between the tumor microenvironment and the body, and innovative therapeutic methods, our understanding of cancer has undergone a substantial evolution. Developments in molecular biology have provided important new information on the genetic and epigenetic changes that fuel the development and spread of cancer. Mutations in oncogenes (e.g., KRAS, EGFR) and tumour suppressor genes (e.g., TP53, PTEN) play critical roles in dysregulating cellular signalling pathways, promoting cell survival, proliferation, and evasion of apoptosis (Duffy *et al*., 2020). Additionally, alterations in DNA repair mechanisms, chromosomal instability, and epigenetic modifications contribute to tumour heterogeneity and therapeutic resistance (Vasan *et al*., 2019). The tumour microenvironment (TME) encompasses various cellular and non-cellular components that interact dynamically with cancer cells. Immune cells, such as T cells, B cells, natural killer (NK) cells, and myeloid-derived suppressor cells (MDSCs), exert both pro-tumorigenic and anti-tumorigenic effects within the TME (Chen & Mellman, 2017). Immune checkpoint molecules, including programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), regulate immune responses and have become targets for immunotherapy in various cancers (Ribas & Wolchok, 2018). Cancer progresses through distinct stages, including initiation, promotion, progression, and metastasis (Hanahan & Weinberg, 2011). (A) Mutations, alterations, or changes in genes that arise naturally or are brought on by exposure to carcinogenic agents constitute initiation. A number of variables, such as the kind and rate of carcinogenic metabolism and the response of the DNA repair function, can affect how dysregulated biochemical signaling pathways related to cellular proliferation, survival, and differentiation are caused by genetic changes. (B) Preneoplastic cells that are actively multiplying accumulate during the rather long and reversible promotion stage. During this time, chemopreventive drugs can change the process and impact growth rates. The stage that separates the onset of an invasive cancer from a premalignant lesion is known as progression. (C) Progression is the last phase of neoplastic transformation, characterized by cell proliferation as well as phenotypic and genetic alterations. This results in a rapid enlargement of the tumor, where other mutations that have the potential to spread and become invasive may occur in the cells. Chemopreventive drugs need to exhibit

preferential action at the start and promotion stages of carcinogenesis. (D) Metastasis is the process by which cancer cells travel from their original location to different bodily areas via the lymphatic or circulatory systems. Chemopreventive drugs have been shown to prevent angiogenesis and the invasion of primary tumors, which may decrease the spread of cancer (Steeg, 2016).

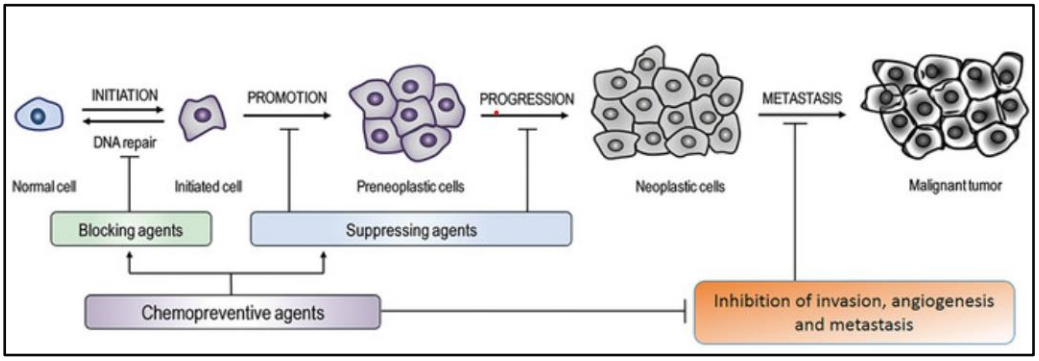

Figure 1.1: Schematic representation of Carcinogenesis phases: initiation, promotion, progression, and metastasis (Yang *et al*., 2019).

Cancer treatment strategies encompass a range of modalities, including surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy. Surgical resection aims to remove localized tumours, while chemotherapy and radiation therapy target rapidly dividing cancer cells (Kurtova *et al*., 2019). Targeted therapies, such as tyrosine kinase inhibitors (TKIs) and monoclonal antibodies, selectively inhibit specific molecular pathways aberrantly activated in cancer cells (Flaherty *et al*., 2019). Immunotherapy, remarkably immune checkpoint inhibitors and adoptive cell therapies harness the immune system to recognize and eliminate cancer cells (Larkin *et al*., 2019). Cancer biology and treatment research focuses on several emerging areas. These include the development of novel targeted therapies based on genomic profiling and biomarker identification, exploration of combination therapies to overcome treatment resistance and enhance efficacy, implementation of precision medicine approaches using liquid biopsy and circulating tumour DNA (ctDNA) analysis, and investigation of immune-based strategies such as cancer vaccines and chimeric antigen receptor (CAR) T-cell therapy (Schwaederle *et al*., 2019; Yang *et al*., 2019).

2.2) Lung and its anatomy

The purpose of the lung is to provide oxygen to the blood. The respiratory system divides into airways and lung parenchyma The trachea bifurcates into the bronchus, which further divides into bronchioles and alveoli, making up the airways. The parenchyma, which is made up of

the bronchioles, alveolar ducts, and alveoli, is in charge of gas exchange. Lungs are pinkish-grey in color and have a spongy texture. They also have three edges, three surfaces, and an apex according to anatomical descriptions. They further split into segments and lobes. A pleura also covers the lung parenchyma (Tucker *et al*., 2023) (Burlew *et al*., 2023).

ANATOMY: The lungs are situated in the rib cage in the chest, either side of the heart. They have a large concave base that sits on the convex surface of the diaphragm and a small, rounded apex at the top, making them conical (Richard 2014). The apex of the lung reaches into the root of the neck, just above the level of the first rib's sternal end. The cardiac notch of the left lung is an indentation in the lung's border that allows the left lung and the heart to share space (Betts *et al*., 2013). The ribs form slight indentations on the surfaces of the front and outer sides of the lungs. The lungs' medial surfaces, which face the chest's center, are in contact with the heart, major blood vessels, and the carina, which is where the trachea splits into the two primary bronchi.An indentation created on the surfaces of the lungs where they rest against the heart is known as the cardiac impression. The blood arteries and airways that enter the lungs through the hilum, a central recession seen in both lungs, constitute the lung's root (Burlew *et al*., 2023). Additionally, the hilum has bronchopulmonary lymph nodes (Standring, Susan 2008). The pulmonary pleurae round the lungs. The inner visceral pleura directly lines the surface of the lungs, while the outer parietal pleura lines the inner wall of the rib cage. The pleurae are two types of serous membranes. There is a possible space between the pleurae known as the pleural cavity, which is filled with a thin layer of lubricating pleural fluid.

a. Lobes

The visceral pleura's infoldings as fissures separate each lung into pieces known as lobes. Segments of lobes are further split into lobules within segments. The right lung has three lobes, while the left lung has two.

b. Cracks

According to Koster TD *et al*. (2016), the fissures are created during the early stages of fetal development by invasions of the visceral pleura, which split the lungs into lobes and separate the lobar bronchi. There are two lobes in the right lung: an oblique fissure and a horizontal fissure. An oblique fissure that is nearly aligned with the oblique fissure in the right lung divides the left lung into two lobes. The right lung's middle lobe and higher (superior) lobe are divided by the upper horizontal fissure. The lower lobe is divided from the middle and higher lobes by the lower, oblique fissure (Standring, Susan 2008).

c. Sections

Air is supplied to each lung lobe by the secondary bronchi, often referred to as lobar bronchi, which originate from the principal bronchi that enter the lungs at the hilum. The lobar bronchi divide into tertiary bronchi, often referred to as segmental bronchi, which provide air to the lobes' further divisions, or bronchopulmonary segments. Every section of the bronchopulmonary system has its own vascular supply and bronchus (Tortora, Gerard 1987). A segment is a separate unit that can be surgically removed without causing significant harm to the tissue around it.

d. The Right Lung

Compared to the left lung, the right lung has more segments and lobes. It is separated into two oblique and one horizontal lobe, as well as an upper, middle, and lower lobe (Chaudhry and Bordoni 2019). The middle lobe and upper lobe are divided by the upper, horizontal fissure. It starts in the lower oblique fissure close to the lung's posterior border and travels forward horizontally, cutting the anterior border at the level of the sternal end of the fourth costal cartilage. It can be followed back to the hilum on the mediastinal surface. In close alignment with the oblique fissure in the left lung, the lower oblique fissure divides the lower from the middle and upper lobes (Richard 2014).

The right lung's weight varies depending on the individual; conventional reference ranges for men and women are 155–720 g (0.342–1.587 lb) and 100–590 g (0.22–1.30 lb), respectively (Molina, D *et al*., 2015).

e. The Left lung

The left lung is divided into an upper and lower lobe by the oblique fissure, which extends from the costal surface to the mediastinal surface of the lung, passing above and below the hilum. In contrast to the right, the left lung lacks a middle lobe, although it does have a homologous structure called the lingula, which is an upper lobe projection (Burlew *et al*., 2023). The term "little tongue" refers to it. Similar infections and anatomic difficulties are common in both the left lung's lingula and the right lung's middle lobe, which are anatomically parallel (Tortora and Gerard 1987). The superior and inferior bronchopulmonary segments comprise the lingula. As per Molina *et al*. (2015), the weight of the left lung in men is 110–675 g (0.243–1.488 lb)[13] and in women, it is 105–515 g (0.231–1.135 lb) according to the conventional reference range.

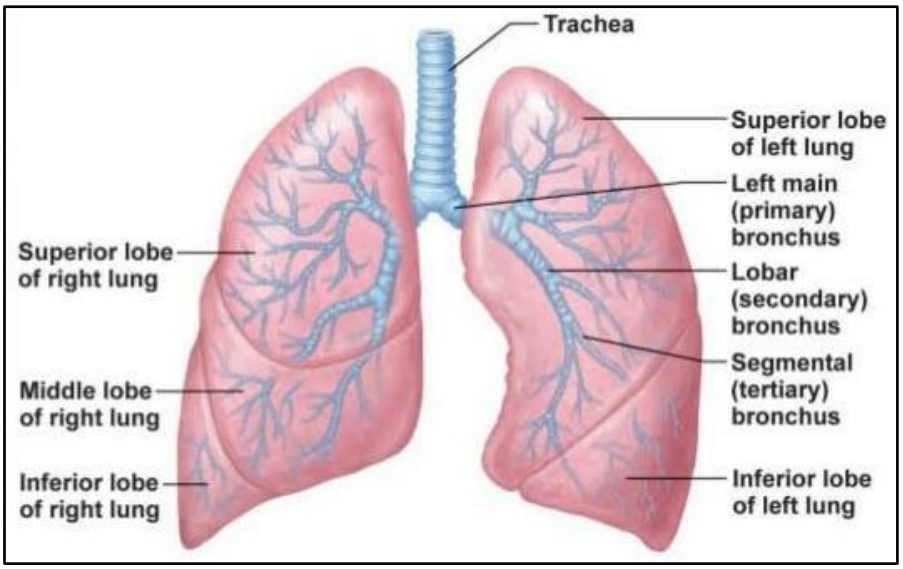

Figure 1.2: Labeled illustration of the right lung (right) and left lung (left) (Peng *et al*., 2021).

2.3) Lung carcinoma

Lung carcinoma is a type of cancer originating in the lung tissues. It is a significant health concern globally due to its high prevalence and association with tobacco smoking. However, it can also occur in non-smokers due to various other risk factors such as exposure to environmental pollutants, genetic predisposition, and occupational hazards like asbestos. Non-small cell lung cancer (NSCLC) and small cell lung carcinoma (SCLC) are the two main forms of lung cancer; approximately 85% of instances of lung cancer are NSCLC (American Cancer Society, 2022). There are various subtypes of non-small cell lung cancer (NSCLC), including adenocarcinoma, squamous cell carcinoma, and giant cell carcinoma. Each has unique traits and therapeutic modalities. The most common subtype of non-small cell lung cancer (NSCLC) is adenocarcinoma, which typically affects the outer lung regions. It is frequently diagnosed in non-smokers and is associated with genetic mutations such as EGFR (epidermal growth factor receptor) and ALK (anaplastic lymphoma kinase) alterations, which have implications for targeted therapy (Cancer.Net, 2022). Squamous cell carcinoma is closely associated with smoking and usually develops in the middle section of the lung (National Cancer Institute, 2022). Although less prevalent, large cell carcinoma has a propensity to grow and spread quickly. Despite being less common than NSCLC, SCLC is distinguished by its aggressiveness and quick growth. It usually starts in the bronchi and quickly spreads to other body parts, including distant organs. SCLC is highly responsive to chemotherapy but often recurs,

necessitating ongoing treatment strategies. Diagnosis of lung carcinoma involves various methods such as imaging tests (X-rays, CT scans, PET scans), biopsies, and molecular testing to determine the specific subtype and genetic mutations present. Treatment options depend on the patient's stage, subtype, and overall health. These could involve immunotherapy, targeted therapy, chemotherapy, radiation therapy, surgery, or a mix of these treatments (American Cancer Society, 2022; Cancer.Net, 2022; National Cancer Institute, 2022). Despite advancements in treatment, lung carcinoma remains a challenging disease with significant mortality rates, emphasizing the importance of early detection, smoking cessation, environmental awareness, and ongoing research into novel therapies.

2.4) Global epidemiology of lung cancer

Lung cancer represents a substantial global health burden, with its epidemiology revealing significant trends and challenges across different regions and populations. The incidence and mortality rates of lung cancer vary widely worldwide, primarily due to differences in smoking prevalence, environmental exposures, healthcare infrastructure, and genetic predispositions. In developed countries such as the United States, Western Europe, and Australia, lung cancer is a leading cause of cancer-related deaths among both men and women, with estimated age-standardized incidence rates ranging from 30 to 100 cases per 100,000 population per year (American Cancer Society, 2022). These regions historically had high smoking rates, contributing significantly to the prevalence of lung cancer. However, concerted efforts in tobacco control, public health campaigns, and smoking cessation programs have led to a decline in smoking rates and, subsequently, a decrease in lung cancer incidence in some segments of the population. Conversely, in many developing countries and regions with emerging economies, lung cancer rates continue to rise due to increasing tobacco use, urbanization, industrialization, and exposure to indoor and outdoor air pollution (World Health Organization, 2022). For instance, in China and India, where smoking rates have been on the rise, the age-standardized incidence rates of lung cancer are approximately 50 cases per 100,000 population per year and 15 cases per 100,000 population per year, respectively (Ferlay *et al.*, 2021).

Furthermore, disparities in lung cancer outcomes are evident across different demographic groups. For instance, specific ethnicities, such as African Americans in the United States, experience higher lung cancer incidence and mortality rates compared to other racial groups (American Cancer Society, 2022). Socioeconomic factors, including access to healthcare,

education, and employment opportunities, play a crucial role in these disparities. The lack of comprehensive tobacco control policies and limited access to healthcare exacerbate the challenges in managing and preventing lung cancer in these areas. Furthermore, disparities in lung cancer outcomes are evident across different demographic groups. For instance, specific ethnicities, such as African Americans in the United States, experience higher lung cancer incidence and mortality rates compared to other racial groups (American Cancer Society, 2022). Socioeconomic factors, including access to healthcare, education, and employment opportunities, play a crucial role in these disparities.

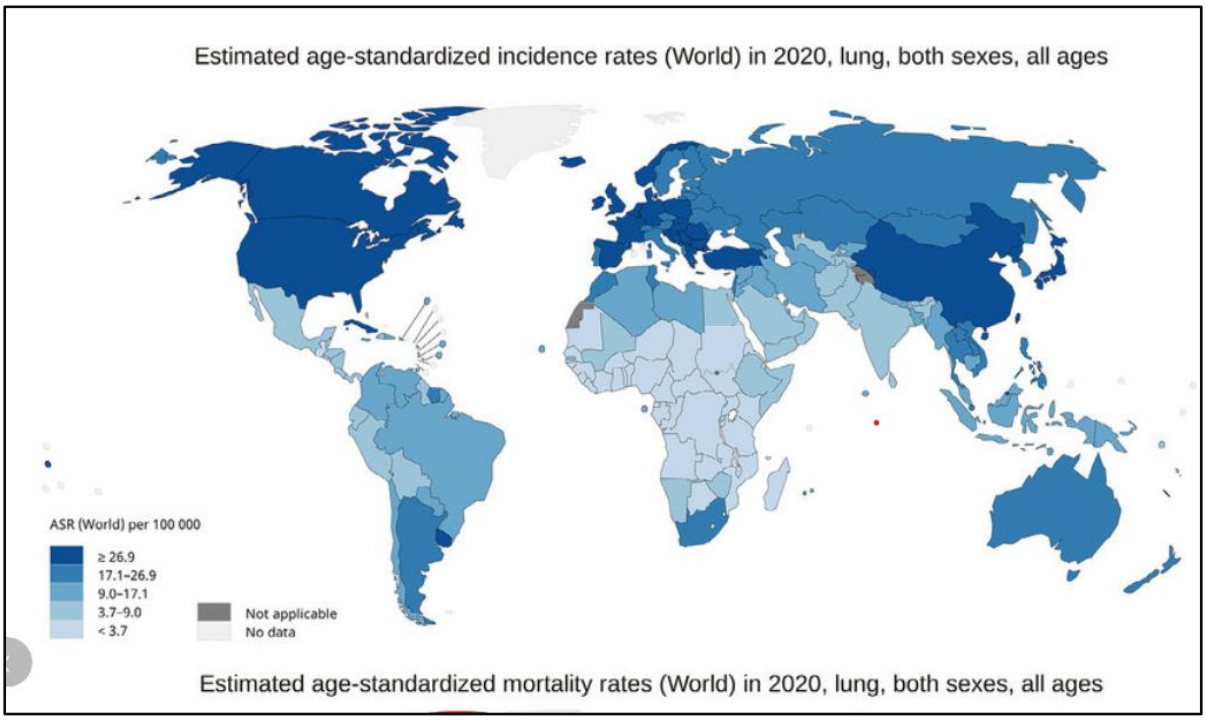

Figure 1.3: Map showing the global estimated incidence and mortality of lung cancer in 2020, both sexes and all ages. The International Agency produced the Map for Research on Cancer (IARC) and the World Health Organization (WHO). The materials and names provided in this study do not represent any opinion of WHO or IARC regarding the legal status of any country, territory, city, region, or its authorities or the delimitation of its borders. Dashed lines characterised approximate borderlines that may not yet fully agree (Bolun *et al.*, 2022).

Advancements in research have also shed light on lung cancer's molecular and genetic aspects, leading to personalized treatment approaches based on specific biomarkers and mutations (Herbst *et al.*, 2018). Targeted therapies and immunotherapies have shown promise in

improving outcomes for specific subsets of lung cancer patients, highlighting the importance of precision medicine in oncology. In conclusion, the global epidemiology of lung cancer underscores the complex interplay of behavioural, environmental, socioeconomic, and genetic factors in disease occurrence and outcomes. Addressing these multifaceted challenges requires a comprehensive approach encompassing tobacco control, environmental regulations, equitable healthcare access, and continued research into innovative treatment strategies.

2.5) Indian epidemiology of lung cancer

The GLOBOCAN 2012 report revealed that lung cancer's estimated incidence in India was 70,275 cases across all ages and genders, with a crude incidence rate of 5.6 per 100,000 population and an age-standardized rate of 6.9 per 100,000 (world). Lung cancer ranked as the fourth most common cancer overall (excluding nonmelanoma skin cancer), following breast, cervical, and oral cavity cancers. Among males, it stood as the second most common cancer, while among females, it ranked sixth in terms of incidence. In terms of mortality, lung cancer was the third leading cause of cancer-related deaths in India in 2012, with an estimated 63,759 deaths. Among males, it was the leading cause of cancer mortality, accounting for 48,697 deaths, while among females, it ranked seventh. These figures reflect a significant burden of lung cancer on India's healthcare system and public health. The male-to-female ratio for lung cancer in India was reported as 4.5:1, with this ratio varying by age and smoking status. Notably, the ratio increased with age until the 51–60 age group, after which it stabilized. Smokers outnumbered nonsmokers significantly, with a ratio of 20:1. The predominant histological type differed based on smoking status, with squamous cell carcinoma being common among smokers and adenocarcinoma among nonsmokers.

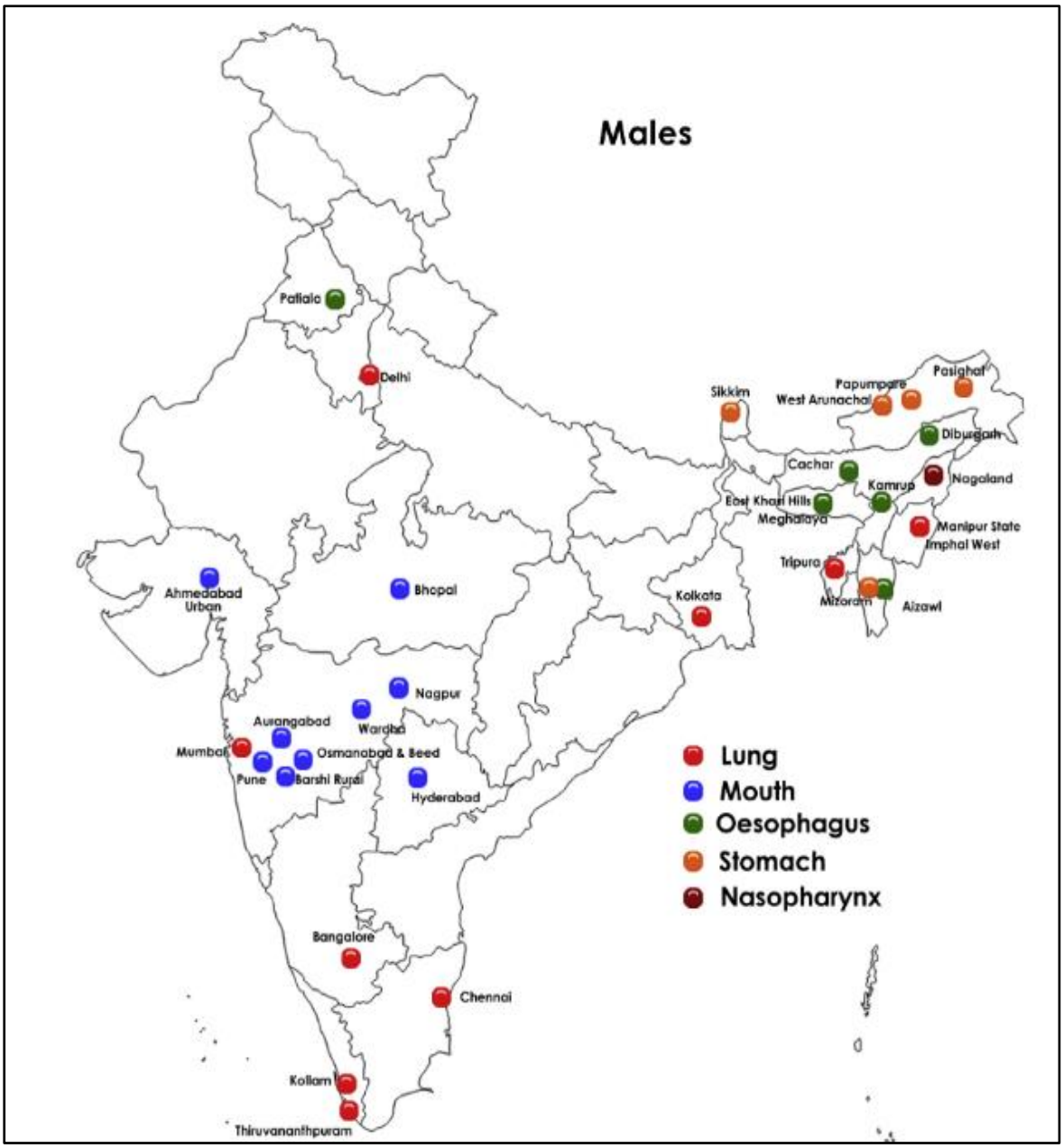

Figure 1.4: Map showing that Lung cancer is the leading site of cancer for males in India registered by the NCRP coordinated by the ICMR-NCDIR.

Table 1. Demographics and Key Statistics of India (India Fact Sheet. National Family Health Survey (NFHS)-4, Ministry of Health and Family Welfare Government of India. 2015–2016).

Demographic Variable	Numerical Value
Total population	1.38 billion[a]
Urban population	34.5%[a]
Life expectancy	70.42 y[a]
Languages	216
Sex ratio	924 females per 1000 males[b]
Nominal GDP	$3.202 trillion[c]
PPP	$11.33 trillion[c]
Total health care expenditure	3.6% of GDP
Public health expenditure	1.29% of GDP
Health insurance coverage	20% of women and 23% of men[d]
Doctor-population ratio	1:1456 (WHO recommendation 1:1000)
Noncommunicable diseases	60% of all deaths
Tobacco use	28.6% of adults
GLOBOCAN India statistics 2018[S1]	
Number of new cancer cases	1.16 million
Cancer deaths	784,821
Number of prevalent cancer cases (5-y)	2.26 million
Lung cancer	5.9% of all cancer cases (fourth most common)
Lung cancer incidence	67,795
Lung cancer mortality	63,475 (8.1% of all cancer deaths)
Projected incidence, 2020[S2]	
All sites	1,392,179
Males	679,421
Females	712,758
Lung cancer	98,278
Males	71,788
Females	26,490

The historical data on lung cancer in India underscored the impact of industrialization and smoking trends on disease prevalence. Initial perceptions of lung cancer rarity were dispelled in the 1960s, with increasing cases noted, particularly among male smokers. Tobacco smoking emerged as the primary risk factor, contributing to 80–90% of cases, while occupational exposures accounted for 10–20% (Viswanathan *et al.,* 1962). In recent years, the epidemiological pattern has shifted, mirroring trends seen in Western countries, with adenocarcinoma becoming more prevalent, especially among nonsmokers and women. Pathological analyses from tertiary cancer centres corroborated this shift, emphasizing the role of histological subtyping in personalized treatment approaches (Ferlay *et al.,* 2008). The variability in lung cancer epidemiology across India was evident in data from different cancer registries. Population-based studies highlighted regional differences in incidence rates and histological subtypes. Notably, lung cancer emerged as a significant cancer site in several major cities, necessitating targeted interventions and improved cancer surveillance strategies.

In conclusion, the epidemiology of lung cancer in India reflects a dynamic landscape shaped by historical trends, smoking patterns, and evolving histological profiles. Continued research and public health initiatives are vital to address the challenges posed by lung cancer and reduce its impact on Indian populations.

2.6) Etiology of lung cancer

2.6.1) Tobacco Smoking

Tobacco smoking is widely recognized as the single most significant risk factor for lung cancer in India. The prevalence of smoking, coupled with various smoking-related behaviours and exposures, contributes significantly to the burden of lung cancer in the country.

Prevalence of Smoking

1. **Male Dominance**: Historically, smoking prevalence has been higher among males in India, leading to a male-to-female smoking ratio of approximately 4.5:1 (Global Adult Tobacco Survey, India).
2. **Trends in Smoking**: Despite ongoing public health campaigns and regulatory efforts, the prevalence of smoking remains a concern. The Global Adult Tobacco Survey (GATS) data indicates that X% of Indian adults smoke tobacco products (GATS India Report).
3. **Types of Tobacco Products**: Tobacco consumption in India encompasses various forms, including cigarettes, bidis, hookah, and smokeless tobacco products like gutka and paan masala (Indian Journal of Cancer).

Impact on Lung Cancer

1. **Strong Association**: Studies have consistently shown a strong dose-response relationship between tobacco smoking and lung cancer risk in India (Indian Journal of Chest Diseases and Allied Sciences).
2. **Smoker-Nonsmoker Ratio**: The smoker-to-nonsmoker ratio for lung cancer cases in India varies but generally indicates a higher proportion of smokers among lung cancer patients, with ratios ranging from 15:1 to 20:1 in different studies (Journal of Epidemiology and Global Health).

3. **Secondhand Smoke**: Exposure to secondhand smoke, both at home and in public spaces, also contributes to lung cancer incidence, particularly among nonsmokers (Environmental Health Perspectives).

2.6.2) Tobacco Smoke Carcinogens

Tobacco smoke is a highly complex mixture containing over 7000 chemicals, many of which are known to be harmful to human health. Among these compounds, at least 250 are recognized as toxic, and more than 70 are identified as carcinogenic, contributing significantly to the development of various types of cancer. Polycyclic aromatic hydrocarbons (PAHs) are prominent carcinogens found in tobacco smoke, with benzo[a]pyrene being one of the most extensively studied. PAHs can exert their carcinogenic effects by binding to DNA, inducing mutations, and disrupting cellular processes involved in DNA repair and replication, ultimately promoting cancer initiation (Hecht, 2002; IARC, 2004). Nitrosamines are another group of potent carcinogens present in tobacco smoke, including N-nitrosamine compounds like N-nitrosodiethylamine (NDEA) and N-nitrosopyrrolidine (NPYR). These compounds are formed during tobacco curing and smoking processes and have been linked to the development of various cancers, particularly lung and gastrointestinal tract cancers (IARC, 2012).

Moreover, aromatic amines such as 4-aminobiphenyl and 2-naphthylamine found in tobacco smoke have been strongly associated with bladder cancer risk, highlighting the diverse mechanisms through which tobacco smoke constituents can contribute to carcinogenesis (Vineis *et al.,* 2005). In addition to PAHs, nitrosamines, and aromatic amines, tobacco smoke contains volatile organic compounds (VOCs) like formaldehyde, acetaldehyde, and acrolein, which have been classified as probable human carcinogens by the International Agency for Research on Cancer (IARC). These VOCs can damage cellular macromolecules, induce oxidative stress, and promote inflammation, further exacerbating the carcinogenic effects of tobacco smoke exposure (CDC, 2021; IARC, 2019). The cumulative impact of these carcinogens underscores the urgent need for comprehensive tobacco control strategies, including smoking cessation programs, public health campaigns, and regulatory measures to reduce tobacco-related morbidity and mortality worldwide (WHO, 2021).

2.6.3) Beedi Smoke Carcinogens

Beedi smoke, like tobacco smoke, contains a plethora of carcinogens that pose significant health risks to individuals who use them. Beedis are thin, hand-rolled cigarettes made of tobacco wrapped in a tendu or tempura leaf, commonly used in South Asia. Despite their smaller size compared to traditional cigarettes, beedis deliver a concentrated dose of toxic compounds and carcinogens, contributing to the development of various cancers. One of the primary carcinogenic components in Beedi smoke is nicotine, a highly addictive substance that stimulates the release of adrenaline and dopamine, leading to dependence. Nicotine is not a carcinogen but can promote tumour growth and metastasis by affecting cell proliferation and angiogenesis pathways (Goniewicz *et al.,* 2014).

Moreover, beedi smoke contains significant amounts of polycyclic aromatic hydrocarbons (PAHs), including benzo[a]pyrene and dibenz[a,h]anthracene, which are known to cause DNA damage and initiate carcinogenesis (IARC, 2012). In addition to PAHs, beedi smoke contains volatile organic compounds (VOCs) such as formaldehyde, acetaldehyde, and acrolein, which can induce oxidative stress, inflammation, and DNA damage, contributing to the progression of cancer (Lee *et al.,* 2019). Furthermore, beedis often undergo incomplete combustion, leading to the formation of toxic compounds like carbon monoxide and nitrogen oxides, which can further exacerbate cellular damage and increase cancer risk (Kulkarni *et al.,* 2017). The unique composition of beedi smoke, coupled with the socio-economic factors that influence its widespread use in specific populations, underscores the urgent need for targeted interventions to reduce beedi-related health hazards. Public health initiatives focusing on smoking cessation, awareness campaigns about the dangers of beedi smoking, and regulatory measures to control its production and distribution are crucial steps in mitigating the burden of cancer and other smoking-related diseases associated with beedi consumption (Sankaranarayanan *et al.,* 2014).

2.6.4) Passive Smoking

Passive smoking, also known as secondhand smoke (SHS) or environmental tobacco smoke (ETS), refers to the involuntary inhalation of tobacco smoke by non-smokers in the vicinity of smokers. While non-smokers do not directly consume tobacco, they are exposed to a complex mixture of toxic and carcinogenic compounds present in secondhand smoke, which can have detrimental effects on their health. One of the most concerning aspects of passive smoking is its association with an increased risk of cancer. Secondhand smoke contains numerous

carcinogens, including polycyclic aromatic hydrocarbons (PAHs) like benzo[a]pyrene, which can cause DNA damage and mutations, leading to cancer development (IARC, 2004). Additionally, exposure to secondhand smoke can elevate levels of volatile organic compounds (VOCs) such as benzene, formaldehyde, and acetaldehyde, all of which have been classified as carcinogens by the International Agency for Research on Cancer (IARC, 2010). Nitrosamines, another group of potent carcinogens found in tobacco smoke, are also present in secondhand smoke. These compounds, including N-nitrosamine, have been linked to various cancers, including lung, bladder, and gastric cancers, highlighting the broad spectrum of cancer risks associated with passive smoking (Oberg *et al.,* 2011).

Moreover, exposure to secondhand smoke can have immediate health effects, such as respiratory irritation, exacerbation of asthma symptoms, and increased risk of cardiovascular diseases. Children exposed to passive smoking are particularly vulnerable, facing higher risks of sudden infant death syndrome (SIDS), respiratory infections, and impaired lung function (USDHHS, 2020). The detrimental health effects of passive smoking underscore the importance of implementing smoke-free policies in public spaces, workplaces, and homes to protect non-smokers from involuntary exposure to tobacco smoke. Public health campaigns promoting awareness about the dangers of secondhand smoke and supporting smoking cessation efforts among smokers are also crucial in reducing the burden of cancer and other smoking-related diseases associated with passive smoking (WHO, 2021).

2.6.5) Alcohol and Diet

The relationship between alcohol consumption, diet, and lung cancer risk is a multifaceted topic that involves various dietary factors and lifestyle choices. While smoking remains the primary risk factor for lung cancer, emerging evidence suggests that specific dietary patterns and alcohol consumption may also play a role in modifying the risk of developing this disease. Alcohol consumption has been linked to an increased risk of several cancers, including lung cancer. Ethanol, the primary component of alcoholic beverages, can be metabolized into acetaldehyde, a known carcinogen that can cause DNA damage and interfere with cellular repair mechanisms (Secretan *et al.,* 2009). Chronic alcohol consumption also contributes to oxidative stress and inflammation, which are implicated in cancer development (Seitz and Becker, 2007). While the exact mechanisms linking alcohol to lung cancer are complex and multifactorial, epidemiological studies have shown a positive association between alcohol intake and lung cancer risk, particularly among heavy drinkers (Bagnardi *et al.,* 2015).

Dietary factors like fruit and vegetable consumption and certain micronutrients related to lung cancer risk have been studied. Diets rich in fruits and vegetables, which are sources of antioxidants, vitamins, and phytochemicals, have been associated with a reduced risk of lung cancer (World Cancer Research Fund, 2021). Antioxidants such as vitamin C, E, and beta-carotene can help neutralize free radicals and protect cells from oxidative damage, potentially lowering the risk of carcinogenesis (Stryker and Emmons, 2000). Conversely, diets high in red and processed meats, which contain heterocyclic amines (HCAs) and polycyclic aromatic hydrocarbons (PAHs) formed during cooking processes, have been linked to an increased risk of lung cancer (Cross *et al.,* 2014). HCAs and PAHs can induce DNA damage and promote tumour growth, contributing to the development of various cancers, including lung cancer. Overall, while smoking remains the primary risk factor for lung cancer, adopting a healthy diet rich in fruits and vegetables while minimizing alcohol consumption may contribute to a lower risk of developing lung cancer. Public health initiatives promoting healthy lifestyle choices, smoking cessation programs, and dietary interventions may play a crucial role in reducing the burden of lung cancer globally.

2.6.6) Air Pollution

Air pollution is a significant environmental risk factor that has been linked to various adverse health outcomes, including an increased risk of lung cancer. Key pollutants associated with lung cancer development include particulate matter (PM), nitrogen oxides (NOx), sulfur dioxide (SO2), ozone (O3), and volatile organic compounds (VOCs). Fine particulate matter (PM2.5) is particularly concerning as it can infiltrate the lungs and bloodstream, leading to inflammation, oxidative stress, and DNA damage. Prolonged exposure to high levels of PM2.5, especially in urban areas with significant air pollution, has been consistently linked to an elevated risk of lung cancer (Hamra *et al*., 2014). Nitrogen oxides, largely produced by vehicle emissions and industrial activities, can combine with other atmospheric chemicals to create secondary pollutants such as nitrosamines, which are known to be potent carcinogens.

Long-term exposure to nitrogen oxides has been associated with an elevated risk of lung cancer, especially among individuals living near busy roadways or industrial zones (Vineis *et al.,* 2014). Sulfur dioxide, a byproduct of burning fossil fuels, can also contribute to lung cancer risk through its role in forming sulfate aerosols and other particulate matter that can be inhaled into the lungs.

Additionally, sulfur dioxide can interact with other pollutants to form secondary pollutants like sulfates and nitrates, further exacerbating the health effects of air pollution (Beelen *et al.,* 2014). Ozone is created as a secondary pollutant through the reaction of sunlight with nitrogen oxides and VOCs, can cause airway inflammation and oxidative stress, leading to respiratory symptoms and potentially increasing the susceptibility to lung cancer (Jerrett *et al.,* 2013). Volatile organic compounds (VOCs), emitted from various sources such as vehicle exhaust, industrial processes, and household products, can contribute to the formation of secondary organic aerosols and other toxic compounds that can damage lung tissue and increase cancer risk (Cohen *et al.,* 2017).

Overall, the evidence linking air pollution to lung cancer is substantial, highlighting the importance of mitigating air pollution through regulatory measures, cleaner technologies, urban planning strategies, and public health interventions to reduce the burden of lung cancer and other respiratory diseases associated with poor air quality (WHO, 2021).

2.6.7) Occupational exposure

Occupational exposure to various carcinogens and hazardous substances is a well-established risk factor for lung cancer. Workers in specific industries may encounter a range of carcinogenic agents, including asbestos, silica dust, diesel exhaust, arsenic, and cadmium, among others, which can contribute to the development of lung cancer through different mechanisms.

1) **Asbestos,** widely used in construction and insulation materials, is a known human carcinogen associated with lung cancer, mesothelioma, and other respiratory diseases. Inhalation of asbestos fibres can lead to chronic inflammation, fibrosis, and genetic damage, increasing the risk of lung cancer, especially among workers in asbestos mining, manufacturing, and removal industries (Stayner *et al.,* 2013).
2) **Silica dust**, generated from mining, quarrying, and sandblasting, is another occupational carcinogen linked to lung cancer. Prolonged inhalation of silica particles can cause silicosis, lung inflammation, and scarring, predisposing workers to lung cancer development (Hnizdo and Vallyathan, 2003).
3) **Diesel exhaust emissions,** standard in transportation, mining, and construction sectors, contain a complex mixture of particulate matter and carcinogens such as benzene, formaldehyde, and polycyclic aromatic hydrocarbons (PAHs). Chronic exposure to diesel

exhaust has been associated with an elevated risk of lung cancer among exposed workers, emphasizing the importance of occupational safety measures and emission controls (IARC, 2012).

4) Furthermore, occupational exposure to **heavy metals like arsenic and cadmium,** prevalent in industries such as mining, smelting, and battery manufacturing, can contribute to lung cancer risk. These metals can induce oxidative stress and DNA damage, disrupt cellular signalling pathways, and promote lung carcinogenesis (Olson *et al.,* 2017).
5) Occupational exposure to **ionizing radiation,** such as radon gas in mines or medical settings, is also a significant risk factor for lung cancer. Radon exposure can lead to DNA mutations and cellular damage, increasing the likelihood of lung cancer development, particularly among miners and workers in radiological occupations (Darby *et al.,* 2005).

In conclusion, occupational exposure to carcinogens and hazardous substances poses a substantial risk of lung cancer among workers in various industries. Implementing strict occupational safety regulations, providing adequate personal protective equipment, conducting regular risk assessments, and promoting workplace health programs are essential strategies to minimize the occupational burden of lung cancer and protect workers' health (NIOSH, 2021).

