Diploma Thesis

Immune Checkpoint Inhibitor Therapy in Non-Small Cell Lung Cancer Patients

Submitted by

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In Partial Fulfillment of the Requirements

For the Academic Degree of Doctor medicinae universae

(Dr. med. univ.)

In Medicine

Medical University Graz

Otto Loewi Research Center, Pharmacology

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Graz, May 2018

„Immune manipulation may turn out to be an even more important intervention than chemotherapy was – maybe the most important ever.“

- Roger Perlmutter

Statutory Declaration

I declare that I have authored this thesis independently, that I have not used other than the declared sources/resources, and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

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Acknowledgments

This thesis was drafted in the working group of Univ.-Ass. Dr. Kargl Julia and Prof. Akos Heinemann at the Otto Loewi Institute, Department of Pharmacology, Medical University Graz, in the time period from February 15, 2018, until May 25, 2018.

I would like to thank my thesis advisor Univ.-Ass. Dr. Kargl Julia, who provided the topic and readily agreed to supervise and oversee this thesis. The door to her office was always open whenever I ran into a trouble spot or had a question about my research or writing. She consistently allowed this thesis to be my own work but steered me in the right direction whenever she thought I needed it.

Finally, I must express my very profound gratitude to my parents for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this thesis. This accomplishment would not have been possible without them. Thank you.

Bundschuh Christian

Abstract

This diploma thesis with the title ‘Immune Checkpoint Inhibitor Therapy in Non-Small Cell Lung Cancer Patients’ deals with the topic of lung cancers, focused on non-small cell lung cancers (NSCLCs). Therefore, their incidence numbers, the anatomical and histological characteristics of the pulmonary systems, risk factors for the development of lung cancers, classification and staging of NSCLCs, diagnostic and therapeutic approaches, with the emphasis on immunotherapeutics were investigated. This thesis focuses on a systematic review of the recent developments and currently ongoing clinical trials in the field of immune checkpoint inhibitors.

The literature researched for this topic includes books, journals, and current treatment guidelines. For this purpose, the databases PubMed, ClinicalTrials and UpToDate, as well as the guidelines of the ICDO, TNM, and ESMO, were used. If possible, current literature (not older than 5 years) was used, especially in the immunotherapy chapter.

The results of this thesis include the systematic processing of the anatomical and histological bases of the pulmonary system, the current risk factors, systematic classifications, diagnostic and therapeutic guidelines for lung cancers, in particular, NSCLCs. The chapter on immunotherapy provides a brief outline of the history of immunotherapy and an overview of the different types of immunotherapy. The systematic review deals with immune checkpoint inhibitors. This chapter includes a presentation of the already Food and Drug Administration (FDA) approved substances, as well as a delineation of (early) clinical trials.

Due to advances in immunotherapy research, which resulted in an approval of various immunotherapeutic agents, such as PD-L1 inhibitors, immunotherapy has already become an integral part of the recommended treatment plan for certain indications. In the future, immune checkpoint inhibitors are expected to play an increasingly important role as part of combination therapies in the treatment of NSCLC (in addition to established surgical, chemo- and radiotherapy treatment plans).

Zusammenfassung

Die vorliegende Diplomarbeit mit dem Titel „Immune Checkpoint Inhibitor Therapy in Non-Small Cell Lung Cancer Patients" beschäftigt sich mit der Therapie von Bronchialkarzinomen, insbesondere mit der Therapie von nicht-kleinzelligen Bronchialkarzinomen (NSCLCs). Hierfür wurden deren Inzidenz, die Anatomie und Histologie der Lunge, die Risikofaktoren für die Entstehung von Lungenkrebs, die Klassifikation und das Staging von NSCLC, die diagnostischen und therapeutischen Möglichkeiten, mit dem Schwerpunkt auf Immuntherapie, recherchiert. Im Fokus der vorliegenden Arbeit steht ein systematischer Review über die jüngsten Entwicklungen und derzeit laufenden klinischen Studien auf dem Gebiet der immunologischen Checkpoint-Inhibitoren.

Die für dieses Thema recherchierte Literatur umfasst Bücher, Zeitschriften und aktuelle Behandlungsrichtlinien. Hierfür wurden als Datenbanken PubMed, ClinicalTrials und UpToDate sowie die Leitlinien der ICDO, TNM und ESMO verwendet. Sofern möglich, wurde, insbesondere im Kapitel der Immuntherapie, aktuelle Literatur (nicht älter als 5 Jahre) herangezogen.

Die Ergebnisse der vorliegenden Arbeit umfassen neben der systematischen Aufarbeitung der anatomischen und histologischen Grundlagen der Lunge die aktuellen Risikofaktoren, Klassifikationen, systematischen Einteilungen, Diagnostik- und Therapievorgaben bei Bronchialkarzinomen, insbesondere NSCLCs. Im Kapitel Immuntherapie wird ein kurzer Abriss über die Geschichte der Immuntherapie und ein Überblick über die verschiedenen Arten der Immuntherapie gegeben. Die Aufarbeitung als systematischer Review erfolgte für die Gruppe der Immun-Checkpoint-Inhibitoren. Hier werden bereits von der Food and Drug Administration (FDA) zugelassene Substanzen vorgestellt, es werden jedoch auch (frühe) klinische Studien präsentiert.

Durch die Fortschritte in der Immuntherapieforschung, welche bereits zur Zulassung von diversen immuntherapeutischen Substanzen, z.B. PD-L1 Inhibitoren geführt hat, sind Immuntherapien für bestimmte Indikationen bereits integraler Teil des empfohlenen Therapieschemas geworden. Im Hinblick auf die Zukunft ist damit zu rechnen, dass Immun-Checkpoint-Inhibitoren als Bestandteil von Kombinationstherapien zur Behandlung von NSCLC eine zunehmend wichtigere Rolle einnehmen werden (neben den etablierten chirurgischen, Chemo- und Radiotherapien).

