A Review on Targeted Therapies for Lung Cancer

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled “A Review on Targeted Therapies for Lung Cancer” submitted by Anushey Ashraf, ID: 17346033 of Summer, 2017 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy on October, 2022

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Ethics Statement

This study does not involve any kind of animal trial or human trial.

Abstract

Lung cancer is one of the leading causes of cancer related deaths. The malignancy is marked by a bleak prognosis and poor clinical result, owing primarily to advanced stage at diagnosis, imposing a significant cost on public health worldwide. Lung cancer genomic analysis revealed genetic variety and complexity, as well as multiple targetable oncogenic driver changes. These molecular profiling efforts have enabled the potential of molecularly tailored medicines to be realized. In this review, an insight has been provided on the current landscape of targeted therapy for lung cancer treatment, the obstacles associated with this approach, and tactics that could be used to overcome those challenges.

Keywords: Lung cancer; antibodies; targeted therapy; vaccines

Dedication

This thesis project is dedicated to my beloved parents

Acknowledgement

First, I would like to thank the Almighty, for providing me with patience, and strength to successfully complete this thesis.

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List of Acronyms

ADC	Adenocarcinoma
ADCs	Anti-Drug Conjugates
ATP	Adenosine Tri Phosphate
AC	Atypical Carcinoid
ALK	Anaplastic Lymphoma Kinase
ACT	Activated Clotting Time
APC	Anti-tumor Effector Cells
BCT	Bronchial Carcinoid Tumor
BRAF	B-Raf proto-oncogene serine/threonine Kinase
BiTEs	Bi-specific T Cell-Engaging Antibodies
CT	Chest Tomography
CAR	Chimeric Antigen Receptor
CRS	Cytokine Release Syndrome
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
DSBs	Double Stand Breaks
EGFR	Epidermal Growth Factor
ERK	Extracellular signal Regulated Kinase
EPR	Enhanced Permeation and Retention
EDA	Ectodysplasin A

FAK	Focal Adhesion Kinase
FDA	Food and Drug Administration
GCC	Giant Cell Carcinoma
GGO	Ground Glass Opacity
HR	Hazard Ratio
HGF	Hepatocyte Growth Factor
ICB	Immune Checkpoint Blockade
IPAS	Iressa Pan-Asia Study
IMRT	Intensity-Modulated Radiation Therapy
IHC	Immunohistochemistry
LUAD	Lung Adenocarcinoma
LUSC	Lung Carcinomas of Squamous Cells
LC	Lung Cancer
MAPK	Mitogen Activated Protein Kinase
MEK	Motogen-activated Extracellular Kinase
MET	Mesenchymal Epithelial Transition
MAGE-A3	Melanoma Associated Antigen-A3
NTRK	Neurotrophic Tyrosine Kinase
NCI	National Cancer Institute
NGS	Next Generation Sequencing

NK	Natural Killer
NPS	Nano Pulse Stimulation
NSCLC	Non-Small Cell Lung Cancer
OS	Overall Survival
ORR	Objective Response Rate
PCR	Polymerase Chain Reaction
PFS	Progression Free Survival
PET	Positron Emission Tomotherapy
PCI	Prophylactic Cerebral Irradiation
RAT	Robot Assisted Thoracoscopic Surgery
RTOG	Radiation Therapy Oncology Group
RTK	Receptor Tyrosine Kinase
SCLC	Small Cell Lung Cancer
SVC	Superior Vena Cava
SABR	Stereotactic Ablative Body Radiation
SCC	Small cell lung cancer
SCID	Severe Combined Immunodeficiency
TNM	Tumor lymph node metastasis
TC	Typical Carcinoid
TKI	Tyrosine Kinase Inhibitors

TrK	Tropomyosin Related Kinase
TME	Tumor Micro-Environment
TIL	Tumor Infiltrating Lymphocytes
TCR	T-Cell Receptor
TME	Tumor Micro-Environment
TALENs	Transcription Activator-like Effector Nuclease
VAT	Video Assisted Thoracoscopic Surgery
WHO	World Health Organization
ZFNs	Zinc Finger Nucleases
4DCT	Four Dimensional Computed Tomography

Chapter 1

Introduction

1.1 Background

Lung cancer (LC) accounts for one of the major causes of cancer-related deaths. A bleak prognosis marks the malignancy and poor clinical results, owing mostly to the advanced stage at diagnosis, imposing a significant cost on public health globally. Lung cancer is the second most frequent cancer, with the highest cancer death rate in both sexes, and its dismal prognosis can be partly linked to a lack of early detection measures. Moreover, it is one of the most actively investigated malignancies in immune-oncology, with a significant need to create screening tools for early signs of lung cancer development and innovative therapeutic techniques to target the illness at its earliest stages (Nasim et al., 2019).

Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two most prevalent histological subtypes of lung cancer (NSCLC). Multiple histologically and molecularly distinguishable subtypes exist within NSCLC. Small cell lung cancer (SCLC) is the most prevalent form of lung cancer in the United States. Smoking is the leading cause of small cell lung cancers (SCLCs), which comprise 15–20% of all primary lung cancers. In SCLC, *MYC* gene amplifications and paraneoplastic diseases are prevalent. Lung adenocarcinoma (LUAD), carcinomas of squamous cells (LUSC), giant cell carcinoma (GCC), and bronchial carcinoid tumour (BCT) are the four subtypes of NSCLC. LUAD is the most prevalent form of non-small cell lung cancer and the most prevalent primary lung tumor. Cancer often occurs in females who have never smoked and has a glandular histological pattern and activating mutations that affect driver genes such as *KRAS*, *EGFR*, and *BRAF*, as well as *ALK* fusions and other genetic changes. In most cases, patients have

never smoked (Saab et al., 2020). Once lung cancer has been identified, a diagnosis and staging must be undertaken, and new guidelines are available to aid in this process. Patients with lung cancer may opt for surgery, radiation therapy, chemotherapy, or targeted therapy to treat their illness. The treatment a patient receives for cancer varies on the illness's subtype and stage. A few years ago, several individualized medications were unavailable. There are a variety of malignancies, and the cells that comprise each type of cancer are diverse, even among individuals with the same type of cancer overall. Because cancer cells might have different gene mutations, the exact type of lung cancer a person gets can vary from person to person. In addition, scientists have discovered that the environment in which particular tumors develop, grow, and thrive is not always the same. This was one of the most important observations that they came across during their research. Several malignancies, for instance, have proteins or enzymes that, when triggered, deliver specific messages to cancer cells, instructing them to proliferate. Due to this knowledge, pharmaceutical companies have developed drugs that can "target" specific proteins or enzymes in the body and inhibit them from transmitting signals. Targeted therapies have the potential to either block or turn off signals that stimulate the formation of cancer cells, or they have the ability to deliver messages to cancer cells commanding them to destroy themselves (Nasim et al., 2019).

A comprehensive understanding of these challenging kinds of cancer biology could lead to developing more effective and possibly targeted therapies. This study seeks to provide a succinct assessment of the most recent advances in lung cancer biology and its therapeutic approaches, as well as a discussion of the most recent developments in targeted therapy, including drugs currently undergoing clinical testing, as well as the possibilities of precision medicine, with an emphasis on personalized drugs (Hirsch et al., 2017).

1.2 Objectives of the Study

The objectives of the study are-

- To provide an overview on lung cancer
- To provide an insight on the existing targeted therapies for lung cancer and their clinical outcomes
- To identify the challenges associated with targeted therapies

1.3 Rationale of the Study

Cancer in general is one of the most difficult diseases to cure and when it comes to lung cancer the initial detection in the early stage becomes more difficult than the rest which creates irreversible health complexity. Studies show that lung cancer is the 11^{th} most common cancer and 7^{th} leading cause of cancer related deaths (Lupo & Spector, 2020). Throughout the years scientists have discovered a plethora of treatments to cure or at the very least confine its effect on the human body which improved the survival rate marginally. The purpose of this review is to gather information on the targeted therapies for lung cancer so it can paint a better picture on the current developments in lung cancer biology which might help to get to more efficacious and perhaps more specific drugs.

Chapter 2

Methodology

The information in this review paper were collected from the journals such as MDPI, PubMed, Frontiers, Nature, Elsevier, ScienceDirect, Cell, etc. The information prioritized were mostly from recent years and consisted of research articles, review articles, and reports. The keywords used in searching for relevant information pertaining to the topic included "Targeted therapy", " Targeted therapy in lung cancer treatment", "Lung cancer classification", "Immunotherapy for lung cancer", "Biomarkers for cancer screening", "Gene editing technique", "Adoptive cell therapy", "Lung cancer screening", "Chemotherapy", "Radiotherapy" "Symptoms of lung cancer", etc. These articles were then reviewed and those with relevant information were included in this review paper. In order to be respectful of the work or the original writers, I have used the Mendeley tool for referencing purpose. Duplicate articles were also removed manually from the list before the paper was finalized. The aim of this review paper is to briefly relay the recent findings on targeted therapy for lung cancer treatments.

Chapter 3

Lung Cancer

3.1 Types of Lung Cancer

3.1.1 Small-cell Lung Cancer (SCLC)

Among all cancers, small cell lung cancer (SCLC) is the leading cause of death in men and the second most frequent reason for death in women nationwide. It accounts for approximately 15% of all cases of lung cancer. Individuals with small cell lung cancer have a poor chance of survival, with a 5-year survival probability of fewer than 5% and an average overall survival duration of only 2–4 months if they do not receive any active therapy. The prognosis for these patients is extremely bleak. The use of tobacco products continues to be the leading risk factor for small cell lung cancer, which is also associated with a high mutation burden in this illness. The absence of specific symptoms and the rapid growth of the tumour make it difficult to detect small cell lung cancer at an early stage. Because of this, the screening methods that are currently used are inefficient in diagnosing patients in the early stages of the disease. According to the staging criteria established by the veteran affairs lung group, SCLC might be either in the limited or widespread stage. Approximately 65–70% of newly diagnosed cases will progress to the severe stage. For SCLC, there aren't many available treatment alternatives. For patients with tumor lymph node metastasis (TNM) stage I disease with no evidence of mediastinal or supraclavicular involvement, surgery in the form of lobectomy may be an option. Combining etoposide or irinotecan with platinum is the conventional first-line treatment for cancer patients undergoing chemotherapy. In the restricted stage, radiotherapy to the thorax and the mediastinum must also be administered concurrently or sequentially. If a complete response was accomplished, prophylactic cerebral

irradiation (PCI) is recommended to forestall the subsequent development of brain metastases. Chemotherapy is the primary treatment used in the extensive stage of the disease in the first-line setting. However, the value of thoracic radiation and PCI is debated, and it is not a treatment given to all patients. Only about ten months is the median overall survival (OS) for patients with extensive stage SCLC treated with standard frontline chemotherapy. SCLC is normally responsive to the initial treatment; however, after initial treatment, most patients experience recurrent disease, frequently with new sites of metastasis. Unfortunately, the FDA has only approved a relatively limited number of medicines for the second-line therapy of SCLC. Topotecan is typically considered a standard choice for usage in the second line of treatment; nevertheless, it is not always prescribed to patients, partly because of its moderate efficacy and considerable hematologic toxicity. The overall survival (OS) rate for patients treated with topotecan is only 26 weeks, whereas the overall survival rate for patients with the greatest supportive care alone is 14 weeks. Because the efficacy of available conventional salvage treatments is only moderate, as determined by progression-free survival rates and overall survival, the search for new effective therapeutic approaches has not ceased. In phase II clinical trials, single-agent regimens of conventional cytotoxic drugs such as paclitaxel, docetaxel, gemcitabine, and vinorelbine have been investigated as potential second-line treatments; nevertheless, the outcomes of these studies have been somewhat unimpressive. In more recent years, targeted therapy has also been actively studied, with mixed outcomes, including several disappointing findings and a few positive ones (Bernhardt & Jalal, 2016). Patients often arrived with symptoms that had only been present for a brief period, on average, three months. Coughing, wheezing, shortness of breath or post-obstructive pneumonia are some symptoms that endobronchial tumours may cause. Because of the central nature of these tumours, patients with regional extension of the disease may have hoarseness in their voices, pain in their chests or throats, difficulty swallowing, or

superior vena cava syndrome. Patients with metastatic disease may appear with symptoms such as stomach discomfort, pain in the bones, nausea, vomiting, anorexia, weight loss, or neurologic impairments in localized areas. Paraneoplastic syndromes can reveal themselves in patients at any stage of the disease. The majority of SCLC cells are very responsive to the chemotherapy that is administered. When treated with effective chemotherapy, individuals with a substantial amount of tumour burden run the risk of developing tumour lysis syndrome. Unfortunately, these tumour cells are heterogeneous, and some clones within them are resistant to the chemotherapy being administered. This leads to the disease returning and eventually leading to death (Kalemkerian & Schneider, 2017).

3.1.2 Non-small Cell Lung Cancer (NSCLC)

Adenocarcinoma (ADC): Adenocarcinoma (ADC) is the most common non-small cell lung cancer, accounting for 38.5% of all lung cancer cases. ADC is described as a malignant epithelial tumor with glandular differentiation that has mucin synthesis detectable by mucin stainings, such as mucicarmin, or pneumocyte marker expressions, such as napsin A or thyroid transcription factor 1 (TTF1). ADC is generally found in the lung's periphery. Lepidic, acinar, papillary, micropapillary, and solid histology patterns can coexist in a single ADC tumor alongside lepidic, acinar, papillary, and micropapillary patterns. While the lepidic pattern is connected with a positive prognosis, the micropapillary and solid patterns are linked to more aggressive behavior. Solid ADC can be mistaken for SqCC or LLC; in difficult situations, mucin production and immunochemical (IHC) expression of TTF-1 or napsin can aid in the identification of solid ADC (Langfort & Szołkowska, 2012).

Adenocarcinoma (ADC) is the most prevalent kind of LC, comprising over 40% of all LC. Lung ADCs originate from type II alveolar epithelial cells that release mucus and other chemicals. 5–10% of LC is composed of undifferentiated large-cell carcinoma. This kind of

cancer lacks squamous or glandular maturation and, as a result, is frequently detected by eliminating all other possibilities. The discovery of mutant oncogenes, which encode active signaling molecules that promote cellular proliferation and tumor growth, has led to the development of more effective and less harmful targeted therapies for patients with LC. However, similar to conventional chemotherapies, these new targeted medications tend to fail due to the development of resistance. Gene alterations and localized amplification modulate the sensitivity of malignancies to the induction of cell death; hence, differences in treatment sensitivity may depend on the susceptibility of LC cells and lung ADC cells to undergo cell death (Langfort & Szołkowska, 2012).

Recent breakthroughs in understanding the molecular processes controlling tumor progression and the related targeted therapy for lung ADCs are discussed. In addition, the processes of cell death generated by various treatment strategies and their contribution to therapy resistance are examined. Focus is placed on strategies for overcoming drug resistance to enhance future treatment decisions. Influencing mutations typically, lung ADCs have a heterogeneous mixture of histological development patterns, defined as "mixed type." Recent innovations in sequencing technologies have resulted in an improved understanding of tumor heterogeneity. They have enabled the further separation of lung ADC into genetic sub sets based on a classification based on such driver mutations. These mutations represent the genetic alterations necessary for the initiation and progression of tumors. They are frequently found in genes that regulate cellular proliferation and survival. Consequently, cancers may rely on the production of these single-mutant oncogenes to support tumor growth and survival; this concept is known as oncogene addiction. As tumor cells rely on the abnormal activity of a specific mutant gene or pathway for survival and proliferation, inactivating these genes or pathways is often sufficient to induce growth arrest or cell death. An intriguing explanation for the phenomena of oncogenic addiction has been offered. According to this

theory, the apoptotic response observed in tumors following the abrupt disruption of an oncogene product is caused by the differential degradation of several pro-survival and pro-apoptotic signals originating from the oncoproteins (Denisenko et al., 2018).