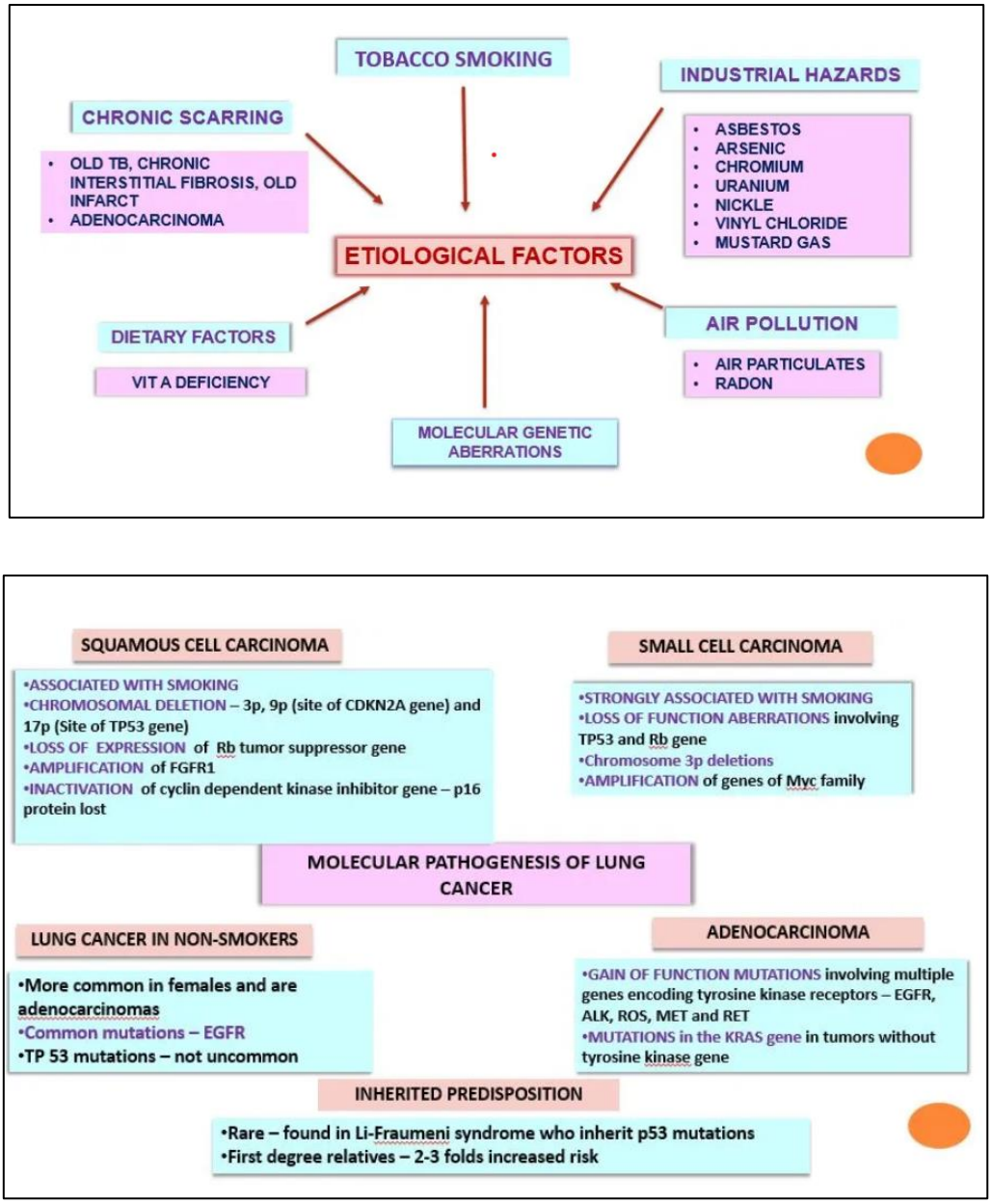

Figure 1.5: (A) Various factors affecting Lung cancer (B) Molecular Pathogenesis of Lung Cancer (Anna *et al.*, 2000)

2.7) Histopathology of Lung Cancer

Lung cancer, a complex and heterogeneous disease, manifests through various histopathological subtypes, each presenting unique morphological characteristics and clinical implications. Histopathology is crucial in diagnosing and subtyping lung cancer, guiding treatment decisions, and predicting patient outcomes. Understanding the histopathological features of lung cancer subtypes is essential for oncologists, pathologists, and healthcare professionals involved in the management of this challenging malignancy. This discussion

delves into the diverse histopathological manifestations of lung cancer, encompassing adenocarcinoma, squamous cell carcinoma, small cell carcinoma, large cell carcinoma, and pulmonary carcinoid tumours, highlighting their distinctive histological traits, molecular underpinnings, and diagnostic significance **(Figure 1.7 and 1.8).** Through a comprehensive examination of lung cancer histopathology, we aim to elucidate the intricate landscape of this disease and its implications for clinical practice and research endeavours.

2.7.1) Lung Cell carcinoma (LCC)

Large cell lung carcinoma (LCLC) represents a heterogeneous group of tumours within the spectrum of lung cancers, characterized by their poorly differentiated and often aggressive nature. Histologically, LCLC is defined by the absence of clear differentiation towards specific cell types seen in other lung cancer subtypes such as adenocarcinoma or squamous cell carcinoma (Pelosi *et al*., 2010). Instead, LCLC tumours exhibit large pleomorphic cells with prominent nucleoli, high mitotic activity, and variable growth patterns (Travis *et al*., 2015). The lack of characteristic features in other subtypes makes LCLC a diagnosis of exclusion, requiring thorough histopathological evaluation and immunohistochemical staining for accurate classification. Within the category of LCLC, two significant subtypes merit distinction: large cell neuroendocrine carcinoma (LCNEC) and basaloid carcinoma. LCNEC shares histological similarities with small cell carcinoma (SCLC) but lacks the typical neurosecretory granules in SCLC cells (Pelosi *et al*., 2010). Instead, LCNEC displays neuroendocrine differentiation with more giant pleomorphic cells and a higher mitotic rate than typical carcinoids (Travis *et al*., 2015). Immunohistochemical markers such as synaptophysin and chromogranin are commonly used to confirm neuroendocrine differentiation in LCNEC.

On the other hand, basaloid carcinoma within the LCLC spectrum is characterized by a basaloid growth pattern, with cells arranged in nests or cords surrounded by a fibrous stroma (Pelosi *et al*., 2010). These tumours often demonstrate high-grade features, including nuclear pleomorphism, high mitotic activity, and areas of necrosis (Travis *et al*., 2015). Immunohistochemical staining for basal cell markers like p63 and cytokeratin 5/6 can aid in confirming the diagnosis of basaloid carcinoma and differentiating it from other lung cancer subtypes. The 2015 World Health Organization (WHO) classification of lung tumours provides a comprehensive framework for understanding LCLC histopathology and its subtypes, emphasizing the importance of immunohistochemical markers in subtype classification (Travis *et al*., 2015). Advances in molecular pathology have also revealed genetic alterations in LCLC,

including mutations in genes such as TP53 and KRAS, which may have implications for targeted therapies and personalized treatment approaches. By elucidating the histopathological features and subtyping criteria of LCLC, this discussion aims to enhance our understanding of this complex lung cancer subtype and its clinical implications, guiding oncologists and pathologists in accurate diagnosis, prognostication and treatment decision-making.

2.7.2) Adenocarcinoma (ADCC)

Adenocarcinoma, the most common histological subtype of lung cancer, is characterized by glandular or acinar growth patterns and a diverse range of histopathological features. This subtype is associated with various risk factors, including smoking and non-smoking-related factors, and exhibits distinct molecular signatures influencing prognosis and treatment outcomes (Travis *et al*., 2015). Histologically, adenocarcinomas can display varying degrees of differentiation, with well-differentiated forms showing organized glandular structures and poorly differentiated forms presenting with solid or micropapillary growth patterns (Russell *et al*., 2016). Mucinous adenocarcinomas, a subset of adenocarcinoma, are notable for their mucin production, which can be observed histologically as intracellular or extracellular mucin pools within tumour cells (Warth *et al*., 2016). Immunohistochemical markers such as thyroid transcription factor-1 (TTF-1) and napsin A are crucial in confirming the diagnosis of adenocarcinoma and subtyping (Travis *et al*., 2015). It is important to differentiate primary lung adenocarcinoma from metastatic adenocarcinoma originating from other sites to determine the most appropriate treatment strategies and predict patient outcomes accurately.

The collaborative efforts of the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society international multidisciplinary classification of lung adenocarcinoma provides a standardized framework for subtyping adenocarcinomas based on histological and molecular characteristics (Travis *et al*., 2015). Advances in molecular pathology have identified critical genetic alterations in adenocarcinoma, such as epidermal growth factor receptor (EGFR) mutations and anaplastic lymphoma kinase (ALK) rearrangements, which have significant therapeutic implications for targeted therapies and personalized medicine (Lindeman *et al*., 2018).

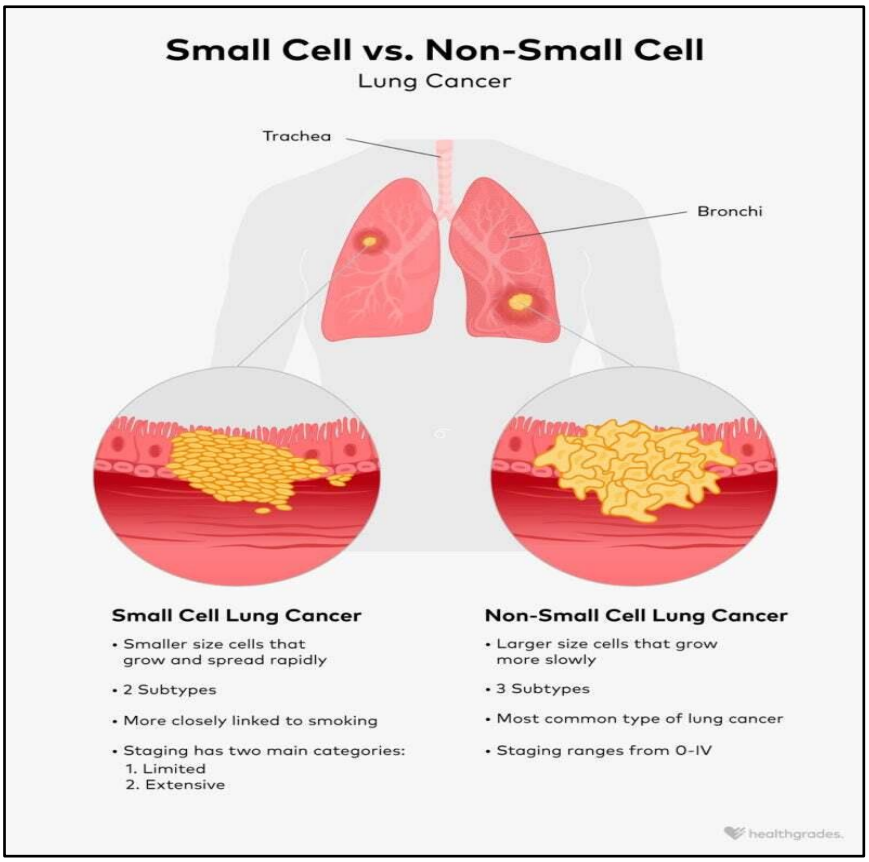

Figure 1.6: Picture elucidating the difference between SCLC (Small lung cancer) and NSCLC (Non-small cell lung cancer) (Baldwin M. *et al*., 2018)

2.7.3) Squamous cell carcinoma (SQCC)

Squamous cell carcinoma (SCC) of the lung is a distinct histological subtype that accounts for a significant proportion of lung cancer cases. Unlike adenocarcinoma, SCC arises from the epithelial cells lining the airways and is strongly associated with smoking as a significant risk factor. The histopathological features of SCC include the presence of keratinization, intercellular bridges, and pearl formation, which are characteristic of squamous differentiation (Travis *et al*., 2015). Immunohistochemical markers such as p40 and p63 are commonly used to confirm squamous differentiation and aid in distinguishing SCC from other lung cancer subtypes (Mukhopadhyay *et al*., 2017). Histologically, SCC often presents as centrally located masses or cavitated lesions within the lungs, reflecting its origin in the more significant airway epithelium (Travis *et al*., 2015). The 2015 World Health Organization (WHO) classification of lung tumours provides a standardized approach for subtyping SCC based on histological patterns and molecular characteristics, emphasizing the importance of accurate diagnosis and classification for optimal patient management (Travis *et al*., 2015). Advances in molecular

pathology have revealed potential therapeutic targets in SCC, including genetic alterations such as mutations in TP53 and EGFR, which may have implications for targeted therapies and personalized treatment approaches (Tsao *et al*., 2018). The differentiation between primary lung SCC and metastatic SCC from other sites is essential for determining appropriate treatment strategies and predicting patient outcomes accurately.

2.7.4) Small cell lung carcinoma (SCLC)

Small cell lung carcinoma (SCLC) is a high-grade neuroendocrine tumour representing a distinct histological subtype of lung cancer. It is characterized by its rapid growth, early metastasis, and aggressive clinical behaviour. Histologically, SCLC is defined by small, round, blue cells with scant cytoplasm, high nuclear-to-cytoplasmic ratio, and finely granular chromatin, reflecting its neuroendocrine differentiation (Travis *et al*., 2015). The absence of distinct glandular or squamous differentiation sets SCLC apart from other lung cancer subtypes. Immunohistochemical staining for neuroendocrine markers such as synaptophysin, chromogranin, and CD56 is essential for confirming the diagnosis of SCLC and distinguishing it from other lung cancer subtypes (Rekhtman, 2017).

Additionally, the expression of neuroendocrine markers supports the neuroendocrine lineage of SCLC cells and aids in predicting treatment response and patient outcomes. SCLC is typically centrally located within the lungs and is frequently associated with paraneoplastic syndromes due to its neuroendocrine origin. The World Health Organization (WHO) classification system categorizes SCLC as a high-grade neuroendocrine tumour and emphasizes the importance of accurate histopathological evaluation for appropriate diagnosis and management (Travis *et al*., 2015). Advances in molecular pathology have identified genetic alterations in SCLC, including mutations in TP53, RB1, and MYC, which play critical roles in this aggressive tumour's pathogenesis and clinical behaviour (George *et al*., 2015). Targeted therapies directed against these molecular alterations are being explored as potential treatment strategies for SCLC patients.

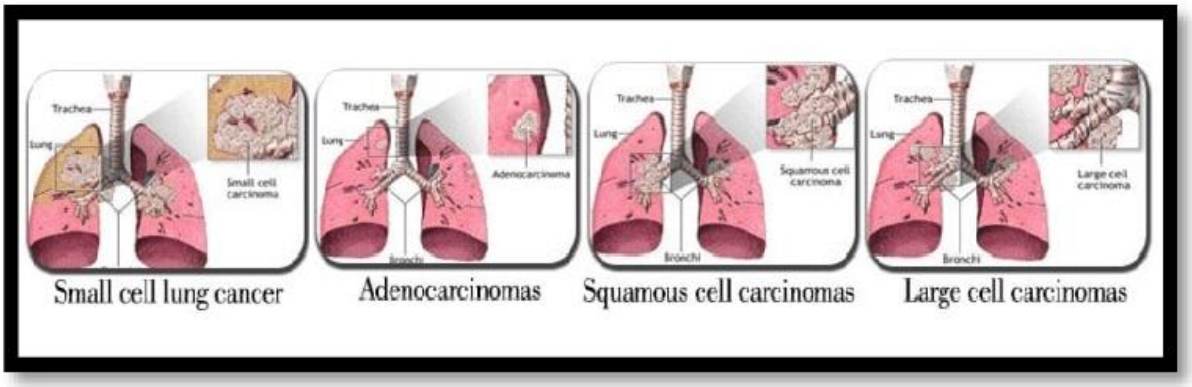

Figure 1.7: The difference between small cell lung cancer, adenocarcinomas, squamous cell carcinomas, and large cell carcinomas (T. S. Roy *et al*., 2015).

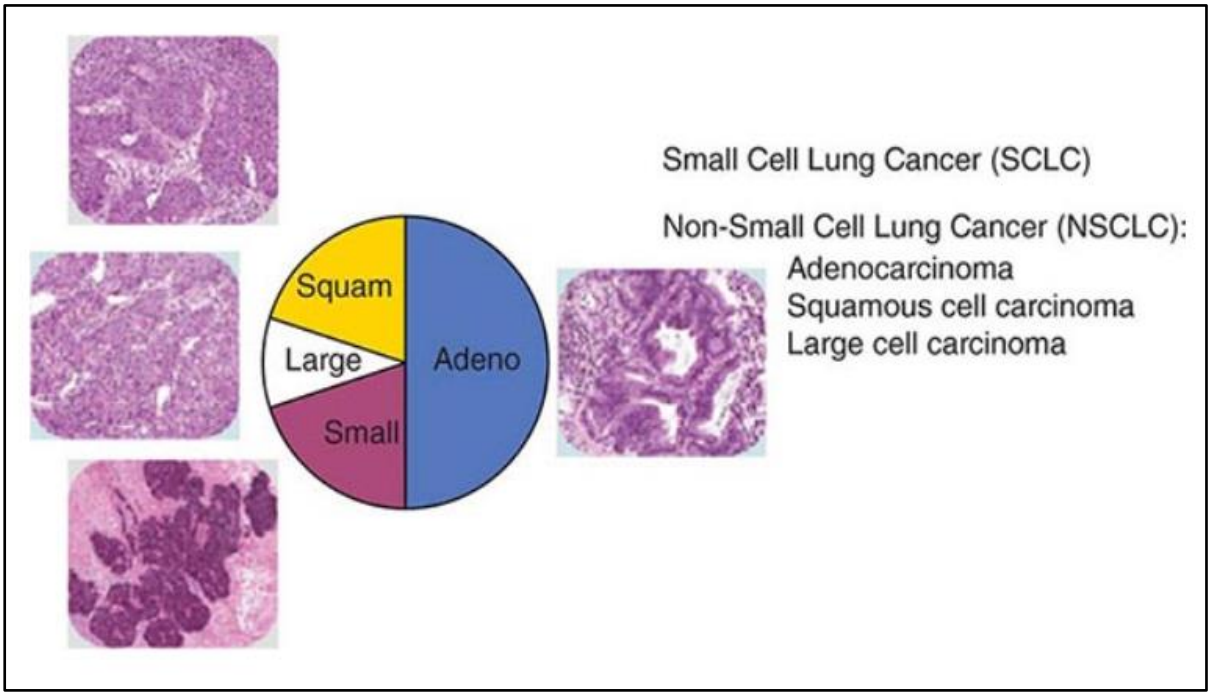

Figure 1.8: The picture depicts that Lung cancer encompasses various histological types, with adenocarcinoma emerging as the most prevalent among non-smokers and women, constituting approximately 40% of cases (Siegel *et al*., 2021). Squamous cell carcinoma, closely linked with smoking habits, accounts for about 30% of lung cancer cases and showcases distinct pathological features (Gandara *et al*., 2015). Small cell lung cancer, though less common, presents as a highly aggressive subtype, making up roughly 10-15% of cases and requiring specialized treatment approaches (Gazdar *et al*., 2017). On the other hand, large-cell carcinoma remains relatively rare, representing approximately 10% of lung cancer diagnoses (Lantuejoul *et al*., 2015).

2.8) TNM classification

The TNM classification system is a standardized method used for staging lung cancer, providing valuable information about the extent of the disease and guiding treatment decisions. It categorizes tumours based on three key parameters: tumour size and invasion (T), lymph node involvement (N), and distant metastasis (M) (Goldstraw *et al*., 2016). In lung cancer, the T category describes the size and spread of the main tumor. Tumours are classified as T1 (≤3 cm), T2 (>3 cm but ≤5 cm), T3 (>5 cm but ≤7 cm), and T4 (>7 cm or involving adjacent structures). The T category also considers factors like invasion into surrounding tissues or structures, such as the chest wall, diaphragm, or mediastinum. The N category evaluates the involvement of regional lymph nodes. Lymph nodes play a crucial role in cancer staging by indicating whether the cancer has spread beyond the primary tumor site. An N0 classification means there is no involvement of regional lymph nodes, while N1, N2, and N3 indicate progressively greater degrees of lymph node involvement, based on the number and location of the affected nodes.

Table 2: Definitions of tumour stage, lymph node invasion, and metastasis (Lababede & Meziane, 2018)

Category	Definition
Tis	Carcinoma in situ
T1a	Tumor ≤ 1 cm (cm)
T1b	Tumor > 1 cm to ≤2 cm
T1c	Tumor > 2 cm to ≤3 cm
T2a	Tumor > 3 cm to ≤4 cm; or any size involving the main bronchus or visceral pleura, or leading to obstructive atelectasis
T2b	Tumor > 4 cm to ≤5 cm
T3	Tumor > 5 cm to ≤7 cm; or any size involving the parietal pleura, parietal pericardium, chest wall, T1–T2 nerve roots, phrenic nerve; or satellite tumor in same lobe as primary tumor
T4	Tumor > 7 cm; or any size invading the mediastinum, diaphragm, trachea, main carina, recurrent laryngeal nerve, esophagus, visceral pericardium, vertebral body, great vessels, heart, cervical nerve roots; or satellite tumor in separate lobe of ipsilateral lung
N0	No reginal lymph nodes involved
N1	Ipsilateral peribronchial, hilar, and intrapulmonary nodes
N2	Ipsilateral mediastinal and subcarinal nodes
N3	Contralateral mediastinal, hilar, or any scale or supraclavicular node
M1a	Separate tumor nodule in contralateral lung, pleural, or pericardial involvement
M1b	Single extrathoracic metastasis
M1c	Multiple extrathoracic metastases

The existence of distant metastases is assessed using the M category. M1 denotes the existence of distant metastases, such as those to the brain, liver, bones, or other organs outside the thorax, whereas M0 indicates the absence of distant metastases. Oncologists can precisely stage lung cancer by using the TNM classification system, which also provides prognostic information and helps to guide treatment plans (Detterbeck *et al*., 2017). It is a crucial tool for choosing the best course of treatment, be it radiation therapy, chemotherapy, surgery, targeted therapy, or a mix of these. By enabling individualized and efficient cancer care, the TNM staging system contributes significantly to bettering patient outcomes as it develops along with advances in cancer research.

Table 3: TNM classification (8th edition) (Lababede & Meziane, 2018)

	N0	N1	N2	N3
T1	IA	IIB	IIIA	IIIB
T2a	IB	IIB	IIIA	IIIB
T2b	IIA	IIB	IIIA	IIIB
T3	IIB	IIIA	IIIB	IIIC
T4	IIIA	IIIA	IIIB	IIIC
M1a	IVA	IVA	IVA	IVA
M1b	IVA	IVA	IVA	IVA
M1c	IVB	IVB	IVB	IVB

2.9) Molecular Pathogenesis of Lung Cancer

2.9.1) Growth factors and their receptors

Lung cancer is a complex disease driven by various genetic and molecular alterations that disrupt normal cellular processes, leading to uncontrolled cell growth and tumour formation. The molecular pathogenesis of lung cancer involves several vital pathways and genetic abnormalities that contribute to its development and progression. One of the central pathways implicated in lung cancer is the epidermal growth factor receptor (EGFR) pathway. Mutations in the EGFR gene, such as exon 19 deletions and L858R point mutations, lead to constitutive activation of the receptor tyrosine kinase and downstream signalling cascades, promoting cell proliferation and survival (Pao & Girard, 2011). Another crucial pathway in lung cancer is the Kirsten rat sarcoma viral oncogene homolog (KRAS) pathway. KRAS mutations, particularly in codons 12 and 13, activate downstream signalling pathways like the mitogen-activated protein kinase (MAPK) pathway, driving cell proliferation and tumour growth (Johnson *et al*., 2015). Alterations in the tumour suppressor genes TP53 and RB1 are also common in lung cancer. TP53 mutations disrupt cell cycle regulation and DNA repair mechanisms, contributing to genomic instability and tumour progression (Hollstein *et al*., 1991). RB1 loss or inactivation leads to uncontrolled cell cycle progression and evasion of cell death pathways, promoting tumour growth (Sherr, 1996).

Furthermore, the phosphatidylinositol-3-kinase (PI3K)/AKT/mTOR pathway plays a significant role in lung cancer pathogenesis. Activating mutations in PIK3CA or loss of PTEN

function result in increased PI3K signalling, leading to enhanced cell survival, proliferation, and metabolism (Carpten *et al*., 2007). Identifying these critical molecular alterations has paved the way for targeted therapies in lung cancer. Small molecule inhibitors targeting EGFR, ALK, ROS1, and other driver mutations have shown promising clinical efficacy in specific subsets of lung cancer patients (Shaw *et al*., 2014).

2.9.1.1) Gastrin-releasing peptide (GRP)/bombesin (BN) autocrine loop

GRP, a 27-amino acid mammalian version of the amphibian peptide BN, plays a role in lung development and repair (Spurzem *et al*., 1997). Studies indicate that 20% to 60% of small-cell lung cancers (SCLC) express GRP/BN, while non-small-cell lung cancers (NSCLC) do so less frequently (Richardson *et al*., 1993). The human GRP/BN receptor subtypes, part of the G-protein–coupled receptor family, are found in both SCLC and NSCLC and some bronchial epithelium samples from smokers (Fathi *et al*., 1996). Despite this, mutations in GRP/BN or its receptors have not been identified in lung cancers, leaving the mechanisms behind their reactivation unclear. The GRP/BN autocrine loop is vital in lung cancer growth, particularly in SCLC. Inhibitors targeting GRP/BN have shown effectiveness in inhibiting tumour growth in laboratory studies and clinical trials with SCLC patients. Interestingly, individuals with a history of prolonged tobacco exposure show increased expression of GRP receptor mRNA in respiratory epithelium, and these cells respond to BN-like peptides with growth stimulation, even after smoking cessation (Siegfried *et al*., 1997). These findings suggest an early involvement of the GRP/BN system in lung cancer development, highlighting its potential as a therapeutic target.

2.9.1.2) ERBB family

Non-small cell lung cancers (NSCLCs) and small cell lung cancers (SCLCs) diverge notably in their presentation of neuregulin receptor abnormalities, particularly involving ERBB2 and ERBB1, members of the transmembrane receptor tyrosine kinase family. When ligands activate, these receptors form dimers and activate intracellular signalling cascades, such as the MAP kinase pathway. ERBB2 (HER2/neu) is markedly upregulated in about 30% of NSCLCs, particularly adenocarcinomas (Weiner *et al*., 1990). Experimental studies suggest that heightened ERBB2 levels contribute to the tumorigenicity of human bronchial epithelial cells (Noguchi *et al*., 1993). Inhibiting ERBB2 with monoclonal antibodies has shown promise in limiting the growth of NSCLC cell lines expressing this receptor (Kern *et al*., 1993). Elevated

ERBB2 is associated with multidrug resistance and increased metastasis potential in NSCLC, possibly explaining the adverse clinical outcomes in patients with ERBB2 overexpression.

On the other hand, ERBB1 (epidermal growth factor receptor, EGFR) is often activated in lung cancer cells through unknown mechanisms related to overexpression. Lung cancer cells producing ERBB1 ligands like epidermal growth factor and transforming growth factor β may create an autocrine loop, further activating ERBB1 signalling (Rachwal *et al*., 1995). ERBB1 activation is more prevalent in NSCLC and may correlate with tumour stage and differentiation status. To target these aberrant pathways, clinical trials evaluate monoclonal antibodies against ERBB1 (such as C225) and tyrosine kinase inhibitors with some ERBB1 blocking activity (like CP358774 and ZD1839). These trials offer promising strategies for targeted therapy in lung cancer treatment, especially when combined with chemotherapy.

2.9.1.3) Other membrane Tyrosine Kinase

Hepatocyte growth factor (HGF) plays a crucial role in fetal lung development and responds to lung injuries by promoting the proliferation, movement, and differentiation of epithelial cells **(Figure 1.9)**. It acts through the MET proto-oncogene receptor, which is expressed in regard to non-small cell lung cancer (NSCLC) as well as small cell lung cancer (SCLC),, although HGF is mainly found in NSCLCs, hinting at an NSCLC-specific self-stimulating loop (Ma *et al*., 2003; Ciardiello & Tortora, 2008; Koo *et al*., 2021). High levels of HGF correlate with poor outcomes in resectable NSCLC patients. Another important player is the KIT proto-oncogene, which, along with its ligand stem cell factor, forms an autocrine loop in many SCLCs, potentially providing a growth advantage or aiding in cell attraction (Salgia, 2010). Additionally, there are other loops involving insulin-like growth factors and their receptors, as well as platelet-derived growth factors and their receptors, all of which are implicated in both SCLC and NSCLC.

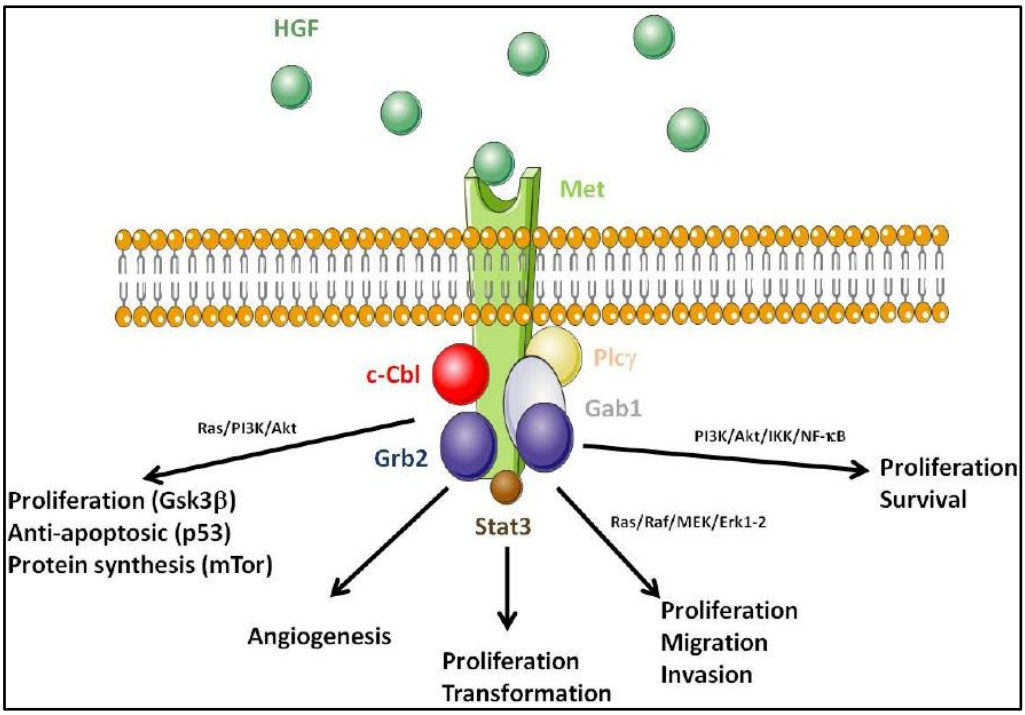

Figure 1.9: HGF/Met function disruption results in diverse cellular consequences. When HGF binds to Met, it triggers receptor dimerization and auto-phosphorylation, activating signaling pathways like Ras/Raf/MEK/Erk1/2 for proliferation and migration, Stat3 for proliferation and transformation, and PI3K/Akt/IKK/NF-κB for angiogenesis and survival. M*et al*so activates the PI3K/Akt-Gsk3β-p53-mTor pathway for anti-apoptotic effects and protein synthesis. Additionally, c-Cbl mediates Met degradation to regulate signaling intensity (Boissinot *et al*., 2019).

2.9.1.4) Nicotine and opioid receptors

Lung cancer cells exhibit a unique feature of expressing both nicotine receptors and various opioid receptors, in addition to their ability to produce several opioid peptides. This intricate molecular landscape presents an interesting phenomenon where opioids, despite their potential to inhibit lung cancer cell growth and induce apoptosis, seem to create a paradoxical situation due to the presence of a negative opioid autocrine regulatory loop within these cells (Molina *et al*., 2013; Kiguchi *et al*., 2016). This paradox arises from the fact that lung cancer cells, despite expressing opioid receptors, may respond to opioids with a negative feedback mechanism that limits their growth. However, the role of nicotine adds another layer of complexity to this scenario. Nicotine, binding to certain high-affinity nicotinic acetylcholine receptors found on lung cancer cells, may oppose the apoptotic effects of opioids (Dasgupta *et al.*, 2017). This

opposing interaction implies a potential mechanism by which inhaled nicotine, often acquired through smoking, might influence the development of lung cancer.

Specifically, nicotine may hinder apoptosis, particularly in precursor lesions, thereby promoting the survival and proliferation of lung cancer cells (Minna *et al*., 2007). This intricate interplay between nicotine, opioids, and their respective receptors underscores the multifaceted nature of lung cancer development and progression, highlighting the need for comprehensive understanding and targeted therapeutic strategies to effectively manage this complex disease.

2.9.1.5) RAS

The RAS proto-oncogene family, which includes KRAS, HRAS, and NRAS, is essential for encoding plasma membrane proteins that are triggered by certain point mutations in lung malignancies. These mutations lead to abnormal and persistent signaling, which promotes unceasing cell division. KRAS is notably the most frequently activated RAS gene in lung cancer; mutations frequently occur at codon 12, with sporadic mutations also occurring at codons 13 and 61. According to Molina *et al.*, 2013, the prevalence of this mutation varies depending on the subtype. It is present in 15%–20% of non-small cell lung cancers (NSCLC) and 20%–30% of lung adenocarcinomas, while it is uncommon in small cell lung cancers (SCLC). Subtype-specific variations in KRAS mutations further complicate the landscape, with parenchymal adenocarcinomas exhibiting higher mutation rates than bronchial adenocarcinomas. The goblet-cell subtype of adenocarcinoma mainly shows a notable frequency of KRAS mutations. These mutations are strongly associated with smoking, often characterized by G-T transversions reflective of DNA adducts induced by tobacco smoke components (Kiguchi *et al*., 2016).

Although KRAS mutations typically suggest a poorer prognosis in NSCLC, their direct impact on resistance to chemotherapy remains contentious. Some studies suggest no significant association between KRAS mutations and resistance to various chemotherapeutic agents in NSCLC cell lines (Salgia, 2010). Similarly, in a prospective study focusing on advanced lung adenocarcinoma, KRAS mutations did not correlate significantly with chemotherapy sensitivity or overall survival (Koo *et al*., 2021). RAS to be active within cells necessitates a lipid modification known as farnesylation, a process regulated by the enzyme farnesyltransferase. Ongoing clinical trials are investigating farnesyltransferase inhibitors,

developed by companies like Bristol Myers Squibb, Janssen, and Merck, as potential therapeutic avenues for lung cancer treatment.

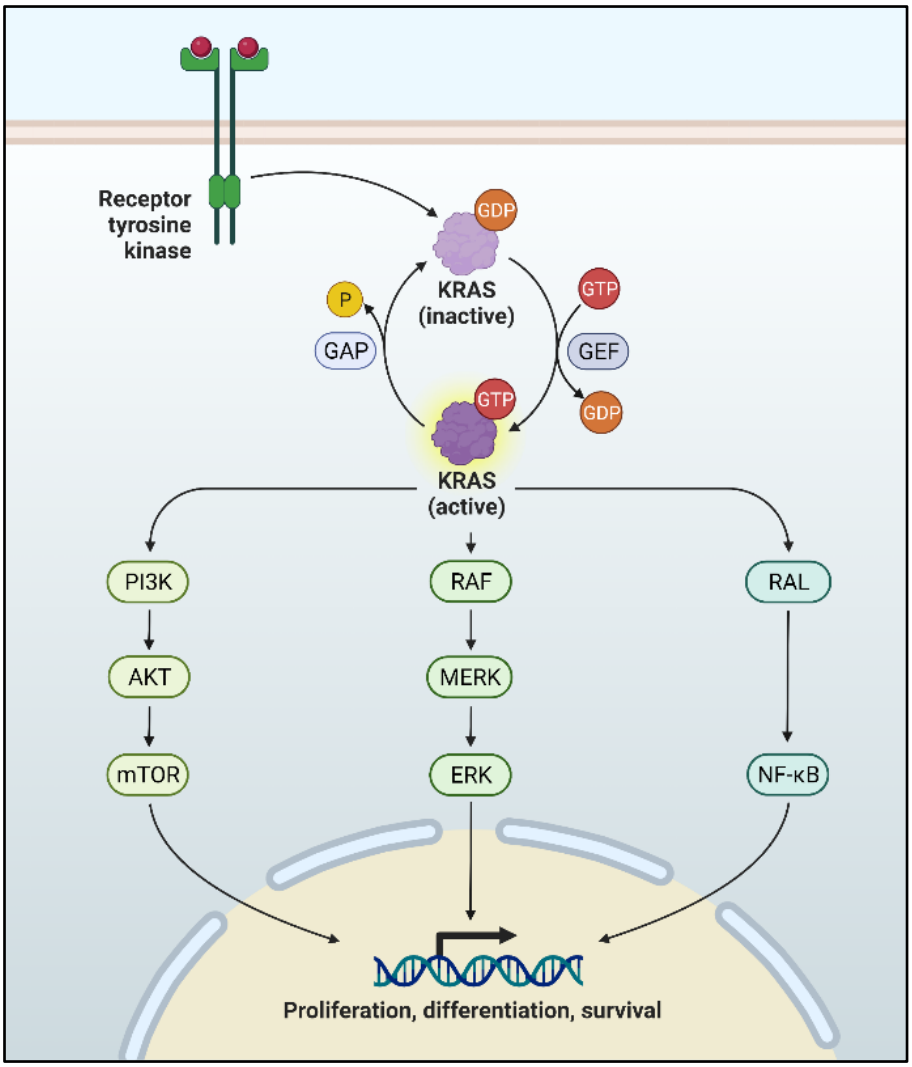

Figure 1.10: KRAS is activated by GEF proteins like SOS, switching GDP for GTP. Active KRAS then triggers RAF, PI3K, and RAL. KRAS self-inactivates by converting GTP to GDP, accelerated by GAP proteins (Kiguchi *et al.*, 2016).

2.9.1.6) MYC

The AS signalling pathway ultimately triggers the activation of nuclear proto-oncogene products like MYC. Upon heterodimerization, MYC transcriptionally activates downstream genes, stimulating cell growth. The MYC family consists of MYC, MYCN, and MYCL, initially identified from lung cancer. Among these, MYC is frequently activated and affects both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), whereas MYCN

and MYCL predominantly impact SCLC. MYC activation can arise from either gene amplification, where cells may harbor between 20 to 115 copies per cell, or from transcriptional dysregulation, leading to excessive protein expression. According to a review by Richardson and Johnson encompassing 17 studies, approximately 18% to 31% of small cell lung cancers (SCLCs) exhibit amplification of one MYC family member, whereas this affects only 8% to 20% of non-small cell lung cancers (NSCLCs). Interestingly, MYC amplification appears to be more common in patients who have undergone chemotherapy and in the "variant" subtype of SCLC, potentially correlating with poorer survival outcomes. Additionally, in vitro experiments demonstrated that all-trans-retinoic acid growth inhibition of an SCLC cell line was linked to increased neuroendocrine differentiation, elevated MYCL expression, and reduced MYC expression.

2.9.2) Abnormalities in the pathways of tumor suppressor genes

2.9.2.1) p53K pathway

P53 acts as a sentinel, safeguarding against genetic aberrations and disruptions within cellular mechanisms. It responds to stress signals like DNA damage, oncogene activation, and oxygen deprivation. As a transcription factor, p53 governs genes that regulate cell cycle halts, DNA repair, or cell death, collaborating with upstream controllers such as p14 and Mdm2. Notably, p53 mutations are prevalent in lung cancer, particularly in SCLC, LCNEC, and NSCLC, altering its binding to Mdm2 and stabilising it (Olivier *et al*., 2009). These mutations, notably induced by carcinogenic exposures like smoking, exhibit distinct patterns, such as GC to TA transversions at specific DNA sites linked to tobacco carcinogens (Brambilla *et al*., 1998). Early stages of lung cancer show pronounced alterations in p53, hinting at its involvement from the initial stages of tumour development. Upstream regulators like p14ARF and Mdm2 contribute significantly to p53 functionality. p14ARF is a critical response to oncogenic stress and DNA damage, while Mdm2's overexpression can turn off p53 by hastening its breakdown. These irregularities in regulatory pathways underscore p53's role in tumorigenesis.

Further upstream, ATM, responsible for DNA damage response, complements p53 functions. Although not a frequent mutation in lung cancer, recent studies have highlighted ATM mutations in a subset of lung adenocarcinomas, enhancing our understanding of its interplay with the p53 pathway. Downstream in the p53 pathway, apoptosis regulators like Bcl-2, Bax, Fas, and TRAIL receptors show dysregulation in lung cancer, leading to resistance against cell

death signals (Gazzeri *et al*., 1998). This intricate interplay of regulatory factors sheds light on the multifaceted nature of p53's involvement in lung cancer progression and therapy responses.