Table of Contents

List of Abbreviations

ADCC	Antibody-dependent cell-mediated cytotoxicity
AJCC	American Joint Committee on Cancer
ALK	Anaplastic lymphoma kinase
APC	Antigen presenting cell
BCG	Bacillus Calmette-Guérin
BTLA	B and T cell lymphocyte attenuator
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
CTLA-4	Cytotoxic T-lymphocyte-associated antigen 4
DIPNECH	Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
DLCO	Diffusing capacity of the lung for carbon monoxide
EGFR	Epidermal growth factor receptor
ESMO	European Society for Medical Oncology
FDA	United States Food and Drug Administration
FEV_1	Forced expiratory volume in the first second
GITR	Glucocorticoid-induced tumor necrosis factor-like receptor
ICDO	International Classification of Diseases for Oncology
iCPD	Immune confirmed progressive disease
iCR	Immune complete response
IFN	Interferon
Ig	Immunoglobulin
IHC	Immunohistochemistry
IL	Interleukin
imRECIST	Immune-modified Response Evaluation Criteria In Solid Tumors
iPR	Immune partial response
irRC	Immune-related response criteria
iSD	Immune stable disease
iUPD	Immune unconfirmed progressive disease
LAG3	Lymphocyte activation gene 3
LCC	Large cell carcinoma
MHC	Major histocompatibility complex
NKs	Natural killer cells
(N)SCLC	(Non-)small cell lung cancer
OSR	Overall survival rate

PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death protein 1 ligand
PET	Positron emission tomography
(i)RECIST	(Immunotherapy) Response Evaluation Criteria In Solid Tumors
SABR	Stereotactic radiotherapy
SRS	Stereotactic radiosurgery
TIM-3	T cell immunoglobulin and mucin domain 3
TKI	Tyrosine kinase inhibitors
TNM	Union for International Cancer Control Tumor/Node/Metastasis system
WBRT	Whole brain radiotherapy
WHO	World Health Organization

1 Introduction

Pulmonary cancer, also termed bronchogenic carcinoma, refers to malignancies originating within the airways or the pulmonary parenchyma. Numerous tumor subtypes of the pulmonary system are described. They can be differentiated by their localization, their prognostic aspects and in respect to their cellular origin, composition and growth behavior. This distinction is necessary for the staging, prognosis, and treatment of pulmonary neoplasms.

Non-small cell lung cancer (NSCLC), which comprises adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, and small cell lung cancer (SCLC) accounts for about 95% of all lung cancers. Other histological pulmonary cancers, such as bronchial adenoma, comprise about 5 percent of malignancies developing in the lung (Midthun, 2018a).

The most common secondary pulmonary cancers are metastatic malignant neoplasms and are found in 30-55% of all cancer patients (Dishop and Kuruvilla, 2008).

The incidence rate of pulmonary cancer in 2012 adds up to 1.8 million new cases worldwide, accounting for 12.9% of all new cancer diagnoses, making it the most frequently diagnosed malignancy (Ferlay et al., 2015). The incidence of pulmonary cancer is about 1 in 15 in men and 1 in 17 in women, with a higher frequency in smokers compared to non-smokers and ethnic and racial differences in relative risk (Haiman et al., 2006). Wong and his research team found that among men, the highest rates were found in Europe, Eastern Asia, and Micronesia, whereas the lowest incidence rates were found in Africa and Central America. In women, the highest incidence rates were found in Northern America, Europe, Micronesia and Australia/New Zealand, whereas the lowest numbers were found in Africa (Wong et al., 2017). The five-year overall survival rate (OSR) is only 17.8% but largely depends on age, histology, stage, and growth behavior (Howlader et al., 2017, Malvezzi et al., 2015).

For the year 2018 about 234,030 new pulmonary cancer cases (SCLC and NSCLC), 121,680 in men and 112,350 in women, and 154,050 lung cancer-associated deaths, 83,550 in men and 70,500 in women are estimated for the United States alone. Therefore, pulmonary cancer is the second most common cancer worldwide, second only to prostate cancer in men and breast cancer in women, accounting for 14% of all new cancer cases and is the reason for 25% of all cancer fatalities in the United States (American Cancer Society, 2018a, Howlader et al., 2017, National Cancer Institute, 2018).

Alterations in the cell's proliferation, differentiation and apoptosis can lead to a formation of neoplastic cells. These cellular processes are usually tightly regulated by messenger substances; hence an imbalance of these regulation factors, such as proapoptotic substances or growth factors, results in an interrupted signaling pathway, blocking the cell's downregulation or apoptosis. The regulation of the tissue homeostasis is presented in Figure 1. A mutation of the receptors, the signaling cascades, and the transcription factors are key points in the cell's regulation process and thus possible protooncogenes.

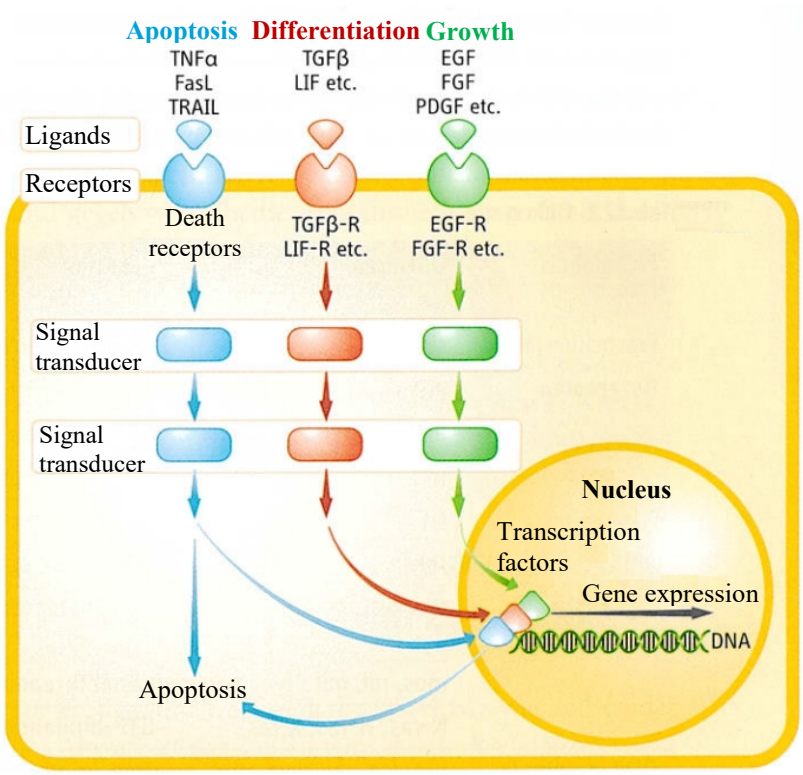


Figure 1 Regulation of tissue homeostasis (Steinhilber et al., 2010)

The mutations of these protooncogenic genes or gene products are presented in Figure 2 and lead to an increased growth of the cell or a protection of the cell from cell death mechanisms such as apoptosis. The genetic changes extend from point mutations, gene amplification, and chromosomal translocations to chromosomal deletions. For human tumorigenesis mutations in 4 to 7 different genes were identified to modify the cell's growth and survival behavior, leading to a number of about 60 mutations in tumor-associated tissues (Bundschuh, 2016, Hanahan and Weinberg, 2000, Steinhilber et al., 2010).

The most important risk factor identified for the development of lung cancer is tobacco smoking, which is estimated to account for approximately 90 percent of all lung cancers. One pack per day for 40 years increases the risk for a lung tumor about 20 times compared to a non-smoker. Other risk factors for lung cancer development include environmental toxins such as radon, asbestos, pulmonary fibrosis, and oncogenic viruses (Midthun, 2018b).