The disruption of the equilibrium between pro-apoptotic and pro-survival signals may induce oncogenic shock, eventually resulting in the death of tumor cells. The first therapeutically useful mutation found in lung ADC was a somatic mutation deletion in exon 19 or an L858R point mutation in the transmembrane receptor tyrosine kinase (RTK) such as epidermal growth factor receptor (EGFR). EGFR mutations close to the ATP (adenosin triphosphate) cleft of the tyrosine kinase (TK) domain boost receptor activation and function as carcinogenic drivers. Binding with ligands (EGF and TGF) modifies EGFR's shape and causes homodimerization or heterodimerization with other HERs. Subsequent auto-phosphorylation of the cytoplasmic TK domain by adapter proteins (e.g., SHC and GRB-2) initiates the following signaling pathways: (1) the rat sarcoma (RAS)/rapidly accelerated fibrosarcoma (RAF)/mitogen-activated protein kinase (MAPK) pathway; (2) the phosphatidylinositol-3-kinase (PI3K) accelerates mitosis, resulting in cell proliferation and apoptosis inhibition. These pathways are crucial for normal cell development. Additionally, EGFR stimulates cancer growth (Denisenko et al., 2018).

Squamous Cell Carcinoma (SqCC): Approximately 20% of all lung malignancies are SqCC. SqCC typically manifests centrally, originating in the main or lobar bronchus. The World Health Organization (WHO) defines SqCC histologically as a malignant epithelial tumor with keratinization and intercellular bridges expressing IHC markers of squamous cell differentiation. Although keratinization is the hallmark of SqCC, many SqCC may lack keratinization's morphological characteristics. Lung cancer diagnosis and molecular classification, poorly differentiated SqCC can have a pseudo glandular appearance, and

poorly differentiated adenocarcinomas might have papulosquamous characteristics, interpreting tiny biopsies or cytological specimens particularly difficult. In challenging situations, IHC assays comprising markers of squamous cell differentiation, such as p40 or p63 and cytokeratins 5/6, are useful tools for identifying SqCC. Basaloid squamous cell carcinoma is a distinct entity; it is a poorly differentiated malignant tumor without morphological features of squamous cell differentiation that can be confused with small-cell lung carcinoma; however, it is characteristically positive for immunomarkers of squamous cell differentiation, including p40, p63, and cytokeratins 5/6, while TTF-1 is negative (Rodriguez-Canales et al., 2016).

Large-cell lung carcinoma (LLC): LLC represents 2.9% of all lung malignancies. LLC is described as an undifferentiated NSCLC carcinoma that lacks signs of squamous cell, glandular, or small-cell differentiation in histology or immunohistochemistry. After ruling out SqCC, ADC, and SCLC, the diagnosis of LLC needs extensive sampling of a surgically removed specimen; consequently, it cannot be done with core needle biopsies or cytology samples. Absence of mucin synthesis as indicated by mucin staining with mucicarmine. LLC may be immunohistochemically positive for cytokeratins but negative for TTF-1 and p40. LLC must be distinguished from large-cell neuroendocrine (typically expressing TTF-1 and neuroendocrine markers), a solid pattern of ADC (TTF-1 positive), non-keratinizing SqCC (p40 positive), and infrequently adenosquamous carcinoma (exhibiting both ADC and SqCC differentiation). As noted, tumors having NSCLC characteristics and a null IHC phenotype are designated NSCLC-NOS in small biopsies. 2-3% of all lung cancers are lung large cell neuroendocrine carcinomas (L-LCNEC), a rare yet aggressive tumor subtype. This tumor is more prevalent in heavy male smokers than in nonsmokers. Common in non-smoking females. L-LCNEC develops specifically from neuroendocrine lung cells. Since it is now recognized as a neuroendocrine subtype of large cell lung carcinoma, it is often classified as

non-small cell lung carcinoma. However, it also belongs to neuroendocrine lung tumors (NET). Lung NETs accounts for 20–30% of all NETs and account for around 25% of lung malignancies. The World Health Organization (WHO) categorized tumors which were mentioned above into four subtypes with increasing biological aggressiveness: typical carcinoid (TC), atypical carcinoid (AC), L-LCNEC, and small-cell lung cancer (SCLC). 2015 WHO classification of lung NETs mostly retained the same terminology but removed LLCNECs from the group of LLC and grouped all lung NETs into a single entity, abandoning the prior subdivision of tumors into several subgroups. In particular, although L-LCNEC is conventionally categorized as a subtype of NSCLC, its advanced biochemical, clinical, and prognostic characteristics resemble those of SCLC. Due to the scarcity of literature data regarding these neoplasms and their unique characteristics, there is a growing need for a consensus on the optimal treatment strategy for managing L-LCNEC (Lo Russo et al., 2016).

3.2 Symptoms

Most lung malignancies do not cause symptoms until after they have spread. However, some patients with early lung cancer do. The majority of these symptoms are caused by something other than lung cancer. Cancer may be detected earlier if a person goes to the doctor as soon as they notice symptoms. At this stage, treatment is more likely to be curative if received early. The following are the most prevalent symptoms of lung cancer:

- Coughing blood or rust-colored sputum (spit or phlegm)
- Coughing, Chest pain that is typically exacerbated by deep breathing, coughing, or laughing
- Hoarseness, unexplained weight loss, appetite loss, shortness of breath
- Infections such as bronchitis and pneumonia do not go away or keep recurring.

If lung cancer spreads to other parts of the body, in that case, it can cause the following symptoms:

- Changes in the nervous system, (such as headaches, arm or leg weakness or numbness, dizziness, balance issues, or seizures) caused by cancer spread to the brain
- Yellowing of the skin and eyes (jaundice) caused by cancer spread to the liver Lymph node swelling (collection of immune system cells) in the neck, above the collarbone Some lung tumors can result in syndromes.
- Horner disease Pancoast tumors are cancers that develop in the upper section of the lungs. Small cell lung cancer is less likely to be present in these tumors than in NSCLC. Pancoast tumors can impact particular nerves in the eye and part of the face, resulting in Horner syndrome: Drooping or weakening of one upper eyelid; a smaller pupil (the black spot in the middle of the eye) in the same eye
- Sweating very little or not at all on the identical side of the face Pancoast cancers occasionally also result in excruciating shoulder pain. Syndrome of the superior vena cava (SVC) is a major vein that connects the head and arms to the heart. It runs alongside the right lung's upper portion and the chest lymph nodes. This location's tumor can press on the SVC, causing blood to pool in the veins. Inflammation in the face, neck, arms and upper chest might result from this (sometimes with bluish-red skin color). If it affects the brain, it can also induce headaches, dizziness, and a shift in awareness. SVC syndrome can occur spontaneously or progressively over time; it can become life-threatening in some individuals and must be treated immediately.
- Syndromes of paraneoplastic origin some lung tumors produce hormone-like chemicals that reach the bloodstream and cause difficulties in distant tissues and organs. However, the disease has not progressed to those locations. These are known as paraneoplastic syndromes. These disorders are sometimes the first signs of lung cancer. Because the

symptoms impact other organs, a condition other than lung cancer may be blamed. Paraneoplastic syndromes can occur with any lung cancer; however, they are more commonly related to SCLC. Although, many of these symptoms are caused by conditions other than lung cancer. Still, if someone has any of these symptoms, they should seek medical help immediately so that the cause may be identified and treated, if necessary (Lei and colleagues, 2020; Verma et al., 2020).

3.3 Biomarkers

Numerous biomarkers for lung cancer are currently in development, with the majority in their earliest stages and few having advanced beyond clinical validation tests. The majority of risk prediction and diagnostic biomarkers have been generated using cohorts that are histologically agnostic. However, the majority of available specimens represent NSCLCs, with adenocarcinomas and squamous cell carcinomas as the most common histologic subtypes. Small cell and other less common lung malignancies are less frequently the subjects of biomarker discovery or use. Numerous lung cancer biomarkers have been used to guide therapy, for example, when genetic alterations driving tumor growth can be targeted by available drug therapies (EGFR, ALK, ROS1, HER2, BRAF/ MEK, MET, and RET mutations and aberrations) (Saarenheimo et al., 2019).

The majority of additional clinically validated molecular indicators were constructed for patient selection in LCS or lung nodule therapy. These biomarkers measure various substances, typically in panels that comprise proteins, autoantibodies, methylation DNA, mRNA, miRNA, and single nucleotide polymorphisms, either alone or in conjunction with clinical factors. The majority of early biomarkers were derived from blood components taken during normal blood samples and stored, with systemic changes produced by lung cancer serving as their basis (Silvestri et al., 2018). Two assays based on changed genetic and

epigenetic features of noncancerous bronchial epithelial cells in patients with lung cancer were developed for use in bronchial epithelial specimens acquired during diagnostic bronchoscopy conducted to examine pulmonary nodules (field cancerization effect). Some of these tests are now commercially accessible, while others are nearing clinical viability testing (Silvestri et al., 2015).

Diagnostic practices for lung cancer have shifted due to the advent of predictive biomarkers for determining which cancers may be amenable to targeted therapy. The role of pathologists in the fight against lung cancer is shifting due to this paradigm shift. Multiplex genotyping platforms are now being developed and transitioned into clinical use to identify oncogene mutations, gene amplifications, and rearrangements. Genome-wide molecular investigations have assessed next-generation sequencing (NGS) methods, and the outcomes have been encouraging. Additional research into NSCLC is necessary to fully comprehend the functions of tumor suppressor genes and epigenetic events in the absence of recognized driver mutations and the consequences of intratumor heterogeneity. As NGS only requires a little sample of tissue for analysis, it can be used in a clinical environment with great efficiency. Accurately preserving tumor cells to identify molecular changes requires pathologists to grasp tissue adequacy in terms of number and quality. Recent clinical successes of immunotherapy approaches to lung cancer have heightened the need for proper procurement and processing of tissue specimens from patients with lung cancer and posed additional challenges to the scientific community and pathologists in developing predictive biomarkers of response to these therapies (Walcher et al., 2020).

EGFR or the epidermal growth factor receptor, is an ERBB family tyrosine kinase receptor. The EGFR gene is found at location 12 on the short arm of chromosome 7. When an extracellular ligand binds to EGFR, it causes homodimerization or heterodimerization of the

receptor, which results in phosphorylation of cytoplasmic tyrosine kinase sites and activation of various intracellular pathways, including the PI3K/AKT/mammalian target of rapamycin (mTOR) and RAS/RAF/mitogen-activated protein kinase (MAPK) pathways (Sholl, 2015). EGFR is ubiquitously expressed in 62% of NSCLCs, and it has been linked to a poor prognosis. EGFR mutations are found in 10% of patients with lung adenocarcinoma in the United States and (30% -50%) in East Asia. These mutations occur within exons 18 to 21, which encode for a component of the EGFR kinase domain. Approximately 90% of all EGFR mutations are in-frame deletions in exon 19 or missense mutations in exon 21 (41% and 44%, respectively) (Chatziandreou et al., 2015).

Modifying the kinase domain of EGFR activating elicits ligand-independent tyrosine kinase activation, resulting in the hyperactivation of downstream antiapoptotic signaling pathways. EGFR mutations are more prevalent in adenocarcinomas with lepidic characteristics in never-smoking females. The high response rates (55–78%) to treatment with EGFR tyrosine kinase inhibitors (TKIs), such as gefitinib, erlotinib, and afatinib, in patients with EGFR-mutant tumors, and the significantly longer progression-free survival (PFS) of these patients, have made EGFR TKIs the standard treatment for patients with these mutations. However, the majority of these patients acquire resistance and relapse rapidly due to the emergence of a novel mutation (T790 M) in exon 20 of the EGFR kinase domain (50 percent), amplification of the MET oncogene (21 percent), or mutations in PI3KCA. The majority of EGFR mutations are detected using gene sequencing techniques and true polymerase chain reaction (PCR)-based tests. It has been shown that both approaches detect these mutations in formalin-fixed and paraffin-embedded tissues with great performance and sensitivity. It has been attempted to detect EGFR mutations using an IHC-based technique with specific antibodies against mutant proteins. However, the sensitivity and variability of these investigations have been inconsistent (Fujimoto & Wistuba, 2014).

Position 34 on chromosome 7's long arm is linked to the B-RAF proto-oncogene, also known as the serine/threonine kinase (BRAF) oncogene. Serine/threonine kinase, a component of the RAS/RAF/MEK/ERK signaling cascade, is what it encodes. BRAF phosphorylates MEK and stimulates cell growth, multiplication, and survival when oncogenic mutations activate it. Malignant melanoma (27%–70%), papillary thyroid cancer, colorectal cancer, and serous ovarian cancer have the greatest rates of BRAF mutation (Planchard et al., 2016).

Compared to melanoma, only half of the BRAF mutations in NSCLC are the V600 E variants. BRAF mutations have also been documented in 1% to 3% of NSCLCs. There have also been reports of the non-V600 E mutations in NSCLC. The mutations of EGFR, KRAS, and ALK, as well as all BRAF mutations, are mutually exclusive. According to reports, adenocarcinoma makes up the majority of BRAF-mutated NSCLC. Unlike patients with EGFR mutations or ALK rearrangements, who are typically never smokers, patients with BRAF mutations are primarily current or former smokers. Despite this, patients with NSCLC and BRAF V600 E mutations have an inferior prognosis and lack of response to platinum-based treatment than those with wild-type BRAF. BRAF inhibitors have been beneficial for these patients, with response rates ranging from 33% to 42%. BRAF inhibitors like vemurafenib and dabrafenib exhibit significant and selective action against the V600E-mutant BRAF kinase. Clinical trials actively evaluate BRAF and MEK inhibitors targeting BRAF mutation-positive NSCLC, for example: selumetinib, trametinib and dasatinib among others (Hyman et al., 2015).

EB Biomarkers Growing data suggest that EB, specifically EB condensate (EBC), can be used to diagnose malignancy. It is possible that EGFR-resistant clones can be found with the use of EBC, which consists of cells and DNA fragments (Seijo et al., 2019). Predictive Biomarkers for Lung Cancer Screening in 2019 7 EB volatile fragments are sensitive

indicators for lung cancer. Gas chromatography/mass spectrometry, nanosensors, colorimetric sensors, and other technologies can all be used to detect and evaluate volatile organic molecules. Breath samples from 1404 people were utilized to identify 17 different diseases with an 86% accuracy using an artificially intelligent nanoarray sensor. In addition to predicting response to therapy and recurrence, some research suggests that this array can distinguish benign from malignant pulmonary nodules. Additionally, it may differentiate histological type or predict molecular analysis outcomes. And interestingly, it may be able to tell the difference between certain cancers (breast, lung, prostate and colorectal) (Walcher et al., 2020).

The growing use of chest computed tomography (CT) images for both LCS and diagnostic purposes has resulted in a surge in the detection of pulmonary nodules. Although some of these nodules are initially classified as high or low risk of lung cancer based on imaging features, many are classified as intermediate risk (Sawada et al., 2017**)**. Subsolid nodules carry a greater risk of malignancy than solid nodules, but they often exhibit a more placid disposition. As a result, they can be monitored radiologically for the formation of a solid component, at which point surgical resection is the chosen treatment option. A biomarker capable of distinguishing benign from malignant nodules measuring 8 to 30 mm in diameter might be useful for solid, noncalcified nodules. Several lung nodule risk calculators have been created to assess the risk of these intermediate-risk nodules, with utility varying depending on the cohort. Malignancy risk, as assessed by nodule risk calculators or physician estimation, is then utilized to stratify early care methods into very low (10%), intermediate (w11%-64%), and high risk (>65%), with cutoffs, varying depending on patient preference and contraindications **(**Gould et al., 2015).

Typically, extremely low-risk nodules are treated with close radiologic monitoring. High-risk nodules are treated with decisive therapy (surgical resection or stereotactic radiotherapy) (Gould et al., 2015)**.**Intermediate-risk nodules necessitate extra assessment, such as additional imaging (e.g., PET) or a biopsy (percutaneous or bronchoscopic). Despite its high sensitivity (w90%), PET paired with CT has a lower specificity (61%–77%), which is significantly lower in regions with a high prevalence of endemic mycoses. Percutaneous nodule biopsies offer a good diagnostic yield but are associated with a risk of complications, including pneumothorax, especially in this patient group, who may have tobacco-related emphysema. Despite having a decreased risk of pneumothorax, bronchoscopy biopsy is conducted on a subset of patients based on the nodule's characteristics. It has varying diagnostic results, even when performed by highly trained treatment specialists. These intermediate-risk nodules would benefit the most from additional risk classification using a pulmonary nodule biomarker (Katki et al., 2016; Gould et al., 2013).

