Chapter 1

Introduction

1. Introduction

Over the course of human history, changes in the external environment have been a major driver of the appearance of new diseases. Throughout the beginning of time, humans have engaged with the natural environment around them, and as our societies have grown and evolved, so have the effects we have on the environment and vice versa. The onset of diseases can also be influenced by other alterations. For instance, the increase in industrialization in the 19th and 20th centuries which brought more pollution and exposure to chemicals, which in turn aided in the emergence of malignancies and respiratory illnesses[1]. The emergence of new diseases can also be influenced by changes in human behaviour. For instance, greater travel and international trade were factors in the 1980s HIV/AIDS epidemic, which made it possible for the virus to spread quickly around the globe[2]. The trading and consumption of wild animals in China is believed to have contributed to the COVID-19 outbreak in 2019, giving the virus a chance to spread from animals to people[3]. New illnesses can also develop as a result of changes in animal populations. For instance, the outbreak of COVID-19 in 2019 and SARS in 2002 were both attributed to zoonotic transmission, which is the spread of viruses from animals to people[3]. Economic factors also contribute to the development of diseases, including communicable and non-communicable diseases which are more in Low-income countries[4]. Non-communicable diseases (NCDs) are chronic illnesses that are typically not brought on by infectious agents, as compared to communicable diseases, which are those that are brought on by infectious agents like bacteria, viruses, and parasites. Several popular and scholarly articles note that the burden of noncommunicable diseases (NCDs) is gradually increasing in India and that these are the leading causes of death, outpacing communicable diseases by a wide margin[5]. Non-communicable diseases such as **cancer**, heart disease, and diabetes are often associated with lifestyle factors such as diet, physical activity, and tobacco and alcohol use[6].

Cancer's rising incidence and mortality rates have made it a huge burden on society. In the past, infectious diseases were the leading cause of death, but as people are now living longer, chronic diseases like cancer are now more common. According to the World Health Organization (WHO), cancer is now the leading cause of death globally[7] second to Ischemic heart disease, with an estimated 9.6 million deaths in 2018 and 10 million deaths from cancer in 2020 alone[8]. The prevalence of cancer is predicted to increase in the coming years due to an ageing population, increased rates of obesity and physical inactivity, and changing patterns of cigarette use[6].

1.1 Cancer

1.1.1 Overview of Cancer Prevalence

Cancer is one of the most devastating diseases to affect people. Since cancer is the second-leading cause of death globally, it poses a significant public health and economic burden. Almost half of individuals who are diagnosed with it die from it each year, and it affects many people worldwide. According to the International Agency for Research on Cancer (IARC), 1 in 5 people will eventually get cancer in their lives and that 1 in 8 men and 1 in 11 women will pass away from it[9].

More than 19.3 million new cases and 10 million cancer deaths have been reported by GLOBOCAN 2020[8,10], as shown in **Figure 1**. With an estimated 0.8 million new instances of cancer per year, the cancer burden has steadily increased. More than 1 million people will die from cancer and close to 2 million additional cases are anticipated in 2040[10,11].

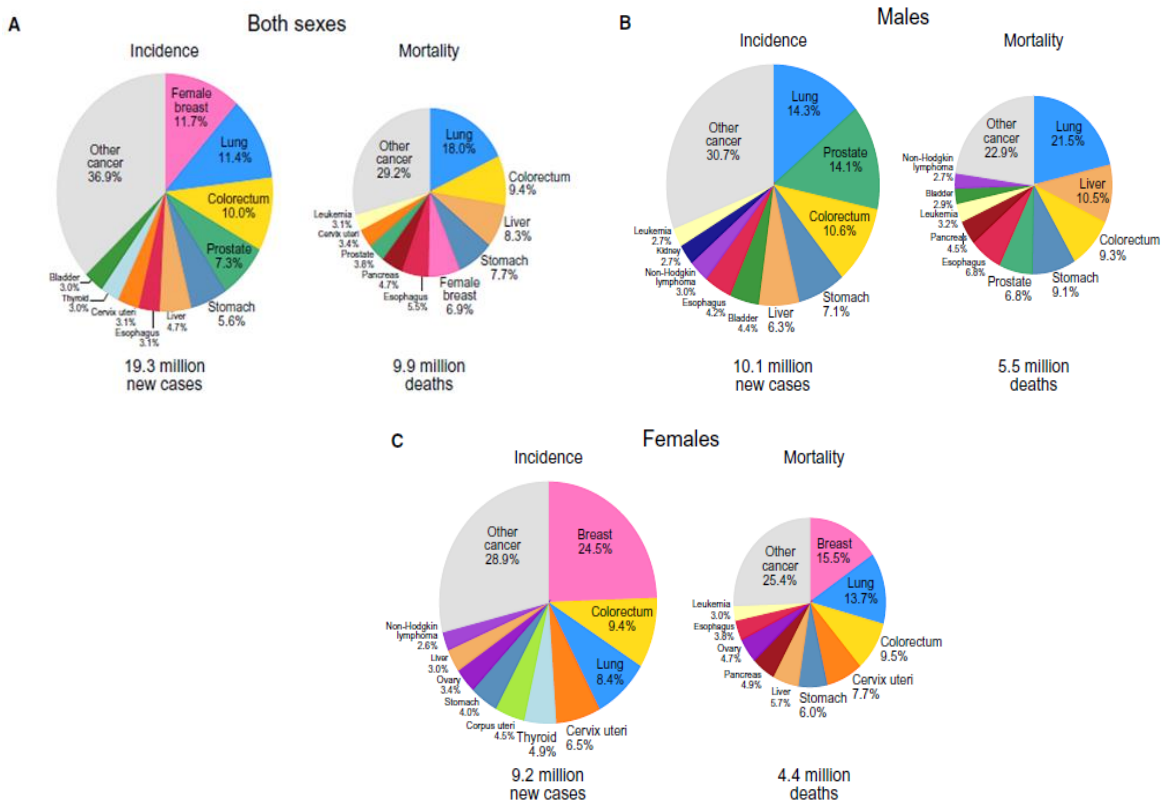

Figure 1 Pie chart showing the number of new cases of cancer and deaths in a) Both sexes b) Men c) Women of top 10 most prevalent cancers - GLOBOCAN 2020.

As per data from National Cancer Registry of Indian Council of Medical Research (ICMR), 14.6 lakhs new cases were reported in 2022, from 14.2 lakhs in 2021 and 13.92 lakhs in 2020, as shown in **Figure 1.1**.

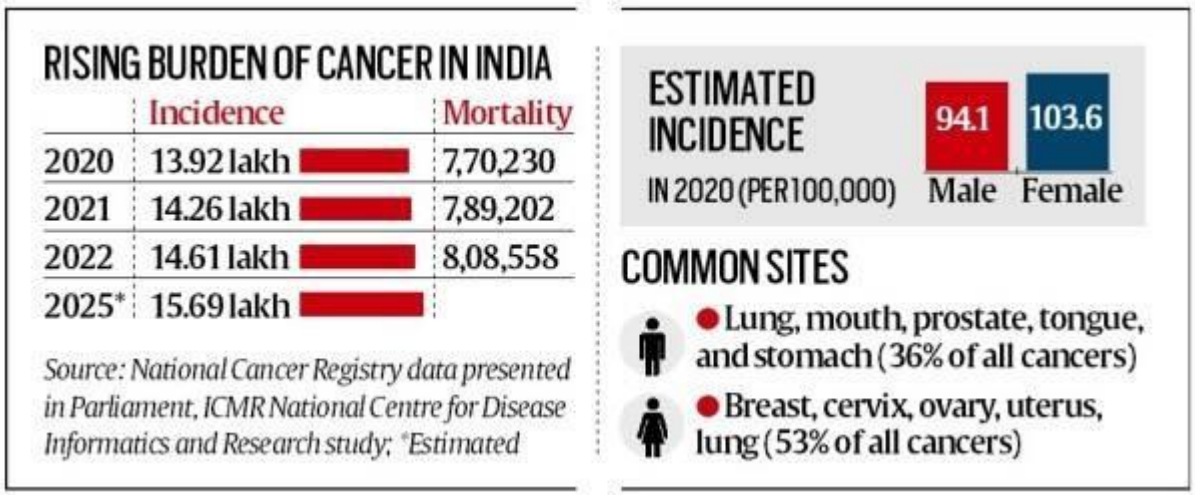

Figure 1.1 Estimated cancer incidences in India according to ICMR

Cancer types involving **Lung**, colorectal and stomach cancers, which are leading cause of cancer deaths, are higher in males than females[10, 12,13]. In Females, breast, ovarian and uterine cancers are more prevalent and cause higher mortality[10,12,13] as shown in **Figure 1.2**.

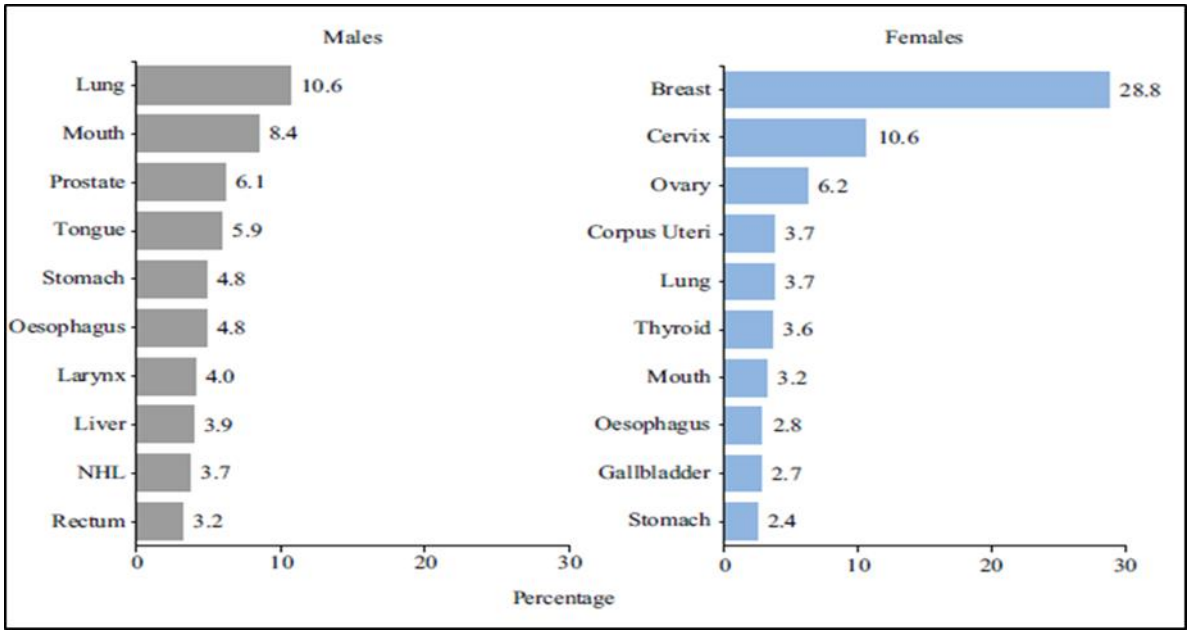

Figure 1.2 Estimated proportion of most common types of cancers in India by sex -2022 (**Kumar** *et. al*., 2022).