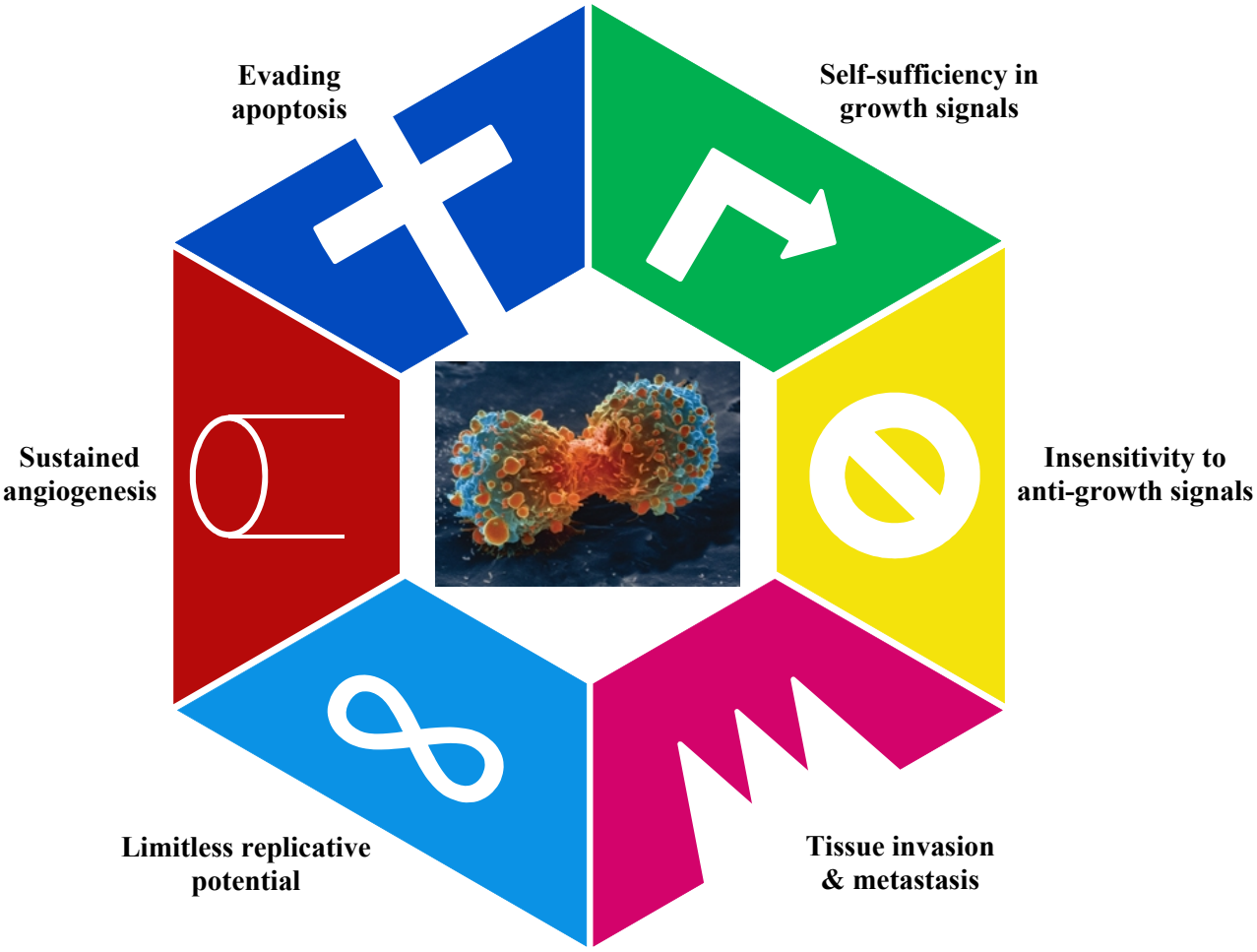


Figure 2 Genetic modifications inducing neoplastic cell properties

Compared to other cancers, such as colon, bladder and ovarian cancers, only 8% of pulmonary cancers showed hereditary predisposition (Kanwal et al., 2017). These pulmonary neoplasms showed chromosomal abnormalities such as extensive aneusomy of the chromosomes 5p, 6, 7 and 8, the most common chromosomal aberrations in NSCLC patients, allelic loss, isochromosomes, unbalanced translocations and loss of heterozygosity (Kang et al., 2007, Kettunen et al., 2006, Liu et al., 2008). Oncogenes associated with pulmonary cancer are the epidermal growth factor receptor (EGFR) and the anaplastic lymphoma kinase (ALK) (Zochbauer-Muller et al., 2002).

2 Methods

This thesis was drafted as a systematic review of the recent developments of immunotherapy in the treatment of pulmonary cancer. Because it represents the most frequent cancer of the pulmonary system and the resulting scientific interest NSCLC was chosen as the main focus of this work.

The relevant literature for this thesis was selected after a comprehensive research of scientific literature such as books, journals and guidelines using the PubMed, ClinicalTrials.gov and UpToDate databases as well as the ICDO, TNM and ESMO guideline publications. To ensure a high-quality review of the current literature addressing the topic of immunotherapy in NSCLC patients, literature published within the last five years was used to achieve an up-to-date information value of this thesis, whenever possible, especially in the immunotherapy chapter of this thesis. Citations of the used literature were done using EndNote X8 and the Harvard citation style.

3 *Anatomy and Histology of the Pulmonary System*

3.1 *General information about the pulmonary system*

The pulmonary system's main function is the gas exchange, comprising oxygen uptake and removal of carbon dioxide from the circulation. Therefore, the pulmonary anatomic compartments closely interact with the blood flow, allowing rapid adjustments to physiological changes due to cyclical volume and pressure fluctuations. The breath rate is inverse to the organism's size, hence children have a higher breath rate (16-30 breaths per minute) compared to adults (12-15 breaths per minute) (Tomashefski and Farver, 2008).
The lung is embedded in the visceral pleura and can be divided into a right and left lung, which are separated by the heart and mediastinal structures, its caudal border is the diaphragm. Furthermore, the lung is supported by a net-like connective tissue skeleton and protected by the thoracic cage (Grant, 1972). The weight of a physiological, adult lung is usually between 300 and 450 grams, its volume ranges from 3.5 to 8.5 liters, an increased weight or volume might indicate pathologies like congestion, edema or inflammatory exudates (Thurlbeck, 1979). Because of the situs of the heart (cardiac notch) the right lung is usually larger than the left one by about 6%. Besides the cardiac notch at the lingula, the pleura has prominent indentations for the esophagus and the superior vena cava, additionally, an aortic groove is located superiorly and posteriorly to the left hilum (Sobonya, 1989).