A nodule management biomarker's clinical utility requires two objectives. First, lung cancers are diagnosed earlier without significantly increasing procedures performed on benign nodules. Second, fewer procedures are performed on benign nodules without a clinically significant delay in diagnosing lung cancer in those with malignant pulmonary nodules. Even if these conditions are met, a lung nodule biomarker may be of limited clinical usefulness. If a lung nodule biomarker properly identifies a patient's tumor as malignant, the patient may have unnecessary surgery or other intervention to treat a cancer that would otherwise stay indolent (i.e., never cause the patient's death). A false negative biomarker result on a very small, early lung cancer followed by close radiologic surveillance, on the other hand, may result in a delay in diagnosis that is not related with an increase in mortality. Therefore, it is critical that these biomarkers are applied to the desired group and that clinical utility trials are planned keeping these specific objectives in mind (Mazzone et al., 2017).

3.4 Available Treatment Options for Lung Cancer

3.4.1 Chemotherapy

Chemotherapy is a cytotoxic therapy that interferes with fundamental cellular functions like maintenance, proliferation, metastasis, angiogenesis, and apoptosis in all cells, not only those with oncogenic drivers (Sawant et al., n.d.). It is effective because cancer cells are more dependent on these activities than healthy cells. They may also have a diminished capacity to survive cytotoxic stress compared to normal cells. In reality, all chemotherapies are targeted agents; yet, we lack a comprehensive understanding of their targets in normal and cancerous cells (Thomas et al., 2013).

Recent studies have revealed that specific chemotherapies routinely used to treat lung malignancies, such as paclitaxel, cisplatin, and gemcitabine, can enhance the immune system's response to cancer. Paclitaxel plus cisplatin made tumor cells more susceptible to destruction by tumor-specific cytotoxic T lymphocytes in mice (Hellmann et al., 2014). Gemcitabine can boost T cell responses to malignancies by preferentially diminishing immunosuppressive cells in the tumor microenvironment (such as myeloid-derived suppressor cells and regulatory T cells) and augmenting effector T cells, according to another research. Two clinical studies have assessed the efficacy of combining cytotoxic chemotherapy with T-cell checkpoint inhibitors in lung cancer patients. 204 patients with advanced lung malignancies were randomly given carboplatin and paclitaxel with or without ipilimumab. Ipilimumab was administered either simultaneously with chemotherapy or progressively, beginning after two cycles of chemotherapy. Median progression-free survival was significantly longer in the phased group compared with chemotherapy alone (5.6 vs. 4.6 months, HR (Hazard ratio) = 0.72, p= 0.05); although, progression-free survival was numerically longer in the contemporaneous treatment arm (5.5 vs. 4.6 months, HR= 0.81, p=

0.13). In a second research, platinum-based chemotherapies were coupled with nivolumab (anti-PD-1). The overall response rate was between 30% and 40%, which was not significantly higher than the response rates observed in previous studies of the first treatment—awaiting long-term follow-up data to see whether the inclusion of nivolumab increases the duration of response or survival relative to chemotherapy by itself (Kris et al., 2019).

In recent years, metronomic chemotherapy has drawn significant interest. In Japanese patients with sensitive recurrent SCLC, a metronomic chemotherapy regimen of cisplatin, etoposide, and irinotecan was compared to single-agent topotecan **(**Goto et al., 2016)**.** A total of 180 participants were randomly assigned to either a metronomic or a control group. Patients receiving the three-drug metronomic regimen had a considerably longer OS than those receiving topotecan alone (18.2 vs. 12.5 months, HR = 0.67, P = 0.0079). This result marks a significant advancement in the second-line treatment for SCLC. However, it is impossible to disregard the toxicity of the three-drug metronomic regimen. It has to be determined and examined in additional patient groups whether metronomic chemotherapy could be a future second-line therapeutic option (Yang et al., 2019).

Compared to non-targeted areas, localized chemotherapy can deliver anticancer medicines to afflicted tumor tissues in higher concentrations. Numerous forms of cancer, including colorectal and ovarian tumors, have been shown to respond favorably to chemotherapy when administered locally (Liyanage et al., 2019). The administration of inhaled medications enables the direct delivery of pharmaceuticals to the lungs via nasal or oral inhalation. The effectiveness of inhalation chemotherapy for lung cancer has been established. Compared to parenteral administration, inhalation can affect the biodistribution of medicines and increase their very high fraction accumulation in the lungs. Additionally, lung cancers that have

spread to the lungs benefit greatly from inhaled chemotherapy because the lungs are distant from the body's main blood vessels yet still get their supply of oxygenated blood through these channels. While inhalational chemotherapy has significant pharmacokinetic advantages over systemic delivery and oral, drug deposition in the local tumor remains a daunting challenge. The clinical effectiveness of inhalation chemotherapy is dependent on a variety of variables, including patient condition, disease stage, tumor size, drug penetration into the tumor, local side effects, and the physicochemical features of the medicines (Mangal et al., 2017).

The manufacturing of nanocarrier-based medication delivery devices has increased dramatically over the past decade. Researchers created highly stable, sustainable, and efficient nanocarrier systems for very sensitive and selective imaging as well as better therapeutic applications (Ahmad et al., 2015)**.** Furthermore, nanocarriers have the potential for surface functionalization with targeting ligands, allowing them to efficiently deliver their loaded therapeutic material to malignancies. This translates to more selective drug accumulation, resulting in improved chemotherapeutic effects and fewer negative off-target side effects (Senapati et al., 2018). Similarly, nanocarriers can be constructed to release their laden contents in a regulated manner, preserving the therapeutic amount of pharmaceuticals at the target areas for a longer period of time. As a result, the clinical effectiveness of medications at low doses increases, as does patient satisfaction (Patra et al., 2018).

There are various concerns with nanocarriers used to treat lung cancer, including biological obstacles caused by the physiology and anatomy of the lungs and incorrect physicochemical qualities of the particles. In clinical practice, drug delivery methods based on nanocarriers present significant challenges, such as increased circulatory clearance, immunological response, and reduced targeting effectiveness (Ahmad et al., 2015). To achieve the most

effective drug delivery, a thorough understanding of the biological function of nanocarriers is required. Like many other malignant tissues, the inadequate lymphatic flow and development of delicate blood vasculature in the lungs promote the enhanced permeation and retention (EPR) effect, boosting nanocarrier entrance into tumors. On the other hand, NPS (nanopulse stimulation) smaller than 50 nm are less likely to persist in tumor tissue for an extended period. Active targeting entails altering the surface of the nanocarrier with specific ligands to promote contact with overexpressed receptors on tumor cells. Different ways are employed to optimize the size of nanocarriers, but the ultimate result is a skewed explosion of encapsulated medicines. As a result, sustaining the size up to 200 nm in the production process is difficult. Surface charge is critical in determining nanocarrier destiny *in vivo*; the nanocomposites' zeta potential mostly determines particle contact and aggregation. Even so, it causes hemolysis and presents the issue of safe delivery (Lee et al., 2015).

3.4.2 Radiation Therapy

The treatment of lung cancer is typically multimodal involving; surgery, radiotherapy, systemic therapies (chemotherapy, immunotherapy, and targeted medicines), interventional radiology, and palliative care. Radiotherapy is the only treatment modality with indications in all disease stages and across all patient performance status categories (Delaney & Barton, 2015). Modeling demonstrates that 77% of lung cancer patients have an evidence-based rationale for radiotherapy at some stage during their cancer journey. Nonetheless, radiation is underutilized in many regions of the world. At the population level, optimal radiation utilization might result in a 5-year local control increase of 8.3% and a 4% survival gain. Recent advancements in radiotherapy have improved lung cancer treatment outcomes (Vinod, 2015).

Radiotherapy technology is continually advancing, resulting in more precise, faster, and less harmful treatments. Imaging is a crucial aspect of the precision of radiotherapy delivery. Using four-dimensional computed tomography (4DCT) is now commonplace for radiotherapy planning. It permits the assessment of patient-specific tumor movement, which can be included in radiotherapy planning to ensure that the required dose is provided to the tumor regardless of its position. The usual configuration of cone beam computed tomography on linear accelerators also permits tumor position verification before treatment. The introduction of these imaging technologies, coupled with enhanced patient immobilization techniques, has facilitated the delivery of stereotactic ablative body radiation (SABR) (Shafiq et al., 2016). SABR is the delivery of substantial ablative doses of radiation with geometric precision and accuracy in fewer fractions. For traditional radiotherapy, enhanced imaging has reduced the hitherto broad margins given to account for tumor movements and uncertainty, hence reducing the accidental dose to adjacent normal tissues and, consequently, radiotherapy toxicity. Intensity-modulated radiation (IMRT), in which numerous beams of non-uniform intensity are focused on the tumor, also permits increased conformity of treatment and decreased doses to normal tissue. Respiratory gating is an alternate approach to account for tumor migration in which the radiation beam is only activated when the tumor is in a specified place. It may be effective for tumors with significant respiratory excursion, such as those in the lower lobes. In order to achieve gating, breath-hold techniques can be utilized. In this technique, the patient temporarily stops breathing at a predetermined point in the respiratory cycle, and therapy is only delivered while the patient is in the breath-hold position. However, this can be difficult for those suffering from underlying pulmonary illness. In addition to detecting tumor mobility, gating can be achieved by activating treatment only when the tumor is in a predetermined position. Typically, this requires the installation of fiducial markers or specialized tracking technology. These technologies have

increased the effectiveness of current radiation across all lung cancer stages (Vinod & Hau, 2020).

Patients with stage I–IIA NSCLC who are either unable to undergo surgery or refuse surgery are now routinely offered SABR as the primary treatment option. This method is superior to conventional radiation in two randomized clinical trials (Nyman et al., 2016). Most patients in both studies were older than the median age of 70 years for patients with NSCLC and had comorbidities. The SPACE trial demonstrated a reduction in toxicity and an improvement in health-related quality of life, with comparable survival rates between groups. The CHISEL trial was the first to demonstrate better overall survival (OS) in patients treated with SABR (2-year OS: 77% vs. 59%, P = 0.027), with comparable adverse effects and quality of life. The differences in survival outcomes between trials may be attributable to the lower rate of pathological confirmation, positron emission tomography (PET) staging, and 4DCT in the SPACE trial, as well as the different radiotherapy doses selected for the conventional arms. SABR has revolutionized the radiation treatment of stage I-IIA NSCLC since the schedule is significantly more accommodating, particularly for elderly patients with comorbidities. According to a Dutch population-based analysis, the introduction of SABR was associated with a 16% rise in radiation use, a comparable drop in the number of untreated elderly patients, and an improvement in mortality (Boldrini et al., 2019).

Moreover, in patients with operable disease, a pooled analysis of two randomized controlled trials, STARS (NCT00840749) and ROSEL (NCT00687986), suggests that SABR may be an option given the surgical mortality rate of 4% and the grade 3–4 post-operative complication rate of 44%. However, additional research is necessary before this becomes the standard of care. For centrally positioned tumors, toxicity, including treatment-related death (3.7–8.5%), is higher than for peripherally located tumors; however, a comprehensive evaluation of 563

tumors in 315 individuals revealed a treatment-related mortality rate of 2.7% (Bezjak et al., 2019). The Radiation Therapy Oncology Group (RTOG) 0813 dose escalation trial demonstrated that with a five-fraction regimen, it is possible to give 12 Gray (Gy) per fraction with a dose-limiting toxicity rate of 7.2% and outcomes equivalent to peripheral tumors. Conventionally, fractionated radiation is still the primary potential treatment for inoperable patients who are not candidates for stereotactic body radiation therapy (SABR) due to the position of the tumor or the existence of lymph node metastases. Normal dosing is 60 Gy in 30 fractions, but hypo-fractionated regimens (e.g., 55 Gy in 20 fractions) may be an acceptable alternative, particularly for patients receiving radiation alone (Senthi et al., 2013).

Over the past two decades, radiotherapy technology has grown significantly, resulting in increased treatment eligibility, decreased toxicity, and improved survival results. The MRI-linac is a promising new technology that allows for the viewing of tumors during treatment, allowing for the real-time customization of treatment in response to changes in tumor or normal tissue. In SABR, adaptive MRI-based radiation for peripheral and ultra-central tumors has been evaluated. It improves the therapeutic ratio for central tumors by ensuring adequate radiation dose coverage of the tumor while adhering to dose restrictions for adjacent mediastinal tissues. It may lessen the severe toxicity observed with SABR when conventional linear accelerators are used to treat central tumors (Finazzi et al., 2020).

3.4.3 Targeted Therapy

Several definitions have been developed for the treatment known as targeted therapy. Sledge provided the following synopsis of targeted therapy: A targeted therapy should address a biologically significant process (typically, though not necessarily a single molecule), preferably one central to a characteristic of malignancy. During its time, 5-fluorouracil (5-FU) was considered a targeted therapy. Heidelberger et al. referred to 5-FU as a targeted

therapy since this medication disrupts metabolic pathways that are unique to a tumor. The definition of "targeted therapy" has changed throughout time and even within different sections of the same resource. In the lexicon published by the National Cancer Institute (NCI) in 2012, targeted therapy was defined as "a treatment that uses medications to attack specific cancer cells." However, the definition published in 2019 includes similar but additional explanations: few variations help the immune system to kill cancer cells and other variations assist the immune system in killing cancer cells, however the majority of targeted drugs are either small-molecule drugs or monoclonal antibodies (Frigerio et al., 2019).

A medicine like 5-FU has a molecular weight of 131, whereas gemcitabine weighs 266, methotrexate weighs 454, doxorubicin weighs 543, and imatinib has a weight of 494. It is essential to recognize that the term "small molecule" is incredibly vague. The National Cancer Institute provides the following synopsis of the key distinctions between targeted therapies and conventional chemotherapy in its definition of the terms: Targeted therapies act on specific molecular targets, are designed to interact with their target, and often block proliferation; standard conventional chemotherapy is designed to kill rapidly dividing tumour cells, may also affect dividing normal cells, and is cytotoxic. On the other hand, targeted therapies act on specific molecular targets designed to interact with their target and often block proliferation (kill tumour cells). In practice, these distinctions are not as clear-cut as they may seem, given that it is now common knowledge that most TKI-based targeted therapies can affect normal cells, which can result in toxicity that is often fatal (Peters, 2019).

The general mechanism of tyrosine kinases receptor is that it must be activated by a particular ligand, which binds to the RTK on the exterior of the cell membrane. This usually results in autophosphorylation of the intracellular TK domain, which initiates a cascade of activation of RTKs, which can range from one to multiple TKs and frequently involve parallel pathways.

In the end, this results in activating an effector, which is typically a transcription factor. This eventually causes TKIs to be classified as DNA-targeted medicines. When one of the RTKs is triggered in a tumour cell, this frequently results in the tumour cell developing an "addiction" to the activated route (Van Der Steen et al., 2019). As a result of this addiction, the tumour cells become desirable targets for RTK-targeted medicines, such as erlotinib and gefitinib for amplified or mutated (activation mutations) EGFR-TK and crizotinib for amplified or mutated c-MET receptors. Patients with NSCLC activating mutations in the EGFR gene (such as the in-frame deletion in exon 19, delE746-A750 and the missense point mutation L858R) respond well to treatment with first and second generation EGFR-targeted drugs. However, these patients must not have mutations in intracellular pathways such as KRAS, PTEN, or AKT. Nearly all patients diagnosed with NSCLC will acquire resistance to erlotinib or gefitinib treatment within one year. This is typically the result of a mutation in the active kinase domain known as the T790M mutation. Several specific inhibitors were developed based on the three-dimensional structure of the mutated TK domain (Avan et al., 2013). These inhibitors include the third-generation agents. rociletinib and osimertinib. Osimertinib was initially registered for second-line therapy of patients with the T790M mutation, but it is now indicated for first-line treatment. Osimertinib is an example of a new, genuinely targeted TKI because of its particular activity against the mutant RTK domain, which makes it a promising choice for cancer treatment (Péron et al., 2019).