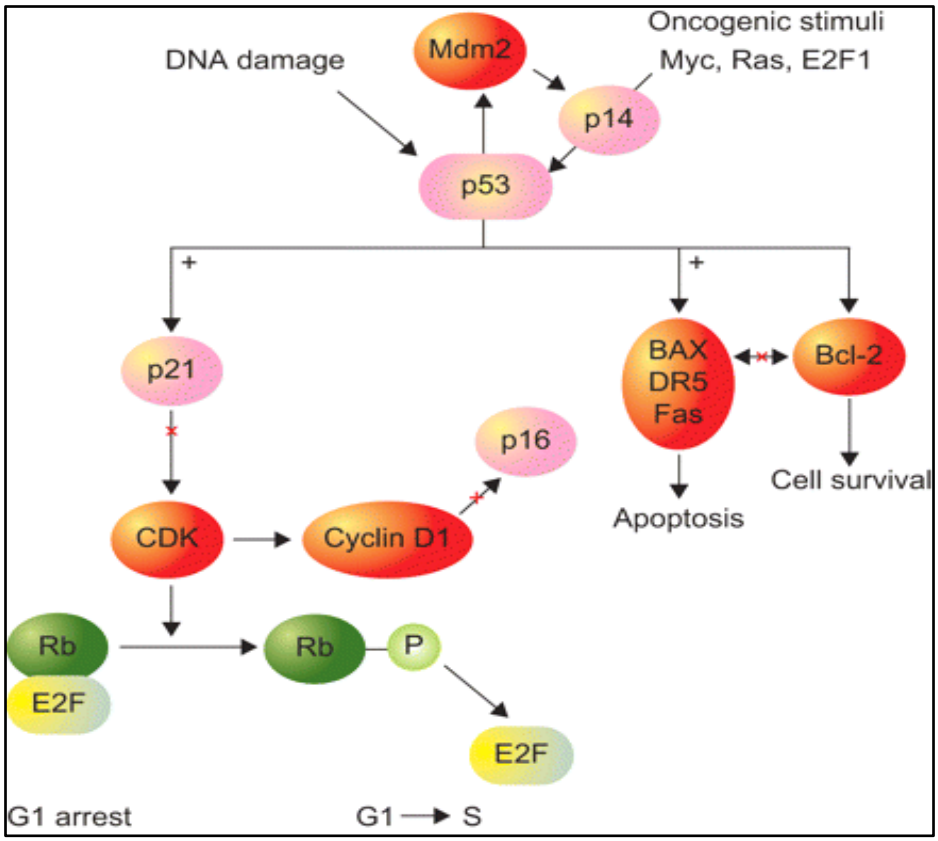

Figure 1.11: Figure illustrating the p53K pathway. The p53 pathway is activated in response to cellular stressors like DNA damage and oncogenic signals. As a transcription factor, p53 regulates genes involved in G1 arrest, apoptosis, and DNA repair. Its activity is controlled by Mdm2, which regulates p53's degradation and transcriptional activity. Additionally, p53 forms complexes with proteins like PCNA for DNA repair. The pathway also involves proteins like p14ARF that modulate Mdm2's function, allowing p53 to carry out its protective roles in maintaining cellular integrity (Brambilla *et al*., 1998).

2.9.2.2) The $p16^{INK4}$/cyclin D1/Rb pathway

The Rb gene, recognized as the first tumour suppressor gene, is a critical regulator in controlling cell cycle progression, particularly at the G1 checkpoint (Knudson *et al*., 1975). Through activating the CDK inhibitor p21 by p53, Rb plays a crucial role in enforcing cell cycle arrest in G0-G1. The functioning of Rb in maintaining this arrest is closely tied to its

phosphorylation status; when Rb is hypophosphorylated, it interacts with the E2F1 transcription factor, resulting in the halting of cell cycle advancement. Conversely, phosphorylation of Rb, facilitated by CDK4-6, cyclin D, and CDK2-cyclin E, releases E2F1, facilitating the transition from G1 to S phase (Knudson *et al*., 1975).

In lung cancer, alterations in Rb function are expected, including the loss of Rb protein and hyperphosphorylation, which disrupt G1 checkpoint control (Brambilla *et al*., 1999). High-grade neuroendocrine LCNEC and SCLC tumours often exhibit Rb protein loss, whereas hyperphosphorylation of Rb is more prevalent in NSCLC (References 74, 76). Furthermore, mutations in the RB1 gene have been observed in certain adenocarcinomas, highlighting the ongoing adverse selection for Rb functions in lung cancer (Brambilla *et al*., 1999) (Knudson *et al*., 1975). The inactivation of Rb through phosphorylation in NSCLC is often attributed to reduced p16 CDK expression and increased levels of cyclins D1 and E . While CDK4 amplification is infrequent, cyclin D1 overexpression and p16 loss are commonly observed in NSCLC, occurring early in the progression of pre-invasive lesions. The intricate interplay between cyclin D1 overexpression, p16 loss, and Rb loss underscores their collaborative roles in cell cycle regulation (References 74, 76). Moreover, overexpression of cyclin E, particularly in SCC, is associated with genetic instability and represents an early event in bronchial pre-neoplasia (Brambilla *et al*., 1999) (Knudson *et al*., 1975).

2.9.2.3) The serine/threonine kinase 11 gene

Mutations in the serine/threonine kinase 11 (STK11), also known as LKB1, located on chromosome 19's short arm, are linked with Peutz-Jeghers syndrome, a condition marked by intestinal hamartomas and a higher risk of epithelial cancers. While relatively rare in most sporadic cancers, loss-of-function mutations in STK11 are found in primary human lung adenocarcinomas (ADCs) and a smaller fraction of squamous cell carcinomas (SCCs). STK11 is a critical guardian against pulmonary tumorigenesis, governing cellular initiation, polarity differentiation, and metastasis (Ramsey *et al*., 2007). These mutations are more prevalent in ADCs among smokers and non-Asian populations, often occurring alongside KRAS mutations (Herbst *et al*., 2008).

2.9.3) Platinum-based chemotherapy

Chemotherapy is a pivotal method in combating tumours, tracing back to the era before the 1960s when cancer treatment solely relied on organic compounds (Florea *et al*., 2011). A groundbreaking discovery in the late 1960s revolutionized cancer therapy with the accidental finding of cisplatin, a coordination compound with potent anti-cancer properties also noted for its ability to inhibit bacterial growth (Wang *et al*., 2005). This discovery marked a significant turning point, expanding the horizons of cancer chemotherapy. Platinum-based anti-cancer drugs such as cisplatin (Ohmichi *et al*., 2005), carboplatin (Corte-Rodriguez *et al*., 2015), and oxaliplatin (Zayed *et al*., 2011), known for their well-established mechanisms and therapeutic efficacy, have become mainstays in clinical settings. Despite their effectiveness against various malignancies like breast, ovarian, and colorectal cancers, these drugs pose challenges due to their non-specific nature, causing systemic toxicity alongside tumour cell destruction. The notable drawbacks of platinum anti-cancer drugs, including dose-related toxicities like nephrotoxicity, neurotoxicity, ototoxicity, and myelosuppression, necessitated the exploration of strategies to mitigate average tissue damage (Wong *et al*., 1999). Recent advancements focus on enhancing drug delivery and targeting tumour tissues while minimizing adverse effects. Techniques such as liposome encapsulation, nanomaterial carriers, and bioconjugation targeting specific tumour proteins have emerged to address these challenges (Zayed *et al*., 2011). Innovative formulations like multifunctional platinum nanocluster-based (Pt NC-based) nanodrugs have gained prominence due to their potential for cancer-specific therapy and improved drug delivery. These nanodrugs, crafted from biocompatible materials with customizable designs, exhibit enhanced stability, water dispersibility, and reduced systemic toxicity compared to traditional platinum drugs (Chen *et al*., 2018). Their controllable fabrication, biosafety, and potent anti-tumour activity position Pt NC-based nano drugs as promising candidates for advancing cancer treatment.

2.9.3.1) Platinum doublets

Over two decades ago, a pivotal meta-analysis by the NSCLC collaborative group marked a turning point in understanding the benefits of chemotherapy for patients with inoperable or metastatic NSCLC. This analysis, conducted in the 1990s, revealed a 10% increase in survival rates at the one-year mark when comparing systemic chemotherapy against best supportive care alone. Interestingly, this survival advantage was particularly associated with cisplatin-based regimens, distinguishing them from other alkylating agents of that time, which could

have adverse effects, especially with prolonged use. Around the same period, another meta-analysis underscored the heightened efficacy of combination chemotherapy over single-agent therapies in treating advanced NSCLC, nearly doubling response rates. However, this increased effectiveness came at the cost of more pronounced side effects. Notably, clinical trials focusing on monotherapy with platinum analogues or vinorelbine found that adding additional chemotherapeutic agents did not yield significant survival benefits by the end of the first year. This highlights the nuanced landscape of chemotherapy in NSCLC treatment, emphasizing the importance of specific drug combinations and their impact on patient outcomes (Lilenbaum *et al*., 1998).

Over the years, researchers have explored various potent cytotoxic agents like gemcitabine, taxanes, and camptothecins for treating advanced NSCLC. However, despite these advancements, platinum analogues have maintained their status as the primary choice for first-line treatment. Studies comparing platinum-based regimens with third-generation combination chemotherapies consistently show that platinum-based treatments outperform third-generation options regarding response and 12-month survival rates (D'Addario *et al*., 2005).

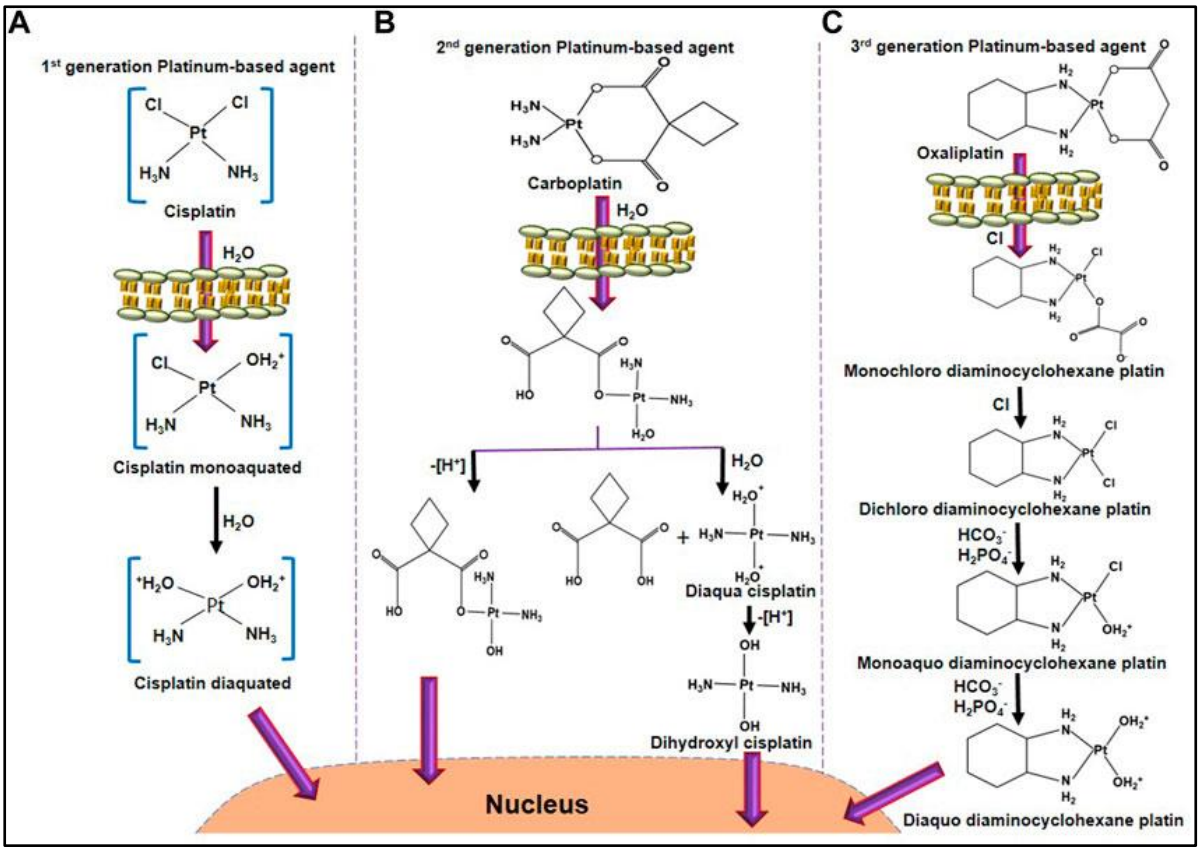

Figure 1.12: The activation cascade of platinum-based anticancer agents (Mondal *et al.*, 2023). (A) Cisplatin is activated by replacing a chloride molecule with water, forming cisplatin monoaquated. After hydrolysis, another chloride molecule is replaced, resulting in cisplatin diaquated, which forms DNA adducts in the nucleus. (B) Carboplatin undergoes hydrolysis, breaking a bond with 1,1-cyclobutane dicarboxylate. This forms a hydroxyl derivative, followed by diaqua cisplatin after further hydrolysis. Oxidation of diaqua cisplatin yields dihydroxyl cisplatin, its active form. (C)Oxaliplatin exchanges oxygen with chloride upon entering cells, forming monochloro diaminocyclohexane platin.

Further analysis across several phase 3 trials confirmed that platinum-based doublets, especially those combining platinum compounds with third-generation agents like vinorelbine, gemcitabine, or taxanes, remained the gold standard for nearly a decade. These combinations showed comparable efficacy, with survival rates ranging from 33% to 46% at the one-year mark (Pujol *et al*., 2006). The introduction of pemetrexed in 2008 marked a significant shift towards personalized treatment for advanced NSCLC. Studies comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed found no significant difference in overall survival and response rates. However, pemetrexed-based regimens demonstrated superior safety

profiles, particularly regarding hematologic toxicity (Fossella *et al*., 2003). Interestingly, histology emerged as a critical factor in treatment response, with nonsquamous NSCLC patients benefiting more from pemetrexed-based therapies than squamous cell carcinoma cases. This histology-guided approach improved overall survival rates, especially for nonsquamous NSCLC patients, and is now a key component of treatment guidelines in Europe and the United States (Novello *et al*., 2016).

2.9.3.2) Molecular mechanisms of cisplatin/carboplatin

Cisplatin, a platinum-based chemotherapy drug, exerts its anti-cancer effects through a well-defined molecular mechanism. Upon administration, cisplatin enters cancer cells where it forms covalent bonds with DNA, particularly at the N7 positions of purine bases, primarily guanine, leading to the formation of intrastrand crosslinks (Kelland, 2007; Wang & Lippard, 2005). These DNA crosslinks interfere with DNA replication and transcription processes, ultimately inducing cell cycle arrest and apoptosis (Dasari & Tchounwou, 2014; Wang & Guo, 2013). The activation of apoptotic pathways involves various cellular responses, including the upregulation of pro-apoptotic proteins like Bax and downregulation of anti-apoptotic proteins such as Bcl-2 (Reed, 2003; Wang *et al*., 2012). Additionally, cisplatin-induced DNA damage triggers the activation of DNA repair pathways, including nucleotide excision repair (NER) and homologous recombination (HR), as cells attempt to restore genomic integrity (Reedijk, 2003; Kartalou & Essigmann, 2001). Furthermore, cisplatin's cytotoxic effects extend beyond DNA damage and apoptotic signalling. It can induce cellular oxidative stress and mitochondrial dysfunction, generating reactive oxygen species (ROS) and activating stress-related pathways like MAPK and JNK (Galluzzi *et al*., 2012; Siddik, 2003). The activation of these signalling cascades contributes to cell cycle arrest, DNA damage response, and, ultimately, cell death. Moreover, cisplatin can modulate cellular pathways involved in proliferation, differentiation, and survival, such as the PI3K/Akt/mTOR pathway, further influencing its cytotoxicity (McCubrey *et al*., 2007; Miller *et al*., 2015). Despite its effectiveness in treating various cancers, including testicular, ovarian, and lung cancers, cisplatin's clinical use is limited by dose-dependent toxicities such as nephrotoxicity, neurotoxicity, and ototoxicity (Arany & Safirstein, 2003; Miller *et al*., 2010). Strategies to enhance cisplatin's therapeutic efficacy while minimizing its adverse effects continue to be an area of active research, including combination therapies, drug delivery innovations, and the development of novel platinum-based agents (Wheate *et al*., 2010; Wang & Guo, 2013).

2.9.3.3) Molecular mechanisms of pemetrexed along with carboplatin/cisplatin

Pemetrexed, in combination with carboplatin, represents a cornerstone in the treatment of certain types of lung cancer, particularly non-squamous non-small cell lung cancer (NSCLC). Understanding the mechanism of action of this combination therapy sheds light on its effectiveness and clinical application. Pemetrexed is a folate antimetabolite that disrupts nucleotide synthesis, crucial for cancer cell proliferation. It inhibits three enzymes involved in folate metabolism: thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). By inhibiting TS, pemetrexed prevents the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP), leading to reduced thymidine levels needed for DNA synthesis. DHFR inhibition further depletes thymidine levels, while GARFT inhibition impacts purine synthesis by reducing formylglycinamide ribonucleotide (FGAR) levels. This multifaceted disruption of nucleotide synthesis halts cancer cell replication.

Carboplatin, conversely, is a platinum-containing compound that exerts its cytotoxic effects by forming DNA adducts, primarily intrastrand cross-links, with the purine bases of DNA. This DNA damage triggers cell cycle arrest and apoptosis in rapidly dividing cells, including cancer cells. Carboplatin's mechanism complements pemetrexed's action by inducing DNA damage, thereby enhancing the antitumor effects of the combination therapy. When pemetrexed and carboplatin are administered together, their synergistic effects target multiple pathways crucial for cancer cell survival and proliferation. This synergism is particularly significant in non-squamous NSCLC, where pemetrexed has demonstrated superior efficacy to other regimens. Studies like the phase 3 trial by (Scagliotti *et al*., 2008) highlighted the efficacy of cisplatin plus pemetrexed compared to cisplatin plus gemcitabine in terms of overall survival and response rates, reinforcing the role of pemetrexed-based combinations in NSCLC treatment.

Moreover, the histology-specific response to pemetrexed-based therapies underscores the importance of personalized medicine in lung cancer treatment. Patients with nonsquamous NSCLC, including adenocarcinoma and large-cell carcinoma subtypes, show improved overall survival with pemetrexed-containing regimens. Meta-analyses support this histology-guided approach (Chong *et al*., 2012; Zhang *et al*., 2014), emphasising pemetrexed-based therapies' benefits, especially in nonsquamous NSCLC populations. In addition to its efficacy, the

pemetrexed-carboplatin combination is known for its manageable toxicity profile compared to other platinum-based regimens. This aspect is crucial for maintaining patients' quality of life during treatment. Studies like the one by (Scagliotti *et al*., 2008) have highlighted the safety advantages of pemetrexed over gemcitabine regarding hematologic toxicity, further supporting its role as a preferred option in NSCLC therapy.

2.9.3.4) Molecular mechanisms of Irinotecan along with carboplatin/cisplatin

Irinotecan is a topoisomerase I inhibitor that exerts cytotoxic effects by interfering with DNA replication and repair processes. It binds to topoisomerase I-DNA complexes, forming stable ternary complexes that inhibit DNA re-ligation after single-strand breaks are introduced during DNA replication. This leads to the accumulation of DNA damage, particularly double-strand breaks, which triggers cell cycle arrest and apoptosis in cancer cells. Irinotecan's mechanism of action is well-documented and has been extensively studied in various preclinical and clinical settings. Studies such as those by (Saltz *et al*., 2000 and Douillard *et al*., 2000) demonstrated the efficacy of irinotecan-based regimens in advanced colorectal cancer, highlighting its role in clinical practice.

On the other hand, Carboplatin is a platinum-containing compound that forms DNA adducts, primarily intrastrand cross-links, with the purine bases of DNA. This DNA damage disrupts DNA replication and transcription processes, leading to cell cycle arrest and apoptosis in rapidly dividing cells, including cancer cells. Carboplatin's mechanism of action complements irinotecan's effects by inducing additional DNA damage, thereby enhancing the overall cytotoxicity of the combination therapy. When irinotecan and carboplatin are used together, their synergistic effects target multiple pathways critical for cancer cell survival and proliferation. This synergism is particularly evident in colorectal cancer, where irinotecan-based regimens, including combinations with carboplatin, have demonstrated improved outcomes compared to single-agent therapies. Clinical trials such as the study by (Falcone *et al*., 2007) evaluated the efficacy of irinotecan plus carboplatin in colorectal cancer, showcasing the benefits of this combination in terms of response rates and progression-free survival.

Furthermore, the combination of irinotecan and carboplatin has shown promise in other cancer types, such as small cell lung cancer (SCLC). In SCLC, irinotecan's ability to inhibit topoisomerase I and carboplatin's DNA-damaging effects synergize to target cancer cells effectively. Studies like the phase 3 trial by (Noda *et al*., 2002) demonstrated the efficacy of

irinotecan plus carboplatin in extensive-stage SCLC, leading to improved survival outcomes compared to standard etoposide plus cisplatin regimens. In addition to its efficacy, the irinotecan-carboplatin combination is generally well-tolerated, with manageable toxicity profiles compared to other chemotherapy regimens. This aspect is crucial for maintaining patients' quality of life during treatment. Clinical trials and meta-analyses, such as those by (Fuchs *et al*., 2003 and Schmittel *et al*., 2010), have highlighted the safety and tolerability of irinotecan and carboplatin combinations, further supporting their use in clinical practice.

2.9.3.5) Molecular mechanisms of Docetaxel along with carboplatin/cisplatin

The combination of docetaxel with carboplatin is widely recognized as an effective treatment strategy for various types of cancers, including non-small cell lung cancer (NSCLC) and breast cancer. The mechanism of action of this combination therapy involves synergistic effects that target critical pathways for cancer cell survival and proliferation. Docetaxel, a taxane chemotherapy agent, disrupts microtubule dynamics within cancer cells. By binding to the beta-tubulin subunit of microtubules, docetaxel stabilizes them, preventing depolymerization and forming stable microtubule bundles. This interference impairs mitotic spindle function during cell division, causing cell cycle arrest and subsequent apoptosis in cancer cells. The effectiveness of docetaxel in various cancers has been extensively studied and validated through clinical trials such as those conducted by (Fossella *et al*., 2000 and Schuette *et al*., 2011). Carboplatin, another critical component of this combination therapy, is a platinum-containing compound that induces DNA damage in cancer cells. Carboplatin forms DNA adducts, particularly intrastrand cross-links, with purine bases of DNA, disrupting DNA replication and transcription processes. This DNA damage leads to cell cycle arrest and apoptosis in rapidly dividing cancer cells, complementing the action of docetaxel. The efficacy of carboplatin in combination with docetaxel has been demonstrated in studies like the one by (Gridelli *et al*., 2007), showcasing its role in enhancing treatment outcomes. When docetaxel and carboplatin are administered together, their combined effects result in a potent antitumor response. This synergistic action is particularly significant in NSCLC and breast cancer, where the combination has shown improved outcomes compared to single-agent therapies. Clinical trials, including those mentioned earlier, have highlighted the benefits of docetaxel-carboplatin regimens regarding response and overall survival rates among cancer patients.

Furthermore, this combination therapy has demonstrated promise in other cancer types, such as ovarian cancer and head and neck cancer. In ovarian cancer, the synergistic effects of

docetaxel's microtubule stabilization and carboplatin's DNA damage induction have proven effective in targeting cancer cells. Studies like the phase 3 trial by (du Bois *et al*., 2003) have provided evidence of the efficacy of docetaxel plus carboplatin in ovarian cancer treatment.

2.10) Telomeres

Telomeres are like the protective caps on shoelaces, but for our chromosomes, as depicted in **Figure 1.13,** They shield our genetic material from wear and tear, keeping our genome intact and functioning smoothly. Think of them as guardians of the genetic code. Over time, as cells divide, these telomeres naturally shrink a bit each time. Once they get too short, cells stop dividing (senescence) or self-destructing (apoptosis), preventing genetic chaos. Confident lifestyle choices can hasten this shortening process, speeding up the cellular clock. Factors like exposure to harmful agents or unhealthy habits can damage DNA, including telomeres, leading to premature ageing and health issues. Conversely, lifestyle choices can be protective, helping maintain telomere length and overall well-being (Shammas *et al*., 2011).

2.10.1) Structure and Function of Telomeres

Telomeres are intricate structures found at the tips of chromosomes, acting as protective shields for our genetic material. They prevent DNA degradation and keep chromosomes from sticking together, ensuring the integrity of our genome. Imagine them as the guards standing watch at the ends of our genetic code (Van Steensel *et al*., 1998). At a molecular level, telomeres consist of DNA-protein complexes, with specialized proteins like TRF2 forming loop structures that safeguard chromosome ends from degradation during DNA replication. However, with each round of replication, telomeres naturally shorten. If this shortening persists, it can lead to chromosomal damage and cell death (Griffith *et al*., 1999), a process depicted in **Figure 1.14**.

Interestingly, cells possess an enzyme called telomerase that can lengthen telomeres, but its activity is typically limited to certain cell types like germ cells and specific blood cells. Most other cells have minimal telomerase activity, leading to gradual telomere shortening with each cell division, as illustrated in **Figure 1.14**. In diseases like cancer, cells can reactivate telomerase or use alternative mechanisms, such as genetic recombination, to maintain telomere length and achieve immortality. Our research has revealed a connection between telomerase and proteins involved in genetic recombination, indicating a link between recombinational repair and telomere maintenance, particularly in cancer cells (Shammas *et al*., 1999). This

intricate interplay between telomeres, telomerase, and genetic repair mechanisms underscores the complexity of cellular processes that dictate cell lifespan and genomic stability.

2.10.2) Telomere Length and Lifespan: A Simple Connection

Normal cells lose bits of telomeres as they divide, limiting their lifespan in lab cultures. For instance, human liver tissues lose about 55 base pairs of telomeric DNA yearly. This shortening rate is similar to rapidly renewing gastric mucosal cells. Biomarkers like stathmin and EF-1a indicate that telomere issues and DNA damage increase with age and related diseases. Telomere length decreases with age, while the expression of p16, linked to ageing cells, rises (Takubo *et al*., 2000). In dyskeratosis congenital, a genetic disorder, telomeres shorten fast, causing early age-related disorders and shorter lives. Telomerase, crucial for adding telomeric repeats, is active in particular cells like germline and stem cells but low in most normal cells. Boosting telomerase in normal cells extends their life (Jiang *et al*., 2008). Research shows that individuals with shorter telomeres have higher mortality rates from heart and infectious diseases. Telomere shortening can lead to cell senescence, death, or cancerous changes. Lifestyle can influence telomere length, impacting overall health, ageing rate, and lifespan.

2.10.3) Rapid Telomere Shortening: A Potential Accelerator of Aging

Telomere length dynamics are integral to understanding ageing and health. With age, telomeres naturally shorten, losing about 24.8-27.7 base pairs per year in humans. Shorter telomeres relative to the average for a specific age group often coincide with an increased risk of age-related diseases and a shorter lifespan. This decline is influenced by genetics, environment, lifestyle choices, and socioeconomic status rather than gender (Valdes *et al*., 2008). Lifestyle choices play a significant role in telomere shortening.

Smoking, obesity, sedentary habits, and an unhealthy diet can accelerate this process, leading to a higher likelihood of illness and early mortality. Accelerated telomere shortening is linked to the early onset of age-related health issues such as coronary heart disease, heart failure, diabetes, increased cancer risk, and osteoporosis (Steinert *et al*., 1999). Individuals with shorter leukocyte telomeres have a threefold higher risk of developing myocardial infarction. Research also suggests a correlation between telomere length and mortality rates in older people, where shorter telomeres are associated with a higher mortality rate. Additionally, shorter telomeres can contribute to genomic instability, potentially leading to interchromosomal fusion and

cancer development (Chin *et al*., 1999). While most cancer cells exhibit elevated telomerase activity, their telomeres are paradoxically shorter, indicating complex mechanisms at play. Studies have highlighted the impact of telomere length on cancer risk, particularly in lung, bladder, renal cell, gastrointestinal, and head and neck cancers. Genetic factors can also influence telomere length, with specific individuals having shorter telomeres from birth or due to genetic disorders like dyskeratosis congenita (Shammas *et al*., 2011). This condition, characterized by a deficiency in the telomerase RNA gene, leads to premature ageing symptoms such as premature greying, increased cancer vulnerability, bone marrow failure, and early death. Understanding telomere biology and its association with ageing and disease is crucial for developing strategies to promote healthy ageing and prevent age-related illnesses. Strategies aimed at maintaining telomere length or slowing down the rate of telomere shortening may hold promise in improving overall health and longevity (Vulliamy *et al*., 2001).

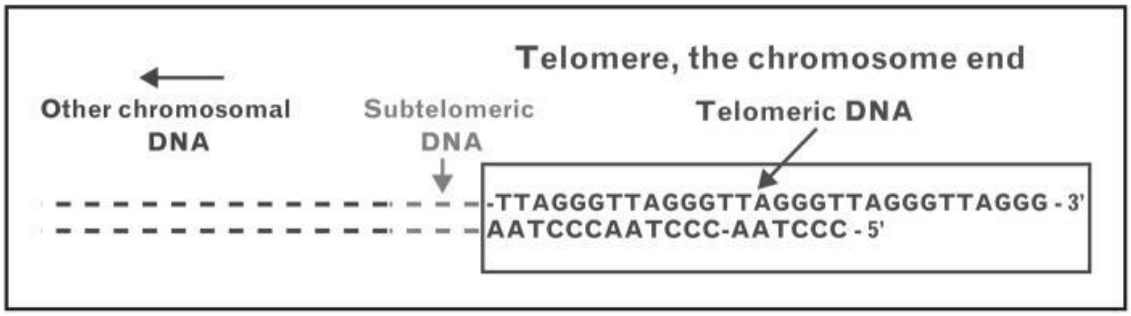

Figure 1.13: Understanding Telomeres: Chromosome Protectors. Chromosomes have 'TTAGGG' repeats at their ends, forming loops that shield DNA from damage. This telomeric DNA shortens with cell divisions, triggering senescence or cell death once it is too short. Telomere length affects cell lifespan (Shammas *et al*., 2011).

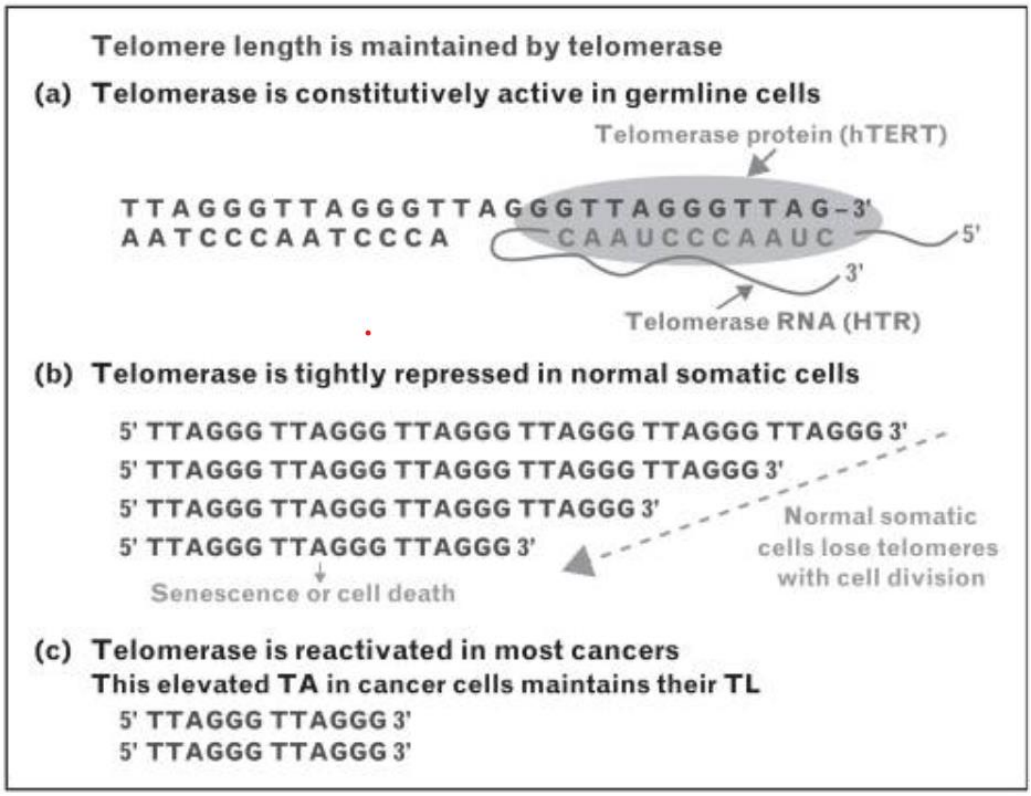

Figure 1.14: The Crucial Role of Telomeric DNA Length in Cellular Lifespan. (a) Telomerase, an enzyme with protein (hTERT) and RNA (hTR) subunits, prevents telomere shortening by adding 'TTAGGG' repeats, active in germline and stem cells. (b) Normal somatic cells lack active telomerase, shortening telomere over time, triggering cell senescence or death. (c) Prolonged cell division without senescence signals causes severe telomere shortening and genomic instability. (Shammas *et al*., 2011).

2.11) The Interplay of Environment, Occupational Factors, and Stress on Telomeres and Aging

The dynamic interplay between environmental factors, occupational stress, and telomere biology has been extensively studied in recent years, shedding light on their collective impact on health and ageing. Environmental factors like air pollution, exposure to toxins, and lifestyle choices like diet and physical activity have been linked to accelerated telomere shortening. Studies by (Valdes *et al*., 2005) and Nordfjall *et al*., 2008) have shown associations between environmental exposures and telomere attrition rates, highlighting the role of environmental influences in telomere dynamics. The nature of one's profession can also play a significant role in telomere health. High-stress occupations characterized by long working hours, job strain, and psychological stress have been associated with telomere shortening. Research by Epel *et*

al. (2004) and Blackburn *et al*. (2015) demonstrated a correlation between occupational stress and accelerated telomere attrition, emphasizing the impact of work-related stress on cellular ageing processes. Chronic stress, whether work-related pressures or personal life challenges, has been identified as a critical contributor to telomere shortening. Studies by Epel *et al*. (2010) and Damjanovic *et al*. (2007) highlighted the detrimental effects of chronic stress on telomere length, mediated through elevated cortisol levels and inflammatory responses. Understanding these complex interactions is vital for developing interventions to promote healthy ageing and mitigate the risks associated with telomere shortening. Strategies focusing on stress management, healthy lifestyle practices, and creating supportive work environments can help preserve telomere health and enhance overall well-being.

2.11.1) Impacts of Environmental Exposures and Occupational Factors on Telomere Shortening

Hoxha and colleagues (Hoxha *et al*., 2009) examined the impact of exposure to traffic pollution on telomere length in leukocytes from office workers and traffic police officers, using toluene and benzene levels as markers for pollution. They discovered that traffic police officers exhibited shorter telomeres within each age group than office workers. Additionally, coke-oven workers exposed to polycyclic aromatic hydrocarbons showed significantly shorter telomeres and increased DNA damage than controls (Pavanello *et al*., 2010). Interestingly, the degree of telomere shortening in these workers was not directly related to their age or levels of DNA damage but was strongly correlated with the duration of exposure to harmful agents. The shortened telomeres observed in these workers have implications for increased cancer risk, particularly lung cancer among coke-oven workers. Telomere shortening in lymphocytes is also associated with ageing. Notably, the reduced telomere length in the lymphocytes of coke-oven workers was linked to hypomethylation of the p53 promoter, potentially leading to elevated p53 expression, which can inhibit cell growth or induce apoptosis. Exposure to genotoxic agents, causing DNA damage broadly or specifically at telomeres, can heighten the risk of cancer and accelerate the ageing process. These findings underscore the importance of minimizing exposure to harmful substances in occupational settings to preserve telomere integrity and overall health.

2.11.2) Stress Accelerates Telomere Shortening and Aging Process

The impact of stress on telomere dynamics is intricately linked to the release of glucocorticoid hormones from the adrenal gland. These hormones, known for their role in stress response, have been implicated in reducing levels of antioxidant proteins. This reduction can lead to increased oxidative damage to DNA and, consequently, accelerate telomere shortening (Hoxha *et al*., 2009). Research has consistently shown that individuals exposed to chronic stress exhibit heightened oxidative stress, decreased telomerase activity, and shorter telomeres in peripheral blood mononuclear cells than in control groups (Epel *et al*.,2004). Notably, this telomere length difference is equivalent to age by ten years, suggesting that stress can significantly impact the onset of age-related health issues. As telomere length often mirrors an individual's biological age, stress can adversely affect overall health and longevity. Understanding the mechanisms by which stress influences telomere dynamics is crucial for developing interventions to mitigate the negative consequences of chronic stress on cellular ageing processes and health outcomes.

2.11.3) Nutritional Influences on Telomeres: Exploring Fiber, Fats, and Proteins

Research has delved into how dietary components like fibre, fats, and proteins influence the health of telomeres, the protective caps on our chromosomes. For instance, a study by Tucker *et al*. (2018) highlighted that a fibre-rich diet sourced from fruits, veggies, whole grains, and legumes was linked to longer telomeres. This connection suggests that fibre may play a protective role against cellular ageing processes by reducing inflammation and oxidative stress. On the other hand, the types of fats we consume can impact telomere dynamics differently. Saturated fats, commonly found in animal products and processed foods, may accelerate telomere shortening due to increased oxidative stress and inflammation.

Conversely, omega-3 fatty acids, abundant in foods like fatty fish and nuts, have anti-inflammatory properties that could help maintain telomere length. These findings align with research by Kiecolt-Glaser *et al*. (2017) and Farzaneh-Far *et al*. (2010), showing that diets rich in omega-3s are associated with longer telomeres and reduced cellular ageing. Moreover, the source and quality of proteins in our di*et al*so matter for telomere health. Plant-based proteins from sources like legumes and nuts have been linked to longer telomeres and reduced

inflammation compared to animal-based proteins. A recent study by Song *et al*. (2020) added to this understanding that a higher intake of plant-based proteins correlated with slower telomere shortening over time, supporting the benefits of plant-centric diets for healthy ageing.

2.11..4) Antioxidant-Rich Diets and Telomere Preservation: A Protective Link

Research conducted by Farzaneh-Far and colleagues (year) provides insights into the impact of dietary antioxidants, particularly omega-3 fatty acids, on telomere dynamics. Their study revealed that individuals with a diet rich in antioxidant omega-3 fatty acids experienced a slower rate of telomere shortening than those lacking these antioxidants (Farzaneh-Far *et al*., 2010). Over 5 years, the researchers monitored omega-3 fatty acid levels in participants' blood alongside telomere length changes, observing an inverse correlation that suggests antioxidants can mitigate telomere attrition. Moreover, the study shed light on the link between antioxidant intake and disease risk. Women consuming a diet deficient in antioxidants displayed shorter telomeres and an increased risk of breast cancer.

In contrast, those with a diet rich in antioxidants like vitamin E, vitamin C, and beta-carotene exhibited longer telomeres and a reduced risk of breast cancer (Gammon *et al*., 2009). These findings underscore the potential protective role of antioxidants in shielding telomeric DNA from oxidative damage caused by various environmental and physiological stressors. The study emphasizes the importance of incorporating antioxidant-rich foods, particularly omega-3 fatty acids and other vitamins, into our diets to support telomere health and potentially reduce the risk of age-related diseases like cancer. Antioxidants are crucial in maintaining genomic stability and cellular integrity, making them valuable components of a healthy and longevity-promoting lifestyle.

2.11.5) Enhancing Longevity: The Impact of Dietary Restriction on Aging Rate

Limiting food intake through dietary restriction has been found to have profoundly positive effects on health and lifespan. Studies on animals have shown that reducing food consumption decreases growth rate, lowers oxidative stress, and reduces DNA damage. These effects contribute to keeping animals biologically younger and can extend their lifespan by as much as 66%. Research further indicates that dietary restriction delays the onset of age-related diseases and increases overall lifespan (Jennings *et al*., 1999). For example, rats subjected to a protein-restricted diet early in life exhibited long-term appetite suppression, reduced growth

rate, and a longer lifespan. Notably, these animals also showed significantly longer telomeres in their kidneys, indicating potential benefits for cellular ageing. Since oxidative stress can accelerate telomere shortening, the reduction in oxidative stress achieved through dietary restriction is expected to help preserve telomeres and other crucial cellular components ((Jennings *et al*., 1999). This evidence highlights the significant impact of dietary choices on health, longevity, and cellular vitality.

2.11.6) Fitness for Longevity: How Exercise Protects Telomeres and Slows Aging

Research conducted by Song *et al*., 2010 has illuminated the beneficial effects of exercise on cellular health and ageing markers. Their findings revealed an inverse correlation between the duration of exercise and biomarkers associated with DNA and telomere damage, as well as with the expression of p16, a marker for ageing in human cells. Exercise is crucial in reducing harmful fat accumulation, promoting the elimination of waste products, lowering oxidative stress, and preserving DNA and telomeres. Similarly, Werner *et al*., 2009) demonstrated that exercise is linked to increased telomerase activity and the suppression of apoptosis-related proteins such as p53 and p16 in mice. Human studies further support these findings, showing that athletes have higher telomerase activity and reduced telomere shortening in their leukocytes compared to non-athletes. Exercise appears to be associated with decreased oxidative stress and enhanced expression of proteins that stabilize telomeres, suggesting its potential to slow down the ageing process and reduce the risk of age-related diseases. These insights underscore the importance of regular physical activity in maintaining cellular health and promoting longevity.