3.2 *Lobes and segments*

The right lung is divided into the upper, middle and lower lobes by a major and minor diagonal fissure, whereas the left lung is composed of an upper and lower lobe, demarcated by a single diagonal fissure. The lingula represents the anterior-inferior division of the left upper lobe, overrides the left cardiac ventricle, and is the counterpart of the right middle lobe. Anatomical variations of the fissures such as accessory and partial fissures are common. Frequently encountered variations are horizontal fissures separating the lingula from the rest of the left upper lobe, which leads to the formation of a trilobed left lung, or a retrocardiac lobe, an isolation of the medial basal segment of the right lower lobe (Godwin and Tarver, 1985). These anatomical variations become important in patients with a planned lung resection. Another systematic division of the lung is established by the before mentioned segments, which are parts of the lung with their own supply provided by a segmental bronchus, but no defined anatomic boundaries (Felson, 1973). For a better overview of the

bronchopulmonary segments, the segmental distribution, as proposed by Jackson and Huber (Jackson and Huber, 1943), is presented in Table 1.

Table 1 Lobes and segments of the lung

Right Lung		**Left Lung**	
Lobe	**Segment**	**Lobe**	**Segment**
Upper	Apical	**Upper**	Apical
	Posterior		Posterior
	Anterior		Anterior
Middle	Lateral	**Lingular**	Superior
	Medial		Inferior
Lower	Superior	**Lower**	Superior
	Medial-basal		Medial-basal
	Anterior-basal		Anterior-basal
	Lateral-basal		Lateral-basal
	Posterior-basal		Posterior-basal

The most prominent difference between the left and right lung exists in the form of the lingula (superior and inferior segment) and the right middle lobe (medial and lateral segments). This results from the different bifurcation angles of the right and left main bronchi. While the left main bronchus angles at 40-60° off the trachea's course, extending longer and circumventing the left side of the heart, the right main bronchus angles at 20-30° off the trachea's course, leading almost straight into the right lower lobe bronchus. The right main bronchus branches into the right upper lobe bronchus and the intermediate bronchus, which subdivides into the middle and lower lobe bronchi. Three segmental bronchi derive from the upper lobar bronchus, whereas the medial and lateral segmental bronchi derive from the middle lobe bronchus, and the short lower lobe subdivides into the lower lobe superior segmental bronchus at the middle lobe bronchial origin and the four basal segmental bronchi. The left main bronchus divides into the upper and lower lobar branches. The left upper lobe bronchus divides into a superior division, which can be divided into the apico-posterior and anterior segmental branches and the lingular lobe, whereas the lower lobe bronchus divides into the superior segmental bronchus and continues to the four basal segmental divisions. On their axial pathway from the main bronchus to the terminal bronchiole, the airway's diameter

narrows after each division. The number of division varies from 5 to 25, depending on the airway‘s length (Jackson and Huber, 1943, Sobonya, 1989).

3.3 *Airways*

The airways are embedded in connective tissue, cartilage, and smooth muscle. The 16-20 tracheal cartilage rings, consisting of hyaline cartilage, encircle the trachea, with a posterior opening allowing the adjacent esophagus‘ expansion (the cartilage rings calcify and ossify with higher age). While most of the rings have a similar anatomy, the first ring shows a bifurcation and attachment to the larynx‘ cricoid cartilage, whereas the last tracheal cartilage has a wider diameter and a triangular-shaped lower border with two semi-ring-shaped areas, which form the cartilages of the two major bronchi. For the recoil, during breathing, the cartilage rings are attached to a fibrous membrane, which is composed of collagen and elastic fibers. Compared to the tracheal cartilage the bronchial cartilage decreases in size and mass as the bronchi extend into the lung, leading to a higher proportion of circular smooth muscle bundles, which results in a more efficient bronchoconstriction. In the terminal bronchiole, the circular smooth muscle amount decreases, allowing for short diffusion pathways (Bannister, 1995).

3.4 *Bronchi*

The bronchial epithelium is covered by a mucous bilayer secreted by seromucous glands, which contain bacteriostatic lysozymes, lactoferrins, antibodies (mainly immunoglobulin (Ig)A) and antiproteases. These seromucous glands tend to be replaced by oxyphil cells in older patients. The bronchial glands‘ secretion is controlled by myoepithelial cells of the autonomic nervous system. The tracheal and bronchial mucosa resembles the larynx’ mucosa and consists of a pseudostratified ciliated epithelium interspersed with goblet cells on a basement membrane, which consists of extracellular matrix providing the epithelium’s support and measures about 7 µm. The components of this bilaminar membrane (basal lamina and lamina reticularis) are collagen IV, fibronectin, laminin-entactin/nidogen complexes, and proteoglycans. The cells found in this epithelium are ciliated cells, goblet cells, basal cells and neuroendocrine cells (Djukanovic et al., 1990, Laitinen and Laitinen, 1994).

Atop the epithelium, the airway surface liquid and cilia are located and protect the bronchiole from particles and organisms via the mucociliary clearance mechanism. These ciliated cells are up to 20 µm in length and 10 µm in width (their size decreases with smaller bronchus’ diameters), with a basal nucleus and a mitochondria-rich cytoplasm. The cilium’s center is

formed by an axoneme, which consists of a central pair and nine peripheral doublets of microtubules with A and B subfibers. These cilia reach the airway surface liquid, a bilayer of low-viscosity, secreted by the mixed seromucous glands in the submucosa, and a high-viscosity layer, secreted by goblet cells (Ng et al., 2004). The cilia's movement (12-16 strokes/second) is achieved by the doublets sliding in opposite directions. While the effective stroke occurs in the gel and moves the top layer forward, the backstroke occurs in the lower layer (Rutland et al., 1982).

The goblet cells are the most frequent non-ciliated cells of the pulmonary system and are present in the bronchi, but not the bronchiole. They have a basal nucleus, a well-developed rough endoplasmic reticulum, Golgi apparatus. Furthermore, they have abundant apical collections of mucous granules and release mucous granules into the bronchial lumen (merocrine-type secretion). The number of goblet cells increases in acute bronchial pathologies, thereby replacing ciliated cells within a few days, which can lead to a disruption of the ciliary flow's continuity. Goblet cells support the submucosal glands in their mucus production (Tomashefski and Farver, 2008).

Ciliated as well as non-ciliated cells in the bronchial mucosa derive from basal cells, which have a triangular shape and are attached to the basement membrane by hemidesmosomes. They contain a large nucleus and their cytoplasm is low in organelles. The basal cells are responsible for the reconstruction of the bronchial epithelium following a prolonged irritation. Deranged basal cells can lead to the formation of a dysplastic or neoplastic epithelium (Tomashefski and Farver, 2008).