However, treatment with third-generation TKIs still leads to resistance, either in the kinase target itself, which is typically a C797 mutation or by activating other pathways such as the NF-kB pathway or c-MET. Inhibitors that are targeted specifically at C797 are currently under development. Because c-MET amplification is present, one can combine erlotinib or osimertinib with the c-MET inhibitor crizotinib. Crizotinib and the analogues produced from these are the other illustrations of the successful creation of a truly targeted TKI. Crizotinib

works by inhibiting the c-MET receptor, and a protein is turned on when hepatocyte growth factor is present. This increases cell proliferation, transformation, migration, and tubulogenesis. Because of this, the c-MET receptor is an appealing target, particularly in cancers with an amplified or mutant c-MET receptor. An overexpression of c-MET was discovered in a few cases of pancreatic ductal adenoma cancer. Orthotopic patient-derived xenografts were responsive to crizotinib, and the combination of crizotinib and gemcitabine was synergistic (Péron et al., 2019). It increases the amount of the active gemcitabine metabolite dFdCTP and the amount of crizotinib accumulated in the tumour. This is a great illustration of how a TKI and the traditional chemotherapy medication gemcitabine can work together to produce a synergistic effect on cancer treatment (Giovannetti et al., 2017).

3.4.4 Surgery

In 1933, Graham performed the very first effective one-stage pneumonectomy for bronchial carcinoma, which marked the beginning of surgical treatment for lung cancer. Pneumonectomy became the usual treatment for lung cancer throughout the next two decades. Since Ochsner's findings, regional lymph nodes throughout the bronchial tree have been known to be easily implicated by lung cancer. Koletsky's 100 autopsies revealed the features of lung cancer cells (Goldstraw et al., 2015). On the basis of these findings, the authors defined pneumonectomy with hilar and mediastinal lymph node dissection as radical pneumonectomy and pneumonectomy without lymph node dissection as simple pneumonectomy. A 1960 publication also defined radical lobectomy as a technique in which lobe units are removed along with their regional hilar and mediastinal lymphatics. This seminal paper unquestionably marked a watershed moment in the history of lung cancer surgery. Radical lobectomy has been the standard operation for more than half a century despite the lack of convincing evidence for lobectomy's superiority over pneumonectomy.

Meanwhile, in 1973, lung cancer surgery adopted the segmental excision of a smaller portion of the lobe that was established for benign lung illnesses (Cancer, 2015).

Wedge resection, segmentectomy, lobectomy, and pneumonectomy are all types of surgery that can be performed to treat lung cancer. Both wedge resection and segmentectomy can be performed with minimally invasive methods, such as video-assisted thoracoscopic surgery (VATS) or robotic-assisted thoracoscopic surgery (RATS). The instrument is held by the surgeon during VATS, whereas with RATS, the instrument is held by the robot. This is the primary distinction between the two surgical techniques. Both of these approaches are considered less invasive, and patients who undergo them experience fewer adverse effects due to the surgical procedure. From the research of many years surgery has shown the highest promise for curing early-stage lung cancer and is the treatment of choice (Hoy et al., 2019).

Wide-wedge resection is characterized as non-anatomically minor lung parenchymal excision. This operation is performed without confirming the bronchial and pulmonary vascular architecture, and the effectiveness of lung cancer treatment largely depends on the tumor's surgical margin and histological grade. Along the bronchovascular bundle and lymphatics, cancer cells spread towards the hilum of the lung lobe. Even when a tumor appears to be local and minor, lymph node involvement is frequently present (Yoshida et al., 2015). In approximately 20% of patients, solid nodules devoid of ground-glass opacity (GGO) are known to be associated with lymph node metastases or lymphatic penetration to more central locations via lymphatics. Despite wedge resection for these tumors, it is likely that local recurrence will occur. In retrospective studies, researchers have reported on the efficacy of wedge resection for peripheral small lung carcinoma. However, wedge resection for small lung cancer should only be performed on patients with cardiopulmonary impairment. Even after R0 resection, the late recurrence occurred following wedge resection

of pulmonary ground-glass nodules. According to these findings, wedge resection is insufficient for healthy patients with lung cancer, even if the tumors are minor and peripheral (Speicher et al., 2015).

In most cases, patients with stage I to stage II (and even stage III) non-small cell lung cancer are eligible for surgical treatment options for lung cancer; individuals with stage SCLC very seldom qualify. The role of surgical intervention in non-small cell lung cancer (NSCLC) N2 disease, defined as metastases in ipsilateral mediastinal or subcarinal nodes, is still debatable. The risks of complications following surgery for lung cancer are comparable to those following other types of thoracic operations. To facilitate a deeper level of comprehension, these difficulties can be broken down into two broad categories: thoracic and nonthoracic. Atelectasis, pulmonary edema, pneumonia, and respiratory failure are some of the most common issues in the chest. It's vital to remember that nursing care, which includes movement and careful airway clearance, can have a big impact on all of these potential issues (Howington et al., 2013).

Injury to the phrenic nerve and postpneumonectomy syndrome are two additional thoracic complications following lung cancer surgery. An early complication that can arise after thoracotomy is an injury to the phrenic nerve, which can be brought on by the use of cold packs or by direct mechanical stress. This damage can make it more difficult to wean patients off mechanical ventilation and contribute to decreased coughing and secretion clearance. This can make transitioning patients off artificial ventilation more complex. Postpneumonectomy syndrome is a late postsurgical complication resulting from a mediastinal shift toward the side of the damaged lung following surgical resection. This shift occurs as a result of the resection of the lung. After lung resection surgery, a high index of suspicion for post pneumonectomy syndrome should be kept throughout the recovery process. Damage to the

vocal cords and cardiac herniation are two examples of nonthoracic issues that are distinct from the venous thromboembolism and pulmonary embolism. (Hoy et al., 2019).

It is anticipated that the robotic surgical system would mitigate the disadvantages of traditional endoscopic surgery. Surgical techniques for thoracic disorders have developed from thoracotomy to endoscopic surgery, such as video-assisted thoracoscopic surgery (VATS) and, more recently, robotic-assisted thoracoscopic surgery due to technology improvements (RATS). In 2009, the da Vinci® robotic surgical system (Intuitive Surgical, Sunnyvale, California, United States) was introduced globally, but mostly in the United States. Subsequently, permission was granted based on the Pharmaceutical Affairs Law in Japan, and it is primarily employed in university hospitals (Brooks, 2015). In 2012, Japan's national health insurance began to cover robot-assisted complete prostatectomy procedures. Since 2018, the national health insurance has covered RATS for malignant lung tumors, benign mediastinal tumors, and malignant mediastinal tumors; thus, the number of domestic robotic surgical procedures has increased. In addition to being minimally invasive, the da Vinci® surgical system has additional benefits, such as allowing surgeons to use instinctive maneuvers, providing wide visibility through high-quality three-dimensional (3D) images, and allowing complex operations to be performed using delicate instruments. The da Vinci® surgical system gives surgeons better tool dexterity and a higher range of motion than VATS, which uses long, straight instruments. Several studies have utilized stereoscopic endoscopic video to obtain higher-quality images than monocular video. Even though traditional VATS refers to a two-dimensional (2D) image on display, thoracic surgeons derive three-dimensional (3D) structures from their experience. In the da Vinci® surgical system, a 3D high-vision camera can capture clear 3D image data. Under a 3D-visual field, operational movements become clearer and easier, the surgical learning curve is shortened, and straightforward surgeries may be accomplished reasonably quickly (Moore et al., 2015).

Chapter 4

Targeted Therapies for Lung Cancer

4.1 Targeted Therapy by Small Molecules

Epidermal growth factor receptor:

EGFR belongs to the family of receptor tyrosine kinases, which also contains ERBB3/HER3, ERBB2/HER2, and HER4/ERBB4. Activation of effector transcription factors, including MAPK-RAS, STAT, and AKT-PI3K, is caused by the binding of ligands to EGFR, which includes EGF (Roskoski, 2014). The discovery that EGFR is overexpressed in some lung malignancies stimulated interest in molecularly targeting the protein. Gefitinib, a small-molecule inhibitor of the EGFR, was studied in drug testing; nevertheless, the results suggested that it had only a moderate effect on patients with advanced NSCLC. However, this led to the expedited approval of gefitinib by the FDA for those who had advanced NSCLC and had previously been treated with standard chemotherapy but not been successful. Patients who had been unsuccessfully treated with chemotherapeutic drugs. Nonetheless, detailed analysis of these studies found that certain individuals with genetic mutations in the EGFR gene respond better to EGFR inhibitors. This was only a small percentage of patients, though (Paz-Ares et al., 2017).

This promoted the adoption of biomarker-driven targeted therapies for lung cancer. In addition, it justifies directing efforts toward identifying the larger genetic landscape of lung tumors to identify more lung cancer therapeutic targets (Mok et al., 2017). EGFR gene mutations are found in 10–15 percent of lung cancer patients, the majority of whom are young people who have never smoked. The breakthrough IPASS (Iressa Pan-Asia Trial)

study that was conducted in East Asia offered significant support for the adoption of gefitinib as the first-line treatment for EGFR mutant lung adenocarcinomas. This study found that individuals with treatment-naive NSCLC who had activating EGFR mutations had significantly longer PFC or progression-free survival when they were treated with gefitinib rather to paclitaxel/carboplatin (Collisson et al., 2014).

As a consequence of this, the EGFR mutational status was discovered to be a predictive biomarker for response to EGFR inhibitors like gefitinib during the IPASS study. The first-generation EGFR inhibitors were unable to elicit a response that was long-lasting, despite the fact that they were more effective than conventional cytotoxic treatment. Over fifty percent of patients who had relapsed were found to have the EGFR gatekeeper mutant T790M, which was found to be the cause of EGFR antagonist therapy resistance. In order to inhibit EGFR signaling in a manner that is more efficient, second-generation EGFR inhibitors that are more powerful and irreversible, like afatinib. During the LUX-Lung 3 and 6 studies, the administration of afatinib was found to improve patient mortality for patients who had the EGFR del 19 mutation, however this was not the case for those who had the EGFR L858R mutation. Accordingly, despite the fact that both the EGFR exon 19 deletion and the EGFR L858R mutations are EGFR activating mutations, differences in their ability to activate signaling pathway may lead to different responses to targeted therapy. This is something that has been recommended by these and other studies. When treating EGFR-mutation-positive, the LUX-Lung7 research found no significant differences in prognostic factors between afatinib and gefitinib (Sullivan & Planchard, 2016).

In contrast, the second-generation EGFR inhibitor, dacomitinib indicated better response than gefitinib in progression-free survival (14.7 vs. 9.2) but not in overall response rate (75% vs. 72%) in treatment-naive patients with advanced NSCLC who had EGFR-activating

mutations. The trial was called ARCHER 1050. The fundamental impetus behind the development of these drugs was the finding, through preclinical research, that second-generation EGFR inhibitors are effective against T790M mutant protein. However, the therapeutic applicability of these inhibitors is restricted because of the toxicity that is caused by the irreversible suppression of wild-type EGFR. In order to solve these problems, the EGFR inhibitor osimertinib, which is a wild-type sparing, irrevocable, third-generation drug, was developed. Osimertinib showed significant performance in phase I (ORR 61%) and phase II (ORR 66%) of the AURA study in EGFR mutant NSCLC patients, leading to rapid Food and drug administration approval for the treatment of EGFR T790M-positive patients who had progressed on EGFR-TKI therapy (Greig, 2016; Paz-Ares et al., 2017).

ALK:

In 2007, it was shown that oncogenic drivers in non-small cell lung cancer include ALK translocations, which encode fusion proteins of the ALK kinase domain with 5' partners such as NPM, EML4, and TFG. The ALK protein becomes overactive as a result of the formation of these fusion proteins. ALK fusions are expressed in roughly 3–7 percent of NSCLC patients. The vast majority of patients who have this type of fusion are young and have never smoked. The medicine crizotinib, which had first been developed to target c-MET but was later revealed also to target ALK, was reprocessed and attempted in medical studies to seek treatment with ALK-positive lung cancer shortly after the discovery of the EML4-ALK fusions (Lehmann-horn et al., 2014).

After crizotinib quickly demonstrated promising success, the FDA swiftly granted it approval as a treatment for ALK-positive locally advanced or metastatic NSCLC. This success included an objective response rate (ORR) in 61percent in terms of the 149 heavily pretreated ALK-positive patients who participated in the trial and a median progression-free survival

(PFS) of 10 months. For the purpose of comparing crizotinib to conventional chemotherapy as a first-line treatment, the median progression-free survival (PFS) was determined to be 10.9 months, and the overall response rate (ORR) was calculated to be 74%. On-target mechanisms such as the C1156Y substitution, the solvent-exposed mutation G1202R, the gatekeeper mutation L1196M, and the ALK copy number were identified as the cause of crizotinib drug resistance. In addition to this, there was a lack of penetration of crizotinib across the blood-brain barrier, which led to the development of brain metastases. As a direct consequence of this, research into the development of ALK inhibitors that are more efficient continued (Dagogo-Jack & Shaw, 2016).

The first-generation ALK inhibitor crizotinib is less effective than the second-generation inhibitors ceritinib and alectinib. They have the capability of inhibiting on-target resistance pathways to crizotinib. In addition to this, these ALK inhibitors of the second generation are more able to pass across the blood-brain barrier. Patients with metastatic ALK-positive NSCLC who are unable to tolerate crizotinib or whose disease progressed after treatment with crizotinib were qualified for expedited FDA approval of ceritinib. Patients must also have a high risk of developing a resistance to crizotinib. Patients who satisfied both of these requirements were eligible to receive this approval. This approval was prompted by the encouraging results from the ASCEND-1 trial, which demonstrated an impartial response rate (ORR) of 72percent of total (60 of 83) and a median progression-free survival (PFS) of 18.4 months in ALK inhibitor-naive patients treated with ceritinib, and an ORR of 56% (92 of 163) and a median PFS of 6.9 months in ALK inhibitor-pretreated patients treated to ceritinib. These results were encouraging enough to This approval was a direct result of one of the most exciting developments to emerge from the ASCO meeting this year, which was the astounding success of alectinib (Kim et al., 2016). Alectinib was shown to have exceptional efficacy over crizotinib in the ALEX clinical trials, which compared the efficacy

of crizotinib with alectinib in advanced stage, crizotinib-naive ALK-fusion positive patients. This resulted in a lengthening of the median progression-free survival to 25.7 months. After demonstrating remarkable performance in crizotinib-resistant ALK-positive NSCLC patients, the FDA granted permission to brigatinib, a second-generation ALK inhibitor. In order for the FDA to make its determination, they needed this information. With toxicity levels that were acceptable, the higher dose regimen of brigatinib obtained an objective response rate of 54% and a median progression-free survival of 12.9 months correspondingly. Patients who had brain metastases also demonstrated a considerable response, and their overall response rate was 67%. Patients' overall response rate. ALK inhibitors of the second generation, on the other hand, are ineffective when utilized to address the G1202R substitution seen in ALK. ALK inhibitor of the third generation lorlatinib is capable of not only targeting the ALK mutant G1202R but also blocking a number of additional ALK mutations that give resistance to existing ALK inhibitors. In addition to this, its capability of penetrating the blood-brain barrier has been considerably improved. The ALK substitution L1198F, on the other hand, can confer resistance to lorlatinib while making the patient responsive to crizotinib and increasing the binding of crizotinib to ALK (Thress et al., 2016).

lorlatinib is a drug that inhibits the activity of a protein called ALK. It is possible to create an efficient sequential therapy by making use of the fact that lorlatinib and crizotinib do not share any on-target resistance mechanisms in common with one another. This was demonstrated in a patient who was already being treated with various ALK inhibitors before enrolling in the clinical trial investigating the effectiveness of lorlatinib (NCT01970865). Lorlatinib was able to help overcome acquired resistance to crizotini (Hrustanovic et al., 2015). A therapeutic advantage may be achieved with lorlatinib in ALK-positive NSCLC patients who have previously been treated with one or more ALK inhibitors, according to the preliminary findings of clinical trials (ASCO 2017, Abstract 9006) (Kim et al., 2017).