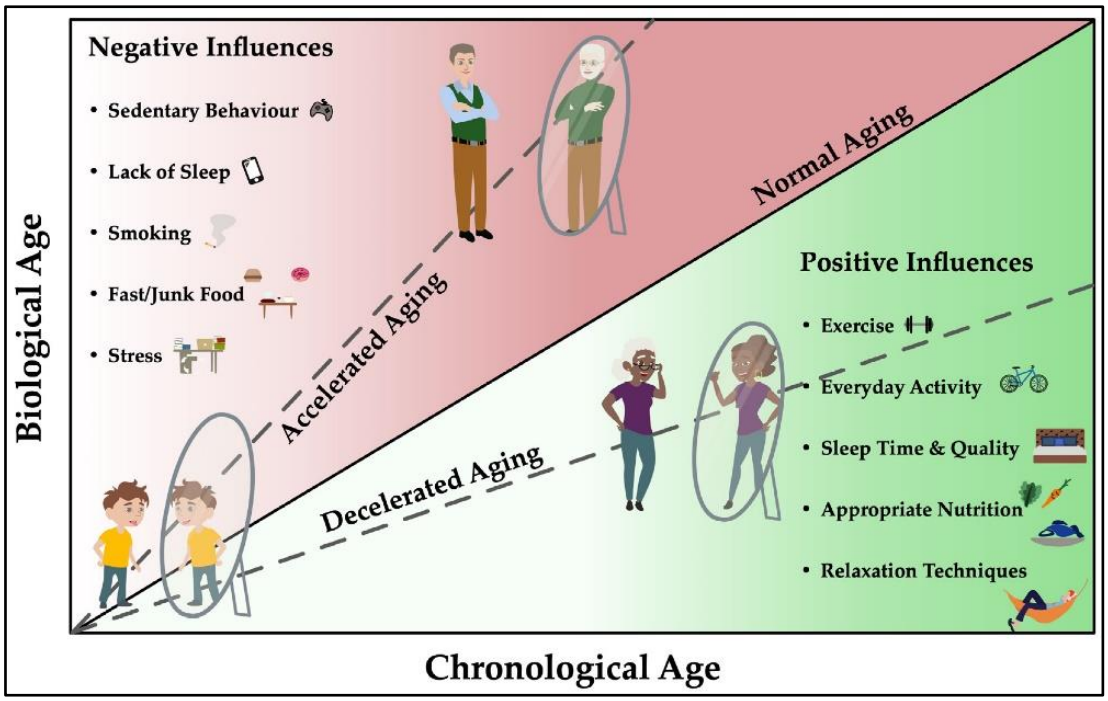

Figure 1.15: Biological age, influenced by genetics, phenotypic alterations, and epigenetic changes, correlates with a person's chronological age. Factors like nutrition, environment, psychosocial aspects, exercise, and weight can either slow down or speed up ageing, impacting disease risk. Biomarkers indicative of biological age can help assess the risk of age-related diseases, highlighting pathways for intervention. Identifying these differences among individuals of the same age can guide strategies to target negative epigenetic changes and promote healthy ageing (Haupt *et al*., 2021).

2.12) Telomerase: Preserving Telomere Length for Cellular Longevity

2.12.1) Understanding Telomerase: Guardian of Telomere Length

Telomerase is a complex ribonucleoprotein that plays a critical role in maintaining telomere length by synthesizing telomeric DNA repeats (TTAGGG) at chromosome ends. This process counters the natural loss of DNA during replication cycles. The enzyme is composed of two key components: telomerase reverse transcriptase (TERT), encoded by the hTERT gene on chromosome 5p15.33, and human telomerase RNA (hTR or hTERC), encoded by the hTERC gene on chromosomal region 3q26. hTR serves as a template for telomere DNA synthesis and is vital for telomerase activity and assembly (Akincilar *et al*., 2016). Recent research has revealed the diverse functions of telomerase that extend beyond its traditional role in regulating telomere length. Telomerase is now understood to influence gene expression, affecting cellular

processes such as proliferation and apoptosis. Additionally, it interacts with signaling pathways like WNT/β-catenin and NF-kB. Telomerase's involvement in these activities underscores its broader biological significance beyond telomere maintenance. Telomerase is also implicated in MYC-driven oncogenesis, DNA damage response (DDR), cell adhesion, migration, and epithelial-mesenchymal transition (EMT) (Ghosh *et al*., 2012). These diverse functions underscore its significance in cellular biology and oncogenic processes. Telomerase-mediated telomere length (TL) maintenance involves a multifaceted molecular journey. It encompasses several vital stages, including transporting the hTERT protein into the nucleus and assembling hTR and hTERT with accessory components (Liu *et al*., 2013). This process occurs in the cell nucleus, where telomerase is recruited to telomeres during DNA replication. In vitro studies have underscored the critical roles of hTERT and hTR in telomerase reverse transcriptase activity. In vivo, however, the telomerase complex comprises not only hTERT and hTR but also additional proteins—dyskerin, NHP2, NOP10, and GAR1—associated with H/ACA small nucleolar RNAs, essential for RNA pseudouridylation during post-transcriptional modification.

Furthermore, TCAB1, a protein containing WD repeats, binds to the CAB-box sequence in hTR, directing the telomerase complex to Cajal bodies in the nucleolus (Venteicher *et al*., 2009). Other factors, including chaperones like HSP90 and p23, as well as ATPases pontin and reptin, interact with telomerase subunits to assemble the functional telomerase complex in vivo. While their roles in telomerase assembly are acknowledged, the precise mechanisms of their interactions remain incompletely understood.

2.12.2) Telomerase Biogenesis, Recruitment, and Functionality

The biogenesis of telomerase, an essential enzyme for telomere maintenance and cellular longevity, involves a complex interplay of various proteins and ribonucleoproteins. Central to this process is the assembly of the H/ACA motif-binding complex. Dyskerin, pontin, and reptin form a critical scaffold for nascent human telomerase RNA (hTR) transcripts. This framework facilitates the assembly of a larger ribonucleoprotein complex that includes dyskerin, NHP2 ribonucleoprotein, NOP10 ribonucleoprotein, nuclear assembly factor 1 (NAF1), and the telomerase ribonucleoprotein (RNP) particle. These coordinated molecular processes lead to the formation of a stable hTR-H/ACA-RNP complex, critical for the maturation and catalytic activity of telomerase RNP.

Additionally, TCAB1, a protein associated with Cajal bodies, plays a role in guiding telomerase to these nuclear sub-organelles, thereby influencing its intracellular trafficking. Although the precise functions of TCAB1 in telomerase regulation are not fully elucidated, it is evident that TCAB1 plays a significant role in the spatial and temporal regulation of telomerase assembly and function (Hockemeyer *et al*., 2015). The recruitment of telomerase to telomeres is a tightly regulated process that occurs during the S phase of the cell cycle, synchronized with the replication of DNA 3′ ends. This crucial step involves intricate protein-protein interactions, where TPP1, POT1, and the DAT domain of hTERT play pivotal roles. TPP1, an essential component of the shelterin complex, orchestrates this process in several ways: its N-terminal OB-fold domain contains the Tel patch motif, which directly interacts with the DAT domain of hTERT, facilitating the localization of telomerase to the telomere. Moreover, TPP1's central domain binds to POT1, a protein crucial for safeguarding telomere integrity, while its C-terminal domain interacts with TIN2, another essential shelterin complex protein. These coordinated interactions create a stable and functional platform that ensures precise positioning of telomerase at the telomere, optimizing its ability to elongate telomeric DNA effectively (Schmidt *et al*., 2014). Recent studies have expanded our understanding by identifying additional factors involved in telomerase recruitment. For instance, research has highlighted SRSF11 as a novel protein that binds to TERC (telomerase RNA component), providing further insights into the complex mechanisms governing telomerase loading onto telomeres. These findings underscore the intricate regulatory network that governs telomere maintenance and its implications for cellular aging and cancer development. SRSF11 facilitates the loading of telomerase onto the telomere overhang, a critical step for efficient telomere synthesis. This interaction leads to the stable association of telomerase with the telomere, ensuring that the DNA 3′ end is correctly positioned at the enzyme's active site. This precise positioning is crucial for efficiently adding nucleotides, which extends the telomeric DNA and protects chromosome ends from degradation and fusion. The function of SRSF11 emphasizes the significance of extra regulatory layers in telomerase function, highlighting the fact that telomerase activity is dependent on a variety of accessory proteins in addition to its core components, which guarantee appropriate localization and activation. In conclusion, the biogenesis, recruitment, and functionality of telomerase are orchestrated by a sophisticated network of protein and RNA interactions. Each step is tightly regulated to maintain telomere integrity and cellular longevity, from the assembly of the hTR-H/ACA-RNP complex to the precise recruitment of telomerase to telomeres and the facilitation of its nucleotide addition activity. A comprehensive understanding of these mechanisms at a molecular level is essential

for gaining insights into how telomerase is regulated and its significance in cellular aging and cancer. Dysregulation of telomere maintenance mechanisms frequently occurs in cancer, underscoring the importance of studying these processes in detail. This knowledge not only enhances our understanding of fundamental cellular processes but also informs potential therapeutic strategies targeting telomerase in cancer treatment and age-related diseases.

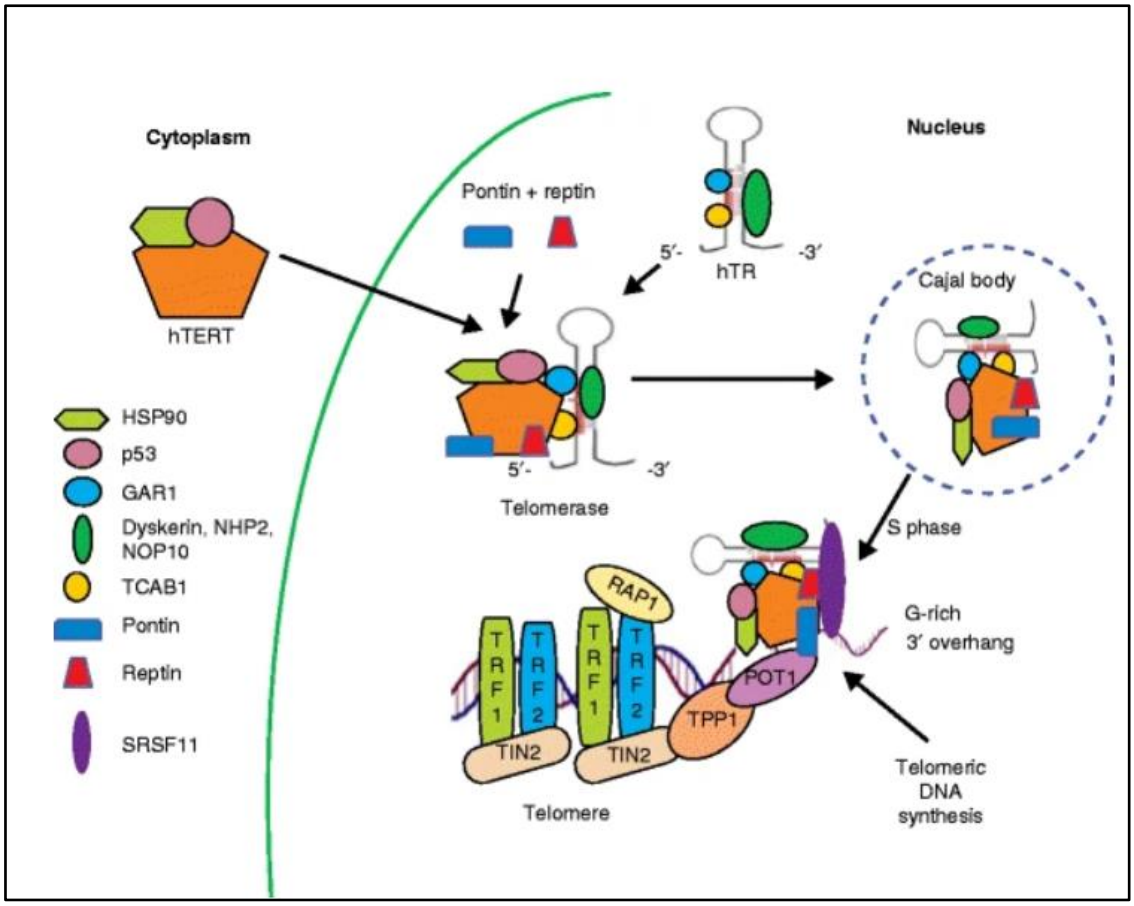

Figure 1.16: Telomerase is a complex enzyme crucial for extending telomeric DNA in cells. It assembles in the cytoplasm with hTERT, moves to the nucleus with hTR, and forms a complex with accessory proteins like dyskerin, NOP10, NHP2, and GAR1. TCAB1 guides this complex to Cajal bodies in the nucleus. During cell replication, telomerase is recruited to telomeres by interacting with TPP1, POT1, and the DAT domain of hTERT. SRSF11 stabilizes the complex at the telomere for DNA synthesis.

2.13) Telomerase and Cancer: Unveiling TERT Promoter Mutations and Telomerase Reactivation

2.13.1) Telomerase Activation and Regulation in Cancer

Telomerase activation is a critical feature in cancer, with over 90% of cases exhibiting upregulation or reactivation of the enzyme, highlighting its significance in oncogenesis (Buseman *et al*., 2012). Human telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase, plays a pivotal role in this process. The hTERT gene spans approximately 40 kilobases on chromosome 5 (5p15.33) and includes 15 introns and 16 exons. Remarkably, it is located about 1.2 megabases from the telomere within a chromatin domain resistant to nucleases, suggesting a tightly regulated genomic context. Unlike many other genes, the hTERT promoter is rich in GC content and lacks traditional TATA and CAAT boxes. Instead, it possesses multiple binding sites for various transcription factors, indicating its multifaceted and context-dependent regulation. At the core of hTERT's transcriptional regulation is a 260-base pair region crucial for its promoter activity. This region contains GC boxes that facilitate binding with SP1, a zinc finger transcription factor essential for hTERT promoter activity.

Additionally, the core region includes E-boxes, which interact with MYC/MAX/MXD1 and USF1/2. These E-boxes play a dual role in regulating hTERT, being involved in both the activation and repression of its transcription. The dynamic regulation of hTERT transcription is further orchestrated by various transcription factors. Activators such as c-MYC, SP1, ETS family members, and NF-kB upregulate hTERT expression, promoting its activity. Conversely, transcription factors such as p53, MAD, WT1, and others act as repressors, dampening hTERT transcription and maintaining a regulatory balance (Hsu *et al*., 2016).

Recent research has unveiled the importance of specific mutations within the core promoter region of hTERT. Two prevalent mutations at positions −124 bp and −146 bp from the ATG start site involve C to T transitions (C228T and C250T). These mutations create an identical 11-base pair nucleotide stretch, including a binding motif for ETS transcription factors. The presence of these mutations has been associated with enhanced telomerase activity and is commonly observed in various cancers, suggesting a potential mechanism for hTERT activation in tumorigenesis. The role of ETS factors in telomerase activation is complex and remains under investigation, with evidence indicating they can function as both repressors and activators depending on the context (Heidenreich *et al*., 2014). Understanding the regulation

of hTERT is crucial because its expression and activity are tightly linked to cellular immortalization and oncogenesis mechanisms. In normal somatic cells, telomerase activity is typically repressed, leading to progressive telomere shortening and eventual cellular senescence or apoptosis. However, in cancer cells, the reactivation or upregulation of telomerase allows telomere length maintenance, enabling these cells to bypass senescence and continue proliferating indefinitely. This underscores the significance of hTERT regulation in cancer biology and highlights the potential for targeting telomerase as a therapeutic strategy in oncology.

2.13.2) Influence of hTERT Promoter Mutations in Cancer

These recurrent hTERT promoter mutations have emerged as prominent features in various cancers, particularly melanomas, glioblastomas, hepatocellular carcinomas, bladder cancers, basal cell carcinomas, and others. They are linked with heightened hTERT promoter activity, contributing to increased telomerase expression in many cancer cases. While specific mutations like −57 A>C, −124 C>T, and −146 C>T exhibit moderate effects on hTERT transcription, others such as C228T and C250T lead to substantial increases in hTERT expression and telomerase activation (Li *et al*., 2015). However, the direct correlation between enhanced transcription and functional telomerase enzyme activity in tumour cells remains a topic of exploration **(Figure 1.17).** Studies have delved into the broader implications of hTERT promoter mutations in transformed tumour cells and human embryonic stem cells (hESCs) (Hsu *et al*., 2016). While specific mutations displayed augmented hTERT mRNA levels in undifferentiated hESCs, their effect on telomerase activity varied in differentiated fibroblasts, hinting at context-dependent regulatory mechanisms. Beyond genetic mutations, telomerase expression undergoes multifaceted regulation at transcriptional, post-transcriptional, and epigenetic levels. Processes such as mRNA splicing, hTR and hTERT synthesis, post-translational modifications, and telomerase recruitment to telomeres contribute to the intricate control of telomerase activity in cellular contexts. Epigenetic modifications, including chromatin remodelling and histone modifications, also play crucial roles in hTERT transcriptional regulation, underscoring the complexity of telomerase expression mechanisms.

Table 4: Diverse Cancer Types: Exploring the Frequency of hTERT Promoter Mutations

Cancer type	Mutation frequency (%)
Bladder carcinoma	47–85
Renal pelvic carcinoma	60–64
Urothelial carcinoma	47
Hepatocellular carcinoma	24–59
Melanoma	67–85
Skin basal cell carcinoma	39–74
Thyroid cancer (papillary and poorly differentiated carcinomas)	50–52
Myxoid liposarcoma	74–79
Glioblastoma	28–84
Medulloblastoma	19–42
Oligoastrocytoma Oligodendroglioma	25–53 72
Breast cancer, colorectal cancer, medullary thyroid carcinoma, ovarian cancer, esophageal adenocarcinoma, acute myeloid leukemia, chronic lymphoid leukemia, pancreatic cancer, prostate cancer, testicular carcinoma, uterine cervix cancer	0–5

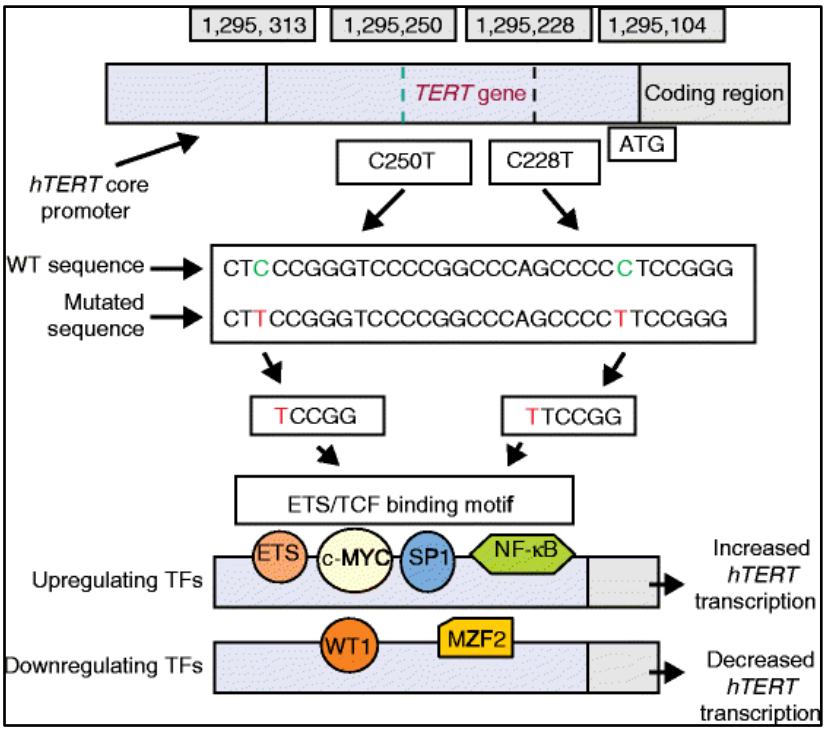

Figure 1.17: hTERT transcription and promoter mutations. The hTERT gene, usually tightly silenced, undergoes specific promoter mutations during cancer progression. These mutations create new ETS/TCF binding sites, increasing hTERT transcription. TFs like ETS, c-MYC, SP1, and NF-kB bind to these sites, promoting transcription. A permissive chromatin environment is also crucial. Downregulating TFs like WT1 and MZF2 can decrease hTERT transcription.

2.14) Targeting Telomerase for Cancer Therapy

Targeting telomerase for cancer therapy has been a significant focus in developing effective treatments, given its widespread expression in cancer types and stem-like cells. Unlike normal cells, which maintain longer telomeres and lower telomerase activity, cancer cells exhibit higher telomerase activity, making them vulnerable to targeted therapies that exploit this difference without affecting normal cells (Buseman *et al*., 2012). The primary objective of anti-telomerase therapeutics is to induce apoptosis and cell death specifically in cancer cells while preserving normal cell function. Several strategies have been investigated, including vaccines, antisense oligonucleotides, and small-molecule inhibitors that target either hTERT or hTR. Imetelstat (GRN163L), a leading telomerase inhibitor, has emerged as a promising candidate in clinical trials for its ability to selectively disrupt telomerase function in cancer cells. Another inhibitor, BIBR1532, disrupts TERT–RNA binding, inhibiting telomerase activity effectively, but has not yet progressed to clinical trials. Strategies like G-quadruplex stabilizers, tankyrase inhibitors, HSP90 inhibitors, and T-oligo are also being explored to kill cancer cells selectively (Bryan *et al*., 2015). Immunotherapies utilizing dendritic cells (GRVAC1), hTERT peptide (GV1001), and cryptic peptides (Vx-001) are currently undergoing clinical trials, as noted by Cruz *et al*. (2014). These approaches aim to harness the immune system's ability to target telomerase and inhibit its activity in cancer cells, offering promising avenues for cancer treatment.

2.15) Evolution of Imetelstat: Milestones in Oligonucleotide Inhibitor Development

Imetelstat represents a groundbreaking approach in cancer therapy as a competitive inhibitor targeting telomerase. Unlike traditional antisense oligonucleotides that bind to mRNA, Imetelstat features a distinctive structure—a 13-mer N3′–P5′ thio-phosphoramidate oligonucleotide linked to a palmitoyl (C16 lipid) moiety—that provides exceptional properties. The thio-phosphoramidate backbone ensures high solubility, stability against acidic conditions and metabolism, resistance to nucleases, and the ability to form stable RNA duplexes (Jackson *et al*., 2007). Concurrently, the lipid component enhances cellular uptake, retention, and overall drug efficacy by facilitating its entry into target cells (Herbert *et al*., 2005).

Imetelstat functions by binding specifically and with high affinity to a 13-nucleotide segment of hTR, a critical component of the telomerase holoenzyme, at its active site, thereby completely inhibiting telomerase activity. Extensive preclinical studies across diverse cancer

types, including bladder, breast, lung, liver, prostate, and pancreatic cancers, have demonstrated its potent inhibition of telomerase activity and subsequent telomere shortening in cell lines and animal models (Hu *et al*., 2015). In vivo studies using mouse xenograft models of human tumors have provided compelling evidence of Imetelstat's efficacy. It has been shown to significantly reduce tumor growth, prevent metastasis, and enhance the sensitivity of tumors to standard chemotherapy regimens. Notably, its ability to penetrate the blood-brain barrier has been crucial in effectively targeting and inhibiting the growth of glioblastoma tumors in experimental models (Marian *et al*., 2010). Moreover, combination therapy studies have highlighted Imetelstat's potential when used in conjunction with other treatments, suggesting synergistic effects that could improve overall treatment outcomes for cancer patients. Ongoing clinical trials continue to evaluate Imetelstat's safety, efficacy, and potential benefits across various cancer types, paving the way for its potential approval as a novel therapeutic option in oncology.

Combined with nilotinib, a tyrosine kinase inhibitor, it showed enhanced efficacy in suppressing homologous recombination and telomerase activity in a mouse model of Barrett’s adenocarcinoma compared to either compound alone. While clinical trials for imetelstat have been ongoing for several years, some trials were halted by the FDA due to haematological toxicity concerns, particularly in breast and lung cancer, lymphoproliferative disorders, and polycythemia vera. However, ongoing trials targeting myelofibrosis and myelodysplastic syndrome patients actively recruit participants across multiple USA, Europe, and Asia centres, indicating continued interest and development in this promising therapeutic agent.

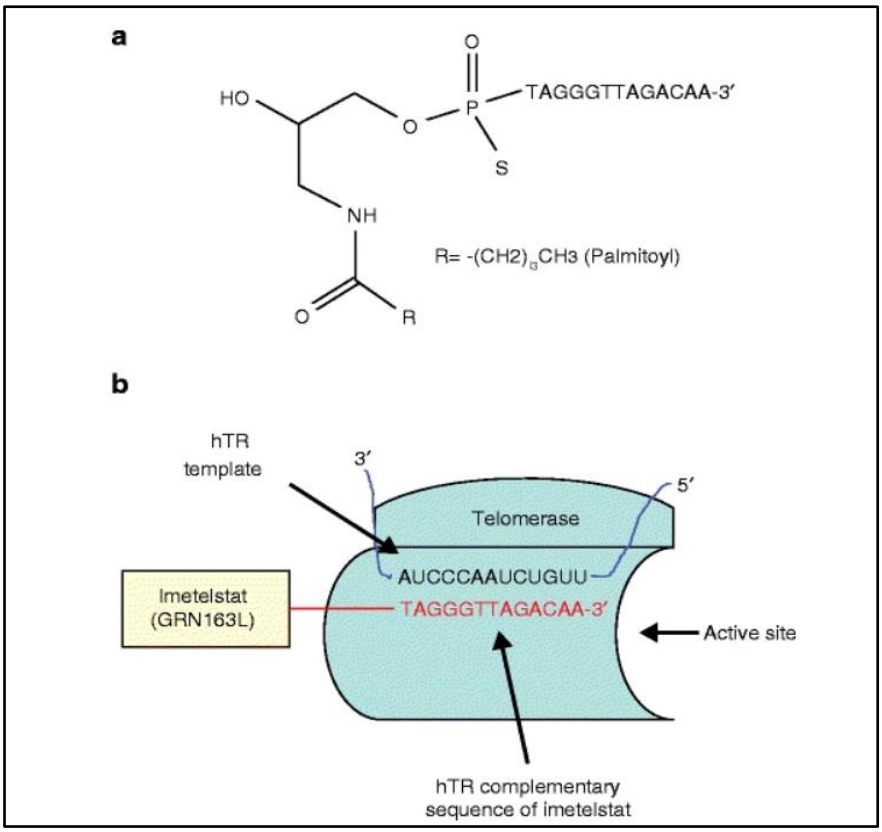

Figure 1.18: Structure and action of imetelstat (GRN163L). (a) Imetelstat (GRN163L) Structure: Imetelstat is a lipid-conjugated 13-mer oligonucleotide with a thio-phosphoramidate backbone, providing stability and resistance to nucleases. The lipid component enhances cellular uptake and retention. (b) Imetelstat Action: Imetelstat binds to the template region of hTR, disrupting hTERT recruitment to telomeric DNA. This competitive antagonism inhibits telomere elongation, leading to shortened telomeres in cancer cells and impairing their proliferation (Hu *et al*., 2015).

2.16) Exploring Anti-Telomerase Immunotherapeutics

Telomerase presents an enticing target for developing immunotherapeutic approaches against cancer. Within cancer cells, the breakdown of telomerase by proteasomes generates protein fragments or peptides that surface as antigens via the HLA class I pathway (Vonderheide *et al*., 1999). These telomerase antigenic epitopes become targets for cytotoxic T cells, triggering the destruction of tumour cells. This rationale underpins anti-telomerase immunotherapy, aimed at sensitizing the immune system to hTERT-expressing tumour cells and activating hTERT-specific CD8+ cells for enhanced anti-tumour effects (Vonderheide *et al*., 2008). Two primary strategies have emerged in developing telomerase-based immunotherapy: the hTERT vaccine

approach and the dendritic cell approach for ex vivo priming of antigen-presenting cells. Notable vaccines like GV1001, Vx001, and GRNVAC1 have been pivotal in eliciting anti-telomerase immune responses in cancer patients. GV1001, an HLA class II-restricted hTERT peptide vaccine, induces CD4+ and CD8+ responses through endogenous processing bolstered by GM-CSF or TLR7 adjuvants. Vx001, a cryptic peptide-based vaccine, targets HLA class I with high affinity, showcasing significant immune response rates.

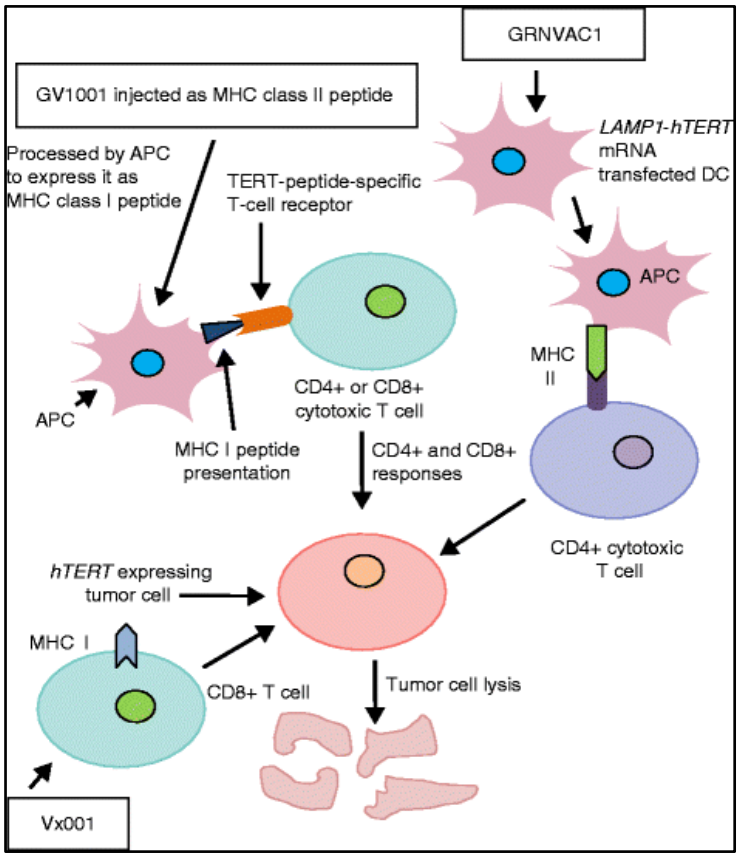

Figure 1.19: Anti-telomerase immunotherapy utilizes telomerase-based vaccines to sensitize immune cells to cancer cells expressing hTERT peptides as surface antigens via HLA class I and II pathways. As a result, cancer patients have an increase in telomerase-specific CD4+ and CD8+ cytotoxic T lymphocytes (CTLs), which allow T cells to specifically target and eradicate telomerase-positive tumor cells. GV1001, an MHC class II-restricted hTERT peptide, is processed by antigen-presenting cells (APCs) to present as an MHC class I peptide, generating CD4+ and CD8+ immune responses. GRNVAC1 activates CD4+ T cells against hTERT-expressing tumours, while Vx001's action is mediated by CD8+ T cells (Mender *et al.*, 2015).

Conversely, mature autologous dendritic cells transduced with mRNA expressing hTERT and LAMP1 are used in GRNVAC1, a dendritic-cell-based vaccination. This approach fosters a polyclonal immune response specific to all hTERT epitopes patient tumours express (Ruden *et al*., 2013) **(Figure 1.19).** Clinical trials actively evaluate the efficacy and safety of these vaccines (GV1001, GRNVAC1, and Vx001) in cancer patients. The hTERT-specific immune responses induced by these vaccines have been well tolerated, paving the way for promising advancements in anti-telomerase immunotherapy **(Table 3).**

Identifier code/phase	Indication	Objective	Start/ completion date	Results	Sponsor/reference
NCT00510133/ GRNVAC1 phase II	Acute myelogenous leukemia	Efficacy	July 2007/August 2014	GRNVAC1 was found to be safe and well tolerated. Positive immune responses in 55 % of patients. Toxicity: thrombocytopenia.	Asterias Biotherapeutics (http:// asteriasbiotherapeutics.com/pipeline/ast-vac1/)
NCT01579188/GV1001 phase III	Non-small-cell lung cancer	Efficacy	May 2012/May 2016	Ongoing.	Kael-GemVax
NCT00425360/GV1001 phase III	Metastatic pancreatic cancer	Efficacy in combination with chemotherapy	September 2006/ March 2013	Adding GV1001 vaccination to chemotherapy did not improve overall survival.	[108]
NCT01935154/Vx001 phase II	Non-small-cell lung cancer	Efficacy	August 2012/ December 2016	Active.	Vaxon Biotech (http://www.clinicaltrials.gov)

Table 5: Status Update on Clinical Trials for Anti-Telomerase Vaccines: Past and Present

2.17) Using Telomerase Activity to Kill Cancer Cells Specifically

One of the significant challenges in anti-telomerase therapies is the time it takes for telomeres to shorten enough to kill cancer cells. This process needs several cell division cycles, and treatment might have to be continuous for months to see significant tumour reduction. While waiting for this effect, most tumour cells can still grow, so additional treatments might be needed to achieve better results. With direct telomerase inhibitors like imetelstat, if there are any toxicities (like blood-related issues), stopping treatment for a while could reverse some benefits gained. Deciding to stop treatment or take breaks depends on balancing effectiveness against potential side effects. Hence, there is a growing need for fast-acting therapies that can block telomerase activity effectively. One innovative approach does not directly target telomerase but introduces a modified nucleoside into cells. This modified nucleoside is designed to be preferentially incorporated into telomeric DNA by telomerase. Once incorporated, this altered nucleotide does not bind efficiently to shelterin proteins, leading to telomere dysfunction and swift cell death. Recent research by Mender and team showcased the effectiveness of 6-thio-2′-deoxyguanosine (6-thio-dG), a nucleoside analogue of an approved drug, in telomerase-positive cells. This compound was recognized by telomerase and integrated

into telomeres, resulting in disrupted telomere organization and activation of telomere-associated DNA damage signals. This ultimately led to rapid cell death (Mender *et al*., 2015). Experiments with 6-thio-dG against cell lines and in animal models demonstrated its ability to induce swift cell death in telomerase-positive cells while sparing normal telomerase-silent cells. In vivo studies also showed significant reductions in tumour growth rates without causing adverse effects in mice. This telomerase-mediated telomere-disrupting approach shows promise as a safe and effective option for cancer treatment (Mender *et al*., 2015).

2.18) Exploring the Functional and Genetic Complexity of (MNS16A)-a minisatellite

(MNS16A)A minisatellite is a variable number tandem repeat (VNTR) found in the human genome. VNTRs are sections of DNA consisting of a short series of nucleobases, typically ranging from 10 to 60 base pairs. Minisatellites, including (MNS16A), are longer compared to microsatellites, another class of VNTRs consisting of only 2 to 6 base pairs (Wang *et al*., 2003). Minisatellites are distinguished from microsatellites by their size and complexity. They are often called VNTRs due to their variability in the number of tandem repeats occurring at more than 1,000 locations in the human genome. This variability contributes to genetic diversity and can have functional implications. The (MNS16A)- a minisatellite, specifically, has been studied for its promoter activity, which depends on the number of tandem repeats present. The structure of MNS16A is characterized by two repeat elements forming a core sequence of either 23 base pairs or, when separated by a CAT trinucleotide insertion, a 26-base pair sequence. This CAT insert creates a transcription factor binding site for GATA-1, a protein involved in gene regulation (Wang *et al*., 2003). Several variable numbers of tandem repeats (VNTRs) within MNS16A have been identified, including VNTR-243, VNTR-274, VNTR-302, and VNTR-333, named based on their PCR fragment sizes. These VNTRs contribute to the overall variability and functional diversity of the MNS16A minisatellite.

Research on MNS16A and its VNTRs has focused on understanding their role in gene expression regulation, particularly in cancer and other diseases. The variability in tandem repeats within MNS16A can influence gene activity, including promoter activity and transcription factor binding, which may impact disease susceptibility, progression, and response to treatments (Wang *et al*., 2003). Studies have also explored the association between MNS16A polymorphisms and various traits or conditions, such as cancer risk, neurological disorders, and immune system function. The functional implications of different VNTR configurations within MNS16A are an area of ongoing research, with potential implications

for personalized medicine and disease prevention strategies. In summary, (MNS16A)-a minisatellite is a complex VNTR with variable tandem repeats contributing to genetic diversity and gene regulation. Its structure and functional properties, including promoter activity and transcription factor binding, make it a subject of interest in genetics, genomics, and disease research (Wang *et al*., 2003). Further studies are needed to elucidate the specific roles and implications of MNS16A VNTRs in human health and disease.

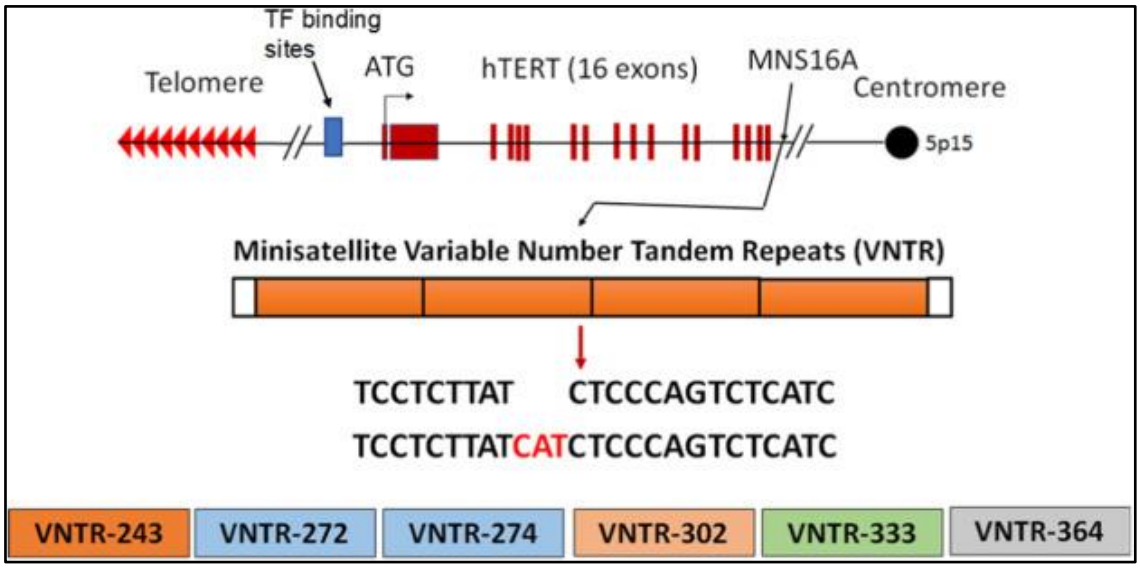

Figure 1.20: MNS16A is a variable number tandem repeat (VNTR) located downstream of exon 16 in the hTERT gene promoter. It has two core sequences, 23 or 26 base pairs, with CAT insertions binding to GATA-1. The VNTR alleles, like VNTR-364, vary in tandem repeats and CAT insertions, contributing to gene regulation complexity (Huda *et al*., 2021).