1.1.2 Understanding Cancer: What It Is and How It Develops

The process of cell division is crucial for the organism's ability to maintain its tissues. There is a balance between cell death and growth under normal conditions. Yet, throughout the process, cells may have mutations (and/or epimutations), which causes their uncontrolled proliferation. One key feature of the group of disorders known as **cancer** is this aberrant cell proliferation. Cancer typically results from many cell mutations, and only mutations in a certain group of genes (known as **oncogenes**) can cause this. A tumour is a collection of cells that results from unchecked proliferation as shown in **Figure 1.3a**. These tumours could be benign or malignant.

Benign tumours continue to be confined to a single area. They frequently disappear on their own or usually doesn't reappear. These cancers' cells do not disseminate to other areas. **Malignant tumours** are made of cells which invade neighbouring tissues and spread to other regions. Cells spread from the original (primary) cancer site to other regions (secondary sites). This process of spreading is termed **Metastasis** as shown in Figure **1.3b.**

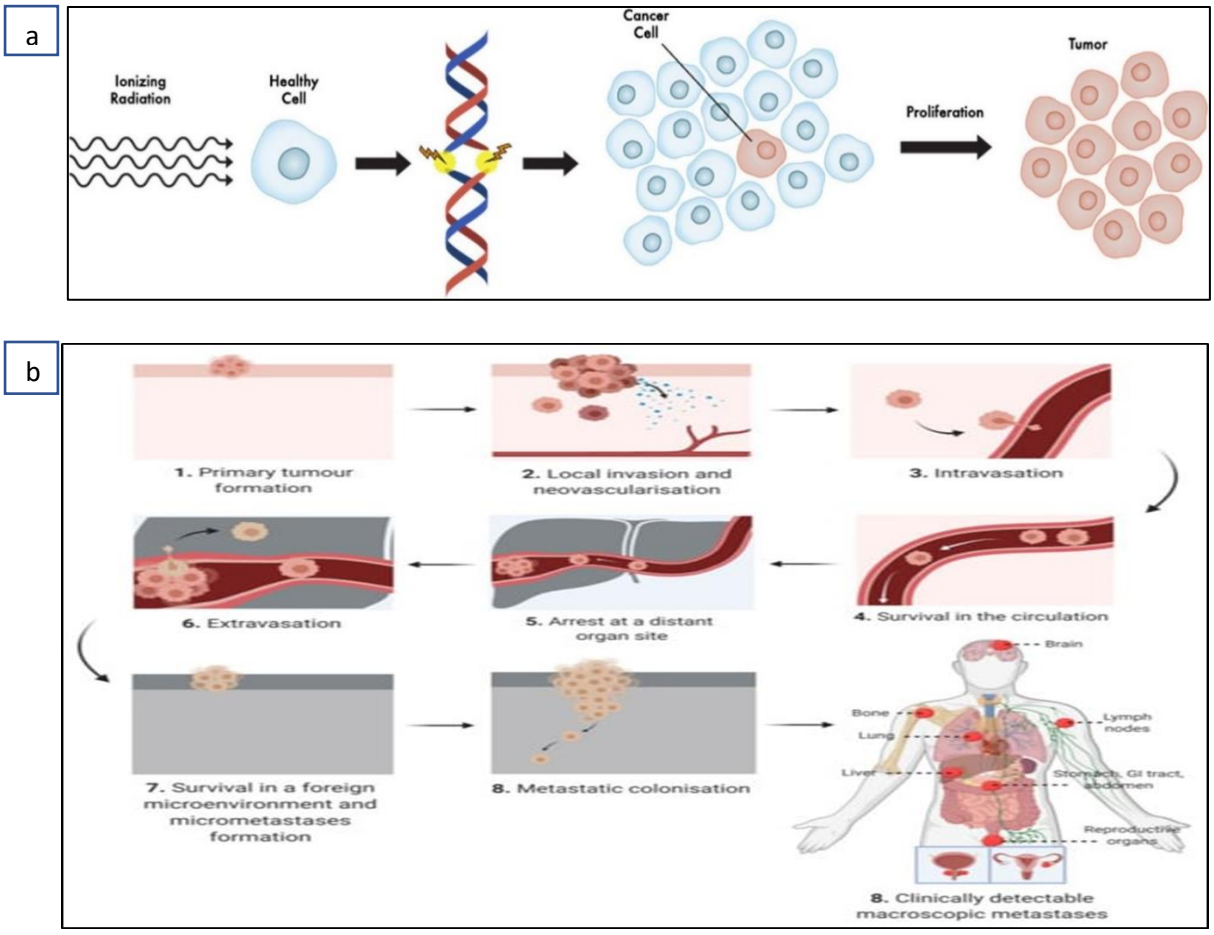

Figure 1.3 a) Cancer cell proliferation b**)** Key steps of Metastatic Cascade (**Annett** *et. al*., 2020)

1.2 Classification of Cancer

Cancers are classified into two categories, one by the primary site and another by the type of tissue from which the cancer originated (histological type). According to histology, there are hundreds of different types of cancers, which are grouped into six main categories:

- **Carcinoma** Cancer that develops from epithelial cells is referred to as a carcinoma. It covers cancers like breast, prostate, lung, colon, and others. These tumours make up most cases. There are four subtypes of carcinomas:
- **Adenocarcinoma** is a tumour that starts in glandular cells.
- **Squamous cell carcinoma** - originates in the squamous epithelial cells.
- **Transitional cell carcinoma** – originates in transitional epithelium.
- **Basal cell carcinoma** – originates from basal epithelial cells.
- **Sarcoma** - Cancer that develops in supporting and connective tissues, such as bones, tendons, cartilage and muscle, is referred to as a sarcoma.
- **Myeloma** - A form of cancer called myeloma develops in plasma cells of bone marrow, a type of white blood cell.
- **Lymphoma** - Lymphomas are those cancers which originate in the glands or nodes of the lymphatic system (such as spleen, tonsils, and thymus). It is divided into two types – Hodgkin Lymphoma and Non-Hodgkin Lymphoma.
- **Leukaemia** - Leukaemia is a cancer of the bone marrow (where haematopoiesis takes place) Other categories include brain and spinal cord cancers and cancers of mixed type.
- **Mixed types** – The components may be within one category or from different categories.

1.3 Lung Cancer

Lung cancer is a type of carcinoma in which there is a presence of a malignant lung tumour characterized by uncontrolled cell proliferation in the cells and tissues of the lung. Lung cancer is the most common kind of cancer among Men both Globally and in Indian population[14,15]. It is also the third most common cancer among Women, Globally.

1.3.1 Causes and Occurrence of Lung Cancer

1.3.1.1 Causes of Lung Cancer

Lung cancer causes include tobacco usage as its main contributor. Exposure to second-hand smoke, air pollution, and workplace exposure to certain chemicals such as asbestos and radon can also increase the risk of developing lung cancer to people who don't smoke. Also, people received chest radiation treatments in the past (for instance, for breast cancer or lymphoma) and having a family history of lung cancer are at a greater risk to develop lung cancer as shown in Figure 1.4.

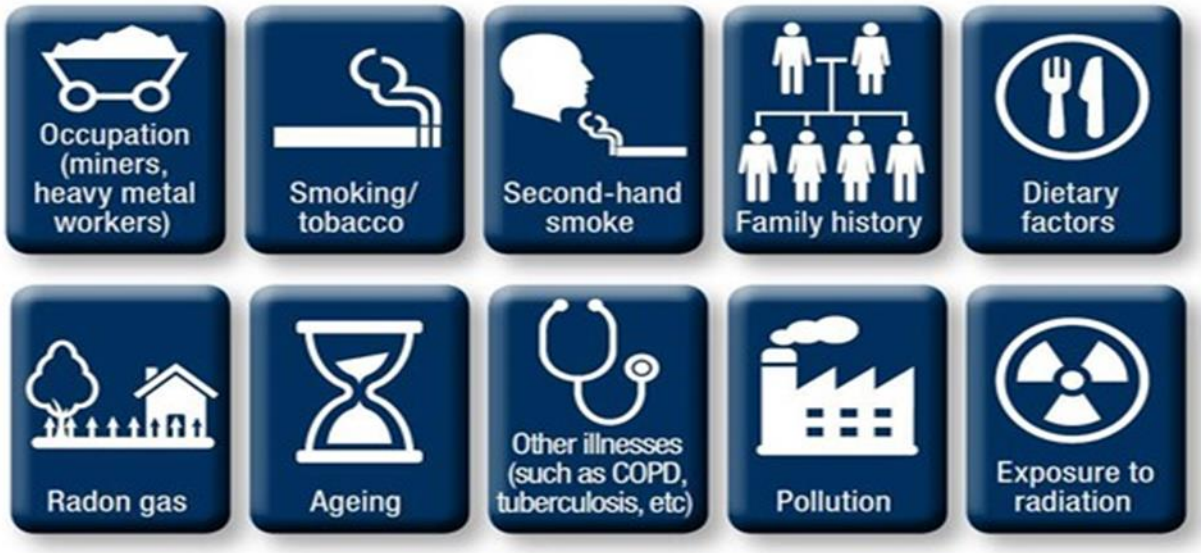

Figure 1.4 Common risk factors for Lung Cancer (Source: https://scdhec.gov/health/diseases)

1.3.1.2 Prevalence Rate of Lung Cancer

Lung cancer is the primary reason for cancer-related mortality in both men and women worldwide[16] as shown in **Figure 1.5**. According to the World Health Organization (WHO), the estimated number of new lung cancer cases worldwide in 2018 was 2.09 million, and the estimated number of deaths from lung cancer was 1.76 million[15].

In India, the estimated number of new lung cancer cases in 2020 was 67,000, with an estimated 48,000 deaths from lung cancer[16,17,14], according to the Indian Council of Medical Research (ICMR). The prevalence rate of lung cancer in India has been increasing over the past few years.

Here are some prevalence rates of lung cancer globally and in India over the last five years:

Global Prevalence Rate of Lung Cancer:

- In 2019, the estimated age-standardized incidence rate of lung cancer was 24.8 per 100,000 population worldwide.
- In 2020, the estimated age-standardized mortality rate of lung cancer was 19.3 per 100,000 population worldwide.

Prevalence Rate of Lung Cancer in India:

- In 2017, the age-adjusted incidence rate of lung cancer in India was 7.2 per 100,000 population.
- In 2018, the age-adjusted mortality rate of lung cancer in India was 6.8 per 100,000 population.
- In 2019, the age-adjusted incidence rate of lung cancer in India was 7.4 per 100,000 population.
- In 2020, the age-adjusted mortality rate of lung cancer in India was 7.0 per 100,000 population.