ROS1:

ROS1 is a protein that belongs to the family of proteins that are known as insulin receptor tyrosine kinases. As part of a study that looked at tyrosine kinase signaling in NSCLC cell lines and over 150 NSCLC tumors, ROS1 fusions with SLC34A2 or CD74 were found in 2007 in an NSCLC cell line called HCC78 as well as in a patient's tumor. This discovery was made over the course of the investigation. The AKT-PI3K, STAT3, and MAPK-RAS pathways are activated when there is carcinogenic activity involving ROS1. ROS1 fusions are detected in 1% to 2% of NSCLC patients, the majority of which are younger people who have never smoked. The CD74-ROS1 fusion is by far the most common type of ROS1 fusion. In addition to SLC34A2 and CD74, several other 5' fusion partners of ROS1 have been discovered in NSCLC. These partners include FIG, CDC4, EZR, and CCDC6. Crizotinib and ceritinib are examples of ALK inhibitors, and both of these drugs have been shown to reduce ROS production. Crizotinib showed promise as an anti-tumor agent in a clinical trial that investigated the effects of the drug on patients with ROS1 fusion-positive NSCLC. The overall response rate that was seen was 72%, and the median PFS was 19.2 months. As a result of this achievement, the FDA granted crizotinib approval in 2016 for the treatment of patients whose advanced-stage NSCLC tumors were positive for ROS1 fusion. It has been shown that resistance to crizotinib can be conferred by a mutation in the CD74-ROS1 G2032R gene that occurs in the solvent front residue. A CD74-ROS1 positive NSCLC patient who had relapsed 13 months after receiving crizotinib treatment was treated with ceritinib, an ALK inhibitor that was shown to block the crizotinib-resistant gatekeeper mutation L2026M. It was claimed that ceritinib provided therapeutic benefit to the patient (Zou et al., 2015). A drug called cabozantinib, which is more selective for ROS1 than ALK and has shown efficacy in inhibiting the clinically-reported crizotinib-resistant CD74-ROS1 G2032R protein as well as preclinically demonstrated crizotinib-refractory ROS1

substitutions such as the solvent front L1951R substitution and the gatekeeper mutation L2026M, is currently being tested on ROS1 fusion-positive NSC On the basis of the remarkable efficacy of lorlatinib in pre-clinical investigations against CD74-ROS1 mutations FIG-ROS1, L2026M, and G2032R, clinical trials are currently being conducted to investigate the efficacy of the third generation ALK inhibitor prolactin in ROS1-fusion-positive advanced NSCLC patients. In addition, the ROS1 inhibitor entrectinib has demonstrated anti-tumorogenic advantages in patients diagnosed with ROS1-positive non-small cell lung cancer (Drilon et al., 2017).

RET:

RET proteins are discovered in just 1% to 2% of NSCLC patients, and they are most prevalent in people who are young and have never smoked. It has been determined that NCO4, KIF5B, EML4, CCDC, and TRIM are the 5' fusion partners of RET in NSCLC (Gautschi et al., 2017). 165 RET-fusion positive patients participated in a global clinical trial that evaluated the effectiveness of multi-kinase inhibitors which have been approved by the EDA or FDA. These inhibitors included cabozantinib (21 patients), vandetanib (11 patients), sunitinib (10 patients), sorafenib (2 patients), alectinib (2 patients), lenvatinib (2 patients), nintedanib (2 patients), ponatinib (1 patient). This trial showed that the targeted therapy had limited efficacy in treating lung cancer when compared to other targeted therapies. The median progression-free survival was 2.3 months, and the median overall survival was 6.8 months. It is speculated that the absence of a robust response is caused by the toxicities created by the off-target effects of these kinase inhibitors, which result in the suboptimal dose and, as a result, insufficient RET inhibition or the effect of 5' fusion partners on the degree of inhibition (Li et al., n.d.,2018).

NTRK:

Chromosomal translocations in the genes that code for neurotrophic tyrosine kinase (NTRK) have been discovered in a variety of solid malignancies, including lung cancer, where the incidence is believed to be somewhere between two and three percent. The tropomyosin-related kinase (Trk) proteins TrkA, TrkB, and TrkC are each encoded by their respective NTRK1, 2, and 3 genes (Passiglia et al., 2016). Lung cancer patients have been shown to have NTRK1 and NTRK2 translocations with 5' fusion partners such as CD74, and SQSTM1. An NSCLC patient who had a fusion of SQSTM1 and NTRK1 had a significant response to the anti-tumor effects of entrectinib, which is a pan-Trk inhibitor with actions that inhibit ROS1 and ALK. Three clinical trials (NCT02122913, n=8 adults; NCT02637687, n=12 pediatric patients; and NCT02576431, n=35 adult/adolescent patients) are currently being conducted to investigate the efficacy of the pan-TRK inhibitor larotrectinib (LOXO-101) on NTRK fusion in a variety of malignancies in adult and pediatric patients. These trials are looking at the effectiveness of larotrectin in this particular study, there are a total of five lung cancer patients. The results of the ongoing trial were revealed at the ASCO convention in 2017. The overall response rate (ORR) of 88% in the individuals who were studied provides cause for optimism. On the other hand, the response in four patients has been reduced due to resistance caused by solvent front mutations. On the other hand, LOXO-195, a Trk inhibitor of the second generation, has shown promise in such situations, albeit in an early and preliminary stage of clinical testing (Amatu et al., 2016).

KRAS:

KRAS mutations are found in around 30% of lung cancer patients, most of whom are smokers. Although KRAS mutant adenocarcinomas have hyperactivated the RAS-MAPK pathway, medicines that inhibit the downstream protein, such as selumetinib and trametinib,

have been generally unsuccessful in them. In patients with previously treated advanced-stage KRAS-mutant NSCLC, drug trials evaluating the effectiveness of trametinib/selumetinib with docetaxel combined with docetaxel alone failed to demonstrate the advantage of the combinations over docetaxel alone. These trials compared the efficacy of trametinib or selumetinib with docetaxel combined with docetaxel alone. In studies conducted before clinical trials, the drug ARS853 was shown to have an effective activity against KRAS G12C by sequestering the protein in its GDP-bound inactive state. Research into therapeutic applications of such direct KRAS inhibitors is warranted (Janne et al., 2017; Lito et al., 2016).

4.2 Immunotherapy

Monoclonal Antibodies

The dynamic between genetic and molecular features of cancer cells, especially their interaction with the immune system, plays a crucial role in the start and progression of lung cancer. Vaccines have been the customary focus of immunotherapy for lung cancer patients. However, these treatments have often failed due to insufficient immune activation. Different ligands and receptors damping or heightening the immunological synapse have been the focus of immunotherapy's various techniques. The most important targets for immunotherapy are the checkpoint inhibitors generated upon T-cell activation, such as CTLA-4-B7, which regulates the immunological synapse between T cells and dendritic cells in lymph nodes and suppresses T-cell activation, or PD-1-programmed death-ligand (PD-L2, PD-L1) which inhibits immune rejection or the effector phase. Antibody-directed therapies targeting these checkpoints are already playing a crucial role in the management of advanced lung cancer and other tumors. They have demonstrated amazing early success in many different types of cancer. Numerous monoclonal antibodies targeting the programmed death 1 (PD-1) receptor

or its ligand PD-L1 (durvalumab, atezolizumab, avelumab) have entered clinical use, and others are in the early stages of research and development (Postow et al., 2015).

Patients with advanced NSCLC who had not been treated previously showed quick and persistent responses in early clinical studies with these medicines. Importantly, survival outcomes are outstanding even though progression-free survival rates are not impressive (median 2-4 months; progression-free survival at one year 20%) (Brahmer et al., 2015). Two-year survival was 24% in the overall population, and 42% in the subset of patients treated at the dose selected for further development (3 mg/kg every two weeks) in a study of patients with NSCLC who received nivolumab and were followed for 27 months; three-year survival was 18% in the overall population and 27% in the development dose subset of patients. While clinical effectiveness seems independent of histology, in most trials, smokers and patients with PD-L1-positive expression experienced better improvement. Only 10% of patients experience serious (grades III-IV) side effects; hence these drugs have a rather benign toxic profile. Asthenia, lethargy, loss of appetite, nausea, and diarrhea were the most common side effects (Fehrenbacher et al., 2016).

Rash, colitis, transaminitis, pneumonitis, and endocrinopathies were all immune-related adverse effects in fewer than 10% of patients. Four randomized studies indicate the clinical efficacy and safety of anti-PD-1 or anti-PD-L1 medicines. CheckMate 017, a Phase III study compared nivolumab to docetaxel in patients with SCC who had advanced on platinum-based chemotherapy and compared nivolumab to docetaxel in patients with non-SCC NSCLC, respectively. In patients with SCC, nivolumab enhanced survival (median 9.2 vs. 6.0 months, HR 0.59), and the degree of the survival benefit was associated with PD-L1 expression (HR of 0.53) and PD-L1 percentage (HR of 0.53). If tumor cells express PD-L1 at a frequency of at least 50%, regardless of histology, the FDA has approved pembrolizumab for use in the second or subsequent lines of treatment. Even though studies comparing immune therapy and

chemotherapy as the first-line treatment for advanced NSCLC are still pending (Fehrenbacher et al., 2016).

Atezolizumab fared much better than docetaxel in a randomized phase 2 trial in patient groups with non-small cell lung cancer of any histology subtype. Evidence suggests that PD-1 inhibitors are superior to regular chemotherapy as a second-line treatment for SCC and, at the very least, for PD-L1-positive non-SCC of the lung. However, the most accurate way to define PD-L1 expression for benefit prediction is still unclear. Yet, these medications are not helpful for all individuals with PD-L1-positive non-small cell lung cancer; however, particular malignancies react to them even though they exhibit low amounts of PD-L1 or even when they are PD-L1-negative. Current research compares PD-L1 assays on a standardized group of NSCLC tumors to better understand their similarities and differences. Early trials with anti-PD-1 or anti-PD-L1 inhibitors have shown promising results in previously untreated patients, with 1-year survival exceeding 70% in PD-L1-positive tumors. These medicines are currently being tested in several clinical trials that compare them to platinum combination regimens used as first-line therapy. Early data are positive in other thoracic malignancies, such as small-cell lung cancer (SCLC) and mesothelioma, and trials are also ongoing in earlier disease settings (stage III, post-surgery) (Kerr & Hirsch, 2016).

Cancer Vaccines

A form of immunotherapy known as cancer vaccines aims to treat conditions that are specific to malignancies or that are associated with tumors. This encourages the microenvironment to fight these entities by enhancing the antitumor response that is mediated by T cells or B cells. Both autologous and allogeneic forms of tumor cell vaccines are possible. Autologous vaccinations are made from the patient's own tumor cells, whereas allogeneic vaccines are obtained from human tumor cell lines. These vaccines are separated into a variety of

categories, such as genetic vaccines (also known as DNA vaccines), cell-based vaccines (also known as whole tumor vaccines), protein vaccines, small molecule vaccines, bacteria vaccines and DC-based vaccines. The target entity determines which category the vaccine belongs to (Weir et al., 2014).

Coly was the first person to suggest the idea of cancer vaccinations. He did this by administering intra tumoral injections of inactive versions of the bacteria Streptococcus pyogenes and Serratia marcescens to patients who were suffering from sarcoma. Because of the inflammatory response that the bacteria provoked, the sarcomas were able to become smaller in size. Later on, tumor vaccines that were made up of melanoma-associated antigen-A3 (MAGE-A3) were used in a large scale phase III clinical trial as an adjuvant to surgery for the treatment of early stage MAGE-A3+ NSCLC patients. According to the results of the trial, there was no difference in survival rates between patients who were given the vaccine and those who were given a placebo. Tecemotide, which is an analog of mucin-1 and a glycoprotein that was discovered to be overexpressed in NSCLC, was another vaccination that was tested. Patients who received the Tecemotide tumor vaccine following chemotherapy and radiation therapy had the same survival rates as patients who acted as controls (Oliveres et al., 2018). Antibodies that have been modified to identify particular cancer cell antigens are referred to as targeted antibodies. In addition to the use of cancer-specific monoclonal antibodies, also known as mAb, two potent customizations known as antibody-drug conjugates (ADCs) and bi-specific T cell-engaging antibodies (BiTEs) are currently being tested as anti-cancer immunotherapies (LaRocca & Warner, 2018). Several immunotherapies have already been approved, primarily for treating hematological malignancies. ADCs are highly potent constructions of tumor-specific mAbs that are loaded with anti-cancer medicines that are effective once a tumor cell has been taken in the ADC and processed (Yu & Liu, 2019).

Adoptive Cell Therapy

Adoptive cell therapy (ACT) is a method of immunotherapy that involves expanding patient-derived lymphocytes *ex vivo* before reinfusing them back into the patient. This practice is founded on the hypothesis that the expansion of tumor-reactive lymphocytes is made possible when carried away from the suppressive effects of the TME. Patients with various malignancies that had progressed past the point where traditional treatment was effective participated in the earliest trial ever conducted in this sector (Garofalo et al., 2018). The so-called lymphokine-activated cells, were generated by the experts by culturing lymphocytes taken from the peripheral blood of patients with IL2. After then, several doses of IL-2 coupled with LAK cells were administered to the patients. Only one patient with melanoma exhibited a complete response, although 11 out of 25 subjects with LUAD, renal cancer, colorectal cancer, or melanoma showed at least some degree of remission. The findings provided support for the use of immunotherapies that are based on ACT in the treatment of metastatic tumors and opened the door to the investigation of additional forms of immunotherapy. According to research, the ACT is capable of making use of a specific subset of immune cells known as tumor-infiltrating lymphocytes (TILs) (Corraliza-Gorjón et al. 2017).

They are a subset of lymphocytes that are more likely to recognize cancer antigens and react by secreting pro-inflammatory cytokines, which allow them to penetrate the tumor micro-environment. TILs were obtained from tumors that had been surgically excised and cultured outside of the patient's body before being rapidly developed and administered back into the patient. The administration of chemotherapy prior to TIL infusion led to an increase in the quantities of the homeostatic cytokines IL-21, IL-15, and maybe IL-17, while simultaneously leading to a decrease in immune cells that mediate tolerance. This resulted in an improvement in TILs' capacity to produce tumor regression. Additionally, it was demonstrated that this

technique reduced myeloid-derived suppressor cells and T regulatory cells, two elements of the TME's anti-tumor immunity (van Belzen & Kesmir, 2019).

The high mutation rates seen in carcinoma provide tumor-specific antigens, or neo-antigens, that are readily identified by TILs; this observation led researchers to conclude that adoptive call treatment was mostly limited to patients with carcinoma. The potential for adoptively transferring TILs that have been genetically altered to react to mutational epitopes present only in malignancies has therefore been investigated. Next, highly altered cancers like melanoma and lung cancer were targeted by engineering peripheral T cells to express TCRs that target particular neo-antigens seen in tumors. Clinical results for several tumor types have been encouraging after adoptive transfer of genetically modified lymphocytes targeting specific NY-ESO1 epitopes. One of the most well-known cancer-testis antigens is called NY-ESO1. As a component of the modified T cell treatment being researched and conducted in clinical trials for lung cancers such as NSCLC. Due to the MHC-down regulating nature of some cancers, it was discovered through in-depth research of TCR engineered TILs' mechanism of action that the need for MHC recognition is a severe constraint (Kimura et al., 2015).

Combination Therapy

The vast majority of patients who were treated with a single-agent immune checkpoint blockade (ICB) showed durable disease control. Despite this, these patients were unable to activate an antitumor immune response, which usually led to patient recurrence and tumor tolerance to the ICB. As a result, the option of combining the two or more immunotherapies in the goal of generating synergistic interactions became intriguing (Shi et al., 2019).This is because the survival benefits of combination therapy are known to be greater than those of monotherapy in the treatment of some types of cancer. In recent years, the focus of clinical

trials has evolved away from sequential monotherapies and toward evidence-based analyses of the potential of integrating multiple treatments that have non-redundant anticancer activity. In point of fact, the two types of ICBs that have been researched the most, anti-CTLA-4 and anti-PD-1 mAbs, generate antitumor responses that are distinct from one another while simultaneously complementing one another, all while having a suppressive effect on T cells. The proliferation of T cells can be controlled by CTLA-4, which does this by suppressing auto-reactive T cells. This is particularly effective in lymph nodes and during the early priming stages of immune system activation. PD-1, on the other hand, is expressed on a wider variety of cell types than CTLA-4 and inhibits T cell activation during the late effector phase as well as in peripheral regions (Buchbinder & Desai, 2016). Research on dual ICB was prompted by the fact that the mechanisms of the blockers are complimentary to one another. Nivolumab with ipilimumab concurrent therapy demonstrated clinical benefits, including progression-free survival, lower toxicity, a better objective response rate, a steady improvement in survival benefit when compared to nivolumab alone. Although a high TMB and an improved response were substantially related in the dual ICB group, it appeared that the enhanced response to dual ICB outweighed the requirement for abundant PD-L1 expression found in monotherapy. It was also found that combination immunotherapies could not increase survival in NSCLC patients with low TMB, which is in accordance with results from gastrointestinal cancers. While some drug combinations were shown to be less hazardous than the individual medications they were combined with, other immune-related adverse effects were discovered. These side effects might be remedied if the necessary measures were taken (LaRocca & Warner, 2018).