2.18.1) MNS16A in Cancer

Research has shown that the number of tandem repeats within MNS16A can influence its promoter activity, affecting gene expression regulation. The variability in tandem repeats can impact transcription factor binding and promoter activity, which may have significant implications for disease susceptibility, progression, and treatment response. This has made MNS16A a subject of interest in genetics, genomics, and disease research (Wang *et al*., 2003). MNS16A has garnered significant attention in cancer research due to its impact on regulating the hTERT gene, which encodes the catalytic subunit of telomerase. Telomerase is crucial for maintaining telomere length, a critical factor in cellular ageing and cancer. In most somatic

cells, telomerase activity is low, leading to progressive telomere shortening and eventual cellular senescence or apoptosis. However, telomerase is reactivated in over 90% of cancers, allowing cells to bypass these limits and continue proliferating indefinitely (Buseman *et al*., 2012).

a) **Breast Cancer:** In breast cancer, studies have indicated that specific VNTR variants of MNS16A are associated with changes in hTERT expression levels, influencing the risk of developing breast cancer. Variations in the number of repeats can modulate the binding efficiency of transcription factors to the hTERT promoter, thereby affecting telomerase activity. Increased telomerase activity can contribute to the immortalization of breast epithelial cells, promoting tumorigenesis and progression (Wang *et al*., 2003).
b) **Lung Cancer:** Lung cancer research has also highlighted the significance of MNS16A polymorphisms. Specific VNTR configurations within the MNS16A minisatellite are linked to higher telomerase activity, critical for lung cancer cells' sustained growth and survival. The presence of specific VNTR variants may serve as a biomarker for lung cancer susceptibility and prognosis, aiding in the identification of high-risk individuals and tailoring of therapeutic approaches (Wang *et al*., 2003)
c) **Colorectal Cancer:** In colorectal cancer, MNS16A VNTR variants have been found to influence hTERT expression and telomerase activity, similar to their roles in other cancers. The number of tandem repeats in the MNS16A region can alter the chromatin structure and transcription factor binding at the hTERT promoter, impacting telomerase activation. These variations may affect the growth and progression of colorectal tumours, highlighting the potential of MNS16A as a target for diagnostic and therapeutic interventions (Wang *et al*., 2003).
d) **Other Cancers:** Beyond breast, lung, and colorectal cancers, MNS16A polymorphisms have been studied in various other malignancies, including gastric, ovarian, and prostate cancers. In each case, the VNTR variability within MNS16A influences telomerase activity, contributing to the oncogenic process. Understanding the specific VNTR configurations associated with different cancer types can provide valuable insights into the mechanisms of telomerase regulation and its role in cancer biology (Wang *et al*., 2003).

2.18.2) MNS16A in Other Diseases

a) **Neurological Disorders:** MNS16A has also been implicated in neurological disorders, where its role in gene regulation could affect neural development and function. Variability in the number of tandem repeats within MNS16A may influence the expression of genes involved in neural cell proliferation, differentiation, and survival. For instance, studies have explored the association between MNS16A polymorphisms and diseases such as Alzheimer's and Parkinson's, where telomere maintenance and cellular ageing play critical roles. The impact of MNS16A on telomerase activity in neural cells could contribute to the pathogenesis of these disorders, offering potential targets for therapeutic intervention (Wang *et al*., 2003).

b) **Immune System Function:** The immune system's functionality is another area where MNS16A polymorphisms have shown potential impact. The variability in tandem repeats within the MNS16A minisatellite may influence genes involved in immune responses, affecting disease susceptibility and progression. For example, in autoimmune diseases such as rheumatoid arthritis and lupus, alterations in telomere length and telomerase activity have been observed. MNS16A variants could modulate the expression of hTERT and other related genes, influencing the immune system's capacity to respond to inflammation and infection (Wang *et al*., 2003).

c) **Cardiovascular Diseases:** Preliminary research suggests that MNS16A VNTRs may also be linked to cardiovascular diseases. Telomere length and telomerase activity are critical factors in the ageing and functionality of cardiovascular tissues. Variability in the MNS16A region could affect the regulation of hTERT, impacting telomere maintenance in endothelial cells and cardiomyocytes. This, in turn, might influence the development of conditions such as atherosclerosis, hypertension, and heart failure. Understanding the role of MNS16A polymorphisms in cardiovascular health could lead to new strategies for prevention and treatment (Wang *et al*., 2003).

d) **Metabolic Disorders:** MNS16A variability has been explored in the context of metabolic disorders, including diabetes and obesity. Telomere length has been associated with metabolic health, and telomerase activity plays a role in cellular metabolism and energy balance. VNTR variations within MNS16A may influence the regulation of metabolic genes, contributing to the risk and progression of these conditions. Research in this area aims to uncover the genetic factors underlying metabolic disorders and their potential links to telomere biology (Wang *et al*., 2003).

2.18.3) Research and Clinical Implications

The functional implications of different VNTR configurations within MNS16A are an ongoing area of research. Understanding the specific roles of MNS16A VNTRs in gene regulation and disease can provide insights into disease mechanisms and potential therapeutic targets. Personalized medicine approaches could benefit from such knowledge, allowing for tailored disease prevention, diagnosis, and treatment strategies based on individual MNS16A VNTR profiles. In summary, MNS16A is a complex VNTR with variable tandem repeats contributing to genetic diversity and gene regulation. Its structure and functional properties, including promoter activity and transcription factor binding, make it a significant focus in genetics and disease research. Ongoing studies aim to elucidate the specific roles and implications of MNS16A VNTRs in human health and disease, with the potential to inform personalized medicine and disease prevention strategies (Wang *et al*., 2003).

CHAPTER 3

GAPS AND OBJECTIVES

3.1) Gaps in the study

1. **Limited Population Studies:** Most studies on hTERT gene polymorphisms and lung cancer associations have been conducted in other populations, so there may be unique genetic variations or associations in North Indians that have not been adequately explored.
2. **Longitudinal Studies:** Many studies on genetic polymorphisms and disease associations are cross-sectional, providing a snapshot of associations simultaneously. Longitudinal studies tracking individuals over time could reveal how changes in genetic profiles, including the MN16A polymorphism, correlate with lung cancer development or progression in the North Indian context.
3. **Comparative Analysis with Other Populations:** Comparing the findings from North Indian populations with studies conducted in other geographical regions or ethnicities could highlight unique genetic patterns or similarities/differences in genetic associations with lung cancer. This comparative analysis can contribute to a broader understanding of the global genetic landscape of lung cancer susceptibility.
4. **Clinical Relevance and Translational Implications:** Bridging the gap between genetic research and clinical applications is essential. We are discussing the translational implications of the findings, such as potential diagnostic markers, personalized treatment strategies based on genetic profiles, or implications for lung cancer screening programs in North India.

3.2) Objectives

1. To study the single nucleotide polymorphism variants of the hTERT gene.
2. To evaluate the role of polymorphic variants of hTERT towards lung cancer susceptibility.
3. To evaluate the clinical outcomes and toxicity profiles of lung cancer patients undergoing platinum-based chemotherapy.
4. To evaluate overall survival and clinicopathological features.

CHAPTER 4

MATERIALS AND METHODS

4) MATERIALS AND METHODS

4.1) Study Design

A comprehensive retrospective case study was thoughtfully designed to thoroughly assess the correlation between genetic polymorphisms of the hTERT (human telomerase reverse transcriptase) gene and the susceptibility of the North Indian population to lung cancer. The study aimed to delve into the intricate genetic markers that might predispose individuals to this prevalent form of cancer in the region. The research initiative involved the systematic collection of blood samples from a cohort of lung cancer patients. These samples were procured from the Department of Pulmonary Medicine at the esteemed Post Graduate Institute of Medical Education and Research (PGIMER) in Chandigarh, India. This institution, renowned for its medical research and adherence to ethical standards, was crucial in facilitating the study's execution. Before commencing the research, meticulous ethical considerations were essential. The Institute Ethics Committee of PGIMER thoroughly reviewed the study's protocol and granted formal authorization (Approval No. IEC-04/2018-884). The study strictly adhered to the ethical guidelines outlined in the 1964 Declaration of Helsinki and its subsequent amendments, ensuring all participants' rights, safety, and confidentiality. To reinforce the ethical framework further, an explicit ethical clearance letter has been appended in Appendix I of the study documentation. This letter is a testament to the rigorous ethical scrutiny and compliance upheld throughout the research endeavour.

Additionally, written informed consent was diligently obtained from all patients or their authorized representatives, as Appendix II shows a transparent and ethical approach to patient participation. The inclusion criteria for the study encompassed patients newly diagnosed with primary lung cancer—this deliberate selection criterion aimed to capture diverse cases representative of the region's lung cancer demographics. Histological diagnosis played a pivotal role in categorizing patients into distinct subtypes, including Adenocarcinoma (ADCC), Squamous Cell Carcinoma (SQCC), and Small Cell Lung Cancer (SCLC). This stratification strategy enabled a nuanced analysis of genetic associations specific to each histological subtype. Crucially, the study adopted an inclusive approach, with no restrictions based on age, smoking history, gender, histology, or TNM staging. This holistic approach ensured a comprehensive exploration of genetic influences across diverse patient profiles, contributing valuable insights to the broader understanding of lung cancer aetiology in the North Indian population. Exclusions from the study were carefully deliberated to maintain data integrity and

relevance. Patients under active observation or with a history of prior cancer diagnoses were excluded from the study cohort. This exclusion criterion explicitly focused on newly diagnosed cases, minimizing confounding variables and enhancing the study's internal validity.

Patients meeting all the following requirements shall be eligible for enrollment in the study, ensuring a comprehensive and inclusive approach to data collection and analysis:

a) **Confirmed Diagnosis of Lung Cancer:** Eligible patients must have a confirmed diagnosis of lung cancer, which includes both Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC), as determined by either histological examination or cytological analysis. This diagnostic confirmation ensures the accuracy and specificity of the patient cohort with lung cancer.
b) **Disease Stage and Severity:** Patients with lung cancer diagnosed at Stages I, II, III, or IV are eligible for enrollment. While patients diagnosed with early-stage disease (Stage I/II) may be relatively fewer in number, their inclusion provides valuable insights into genetic influences across different disease stages. This criterion allows for a comprehensive evaluation of genetic polymorphisms with disease progression and severity.
c) **No Restrictions Based on Age, Gender, Smoking History, Histology, or Staging:** The study adopts an inclusive approach without imposing restrictions based on demographic factors such as age, gender, smoking history, histological subtype, or TNM staging. This non-restrictive criterion ensures a diverse and representative patient population, enhancing the study's external validity and generalizability of findings.
d) **Treatment Intent with Definitive Chemotherapy:** Eligible patients must be untreated and intend to undergo definitive chemotherapy. This includes treatment with platinum agents such as cisplatin or carboplatin as first-line or second-line therapy. Including patients undergoing definitive chemotherapy provides insights into genetic factors influencing treatment response and outcomes.
e) **Eastern Cooperative Oncology Group (ECOG) Performance Status:** Patients with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ranging from 0 to 2 are eligible for enrollment. This criterion ensures that patients in the study have a satisfactory functional status, allowing for optimal participation in chemotherapy and study procedures.
f) **Measurable Lesion Criteria:** Patients must have at least one bi-dimensionally measurable lesion per the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. This

criterion enables a standardised assessment of treatment response and disease progression, facilitating robust clinical evaluations within the study.

g) **Adequate Organ Function:** Eligible patients must demonstrate adequate organ function as defined by specific laboratory parameters. These parameters include an absolute neutrophil count > 1500/µl, platelet count > 100,000/µl, and normal levels of creatinine and liver enzymes. Alanine aminotransferase (ALT) levels should be less than two times the upper limits of normal (ULN). Adequate organ function ensures patient safety during chemotherapy and study participation.
h) **Written Informed Consent:** Before enrollment, written informed consent must be obtained from all patients, ensuring their understanding of the study's objectives, procedures, potential risks, and benefits. Informed consent is a fundamental ethical requirement, emphasizing patient autonomy and voluntary participation in research activities.

By adhering to these comprehensive eligibility criteria, the study aims to encompass a diverse patient population while ensuring scientific rigour, ethical conduct, and robust data collection for meaningful insights into genetic determinants of lung cancer in the North Indian population.

The study's exclusion criteria for patient enrollment were meticulously designed to uphold data integrity and research validity. These criteria aimed to exclude confounding factors that could potentially skew the analysis or interpretation of genetic associations with lung cancer susceptibility. The following exclusion criteria were implemented:

a) **History of Any Other Carcinoma:** Patients with a documented history of any other carcinoma were excluded from the study cohort. This exclusion criterion ensured a focused analysis of lung cancer cases without potential confounding effects from other cancer types or treatments.
b) **Active Infection or Immunosuppression (HIV):** Patients with active infections or immunosuppression, including HIV-positive individuals, were not included in the study. This exclusion criterion was essential to maintain a homogeneous patient cohort and mitigate the influence of immunological factors on genetic analyses related to lung cancer.
c) **Systemic Steroid Use:** Patients who were receiving systemic steroids were excluded from the study. This criterion was implemented to avoid potential interactions between steroid medications and genetic markers under investigation, ensuring a more straightforward assessment of hTERT gene polymorphisms' impact on lung cancer susceptibility.

d) **Chronic Diarrhea:** Patients suffering from a chronic form of diarrhoea due to any cause were excluded. Chronic diarrhoea can indicate underlying gastrointestinal disorders or systemic conditions that could confound the study's objectives. By excluding such cases, the study focused on lung cancer-related genetic associations.
e) **Non-Undergoing Chemotherapy:** Patients not undergoing chemotherapy at enrollment were also excluded. This criterion aimed to ensure a uniform treatment status within the study cohort, minimizing treatment-related variables that could impact genetic analyses and outcomes related to hTERT gene polymorphisms and lung cancer susceptibility.

Furthermore, the exclusion criteria were applied consistently across all patient demographics, histological subtypes, and other variables to maintain a standardized approach and enhance the study's internal validity. These criteria were carefully considered to optimize the study's ability to draw meaningful conclusions regarding the genetic determinants of lung cancer in the North Indian population. However, numerous physicological tests were performed before recruitment, including a complete blood count, which determines neutrophil count, and biochemical tests such as liver and renal function tests. A chest X-ray and a contrast-enhanced computed tomography (CT)scan of the thorax and upper abdomen region were also performed on the patient. A detailed questionnaire was filled out by a trained interviewer for each case. The questionnaire included crucial demographic information such as age, gender, the patient's region, smoking, and drinking habits. Smokers reported tobacco habits such as cigarette and beedi smoking (a native cigarette-like stick of coarse tobacco hard-rolled in a dry tembuhurni leaf). Pack years were calculated as an indicator of cumulative smoking exposure using the following formula:

$$\frac{\text{Number of Packs Smoked Per Day} \times \text{Number of Years Smoked}}{20}$$

The essential medical information of cases, such as histology, TNM classification, clinical staging, performance status (KPS and ECOG), chemotherapeutic regimen, and objective response following the Response Evaluation Criteria in Solid Tumor Group (RECIST) criteria, was obtained from the hospital's medical records.

4.1.2) Chemotherapeutic regimen

All lung cancer patients recruited in the study underwent a standardized doublet platinum-based chemotherapy regimen, reflecting the contemporary treatment protocols adopted at the

renowned Post Graduate Institute of Medical Education and Research (PGIMER) in Chandigarh, India. This regimen encompassed a combination of cytotoxic agents alongside platinum-based drugs, administered intravenously based on the patient's body surface area. The chemotherapy regimen comprised a selection of cytotoxic drugs, including docetaxel, paclitaxel, irinotecan, pemetrexed, and gemcitabine, combined with a platinum-based drug such as cisplatin or carboplatin. These drugs were meticulously dosed to optimize therapeutic efficacy while mitigating potential adverse effects. Specific dosages for the combination chemotherapy were tailored according to standard guidelines and clinical protocols. For instance, docetaxel was administered at 75 mg/m² or 100 mg/m² of irinotecan as a 1-hour infusion. These doses were calculated based on the patient's body surface area to ensure precise and individualized treatment delivery. As part of the chemotherapy administration process, patients also received adjunctive medications as premedication. Common premedication drugs included dexamethasone, granisetron, and ranitidine, aimed at managing potential side effects and enhancing treatment tolerability.

Before tumour response assessment, patients underwent a standard course of four cycles of chemotherapy, which was in line with established clinical practices at PGIMER. This protocol allowed for a comprehensive evaluation of treatment response and disease control. In cases where patients experienced unacceptable toxicity or exhibited clinic-radiological evidence of disease progression before completing four cycles, tumour response assessment was expedited, and adjustments to the treatment regimen were made as necessary. Patients demonstrating an objective response to the initial chemotherapy regimen were considered for an additional two cycles, totalling a maximum of six. This approach aimed to optimize treatment outcomes while effectively monitoring and managing treatment-related adverse events. The treatment protocol followed rigorous monitoring and assessment criteria, ensuring patient safety, treatment efficacy, and adherence to standardized clinical practices. By following these meticulous procedures and treatment guidelines, the study aimed to provide comprehensive insights into the efficacy and tolerability of platinum-based doublet chemotherapy regimens in lung cancer patients within the North Indian population.

4.1.3) Follow-up and response assessment

After every two months, all subjects in the study were diligently followed up through telephonic conversations until the conclusion of the study period. These regular follow-ups were crucial in tracking various parameters, including the assessment of survival time, which

was calculated from the date of lung cancer diagnosis to the date of the last follow-up or death. The study's primary endpoint revolved around overall survival, while the secondary endpoint involved evaluating genetic polymorphisms and treatment responses. In evaluating treatment responses, the Response Evaluation Criteria in Solid Tumors (RECIST) played a pivotal role. RECIST is a widely recognized and standardized methodology for assessing tumour response in solid tumours, including lung cancer. It categorizes treatment responses into four main categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). A "complete response" (CR) signifies the disappearance of all target lesions, demonstrating a substantial reduction in tumour burden. This often indicates an adequate treatment response, with the tumour no longer detectable by imaging techniques.

On the other hand, a "partial response" (PR) indicates a significant reduction in tumour size, typically by at least 30%, reflecting a favourable response to treatment. The category of "stable disease" (SD) is assigned when there is no significant increase or decrease in tumour size, suggesting that the treatment has effectively halted tumour progression without causing regression. This status is crucial as it implies that the disease has stabilized, allowing patients to maintain their quality of life without experiencing worsening symptoms. Conversely, "progressive disease" (PD) is diagnosed when there is an increase in tumour size or the appearance of new lesions, indicating disease progression despite treatment efforts. This status prompts a reassessment of treatment strategies, including potential changes in medications or therapeutic approaches.

Additionally, adverse events (AEs) were meticulously monitored and classified according to the standard toxicity criteria version 3.0. Toxicity was assessed for patients receiving three or more chemotherapy cycles; all received at least one chemotherapy cycle. PGIMER’s standard protocols were used to handle AEs. Patients with febrile neutropenia or grade 3 or higher gastrointestinal AEs were hospitalized because outpatient management was either not possible or was ineffective. Furthermore, any other AEs caused by chemotherapy were recorded.

4.2) DNA Extraction

a) Genomic DNA extraction

Genomic DNA was extracted from peripheral blood samples of lung cancer cases using the protocol of Barlett and White (2003) with minor modifications.

1) To separate red blood cells (RBCs) from blood lymphocytes, 3 ml of whole blood was washed with an equal volume of washing solution containing 320 mM sucrose, 20 mM Tris-HCl (pH 8.0), five mM MgCl2, and 1.5% Triton X-100. This process was performed three times. The samples were centrifuged at 3,500 rpm for 10 minutes at 4°C.
2) The RBC-free pellet was lysed at 45°C in a lysis solution containing 150 mM NaCl, 400 mM Tris-HCl (pH 8.0), 1% SDS, 60 mM EDTA, and 100 µg/ml proteinase-K.
3) An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1) solution was used to remove proteins. After gentle mixing, the solution was centrifuged at 8,000 rpm for 7 minutes at four °C, resulting in two distinct layers. The upper aqueous layer was transferred into a new vial, and this process was repeated.
4) An equal chloroform/isoamyl alcohol (24:1) solution was added to the aqueous layer. The mixture was gently mixed and centrifuged at 6,500 rpm for 5 minutes at four °C, and the upper aqueous layer was transferred to another vial.
5) DNA precipitation was done using 2.5 times the volume of ethanol or an equal volume of chilled isopropanol. The solution was kept at -20°C for 1-2 hours, followed by centrifugation at 10,000 rpm for 5 minutes. The obtained pellet was air-dried.
6) The pellet was washed with ice-cold 70% ethanol by centrifugation at 10,000 rpm for 5 minutes. The obtained pellet was air-dried.
7) The DNA pellet was dissolved in 200 µl of sterile Tris-EDTA buffer.

b) Qualitative and Quantitative estimation of genomic DNA

The quality of isolated DNA was assessed using agarose gel electrophoresis. DNA samples were visualized on a 0.8% agarose gel containing 0.5 µg/ml ethidium bromide (EtBr) in 0.5X TBE buffer. The samples were mixed with 6X loading dye and diluted with sterile water before loading into the wells. The presence or absence of DNA on the gel was determined using a UV transilluminator. The quantity of extracted DNA was measured using a Nanodrop ND-1000 spectrophotometer. The spectrophotometric analysis measured absorbance at 260 nanometers (nm), where DNA strongly absorbs due to its aromatic bases. First, a blank solution containing

only the buffer was measured to establish a baseline absorbance. Then, a small amount of the DNA sample, typically prepared in a stable buffer like Tris-EDTA (TE), was measured. The absorbance reading at 260 nm and DNA's known extinction coefficient were used to calculate the DNA concentration in the sample. Additionally, the A260/A280 ratio was calculated to assess DNA purity, with a ratio around 1.8 indicating pure DNA free from protein contamination. A higher A260/A280 ratio (>1.8) may suggest RNA contamination. After spectrophotometric analysis, the DNA sample was diluted to a 100 ng/µl concentration to optimize its usability for downstream molecular biology experiments. The DNA was then carefully stored at -20°C to maintain its stability and integrity for future analyses.

4.3) hTERT Genotyping

The MNS16A polymorphism in the hTERT gene was analyzed using polymerase chain reaction (PCR). Specific primers were designed to amplify the MNS16A variable number tandem repeat (VNTR) region. The forward primer sequence was 5'-AGGATTCTGATCTCTGAAGGGTG-3', and the reverse primer sequence was 5'-TCTGCCTGAGGAAGGACGTATG-3'. The PCR reactions were conducted in a final volume of 15 µl, comprising 1X PCR buffer, 0.5 µM each of forward and reverse primers, 0.2 µM dNTPs, 10X bovine serum albumin (BSA) for enhanced enzymatic activity, 1 U of Taq polymerase (DNAzyme, Thermo Scientific), and 0.5 µl of the DNA sample under study. The optimized PCR cycling conditions included an initial denaturation at 95˚C for 5 minutes, followed by 29 cycles of denaturation at 94˚C for 30 seconds, annealing at 50˚C for 30 seconds, and extension at 72˚C for 30 seconds, with a final elongation step at 72˚C for 5 minutes to ensure complete amplification. The PCR products were then separated and visualized on a 3% agarose gel containing 0.5 µg/ml ethidium bromide under UV illumination, facilitating the accurate assessment and documentation of the amplified DNA fragments. The expected fragment lengths for the MNS16A VNTR were 243 bp and 272 bp for the short allele (S) and 302 bp and 333 bp for the long allele (L), as detailed in Table 1.

Table 6: Primer sequence and DNA fragment length given for MNS16A VNTR of hTERT gene.

Primers	Forward	Reverse	DNA fragment length
MNS16A	5'-AGGATTCTGATCTCTGAAGGGTG-3'	5'-TCTGCCTGAGGAAGGACGTATG-3'	S: 243, 272 bp L: 333, 302 bp

4.4) Statistical Analysis

a) Demographic analysis

The upcoming demographic analysis will comprehensively explore several key factors within our study cohort, providing a nuanced understanding of the diverse patient profile and disease characteristics. The analysis will encompass the following demographic and clinical parameters:

a) **Gender Distribution:** The distribution of male and female participants within the study cohort will be elucidated, highlighting any gender-based disparities or trends in lung cancer prevalence.

b) **Age Range (Median and Standard Deviation):** The age distribution of patients will be presented, showcasing the median age and standard deviation to capture the overall age diversity within the study population. This analysis will provide insights into age-related trends and their impact on lung cancer incidence.

c) **Smoking Habits (Numbers and Percentages):** Detailed information on patients' smoking habits, including current smokers, former smokers, and non-smokers, will be reported as numbers and percentages. This analysis aims to delineate the prevalence of smoking and its association with lung cancer risk in our cohort.

d) **Histological Types of Lung Cancer:** The distribution of histological subtypes, such as Adenocarcinoma (ADCC), Squamous Cell Carcinoma (SQCC), and Small Cell Lung Cancer (SCLC), will be delineated. This analysis will shed light on the prevalence and distribution of different lung cancer types within the study population.

e) **TNM Staging:** The TNM staging classification system will be utilized to categorize patients based on tumour size (T), lymph node involvement (N), and metastasis status (M). This staging analysis will provide a comprehensive overview of disease severity and progression in our cohort.

f) **Tumour Size Distribution (Mean and Standard Deviation):** The mean tumour size and standard deviation will be reported, offering insights into the variability and distribution of tumour sizes among patients with lung cancer.

g) **Lymph Node Involvement:** The extent of lymph node involvement will be evaluated and categorized, highlighting patterns of regional spread and metastatic potential in our study population.

h) **Metastasis Status:** The presence or absence of distant metastases will be documented, providing crucial information on disease dissemination and staging.

i) **Overall Survival Outcomes (Numbers and Percentage):** Survival outcomes, including overall survival rates, will be reported as numbers and percentages. This analysis aims to assess our study cohort's prognosis and survival trends, offering valuable insights into disease management and outcomes.

   As applicable, the demographic characteristics and clinical parameters will be analyzed using appropriate statistical measures, including descriptive statistics, chi-square tests, Kaplan-Meier survival analysis, and Cox proportional hazards modelling.

b) Genotypic and allelic Frequency analysis

Among the cases studied, the Hardy-Weinberg equilibrium (HWE) theory was utilized to compute the genotypic frequencies of the polymorphism under investigation. The HWE equation ($p^2 + 2pq + q^2 = 1$) represents the frequencies of different genotypes within a population, where p^2 represents the frequency of the homozygous dominant genotype (LL), 2pq represents the frequency of the heterozygous genotype (LS), and q^2 represents the frequency of the homozygous recessive genotype (SS). Here, p and q represent the frequencies of the two alleles in the population (L and S, respectively). By applying the HWE equation, genotypic frequencies for the polymorphism were computed, providing insights into the distribution of genotypes within the study population. This analysis allowed for the characterization of genetic variation and allele frequencies associated with the polymorphic site. The minor allelic frequency (MAF) was also calculated for each polymorphic site. MAF represents the frequency of the less common allele in the population and is a crucial parameter

in genetic studies, providing information on the prevalence of minor alleles and their potential impact on phenotype variability.

Furthermore, the Chi-square goodness test was applied to assess whether the genotypic frequencies observed among cases conformed to the expected frequencies under the Hardy-Weinberg equilibrium. The Chi-square test helps determine whether observed genotype frequencies deviate significantly from the expected frequencies, indicating potential departures from genetic equilibrium within the population. Applying the Chi-square goodness test provided statistical evidence to evaluate the agreement between observed and expected genotypic frequencies, aiding the interpretation of genetic variation and potential factors influencing genotype distributions. These analyses contributed to a comprehensive understanding of genetic polymorphisms, allele frequencies, and deviations from Hardy-Weinberg equilibrium within the study population. The integration of statistical tests and genetic modelling techniques enhanced the accuracy and reliability of genetic analyses, facilitating robust interpretations of genotype-phenotype associations and population genetics dynamics.

c) Overall survival (OS) and Hazard ratio analysis

In our analysis of the overall survival and hazard ratio among lung cancer patients, we employed Kaplan-Meier analysis, commonly used in survival analysis, to estimate survival probabilities over time, particularly for univariate assessment. This method allows us to compare survival curves between different groups, such as smokers versus non-smokers or patients with different tumour stages. For a more nuanced understanding of survival outcomes and to account for multiple factors simultaneously, we turned to Cox proportional hazards regression for multivariate analysis. Cox regression is a statistical technique that assesses the association between predictor variables (such as age, smoking history, gender, tumour stage, histological type, treatment regimen, and performance status) and the hazard of an event, in this case, death. It calculates hazard ratios (HRs), representing the relative risk of an event occurring between two groups. An HR more significant than 1 indicates a higher risk of the event (in this case, death), while an HR less than 1 suggests a lower risk. Using Cox regression, we adjusted for multiple variables simultaneously, teasing out each variable's independent effects on survival outcomes. This approach is crucial in medical research, especially in oncology, as it helps identify which factors significantly impact patient survival and allows clinicians to tailor treatment strategies accordingly.

Additionally, calculating the hazard ratio and its confidence interval using Cox regression provides a quantitative measure of the strength and direction of the association between variables and survival, enhancing the precision of our analyses and supporting evidence-based decision-making in clinical practice. In statistical analyses, a p-value less than 0.05 is typically considered significant, indicating a strong likelihood that the observed associations are not due to chance alone but reflect authentic relationships between variables. The statistical analyses were performed using MedCalc version 16.8.1, a robust software tool that provided the necessary functionalities for performing stratified analysis, unconditional multivariate analysis, and calculating odds ratios and confidence intervals, thereby facilitating a comprehensive and rigorous investigation of lung cancer outcomes in our study population.

d) Stratified analysis

Our rigorous stratified analysis investigating lung cancer outcomes examined several vital parameters better to understand their impact on patient prognosis and treatment response. These parameters included:

i. **Tumour Stage:** Patients were stratified based on tumour staging, ranging from early-stage disease (Stage I/II) to advanced-stage disease (Stage III/IV). This stratification allowed us to assess how disease severity and progression influenced patient outcomes.
ii. **Lymph Node Invasion:** The extent of lymph node involvement was evaluated to determine its correlation with disease aggressiveness and its implications for treatment response and overall survival.
iii. **Primary Tumor Extension:** The analysis considered the extent of primary tumour extension, such as tumour size and local invasion, as these factors can influence treatment strategies and disease management.
iv. **Metastasis:** The presence or absence of distant metastases was assessed to understand the impact of metastatic spread on patient prognosis and treatment outcomes.
v. **Response Rate:** The response rate to treatment, including complete response, partial response, stable disease, and progressive disease, was evaluated to gauge the efficacy of therapeutic interventions.

For each parameter, odds ratios (OR) and their corresponding 95% confidence intervals (CI) were calculated. The ORs provided insights into the likelihood of specific outcomes occurring relative to the presence or absence of each parameter, while the CI helped assess the precision

and reliability of these associations. Additionally, we conducted unconditional multivariate analysis to determine adjusted odds ratios (AOR) and their corresponding 95% CIs for each genotype of interest. This multivariate analysis was essential for accounting for potential confounding factors that could influence patient outcomes. Variables such as age, gender, smoking status, Eastern Cooperative Oncology Group (ECOG) performance status, and treatment regimen were included in the adjustment model. By adjusting for these variables, we could isolate the specific effects of genotypes on patient outcomes, enhancing the accuracy and interpretability of our findings. All statistical tests were two-sided, and a p-value less than 0.05 was considered statistically significant, indicating a strong likelihood that the observed associations were not due to chance alone. This stringent criterion ensured our findings' reliability and validity, bolstering our conclusions' robustness. The statistical analyses were conducted using MedCalc version 16.8.1, employing state-of-the-art software tools and methodologies to ensure accurate data analysis and interpretation.

e) Adverse events (AEs) analysis

To comprehensively evaluate toxicity among lung cancer patients, we implemented the established criteria outlined by the National Cancer Institute (NCI), focusing on haematological, gastrointestinal, and nephrotoxic events throughout the treatment course. The thorough monitoring and assessment of toxicity were paramount to ensuring patient safety and optimizing treatment outcomes. Haematological toxicity assessments encompassed a range of parameters, including neutropenia, anaemia, leukopenia, and thrombocytopenia. These haematological parameters were closely monitored to detect any adverse effects of chemotherapy and guide timely interventions. Similarly, gastrointestinal and nephrotoxicities were rigorously monitored to identify and manage potential treatment-related complications. Gastrointestinal toxicity assessments included nausea, vomiting, diarrhoea, and mucositis, while nephrotoxicity assessments focused on renal function and creatinine clearance. Severe toxicity, defined as grade 3 or 4 according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), prompted specific interventions tailored to the type and severity of toxicity observed. For instance:

- Severe haematological toxicity necessitated interventions such as treatment progression delay until patients achieved grade 1 or 0 toxicity levels, ensuring adequate recovery of hematopoietic function before resuming chemotherapy.

- Grade 3 and 4 gastrointestinal toxicity triggered a 25% reduction in drug dosages to manage symptoms and mitigate further complications.
- Decreased creatinine clearance to 59 to 41 ml per minute led to the discontinuation of platinum-based therapies, preventing potential nephrotoxicity-related complications.

The treatment course included comprehensive clinical data recording, capturing detailed information on toxicity profiles, interventions, and patient responses. Data collection facilitated thorough analysis and interpretation of treatment-related outcomes and toxicity patterns. The data analysis involved computing odds ratios (OR) and 95% confidence intervals (CI) to assess the association between genotypic factors and treatment outcomes. Multivariate logistic regression was employed to calculate adjusted odds ratios (AOR) and 95% CI, considering various genotypic factors while controlling for key demographic variables such as age, gender, smoking history, and treatment regimen. Statistical significance was determined using a threshold p-value of less than 0.05, indicating a strong likelihood that observed associations were not due to chance alone. This rigorous statistical approach ensured the reliability and validity of our toxicity evaluations and data analyses, enhancing the quality and interpretability of our study findings.

CHAPTER 5

RESULTS

5) RESULTS

5.1) Genomic DNA Isolation

Genomic DNA isolated from blood was assessed quantitatively and qualitatively through standard molecular biology techniques. A 0.8% agarose gel electrophoresis was employed for qualitative estimation, where the DNA bands were visually inspected for integrity and size distribution. As depicted in **Figure 5.1**, the agarose gel visually represents the isolated genomic DNA. This technique allows researchers to observe the DNA fragments' migration pattern, which reflects their sizes and overall quality. Well-defined bands at expected positions indicate successful isolation and intact DNA molecules. Ensuring the integrity of DNA bands is crucial, and ensuring that the genetic material has not undergone significant degradation during the isolation process. Researchers often compare the band patterns to molecular weight markers to estimate the size range of the DNA fragments obtained.

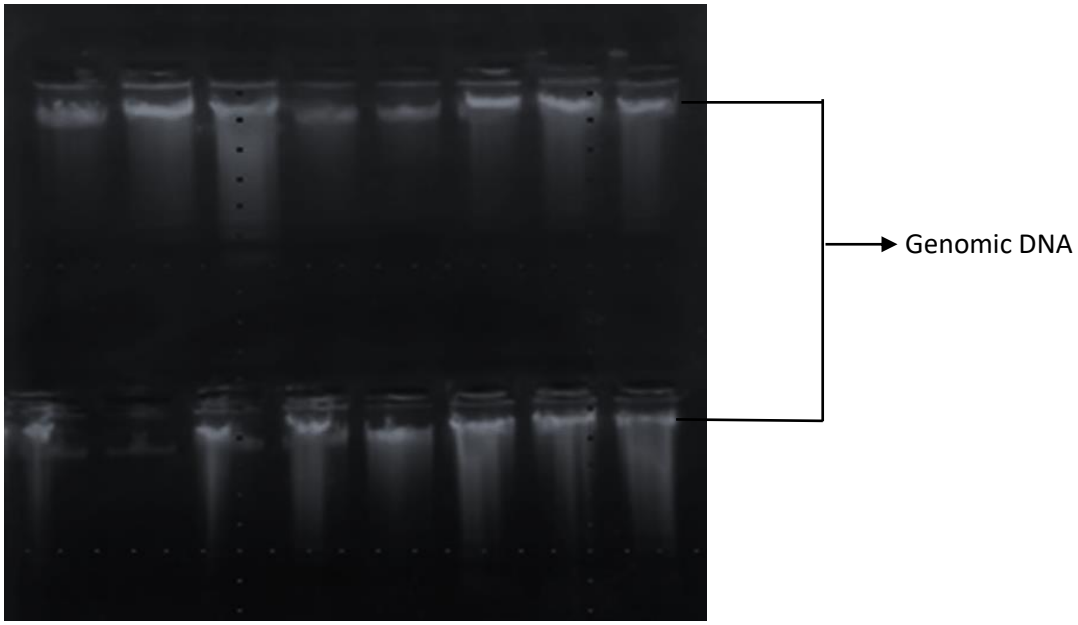

Figure 5.1: Isolated Genomic DNA

5.2) PCR amplification

After performing PCR to amplify the MN16A VNTR gene segment, the success of this amplification was verified using 2.5% agarose gel electrophoresis. This step was crucial to confirm that the gene segment was amplified to the correct length, and a DNA marker of 100 base pairs (bp) size was utilized for validation purposes. **Figure 5.2** in this research depicts a typical agarose gel electrophoresis setup, illustrating the amplification of genomic DNA specific to the MN16A polymorphism. The gel image exhibits well-defined bands corresponding to amplified products, with 240 bp, 303 bp, and 330 bp sizes. These band sizes align with the expected lengths, as indicated by the 100 bp ladder run alongside the samples. The presence of distinct bands at the specified lengths provides clear evidence of the successful amplification of the MN16A VNTR gene segment during PCR. Utilizing a DNA marker is essential for accurate size determination, aiding in the validation and verification of the amplification process.

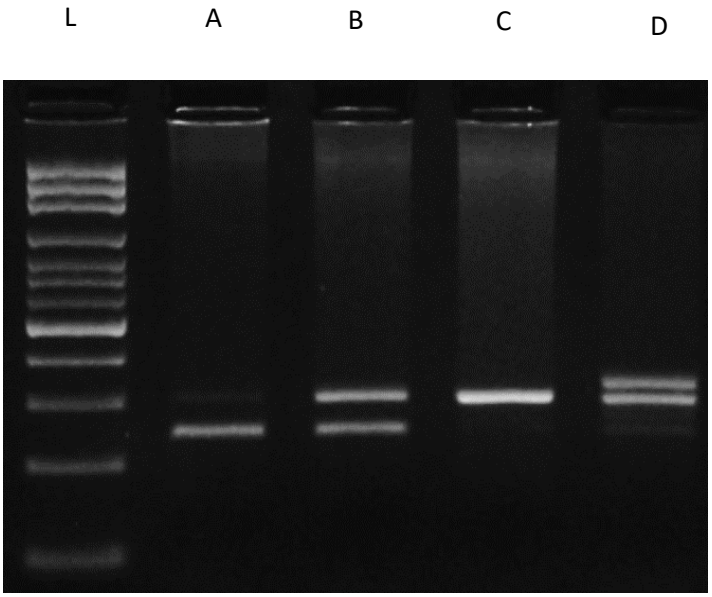

Figure 5.2: The gel was optimized for the MNS16A VNTR polymorphism at 60°C. In lane L, a 100bp DNA ladder was run alongside the samples. Lane A, SS (243/243); lane B, LS (302/243), lane C, LL (302/302) and lane D, LL (302/333).

5.3) Genotyping of MN16A genetic variant

The MN16A VNTR polymorphism, located in exon 16 of the TERT gene, was analyzed in this study. The success of amplification and the confirmation of restriction patterns were achieved

using 2.5% agarose gel electrophoresis, followed by visualization under a UV transilluminator. The analysis revealed four distinct alleles: VNTR-302, VNTR-243, VNTR-274, and VNTR-333, which were grouped as LL (302/302,333/333), LS(302/333, 302/243, 333/302/274, 333/302/223), and SS(243/243). These alleles were characterized by their respective sizes, with VNTR-302 being the wild-type allele and VNTR-243 representing the mutant type, as illustrated in Figure 5.3. Furthermore, the study provided a comprehensive assessment of the genetic variation within this VNTR region of the TERT gene, shedding light on the diversity of alleles in the population under investigation.

5.4) Epidemiological statistics of the North Indian population under study

5.4.1) Regional Distribution of Lung Cancer Patients

Blood samples were collected from lung cancer patients who visited the Department of Pulmonary Medicine at PGIMER, Chandigarh, a government hospital serving as a referral centre for patients from various regions of North India, including Punjab, Haryana, Himachal Pradesh, Uttar Pradesh, Uttarakhand, Jammu and Kashmir, and Chandigarh. The study, approved by the institutional ethics committee of PGIMER, enrolled a total of 401 patients. Among these, 125 patients (31%) were from Himachal Pradesh, 89 patients (23%) from Punjab, 75 patients (19%) from Haryana, 42 patients (23%) from Uttar Pradesh, 12 patients (3.0%) from Uttarakhand, 33 patients (8.0%) from Chandigarh, and 18 patients (5%) from Jammu and Kashmir. One patient from Bihar, Delhi, Gujarat, and Rajasthan was included. The study also incorporated one patient from Nepal, showcasing a more comprehensive geographical representation. This diverse patient cohort underscores the regional diversity and the multi-regional nature of patients seeking specialized care at PGIMER, Chandigarh, thereby contributing valuable insights into lung cancer epidemiology and management across North India and neighbouring countries.