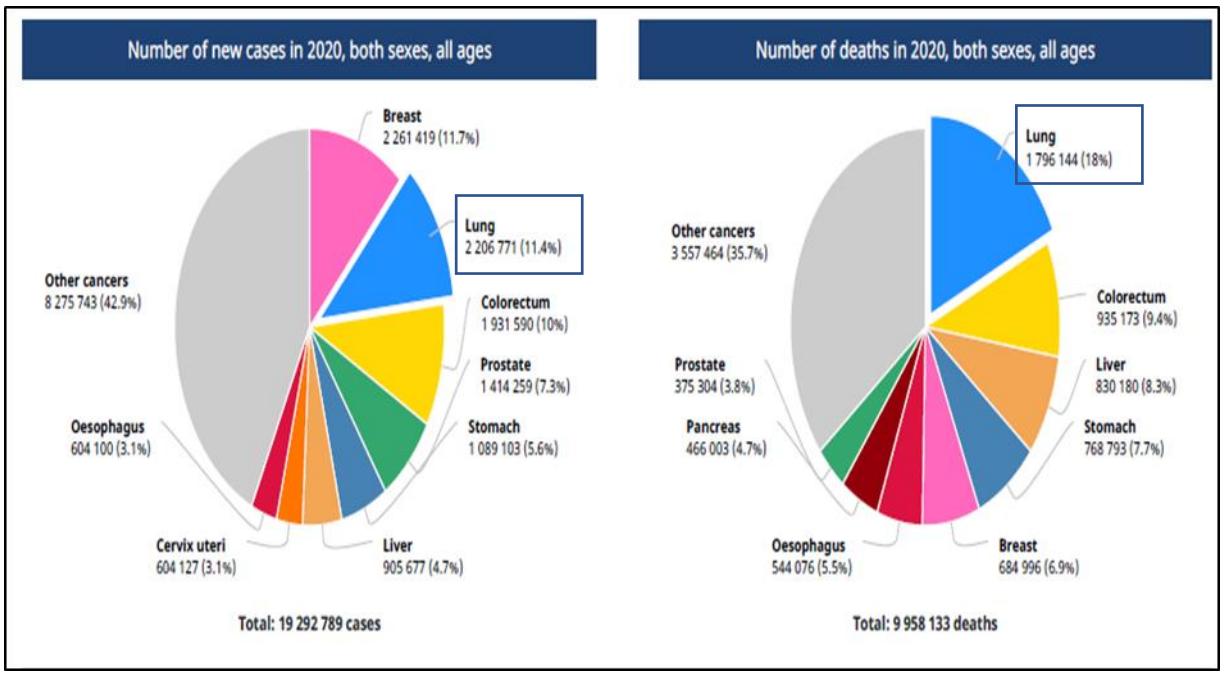

Figure 1.5 Global Cancer Observatory, 2020 (**WHO**)

1.3.2 Types of Lung Cancer

Lung cancer is divided into two main types: NSCLC & SCLC.

a) NSCLC (Non-small cell lung cancer)

NSCLC is the most dominant type of lung cancer, which constitute around 80-85% of lung cancer cases. It frequently develops sluggishly than SCLC and exhibits little or no symptoms.

There are three major types of NSCLC:

i) Adenocarcinoma – It is the most common form of NSCLC, which comprises of 40% of all NSCLC cases as shown in **Figure 1.6**. It generally occurs in the external lining of the lungs (the cells lining the alveoli). It is commonly found cancers between people who never smoked.

ii) Squamous cell Carcinoma – This type of lung cancer occurs substantially in the cells near the bronchi. It accounts for 30 percent of all non-small cell lung cancers and is generally linked to smoking. It spreads gradually.

iii) Large cell carcinoma – It grows and spreads rapidly and can occur anywhere in the lung. This kind of lung cancer generally accounts for 10 to 15 percent of all occurrences of NSCLC.

b) SCLC (Small cell lung cancer)

SCLC recurrently begins in the bronchi before speedily spreading to other body areas, including the lymph nodes. This kind of lung cancer represents lesser than 20 percent of lung cancers and is typically caused by cigarette smoking. It grows aggressively.

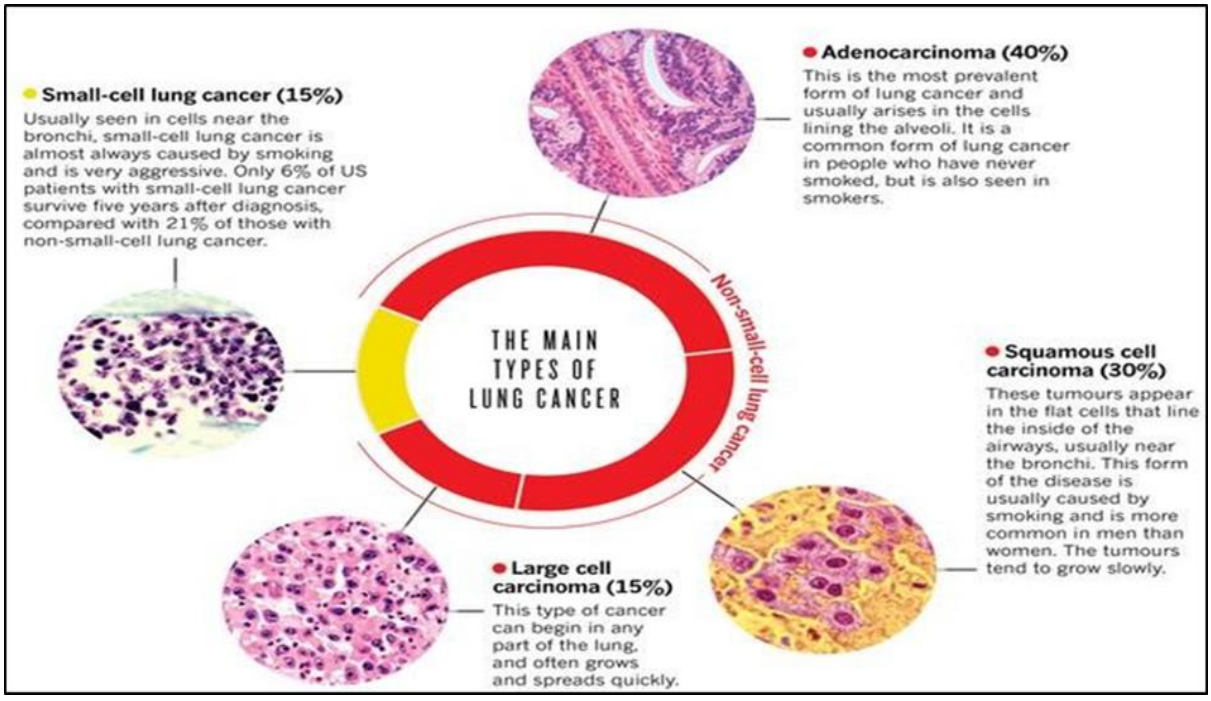

Figure 1.6 Types of Lung Cancer (**Bender**,2014)

1.3.3 Stages of Lung Cancer

1.3.3.1 Stages of Non-small Cell Lung Cancer

Doctors categorise the stages of lung cancer using the Roman numerals I, II, III, and IV are used by doctors to categorise the stages of non-small cell lung cancer. Early-stage cancer is classified as Stage I, while advanced cancer that has spread to other bodily areas, such the bones, is classified as Stage IV. Lung cancer is divided into four stages, depending upon the size of the tumour and how far it has been spread the body:

Occult Stage Lung Tumour: Sputum contains tumour cells, but CT scans and other imaging techniques fail to reveal a lung tumour.

Stage 0 Lung Tumour: The innermost lining of the lung has growth of abnormal cells. Tumour growth has stopped. It is not an invasive cancer.

Stage I Lung Tumour: The tumour is little and restricted to the lung.

Stage II Lung Tumour: The malignancy has intensified and might have spread to close-by lymph nodes.

Stage III Lung Tumour: The disease has progressed to the chest's lymph nodes or other internal organs like the diaphragm or chest wall.

Stage IV Lung Tumour: The cancer has gone to the brain, liver, or bones, among other organs.

1.3.3.2 Stages of Small Cell Lung Cancer

Limited stage: Only one side of the chest has cancer.

Extensive stage: The cancer has spread to tissues on the other side of the chest as well as the lung. Or, lung cancer can be discovered in fluid between the two layers of pleura or in distant organs like the brain.

1.3.4 Lung cancer: Highest death rate and poorest patient survival

Depending on the stage of the cancer at the time of diagnosis, the 5-year survival rate for lung cancer varies. Just 20% of those who are diagnosed with lung cancer go on to survive for five years or more on average, according to the 5-year survival rate for lung cancer[18]. Although studies over the past 30 years have revealed that the projected gain in survival has not yet been attained, developments in early diagnosis and treatment with the prospect of improved survival

are encouraging[19,20].Lung cancer has an 18% 5-year survival rate (15% for men and 21% for women)[19]. Just 16% of patients receive a diagnosis of localised cancer, which has a 56% 5-year survival rate[18,19]. The goal of numerous studies has been to identify prognostic markers in order to more accurately assess therapy success and identify treatment strategies in order to increase survival rates with lesser side effects[21].

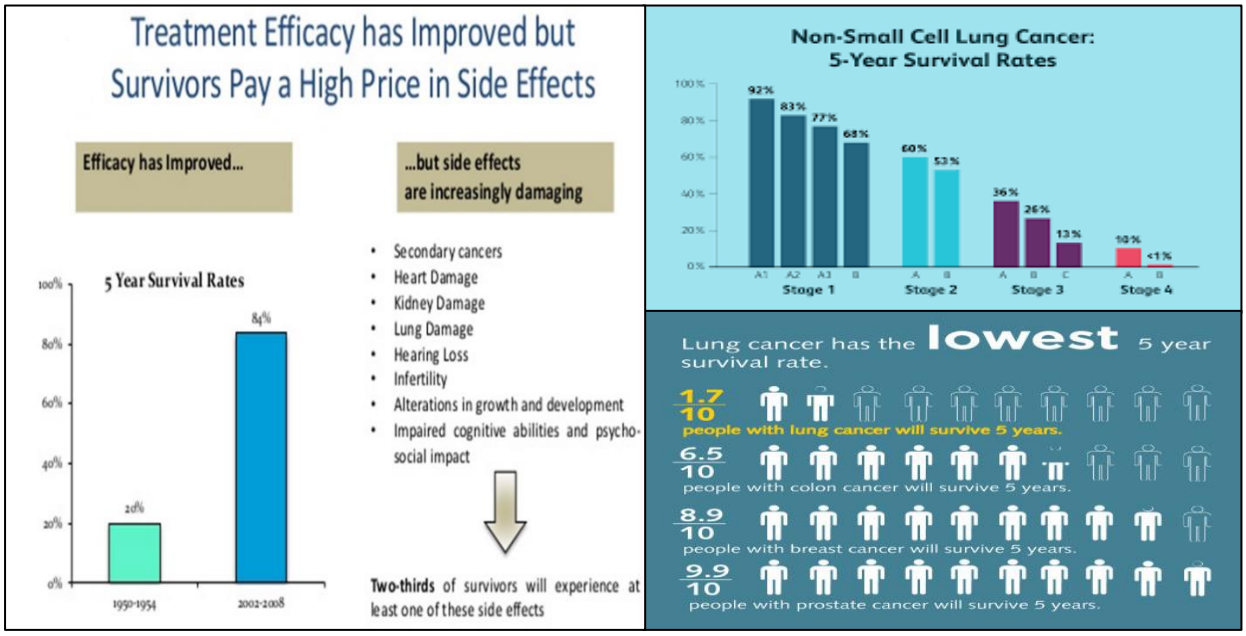

Figure 1.7 5-year Survival rate of Lung Cancer incidences (https://www.verywellhealth.com/what-is-stage-4-lung-cancer-life-expectancy, https://go2.org).

1.4 Diagnosis of Lung Cancer

There are several ways that lung cancer can be diagnosed, including:

1. Imaging tests: X-rays, CT scans, PET scans, and MRI scans can help detect abnormalities in the lungs that may indicate cancer.
    - X-rays: A type of imaging test that uses low levels of radiation to create images of the lungs and detect abnormalities.
    - CT scans: A more detailed imaging test that uses X-rays and computer technology to create 3D images of the lungs, providing more information than X-rays.
    - PET scans: A type of imaging test that uses a small amount of radioactive material to highlight areas of increased metabolic activity, which can indicate cancer.

- MRI scans: A non-invasive imaging test that uses a strong magnetic field and radio waves to create detailed images of the lungs and surrounding tissues, providing a more detailed view than other imaging tests.