4.3 Gene Editing Techniques

To combat cancer, scientists have proposed using genome editing engineering to introduce knock-in and knock-out variants in specific genes. Transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) are two examples of the DNA domain binding conventional techniques that have had a major impact on molecular biology by facilitating the development of animal and cellular cancer models and therapeutic research. However, widespread adoption has been hampered by complexity and prolonged processes associated with these approaches (Kregel et al., 2020).

In molecular biology, the process of editing the genome is typically carried out by fusing the pattern of a specific DNA binding domain with the sequence of a non-specific binding domain. This allows for the facilitation of targeted mutations within a gene of interest through the use of DNA double-strand breaks (DSBs), which in turn activates the DNA repair process. There is substantial evidence that programmable nucleases, which have been used to fix defects in diseases such as cancer, SCID (severe combined immunodeficiency), sickle cell disease and hemophilia, have increased the effectiveness of genome editing. These diseases include sickle cell disease and SCID (severe combined immunodeficiency). Both transcription activator-like effector nucleases (also known as TALENs) and zinc-finger nucleases (also known as ZFNs) are examples of genome editing techniques that have been utilized extensively in the past for the purpose of DNA repair via gene targeting. Specificity and affinity of nucleases are key to the success of these methods. Several oncogenes, including human Papillomavirus oncogenes, have been the traditional targets of TALEN and ZFN editing technologies. Based on a study, Compo-Zr ZFNs substantially impacted the E6 gene in HPV-16 cell cultures with editing activity of 50%, while they exhibited decreased effectiveness in SiHa and CaSki cervical cell lines. To give just one illustration, TALENs

were able to effectively change the E7 genome in SiHa cells but were incapable of doing so in plenty of other cell lines. Recent research has shown that ZFNs may effectively eliminate HIV-1 pro-viral DNA, providing renewed optimism for single- or dual-targeting (TALEN/ZFN) of other viral genomes. Despite the fact that these genome editing methods have shown somewhat better outcomes, they are both extremely time-consuming and less efficient, and they both exert off-target effects (Chen et al., 2020).

To compensate for the inadequacies of earlier methods, a new, more effective, and precise strategy was required. CRISPR is an improvement over other conventional genome editing methods like TALENs and ZFNs, which are just now beginning to make a name for themselves in the molecular field. In comparison to more traditional methods (ZFNs, TALENs, and RNAi), CRISPR-use Cas9's of brief sequence of sg-RNA that is complementary to the target DNA was shown to be not only more effective but also less expensive. Evidence suggests that the adaptive immune system of prokaryotes (*Escherichia coli*) was responsible for discovering CRISPR/Cas9. This system is highly effective against phages and viruses (Fu et al., 2014). Evidence is mounting that the CRISPR/Cas9 tool is superior to RNAi for genome editing due to its ability to selectively target DNA and hence temporarily or permanently silence genes (Cheung et al., 2018). The multi-stage mutational development of cancer necessitates the creation of a novel, multi-purpose therapeutic medicine or technology. However, due to CRISPR and Cas9's ability to edit multiple genes at the same time, it is seen as a parallel and straightforward target and is widely acknowledged as an appropriate analytical focus tool against cancer-associated somatic mutations *in vitro and in vivo* investigations. In addition, it is a target that is seen as a direct and parallel target (Cheung et al., 2018; Ng et al., 2020).

Patients' genetic material is modified by means of gene therapy. Directly targeting certain defective genes allows for the introduction of therapeutic genes into target cells of patients for the goal of curing disease. Antisense gene therapy, tumor-suppressor gene therapy, drug sensitive gene therapy, drug-resistant gene therapy and immunological gene therapy are other methods that make up the field of tumor gene therapy (Jiang et al., 2019). RAS, MYC, ROS1, ERBB2, ERBB1, RAF1, FOS, SRC, and JUN are just some of the oncogenes linked to this particular cancer. Genes including RB, TP53, APH, CDKN2A, NM23, MCC, and APC function as tumor suppressors in lung cancer. Both overexpression of oncogenes and mutations in tumor-suppressor genes were associated with this cancer in a study of people with the disease. In vitro testing of gene-editing therapies for treating cancer has progressed in recent years. In addition, there has been some success with lung cancer treatment for the use of gene editing technology (Zhang et al., 2017).

Oncogenes subvert regular regulatory mechanisms and aid in a cell's malignant development. Eukaryotic cells can develop uncontrollably as a result of oncogene products, as seen in cancer cells. Theoretically, using Cas9/CRISPR technology in cancer treatment is based on the concept of slowing or stopping of tumor cell development that results from the removal of a solitary oncogenic cancerous cells. The rapid application of Cas9/CRISPR gene editing technology has allowed for the accurate recognition of overexpressed or overactivated genes. This method offers fresh suggestions for the treatment of malignancies. The elimination of target tumor genesis that are either abundantly expressed, mutated, or over activated may be beneficial to the therapy of cancer. (Wu & Cao, 2019). In the context of Cas9 and CRISPR gene editing, oncogenes such as the EGFR receptor FAK, NESTIN, CTNND2, RSF1, and IGF1R have been the subject of research in recent years for the lung cancer therapy. These genes, which have been investigated through methods such as mutation or overexpression,

serve as oncogenes that aid in the growth of lung cancer and improve the capacity of lung cancer cells to infiltrate or spread (Bu et al., 2018).

The CRISPR/Cas9 approach has the potential to play a significant part in the screening of therapeutic targets. It is possible to swiftly screen potential target genes throughout the entire genome by combining small guide RNA found in the CRISPR library. Since the development of new tools in the field of molecular biology, conventional chemotherapy has been supplanted as the preferred treatment for advanced non-small cell lung cancer by molecular targeted medicines that are driven by genotyping (Lu et al., 2020). EGFR-TKIs are one example that sees widespread use. In spite of this, EGFR-TKI resistance has garnered an increasing amount of interest over the past few years. A subsequent mutation in the EGFR gene, change in tissue phenotype, amplification of the target gene, activation of the bypass pathway and low expression or detection of anti-oncogenes are the primary causes of resistance. Numerous research published in recent years have shed light on the factors behind patients' susceptibility to targeted therapy for lung cancer. The combination of erlotinib hydrochloride and THZI have been demonstrated to decrease drug resistance in EGFR-dependent PC9 cells in the past. Erlotinib and THZ1 have a synergistic impact, which can be enhanced by inhibiting numerous genes associated to the MED1, EP300, and CREBBP transcription factor complex. On the basis of these findings, genes have been identified that selectively increase or reduce the effectiveness of transcription inhibition in cells that are resistant to a medication. Using the CRISPR/Cas9 technology, a number of experiments have been conducted to investigate genes in lung cancer that are connected to resistance to targeted therapies such as erlotinib and temozolomide. In the human NSCLC cell line HCC827, silencing the IGF1R gene through a technique known as knockout could considerably lower the level of treatment resistance to erlotinib (Xu et al., 2017).

In addition, some progress has been made in the investigation of genes linked to lung cancer inhibitors, such as EGFR, BRAF, ALK, MET, and MEK, through the application of Cas9 and CRISPR technology, which has shown some promise. This research has shown some promise in the fight against lung cancer (Maddalo et al., 2014). In one piece of research, the KEAP1 gene was silenced in lung cancer cell lines HCC364, NCI-H1299, HCC827, and NCI-H1975. These cell lines were used to analyze the disease. According to the findings of this study, inhibiting KEAP1 caused lung cancer cells to become less sensitive to the effects of inhibitors for NRAS, BRAF, EGFR, ALK, and KRAS mutations, which promoted the survival of the cell. In investigations on MET and MEK1 inhibitors in lung cancer cells, the deletion of gene editing of MET might lower cell activity, render the cells sensitive to inhibitors, inhibit colony formation of the cell, and inhibit cell growth. These effects were seen in the studies (Zhan et al., 2019).

Therefore, gene editing with CRISPR/Cas9 is able to also validate and suppress the therapeutic efficacy of targeted medications in lung cancer. During the process of carcinogenesis, the deactivation of genes that suppress cancer also plays a critical role (Lu et al., 2020). The products of expression of tumor-suppressor genes have the potential to promote cell differentiation, block cell migration and decrease cell proliferation in addition to having a detrimental impact on the progression of tumors. Oncogenes are activated when tumor-suppressor genes lose their function, undergo mutation, or are deleted, which ultimately leads to carcinogenesis. Researchers have identified mutated tumor-suppressor genes that are present in a wide variety of cancers and are expressed at low levels. These genes that inhibit the growth of tumors are essential treatment targets for the Cas9/CRISPR gene editing process. Through the use of the Cas9/CRISPR technology, tumor-suppressor genes can be targeted for their function, correction and activity can be restored in order to reduce the risk of developing cancer. It is possible that the Cas9/CRISPR technology for

targeted regeneration of tumor-suppressor genes that have been inactivated will play an important and beneficial part in the treatment of lung cancer as well (Togashi et al., 2015).

Chapter 5

Challenges and Future Directions

The advanced stage of small cell lung cancer (SCLC) is notoriously difficult to treat due to the unique patient and tumor features that are associated with this illness. As a consequence of these characteristics, patients have fewer treatment alternatives open to them; hence, patients typically do not acquire systemic therapy, despite the recommendations of clinical practice guidelines. Due to the interaction of all of these factors, the prognosis for people afflicted with advanced SqCLC is not good. In addition, individuals who have advanced SqCLC want information that is disease-specific in order to better comprehend the many therapy options available to them. Although there is a wealth of knowledge available on adenocarcinoma, information that is explicitly focused on small cell lung cancer has just become available very lately. The newly approved treatments nivolumab, pembrolizumab, atezolizumab, necitumumab, ramucirumab, and afatinib have obtained important survival benefits for patients with SqCLC when compared to previous treatments. These recent treatments also represent the first major updates to the treatment armament for advanced SqCLC in over a decade (Socinski et al., 2018).

Future preventive efforts and research needs to prioritize non–tobacco-related modifiable risk factors and provide more clarity with regard to modern exposures, such as non-cigarette tobacco smoking products. There is likely benefit to maintaining a healthy body weight, increased physical activity, and healthy eating with a diet rich in whole grains, fruit, and vegetables. From a population health perspective, continued measures to promote tobacco smoking avoidance or cessation, protect workers from known inhaled carcinogens, and maintain clean air are needed to facilitate a decreased risk of lung cancer. The challenge in

the future will be to modify the impact of all risk factors while continuing to expand our knowledge of the genetic and molecular basis of carcinogenesis (Bade & Dela Cruz, 2020).

Even though there have been many improvements, the way cancer is treated now is still overly simplistic. Good clinical responses have been achieved by targeting single molecular abnormalities or cancer pathways, which has had a moderate impact on patients' chances of survival in various cancers. We hypothesize that in the not-too-distant future, a potentially fruitful therapeutic approach to treating cancer could involve the use of medication combinations that target many molecular changes or cancer biomarkers, in a manner that is analogous to how we have approached the treatment of HIV. The encouraging effectiveness of immunotherapy, in particular checkpoint inhibitors, suggests that PD-1/L1 blockage could become an essential component of these combos. Combining immunotherapies with either one another (for example, using two checkpoint inhibitors together or using one checkpoint inhibitor in conjunction with an immune-stimulatory antibody) or with other anticancer medicines is a possibility (including targeted agents in oncogene driven cancers). When putting these combination tactics into practice in the clinic, toxicity will be one of the primary limiting variables, and the timely recognition and control of adverse effects will undoubtedly be an essential component for the treatment's overall effectiveness. One of the primary challenges and enticing strategies in the future will be personalized combination strategies according to pathways or key aspects that specifically drive each patient's tumor biology within next generation precision oncology initiatives (Zugazagoitia et al., 2016).

The vast majority of the easily available procedures and approaches that are currently being applied are only capable of detecting cancer in its severe forms, which is when therapy and a remedy may no longer be useful in controlling the condition. The primary diagnostic tools for lung cancer are the bronchoscope and biopsy. It would appear that the level of experience had

by the bronchoscopist is absolutely necessary for making a precise diagnosis when it comes to bronchoscopy. Patients may suffer some discomfort during the bronchoscopy operation, despite the fact that it is a minimally invasive approach. However, difficulties are possible, and this is especially true if biopsies are taken from the area that is in issue. Screening for the initial development of lung cancer is required in order to initiate therapy at an early stage in order to improve the prognosis of the disease. The search for biomarkers in human fluids has become an appealing method in recent years and has made significant headway in the right direction. This development occurred during the past several years. The vast majority of the published biomarkers are detectable by employing technologies such as metabolomics, polymerase chain reaction (PCR). In addition, the experiment on urine samples has demonstrated that the procedure can be completed in a couple of hours and successful result can be achieved. This discovery was made possible by the fact that people with lung cancer have an increased frequency of urination. The development of more reliable tests for the early diagnosis of lung cancer should be a priority so that the appropriate targeted treatment can be provided at the early stage of cancer (Nooreldeen & Bach, 2021).

Chapter 6

Conclusion

In order to properly diagnose, categorize, and treat lung cancer, a multidisciplinary approach is required. This is because lung cancer is a disease that is both complex and diverse. For cancer treatment to be more individualized and effective in the future, the key focus needs to be on the creation of targeted therapy as well as large-scale molecular analysis. Targeted therapy, nonetheless, would help save patients and clinics from expensive personnel, emotional, and pharmaceutical expenditures when factoring in the costs for therapies, including resection and medication techniques, as well as the patient's afflictions due to a re-emerged full-blown malignancy. Even though, this cancer still is a major cause of death. The future is hopeful if lung cancer screening is implemented on a massive scale. Because of the broad clinical presentation, doctors should investigate the diagnosis of any past or current smoker presenting troubling symptoms. There are currently several minimally invasive diagnostic and staging technologies available, and there has been significant progress made in the comprehension of the biology of lung cancer, resulting in numerous new therapeutic options.

References

Amaria, R. N., Reddy, S. M., Tawbi, H. A., Davies, M. A., Ross, M. I., Glitza, I. C., Cormier, J. N., Lewis, C., Hwu, W. J., Hanna, E., Diab, A., Wong, M. K., Royal, R., Gross, N., Weber, R., Lai, S. Y., Ehlers, R., Blando, J., Milton, D. R., … Wargo, J. A. (2018). Neoadjuvant immune checkpoint blockade in high-risk resectable melanoma. *Nature Medicine*, *24*(11), 1649–1654. https://doi.org/10.1038/s41591-018-0197-1

Amatu, A., Sartore-Bianchi, A., & Siena, S. (2016). NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. *ESMO Open*, *1*(2), 1–10. https://doi.org/10.1136/esmoopen-2015-000023

Bade, B. C., & Dela Cruz, C. S. (2020). Lung Cancer 2020: Epidemiology, Etiology, and Prevention. *Clinics in Chest Medicine*, *41*(1), 1–24. https://doi.org/10.1016/j.ccm.2019.10.001

Bezjak, A., Paulus, R., Gaspar, L. E., Timmerman, R. D., Straube, W. L., Ryan, W. F., Garces, Y. I., Pu, A. T., Singh, A. K., Videtic, G. M., McGarry, R. C., Iyengar, P., Pantarotto, J. R., Urbanic, J. J., Sun, A. Y., Daly, M. E., Grills, I. S., Sperduto, P., Normolle, D. P., … Choy, H. (2019). Safety and efficacy of a five-fraction stereotactic body radiotherapy schedule for centrally located non–small-cell lung cancer: NRG Oncology/RTOG 0813 trial. *Journal of Clinical Oncology*, *37*(15), 1316–1325. https://doi.org/10.1200/JCO.18.00622

Brahmer, J., Reckamp, K. L., Baas, P., Crinò, L., Eberhardt, W. E. E., Poddubskaya, E., Antonia, S., Pluzanski, A., Vokes, E. E., Holgado, E., Waterhouse, D., Ready, N., Gainor, J., Arén Frontera, O., Havel, L., Steins, M., Garassino, M. C., Aerts, J. G., Domine, M., … Spigel, D. R. (2015). Nivolumab versus Docetaxel in Advanced Squamous-Cell Non–Small-Cell Lung Cancer. *New England Journal of Medicine*,

373(2), 123–135. https://doi.org/10.1056/nejmoa1504627

Brooks, P. (2015). Robotic-Assisted Thoracic Surgery for Early-Stage Lung Cancer: A Review. *Association of Registered Nurses*, *102*(1), 40–49. https://doi.org/10.1016/j.aorn.2015.04.022

Buchbinder, E. I., & Desai, A. (2016). CTLA-4 and PD-1 pathways similarities, differences, and implications of their inhibition. *American Journal of Clinical Oncology: Cancer Clinical Trials*, *39*(1), 98–106. https://doi.org/10.1097/COC.0000000000000239

Burton, D. R., Mascola, J. R., Diskin, R., Nussenzweig, M. C., Bjorkman, P. J., Morbach, H., Eichhorn, E. M., Liese, J. G., Girschick, H. J., Shih, T. A., Meffre, E., Roederer, M., Nussenzweig, M. C., Porto, J. M. D., Haberman, A. M., Kelsoe, G., & Shlomchik, M. J. (2016). *Re s ear ch | r e p o r t s*. *351*(6280), 1463–1470.