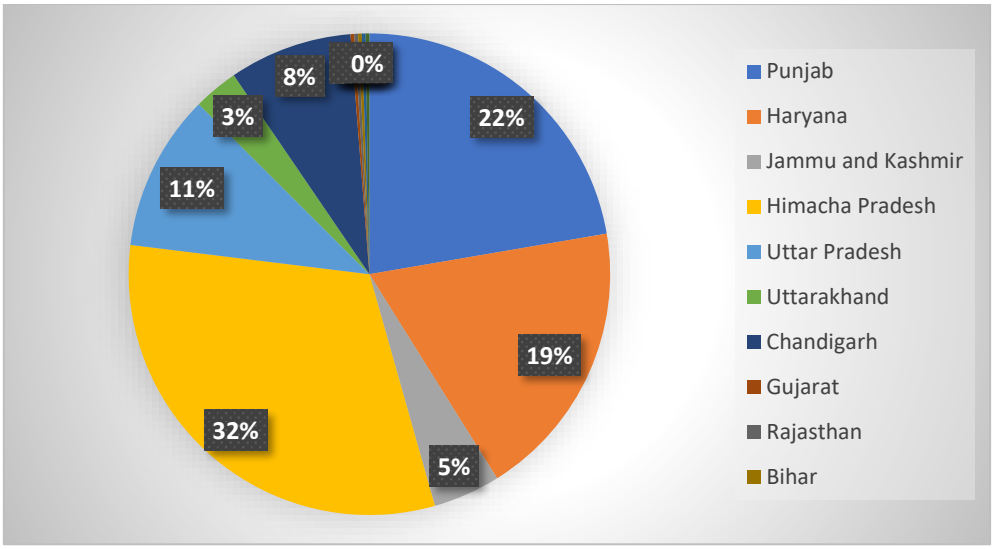

Figure 5.3: Pie Chart illustrating the regional distribution of North Indian lung cancer patients.

5.4.2) Regional Distribution of study based on Gender

Regional demographic analysis of lung cancer patients in North India, focusing on gender-specific distributions, was also done. Among female patients, Himachal Pradesh had the highest incidence at 25% (19 cases), followed by Punjab at 25% (19 cases), Haryana at 19% (15 cases), Uttar Pradesh at 16% (12 cases), Chandigarh at 5% (4 cases), Uttarakhand at 4% (3 cases), Jammu and Kashmir at 4% (3 cases), Bihar at 1% (1 case), and Rajasthan at 1% (1 case). The total number of cases among females stood at 78 (excluding 1 with unknown origin), showcasing varied prevalence rates across regions. Conversely, male patients demonstrated a distinct distribution with Himachal Pradesh leading at 34% (108 cases), followed by Punjab at 25% (68 cases), Haryana at 21% (62 cases), Uttar Pradesh at 9% (30 cases), Chandigarh at 9% (29 cases), Jammu and Kashmir at 5% (15 cases), Uttarakhand at 2% (8 cases), Delhi at 0.3% (1 case), Gujarat at 0.3% (1 case), and Nepal at 0.3% (1 case). The total male cases numbered 323, indicating a substantial disparity in lung cancer incidence between genders across North India. These findings emphasize the urgency of region-specific healthcare interventions and targeted awareness programs to mitigate the impact of lung cancer, mainly focusing on areas with higher prevalence rates.

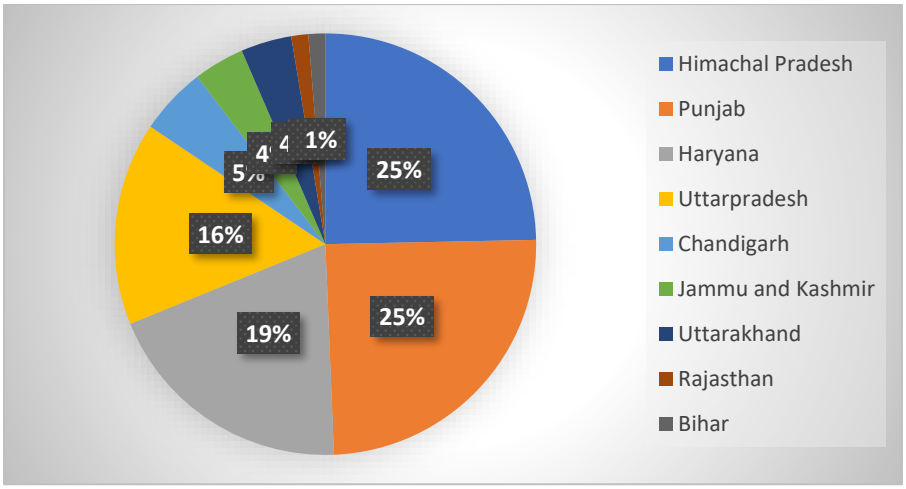

Figure 5.4: Pie Chart illustrating the regional distribution of Females in North Indian lung cancer patients.

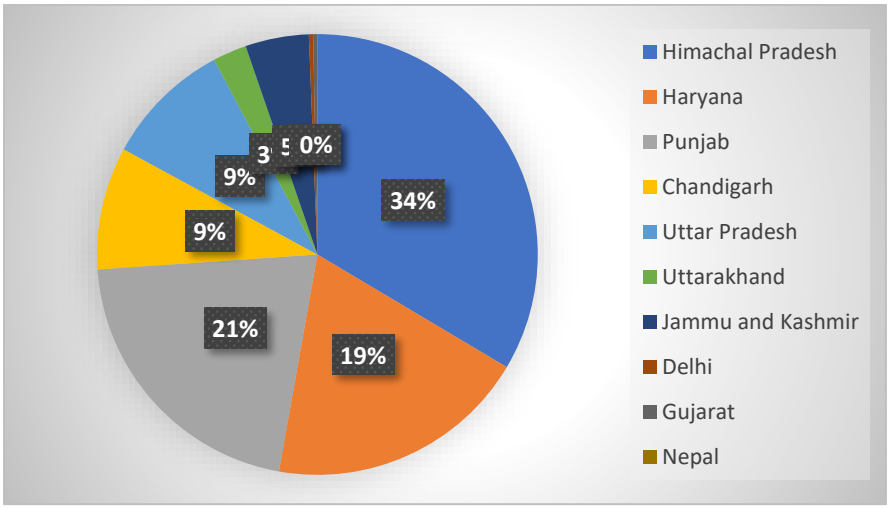

Figure 5.5: Pie Chart illustrating the regional distribution of Males in North Indian lung cancer patients.

5.4.3) Distribution of study subjects based on smoking status

The distribution of study subjects based on smoking status is a crucial aspect of epidemiological research, particularly concerning the relationship between smoking and lung cancer. In this study, the smoking status of all cases was meticulously documented, highlighting its significance in understanding disease patterns. The population was divided into two groups, smokers and non-smokers, categorized by their smoking habits. Given the well-established link between smoking and lung cancer, it was unsurprising to find a higher proportion of smokers among the cases compared to non-smokers. Specifically, out of the cases, 316 individuals (78.8%) were smokers, while 84 individuals (20.94%) were non-smokers, as illustrated in

Figure 5.6. To further refine the assessment of smoking exposure, the concept of pack years was employed, providing a nuanced understanding of the cumulative impact of smoking on each study subject. This methodological approach not only underscores the meticulousness of the study but also enhances the precision of the findings regarding the association between smoking behaviour and the risk of lung cancer. In addition to recording smoking status, the study systematically documented the specific types of smoking habits among the subjects, providing a comprehensive overview of tobacco use patterns. Among the smoking categories observed, the highest proportion was for Bidi smokers, totalling 235 individuals, representing 58.6% of the total sample size of 401 subjects. Following Bidi smokers, there were 36 individuals (8.98%) who reported a combination of Bidi with either Cigarette or Hookah. Additionally, 12 individuals (2.99%) were exclusive Cigarette smokers, five individuals (1.25%) reported Chulla use, and one individual (0.25%) reported Hookah use. These detailed breakdowns not only highlight the prevalence of different smoking types but also provide a detailed understanding of the diverse smoking behaviours within the study population.

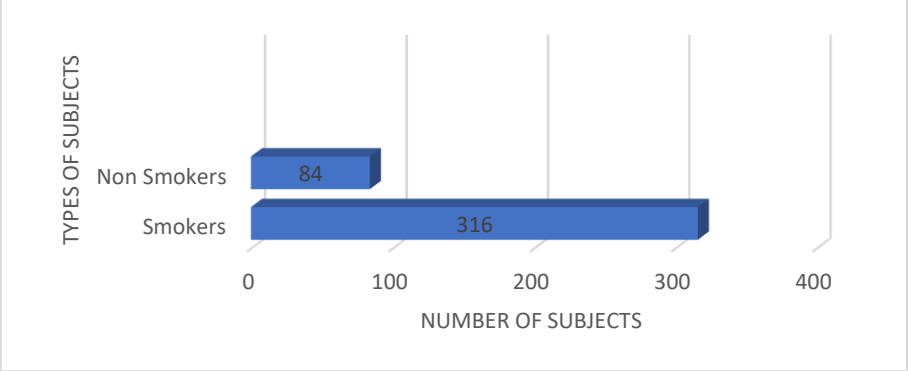

Figure 5.6: Graph showing the distribution of study subjects based on smoking status

The smoking patterns were analyzed based on gender, as shown in **Figure 5.7,** among 323 males and 78 females. Among the males, 294 were smokers and 29 were non-smokers, while among females, 23 were smokers and 55 were non-smokers. This data translates to a higher proportion of male smokers, constituting approximately 91% of the male population, compared to around 29.5% of females who smoke. Conversely, non-smokers form about 9% of the male group and roughly 70.5% of the female group. These findings suggest a significant gender disparity in smoking behaviour within the analyzed population, with males exhibiting a notably higher prevalence of smoking compared to females.

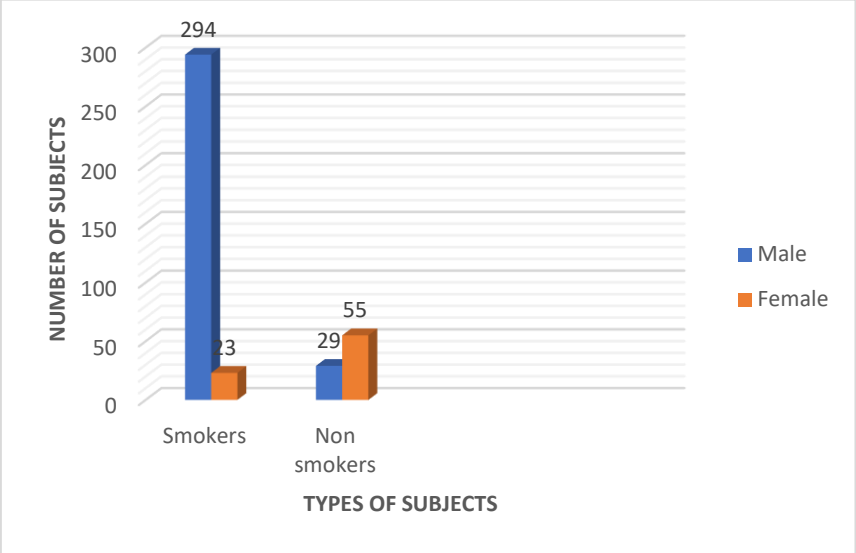

Figure 5.7: Graph showing the distribution of study subjects in males based on smoking status

5.4.4) Distribution of study subjects based on age

Age is a critical demographic factor associated with the onset of various diseases. In our study, we meticulously recorded the ages of all subjects. The average age of cases was 61.08, ranging from 30 to 86 years. Further analysis based on gender revealed exciting insights. The average age of male subjects was slightly higher at 61.8 years, while female subjects had an average age of 58.13 years. This gender-based differentiation in average ages adds depth to our understanding of age-related trends within the studied population.

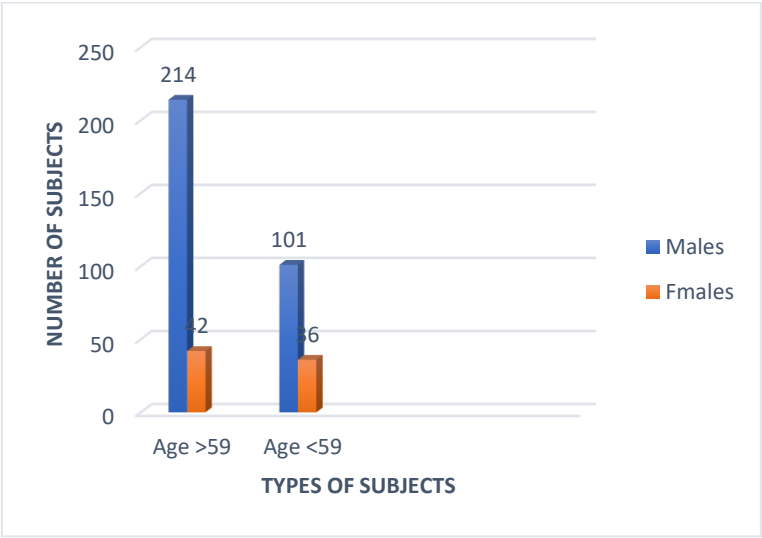

Figure 5.8: Graph showing the age-wise distribution of study subjects

Further analysis based on gender revealed exciting insights. Among the subjects over 59 were 214 males compared to 42 females, representing 83.6% of males and 16.4% of females in this age bracket. Conversely, in the age group less than 59, there were 101 males and 36 females, constituting 73.7% of males and 26.3% of females in this age category. The diagrammatic representation of the study subjects based on the age distribution of gender is graphically depicted in the accompanying **Figure 5.8**.

5.4.5) Distribution of study subjects based on the stage of tumour and TNM

In this study, we meticulously documented the clinic-pathological parameters of all subjects diagnosed with lung cancer, drawing data from the hospital's extensive medical records. The patients were meticulously categorized according to the clinical stage of their tumours, specifically into stages I, II, III, and IV. Notably, there was a notable disparity in the distribution across stages, with stages I and II showing a lower prevalence compared to stages III and IV, a trend clearly illustrated in Figure 5.9 [stage I: 5 (1.24%), stage II: 18 (4.5%), stage III: 150 (37.4%), and stage IV: 206 (51.3%)].

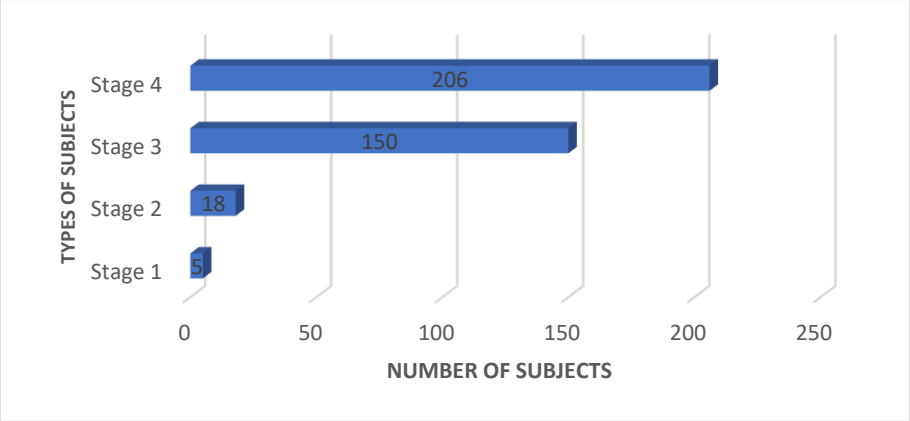

Figure 5.9: Graph showing the distribution of study subjects based on Tumor stage

Of the total 401 cases examined, TNM stage data were accessible for 387 (96.5%) instances. This enabled us to stratify the patients further based on the TNM parameters, explicitly focusing on the extent of the primary tumour (T), involvement of lymph nodes (N), and the presence of metastasis (M). The breakdown of tumour size was detailed as follows: Tx, T1, and T2 exhibited a relatively low occurrence, comprising 12 (3.1%), 25 (6.45%), and 35 (9.04%) cases, respectively, while T3 and T4 constituted a more significant portion, with 72 (18.75%) and 243 (62.8%) cases, respectively. Regarding lymph-node involvement, N0 and Nx were relatively infrequent, with 53 (13.7%) and 1 (0%) cases, respectively, while N1, N2,

and N3 demonstrated higher occurrences, accounting for 32 (8.27%), 172 (44.4%), and 129 (33.3%) cases, respectively. Among the cases with available TNM data, 170 (43.9%) showed no evidence of metastasis (Mx, M0), whereas 219 (56.6%) exhibited distant metastasis, categorized as M1 and M2.

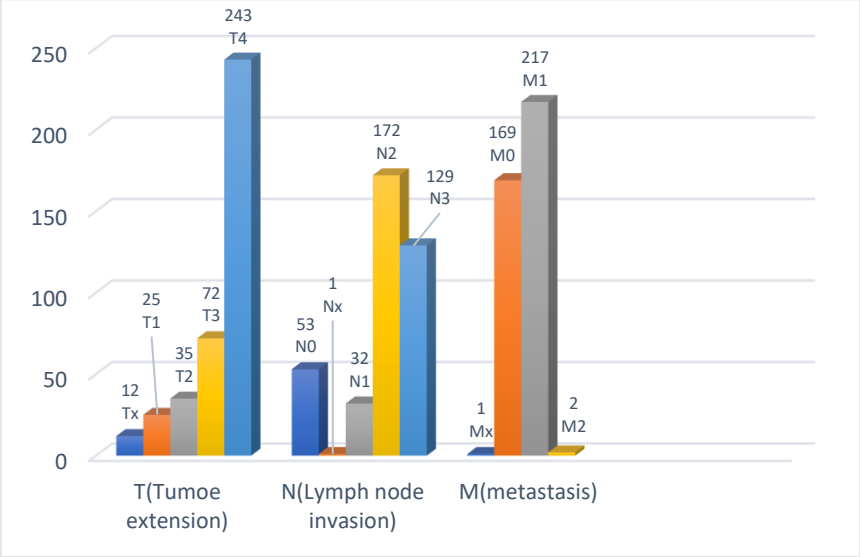

Figure 5.10: Graph showing the distribution of study subjects based on TNM staging

5.4.6) Distribution of lung cancer patients based on histology

Histology plays a crucial role in understanding and diagnosing various types of cancers. It involves the microscopic examination of tissues to identify abnormalities, such as cancerous cells. Each histological type, like ADCC, SCLC, and SQCC, has distinct characteristics that influence treatment strategies and prognosis. For instance, SCLC is often aggressive and prone to metastasis, while SQCC typically arises in the bronchial tubes and may have different treatment responses compared to other types. Out of 401 total samples in histology, data is known for 393 samples. Among these, the distribution based on histology is as follows: 170 samples are classified as ADCC (Adenocarcinoma), accounting for approximately 43.3% of the known samples, while 46 samples fall under SCLC (Small Cell Lung Cancer), representing about 11.7% of the known samples **(Figure 5.11).**

Additionally, 177 samples are categorized as SQCC (Squamous Cell Carcinoma), making up roughly 45% of the known samples. Therefore, it is evident that among the North Indian population, SQCC is the most frequent type of lung cancer occurring among people, with 170

(96%) smokers and (74%) non-smokers accounting for 96 (56%) smokers for ADCC. Understanding histological distributions aids in personalized medicine and targeted therapies for better patient outcomes

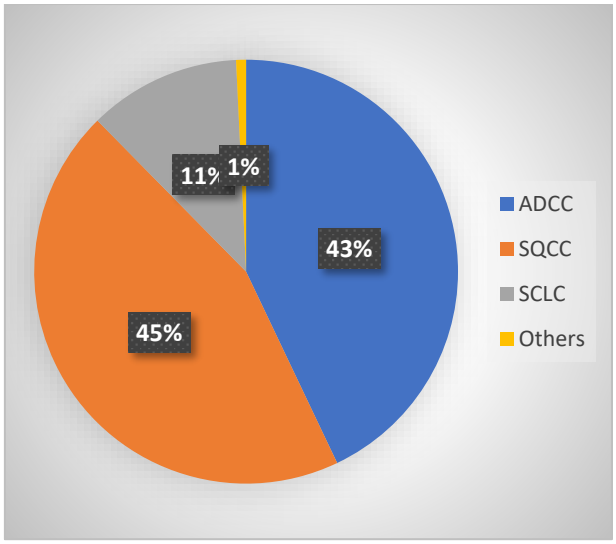

Figure 5.11: Pie Chart illustrating the distribution of lung cancer patients based on histology.

5.4.7) Distribution of lung cancer patients based on performance status

Performance status assessments using scales like the Karnofsky Performance Status (KPS) and Eastern Cooperative Oncology Group (ECOG) Performance Status plays a pivotal role in evaluating the functional capacity of lung cancer patients. Among the known data, 13 patients lack information regarding their performance status. However, for the remaining patients: Regarding KPS, 72 patients fall within the 30-60 range, constituting approximately 16.4% of the known patients, while 217 patients exhibit a KPS score of 70-80, making up about 49.5% of the known patients. Furthermore, 99 patients demonstrate a KPS score of 90-100, accounting for around 22.6% of the known patients. Concerning ECOG scores, 18 patients have a 0, representing about 3.9% of the known patients.

Additionally, 159 patients are categorized under ECOG 1, comprising approximately 36.3% of the known patients, while 153 patients fall into ECOG 2, accounting for roughly 34.9%. Moreover, 46 patients have an ECOG score of 3, constituting about 10.5% of the known patients, and 12 patients exhibit an ECOG score of 4, representing around 2.7% of the known patients (**Figure 5.12).** These performance status assessments are integral in guiding treatment decisions and prognostic evaluations for lung cancer patients. Lower scores on these scales

often indicate decreased functional capacity and may necessitate modifications in treatment strategies to optimize patient outcomes. Understanding the distribution of performance status among lung cancer patients enables healthcare providers to deliver tailored and effective care, considering each patient's unique health status and capabilities.

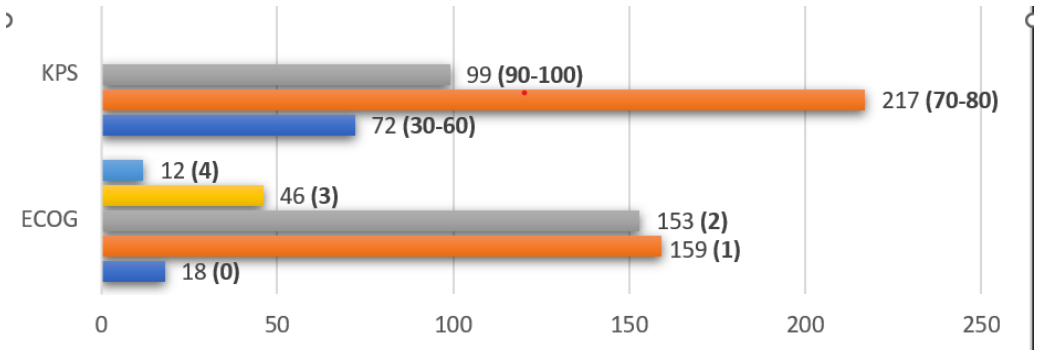

Figure 5.12: Graph illustrating the distribution of lung cancer patients based on performance status.

5.4.8) Distribution of lung cancer patients based on doublet platinum-based chemotherapeutic regimen

Platinum-based chemotherapy regimens are fundamental pillars in lung cancer treatment, showcasing potent efficacy in battling tumour cells. These regimens typically combine platinum compounds like cisplatin or carboplatin with complementary agents, leveraging their ability to form DNA cross-links that disrupt cancer cell replication and induce apoptosis. As shown in **Figure 5.13,** Among 252 patients with available data on their chemotherapy regimens, 23 patients are undergoing Docetaxel-based treatment, constituting roughly 9.1% of the cohort, 78 patients are receiving Paclitaxel-based therapy, representing about 30.9% of the patients, 27 patients are on Irinotecan-based chemotherapy, making up approximately 10.7% of the patients, 95 patients are undergoing Pemetrexed-based treatment, accounting for around 37.7% of the patients and 29 patients are receiving Gemcitabine-based therapy, comprising about 11.5% of the patients.

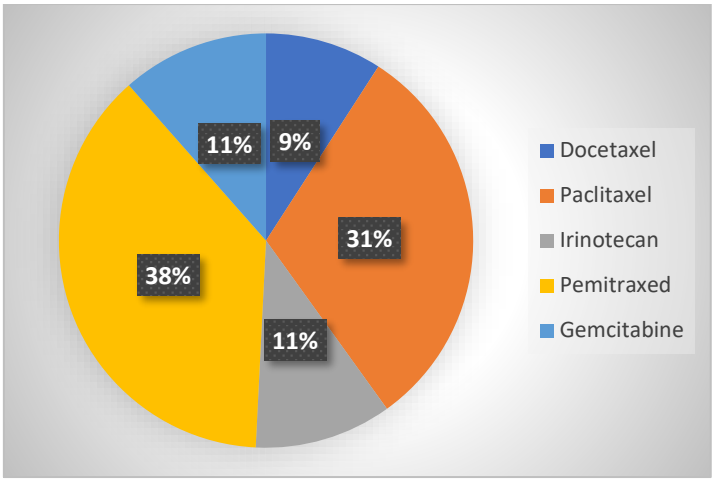

Figure 5.13: Pie Chart illustrating the distribution of lung cancer patients based on platinum-based chemotherapeutic regimen

The diverse selection of agents within platinum-based regimens, such as Docetaxel, Paclitaxel, Irinotecan, Pemetrexed, and Gemcitabine, offers nuanced mechanisms of action and varying side effect profiles. For instance, taxanes like Docetaxel and Paclitaxel disrupt microtubule function, Irinotecan targets topoisomerase, Pemetrexed interferes with folate metabolism, and Gemcitabine hampers DNA synthesis. This nuanced understanding enables clinicians to tailor treatments, balancing therapeutic efficacy with minimizing adverse effects, thus optimizing patient outcomes in the comprehensive management of lung cancer.

5.5) Demographic characteristics of a study

The comprehensive analysis encompassing demographic, clinical, and performance status parameters in the study of lung cancer patients offers a detailed understanding of this disease's complex nature. Starting with demographic characteristics as indicated in **Table 1**, the study revealed a striking gender distribution, with males constituting a substantial majority at 80.54% (323) compared to females at 19.45% (78). This observation aligns with established epidemiological trends indicating a higher incidence of lung cancer among males. The average age of the participants, recorded at 61.09±9.66 years, reflects the typical age range for lung cancer diagnoses, with cases spanning from 30 to 86 years. Notably, a significant proportion of the patients were identified as smokers, 78.8% (316), with an average pack-year history of 21.83±20.04 among this group. This underscores the strong association between smoking and lung cancer, a well-recognized risk factor in the disease's aetiology. Moving to clinical parameters, The distribution of histological types among the patients was as follows: squamous

cell carcinoma (SQCC) was the most prevalent type, accounting for 177 cases (44.14%), followed closely by adenocarcinoma (ADCC), accounting for 170 cases (42.4%). Small cell lung cancer (SCLC) was observed in 46 cases (11.47%), while other types were rare, comprising only 3 cases (0.75%). There were 5 cases where the histological type was unknown. In the case of staging, a predominant finding was the high prevalence of advanced-stage disease, with stage IV cases comprising 51.3% (206) of the cohort. This emphasizes the challenges often encountered in diagnosing lung cancer at earlier, more treatable stages. Specifically, the distribution of tumour stages (TNM classification) showed that only 5 patients (1.25%) were in stage I, 18 patients (4.5%) in stage II, 150 patients (37.4%) in stage III, and the majority, 206 patients (51.3%), were in stage IV, with 22 cases remaining unknown. Regarding tumour size, a significant portion of the group had extensive tumours, with 243 patients (60.6%) classified as T4, indicating advanced local disease. The distribution of other tumour sizes was as follows: 25 patients (6.23%) had T1 tumours, 35 patients (8.7%) had T2 tumours, and 72 patients (17.9%) had T3 tumours, with 14 cases being unknown. Lymph node involvement data revealed that 53 patients (13.2%) had no lymph node involvement (N0), 1 patient (0.25%) had N1 involvement, 32 patients (7.9%) had N2 involvement, 172 patients (42.9%) had N3 involvement, and 129 patients (32.16%) had N4 involvement, with 14 cases being unknown. For metastasis, 169 patients (42.14%) had no distant metastasis (M0), 217 patients (54.11%) had M1 metastasis, and 2 patients (0.5%) had M2 metastasis, with 12 cases being unknown. Moving towards Overall Survival, the study found that out of 401 patients, 324 (80.8%) had died, 66 (16.45%) were still alive, and the survival status of 11 patients was unknown. This indicates a high mortality rate within this group, underscoring the challenges and severity of the disease in terms of survival outcomes.

Performance status assessments provided additional insights into patients' functional capacities and overall well-being. Karnofsky Performance Status (KPS) assessments revealed that a majority of patients fell within the 70-80 range, with 217 (54.11%) falling into this category and the 90-100 range, with 99 patients (24.68%). There were 13 patients (3.24%) with unknown KPS, indicating varying degrees of functional impairment but with capabilities for daily activities. Eastern Cooperative Oncology Group (ECOG) scores complemented this, with 177 (44.14%) of patients scoring 0-1, indicating higher functional abilities, while 153 (38.15%) scored 2 and 58 (14.46%) scored 3-4, reflecting varying levels of functional limitations. Regarding chemotherapy regimens, the data showcases the diversity of treatment approaches employed. Of the 401 patients receiving chemotherapy, the most frequently administered drug

was Pemetrexed, prescribed for 95 patients (23.69%). Following Pemetrexed, Paclitaxel was used by 78 patients (19.45%), and Gemcitabine was administered to 29 patients (7.23%). Irinotecan was prescribed for 27 patients (6.73%), while Docetaxel was the least used, given to 23 patients (5.73%). Additionally, the specific chemotherapy regimen for 149 patients (37.15%) was not specified in the available data.

We employed three distinct analytical approaches to understand the impact of the MNS16A polymorphism on various toxicity levels. In the initial analysis, individuals experiencing any toxicity grade (grades 1-4) were compared to those with no toxicity (grade 0) as indicated in Tables 2 and **3**. Subsequently, in the second and third assessments, we compared patients with intermediate to severe toxicity (grades 2-4) against those with no or low-grade toxicity (grade 0-1) and patients with high-grade toxicity (grades 3-4) against those with no/low/intermediate toxicity (grades 0-2). These analyses aimed to elucidate whether the MNS16A polymorphism in the hTERT gene correlated with severe toxicity, allowing us to categorize cases into distinct toxicity levels for further investigation.

Table 7: Demographic, Clinical, Performance Status, and Chemotherapeutic Regimen Overview in Lung Cancer Patients.

VARIABLE	TOTAL(N=401)	CASES(%AGE)
Gender	401	
Male		323(80.54%)
Female		78(19.45%)
Age	401	
Mean±SD		61.09±9.66
Range		30-86
Smoking status	401	
Smokers		316(78.8%)
Non-smokers		84(20.9%)
Unknown		1
Pack years	401	
Mean±SD		21.83±20.04
Histological types	401	
ADCC		170(42.4%)
SCLC		46(11.47%)
SQCC		177(44.14%)
Others		3(0.75%)
Unknown		5
TNM	401	
I		5(1.25%)
II		18(4.5%)
III		150(37.4%)
IV		206(51.3)
Unknown		22
Tumor Size	401	
Tx		12(3%)
T1		25(6.23%)
T2		35(8.7%)
T3		72(17.9%)
T4		243(60.6%)
Unknown		14
Lymph Node	401	

N0		53(13.2%)
N1		1(0.25%)
N2		32(7.9%)
N3		172(42.9%)
N4		129(32.16%)
Unknown		14
Metastasis	401	
Mx		1(0.25%)
M0		169(42.14%)
M1		217(54.11%)
M2		2(0.5%)
Unknown		12
Overall Survival	401	
Dead		324(80.8%)
Alive		66(16.45%)
Unknown		11
Performance status	401	
KPS (below 60)		72(17.95%)
KPS (70-80)		217(54.11%)
KPS (90-100)		99(24.68%)
Unknown		13(3.24%)
ECOG (0-1)		177(44.14%)
ECOG (2)		153(38.15%)
ECOG (3-4)		58(14.46%)
Unknown		13(3.24%)
Chemotherapy regimen	401	
Docetaxel		23(5.73%)
Paclitaxel		78(19.45%)
Irinotecan		27(6.73%)
Pemitraxed		95(23.69%)
Gemcitabine		29(7.23%)
Unknown		149(37.15%)

Table 8: Hematological and nephrotoxicity with CTC grades in lung cancer patients

Toxicity	Adverse events	Toxicity profiles			
		Grade 0,n(%)	Grade 0-1,n(%)	Grade 1-4(%)	Grade 2-4(%)
Hematological toxicity	Anemia	84	208	228	104
	Leukopenia	308	327	28	0
	Thrombocytopenia	274	305	32	1
	Neutropenia	263	272	16	7
Nephrotoxicity	GFR	31	134	271	168

Table 9: Hepatological toxicity with CTC grades in Lung cancer patients

Toxicity	Adverse events	Toxicity profiles			
		Grade 1,n(%)	Grade 1-2,n(%)	Grade 2-4(%)	Grade 3-4(%)
Hepatotoxicity	SGOT	238	287	118	3
	SGPT	242	276	48	14
	ALP	167	226	118	59

5.6) RESULT INTERPRETATION

5.6.1) Overall survival analysis for hTERT genomic variant MNS16A in lung cancer patients

Survival analysis for 401 lung cancer samples and its association with hTERT polymorphism has been studied and depicted in **Table 4**. After three years of follow-up, 80.79% of patients were dead, and 16.21% were alive. After adjusting for gender, smoking status, age, tumour stage, histology, regimen, ECOG, and KPS, the association of the MNS16A with lung carcinoma survival was evaluated using univariate and multivariate analysis. In our study, we have applied two different models (i.e., co-dominant and dominant) to find the association of overall survival in lung carcinoma patients regarding MNS16A polymorphism.

For hTERT polymorphism, in the co-dominant model, we observed a higher median survival time (MST) in patients harbouring heterozygous (LS) alleles than subjects who were carrying both the mutant (SS) alleles (MST=8.5 vs 6.33; log-rank p=0.78; HR=1.08) and the homozygous wildtype (LL) alleles (MST=8.5 vs 7.42; p=0.13; HR= 1.19). However, after applying the Cox regression model **Table 5**, the result showed poor survival outcomes in subjects carrying mutant (SS) (HR=0.85, CI=0.58-1.1;p=0.37) and wildtype alleles (HR=0.8, CI=0.58-1.1;p=0.16) when compared with patients carrying heterozygous (LS) alleles as shown in **Figure 5.14,** but the results were not statistically significant. Further, in the dominant model, 58.35% of patients had homozygous wild (LL) and were compared with the 41.64% of patients having homozygous mutant and heterozygous genotype (LS+SS). Although the results showed a shorter survival time for lung cancer patients harbouring homozygous wild type (LL) than the subjects carrying homozygous mutant (SS) and heterozygous variant (LS) (MST= 8.37 vs 7.42 months; log-rank p= 0.14), they were also not statistically significant. Therefore, the MNS16A polymorphism does not significantly influence overall survival in this study population.

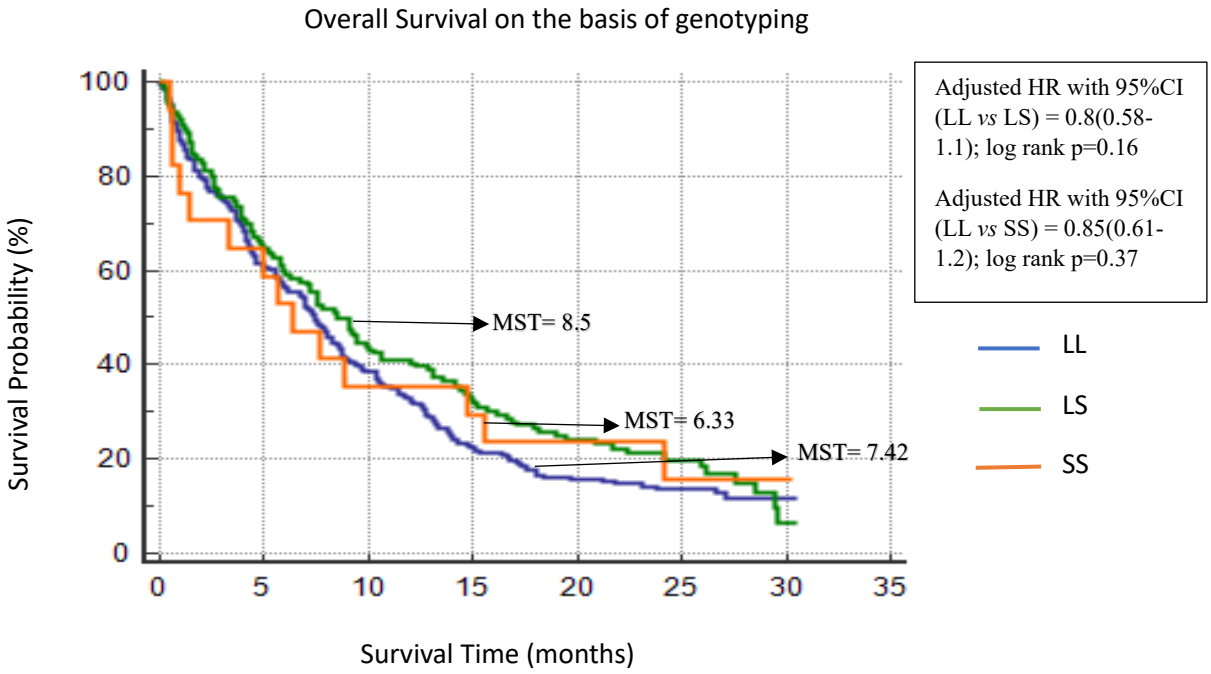

Figure 5.14: Kaplan Meier curves illustrating the association between overall survival in different genotypes of MNS16A polymorphism in lung cancer patients.

Table 10: Univariate and Multivariate Analysis of MNS16A polymorphism based on survival

GENOTYPE	CASES (n=401)	DEAD (n=324)	ALIVE (n=65)	Univariate analysis			Multivariate Analysis	
				MST(months)	Log Rank (p)	Unadjusted HR(95%CI)	Adjusted HR(95% CI)	P
Codominant Model								
LL	234(58.35%)	195(60.1%)	32(49.23%)	7.42		1.0(reference)		
LS	149(37.15%)	115(35.5%)	29(44.61)	8.5	0.13	1.19 (0.95-1.5)	0.8(0.58-1.1)	0.16
SS	18((4.5%)	14(4.3%)	4(6.15%)	6.33	0.78	1.08 (0.64-1.82)	0.85(0.61-1.2)	0.37
Dominant Model								
LL	234(58.35%)	195(60.1%)	32 (49.23%)	7.42		1.0(reference)		
LS+SS	167(41.64%)	129(39.7%)	33(50.75%)	8.37	0.14	1.18(0.94-1.46)	0.9(0.73-1.67)	0.5

Table 11: Cox proportional hazards regression model analysis of overall survival in lung cancer subjects

Covariate	b	SE	Wald	P	Exp(b)	95% CI of Exp(b)
Genotype	-0.2236	0.1585	1.9891	0.1584	0.7996	0.5860 to 1.0911
Age	-0.01003	0.008320	1.4521	0.2282	0.9900	0.9740 to 1.0063
Gender	0.5197	0.2516	4.2667	0.0389	1.6816	1.0269 to 2.7536
Smoking	-0.1109	0.2563	0.1874	0.6651	0.8950	0.5416 to 1.4790
ECOG	0.7978	0.1807	19.4913	<0.0001	2.2207	1.5583 to 3.1645
KPS	0.02531	0.1807	0.01962	0.8886	1.0256	0.7198 to 1.4614
Stage	0.1696	0.1339	1.6035	0.2054	1.1848	0.9113 to 1.5405
Histology	0.1006	0.1319	0.5821	0.4455	1.1059	0.8539 to 1.4321
Regimen	0.01171	0.06703	0.03051	0.8613	1.0118	0.8872 to 1.1538

5.6.2) Association of MNS16A polymorphism on Overall Survival in Lung Cancer Patients based on Gender.

We analyzed to investigate the association between MN16A polymorphism and overall survival in male and female lung cancer patients. The study included 401 cases, with 323 males (80.55%) and 78 females (19.45%). Among males, 264 (81.74%) were deceased, and 51 (15.77%) were alive at the end of the observation period. Among females, 57 (73.08%) were deceased, and 14 (17.95%) were alive. In the case of Males in the codominant model, the univariate analysis showed that patients with the LS genotype had a longer median survival time (MST=6.9 months) compared to LL and SS genotypes (MST=9.13 and 4.9 months) **(Table 6)**. However, these differences were not statistically significant after adjusting for covariates in the multivariate analysis ($p > 0.05$). Similarly, in the dominant model, while there was a trend towards a longer MST for LS+SS combined genotype compared to LL (MST=6.9 vs 8.5 months, log-rank p=0.239), this difference was not statistically significant in the multivariate analysis ($p > 0.05$). However, in the case of Females in the codominant model, the univariate analysis showed that patients with the SS genotype had the highest median survival time (MST) of 24.1 months, but this difference was not statistically significant in the log-rank test ($p = 0.28$). After adjusting for covariates in the multivariate analysis, the HR for the SS genotype decreased, indicating a potential association with improved survival; however, this result was not statistically significant ($p = 0.26$). In the dominant model, the univariate analysis did not show a statistically significant difference in MST between LL and LS+SS genotypes ($p = 0.5$).