2. Biopsy: A tissue sample is taken from the lung and examined under a microscope to determine if cancer cells are present.
3. Sputum cytology: A sample of mucus coughed up from the lungs is examined under a microscope for cancer cells.
4. Bronchoscopy: A flexible tube with a camera on the end is passed through the nose or mouth into the lungs to examine the airways and take a biopsy.
5. Mediastinoscopy: A surgical procedure to examine the lymph nodes in the chest for signs of cancer.
6. Thoracoscopy: A surgical procedure to examine the lining of the lungs and take a biopsy.
7. Blood tests: Certain proteins, such as tumour markers, may be elevated in the blood of people with lung cancer.

The specific diagnostic tests used will depend on the individual's symptoms, medical history, and other factors.

1.5 Treatments for Lung Cancer

Depending on the type, stage of lung cancer and patient health condition, one or a combination of the below approaches to treatment may be undertaken.

1. **Surgery**: Four different of surgery are done to treat lung cancer. In surgery, direct removal of the malignant tissue is involved. The removal of one whole of the lung lobes afflicted by cancer is known as a **lobectomy**. If lobectomy is unsuccessful, the entire afflicted lung is removed during a **pneumonectomy. Wedge resection** is a surgical procedure to remove a tumour along with some surrounding healthy tissue. Surgery to remove a portion of the bronchus is known as a sleeve resection[22].

2. **Chemotherapy**: Chemotherapy is a form of cancer treatment that employs medicines to kill cancer cells or prevent them from proliferating in order to stop the growth of cancer cells. This can be cytotoxic to the normal tissue as well. So, Targeted therapy, which is the use of

medications designed to target specific molecular target, is a growing trend in cancer treatment today[23].

3. **Radiation Therapy**: Ionizing radiation is used to destroy cancerous tissue's DNA, which results in the death of cancer cells. Radiation might be applied externally (external radiation therapy), or radioactive substance can be positioned inside or close to the tumour (Internal radiation therapy)[24].

4. **Drug combinations** are utilised because they work well. Typically, this consists of one platinum-based medication (such as cisplatin or carboplatin)[25] and additional medications like Gemcitabine, Paclitaxel, Doxorubicin, Etoposide, etc[26].

5. **Monoclonal Antibodies**: Monoclonal antibodies can be used to create a variety of illnesses, including cancer. These antibodies can bind to a specific target on cancer cells or other cells that might encourage the growth of cancer cells as a form of cancer therapy[23]. Monoclonal antibodies are administered by infusion.

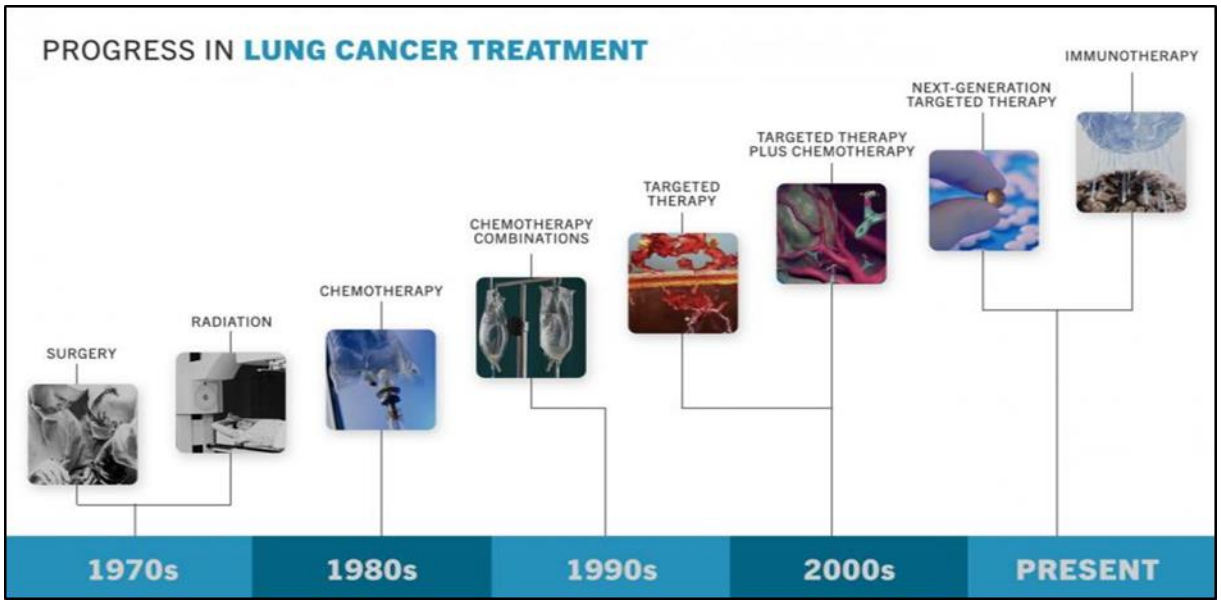

Figure 1.8 Progress in treatments for lung cancer over the last five decades (Chatterji et al,2019).

1.6 Side Effects of Present Treatments of Lung Cancer

Surgery, chemotherapy, radiation therapy, targeted therapy, and immunotherapy are all available as Lung cancer treatments. While these treatments cure lung cancer and improve outcomes, they can also have several negative side effects.

Chemotherapy can lead to side effects like hair loss, nausea, and vomiting. Moreover, chemotherapy medications can harm the body's healthy cells, impairing immunity and raising the risk of infection[27,28]. Radiation therapy can cause skin rashes, fatigue, and difficulty in swallowing[27]. Drugs used for targeted therapy may also result in skin rashes, diarrhoea, and an elevated risk of bleeding[29]. Immunotherapy can result in autoimmune reactions such as exhaustion, diarrhoea, and organ inflammation in addition to other symptoms[30]. Drug resistance can also be a substantial therapy problem for lung cancer[31]. Patients may respond well to a specific treatment at first, but with time, cancer cells may develop medication resistance. This may cause the condition to worsen and necessitate the use of further therapies. Treatment related side effects can affect a patient's quality of life and, in some situations, may even be a factor in the decline in survival rates. Treatment delays or interruptions brought on by treatment-related issues may lower survival rates. Also, the cost of lung cancer treatment is is a deterrent to treatment and can also impact the survival rates of patients. Improving lung cancer patient survival rates will need resolving difficulties with ease of availability of options. ***Therefore, there is a great need to investigate novel therapies and drug combinations that might be able to combat drug resistance, enhance lung cancer patients' prognoses, survival rate and as well as reduce cost of treatment.***

1.7 Relapse or Recurrence of Lung Cancer

Apart from the problem of lack of treatment options; another fear for cancer patients is a relapse. NSCLC has higher relapse rate than SCLC. The five-year recurrence rate for NSCLC is around 40%, while for SCLC, it is around 15%[32] depending upon the stage of lung cancer (Figure 1.9). This means that over a third of patients experienced a recurrence of their cancer within five years of their initial treatment. The factors that can affect the relapse of lung cancer include the presence of genetic mutations, the use of chemotherapy and radiation therapy. For early stage NSCLC patient’s surgery (resection) remains the potential treatment; nonetheless, recurrence rates are still substantial after resection. It has been found that there was presence of occult micro-metastatic cancer cells, which are difficult to identify by conventional staging techniques, are frequently present systemically at the time of surgery[32,33]. These cells can remain dormant for months or years and then become active, leading to a recurrence of cancer. Second, handling the tumour during surgery itself may cause cancer cells to spread. It was also seen that the majority of stage III NSCLC relapses occurs after CRT (Chemoradiotherapy) within a year of finishing treatment[34]. Literature study also showed that the rate of local

recurrence (around 10 – 24%) results in shorter post-recurrence survival times in NSCLC patients[35]. To improve outcomes of lung cancer patients, novel approaches are necessary.

1.8 Prevention for Lung Cancer

Prevention is better than cure. It is advisable to avoid any exposure to cigarette smoke and second hand smoke. All lung cancer patients who smoke should be encouraged to give up. The exposure to radon gas in dwellings must be consequently decreased through suitable technical measures in order to lower the risk of lung cancer from radon exposure. Reduced emissions of diesel exhaust particles are the most efficient way to lower the risk of lung cancer caused by air pollution[36]. It is required to balance the benefits of using ionising radiation for medicinal purposes with any potential dangers associated with radiation exposure. Many clinical studies have shown that consuming a diet high in fruits, vegetables, and tomatoes lowers the chance of developing lung cancer[37].

1.9 ROS - Reactive oxygen species and Apoptosis

ROS (Reactive Oxygen Species) and apoptosis (programmed cell death) have a complex and dynamic relationship in cancer[38,39]. ROS are chemically highly reactive molecules that can damage cellular components, including DNA, lipids, and proteins. They are produced in cells as a by-product of normal cellular metabolism and act as a secondary messenger in cell signalling for various biological processes. While apoptosis is an important mechanism to remove abnormal or damaged cells from the body and its dysregulation can contribute to the progression of apoptosis. Under, physiological condition, ROS can induce apoptosis by activating different signalling pathways that trigger the self-destructive program. At low levels, ROS can act as signalling molecules to stimulate cell proliferation and survival[39,40,41]. However, at high levels, ROS can cause oxidative stress, leading to DNA damage and activation of apoptotic pathways[39,41].

In Cancer, there is an imbalance between ROS production and antioxidant defences (protection against ROS), which lead to cellular stress and DNA damage[42]. Studies have shown that cancer cells have learn to adapt to a high level of ROS by activating antioxidant defence mechanism which results in more ROS clearance[39]. In tumorigenic cells, high ROS concentrations have been shown to cause cytotoxicity and to reverse chemotherapy resistance[40,43]. Several studies point to increased ROS levels as the fundamental cause of cancer[39,44]. Traditional cancer therapies have used this property for exogenous drugs that increase ROS formation or

antioxidant system inhibitors are used to increase ROS levels to target cancerous cells[45]. The advantage behind this strategy is that the normal cells are not affected as they have a basal level of ROS and less dependency on antioxidant defence system.

1.10 Apoptosis

The term "apoptosis" was first coined in 1972 by John F.R. Kerr, Andrew H. Wyllie, and Alastair G. Currie[46]. The Greek term "apoptosis," which meaning "falling off" or "dropping off," is where the name "apoptosis" originates. In multicellular organisms, cell death is an important process development and maintaining homeostasis in the body. Two widely defined mechanisms that are typically involved in cell death are Necrosis and Programmed cell death (PCD). Necrosis is an uncontrolled and often pathological process, where cells die due to cellular damage and stress beyond the point of repair. Programmed cell death refers to cell death that includes a genetically pre-programmed process of cell suicide in response to specific signals[47]. Apoptosis is a programmed cell death process that occurs naturally in multicellular organisms[46]. It is a highly regulated process that involves the activation of specific pathways leading to the controlled disassembly of the cell, without causing damage or inflammation to the surrounding tissues. Different executioner and regulatory molecules carefully control apoptosis. Two main processes that contribute to the induction of apoptosis are the extrinsic pathway and intrinsic pathway. These pathways differ in the stimuli that activate them and the molecular mechanism that lead to cell death.

a) Extrinsic Pathway (also known as the death receptor pathway)

The extrinsic pathway is initiated by the binding of specific ligands to death receptors, such as Fas receptor, tumour necrosis factor receptor (TNFR), or TNF-related apoptosis-inducing ligand (TRAIL) receptor on the cell surface. Binding of ligands to death receptors results in the formation of a death-inducing signalling complex (DISC) which recruits and activates caspase-8. Caspase-8 then activates downstream effector caspases such as caspase-3, leading to the cleavage of key cellular proteins and ultimately cell death as shown in Figure 1.9a.

b) Intrinsic Pathway (also known as the mitochondrial pathway)

The intrinsic pathway can be initiated by various stimuli, such as DNA damage, oxidative stress, or growth factor withdrawal, that cause mitochondrial outer membrane permeabilization (MOMP). This results in the release of cytochrome c and other pro-apoptotic factors from the mitochondrial intermembrane space into the cytosol. Cytochrome c forms a complex with

Apaf-1 and procaspase-9, which activates caspase-9. Activated caspase-9 then cleaves and activates downstream effector caspases, such as caspase-3, caspase-6, and caspase-7, which are responsible for the execution of apoptosis as shown in Figure 1.9. Caspase-3 is the main effector caspase and is responsible for the cleavage of many important cellular proteins, including the nuclear enzyme poly (ADP-ribose) polymerase (PARP), which is involved in DNA repair. Cleavage of PARP leads to the inhibition of DNA repair and the fragmentation of the cell's DNA, ultimately leading to cell death).