3.5 *Bronchioles*

The difference between membranous bronchioles and bronchi is the absence of cartilage in the wall, which usually appears at airway diameters below 1 mm. The terminal bronchioles are lined by ciliated columnar cells and non-ciliated, cuboidal Clara cells and lead into the acinus, which forms the functional gas exchange unit and consists of the respiratory bronchiole, alveolar ducts, alveolar sac, and alveoli. The total number of terminal bronchioles is about

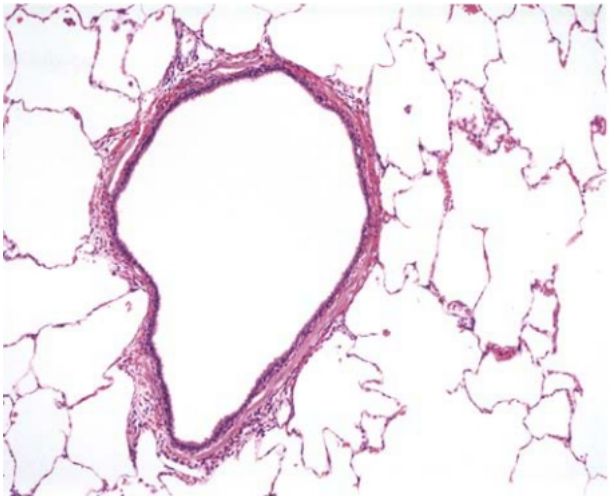


Figure 3 Membranous bronchiole and attached alveoles (Tomashefski and Farver, 2008)

30,000 and each terminal bronchioli contains 10,000 alveoli. The mechanical stability of the bronchioles derives from the tethering effect of the adjacent alveoli, which are present in the respiratory bronchiole's wall, and their connection to the adventitia of the small airways (presented in Figure 3), which prevents their collapse during the final expiration phase. The generations of respiratory bronchioles vary from one to five (most common are 3), as shown in Figure 4 (Tomashefski and Farver, 2008).

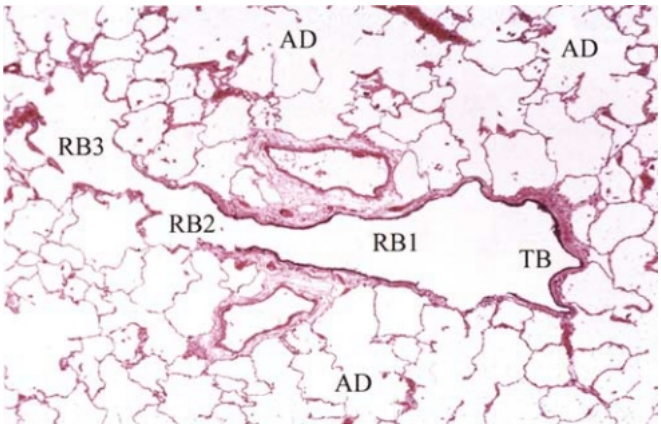


Figure 4 Terminal bronchiole (TB) with respiratory bronchioles (RB1-3) and alveolar ducts (AD) (Tomashefski and Farver, 2008)

Compared to the bronchi, the ciliated cells of the bronchioles are shorter and less frequent, but they maintain the ciliary flow in the bronchioles. The transitional zone adjacent to the ciliary cells is the formation site of new alveoli. Another characteristic of the membranous bronchioles are the canals of Lambert, which are epithelial-lined and are found in the walls between the bronchioles and the adjacent alveoli. These walls are damaged in small-airway diseases, which results in peribronchiolar proliferation and the extension of bronchiolar lining cells into the alveolar region ('Lambertosis') (Tomashefski and Farver, 2008).

Clara cells are the substitutes of the goblet cells in membranous and respiratory bronchioles and have an apical surface, highly active endoplasmic reticulum, mitochondria and Golgi apparatus with apically located secretory granules (Jeffery et al., 1992). Besides the maintenance of the surface tension by secreting apoprotein, the Clara cells serve as the repair cells of the small airways (Bishop, 2004).

The secondary lobule of Miller is contained by fibrous septa and the smallest macroscopically observed unit of lung parenchyma. A single lobule measures between 1 and 2.5 cm and contains three to five acini. Usually, the peripheral subpleura provides the best observation of lobule due to the septa's extension from the visceral pleura. Because the lobular architecture is

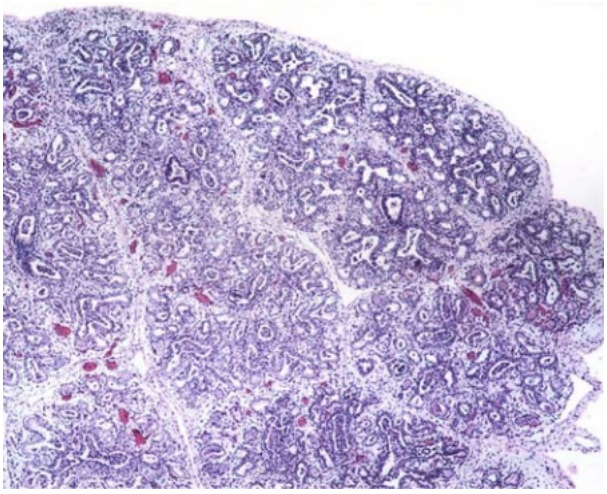


Figure 5 Secondary lobules in fetal lung (Tomashefski and Farver, 2008)

better delineated in fetal lungs compared to adults, Figure 5 presents secondary lobules in a fetal lung (Pump, 1964). While the lobule's center is the entrance point for the airway and pulmonary arteries, the pulmonary veins transport the blood to the interlobular septa (Miller, 1947).

The pulmonary acinus is located distally to the terminal bronchiole, has no septal boundary, comprises three respiratory bronchiole and several alveolar duct divisions and terminates in the alveolar sacs with their alveoli (Raskin, 1982). The usual number of alveoli within a single acinus varies between l500 to 4000 (Miller, 1947, Pump, 1964).

3.6 *Alveoli*

The alveolar sac is a semicircular blind end of the alveolar duct, surrounded by four or more alveoli. Alveoli are the most distal part of the lungs and their diameter varies between 150 and 500 µm. On average a human has about 300 million alveoli, which results in a total alveolar surface of about 143 m^2. With higher age, the alveolar ducts experience a progressive dilatation, which results in flattened alveoli and an aging lung (formerly termed as senile emphysema) (Crapo and Campbell, 1998). The orifices of the alveoli are formed by thick elastic and collagen bundles as well as finer elastic fibers, which are interwoven with capillaries. This network is interconnected with type I alveolar lining cells, allowing the expansion and elastic retraction of the lung in the respiratory process. There are two types of alveolar lining cells. The type I alveolar lining cells are attached to the alveolar wall, have a central flattened nucleus, a large amount of cytoplasm and are connected by tight junctions. Although they make up only 40% of the alveolar lining cells, they are responsible for 90% of the alveolar's surface cover. On the other hand, the type II alveolar lining cells are cuboidal, have a large nucleus with a prominent nucleolus, plenty of cytoplasm, a highly active endoplasmic reticulum and Golgi apparatus, tight junctions with the adjacent type I cells and osmiophilic lamellar inclusion bodies, which are the surfactant's precursors. They account for 60% of the surface cells but they cover only approximately 5% of the alveolar surface. The type II cells also function as reserve cells for the type I cells and are therefore susceptible to hyper- and neoplasticity (Tomashefski and Farver, 2008).