Campbell, J. D., Alexandrov, A., Kim, J., Wala, J., Berger, A. H., Pedamallu, C. S., Shukla, S. A., Guo, G., Brooks, A. N., Murray, B. A., Imielinski, M., Hu, X., Ling, S., Akbani, R., Rosenberg, M., Cibulskis, C., Ramachandran, A., Collisson, E. A., Kwiatkowski, D. J., … Meyerson, M. (2016). Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nature Genetics*, *48*(6), 607–616. https://doi.org/10.1038/ng.3564

Cancer, S. L. (2015). Clinicopathologic Features of Resected. *The Annals of Thoracic Surgery*, 1–8. https://doi.org/10.1016/j.athoracsur.2015.01.034

Carreno, B. M., Magrini, V., Becker-Hapak, M., Kaabinejadian, S., Hundal, J., Petti, A. A., Ly, A., Lie, W. R., Hildebrand, W. H., Mardis, E. R., & Linette, G. P. (2015). A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. *Science*, *348*(6236), 803–808. https://doi.org/10.1126/science.aaa3828

Chatziandreou, I., Tsioli, P., Sakellariou, S., Mourkioti, I., Giannopoulou, I., Levidou, G.,

Korkolopoulou, P., Patsouris, E., & Saetta, A. A. (2015). Comprehensive molecular analysis of NSCLC; Clinicopathological associations. *PLoS ONE*, *10*(7), 1–18. https://doi.org/10.1371/journal.pone.0133859

Chen, S., Yao, Y., Zhang, Y., & Fan, G. (2020). CRISPR system: Discovery, development and off-target detection. *Cellular Signalling*, *70*(December 2019), 109577. https://doi.org/10.1016/j.cellsig.2020.109577

Cheung, A. H. K., Chow, C., Zhang, J., Zhou, Y., Huang, T., Ng, K. C. K., Or, T. C. T., Yao, Y. Y., Dong, Y., Fung, J. M. W., Xiong, L., Chan, A. K. Y., Lung, W. M. R., Kang, W., & To, K. F. (2018). Specific targeting of point mutations in EGFR L858R-positive lung cancer by CRISPR/Cas9. *Laboratory Investigation*, *98*(7), 968–976. https://doi.org/10.1038/s41374-018-0056-1

Collisson, E. A., Campbell, J. D., Brooks, A. N., Berger, A. H., Lee, W., Chmielecki, J., Beer, D. G., Cope, L., Creighton, C. J., Danilova, L., Ding, L., Getz, G., Hammerman, P. S., Hayes, D. N., Hernandez, B., Herman, J. G., Heymach, J. V., Jurisica, I., Kucherlapati, R., … Cheney, R. (2014). Comprehensive molecular profiling of lung adenocarcinoma: The cancer genome atlas research network. *Nature*, *511*(7511), 543–550. https://doi.org/10.1038/nature13385

Corraliza-Gorjón, I., Somovilla-Crespo, B., Santamaria, S., Garcia-Sanz, J. A., & Kremer, L. (2017). New strategies using antibody combinations to increase cancer treatment effectiveness. *Frontiers in Immunology*, *8*(DEC). https://doi.org/10.3389/fimmu.2017.01804

Dagogo-Jack, I., & Shaw, A. T. (2016). Crizotinib resistance: Implications for therapeutic strategies. *Annals of Oncology*, *27*(Supplement 3), iii42–iii50. https://doi.org/10.1093/annonc/mdw305

Davare, M. A., Vellore, N. A., Wagner, J. P., Eide, C. A., Goodman, J. R., Drilon, A., Deininger, M. W., O'Hare, T., & Druker, B. J. (2015). Structural insight into selectivity and resistance profiles of ROS1 tyrosine kinase inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(39), E5381–E5390. https://doi.org/10.1073/pnas.1515281112

De Langen, A. J., & Smit, E. F. (2017). Therapeutic approach to treating patients with BRAF-mutant lung cancer: Latest evidence and clinical implications. *Therapeutic Advances in Medical Oncology*, *9*(1), 46–58. https://doi.org/10.1177/1758834016670555

Delaney, G. P., & Barton, M. B. (2015). Evidence-based Estimates of the Demand for Radiotherapy. *Clinical Oncology*, *27*(2), 70–76. https://doi.org/10.1016/j.clon.2014.10.005

Denisenko, T. V., Budkevich, I. N., & Zhivotovsky, B. (2018). Cell death-based treatment of lung adenocarcinoma article. *Cell Death and Disease*, *9*(2). https://doi.org/10.1038/s41419-017-0063-y

Dijkstra, K. K., Voabil, P., Schumacher, T. N., & Voest, E. E. (2016). Genomics-and transcriptomics-based patient selection for cancer treatmentwith immune checkpoint inhibitors a review. *JAMA Oncology*, *2*(11), 1490–1495. https://doi.org/10.1001/jamaoncol.2016.2214

Drilon, A., Siena, S., Ou, S. H. I., Patel, M., Ahn, M. J., Lee, J., Bauer, T. M., Farago, A. F., Wheler, J. J., Liu, S. V., Doebele, R., Giannetta, L., Cerea, G., Marrapese, G., Schirru, M., Amatu, A., Bencardino, K., Palmeri, L., Sartore-Bianchi, A., … De Braud, F. G. (2017). Safety and antitumor activity of the multitargeted pan-TRK, ROS1, and ALK inhibitor entrectinib: Combined results from two phase I trials (ALKA-372-001 and

STARTRK-1). *Cancer Discovery*, *7*(4), 400–409. https://doi.org/10.1158/2159-8290.CD-16-1237

Duraiswamy, J., Kaluza, K. M., Freeman, G. J., & Coukos, G. (2013). Dual blockade of PD-1 and CTLA-4 combined with tumor vaccine effectively restores T-cell rejection function in tumors. *Cancer Research*, *73*(12), 3591–3603. https://doi.org/10.1158/0008-5472.CAN-12-4100

Fehrenbacher, L., Spira, A., Ballinger, M., Kowanetz, M., Vansteenkiste, J., Mazieres, J., Park, K., Smith, D., Artal-Cortes, A., Lewanski, C., Braiteh, F., Waterkamp, D., He, P., Zou, W., Chen, D. S., Yi, J., Sandler, A., & Rittmeyer, A. (2016). Atezolizumab versus docetaxel for patients with previously treated non-small-cell lung cancer (POPLAR): A multicentre, open-label, phase 2 randomised controlled trial. *The Lancet*, *387*(10030), 1837–1846. https://doi.org/10.1016/S0140-6736(16)00587-0

Forde, P. M., Kelly, R. J., & Brahmer, J. R. (2014). New strategies in lung cancer: Translating immunotherapy into clinical practice. *Clinical Cancer Research*, *20*(5), 1067–1073. https://doi.org/10.1158/1078-0432.CCR-13-0731

Frigerio, B., Bizzoni, C., Jansen, G., Leamon, C. P., Peters, G. J., Low, P. S., Matherly, L. H., & Figini, M. (2019). Folate receptors and transporters: Biological role and diagnostic/therapeutic targets in cancer and other diseases. *Journal of Experimental and Clinical Cancer Research*, *38*(1), 1–12. https://doi.org/10.1186/s13046-019-1123-1

Fu, Y., Sander, J. D., Reyon, D., Cascio, V. M., & Joung, J. K. (2014). Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. *Nature Biotechnology*, *32*(3), 279–284. https://doi.org/10.1038/nbt.2808

Garofalo, M., Villa, A., Rizzi, N., Kuryk, L., Mazzaferro, V., & Ciana, P. (2018). Systemic administration and targeted delivery of immunogenic oncolytic adenovirus encapsulated

in extracellular vesicles for cancer therapies. *Viruses*, *10*(10). https://doi.org/10.3390/v10100558

Gautschi, O., Milia, J., Filleron, T., Wolf, J., Carbone, D. P., Owen, D., Camidge, R., Narayanan, V., Doebele, R. C., Besse, B., Remon-Masip, J., Janne, P. A., Awad, M. M., Peled, N., Byoung, C. C., Karp, D. D., Van Den Heuvel, M., Wakelee, H. A., Neal, J. W., … Drilon, A. (2017). Targeting RET in patients with RET-rearranged lung cancers: Results from the global, multicenter RET registry. *Journal of Clinical Oncology*, *35*(13), 1403–1410. https://doi.org/10.1200/JCO.2016.70.9352

Geukes Foppen, M. H., Rozeman, E. A., Van Wilpe, S., Postma, C., Snaebjornsson, P., Van Thienen, J. V., Van Leerdam, M. E., Van Den Heuvel, M., Blank, C. U., Van Dieren, J., & Haanen, J. B. A. G. (2018). Immune checkpoint inhibition-related colitis: Symptoms, endoscopic features, histology and response to management. *ESMO Open*, *3*(1), 1–9. https://doi.org/10.1136/esmoopen-2017-000278

Goldstraw, P., Chansky, K., Crowley, J., Rami-porta, R., Asamura, H., Eberhardt, W. E. E., Nicholson, A. G., Groome, P., & Mitchell, A. (2015). *The IASLC Lung Cancer Staging Project : Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classi fi cation for Lung Cancer*. *11*(1), 39–51. https://doi.org/10.1016/j.jtho.2015.09.009

Goto, K., Ohe, Y., Shibata, T., Seto, T., Takahashi, T., Nakagawa, K., Tanaka, H., Takeda, K., & Nishio, M. (2016). Combined chemotherapy with cisplatin , etoposide , and irinotecan versus topotecan alone as second-line treatment for patients with sensitive relapsed small-cell lung cancer (JCOG0605): a multicentre , open-label , randomised phase 3 trial. *Lancet Oncology, 2045*(16), 1–11. https://doi.org/10.1016/S1470-2045(16)30104-8

Greig, S. L. (2016). Osimertinib: First Global Approval. *Drugs*, *76*(2), 263–273. https://doi.org/10.1007/s40265-015-0533-4

Haanen, J. B. A. G. (2017). Converting Cold into Hot Tumors by Combining Immunotherapies. *Cell*, *170*(6), 1055–1056. https://doi.org/10.1016/j.cell.2017.08.031

Hellmann, M. D., Chaft, J. E., Jr, W. N. W., Rusch, V., Pisters, K. M. W., Kalhor, N., Pataer, A., & Travis, W. D. (2014). Pathological response after neoadjuvant chemotherapy in resectable non-small-cell lung cancers : proposal for the use of major pathological response as a surrogate endpoint. *Lancet Oncology*, *15*(1), e42–e50. https://doi.org/10.1016/S1470-2045(13)70334-6

Hirsch, F. R., Scagliotti, G. V., Mulshine, J. L., Kwon, R., Curran, W. J., Wu, Y. L., & Paz-Ares, L. (2017). Lung cancer: current therapies and new targeted treatments. *The Lancet*, *389*(10066), 299–311. https://doi.org/10.1016/S0140-6736(16)30958-8

Hrustanovic, G., Olivas, V., Pazarentzos, E., Tulpule, A., Asthana, S., Blakely, C. M., Okimoto, R. A., Lin, L., Neel, D. S., Sabnis, A., Flanagan, J., Chan, E., Varella-Garcia, M., Aisner, D. L., Vaishnavi, A., Ou, S. H. I., Collisson, E. A., Ichihara, E., Mack, P. C., … Bivona, T. G. (2015). RAS-MAPK dependence underlies a rational polytherapy strategy in EML4-ALK-positive lung cancer. *Nature Medicine*, *21*(9), 1038–1047. https://doi.org/10.1038/nm.3930

Jänne, P. A., Van Den Heuvel, M. M., Barlesi, F., Cobo, M., Mazieres, J., Crinò, L., Orlov, S., Blackhall, F., Wolf, J., Garrido, P., Poltoratskiy, A., Mariani, G., Ghiorghiu, D., Kilgour, E., Smith, P., Kohlmann, A., Carlile, D. J., Lawrence, D., Bowen, K., & Vansteenkiste, J. (2017). Selumetinib plus docetaxel compared with docetaxel alone and progression-free survival in patients with KRAS-mutant advanced non-small cell lung cancer: The SELECT-1 randomized clinical trial. *JAMA - Journal of the American*

Medical Association, *317*(18), 1844–1853. https://doi.org/10.1001/jama.2017.3438

Kerr, K. M., & Hirsch, F. R. (2016). Programmed death ligand-1 immunohistochemistry: Friend or foe? *Archives of Pathology and Laboratory Medicine*, *140*(4), 326–331. https://doi.org/10.5858/arpa.2015-0522-SA

Kim, D. W., Mehra, R., Tan, D. S. W., Felip, E., Chow, L. Q. M., Camidge, D. R., Vansteenkiste, J., Sharma, S., De Pas, T., Riely, G. J., Solomon, B. J., Wolf, J., Thomas, M., Schuler, M., Liu, G., Santoro, A., Sutradhar, S., Li, S., Szczudlo, T., … Shaw, A. T. (2016). Activity and safety of ceritinib in patients with ALK-rearranged non-small-cell lung cancer (ASCEND-1): updated results from the multicentre, open-label, phase 1 trial. *The Lancet Oncology*, *17*(4), 452–463. https://doi.org/10.1016/S1470-2045(15)00614-2

Kim, D. W., Tiseo, M., Ahn, M. J., Reckamp, K. L., Hansen, K. H., Kim, S. W., Huber, R. M., West, H. L., Groen, H. J. M., Hochmair, M. J., Leighl, N. B., Gettinger, S. N., Langer, C. J., Rodríguez, L. G. P. A., Smit, E. F., Kim, E. S., Reichmann, W., Haluska, F. G., Kerstein, D., & Camidge, D. R. (2017). Brigatinib in patients with crizotinib-refractory anaplastic lymphoma kinase-positive non-small-cell lung cancer: A randomized, multicenter phase II trial. *Journal of Clinical Oncology*, *35*(22), 2490–2498. https://doi.org/10.1200/JCO.2016.71.5904

Kimura, H., Matsui, Y., Ishikawa, A., Nakajima, T., Yoshino, M., & Sakairi, Y. (2015). Randomized controlled phase III trial of adjuvant chemo-immunotherapy with activated killer T cells and dendritic cells in patients with resected primary lung cancer. *Cancer Immunology, Immunotherapy*, *64*(1), 51–59. https://doi.org/10.1007/s00262-014-1613-0

Kregel, S., Wang, C., Han, X., Xiao, L., Fernandez-Salas, E., Bawa, P., McCollum, B. L., Wilder-Romans, K., Apel, I. J., Cao, X., Speers, C., Wang, S., & Chinnaiyan, A. M.

(2020). Androgen receptor degraders overcome common resistance mechanisms developed during prostate cancer treatment. *Neoplasia (United States)*, *22*(2), 111–119. https://doi.org/10.1016/j.neo.2019.12.003

Kris, M. G., Hellmann, M. D., & Chaft, J. E. (2019). *Chemotherapy for Lung Cancers : Here to Stay*.

Langfort, R., & Szołkowska, M. (2012). International Association for the Study of Lung Cancer , American Thoracic Society , and European Respiratory Society recommendations on the microscopic classification of lung. *Pneumonologia I Alergologia Polska*, *September 2011*, 99–101.