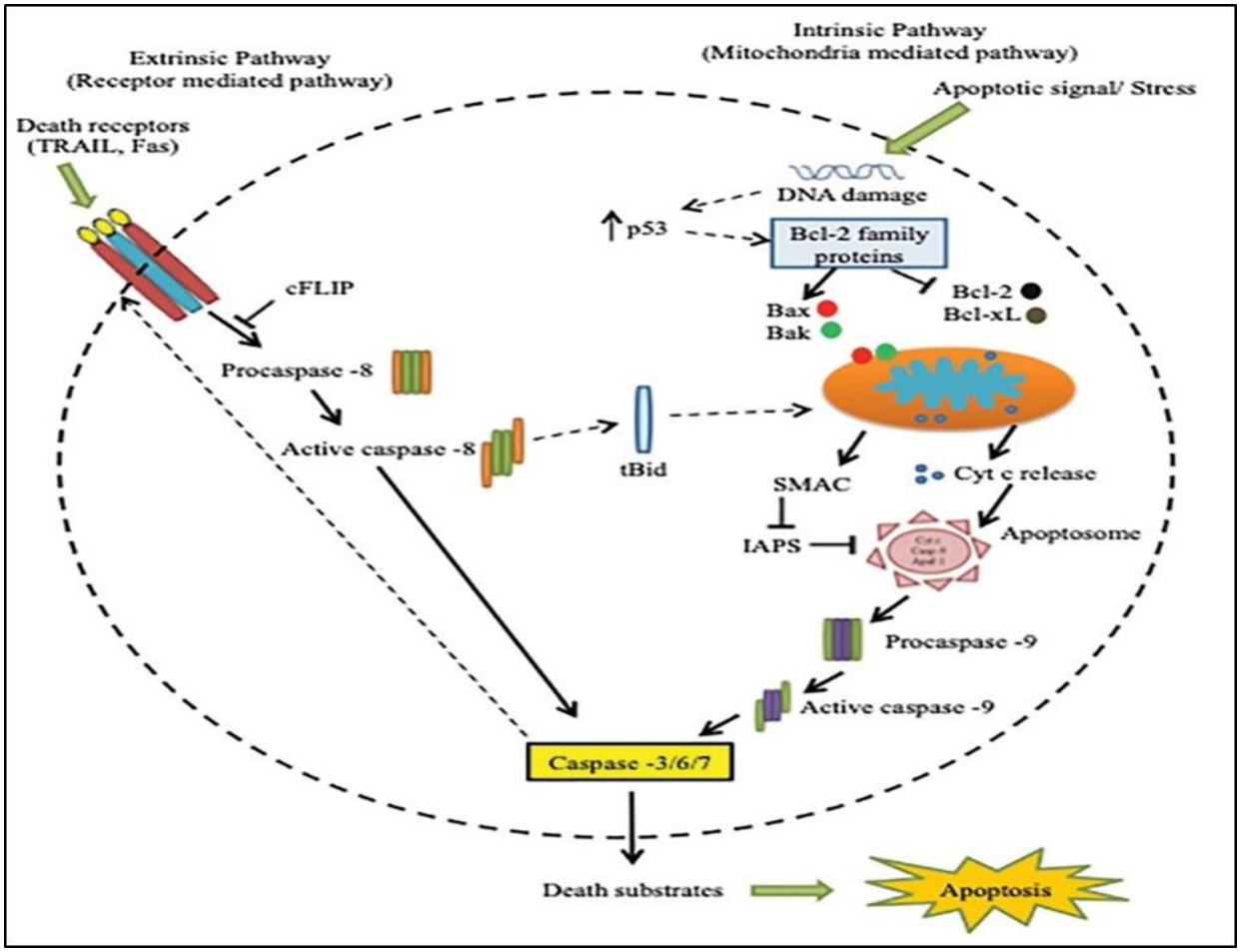

Figure 1.9 Pathways of Apoptosis

tBid: Truncated bid; Cyt C: Cytochrome; Bcl-2: B-cell lymphoma protein 2; cFLIP: cellular FLICE inhibitory proteins; TRAIL: TNF related apoptosis inducing ligand, Bcl-xL: Bcl-2 homologue splice variants; SMAC: Second mitochondrial Caspase Activator; IAPs- Inhibits apoptotic proteins.

Source: Jan et al., 2019.

1.11 Molecular Signalling Pathways in Lung Cancer

Various molecular signalling pathways involved in Lung cancer progression are explained below:

1.11.1 Receptor Tyrosine Kinase – (RTK) Pathway

Receptor tyrosine kinases (RTKs) use cytoplasmic routes to indirectly communicate with the cell nucleus. This process terminates with the translocation of proteins from the cytoplasm to the nucleus, which activate or function as transcription factors orchestrating proliferation through gene expression[49]. Epidermal growth factor receptor (EGFR) is a part of c-ErbB family of receptor TK which also consists of HER2/neu, ErbB-3 and ErbB[50]. EGFR (Epidermal growth factor receptor) is an *RTK* that bind to a specific ligand called epidermal growth factor as ligand[50] and activates downstream pathways including PI3K–PTEN–AKT, MAPK, ERK, and JAK/STAT pathways that promotes proliferation, invasion, angiogenesis, and metastatic spread[51] (Figure 1.10). In cancers like lung, breast, head and neck, altered EGFR expression or mutant receptors have been reported to be perpetrators. According to some studies, it has a role in the growth of tumours and the spread of cancer. Up to 60% of NSCLC cases had EGFR overexpression, which is frequently associated with a poor prognosis[52]. The EGFR protein was shown to be overexpressed in NSCLC squamous cells and ADC subtypes[53]. EGFR activation is frequently associated with cell survival and proliferation via a number of different pathways, including the RAS/MAPK and PI3K/AKT pathway[52]. EGFR is one of the selected pathways for targeted therapy. Mutations including point, insertion and deletion frequently target crucial TK domain areas connected to downstream signalling[52]. However, the point mutation L858R in exon 21 and the deletion in exon 19 account for 85% of mutation. The ligand receptor interaction was initially blocked using monoclonal antibodies. Lately, reversible small-molecule TK inhibitors have been employed. Inhibitors used in preclinical studies are Afatinib (blocks ErbB family in NSCLC), dacomitinib (an irreversible EGFR inhibitor) with erlotinib (reversible EGFR inhibitor) in advanced stage NSCLC patients.

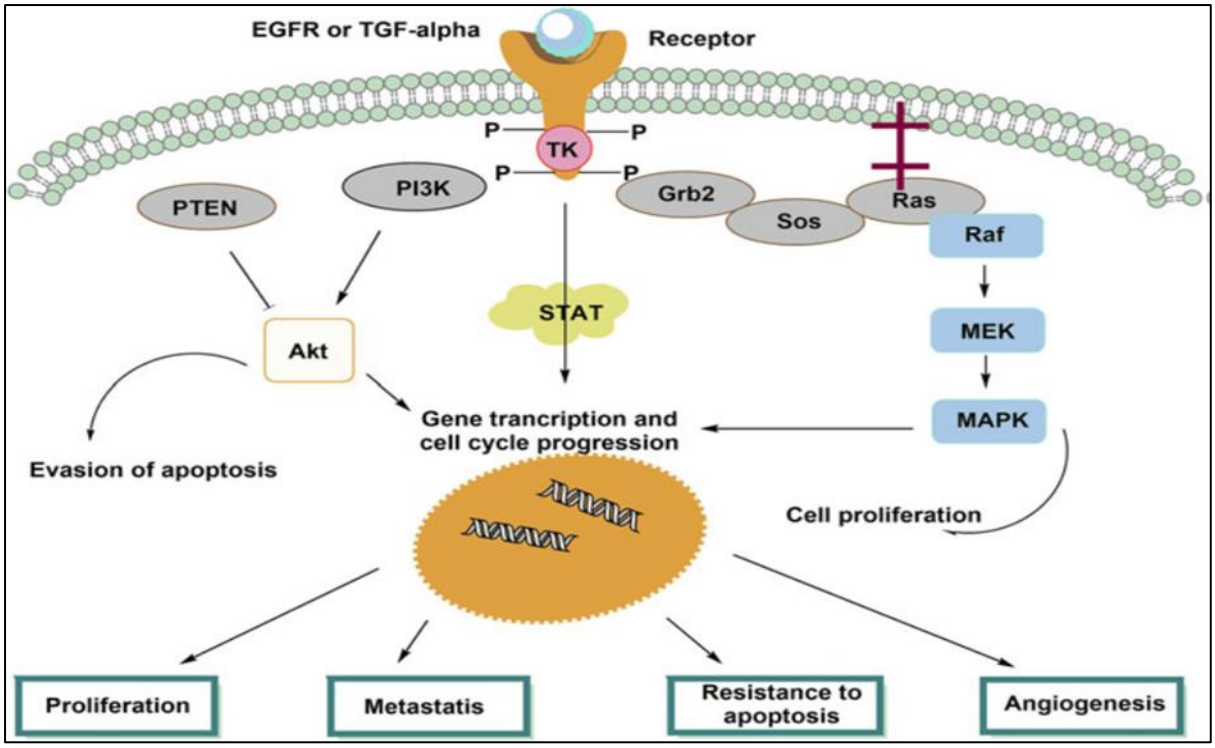

Figure 1.10 Mechanism of EGFR signalling pathway in cancer progression

PI3K: phosphatidylinositol-3-kinase; PTEN: phosphatase and tensin homolog; SOS: son of seven less homolog; MAPK: mitogen-activated protein kinase; EGFR: epidermal growth factor receptor; GRB2:associated binding protein; Raf: v-raf-1 murine leukemia viral oncogene homolog; Ras: rat sarcoma viral oncogene homolog; Akt: v-akt murine thymoma viral oncogene homolog; STAT, signal transducer and activator of transcription; TK- Tyrosine Kinase.

Source: Dubey et al,2021.