Surfactant consists of phospholipids, especially dipalmitoyl lecithin and glycoproteins. A deflation of the alveoli results in a compression and alignment of phospholipids, which reduces the surface tension and prevents the alveolus's collapse. During the alveolar inflation, the phospholipids misalign, which leads to an increased surface tension and assists the elastic recoil of the alveolus during the expiration (Rooney et al., 1994).

Since 85-95% of the alveolar surface is part of the pulmonary capillary network, an average person has a gas exchange surface of about 126 m^2. For the best possible gas exchange of oxygen (air) and carbon dioxide (hemoglobin), the volume of cells and fibers in the alveolar interstitium should be kept as thin as possible, which is achieved by a spread cytoplasm and the fusion of basal laminae, resulting in an air-blood barrier diameter of 0,6 µm. At any given moment 200 ml of blood are within the capillary network, which means that 1.6 ml are spread over 1 m^2 of gas exchange surface. Physical activity can be compensated by utilizing the entire capillary network (resting state only uses about 65%). The capillary endothelium shows a high permeability, resulting from loose junctions, which allows the diffusion of fluids and macromolecules (Renkin, 1992). Besides transport, the capillary endothelium can convert angiotensin I to angiotensin II, inactivate bradykinin and clear serotonin, norepinephrine, prostaglandin E and F and miscellaneous accumulated drugs and metabolites (Tomashefski and Farver, 2008).

Furthermore, mesenchymal cells, such as fibroblasts, pericytes of capillaries, and myofibroblasts can be found in the alveolar septum. These cells play an important role in the maintenance and the metabolism of elastic and collagen fibers as well as proteoglycans. The collagen fibers are mostly found in bronchovascular bundles, intralobular and interlobular septa and pleura. Small numbers of neutrophils, eosinophils, lymphocytes, plasma cells, basophils, and macrophages are present in the alveolar wall and bronchial interstitial space (Tomashefski and Farver, 2008).

3.7 *Blood circulation of the pulmonary system*

Because of its gas exchange function the lung has two different blood supply systems, the first is the pulmonary circulation, a low-pressure system, and the second is the bronchial circulation, a high-pressure system. Because the pulmonary circulation provides no nutritional effect, but only the blood involved in the gas exchange, the lung's supply of nutrients and oxygen depends on the bronchial circulation (Tomashefski and Farver, 2008).

The pulmonary circulation starts at the right ventricle and quickly divides into the left and right pulmonary arteries, which subdivide into the lobar arteries before entering the lung. Compared to the pulmonary vein the pulmonary artery enters the pulmonary hilum superiorly and slightly anteriorly. From the hilum the axial pathway, which forms the main path, leads to the lung's peripheral regions, running near the airways, branching at similar locations and diameters (conventional arteries). The nomenclature of arteries differentiates between preacinar (before the terminal bronchiole) and intraacinar arteries (distal to the terminal

bronchiole) as well as conventional and supernumerary arteries, which don't accompany the airways. The histology of the arteries mainly depends on their diameter, and are termed as elastic, muscular and nonmuscular types. The elastic fibers are found in larger vessels (diameter: 1000 μm) closer to the right ventricle and are important for the pressure reservoir function. The walls of the arteries, which extend into the lung (diameter: 500-1000 μm), contain more muscular fibers until only muscular fibers remain (diameter: 70-500 μm). On the other hand, small vessels, forming the arterioles (diameter: <70 μm), are of the nonmuscular type and transport the blood directly into the capillaries. Compared to the pulmonary arterioles the pulmonary venules have a similar histology, whereas the larger pulmonary veins mainly contain elastic lamina and a thin wall without external elastic lamina and merge with their adventitia counterparts, with intramural muscular fibers being rare. Towards the hilum, larger pulmonary veins, which are formed by the convergence of the lobular veins and venules, follow the bronchi and pulmonary arteries, where they leave the pulmonary system and run as an extrapulmonary vein to the left atrium (Grant, 1972, Reid, 1968).

The nutrient blood supply of the lung is delivered by the bronchial arteries and nourishes bronchi, pulmonary vasculature, and visceral pleura. Usually, the origin of the bronchial arteries is located near the descending portion of the aortic arch at a major branch origin, with one bronchial artery starting at the third intercostal artery, whereas two arise left and directly at the aorta and proceed to the terminal bronchioles. The bronchial artery has intramural muscular fibers with a well-defined internal elastica, but a poorly defined external elastica, unlike the pulmonary arteries. The bronchial veins have a comparable histology to their pulmonary counterparts (Charan, 1984). Other blood supply pathways are the arteries leading from the aorta into the inferior pulmonary ligament and arteries from a superior mediastinal plexus (Parke and Michels, 1965, Sobonya, 1989).

Another special feature of the pulmonary blood system is the existence of physiological shunts between arteries and veins of the pulmonary and bronchial systems, for instance, bronchopulmonary arteries (bronchial arteries draining into alveolar capillaries) or pulmobronchial arteries (pulmonary arteries providing nutrient supply for bronchi or perivascular tissue). Over a lifetime the number of left-to-right shunts increases and can become a significant problem in patients with neoplasms or destructive lung diseases such as tuberculosis (Charan, 1984).

3.8 *Lymphatic system of the pulmonary system*

The pulmonary lymphatic system is divided into the pleural plexus, responsible for the drainage of the peripheral lung parts, and the parenchymal plexus, responsible for the drainage in the bronchovascular bundles. Furthermore, the lymphatics are also present in the in the interlobular septa near the branches of the pulmonary veins. Although the two systems communicate with each other, they function as individual drainage systems towards the hilar nodes. The lymphatic channels are located adjacent to the bronchovascular bundle, distally to the respiratory bronchiole and resemble thin-walled veins with funnel-shaped valves. The lymphatics of the right lung and the left-lower lobe drain into the right lymphatic duct, which leads to the right jugular, subclavian, or innominate veins, whereas the lymphatics of the left upper lobe drain into the thoracic duct, which is connected to the left internal jugular vein. However, cross-drainage is encountered frequently (Fraser and Pare, 1977, Lauweryns, 1970, Sobonya, 1989).

The lung's lymph nodes are differentiated into four groups.