LaRocca, C. J., & Warner, S. G. (2018). Oncolytic viruses and checkpoint inhibitors: combination therapy in clinical trials. *Clinical and Translational Medicine*, *7*(1). https://doi.org/10.1186/s40169-018-0214-5

Lehmann-horn, K., Sagan, S. A., Bernard, C. C. A., Sobel, A., Zamvil, S. S., Wanna, A. G. B., Noble, J. H., Carlson, M. L., Gifford, H., Dietrich, M. S., Haynes, D. S., Dawant, B. M., Considerations, S., Target, M., Inhibitors, R., Complex, T. S., Transplantation, R., Somers, M. J. G., Paul, E., … Dawant, B. M. (2014). This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please c. *The Laryngoscope*, *August*, 2–31.

Lei, Y., Feng, H., Qiang, H., Shang, Z., Chang, Q., Qian, J., Zhang, Y., Zhong, R., Fan, X., & Chu, T. (2020). Clinical characteristics and prognostic factors of surgically resected combined small cell lung cancer: a retrospective study. *Lung Cancer*, *146*, 244–251. https://doi.org/10.1016/j.lungcan.2020.06.021

Li, G. G., Somwar, R., Joseph, J., Smith, R. S., Hayashi, T., Yan, S., Wei, G., Oliver, J. W.,

Patel, R., Multani, P., & Ladanyi, M. (n.d.). *TITLE Antitumor Activity of RXDX-105 in Multiple Cancer Types with*.

Lito, P., Solomon, M., Li, L. S., Hansen, R., & Rosen, N. (2016). Cancer therapeutics: Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science*, *351*(6273), 604–608. https://doi.org/10.1126/science.aad6204

Lo Russo, G., Pusceddu, S., Proto, C., Macerelli, M., Signorelli, D., Vitali, M., Ganzinelli, M., Gallucci, R., Zilembo, N., Platania, M., Buzzoni, R., de Braud, F., & Garassino, M. C. (2016). Treatment of lung large cell neuroendocrine carcinoma. *Tumor Biology*, *37*(6), 7047–7057. https://doi.org/10.1007/s13277-016-5003-4

Lu, Y., Xue, J., Deng, T., Zhou, X., Yu, K., Deng, L., Huang, M., Yi, X., Liang, M., Wang, Y., Shen, H., Tong, R., Wang, W., Li, L., Song, J., Li, J., Su, X., Ding, Z., Gong, Y., … Mok, T. (2020). Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. *Nature Medicine*, *26*(5), 732–740. https://doi.org/10.1038/s41591-020-0840-5

Marshall, H. T., & Djamgoz, M. B. A. (2018). Immuno-oncology: Emerging targets and combination therapies. *Frontiers in Oncology*, *8*(AUG). https://doi.org/10.3389/fonc.2018.00315

Mok, T. S., Wu, Y.-L., Ahn, M.-J., Garassino, M. C., Kim, H. R., Ramalingam, S. S., Shepherd, F. A., He, Y., Akamatsu, H., Theelen, W. S. M. E., Lee, C. K., Sebastian, M., Templeton, A., Mann, H., Marotti, M., Ghiorghiu, S., & Papadimitrakopoulou, V. A. (2017). Osimertinib or Platinum–Pemetrexed in EGFR T790M–Positive Lung Cancer . *New England Journal of Medicine*, *376*(7), 629–640. https://doi.org/10.1056/nejmoa1612674

Moore, L. J., Wilson, M. R., Waine, E., & Vine, S. J. (2015). *Robotic technology results in*

faster and more robust surgical skill acquisition than traditional laparoscopy. 67–73. https://doi.org/10.1007/s11701-014-0493-9

Ng, S. R., Rideout, W. M., Akama-Garren, E. H., Bhutkar, A., Mercer, K. L., Schenkel, J. M., Bronson, R. T., & Jacks, T. (2020). CRISPR-mediated modeling and functional validation of candidate tumor suppressor genes in small cell lung cancer. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(1), 513–521. https://doi.org/10.1073/pnas.1821893117

Nooreldeen, R., & Bach, H. (2021). *Molecular Sciences Current and Future Development in Lung Cancer Diagnosis*. https://doi.org/10.3390/ijms22168661

Nyman, J., Hallqvist, A., Lund, J. Å., Brustugun, O. T., Bergman, B., Bergström, P., Friesland, S., Lewensohn, R., Holmberg, E., & Lax, I. (2016). SPACE – A randomized study of SBRT vs conventional fractionated radiotherapy in medically inoperable stage I NSCLC. *Radiotherapy and Oncology*, *121*(1), 1–8. https://doi.org/10.1016/j.radonc.2016.08.015

Okimoto, R. A., Lin, L., Olivas, V., Chan, E., Markegard, E., Rymar, A., Neel, D., Chen, X., Hemmati, G., Bollag, G., & Bivona, T. G. (2016). Preclinical efficacy of a RAF inhibitor that evades paradoxical MAPK pathway activation in protein kinase BRAF-mutant lung cancer. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(47), 13456–13461. https://doi.org/10.1073/pnas.1610456113

Oliveres, H., Caglevic, C., Passiglia, F., Taverna, S., Smits, E., & Rolfo, C. (2018). Vaccine and immune cell therapy in non-small cell lung cancer. *Journal of Thoracic Disease*, *10*(Suppl 13), S1602–S1614. https://doi.org/10.21037/jtd.2018.05.134

Ott, P. A., Hu, Z., Keskin, D. B., Shukla, S. A., Sun, J., Bozym, D. J., Zhang, W., Luoma, A., Giobbie-Hurder, A., Peter, L., Chen, C., Olive, O., Carter, T. A., Li, S., Lieb, D. J.,

Eisenhaure, T., Gjini, E., Stevens, J., Lane, W. J., … Wu, C. J. (2017). An immunogenic personal neoantigen vaccine for patients with melanoma. *Nature*, *547*(7662), 217–221. https://doi.org/10.1038/nature22991

Ou, S. H. I., Young, L., Schrock, A. B., Johnson, A., Klempner, S. J., Zhu, V. W., Miller, V. A., & Ali, S. M. (2017). Emergence of Preexisting MET Y1230C Mutation as a Resistance Mechanism to Crizotinib in NSCLC with MET Exon 14 Skipping. *Journal of Thoracic Oncology*, *12*(1), 137–140. https://doi.org/10.1016/j.jtho.2016.09.119

Passiglia, F., Caparica, R., Giovannetti, E., Giallombardo, M., Listi, A., Diana, P., Cirrincione, G., Caglevic, C., Raez, L. E., Russo, A., & Rolfo, C. (2016). The potential of neurotrophic tyrosine kinase (NTRK) inhibitors for treating lung cancer. *Expert Opinion on Investigational Drugs*, *25*(4), 385–392. https://doi.org/10.1517/13543784.2016.1152261

Paz-Ares, L., Tan, E. H., O'Byrne, K., Zhang, L., Hirsh, V., Boyer, M., Yang, J. C. H., Mok, T., Lee, K. H., Lu, S., Shi, Y., Lee, D. H., Laskin, J., Kim, D. W., Laurie, S. A., Kölbeck, K., Fan, J., Dodd, N., Märten, A., & Park, K. (2017). Afatinib versus gefitinib in patients with EGFR mutation-positive advanced non-small-cell lung cancer: Overall survival data from the phase IIb LUX-Lung 7 trial. *Annals of Oncology*, *28*(2), 270–277. https://doi.org/10.1093/annonc/mdw611

Péron, J., Marreaud, S., Staelens, D., Raveloarivahy, T., Nzokirantevye, A., Flament, J., Steuve, J., Lia, M., Collette, L., & Schöffski, P. (2019). A multinational, multi-tumour basket study in very rare cancer types: The European Organization for Research and Treatment of Cancer phase II 90101 'CREATE' trial. *European Journal of Cancer*, *109*, 192–195. https://doi.org/10.1016/j.ejca.2018.12.013

Postow, M. A., Callahan, M. K., & Wolchok, J. D. (2015). Immune checkpoint blockade in

cancer therapy. *Journal of Clinical Oncology*, *33*(17), 1974–1982. https://doi.org/10.1200/JCO.2014.59.4358

Rodriguez-Canales, J., Parra-Cuentas, E., & Wistuba, I. I. (2016). Diagnosis and molecular classification of lung cancer. *Cancer Treatment and Research*, *170*, 25–46. https://doi.org/10.1007/978-3-319-40389-2_2

Roskoski, R. (2014). The ErbB/HER family of protein-tyrosine kinases and cancer. *Pharmacological Research*, *79*, 34–74. https://doi.org/10.1016/j.phrs.2013.11.002

Sawant, A., Schafer, C. C., Jin, T. H., Zmijewski, J., Tse, H. M., Roth, J., Sun, Z., Siegal, G. P., Thannickal, V. J., Grant, S. C., Ponnazhagan, S., & Deshane, J. S. (n.d.). *Enhancement of Antitumor Immunity in Lung Cancer by Targeting Myeloid-Derived Suppressor Cell Pathways*. 6609–6620. https://doi.org/10.1158/0008-5472.CAN-13-0987

Senthi, S., Haasbeek, C. J. A., Slotman, B. J., & Senan, S. (2013). Outcomes of stereotactic ablative radiotherapy for central lung tumours: A systematic review. *Radiotherapy and Oncology*, *106*(3), 276–282. https://doi.org/10.1016/j.radonc.2013.01.004

Shafiq, J., Hanna, T. P., Vinod, S. K., Delaney, G. P., & Barton, M. B. (2016). A Population-based Model of Local Control and Survival Benefit of Radiotherapy for Lung Cancer. *Clinical Oncology*, *28*(10), 627–638. https://doi.org/10.1016/j.clon.2016.05.006

Shi, L. Z., Goswami, S., Fu, T., Guan, B., Chen, J., Xiong, L., Zhang, J., Tang, D. N., Zhang, X., Vence, L., Blando, J., Allison, J. P., Collazo, R., Gao, J., & Sharma, P. (2019). Blockade of CTLA-4 and PD-1 enhances adoptive t-cell therapy efficacy in an ICOS-mediated manner. *Cancer Immunology Research*, *7*(11), 1803–1812. https://doi.org/10.1158/2326-6066.CIR-18-0873

Socinski, M. A., Obasaju, C., Gandara, D., Hirsch, F. R., Bonomi, P., Bunn, P. A., Kim, E.

S., Langer, C. J., Natale, R. B., Novello, S., Paz-Ares, L., Pérol, M., Reck, M., Ramalingam, S. S., Reynolds, C. H., Spigel, D. R., Wakelee, H., & Thatcher, N. (2018). Current and Emergent Therapy Options for Advanced Squamous Cell Lung Cancer. *Journal of Thoracic Oncology*, *13*(2), 165–183. https://doi.org/10.1016/j.jtho.2017.11.111

Soularue, E., Lepage, P., Colombel, J. F., Coutzac, C., Faleck, D., Marthey, L., Collins, M., Chaput, N., Robert, C., & Carbonnel, F. (2018). Enterocolitis due to immune checkpoint inhibitors: A systematic review. *Gut*, *67*(11), 2056–2067. https://doi.org/10.1136/gutjnl-2018-316948

Speicher, P. J., Gu, L., Gulack, B. C., Wang, X., Amico, A. D., Hartwig, M. G., & Berry, M. F. (2015). SC. *Clinical Lung Cancer*. https://doi.org/10.1016/j.cllc.2015.07.005

Sullivan, I., & Planchard, D. (2016). Next-generation EGFR tyrosine kinase inhibitors for treating EGFR-mutant lung cancer beyond first line. *Frontiers in Medicine*, *3*(JAN), 1–13. https://doi.org/10.3389/fmed.2016.00076

Thomas, M., Byrne, K. J. O., Moro-sibilot, D., Camidge, D. R., Ph, D., Mok, T., Hirsh, V., Riely, G. J., Ph, D., Iyer, S., Ph, D., Tassell, V., Polli, A., Wilner, K. D., Ph, D., Jänne, P. A., & Ph, D. (2013). *Crizotinib versus Chemotherapy in Advanced*. 2385–2394. https://doi.org/10.1056/NEJMoa1214886

Thress, K. S., Paweletz, C. P., Felip, E., Cho, B. C., Stetson, D., Dougherty, B., Lai, Z., Markovets, A., Vivancos, A., Kuang, Y., Ercan, D., Matthews, S. E., Cantarini, M., Barrett, J. C., Jänne, P. A., & Oxnard, G. R. (2015). Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nature Medicine*, *21*(6), 560–562. https://doi.org/10.1038/nm.3854

van Belzen, I. A. E. M., & Kesmir, C. (2019). Immune biomarkers for predicting response to

adoptive cell transfer as cancer treatment. *Immunogenetics*, *71*(2), 71–86. https://doi.org/10.1007/s00251-018-1083-1

Van Der Steen, N., Leonetti, A., Keller, K., Dekker, H., Funel, N., Lardon, F., Ruijtenbeek, R., Tiseo, M., Rolfo, C., Pauwels, P., Peters, G. J., & Giovannetti, E. (2019). Decrease in phospho-PRAS40 plays a role in the synergy between erlotinib and crizotinib in an EGFR and cMET wild-type squamous non-small cell lung cancer cell line. *Biochemical Pharmacology*, *166*(May), 128–138. https://doi.org/10.1016/j.bcp.2019.05.014

Vinod, S. K. (2015). International Patterns of Radiotherapy Practice for Non-Small Cell Lung Cancer. *Seminars in Radiation Oncology*, *25*(2), 143–150. https://doi.org/10.1016/j.semradonc.2014.11.001

Weir, C., Hudson, A. L., Moon, E., Ross, A., Alexander, M., Peters, L., Langova, V., Clarke, S. J., Pavlakis, N., Davey, R., & Howell, V. M. (2014). Streptavidin: a novel immunostimulant for the selection and delivery of autologous and syngeneic tumor vaccines. *Cancer Immunology Research*, *2*(5), 469–479. https://doi.org/10.1158/2326-6066.CIR-13-0157

Xu, K., Chen, G., Li, X., Wu, X., Chang, Z., Xu, J., Zhu, Y., Yin, P., Liang, X., & Dong, L. (2017). MFN2 suppresses cancer progression through inhibition of mTORC2/Akt signaling. *Scientific Reports*, *7*(January), 1–13. https://doi.org/10.1038/srep41718

Yang, S., Zhang, Z., & Wang, Q. (2019). *Emerging therapies for small cell lung cancer*. *3*, 1–11.

Yoshida, J., Ishii, G., Hishida, T., Aokage, K., Tsuboi, M., Ito, H., Yokose, T., Nakayama, H., Yamada, K., & Nagai, K. (2015). *Limited resection trial for pulmonary ground-glass opacity nodules : case selection based on high-resolution computed tomography — interim results*. 1–5. https://doi.org/10.1093/jjco/hyv057

Yu, B., & Liu, D. (2019). Antibody-drug conjugates in clinical trials for lymphoid malignancies and multiple myeloma. *Journal of Hematology and Oncology*, *12*(1), 1–17. https://doi.org/10.1186/s13045-019-0786-6

Zhang, Z., Zhang, Y., Gao, F., Han, S., Cheah, K. S., Tse, H. F., & Lian, Q. (2017). CRISPR/Cas9 Genome-Editing System in Human Stem Cells: Current Status and Future Prospects. *Molecular Therapy - Nucleic Acids*, *9*, 230–241. https://doi.org/10.1016/j.omtn.2017.09.009

Zou, H. Y., Li, Q., Engstrom, L. D., West, M., Appleman, V., Wong, K. A., McTigue, M., Deng, Y. L., Liu, W., Brooun, A., Timofeevski, S., McDonnell, S. R. P., Jiang, P., Falk, M. D., Lappin, P. B., Affolter, T., Nichols, T., Hu, W., Lam, J., … Fantin, V. R. (2015). PF-06463922 is a potent and selective next-generation ROS1/ALK inhibitor capable of blocking crizotinib-resistant ROS1 mutations. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(11), 3493–3498. https://doi.org/10.1073/pnas.1420785112

Zugazagoitia, J., Guedes, C., Ponce, S., Ferrer, I., Molina-Pinelo, S., & Paz-Ares, L. (2016). Current Challenges in Cancer Treatment. *Clinical Therapeutics*, *38*(7), 1551–1566. https://doi.org/10.1016/j.clinthera.2016.03.026