1.11.2 Ras/Raf/MEK/ERK Pathway

A crucial pathway is the Ras/Raf/MEK/ERK pathway that transmits signals to the nucleus by phosphorylating RAS, RAF, MEK, and ERK in a precise order. This pathway is crucial for controlling biological functions, especially cell proliferation, differentiation, and survival (Figure 1.11). HRAS, KRAS4a, KRAS4b, and NRAS are the four closely similar protein isoforms that the *Ras* genes encode[54]. Cancers most typically involve the KRAS (**Kirstein rat sarcoma)** form. KRAS is a member of a family of small GTPases, or proteins with intrinsic GTPase activity, which control how cells react to external stimuli via the MAPK and PI3K pathways[55]. Ras-regulated signal pathways controls the integrity of cytoskeletal actin, cell motility, differentiation and proliferation. Most of RAS proteins have C-terminal CAAX

tetrapeptide sequence[55]. RAS proteins are initially created in the cytosol, where farnesyltransferases identify their CAAX motif and prenylated. RAS (adding a farnesyl isoprenoid lipid). Following the insertion of RAS into the inner cell membrane, RAS-converting enzyme-1 (RCE1) catalyses the proteolytic cleavage of the AAX sequence and isoprenyl cysteine carboxyl methyltransferase (ICMT) catalyses the carboxymethylation of the farnesylated cysteine residue[56,57]. This process modulates the ability of RAS proteins to integrate into the inner cell membrane and remain activated. Extracellular stimuli, such as an active EGFR, activate RAS proteins, downstream cytosolic effectors such as v-Raf murine sarcoma viral oncogene homolog B (RAF), mitogen-activated protein kinase kinase (MEK), and extracellular signal-regulated kinase (ERK) pathway, along with the phosphoinositide 3-kinase (PI3K)/v-akt murine thymoma viral oncogene (AKT)/mammalian target of rapamycin (mTOR) pathway also get activated[57,58]. KRAS combine with MEK inhibition, it interacts with a PI3 kinase subunit (p110), and the breakdown of this contact results in a regression of tumour growth and development[59]. Approximately 30% of lung adenocarcinomas have KRAS mutations[60]. 26% of smokers are more likely to have KRAS mutations than non-smokers (6%)[60].

KRAS has been unsuccessfully addressed therapeutically despite being present in a widerang e of malignancies; nonetheless, numerous strategies are currently being tried in clinical trials. MEK inhibition is now being studied in several clinical trials because preliminary research suggests that it may be a successful treatment for KRAS cancers. Another strategy for clinically treating KRAS-mutated cancers is Hsp90 inhibition[61].

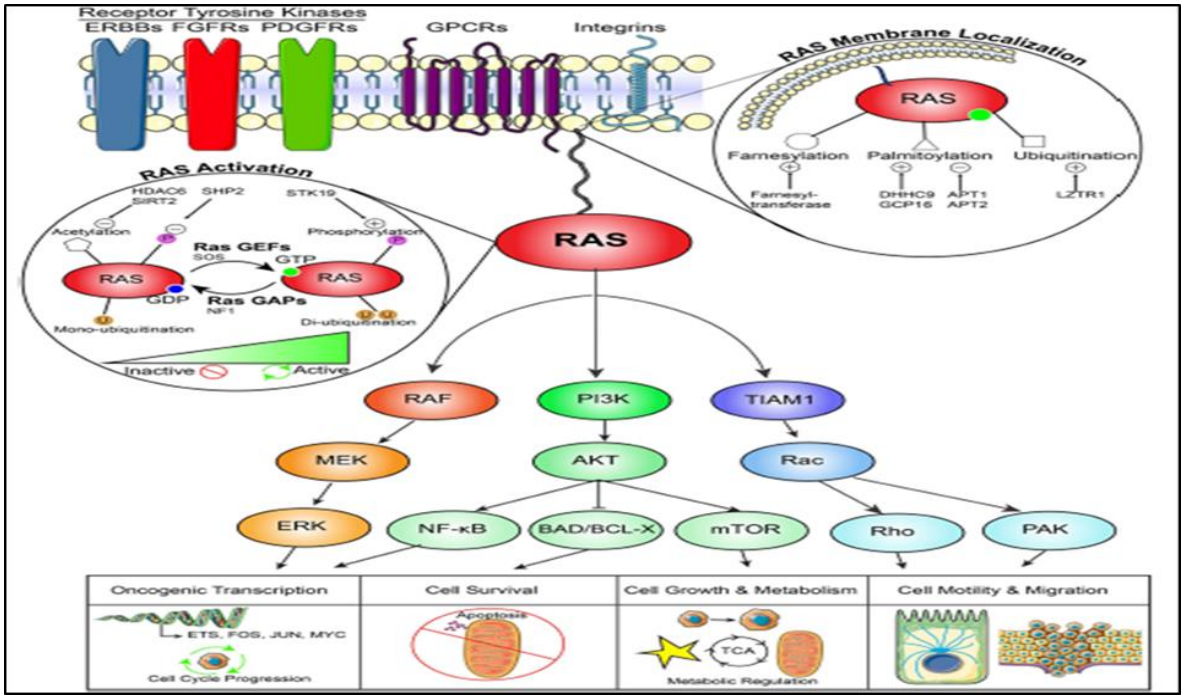

Figure 1.11 The RAS pathway.

RTK: receptor tyrosine kinases; GPCRs: G-protein coupled receptors; MEK mitogen-activated protein kinase kinase; ERK: extracellular signal-regulated kinase; RAF: rapidly accelerated fibrosarcoma; RAS: rat sarcoma; RAS GEF: guanine nucleotide exchange factor; RAS GAP: RAS GTPase-activating protein; ERBB: epidermal growth factor receptor family; FGFR: fibroblast growth factor receptor; PDGF: platelet-derived growth factor; TIAM1: T-cell lymphoma invasion and metastasis 1.

Source: Gimple and Wang et al, 2019.

1.11.3 PI3K Pathway

The phosphatidylinositol 3-kinase (PI3K)/Akt pathway regulates biological functions like cell growth, proliferation, survival, metabolism, and differentiation. Receptor tyrosine kinases interact with growth factors and activates PI3K signaling in cancer[62].These RTKs frequently exhibit abnormal PI3K activation due to mutation, amplification, or overexpression. Epithelial growth factor receptor (EGFR), for instance, activates PI3K in lung malignancies that have somatic activating mutations in EGFR[63]. The PI3K-Akt-mTOR signaling pathway is frequently dysregulated in NSCLC, resulting in constitutive activation and encouraging tumor development, survival, and metastasis. Even in the absence of growth stimuli, genetic changes, such as mutations or amplifications of PIK3CA, PTEN, AKT, or mTOR, result in the constitutive activation of the PI3K-Akt-mTOR signaling pathway. By the activation of downstream targets like S6K and 4E-BP1, the constitutively active pathway encourages cell growth, survival, and metastasis[64]. Constitutive stimulation of mTORC1 also inhibits autophagy, which aids tumor survival and growth. Moreover, chemotherapy and targeted therapies may not be effective in treating NSCLC due to the constitutive activation of the PI3K-Akt-mTOR signaling pathway. Genetic disorders that contribute to the dysregulation of signal transduction in the PI3K-Akt-mTOR pathway include PIK3CA mutation and amplification, PTEN loss, Akt1 and LKB1 mutation, as well as PTEN mutation[62]. In 4.5% of NSCLC, PTEN mutations (in exons 5-8) were found[65]. Somatic activating mutations of p110 (PIK3CA) and deletion of the tumour suppressor PTEN are the two most prevalent genetic variants that directly activate the PI3K signaling pathway[64]. While PIK3CA and PTEN mutations in non-small cell lung cancer (NSCLC) are not prevalent, investigations have shown evidence for loss of PTEN protein expression decrease and PIK3CA amplification[65]. Several PI3K inhibitors,

AKT inhibitors and mTOR inhibitors are in clinical trials phases as potential targets for therapeutic intervention of this pathway against lung cancer.

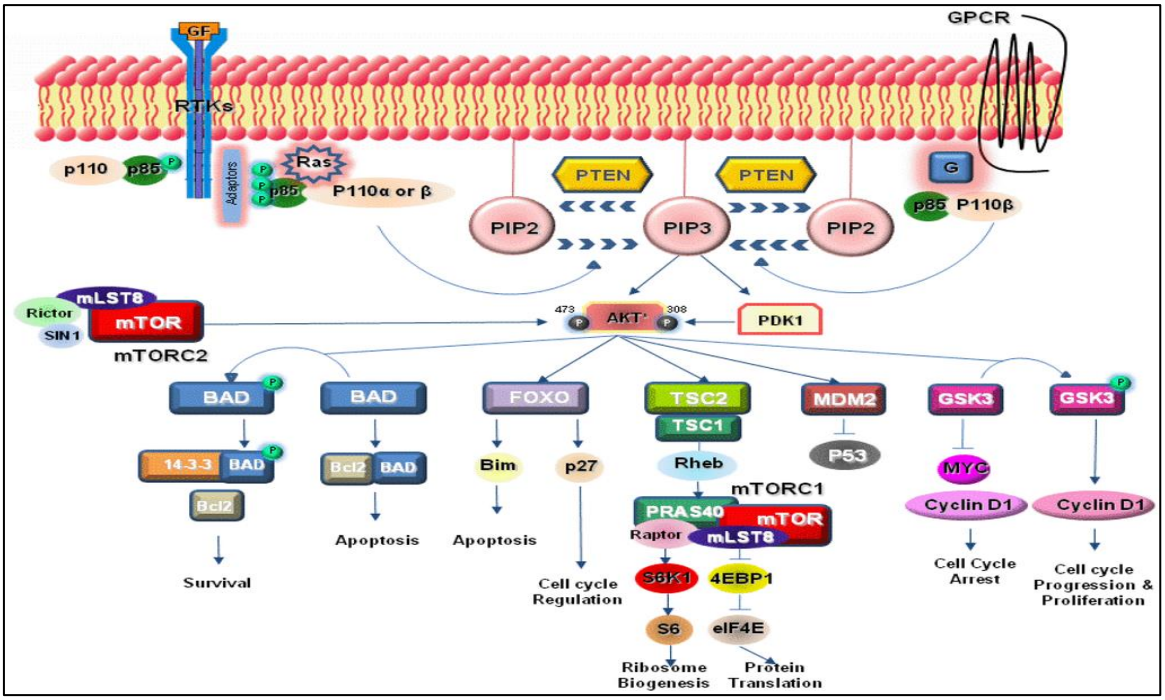

Figure 1.12 The PI3K/Akt/mTOR Signaling Pathway.

PI3K: phosphoinositide-3-kinase; p85: regulatory subunit; p110: catalytic subunit; RTK: receptors tyrosine kinase; GPCR: G-protein coupled receptor; PIP3: phosphatidylinositol-3,4,5-triphosphate;PIP2: phosphatidyl-inositol-4,5-biphosphate; PTEN: phosphatase and tensin homolog; PDK1: phosphoinositide-dependent kinase 1; mTOR: mammalian target of rapamycin; mTORC1:mammalian target of rapamycin complex 1; mTORC2: rapamycin complex 2, serine threonine kinase AKT: protein kinase B; FOXO: fork head box O transcription factors; GSK3: glycogen synthase kinase 3; Rheb: Ras homologue enriched in brain; S6K1: p70S6 kinase; 4EBP1: eukaryotic initiation factor 4E binding protein 1; Bcl-2 family members BAD and BAX; TSC2: tuberous sclerosis 2 protein; MDM2: murine double minute 2 homolog.

Source: Sarris et al., 2012.