- Tracheobronchial lymph nodes
  Adjacent to the trachea and main bronchi
- Subcarinal lymph nodes
  Posterior the main bronchial bifurcation
- Bronchopulmonary lymph nodes
  Adjacent to lobar, segmental, and subsegmental bronchi
- Intrapulmonary lymph nodes

The diameter of intrapulmonary lymph nodes is usually less than 1 cm and they are involved in widespread collateral lymphatic circulation. Histological samples of bronchopulmonary lymph nodes usually contain fine black pigment (the amount depends largely on the pollution exposure, such as silica or dust) and appear black or grey (Gibbs and Wagner, 1998). The presence of small ovoid deep brown hemosiderin bodies usually is an indicator of spread lung cancer (Sobonya, 1989).

Since the lymph node status plays an important role in cancer staging, it will be explained in further detail in the staging chapter of this thesis.

3.9 *Innervation of the pulmonary system*

The pulmonary system is innervated by the autonomic nervous system. The nerve trunks' entrance into the lung is the hilum and they are arranged in the periarterial and peribronchial, which subdivides into the extra- chondral and subchondral, plexuses. While the peribronchial

plexus contains myelinated as well as unmyelinated fibers, the periarterial plexus contains only unmyelinated fibers. While the ganglia are mainly located in the extrachondral plexus at the level of the distal bronchi, the nerve fibers reach the acinus and the visceral pleura, the periarterial nerves even continue to the alveolar capillaries' level. The parasympathetic efferent fibers (vagus nerve, neurotransmitter: acetylcholine) extend to the ganglia in and around the airway as well as blood vessel walls and innervate the airway smooth muscle, glandular epithelium, and blood vessels, leading to airway smooth muscle contraction and increased secretion from the bronchial glands. On the other hand, the sympathetic efferent fibers (upper thoracic and cervical ganglia, neurotransmitter: norepinephrine) innervate bronchial blood vessels and submucosal glands. The additional third system is a neurally mediated bronchodilator pathway controlled by the non-adrenergic, non-cholinergic nervous system with the vasoactive intestinal peptide as its neurotransmitter. The afferent nerves originate in the bronchial and alveolar walls, pleura, and the bronchial mucosa and are connected to the central nervous system via the vagus nerve (Tomashefski and Farver, 2008).
The sensory information is provided by the following receptors and fibers (Belvisi, 2002, Laitinen and Laitinen, 1997, Richardson, 1979).

- Adapting stretch receptors
  Responsible for the Hering-Breuer reflex, which leads to an inspiration pause when the lung is inflated
- Irritant/rapidly adapting stretch receptors
  Triggered by mechanical and chemical irritation
- C fibers
  Unmyelinated nerve fibers that trigger a chemoreflex leading to bradycardia, hypotension and apnea and rapid shallow breathing

A schematic overview of the innervation of the pulmonary system is provided in Figure 6.

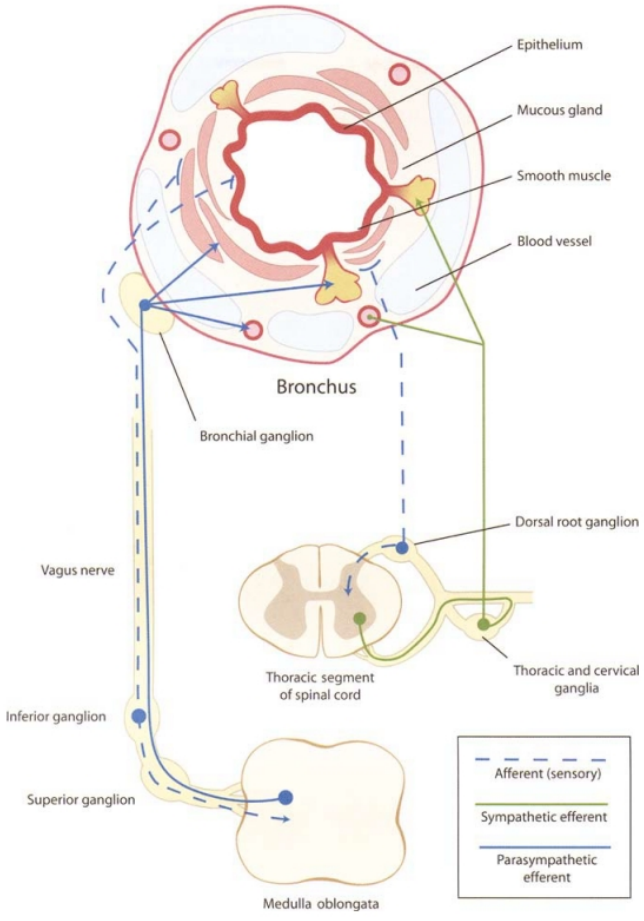


Figure 6 Innervation of the pulmonary system (Tomashefski and Farver, 2008)

3.10 *Pleura, diaphragm, mediastinum*

The visceral pleura covers the surface of the lung as well as the interlobar fissures and delimits the lung from the pleural cavity (the space between the visceral and the parietal pleura). The parietal pleura covers the inner surface of the thoracic cage, mediastinum, and diaphragm, but a transition from visceral to parietal pleura happens at the hilum and pulmonary ligament. The visceral pleura's blood supply derives from the bronchial artery. The microscopic appearance of the pleura is that of a smooth, glistening and semitransparent membrane, with a facultative presence of pigments in the lower parietal pleura. The visceral pleura usually consists of five different layers, but the composition varies regionally (Mitchev et al., 2002, Wang, 1993). The usual composition consists of the following layers.

- Mesothelial layer
- Submesothelial connective tissue layer (thin)
- Superficial elastica layer
- Subpleural connective tissue layer (loose)
- Deep fibroelastic layer (forms the interlobular septa)

The parietal pleura has a similar constitution of mesothelial cells and a layer of fibroelastic tissue underneath. These mesothelial cells are connected by desmosomes, contain pinocytotic vesicles, mitochondria as well as prekeratin fibrils in the cytoplasm and have many microvilli, which trap hyaluronic acid acting as a lubricant to reduce the friction between the moving lung and the chest. These microvilli also allow the microscopic differentiation of mesotheliomas (Charan, 1984, Sobonya, 1989, Tomashefski and Farver, 2008).

The diaphragm is a dome-shaped muscle plate, containing a central tendon and peripheral radiating muscle fibers and separates the thoracic and abdominal cavities. It is innervated by the left and right phrenic nerves and the dome's excursion is about 3.5 cm between in- and expiration in healthy adults (Tomashefski and Farver, 2008, Young and Simon, 1972).

The mediastinum is the intrathoracic median space and is separated into superior, anterior and posterior parts, as well as a middle part containing the heart and the pericardium. These individual regions give rise to different tumors. While lymphomas as well as thymic and thyroid tumors are more frequent in the anterior and superior regions, neural tumors arise preferably in the posterior part and metastatic tumors in the lateral middle mediastinum (Grant, 1972, Tomashefski and Farver, 2008).