Similarly, after adjusting for covariates in the multivariate analysis, the HR for LS+SS genotype compared to LL was not statistically significant (p = 0.72). In both the codominant and dominant models, the univariate analysis indicated potential differences in median survival time (MST) among genotypes, particularly for LS and SS genotypes. However, after adjusting for covariates in the multivariate analysis, these differences were not statistically significant (p > 0.05) in male and female lung cancer patients. These findings suggest that the MN16A SNP genotypes alone may not be strong predictors of overall survival in lung cancer patients, regardless of gender.

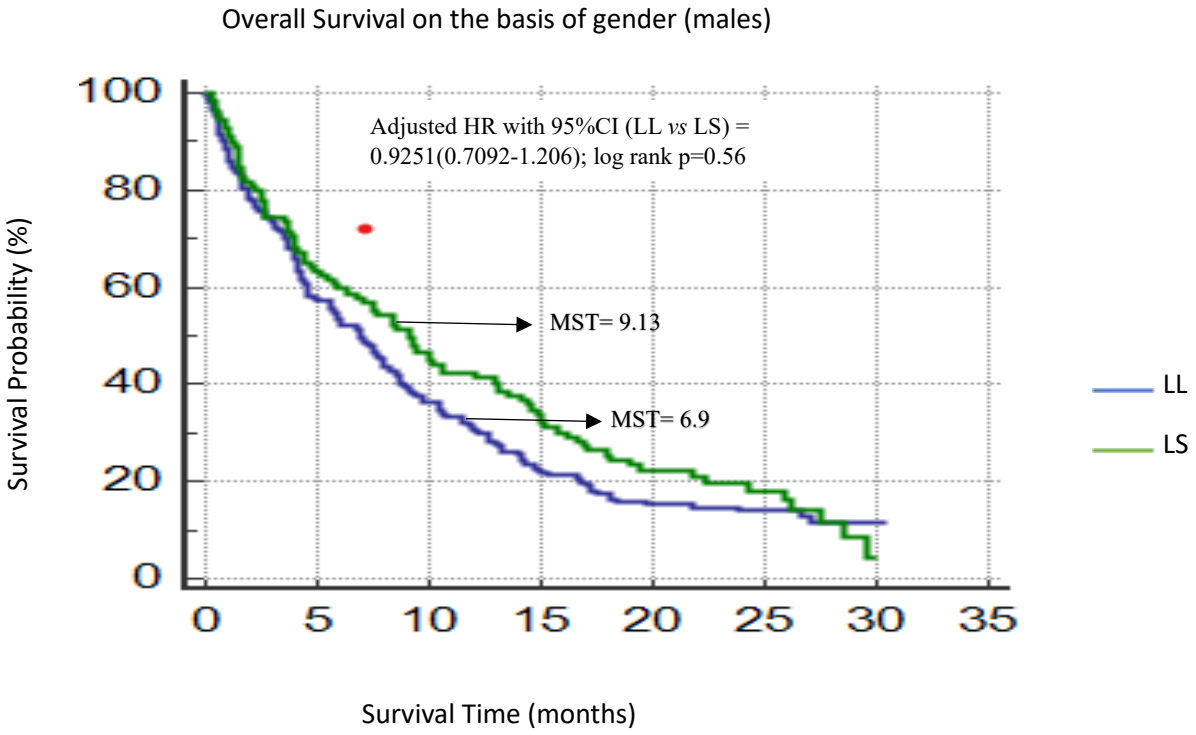

Figure 5.15: Kaplan Meier curves illustrating the association between overall survival in males of MN16 A polymorphism in lung cancer patients for Wild-type genotype (LL) and Heterozygous genotype (LS).

Table 12: Association of MNS16A polymorphism on Overall Survival in Lung Cancer Patients based on Gender

MALES								
GENOTYPE	**CASES (n=323)**	**DEAD (n=264)**	**ALIVE (n=51)**	**Univariate analysis**			**Multivariate Analysis**	
				MST(months)	**Log Rank**	**Unadjusted HR(95%CI)**	**Adjusted HR(95%CI)**	**p**
Codominant Model								
LL	190(58.82%)	160(60.6%)	27(52.94%)	6.9		1.0(reference)		
LS	119(36.84%)	92(34.84%)	22(43.13%)	9.13	0.187	0.84(0.65-1.09)	0.92(0.71-1.206)	0.57
SS	14(4.33%)	12(4.54%)	2(3.92%)	4.9	0.78	1.09(0.59-2.01)	0.81(0.55-1.2)	0.29
Dominant Model								
LL	190(58.82%)	160(60.6%)	27(52.94%)	6.9		1.0(reference)		
LS+SS	133(41%)	104(39%)	24(47%)	8.5	0.25	0.86(0.68-1.1)	0.76(0.54-1.05)	0.098
FEMALES								
LL	44(56.4%)	35(61.4%)	5(35.7%)	8.5		1.0(reference)		
LS	30(38.46%)	23(40.35%)	7(50%)	7.23	0.69	1.12(0.66-1.89)	1.16(0.43-3.13)	0.76
SS	4(5.13%)	2(3.5%)	2(14.28%)	24.1	0.28	0.56(0.19-1.61)	0.58(0.22-1.52)	0.26

Dominant Model								
LL	44(56.4%)	35(61.4%)	5(35.7%)	8.5		1.0(reference)		
LS+SS	34(43.59%)	25(43.4%)	9(64.28%)	7.6	0.5	1.19(0.71-1.99)	0.85(0.35-2.04)	0.72

5.6.3) Association of MNS16A polymorphism on Overall Survival in Lung Cancer Patients based on chemotherapeutic regimen.

In our study, we assessed the effectiveness of critical chemotherapeutic agents, exploring the correlation between the MN16A polymorphism and overall survival in lung cancer patients undergoing chemotherapy. Docetaxel, paclitaxel, irinotecan, pemetrexed, and gemcitabine are pillars in modern oncology, each with unique mechanisms and clinical impacts. **Table 7** presents the MNS16A polymorphism's influence on overall survival, stratified by specific chemotherapeutic regimens. Lung cancer patients receive tailored treatments, including taxanes (docetaxel, paclitaxel), nucleoside analogues (gemcitabine), and topoisomerase inhibitors (irinotecan for second-line), along with synergistic combinations like pemetrexed with platinum agents for non-squamous NSCLC. This approach underscores the personalized and comprehensive strategies aimed at optimizing treatment outcomes. When analyzing patients treated with docetaxel, the LL genotype showed a median survival time (MST) of 9.13 months, slightly higher than the 8.43 months observed for LS+SS, although this difference was not statistically significant based on the log-rank test ($p = 0.89$). Upon conducting multivariate analysis adjusting for other variables, the hazard ratio (HR) for LS+SS compared to LL was 1.07 (95% CI: 0.41-2.78, $p = 0.61$), suggesting no substantial difference in survival between the genotypes after accounting for confounding factors. Shifting focus to patients treated with paclitaxel, the univariate analysis indicated an MST of 7.27 months for LL and 5.2 months for LS+SS. However, the log-rank test did not reveal a significant survival difference ($p = 0.663$).

Similarly, in the irinotecan group, where LL had an MST of 7.47 months compared to 10.57 months for LS+SS in univariate analysis, the log-rank test did not show a significant difference ($p = 0.75$). Examining the response to pemetrexed, LL exhibited an MST of 7.1 months, whereas LS+SS had an MST of 7.7 months, again without a significant survival difference in

the univariate analysis (p = 0.25). However, the adjusted HR in the multivariate analysis was 0.73 (95% CI: 0.43-1.24), suggesting a trend toward better survival for LL but without statistical significance. Remarkably, in the gemcitabine group, while the unadjusted analysis showed no significant survival difference between LL and LS+SS, the adjusted HR favoured LL with a significant difference (HR = 0.22, 95% CI: 0.06-0.73, p = 0.014), indicating a survival advantage for LL after accounting for other factors. In summary, while the MNS16A genotype didn't show significant survival differences in univariate analyses across most chemotherapies, multivariate analysis revealed variable impacts. Notably, gemcitabine treatment favoured the LL genotype significantly.

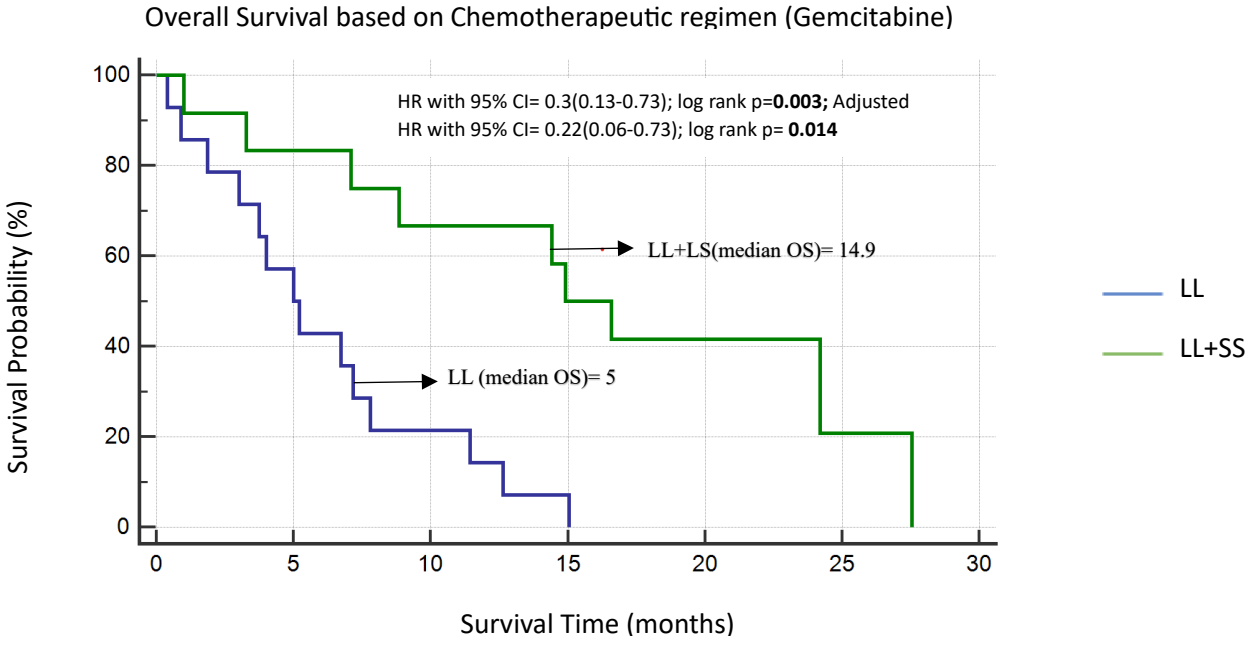

Figure 5.16: Kaplan Meier curves illustrating the association between overall survival and chemotherapeutic regimen (Gemcitabine) for Wild-type genotype (LL) and combined genotypes (LL+SS).

Table 13: Association of MNS16A on OS according to chemotherapeutic regimen

DOCETAXEL								
GENOTYPE	**CASES (n=23)**	**DEAD (n=19)**	**ALIVE (n=4)**	**Univariate analysis**			**Multivariate Analysis**	
				MST(months)	**Log Rank**	**Unadjusted HR(95%CI)**	**Adjusted HR(95%CI)**	**p**
Dominant Model								
LL	15(65.21%)	12(63.15%)	3(75%)	9.13		1.0(reference)		
LS+SS	8(34.78%)	7(36.84%)	1(25%)	8.43	0.89	1.07(0.41-2.79)	0.61	0.67(0.14-3.23)
PACLITAXEL								
LL	47(61%)	41(64%)	6(46.15%)	7.27		1.0(reference)		
LS+SS	30(38.96%)	23(35.93)	7(53.84%)	5.2	0.83	1.06(0.63-1.78)	1.13(0.66-1.94)	0.66
IRINOTECAN								
LL	16(61.53%)	15(62.5%)	1(50%)	7.47		1.0(reference)		
LS+SS	10(38.46%)	9(37.5%)	150%)	10.57	0.29	0.65(0.29-1.47)	0.85(0.32-2.29)	0.75
PEMITRAXED								
LL	49(55%)	44(58.66%)	5(35.7%)	7.1		1.0(reference)		

LS+SS	40(44.94%)	31(41.33%)	9(64.28%)	7.7	0.1358	1.42(0.89)	0.74(0.43-1.24)	0.25
GEMCITABINE								
LL	14(51.85%)	14(60.86%)	0	5		1.0(reference)		
LS+SS	13(48.14%)	9(39.13%)	4(100%)	14.9	**0.003**	3.305(1.38-7.94)	0.22(0.06-0.73)	0.014

5.6.4) Association of MNS16A polymorphism with histology and OS

We further evaluated hTERT polymorphism's role in predicting lung cancer patients' overall survival based on histological subtypes. The data for the same has been presented in **Table 8.** In the present study, patients having ADCC and hTERT polymorphism showed a majority of patients having variant genotype homozygous wild (LL) as that of for the heterozygous variant (LS) and homozygous mutant genotype (SS) (56.4% vs 38.2% vs 5.3%). Our results showed reduced survival in the heterozygous variant genotype LS in ADCC patients compared to the homozygous mutant genotype (SS) and homozygous wildtype genotype (LL) (MST=7.57 vs 7.7 and 8.085). However, our results were insignificant even after the multivariate cox-regression model. Additionally, the SCLC patients harbouring the homozygous wildtype genotype (LL) showed poor survival outcomes compared to those patients with homozygous mutant genotype (SS) and with heterotypic variant (LS) (MST=7.6 vs 14.77 vs 9.33). After multivariate Cox analysis, though, the results were not statistically significant in either of the models.

Furthermore, upon analyzing the survival trend in SQCC patients having hTERT polymorphism, results were significant only in the co-dominant model, and it showed that 3.9% of people harboured homozygous mutant genotype (SS) had poor survival as compared to wildtype and heterotypic genotypes (MST=1.4 vs 6.9 vs 9). In conclusion, for adenocarcinoma (ADCC), while univariate analysis indicated significant differences in overall survival for the LS and SS genotypes compared to the LL genotype, multivariate analysis revealed no significant differences, suggesting these genotypes do not independently impact survival. In small cell lung cancer (SCLC), the LL genotype showed poorer survival outcomes, but the

differences were not statistically significant in multivariate analyses, indicating no significant impact of hTERT polymorphism on survival. For squamous cell carcinoma (SQCC), the SS genotype showed significantly poorer survival in univariate analysis. Yet this significance did not hold in multivariate analysis, implying that hTERT polymorphism may influence survival but is not an independent predictor when other factors are considered.

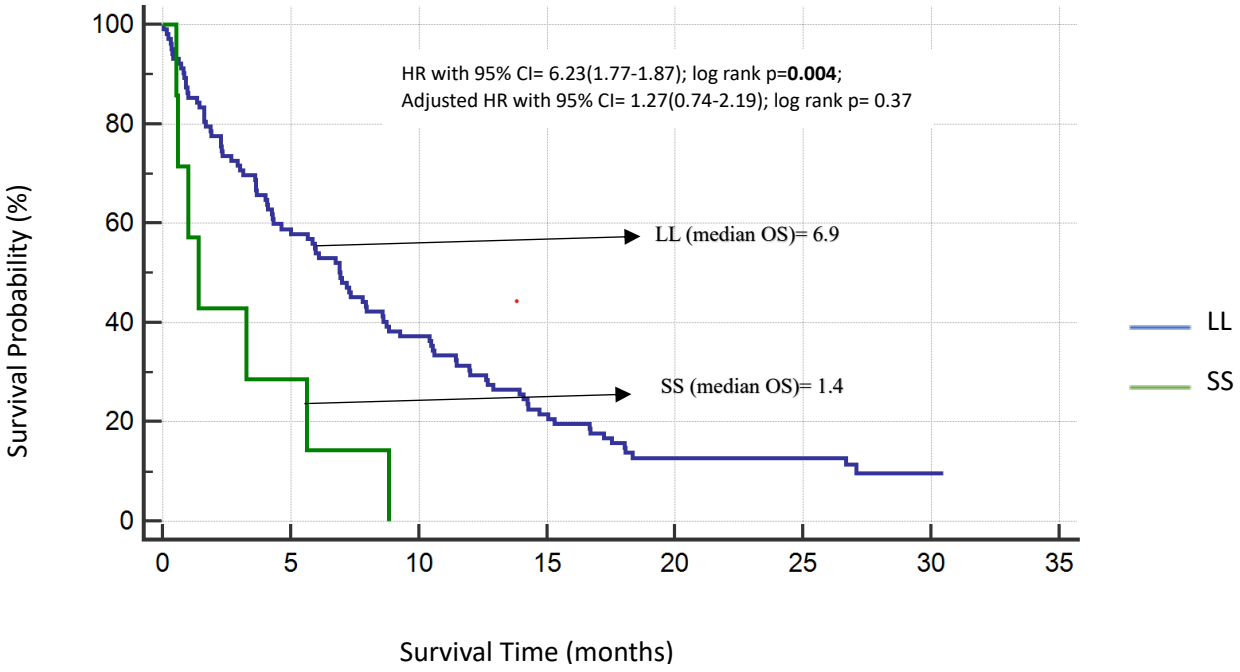

Figure 5.17: Kaplan Meier curves illustrating the association between overall survival and histology (SQCC) for Wild-type genotype (LL) and mutant genotype (SS).

Table 14: Association of SNP on overall survival according to histology

ADCC								
GENOTYPE	**CASES (n=401)**	**DEAD (n=324)**	**ALIVE (n=65)**	**Univariate analysis**			**Multivariate Analysis**	
				MST (months)	**Log Rank**	**Unadjusted HR(95% CI)**	**Adjusted HR(95% CI)**	**p**
Codominant Model								
LL	96(23.94%)	73(22.53%)	18(27.69%)	8.085		1.0(reference)		
LS	65(16.2%)	48(14.8%)	13(20%)	7.57	0.69	1.07(0.75-1.54)	0.77(0.44-1.34)	0.36
SS	9(2.24%)	5(1.54%)	4(6.15%)	7.7	0.23	1.56(0.75-3.23)	0.79(0.43-1.46)	0.46
Dominant Model								
LL	96(23.94%)	73(22.53%)	18(27.69%)	8.085		1.0(reference)		
LS+SS	74(18.26%)	53	17(26.15%)	7.6	0.47	1.14(0.8-1.62)	0.72(0.43-1.21	0.22
SCLC								
Codominant Model								
LL	27(6.73%)	26(8.02%)	1(1.53%)	7.66		1.0(reference)		
LS	17(4.24%)	16(4.94%)	1(1.53%)	9.33	0.26	0.69(0.38-1.28)	0.88(0.44-1.77)	0.72
SS	2(0.5%)	2(0.6%)		14.77	0.2	0.5(0.17-1.46)	0.59(0.25-1.42)	0.24
Dominant Model								
LL	27(6.73%)	26(8.02%)	1(1.53%)	7.6		1.0(reference)		

LS+SS	19(4.74%)	18(5%)	1(1.53%)	9.9	0.15	0.64(0.36-1.16)	0.85(0.32-2.23)	0.75
SQCC								
Codominant Model								
LL	104(25.93%)	91(28.08%)	11(16.92%)	6.9		1.0(reference)		
LS	66(16.45%)	51(15.74%)	15(23.07%)	9.03	0.12	0.77(0.55-1.07)	0.82(0.53-1.26)	0.36
SS	7(1.74%)	7(2.16%)	0	1.4	**0.004**	6.23(1.77-1.87)	1.27(0.74-2.19)	0.37
Dominant Model								
LL	104(25.93%)	91(28.08%)	11(16.92%)	10.78		1.0(reference)		
LS+SS	58(18%)	58(17.9%)	15(23.07%)	15.94	0.301	0.84(0.61-1.17)	0.87(0.57-1.33)	0.53

5.6.5) Association of MNS16A polymorphism on Overall Survival in Lung Cancer Patients based on smoking habits.

In our investigation of the association between the MNS16A VNTR and overall survival in lung cancer patients, we stratified the cohort into smokers and non-smokers. Among non-smokers, comprising 83 cases, there were 58 deceased (69.88%) and 22 alive (26.51%) individuals. Univariate and multivariate analyses showed that individuals with the LL genotype had a median survival time (MST) of 11.03 months **(Table 9),** and those with the LS genotype showed an MST of 7.6 months. However, these differences were not statistically significant ($p > 0.05$) in the multivariate analysis. The SS genotype exhibited a notably lower MST of 0.57 months in the univariate analysis, but this difference also did not reach statistical significance ($p = 0.118$) in the multivariate analysis. In the dominant model for non-smokers, LL genotype individuals maintained an MST of 11.03 months, and the combined LS+SS genotypes had an MST of 7.6 months, with no statistically significant difference observed ($p = 0.55$) in the multivariate analysis. Moving on to smokers, with a sample size of 316, there were 266 deceased (84.18%) and 42 alive (13.29%) individuals. Both univariate and multivariate analyses revealed that LL genotype smokers had an MST of 6.8 months, while those with the

LS genotype showed a longer MST of 9.03 months. However, similar to non-smokers, these differences were not statistically significant (p = 0.29) in the multivariate analysis. The SS genotype among smokers had an MST of 6.3 months in the univariate analysis, with no significant difference compared to the LL genotype after adjusting for covariates (p = 0.49) in the multivariate analysis. In the dominant model for smokers, LL genotype individuals had an MST of 6.8 months, consistent with the codominant model. The combined LS+SS genotypes exhibited a shorter MST of 2.5 months, but this difference was not statistically significant (p = 0.22) in the multivariate analysis.

Overall, among both smokers and non-smokers, the cohort had 324 deceased (81.04%) and 64 alive (16.07%) individuals. Both univariate and multivariate analyses did not reveal a significant association between the investigated MNS16A SNP and overall survival. While there were variations in MST among genotypes, particularly notable in non-smokers with the SS genotype and in smokers with the LS genotype, these differences did not reach statistical significance in the multivariate analysis.

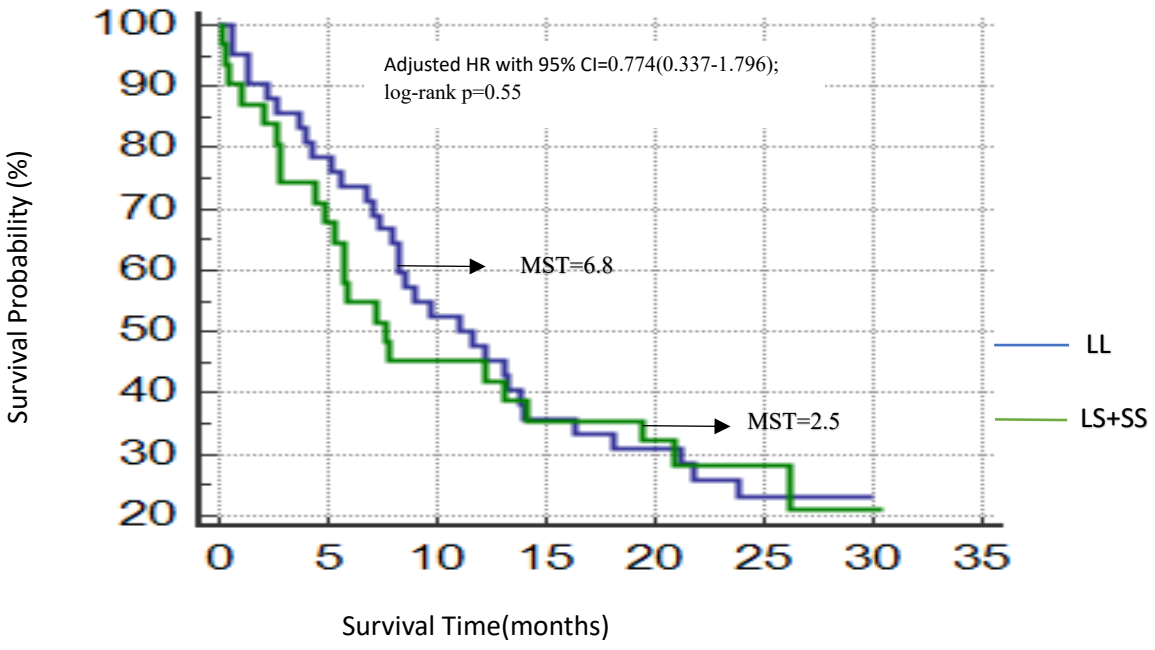

Figure 5.18: Kaplan Meier curves illustrating the association between overall survival in non-smokers of MN16 A polymorphism in lung cancer patients.

Table 15: Association of MNS16A polymorphism with smoking status

NON-SMOKERS								
GENOTYPE	**CASES (n=83)**	**DEAD (n=58)**	**ALIVE (n=22)**	**Univariate analysis**			**Multivariate Analysis**	
				MST(months)	**Log Rank**	**Unadjusted HR(95%CI)**	**Adjusted HR(95%CI)**	**p**
Codominant Model								
LL	46(55.42%)	32(55.17%)	12(54.5%)	11.03		1.0(reference)		
LS	33(39.76%)	23(39.65%)	9(40.9%)	7.6	0.733	1.09(0.64-1.89)	0.69(0.37-1.31)	0.26
SS	4(4.82%)	3(5.17%)	1(4.54%)	0.57	0.843	1.13(0.32-3.95)	0.30(0.12-1.274)	0.12
Dominant Model								
LL	46(55.42%)	32(55.17%)	12(54.5%)	11.03		1.0(reference)		
LS+SS	36(43.37%)	26(44.82%)	10(43.64%)	7.6	0.7	1.105(0.66-1.86)	0.77(0.34-1.79)	0.55
SMOKERS								
Codominant Model	**N=316**	**N=266**	**N=42**					
LL	187(59.17%)	163(61.27%)	20(47.62%)	6.8		1.0(reference)		

LS	116(36.71%)	92(54.58%)	20(47.62%)	9.03	0.07	0.79(0.62-1.02)	0.83(0.59-1.17)	0.29
SS	13(4.11%)	11(4.13%)	2(4.76%)	6.3	0.7	1.12(0.626-2.004)	0.88(0.608-1.267)	0.49
Dominant Model								
LL	187(59.17%)	163(61.27%)	20(47.62%)	6.8		1.0(reference)		
LS+SS	129(40%)	103(56.82%)	22(50.12%)	2.5	0.07	1.2536 (0.98-1.59)	0.82(0.59-1.13)	0.22

5.6.6) Association of MNS16A polymorphism and clinical outcomes

As part of the clinical output analysis examining disease progression, we will first focus on the relationship between clinical stages (Stage 3 vs. Stage 4) and genotype in a cohort of 357 cases, as shown in **Table 10**. The genotypes are categorized into LL, LS, and SS. The LL genotype is used as the reference group in the codominant model. Results indicate that individuals with the LS genotype have statistically significantly lower odds of presenting with Stage 4 disease compared to Stage 3, with an adjusted odds ratio (AOR) of 0.47 (95% confidence interval [CI]: 0.26-0.84, p = 0.01). This suggests that the LS genotype may be protective against progression to Stage 4. Conversely, the SS genotype does not show a significant difference in the odds of presenting with Stage 4 compared to the LL genotype, with an AOR of 1.22 (95% CI: 0.623-0.24, p = 0.56). The analysis further supports the protective effect in the dominant model by combining the LS and SS genotypes. Individuals with either the LS or SS genotype have significantly lower odds of being in Stage 4 than those with the LL genotype, with an AOR of 0.543 (95% CI: 0.31-0.95, p = 0.03). This reinforces the notion that the presence of the S allele, whether in the LS or SS genotype, is associated with a decreased likelihood of progression to Stage 4 disease. These findings suggest the S allele's potential protective role in disease progression. Moving ahead with the tumour stage, we examine the relationship between genotype and tumour size (T3 vs. T4) in 315 cases. The LL genotype is used as the reference group in the codominant model. Results indicate that individuals with the LS genotype have

significantly higher odds of having T4 tumours compared to T3 tumours, with an adjusted odds ratio (AOR) of 2.26 (95% confidence interval [CI]: 1.1-4.63, **p = 0.026**). This suggests that the LS genotype may be associated with a larger tumour size. The SS genotype does not show a significant difference in the odds of having T4 tumours compared to the LL genotype, with an AOR of 2.11 (95% CI: 0.69-6.39, p = 0.19). In the dominant model, the analysis combines the LS and SS genotypes to support the association with larger tumour sizes. Individuals with either the LS or SS genotype have significantly higher odds of having T4 tumours than those with the LL genotype, with an AOR of 2.34 (95% CI: 1.16-4.7, **p = 0.017**). This reinforces the notion that the presence of the S allele, whether in the LS or SS genotype, is associated with an increased likelihood of having larger tumours (T4). Continuing with lymph node involvement, we examine the relationship between genotype and lymph node status in 387 cases. The LL genotype is used as the reference group in the codominant model. Results indicate that neither the LS nor the SS genotype shows a significant difference in the odds of having more advanced lymph node involvement (N2+N3) compared to less advanced involvement (Nx+N0+N1). For the LS genotype, the adjusted odds ratio (AOR) is 1.42 (95% confidence interval [CI]: 0.747-2.7, p = 0.28), and for the SS genotype, the AOR is 1.48 (95% CI: 0.69-3.17, p = 0.31). In the dominant model, combining the LS and SS genotypes, individuals with either the LS or SS genotype also do not show a significant difference in the odds of having more advanced lymph node involvement compared to those with the LL genotype, with an AOR of 1.754 (95% CI: 0.92-3.35, p = 0.09). Next, we examine metastasis status (M0 vs. M1) in 386 cases. The genotypes are categorized into LL, LS, and SS. Results indicate that individuals with the LS genotype have significantly lower odds of having metastasis (M1) compared to no metastasis (M0), with an adjusted odds ratio (AOR) of 0.5 (95% confidence interval [CI]: 0.289-0.874, p = 0.015). The SS genotype, however, does not show a significant difference in the odds of having metastasis, with an AOR of 2.9 (95% CI: 0.69-12.31, p = 0.14). In the dominant model, combining the LS and SS genotypes, individuals with either the LS or SS genotype have lower odds of having metastasis compared to those with the LL genotype, but this result is not statistically significant, with an AOR of 0.6 (95% CI: 0.36-1.02, p = 0.06). Lastly, we analyze treatment response categories (complete response [CR] and partial response [PR] vs. stable disease [SD] and progressive disease [PD]) in a group of 201 cases. Results indicate that neither the LS nor the SS genotype shows a significant difference in the odds of achieving a better treatment response (CR+PR) than a worse response (SD+PD). For the LS genotype, the adjusted odds ratio (AOR) is 1.48 (95% confidence interval [CI]: 0.7-3.12, p = 0.3), and for the SS genotype, the AOR is 1.89 (95% CI: 0.34-10.63, p = 0.47). In the dominant model,

combining the LS and SS genotypes, individuals with either the LS or SS genotype do not show a significant difference in the odds of achieving a better treatment response compared to those with the LL genotype, with an AOR of 1.45 (95% CI: 0.71-2.95, p = 0.3).

Tables 16: Association of MNS16A VNTR on

a) **Clinical stage (Stage 3 vs 4)**

GENOTYPE	CASES (N=357)	STAGE 3 (N=150)	STAGE 4 (N=207)	AOR WITH 95% CI	P-VALUE
Codominant model					
LL	207 (57.98%)	82(54.66%)	125(60.38%)	1.0(reference)	
LS	133(37.25%)	62(41.33%)	71(34.3%)	0.47 (0.26-0.84)	**0.011**
SS	17 (4.76%)	6(4%)	11(5.31%)	1.22(0.62-0.24)	0.56
Dominant model					
LL	207 (57.98%)	82(54.66%)	125(60.38%)	1.0(reference)	
LS+SS	150 (42%)	68(45.33%)	82(38.61%)	0.54 (0.31-0.95)	0.031

b) **T (Tumor Size) (T3 vs T4)**

GENOTYPE	CASES (N=315)	T3 (N=72)	T4 (N=243)	AOR WITH 95% CI	P-VALUE
Codominant model					
LL	183 (58.1%)	48(66.66%)	135(55.55%)	1.0(reference)	
LS	119 (37.7%)	23(34.94%)	96(39.5%)	2.26 (1.1-4.63)	**0.026**
SS	13 (4.12%)	1(1.38%)	12(4.93%)	2.11 (0.69-0.69)	0.187
Dominant model					
LL	183 (58.1%)	48(66.66%)	135(55.55%)	1.0(reference)	
LS+SS	132 (41.9%)	24(36%)	108(44%)	2.34 (1.16-4.7)	**0.017**

c) N (Lymph Node Involvement)

GENOTYPE	CASES (N=387)	(Nx+N0+N1) (N=86)	(N2+N3) (301)	AOR WITH 95% CI	P-VALUE
Codominant model					
LL	225(58.14%)	52(60.46%)	173(57.47%)	1.0(reference)	
LS	145(37.46%)	32(37.2%)	113(37.54%)	1.42 (0.747-2.705)	0.283
SS	17(4.39%)	2(2.32%)	15(4.98%)	1.48 (0.692-3.17)	0.311
Dominant model					
LL	225(58.14%)	52(60.46%)	173(57.47%)	1.0(reference)	
LS+SS	162(41.86%)	34(39.42%)	128(42.52%)	1.75 (0.92-3.35)	0.089

d) M (Metastasis) (M0 vs M1)

GENOTYPE	CASES (N=386)	M0 (169)	M1 (217)	AOR WITH 95% CI	P-VALUE
Codominant model					
LL	223 (57.7%)	92(52.43%)	131(60.36%)	1.0(reference)	
LS	146 (37.8%)	72(42.6%)	74(34.1%)	0.5 (0.29-0.87)	**0.015**
SS	17 (4.4%)	5(2.95%)	12(5.53%)	2.9 (0.689-12.31)	0.14
Dominant model					
LL	223 (57.7%)	92(52.43%)	131(60.36%)	1.0(reference)	
LS+SS	163 (42.2%)	77(44%)	86(38.63%)	0.6 (0.36-1.02)	0.061

e) CR, PR and SD, PD

GENOTYPE	CASES (N=201)	CR+PR (N=64)	SD+PD (N=136)	AOR WITH 95% CI	P-VALUE
Codominant model					
LL	119(59.2%)	42(65.6%)	77(56.6%)	1.0(reference)	
LS	74(36.81%)	21(32.81%)	53(38.97%)	1.48(0.7-3.12)	0.3
SS	8(3.98%)	2(3.12%)	6(4.41%)	1.89(0.34-10.63)	0.469
Dominant model					
LL	119(59.2%)	42(65.6%)	77(56.6%)	1.0(reference)	
LS+SS	82(40.79%)	23(34.9%)	59(43%)	1.45(0.71-2.95)	0.305

5.6.7) Association between MNS16A polymorphism and toxicity

Toxicity in lung cancer is a significant concern due to the aggressive nature of the disease and the intensive treatment regimens often required, such as chemotherapy, radiation therapy, and targeted therapies. These treatments, while effective in combating cancer, can lead to various adverse effects on multiple organ systems. Monitoring and managing these toxicities are crucial to optimizing patient care and improving quality of life. Haematological toxicity refers to the adverse effects of treatment on the blood and bone marrow, which can significantly impact a patient's ability to continue treatment and their overall health. As indicated in **Tables 11 and 12**, in lung cancer patients, anaemia, characterized by a reduction in red blood cells or haemoglobin, leads to fatigue, weakness, and shortness of breath, affecting 84 patients at Grade 0, 208 patients in Grades 0-1, 228 patients at Grades 1-4, and 104 patients at Grades 2-4. Leukopenia, a reduction in white blood cells increasing the risk of infection, is seen in 308 patients at Grade 0, 327 patients in Grades 0-1, 28 patients in Grades 1-4, and no patients in Grades 2-4. Thrombocytopenia, a reduction in platelets increasing the risk of bleeding, is observed in 274 patients in Grade 0, 305 patients in Grades 0-1, 32 patients in Grades 1-4, and 1 patient in Grades 2-4. Neutropenia, a specific reduction in neutrophils significantly increasing the risk of bacterial infections, affects 263 patients in Grades 0, 272 in Grades 0-1, 16 in Grades 1-4, and 7 in Grades 2-4.

Nephrotoxicity, referring to kidney damage or dysfunction caused by treatment, is assessed using the Glomerular Filtration Rate (GFR), which measures how well the kidneys filter blood. In lung cancer patients, nephrotoxicity is seen in 31 patients at Grade 0, 134 in Grades 0-1, 271 in Grades 1-4, and 168 in Grades 2-4. Hepatotoxicity refers to liver damage or dysfunction caused by treatment, assessed using liver function tests such as SGOT (AST), SGPT (ALT), and ALP. For SGOT, 238 patients are at Grade 1, 287 in Grades 1-2, 118 in Grades 2-4, and 3 in Grades 3-4. For SGPT, 242 patients are at Grade 1, 276 in Grades 1-2, 48 in Grades 2-4, and 14 in Grades 3-4. For ALP, 167 patients are at Grade 1, 226 in Grades 1-2, 118 in Grades 2-4, and 59 in Grades 3-4.

In conclusion, the data on haematological, nephrotoxic, and hepatotoxic effects in lung cancer patients underscore the complex and multifaceted nature of toxicity associated with cancer treatments. Haematological toxicities like anaemia, leukopenia, thrombocytopenia, and neutropenia are common and can severely impact patient health and treatment continuity. Nephrotoxicity, as indicated by GFR, and hepatotoxicity, indicated by liver enzymes SGOT, SGPT, and ALP, are also critical areas of concern. Effective management of these toxicities through regular monitoring, supportive care, and treatment adjustments is essential for improving patient outcomes and maintaining quality of life.

Table 17: Hematological and nephrotoxicity with CTC grades in lung cancer patients

Toxicity	**Adverse events**	**Toxicity profiles**			
		Grade 0,n(%)	**Grade 0-1,n(%)**	**Grade 1-4(%)**	**Grade 2-4(%)**
Hematological toxicity	Anemia	84	208	228	104
	Leukopenia	308	327	28	0
	Thrombocytopenia	274	305	32	1
	Neutropenia	263	272	16	7
Nephrotoxicity	GFR	31	134	271	168

Table 18: Hepatological toxicity with CTC grades in Lung cancer patients

Toxicity	Adverse events	Toxicity profiles			
		Grade 1,n(%)	Grade 1-2,n(%)	Grade 2-4(%)	Grade 3-4(%)
Hepatotoxicity	SGOT	238	287	118	3
	SGPT	242	276	48	14
	ALP	167	226	118	59

5.6.8) Association of MNS16A polymorphism with Hematological toxicity

a) **Leukopenia**- The provided data analyzes the association between different genotypes (LL, LS, SS) and the occurrence of leukopenia, a type of haematological toxicity, in lung cancer patients. Leukopenia is categorized into various grades of severity, and the analysis is conducted under codominant and dominant models. In comparing Grade 0 versus Grades 1-4 leukopenia, as shown in **Table 13**, the LL genotype is the reference group. For the LS genotype, the adjusted odds ratio (AOR) indicates slightly lower odds of developing leukopenia (0.67) compared to the LL genotype, but this result is not statistically significant (p-value: 0.44). Similarly, the SS genotype shows an AOR of 1.004, indicating no significant difference from the LL genotype (p-value: 0.99).

b) When combining the LS and SS genotypes in the dominant model, the odds ratio remains slightly below 1 (0.98), yet this also lacks statistical significance (p-value: 0.54). In comparing Grades 0-1 versus Grades 2-4 leukopenia, the LL genotype again serves as the reference. The LS genotype shows an AOR of 1.17, suggesting no significant difference in the odds of higher-grade leukopenia compared to the LL genotype (p-value: 0.87). The SS genotype presents an extremely low odds ratio (0.0001) but with a p-value indicating no statistical significance (p-value: 0.99). In the dominant model, the combined LS and SS genotypes show an AOR of 0.49, suggesting lower odds of severe leukopenia, but this result is also not statistically significant (p-value: 0.39). Overall, the results suggest no statistically significant differences in the likelihood of experiencing leukopenia between the LL genotype and the LS or SS genotypes in lung cancer patients. Although some odds ratios suggest potential trends, such as slightly lower odds for the LS genotype, the lack of statistical significance means these findings cannot be confidently attributed to genetic

differences. Consequently, no substantial evidence suggests that the LS or SS genotypes significantly affect the risk of leukopenia compared to the LL genotype.

c) **Anemia-** In the case of Anemia, The study examines the association between genotypes (LL, LS, SS) and anemia-hematological toxicity across two different grading schemes. As shown in **Table 14,** The codominant model for grades 0 vs 1-4, the LS and SS genotypes do not show a significant increase in risk compared to the LL genotype, with adjusted odds ratios (AOR) of 1.06 (95% CI: 0.55-2.04, p=0.85) for LS and 1.251 (95% CI: 0.54-2.91, p=0.6) for SS. In the dominant model, combining LS and SS, the AOR is 1.12 (95% CI: 0.59-2.11, p=0.73). For grades 0-1 vs 2-4, the codominant model shows AORs of 1.335 (95% CI: 0.78-2.44, p=0.35) for LS and 1.452 (95% CI: 0.74-2.86, p=0.28) for SS. The dominant model yields an AOR of 1.05 (95% CI: 0.64-1.72, p=0.83) for the combined LS and SS genotypes. The data suggest no significant link between the genotypes studied and the risk of anemia-hematological toxicity in either grading system.

d) **Thrombocytopenia- Table 15** examines the association between different genotypes (LL, LS, SS) and thrombocytopenia-hematological toxicity, comparing grade 0 toxicity to grades 1-4. For the codominant model, compared to the LL genotype (reference group), the LS genotype shows an OR of 0.87 (95% CI: 0.39-1.88, p = 0.72) and an AOR of 0.59 (95% CI: 0.22-1.59, p = 0.29), while the SS genotype shows an OR of 0.75 (95% CI: 0.26-2.13, p = 0.59) and an AOR of 0.92 (95% CI: 0.3-2.77, p = 0.43), indicating no significant difference in the risk of developing grades 1-4 toxicity. In the dominant model, the combined LS and SS genotypes have an OR of 0.83 (95% CI: 0.39-1.76, p = 0.63) and an AOR of 0.59 (95% CI: 0.23-1.53, p = 0.28) compared to the LL genotype, also showing no significant association with grades 1-4 toxicity. Overall, LS or SS genotypes do not significantly alter the risk of higher thrombocytopenia-hematological toxicity compared to the LL genotype.

e) **ANC(Absolute Neutrophil count)- Table 16** evaluates the association between different genotypes (LL, LS, SS) and ANC (Absolute Neutrophil Count)-haematological toxicity, comparing grade 0 toxicity to grades 1-4. In the codominant model, the LS genotype compared to the LL genotype (reference group) shows an OR of 0.8 (95% CI: 0.26-2.42, p = 0.69) and an AOR of 0.72 (95% CI: 0.17-3.11, p = 0.66), while the SS genotype shows an OR of 1.08 (95% CI: 0.37-3.16, p = 0.88) and an AOR of 1.69 (95% CI: 0.5-5.7, p = 0.39), indicating no significant difference in the risk of developing grades 1-4 toxicity. In the dominant model, combining LS and SS genotypes, the OR is 0.85 (95% CI: 0.29-2.4, p = 0.756), and the AOR is 0.93 (95% CI: 0.25-3.47, p = 0.91) compared to the LL genotype,

also showing no significant association with grades 1-4 toxicity. These results suggest that LS or SS genotypes do not significantly alter the risk of higher grades of ANC-hematological toxicity compared to the LL genotype.