1.11.4 TP53 PATHWAY

Mutations that make TP53 inactive, interfere with it function in regulating cellular growth and death. Certain TP53 mutations result in "gain of function," and the mutated TP53 protein that results aids in various stages of carcinogenesis or medication resistance. Smoking has a high correlation with TP53 mutations in lung cancer[66]. The most frequent somatogenic alterations

in NSCLC are mutations in the tumour suppressor gene TP53. Around 50% of NSCLC cases and more than 70% of SCLC cases have been documented to have TP53 mutations[67]. One of the most critical developments in lung cancer is TP53 tumour suppressor gene abnormalities, which play a crucial role in lung epithelial cell oncogenesis. Ubiquitin-ligase E3 MDM2 (ubiquitin-proteasome pathway), promotes the degradation of p53 by binding to TP53[61]. MDM2 is increased in 22% of NSCLC patients and is linked to a poor overall survival rate[67]. By preventing binding of MDM2 to TP53 could restore TP53 function in wild- type TP53 tumours[61]. The MDM2-TP53 interaction has drawn significant attention attempts to create certain inhibitors[61].

1.11.5 BRAF/ MAPK Pathway

A BRAF mutation in NSCLC is an oncogenic driver gene that stimulates the downstream effectors MEK and ERK, ultimately encouraging cell growth and survival[68]. BRAF mutations are seen in NSCLC at a prevalence of 3.5–4%[69]. MEK1 and MEK2 in the MAPK pathway are only known substrates of the serine-threonine kinase BRAF[69]. Around 7% of all human malignancies have mutated and activated BRAF, whereas about 50% of melanomas have mutation at V600[70]. Rarely, BRAF mutations occurs in the kinase domain (D594G and L596R) and the G-loop of the activation domain (G465V or G468A) are found in NSCLC cancers[71].

1.12 Targeted Drugs in development or approved for Lung Cancer

Various drugs are approved for the treatment of lung cancer depending upon the type and stage of lung cancer by FDA. Few cancer drugs approved by FDA for lung cancer treatment are:

1.12.1 Erlotinib

Erlotinib (Tarceva) is a US FDA approved small-molecule anti-cancer drug which blocks the epidermal growth factor receptor when taken orally. It is the only EGFR-tyrosine kinase inhibitor (TKI) found to significantly extend survival in non-small-cell lung cancer (NSCLC) patients with recurrent or refractory disease[72]. Erlotinib has also been suggested by the National Comprehensive Cancer Network (NCCN) Medicines & Biologics Compendium for patients with a known active EGFR mutation or gene amplification who have never smoked, either as a single drug or in conjunction with chemotherapy. Erlotinib therapy increased response and progression-free survival in NSCLC patients.

1.12.2 Bevacizumab

Bevacizumab is a type of targeted therapy drug known as a monoclonal antibody. It functions by binding to VEGF, a protein that causes blood vessels to expand. Certain cancer cells produce a lot of VEGF, which stimulates the growth of new blood vessels in the vicinity of the tumour. The tumour receives oxygen and nutrients from the blood, allowing it to develop. Bevacizumab inhibits VEGF and inhibits the growth of tumours. It is used to treat non-squamous non-small cell lung cancer that has relapsed and cannot be surgically removed. As part of the first line therapy, it is used with paclitaxel and carboplatin.

1.12.3 Crizotinib

It is recommended for the treatment of metastatic non-small cell lung cancer (NSCLC), as well as relapsed or refractory cases. It has an aminopyridine structure which interacts competitively with the ATP-binding pocket of target kinases and inhibits protein kinases[73]. Some late-stage (locally progressed or metastatic) non-small cell lung tumours that exhibit the aberrant anaplastic lymphoma kinase (ALK) gene have been given approval by the U.S. Food and Drug Administration to be treated with crizotinib[74].

1.12.4 Afatinib

Afatinib is an orally given, irreversible inhibitor of the ErbB family of tyrosine kinases. An important first-line treatment option for advanced non-small cell lung cancer (NSCLC) with activating epidermal growth factor receptor (EGFR) mutations (i.e., EGFRactMUT+)[75]. It also offers a second treatment option for squamous NSCLC that has progressed after receiving first-line platinum-based chemotherapy. Afatinib extended progression-free survival (PFS) and time to treatment failure (TTF) compared to gefitinib in the first-line treatment of EGFRactMUT+ advanced lung adenocarcinoma, but not overall survival (OS)[76].

1.12.5 Cabozantinib

Cabozantinib is a small-molecule tyrosine kinase inhibitor that targets the MET, VEGFR-2, RET, ROS1, and AXL proteins with specificity. It has shown strong anti-ROS1 activity in a variety of solid tumours, particularly against mutations that cause solvent-front ROS 1 resistance, such as G2032R, D2033N, L1519R, and L2026M. The FDA has given crizotinib full approval to treat advanced ROS1-rearranged lung cancer. But, crizotinib resistance in ROS1-driven NSCLC is unavoidable, and the vast majority of patients experienced disease

progression while receiving crizotinib therapy, which had a significant negative effect on the patients' prognosis and quality of life[77].

1.13 Combination therapy in Lung Cancer

In order to better understand the effects of innovative agents and maximise the usage of traditional treatments, *in vivo* and *in vitro* experiments are now being conducted. Due to patients' growing resistance to single agent therapies, combination therapy is receiving more attention nowadays. Chemotherapeutic combinations typically include the drug **carboplatin** (which is a chemotherapy agent that contains platinum. It damages the DNA of quickly growing cells, including cancer cells, to stop or decrease their growth) and **paclitaxel (Taxol)**, [A taxane is a class of chemotherapy that includes paclitaxel. By inhibiting mitosis, it prevents cell proliferation (cell division). Taxanes disrupt microtubules (cellular structures that help move chromosomes during mitosis). Combinations typically function better than single medications because different drugs destroy cancer cells in various ways. In patients with SCLC, the second-line chemotherapy regimen of carboplatin and paclitaxel is efficient and practical for patients with interstitial lung disease[26]. Another chemotherapy drug in combination against NSCLC is Gemcitabine (kill rapidly growing cancer cells from making DNA) and Cisplatin (contains metal platinum). Gemcitabine and cisplatin are used for patients with stage IIIA, stage IIIB and stage IV lung cancer[26]. Literature study showed that the patients with advanced non-small cell lung cancer had higher survival rates and improved quality of life, with drug combination therapy than with drug alone[78]. ***So, combination therapy can also be helpful as different drugs may have different mode of actions and it can also aid in the overcoming of drug resistance, thus helping in better treatment outcomes.***

1.14 Side Effects of Drugs available for Lung Cancer treatment

All the above-mentioned treatments, meanwhile, come with undesirable side effects like quick clearance, restricted metastasis, low bioavailability, toxicity, and nonspecific behaviour. These drugs on long-term administration shows adverse effects some of which are mentioned below:

- **Erlotinib** major side effects

a. Paronychia (skin infection around nails; [79])

b. Keratoconjunctivitis sicca (dryness of conjuctiva[79])

c. Chronic cough, Diarrhoea and Fatigue ([79])

- Bevacizumab major side effects

hypertension, asymptomatic proteinuria, thromboembolic events, gastrointestinal perforation, thromboembolism etc[80]

- Crizotinib major side effects

visual disturbances, nausea, vomiting, diarrhoea, constipation, edema, reduction in glomerular filtration rate etc [81]

- Afatinib major side effects

Stomatitis/Mucositis, Paronychia, Diarrhoea, skin rashes etc[82]

- Cabozantinib major side effects

- Constipation, decrease urination, redness, swelling, sore, hair loss, blurred vision, hypertension, low platelets etc[83]

Combination of Paclitaxel and Carboplatin showed little side effects like fatigue, vomiting and nausea in different time-periods after treatment[84].

These negative consequences from the usage of drug, drug interactions has led to scientists exploring more therapy options that have lesser or no side effects even after long use. There is yet no perfect treatment for lung cancer and there is a great need to explore for newer therapy options. **Herbal therapy is a rapidly growing area of interest in cancer treatment (since ayurveda) as medicinal plants contain compounds that have potential anti-cancer effects or can act in a complimentary fashion to allopathic drugs or can reduce the side effects of allopathic drugs** Herbal therapy is the use of plants or plant extracts for medicinal purposes, and it is a traditional practice that has been used for thousands of years. They are often more affordable and accessible than synthetic drugs, and they can have fewer side effects. The use of medicinal plants in modern day drug manufacturing is a growing trend as more and more people are turning towards natural-herbal remedies for their healthcare needs, including cancer treatment. There are several reasons for this shift, including the increasing cost of prescription drugs, the potential for side effects from synthetic medications, and the desire for more holistic approaches to healthcare.

The World Health Organization (WHO) supports the use of traditional medicine as they are proven to be efficacious and safe (WHO, 1986). To support the use of traditional medicine, WHO has developed several initiatives and guidelines, including the WHO Traditional

Medicine Strategy 2014-2023.[85] This plan intends to improve access to traditional medicine for people living in underdeveloped nations while also strengthening the development, regulation, and use of traditional medicine globally. The annual market value of products made from medicinal plants surpasses $100 billion worldwide.[86] It has been well recognised that most developing countries employ traditional medicine and medicinal plants as a normative basis for the preservation of good health (UNESCO, 1996). Furthermore, the extraction and creation of numerous medicines and chemotherapeutics from these plants as well as from conventionally used rural herbal remedies has been linked to an increase in the reliance on the usage of medicinal plants in industrialised countries (UNESCO, 1998) [86].

1.15 Herbal Therapy for Lung Cancer

1.15.1 Role of Phytochemicals in Lung cancer therapy

Plants produce a variety of chemical compounds known as **phytochemicals** that can be found in all parts of the plant, including the roots, stem, leaves, flowers, and fruits.[87] These parts of the plant may contain different types and amounts of phytochemicals and the specific phytochemicals present can vary by plant species. Due to their availability, adaptability, and lesser cytotoxicity, natural compounds are used as anticancer agents[88]. In the modern period of drug development, natural compounds have proven an unequalled supply of anticancer medications[89,90]. Natural compounds generated from plants and plant extracts are such attractive prospects for utilisation as anticancer therapies[91].

1.15.2 Some of the medicinal plants mentioned in Ayurveda and used for the treatment of lung cancer are discussed below:

1. *Curcuma longa* (Turmeric): A naturally occurring substance called curcumin (diferuloylmethane) is obtained from the rhizome of the East Indian plant Curcuma longa, also known as turmeric. It belongs to the ginger family Zingiberaceae. Curcumin has been used to treat inflammatory illnesses, including several respiratory conditions with no toxicity[92]. The chemoprevention potential of curcumin is supported by the fact that it affects several biochemical and molecular cascades involved in cell cycle control, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, and inflammation[93]. Studies with curcumin in lung carcinogenesis have shown that it can, through mitochondria-dependent mechanisms, induce apoptosis in human lung adenocarcinoma A549 and non-small cell lung cancer NCI-H460 cells in a dose-dependent manner[94].

***2. Camellia sinensis* (Tea):** It belongs to family Theaceae. The most common polyphenol identified in green tea extract is epigallocatechin-3-gallate. In A549 NSCLC cells, the effects of EGCG on migration, invasion, angiogenesis, and nicotine-induced epithelial mesenchymal transition (EMT) were investigated. Moreover, preliminary molecular mechanisms analysis was done[95]. The results showed that different concentrations of EGCG greatly reduced migration and invasion brought on by nicotine[96].

3. *Moringa oleifera* (drumstick): *Moringa oleifera* Lam. belongs to family Moringaceae, *Moringa oleifera* possess multiple pharmacological properties, but its most protruding one is its anticancer efficacy[97]. Study showed that the alkaloid extract of *Moringa oleifera* Lam. exert antitumor activity in human non-small-cell lung cancer via JAK2/STAT3 Signalling Pathway[98]. Lung cancer has been shown to exhibit growth inhibition and apoptosis induction by *M. oleifera* leaf water extract[97,99].