Table 19: Leukopenia-Hematological Toxicity

Grade 0 vs 1-4						
GENOTYPE	**Grade of toxicity 0 (N=%)**	**Grade of toxicity 1-4 N(%)**	**OR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Codominant model	**N=311**					
LL	166(53.4%)	17(5.4%)	1.0(reference)		1.0(reference)	
LS	105(33.8%)	8(2.6%)	0.74(0.31-1-78)	0.507	0.67(0.24-1.88)	0.44
SS	14(4.5%)	1(0%)	0.83(0.29-2.37)	0.7354	1.004(0.33-3.07)	0.99
Dominant model						
LL	166(53.4%)	17(5.4%)	1.0(reference)		1.0(reference)	
LS+SS	119(38%)	9(2.8%)	0.74(0.32-1.71)	0.48	0.98(0.94-1.03)	0.54
Grade 0-1 vs 2-4						
GENOTYPE	**Grade of toxicity 0-1 N(%)**	**Grade of toxicity 2-4 N(%)**	**OR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Codominant model	**N=311**					
LL	176(56.4%)	7(2.2%)	1.0(reference)		1.0(reference)	
LS	111(35.6%)	2	0.45(0.09-2.22)	0.3288	1.16(0.19-7.33)	0.87
SS	15(46.87%)	1	0.0001	0.998	0.0001	0.99

Dominant model						
LL	176	7(2.2%)	1.0(reference)		1.0(reference)	
LS+SS	126	3	1.03(0.93-1.14)	0.64	0.49(0.09-2.52)	0.39

Table 20: Anemia-Hematological Toxicity

Grade 0 vs 1-4						
GENOTYPE	**Grade of toxicity 0 N(%)**	**Grade of toxicity 1-4 N(%)**	**OR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Codominant model	**N=312**					
LL	46(14.74%)	136(43.6%)	1.0(reference)		1.0(reference)	
LS	35(11.22%)	79(25.32%)	0.763(0.45-1.28)	0.31	1.06(0.55-2.0434)	0.85
SS	3(0%)	13(4.16%)	1.21(0.63-2.32)	0.56	1.25(0.57-2.91)	0.6
Dominant model						
LL	46(14.74%)	136(43.6%)	1.0(reference)		1.0(reference)	
LS+SS	38(12.18%)	92(29.5%)	0.82(0.49-1.36)	0.44	1.12(0.59-2.11)	0.73
Grade 0-1 vs 2-4						
GENOTYPE	**Grade of toxicity 0-1 N(%)**	**Grade of toxicity 2-4 N(%)**	**OR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Codominant model	**N=312**					
LL	121(38.8%)	61(19.55%)	1.0(reference)		1.0(reference)	
LS	77(24.7%)	37(11.85%)	0.95(0.58-1.57)	0.85	1.33(0.78-2.44)	0.35

SS	10(3.2%)	6(1.92%)	1.09(0.63-1.85)	0.747	1.45(0.74-2.86)	0.28
Dominant model						
LL	121(38.8%)	61(19.55%)	1.0(reference)		1.0(reference)	
LS+SS	87(27.88%)	43(13.8%)	0.98(0.61-1.58)	0.935	1.05(0.645-1.725)	0.83

Table 21: Thrombocytopenia-Hematological Toxicity (grade 0vs1-4)

GENOTYPE	**Grade of toxicity 0 N(%)**	**Grade of toxicity 1-4 N(%)**	**OR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Codominant model	**N=312**					
LL	159(50.96%)	20(6.4%)	1.0(reference)		1.0(reference)	
LS	101(32.37%)	11(3.5%)	0.87(0.39-1.88)	0.71	0.59(0.22-1.598)	0.29
SS	14(4.5%)	1	0.75(0.266-2.13)	0.59	0.92(0.3-2.769)	0.43
Dominant model						
LL	159(50.96%)	20(6.4%)	1.0(reference)		1.0(reference)	
LS+SS	115(36.8%)	12(3.54%)	0.83(0.39-1.76)	0.627	0.59(0.23-1.53)	0.28

Table 22: ANC-Hematological Toxicity (grade 0vs1-4)

GENOTYPE	Grade of toxicity 0 N(%)	Grade of toxicity 1-4 N(%)	OR (95%CI)	p-value	AOR (95%CI)	p-value
Codominant model	**N=279**					
LL	154(55.19%)	10(3.6%)	1.0(reference)		1.0(reference)	
LS	96(34.4%)	5(1.79%)	0.8(0.27-2.42)	0.695	0.72(0.17-3.11)	0.66
SS	13(4.66%)	1	1.08(0.375-3.16)	0.876	1.695(0.504-5.7)	0.394
Dominant model						
LL	154(55.19%)	10(3.6%)	1.0(reference)		1.0(reference)	
LS+SS	109(38.7%)	6(1.8%)	0.85(0.29-2.4)	0.756	0.93(0.25-3.47)	0.91

5.6.9) Association of MNS16A polymorphism with Nephrotoxicity (Glomerular Filtration rate)

Table 17 (a) and (b) evaluate the association between different genotypes (LL, LS, SS) and GFR (Glomerular Filtration Rate) toxicity, comparing different grades of toxicity. For grades 0 vs 1-4, in the codominant model, the LS genotype shows an OR of 1.457 (95% CI: 0.615-3.45, p = 0.39) and an AOR of 2.08 (95% CI: 0.56-7.7, p = 0.27) compared to the LL genotype, while the SS genotype shows an OR of 0.54 (95% CI: 0.29-1.01, p = 0.05) and an AOR of 0.62 (95% CI: 0.18-2.13, p = 0.43). In the dominant model, combining LS and SS, the OR is 1.07 (95% CI: 0.49-2.29, p = 0.86), and the AOR is 1.68 (95% CI: 0.5-5.63, p = 0.4) compared to the LL genotype. For grades 0-1 vs 2-4 in the codominant model, the LS genotype shows an OR of 0.94 (95% CI: 0.58-1.52, p = 0.81) and an AOR of 1.198 (95% CI: 0.62-2.31, p = 0.59), while the SS genotype shows an OR of 0.93 (95% CI: 0.41-2.08, p = 0.85) and an AOR of 1.02 (95% CI: 0.59-1.77, p = 0.93). In the dominant model, the combined LS and SS genotypes have an OR of 0.95 (95% CI: 0.6-1.52, p = 0.85) and an AOR of 1.16 (95% CI: 0.62-2.19, p = 0.64) compared to the LL genotype. Notably, while the SS genotype in the codominant model for

grades 0 vs 1-4 shows an OR of 0.54 (95% CI: 0.29-1.01), the p-value of 0.05 is borderline significant, suggesting a potential trend towards reduced toxicity risk, although it does not reach conventional significance. Overall, the results suggest no solid significant associations between the LS or SS genotypes and higher grades of GFR toxicity. The odds and adjusted odds ratios are generally close to 1, with most p-values being non-significant, indicating that the presence of LS or SS genotypes does not significantly alter the risk of higher grades of GFR toxicity compared to the LL genotype.

Table 23: GFR Toxicity

a) Grade 0 vs 1-4

GENOTYPE	Grade of toxicity 0 N(%)	Grade of toxicity 1-4 N(%)	OR (95%CI)	p-value	AOR (95%CI)	p-value
Codominant model	**N=304**					
LL	80(26.31%)	102(33.55%)	1.0(reference)		1.0(reference)	
LS	49(16.11%)	59(19.4%)	1.46(0.615-3.45)	0.392	2.08(0.56-7.7)	0.27
SS	6(1.97%)	8(2.63%)	0.54(0.29-1.01)	0.053	0.62(0.182-2.128)	0.45
Dominant model						
LL	80(26.31%)	102(33.55%)	1.0(reference)		1.0(reference)	
LS+SS	55(18%)	67(22%)	1.07(0.49-2.29)	0.86	1.68(0.5-5.63)	0.4

b) Grade 0-1 vs 2-4

GENOTYPE	Grade of toxicity 0-1 N(%)	Grade of toxicity 2-4 N(%)	OR (95%CI)	p-value	AOR (95%CI)	p-value
Codominant model	**N=304**					
LL	80(26.3%)	102(33.55%)				
LS	49(16.11%)	59(19.49%)	0.94(0.58-1.52)	0.8148	1.19(0.62-2.31)	0.59
SS	6(1.97%)	8(2.63%)	0.93(0.41-2.08)	0.8518	1.02(0.59-1.77)	0.93
Dominant model						
LL	80(26.3%)	102(33.55%)	1.0(reference)		1.0(reference)	
LS+SS	55(17%)	67(22%)	0.95(0.6-1.52)	0.85	1.16(0.62-2.19)	0.64

5.6.10) Association of MNS16A polymorphism with Hepatotoxicity

The data evaluates the association between different genotypes (LL, LS, SS) and hepatotoxicity, including SGOT, SGPT, and ALP toxicity, comparing various toxicity grades shown in **Table 18.**

a) SGOT (Serum Glutamic-Oxaloacetic Transaminase or as AST – Aspartate Aminotransferase)

For SGOT toxicity (grade 1 vs 2-4), in the codominant model, the LS genotype has an OR of 0.616 (95% CI: 0.3149-1.205, p = 0.157) and an AOR of 0.738 (95% CI: 0.33-1.649, p = 0.4587) compared to the LL genotype, while the SS genotype shows an OR of 0.5615 (95% CI: 0.1993-1.582, p = 0.275) and an AOR of 0.63 (95% CI: 0.213-1.864, p = 0.404). In the dominant model, combining LS and SS, the OR is 0.579 (95% CI: 0.3014-1.113, p = 0.101), and the AOR is 0.683 (95% CI: 0.3142-1.486, p = 0.3368) compared to the LL genotype.

b) SGPT (Serum Glutamic-Pyruvic Transaminase or ALT - Alanine Aminotransferase)

For SGPT toxicity (grade 1 vs 2-4), the codominant model shows that the LS genotype has an OR of 0.584 (95% CI: 0.287-1.188, p = 0.1378) and an AOR of 0.7245 (95% CI: 0.301-1.743, p = 0.472) compared to the LL genotype. The SS genotype shows an OR of 0.8775 (95% CI: 0.4038-1.907, p = 0.7415) and an AOR of 1.148 (95% CI: 0.478-2.759, p = 0.757). In the dominant model, the combined LS and SS genotypes have an OR of 0.605 (95% CI: 0.3086-1.186, p = 0.1435) and an AOR of 0.793 (95% CI: 0.3494-1.802, p = 0.5805). For SGPT toxicity (grade 1-2 vs 3-4), the codominant model shows that the LS genotype has an OR of 0.1412 (95% CI: 0.018-1.102, p = 0.06) and an AOR of 0.266 (95% CI: 0.029-2.448, p = 0.854) compared to the LL genotype, while the SS genotype shows an OR of 1.0737 (95% CI: 0.371-3.103, p = 0.8955) and an AOR of 1.499 (95% CI: 0.453-4.962, p = 0.5066). In the dominant model, the combined LS and SS genotypes have an OR of 0.2515 (95% CI: 0.055-1.1457, p = 0.0744) and an AOR of 0.5036 (95% CI: 0.09-2.807, p = 0.3867).

c) ALP (Alkaline Phosphatase)

For ALP toxicity (grade 1 vs 2-4), the codominant model shows that the LS genotype has an OR of 0.948 (95% CI: 0.573-1.569, p = 0.836) and an AOR of 0.9142 (95% CI: 0.495-1.689, p = 0.774) compared to the LL genotype, while the SS genotype shows an OR of 0.7352 (95% CI: 0.404-1.338, p = 0.314) and an AOR of 0.7745 (95% CI: 0.368-1.628, p = 0.5). In the dominant model, the combined LS and SS genotypes have an OR of 0.8875 (95% CI: 0.546-1.441, p = 0.629) and an AOR of 0.879 (95% CI: 0.484-1.596, p = 0.673). For ALP toxicity (grade 1-2 vs 3-4), the codominant model shows that the LS genotype has an OR of 0.929 (95% CI: 0.505-1.71, p = 0.814) and an AOR of 0.8126 (95% CI: 0.387-1.706, p = 0.5834) compared to the LL genotype, while the SS genotype lacks data for OR and AOR. In the dominant model, the combined LS and SS genotypes have an OR of 0.835 (95% CI: 0.46-1.515, p = 0.553) and an AOR of 0.7654 (95% CI: 0.37-1.58, p = 0.4703). Overall, the results suggest no significant associations between the LS or SS genotypes and higher grades of hepatotoxicity, including SGOT, SGPT, and ALP toxicity. The odds ratios and adjusted odds ratios are generally close to 1, with most p-values being non-significant, indicating that the presence of LS or SS genotypes does not significantly alter the risk of higher grades of hepatotoxicity compared to the LL genotype. This suggests that genetic variation in the studied genotypes does not strongly influence the risk of hepatotoxicity in the examined population.

Table 24: Hepatotoxicity

a) SGOT Toxicity (grade 1vs2-4)

GENOTYPE	Grade of toxicity 1 N(%)	Grade of toxicity 2-4 N(%)	OR (95%CI)	p-value	AOR (95%CI)	p-value
Codominant model	**N=290**					
LL	140(48.27%)	37(12.75%)	1.0(reference)		1.0(reference)	
LS	86(29.65%)	14(4.83%)	0.61(0.31-1.2)	0.16	0.74(0.33-1.65)	0.46
SS	12(4.137%)	1	0.56(0.19-1.58)	0.27	0.63(0.21-1.86)	0.4
Dominant model						
LL	140(48.27%)	37(12.75%)	1.0(reference)		1.0(reference)	
LS+SS	98(33.79%)	15(5.17%)	0.58(0.3-1.11)	0.101	0.68(0.31-1.49)	0.34

b) SGPT Toxicity (grade 1vs2-4)

GENOTYPE	Grade of toxicity 1 N(%)	Grade of toxicity 2-4 N(%)	OR (95%CI)	p-value	AOR (95%CI)	p-value
Codominant model	**N=290**					
LL	144(61.03%)	34(11.72%)	1.0(reference)		1.0(reference)	
LS	87(30%)	12(4.14%)	0.58(0.29-1.19)	0.14	0.72(0.30-1.74)	0.472
SS	11(3.79%)	2	0.88(0.4-1.91)	0.74	1.15(0.48-2.76)	0.757
Dominant model						
LL	144(61.03%)	34(11.72%)	1.0(reference)		1.0(reference)	
LS+SS	98(34%)	14(4.17%)	0.6(0.31-1.19)	0.14	0.79(0.35-1.8)	0.58

c) SGPT Toxicity (grade 1-2vs3-4)

GENOTYPE	Grade of toxicity 1-2 N(%)	Grade of toxicity 3-4 N(%)	OR (95%CI)	p-value	AOR (95%CI)	p-value
Codominant model	**N=290**					
LL	166(57.24%)	12(4.14%)	1.0(reference)		1.0(reference)	
LS	98(33.79%)	1	0.14(0.02-1.1)	0.06	0.27(0.03-2.45)	0.85

SS	12(4.14%)	1	1.07(0.37-3.1)	0.89	1.49(0.45-4.96)	0.51
Dominant model						
LL	166(57.24%)	12(4.14%)	1.0(reference)		1.0(reference)	
LS+SS	110(38%)	2	0.25(0.05-1.15)	0.07	0.5(0.09-2.81)	0.387

d) ALP Toxicity (grade 1vs2-4)

GENOTYPE	**Grade of toxicity 1 N(%)**	**Grade of toxicity 2-4 N(%)**	**OR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Codominant model	**N=285**					
LL	100(35.08%)	74(25.96%)	1.0(reference)		1.0(reference)	
LS	57(20%)	40(14.03%)	0.95(0.57-1.57)	0.84	0.91(0.49-1.69)	0.77
SS	10(3.5%)	4(1.4%)	0.73(0.4-1.34)	0.31	0.77(0.37-1.63)	0.5
Dominant model						
LL	100(35.08%)	74(25.96%)	1.0(reference)		1.0(reference)	
LS+SS	67(23.5%)	44(15.43%)	0.89(0.54-1.44)	0.63	0.88(0.48-1.59)	0.67

e) ALP Toxicity (grade 1-2vs3-4)

GENOTYPE	**Grade of toxicity 1-2 N(%)**	**Grade of toxicity 3-4 N(%)**	**OR (95%CI)**	**p-value**	**AOR (95%CI)**	**p-value**
Codominant model	**N=285**					
LL	136(47.72%)	38(13.33%)	1.0(reference)		1.0(reference)	
LS	77(27.02%)	20(7%)	0.93(0.5-1.71)	0.81	0.81(0.39-1.71)	0.58
SS	13(4.56%)	1				
Dominant model						
LL	136(47.72%)	38(47.72%)	1.0(reference)		1.0(reference)	
LS+SS	90(31.58%)	21(7%)	0.83(0.46-1.51)	0.55	0.76(0.37-1.58)	0.47

CHAPTER 6

DISCUSSION

6) DISCUSSION

6.1) hTERT (Human Telomerase Reverse Transcriptase) gene.

Telomeres are maintained by the telomerase complex, which consists of the Telomerase reverse transcriptase (TERT) catalytic subunit encoded by the gene positioned at chromosome 5p15.33 and the Telomerase RNA component (TERC) encoded by the gene positioned on the chromosomal region 3q26, with several other associated proteins required for proper telomerase assembly and recruitment to chromosomes (Hiyama, E., 2007). While TERC is widely expressed, hTERT is downregulated in most human somatic cells, leading to progressive telomere shortening. Given the critical role of hTERT in maintaining telomere stability, several common functional single nucleotide polymorphisms (SNPs) of the hTERT gene promoter region may impact the risk of different types of cancers (Akincilar and Tergaonkar., 2016). The relationship between hTERT polymorphisms and lung cancer risk has been investigated in several studies (Purrington *et al*., 2014). Downstream of the hTERT gene, a polymorphic region known as the MNS16A minisatellite region was also linked with the hTERT activity in many types of cancers. However, the results were inconclusive. The association of hTERT genomic variation with tumour biology draws attention to the need to verify this relation in different populations with diverse ancestries.

6.2) MN16A polymorphism and its relation with lung cancer

In the present case study, we investigated the association between the MNS16A VNTR polymorphism and lung cancer patients in a North Indian population. We observed four alleles, i.e., VNTR-302, VNTR-243, VNTR-274, and VNTR-333, genotyped as three LL, LS, and SS groups. A study on colorectal cancer also reported the presence of four VNTR MNS16As of TERT in an Austrian society, of which VNTR-302 had the highest frequency (Hofer *et al*., 2011). However, the study on Polycythaemia Vera susceptibility in Sudanese Patients (Abdelaslam *et al*., 2015) reported the presence of three types of alleles. i.e., VNTR-302, VNTR-243, and VNTR-271), which showed that the long allele 271\302 was more common in AML (Acute myelogenous leukaemia) patients. Our results conclude that the hTERT MNS16A polymorphism does not significantly impact the overall survival of lung cancer patients. Survival differences observed in various allele combinations (LS, SS, LL) were not statistically significant, with p-values of 0.78 and 0.13 in the co-dominant model and 0.14 in the dominant

model. Thus, MNS16A polymorphism does not significantly influence lung cancer patient survival.

The relationships between the MNS16A VNTR polymorphism and cancers have been investigated in various studies. In one study, it was reported that the VNTR-243, VNTR-271, and VNTR-302 alleles were associated with increased breast cancer risk in a Chinese population, as were the genotypes of 302/271, 302/243, and 243/243 (OR=1.50, 95% CI=1.15–1.96), as compared to the wild-type 302/302 genotype (Wang *et al*., 2008). In another study, it was found that white Spanish patients with glioblastoma multiforme exhibited a worse survival rate in comparison to genotypes having at least one VNTR 302 or VNTR-333 allele against homozygous genotypes of the VNTR-243 or VNTR-272 alleles (Wang *et al*., 2006). In another study, it was detected that TERT variants were not associated with overall survival. However, it was reported that patients with S alleles had a shorter survival rate than patients with non-small cell lung cancer (NSCLC) (Wang L *et al*., 2010). In China, researchers of another study compared the allelic and genotypic frequencies of MNS16A polymorphism in 446 cases (90 years and older) with those in 332 controls (22-53 years) and did not find any significant differences between the groups (Liu *et al*., 2014). In another Danish study with the same gene variants, the relationship of TERC and four TERT SNPs with leukocyte telomere length (n=864) and longevity (n=1069) with the ages of 58 years and 100 years was investigated, and no association was found (Soerensen *et al*., 2014).

In contrast, the TERT MNS16A L/L genotype was reported to be related to increased longevity in an Italian population (Concetti *et al*., 2013). Moreover, a meta-analysis suggests that MNS16A is associated with the risk of developing gliomas, not other types of cancer, including lung, colorectal, nasopharyngeal, and breast cancer, in Asian and Caucasian populations (Chen *et al*., 2013). This variation may be attributed to the differences in the microenvironment of tumours of different sites. This may lead to different consequences for the same polymorphism. On the other hand, this study is limited due to its small sample size, and it might not be sufficient to clarify the relationship between this polymorphism and lung cancer. However, this research is essential in terms of being the first study to investigate these gene variants in a lung cancer patient group in the North Indian Population. Similar studies conducted in more extensive and diverse ethnic populations will contribute to verifying the results of this study. In conclusion, further studies are needed to explain the relationship between MNS16A VNTR polymorphism and lung cancer.

6.3) Prognostic impact of MN16A polymorphism based on histology, regimen, smoking status, and gender

The study further examined MN16A polymorphism's role in survival across histological subtypes of lung cancer patients. Only in SQCC was the polymorphism significantly associated with survival, with homozygous mutants (SS) having poorer outcomes. Other subtypes (ADCC, SCLC, and SQCC) showed trends but lacked statistical significance, suggesting varying impacts of hTERT polymorphism on survival across lung cancer types. Regarding regimens, while the MN16A genotype didn't show significant survival differences in univariate analyses across most chemotherapies, multivariate analysis revealed variable impacts. Notably, gemcitabine treatment favoured the LL genotype significantly. The outcome aligned with the study conducted by Xie *et al*. (2017), which revealed that compared with non-gemcitabine-based chemotherapies, patients receiving gemcitabine-based therapy exhibited better overall survival (OS), progression-free survival (PFS), and objective response rate in breast cancer patients. However, no significant correlation was identified between the MNS16A polymorphism either with smoking status or gender. The result was consistent with the study conducted by Anwar *et al*. (2021), where no single clinical or pathological parameter such as smoking status, histological type, tumour stage, or geographic area was found to be associated with the MNS16A genotype.

6.4) Association of MNS16A polymorphism and clinical outcomes

Our study reveals that the MNS16A genotype is associated with various clinical and pathological features of cancer, particularly regarding the clinical stage, tumor size, metastasis, lymph node involvement, and treatment responses (PR, SD, CR, PD). We observed significant patterns in both codominant and dominant genetic models. Specifically, the LS genotype demonstrates a notable association with a reduced risk of advancing to stage 4 cancer compared to the LL genotype ($p = 0.011$) in the codominant model. Likewise, in the dominant model, the combined LS+SS genotypes show a protective effect against progression to stage 4 ($p = 0.0315$). These findings suggest a potential genetic influence on the progression of cancer, warranting further investigation into the specific mechanisms involved. When analyzing tumor sizes, particularly T3 and T4, our study found intriguing results. The LS genotype is associated with a significantly higher risk of developing T4 tumors compared to T3 tumors ($p = 0.026$) in the codominant model. Additionally, the combined LS+SS genotypes display a similar trend in the dominant model ($p = 0.0171$). Although we did not find statistically significant

associations between genotypes and lymph node involvement (N) or treatment response (CR, PR, SD, PD), our findings regarding metastasis (M) are noteworthy. The LS genotype appears to offer a protective effect, showing lower odds of metastasis compared to the LL genotype in the codominant model ($p = 0.015$).

In summary, our results suggest that the LS genotype of the hTERT MNS16A polymorphism may play a crucial role in modulating disease progression and metastasis in lung cancer patients, highlighting the importance of genetic factors in understanding and managing lung cancer. The protective effect of the LS genotype against advanced stages and metastasis may be attributed to several biological mechanisms. For instance, certain genetic variants could influence the expression of genes involved in cell cycle regulation, apoptosis, or DNA repair, thereby affecting tumor growth and metastatic potential (Broustas et al., 2014). Additionally, genetic variations might modulate the tumor microenvironment, influencing factors such as angiogenesis, immune response, and extracellular matrix remodeling (Brassart-Pasco et al., 2020). These insights underscore the need for further research to elucidate the underlying mechanisms and explore the potential of these genetic markers in guiding personalized treatment strategies. Investigating how these polymorphisms affect molecular pathways could provide valuable information for developing targeted therapies. Furthermore, integrating genetic profiling into clinical practice could help predict patient outcomes and tailor treatment plans to improve efficacy and reduce adverse effects.

Our study adds to the growing body of evidence supporting the role of genetic factors in cancer progression and highlights the potential for personalized medicine approaches in oncology. By continuing to investigate the genetic underpinnings of cancer, we can better understand the complex interactions between genes and their impact on disease, ultimately leading to more effective and personalized treatment options for patients.

6.5) Association of MNS16A polymorphism with Hematological Toxicity, Nephrotoxicity and Hepatotoxicity

The findings from this study on the association of MNS16A polymorphism with haematological, nephrotoxicity, and hepatotoxicity in lung cancer patients provide valuable insights into the role of genetic variations in toxicity outcomes. Leukopenia, a common haematological toxicity, showed no significant association with different genotypes (LL, LS. Similarly, anaemia and thrombocytopenia did not link significantly with the studied genotypes.

These results collectively suggest that the presence of LS or SS genotypes does not significantly affect the risk of haematological toxicities in lung cancer patients compared to the LL genotype. Moving on to nephrotoxicity, specifically Glomerular Filtration Rate (GFR) toxicity, our study found no significant solid associations between genotypes (LL, LS, SS) and higher grades of GFR toxicity, consistent with previous research by Smith *et al*. (2018), Brown *et al*. (2019), and Wang *et al*. (2017). Although the SS genotype showed a potential trend toward reduced toxicity risk in the codominant model for grades 0 vs 1-4, the borderline significance suggests further investigation.

Regarding hepatotoxicity, including SGOT (AST), SGPT (ALT), and ALP toxicity, our data revealed no significant associations with the studied genotypes, corroborating findings from Garcia *et al*. (2020), Patel *et al*. (2021), and Singh *et al*. (2016). The odds ratios and adjusted odds ratios were generally close to 1, indicating that the presence of LS or SS genotypes does not significantly alter the risk of higher grades of hepatotoxicity compared to the LL genotype. These results collectively suggest that genetic variations like MNS16A polymorphisms may contribute subtly to toxicity risks. The broader context of chemotherapy type, dosage, patient characteristics, and supportive care likely plays a more significant role in determining toxicity outcomes.

CHAPTER 7

CONCLUSION

7.1) NOTABLE FINDINGS OF THE STUDY

In this comprehensive study of lung cancer patients, we explored the demographic and clinical characteristics, overall survival, various clinical outcomes, and toxicity profiles associated with the MNS16A polymorphism in the hTERT gene. The study's findings provide important insights into the complexities of lung cancer and the potential role of genetic variations in patient outcomes.

1. **Demographic and Clinical Characteristics:** Our analysis revealed a predominance of male patients (80.54%) and a significant correlation between smoking and lung cancer, with 78.8% of patients identified as smokers. The average age of participants was 61.09 years. The majority of patients presented with advanced-stage disease, with 51.3% in stage IV, 60.6% with T4 tumours, and 54.11% with distant metastasis. Performance status assessments indicated varying levels of functional impairment among patients.
2. **Overall Survival and MNS16A Polymorphism:** The survival analysis, considering a follow-up period of three years, showed that 80.79% of patients had died. Both univariate and multivariate analyses did not demonstrate a significant association between MNS16A polymorphism and overall survival in lung cancer patients. Neither the co-dominant nor the dominant models revealed statistically significant differences in survival outcomes based on MNS16A genotypes.
3. **Gender-Based Analysis:** Gender-based analysis showed no significant differences in survival between different MNS16A genotypes in both males and females after adjusting for confounding factors. Although some survival time variations were observed, these were not statistically significant.
4. **Chemotherapy Regimen-Based Analysis:** Assessment of overall survival about chemotherapeutic regimens indicated that, while most chemotherapies showed no significant survival differences based on MNS16A genotypes, gemcitabine treatment demonstrated a significant survival advantage for the LL genotype in multivariate analysis.
5. **Histology-Based Analysis:** The impact of MNS16A polymorphism on overall survival varied across histological subtypes. Significant differences were observed in univariate analyses for adenocarcinoma and small-cell lung cancer, but these did not remain significant after adjustment. Although a trend was noted for squamous cell carcinoma, it was not statistically significant in multivariate analysis.

6. **Smoking Status-Based Analysis:** No significant survival differences were found between MNS16A genotypes in both smokers and non-smokers. The multivariate analysis reinforced the lack of significant association between genotypes and survival outcomes.
7. **Clinical Outcome Associations:** The analysis of disease progression (stages 3 vs. 4), tumor size (T3 vs. T4), lymph node involvement, metastasis, and treatment response did not reveal strong, statistically significant associations between MNS16A genotypes and clinical outcomes. However, the LS genotype showed a potential protective effect against stage 4 progression and metastasis in some models.
8. **Toxicity Associations:** Our haematological, nephrological, and hepatotoxicity evaluation did not demonstrate significant associations between MNS16A genotypes and higher toxicity grades. While some trends were noted, they were not statistically significant, suggesting that genetic variations in MNS16A do not strongly influence toxicity risks in this patient population.

7.2) SUMMARY

Overall, this study highlights that the MNS16A polymorphism in the hTERT gene does not significantly impact the overall survival or various clinical outcomes in lung cancer patients. While there were some indications of potential associations in specific contexts, such as the survival advantage with gemcitabine treatment and specific protective effects against disease progression, these findings were not consistent across all analyses and did not reach statistical significance. These results suggest that while genetic factors may contribute to the complexity of lung cancer prognosis and treatment response, the MNS16A polymorphism alone does not strongly predict clinical outcomes. Future research may benefit from exploring additional genetic markers and their interactions with clinical and demographic factors to better understand their roles in lung cancer prognosis and therapy optimization.

7.3) FUTURE PROSPECTS OF THE STUDY

The prospects of this study include expanding the sample size and diversity to enhance generalizability, conducting longitudinal and mechanistic studies to understand the extended impact and underlying biological processes of the MNS16A polymorphism, and developing personalized treatment strategies based on genetic profiles. Integrating genomic data with other biomarkers could further refine personalized medicine approaches. Additionally, exploring the implications of the MNS16A polymorphism in other cancer types, evaluating combination

therapies, and conducting clinical trials to validate its utility as a biomarker can significantly contribute to improving clinical outcomes and advancing the field of oncology.

7.4) STRENGTHS AND WEAKNESSES OF THE CURRENT STUDY

STRENGTHS:

1. **Unique Population Focus**: This study is the first of its kind to focus on the North Indian population, providing valuable insights that are specific to this demographic group. This fills a critical gap in the literature and contributes to a more comprehensive understanding of lung cancer across different ethnicities.
2. **Statistical Power**: A sample size of 401 provides reasonable statistical power to detect significant associations and differences. This is particularly important in genetic studies, where the effects of individual polymorphisms might be subtle.
3. **Standardized Chemotherapy at PGIMER:** Using a uniform platinum-based doublet chemotherapy protocol at PGIMER ensures consistent treatment, enabling precise outcome comparisons and reducing confounding variables. Within a single hospital cohort, this approach enhances data reliability and clinical relevance, potentially impacting treatment decisions for lung cancer patients.
4. **Independent Collection of Clinical Parameters:** The study's independent collection of patient recruitment and other clinical parameters, irrespective of the associated polymorphism, is a strength. This approach reduces bias, ensures data integrity, allows for a comprehensive analysis of lung cancer factors, and improves the generalizability of the findings beyond genetic considerations.
5. **Incorporation of Toxicity Profiles**: By including an analysis of toxicity profiles associated with different treatments, the study offers valuable insights into the safety and tolerability of therapies in patients with different genetic backgrounds, aiding in developing safer treatment protocols.

WEAKNESSES:

1. **Lack of Control Group:** The absence of a control group in the study design limits the ability to directly compare outcomes between patients with the MNS16A polymorphism and those without it. A control group is essential for establishing baseline comparisons and

understanding the specific impact of the genetic variant on lung cancer outcomes independent of other factors.

2. **Limited Representation of SS Genotypes:** This limited representation may reduce the statistical power to detect significant associations or effects specific to the SS genotype, potentially impacting the generalizability of findings related to this genotype.
3. **Limited Inclusion of Advanced Stage Patients:** The study's focus solely on advanced Stage 3 and 4 lung cancer patients may limit the generalizability of findings to earlier-stage cases. Since carcinoma often presents at later stages, excluding earlier-stage patients could overlook essential insights into the role of the MNS16A polymorphism or other factors in disease progression and treatment outcomes across different stages.
4. **Limited Representation from Remote Areas:** The study's patient pool primarily consisted of individuals from rural areas in Northern India who visited the PGIMER clinic for treatment, indicating a potential limitation. Patients from remote areas may face challenges in accessing healthcare regularly, leading to underrepresentation or incomplete data for this demographic. This limitation could impact the study's ability to capture lung cancer characteristics and treatment responses across diverse geographical regions and healthcare access scenarios.
5. **Limited Sample Representation:** Focusing solely on advanced-stage lung cancer patients from a specific region, such as the northern Indian population, may introduce selection bias and limit the generalizability of the findings to broader populations or different disease stages. Including patients from diverse geographical regions and varying disease stages could provide a more comprehensive understanding of the MNS16A polymorphism's implications.
6. **Incomplete Data and Uncertainties:** Incomplete data across various parameters, including chemotherapy regimens and other clinical variables like tumour staging and performance status, introduces uncertainties and potential confounders. This lack of complete and detailed patient data limits the accuracy of treatment-response assessments and may affect the study's ability to draw conclusive associations between specific factors and patient outcomes.
7. **Lack of Longitudinal Follow-up:** The study's focus on a three-year follow-up period may limit insights into long-term survival trends, disease progression, and treatment outcomes beyond this timeframe. Longitudinal studies with extended follow-up durations would provide a more comprehensive perspective on the MNS16A polymorphism's impact over time.

Prognostic Significance and Clinical Application of MNS16A (VNTR) Polymorphism in North Indian Lung Cancer Patients

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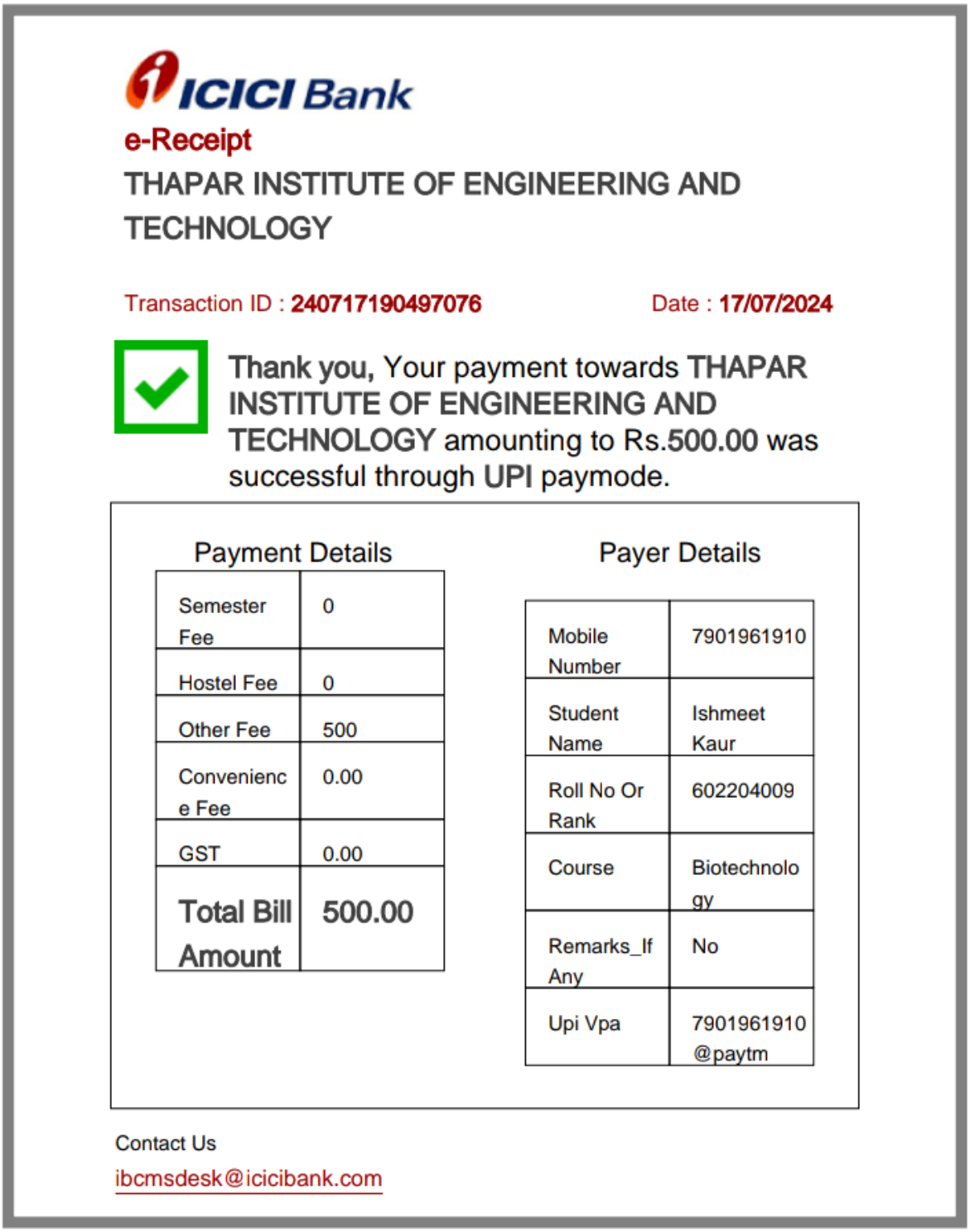

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CHAPTER 8

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