4. *Taxus brevifolia* (Taxanes): It belongs to the family Taxaceae, of yew tree species. Paclitaxel (PTX) belongs to the class of drugs made from the needles of the Pacific yew (*Taxus brevifolia*) tree. It is a member of the class of substances called taxanes. It works by inhibiting mitosis and promoting cell death. Non-small cell lung cancer, Kaposi's sarcoma, breast cancer, and ovarian cancer are all commonly treated with paclitaxel[100]. It is possible to utilise paclitaxel either by itself or in conjunction with other anticancer medications like cisplatin or carboplatin[25]. It induce apoptosis in lung cancer cell lines by increasing the caspase-3 activity[100]. Phase III studies conducted since 1993 have demonstrated that paclitaxel increases survival in several solid tumour diseases, such as ovarian and non-small cell lung cancer (NSCLC)[101].

5. *Catharanthus roseus* (Vinca): It is commonly known as Madagascar periwinkle or vinca. It belongs to the Apocynaceae family. This plant contains several significant bioactive elements that have a significant impact on the herbal medicine sector. Alkaloids as vinblastine, vincristine (named after Vinca), and their derivatives are found in *Catharanthus roseus* which are responsible for anticancer activity of this plant[102]. Furthermore, *catharanthus* alkaloids cause apoptosis (programmed cell death). They are created by the plant's stem. Although vinblastine is produced in greater quantities by the plant, vincristine is utilised more frequently. The *catharanthus* alkaloids stop the proliferation of cancer cells by binding to tubulin in the mitotic spindle[103]. Vincristine and vinblastine and their synthetic analogues are used in combination with other chemo- therapeutic drugs against lung cancer[102,103].

6. *Glycyrrhiza glabra* (Licorice): It is a member of Fabaceae family. One of the primary substances present in licorice is glycyrrhizin, which is a triterpenoid- saponin. It is extracted from the roots of the plant. The plant also contains liquiritin, glabridin, and isoliquiritigenin as additional substances. Researchers have discovered that these substances possess anti-inflammatory, antioxidant, and anticancer activities[104]. It was shown that glycyrrhizin inhibits lung adenocarcinoma A549 cell growth via triggering cancer cell apoptosis by reducing the activity of the thromboxane synthase pathway[105]. Combination of glycyrrhizin with cisplatin against the growth of lung tumour tissue, showed lesser toxicity and side effects in the PDX mice model. Another study showed that Isoliquiritigenin shows an antiproliferative activity against lung cancer[106].

7. *Bauhinia variegata* Linn: It is commonly known as Kachnar in Indian languages or Mountain Ebony in English, belongs to family Leguminosae. In Ayurvedic system, various *Bauhinia* species are recognised and utilised as Kanchnara.In Ayurveda, the medication is also known as Grahi, Krimighna, Kushtaghna, Ga ndamalanashaka, Vranaropaka, Mehaghna, and Raktapittashamak. Kanchanara is a key component of many significant Ayurvedic formulas, including *Kanchanara Guggulu, Kanchan Gutika, Gandamala Kundan Rasa, Gulkand Kanchanara*, and *Kanchanaradi Kwatha*[107]. The pharmacological properties of *Bauhinia variegata* includes anticancer, antioxidant, hypolipidemic, antibacterial, anti-inflammatory, nephroprotective, hepatoprotective, antiulcer, immunomodulating, molluscicidal, and wound healing activities[107,108]. The varied pharmacological properties of *Bauhinia variegata* bark is due to the presence of flavonoids, terpenes, steroids and alkaloids, fatty acids, 5, 7-Dihydroxy flavanone - 4'-O-a- L-rhamnopyranosyl b-D- glucopyranoside, sitosterol. Stigmasterol, neringenin-5,7-dimethylether-4'-rhamnoglucoside, lupeol, 5,7,3',4'-tetrahydroxy-3-methoxy-7-O-alpha-L-rhamnopyranosyl (l->3)-O-beta-galactopyranoside, 2,7-dimethoxy-3-methyl-9, l0-dihydrophenanthrene -l,4-dione named as bauhinione,[109,110,111]. Although the traditional medical system has documented its ethnopharmacological use, there is a paucity of comprehensive scientific data. Despite extensive research on the pharmacological properties of *Bauhinia variegata* bark, its potential effect on lung cancer remains unexplored, thus leaving a significant gap in our understanding of its therapeutic potential. Based on above literature study, plant of interest for the study is ***Bauhinia variegata* (bark)**. **So, the study focus is to explore the anticancer effect of phytocomponents in bark of *Bauhinia variegata* on lung cancer cell lines and to delineate the biochemical mechanism involved in this process.**

1.16 Choice of Lung Cancer Cell lines for the study

The choice of using A549 and H460 cells for this study on lung cancer research is well-supported by the literature, as these cells are widely studied *in vitro* models for investigating NSCLC and large cell lung carcinoma, as well as drug discovery and development research. A549 cells are commonly used as an *in vitro* model for studying lung epithelial cell biology and physiology, as they retain many of the characteristics of normal lung epithelial cells. A549 and H460 cells are both representative of non-small cell lung cancer (NSCLC), which accounts for approximately 85% of all lung cancer cases and hence justifying their use in this study.

1.16.1 A549 Adenocarcinoma Human Alveolar Basal Epithelial Cells

In 1972, D. J. Giard and colleagues isolated A549 cells from the lung cancer tissue of a 58-year-old male (Caucasian) and developed those cells as a cell line. A549 cells have an epithelial-like appearance like squamous cells seen in lung tissue, are adhesive, form monolayers, and are adherent. It distributes substances like water and electrolytes across the lungs' alveoli. The cells produce lecithin and have high quantities of unsaturated fatty acids, which are crucial for maintaining membrane phospholipids. The A549 cell line serves as a useful drug screening model. The type II alveolar epithelium is precisely mimicked by the A549 cell line. This makes it a useful tool for researching drug distribution to the pulmonary tissue and analysing metabolic processes in lung tissue.

1.16.2 NCI-H460 Human Lung cancer Cells

The NCI-H460 cell line is a commonly used human cancer cell line that was derived from a non-small cell lung carcinoma. It was established by the National Cancer Institute (NCI) in 1982 from the pleural effusion of a patient with large cell carcinoma of the lung. NCI-H460 cells are adherent cells. NCI-H460 cells are known to be highly resistant to chemotherapy and radiation, which makes them a valuable tool for studying drug resistance in lung cancer. The NCI-H460 cell line is known to be a highly tumorigenic and metastatic cell line and is frequently used in cancer research for studying lung cancer biology and for testing anti-cancer drugs

1.17 Plant derived anti-cancer drugs used in the study

1.17.1 Paclitaxel

Paclitaxel (Taxol) is a tricyclic diterpenoid formed naturally in the bark and needles of *Taxus brevifolia*. Its molecular formula is $C_{47}H_{51}NO_{14}$ and chemical structure is depicted and Figure 1.13. Paclitaxel (PXT) facilitates the formation of microtubules, which are repeating structures made of α/β-tubulin heterodimers. It increases the amount of assembled tubulin subunits and decreases the concentration of assembled tubulin subunits[112]. As a result, paclitaxel treatment encourages tubulin polymerization and inhibits the progression of mitosis. Paclitaxel encourages the assembly of tubulin into microtubules and prevents the dissociation of microtubules, inhibiting the growth of cancer cells by stopping the cell cycle's progression and mitosis[113]. Based on detailed antitumor activity studies, it was concluded that paclitaxel has been shown to initiate cell cycle arrest, suppress metastasis, and induce apoptosis in cancer cells, including those of non-small cell lung cancer (NSCLC)[114,115].

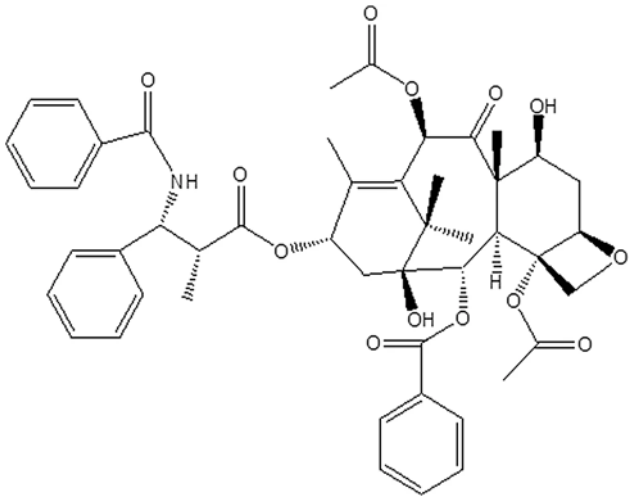

Figure 1.13 Chemical Structure of Paclitaxel

1.17.2 Gemcitabine

Gemcitabine (Gemzar) is a synthetic anticancer drug that is commonly used to treat non-small cell lung cancer (NSCLC) as well as other cancers, such as pancreatic cancer, bladder cancer, and breast cancer[116]. Gemcitabine is a nucleoside analogue, meaning that it mimics the structure of the building blocks of DNA and RNA, the nucleosides. Once inside the cell, gemcitabine is phosphorylated into an active form that inhibits DNA synthesis and causes cell death. Specifically, gemcitabine acts by inhibiting the enzyme ribonucleotide reductase, which

is responsible for the synthesis of deoxynucleotides, the building blocks of DNA. By inhibiting this enzyme, gemcitabine reduces the amount of deoxynucleotides available for DNA synthesis and causes the cells to accumulate in the S phase of the cell cycle. This accumulation ultimately leads to cell death[116,117]. The chemical formula for gemcitabine is $C_9H_{11}F_2N_3O_4$. The chemical structure of Gemcitabine is shown in Figure 1.14. It is used in the study as it is well known chemotherapy drug that has been extensively studied and characterized for its mechanism of action, efficacy, and safety[117].

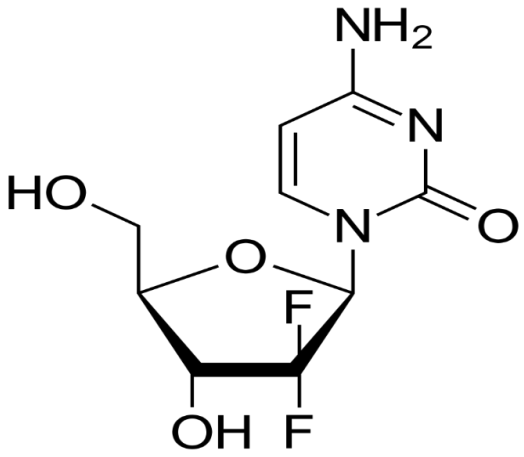

Figure 1.14 Chemical Structure of Gemcitabine

1.18 Rationale of the current study

This study seeks to investigate the effect of Bauhinia variegata bark extracts and its phytocomponents on lung cancer, with the hope for identifying a new molecule for the treatment of this devastating disease which is at the top of cancer mortality. By shedding light on the potential anti-cancer properties of Bauhinia variegata, this research may contribute to the development of novel therapies for lung cancer and pave the way for future investigations into the medicinal benefits of this plant. Hence, we hypothesize that the extract of Bauhinia variegata bark and its individual phytocomponents will activate key apoptotic signalling pathways within lung cancer cells, leading to a significant reduction in cancer cell viability and proliferation. These findings may have important implications for lung cancer treatment.

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