4 Risk Factors for Pulmonary Tumors

The main risk factor for pulmonary tumor development is cigarette smoking. This also reflects the epidemiologic distribution of lung cancer incidences, because when the smoking habit of women changed to similar levels to those in men, the number of pulmonary cancer cases in women increased. For instance, within the European Union in 2013, lung cancer mortality in men decreased by 6% compared to 2009, whereas mortality in women increased by 7% (Malvezzi et al., 2015, Thun et al., 2013). A comparison of long-term smokers and non-smokers showed an increased relative risk of lung cancer varying from 10- to 30-fold, while studies investigating smoking cessation, estimated a risk reduction between 20 and 90 percent, depending on the abstinence time (Peto et al., 2000, Samet, 1991). Even if cessation is not an option, the reduction of smoking proved to lead to a decreased incidence of pulmonary cancer as well (Godtfredsen et al., 2005). Although the lung tumor incidence risk due to secondhand smoke is less than in active smokers, it poses a significant problem because of the possible exposure at a younger age and for longer time periods. Some studies suggested that cigar and pipe smoking might have a lower risk of pulmonary cancer compared to cigarette smokers, but also that they have a higher risk for death from lung cancer (Henley et al., 2004). Another possible risk factor is marijuana and cocaine smoking, which seems to lead to an increased risk for pulmonary cancer, but the magnitude has yet to be properly quantified (Sridhar et al., 1994).

Besides smoking, another identified risk factor is higher age. Over 65 years the number of diagnosed lung tumors is significantly higher, with a median age around 70 years (Ferlay et al., 2015, Howlader et al., 2017).

Furthermore, there are numerous environmental carcinogens, which lead to an increased risk of pulmonary cancer development. The best-known factors are asbestos and radon. Other substances that have been associated with lung cancer include arsenic, bis-chloromethyl ether, chromium, formaldehyde, ionizing radiation, nickel, polycyclic aromatic hydrocarbons, hard metal dust, and vinyl chloride (Beckett, 1993).

Asbestos exposure is an established risk factor for pulmonary cancer, especially in patients suffering from interstitial fibrosis (Mossman and Gee, 1989). The incidence risk was proven to be dose-dependent but seems to depend on the type of asbestos fiber (serpentine fibers vs. the more troublesome amphibole fibers) as well (Mossman and Churg, 1998).

Radon is a gaseous decay product of uranium-238 and radium-226, which emits alpha particles damaging the respiratory epithelium. Increased radon concentrations were proven to

show a significantly increased lung cancer risk (Grosche et al., 2006). Since radon accumulation is possible in the soil and groundwater, the in-home radon exposure might be the reason for 2% of lung cancer deaths in Europe (Darby et al., 2005).

Other pollutants associated with multiple respiratory diseases, including an increased incidence number of pulmonary cancer, are unprocessed biomass fuels such as wood or coal. For instance, a retrospective study found that the lifetime risk for lung cancer development was about 20 percent in people who used bituminous coal compared with 0.5 percent for those who used anthracite coal, while another study found that smoke from wood-burning increases incidence risk of pulmonary cancer as well (Arrieta et al., 2008, Barone-Adesi et al., 2012).

Furthermore, studies found that air pollution, as well as diesel exhaust exposure (Silverman et al., 2012), are associated with an increased lung cancer risk, although their impact is relatively small compared to the effects of cigarette smoking (Raaschou-Nielsen et al., 2013).

Other factors that might lead to an increased risk for pulmonary cancer development include radiation therapy, inflammation, benign lung diseases, chronic obstructive pulmonary disease (COPD), genetic factors, dietary factors, endocrine factors as well as oncogenic viruses. A study by Lorigan et al. showed that radiation therapy for Hodgkin lymphoma increased the risk of secondary lung cancer (Lorigan et al., 2005). Patients with a history of emphysema, chronic bronchitis, pneumonia, and tuberculosis had a higher risk for pulmonary cancer development (Brenner et al., 2012). While COPD is commonly associated with smoking, it was proven to be most common independent risk factor besides smoking for an increased lung cancer risk (Hopkins et al., 2017). Genetic factors and their influence on pulmonary cancer development are poorly understood, but studies found that blood-related persons of patients with early-onset lung cancer show a higher risk for non-lung malignancies (Naff et al., 2007). Endocrine factors, such as estrogen and progesterone have already been proven to increase the risk of tumor development in various organ systems, but an increased risk for pulmonary cancer has yet to be proven. The investigation of oncogenic viruses in NSCLC patients, especially the human papillomavirus, and their association with an increased risk of lung tumors, has yielded mixed results (Anantharaman et al., 2014, Beutner and Tyring, 1997, Klein et al., 2009).

5 *Classification of Tumors of the Pulmonary System*

5.1 *International Classification of Diseases for Oncology (ICDO) system*

The classification of pulmonary tumors is based on their histological subtype and provides essential information required for a targeted treatment. Usually, the foundation for lung tumor classification is the ICDO system, which is published by the World Health Organization (WHO) and is an extension of the ICD-10 system for oncological diseases. It is constituted of a dual-axis classification, comprising a topography axis, an acronym of the tumor's origin tissue type and location, and a morphology axis, which indicates the tumors' histology. The topographical key is coded with a four-digit number, while the morphological key only uses a single-digit number, the two axes are separated by a forward bar. An overview of the ICDO system for tumors of the lung as well as an explanation for the morphological axis is provided in Table 2 and Table 3. The advantage of the ICDO system is the introduction of standardized criteria and terminology for pulmonary tumors according to their pathophysiology, which allows the objective assessment of high-stage diseases and the establishment of treatment guidelines for resectable and non-resectable tumors. The latest edition of the WHO classification from 2015 has included new targeted pathway treatments for a higher antitumor level, a more advanced distinction of NSCLC for a more advanced stage-disease outcome and new criteria for surgical resection to improve its clinical significance (Travis et al., 2015).
Tumors are differentiated according to their origin, which results in a classification of epithelial, mesenchymal and lymphohistiocytic tumors as well as tumors of ectopic origin and metastatic tumors. Since this thesis' topic is the treatment of NSCLC only a compendium of lung tumors will be discussed in detail in this chapter.

Table 2 WHO/ICDO classification (Travis et al., 2015)

ICDO Code	Histological Type and Subtypes
	Epithelial tumors
8140/3	Adenocarcinoma
8250/3	Lepidic adenocarcinoma
8551/3	Acinar adenocarcinoma
8260/3	Papillary adenocarcinoma
8265/3	Micropapillary adenocarcinoma
8230/3	Solid adenocarcinoma
8253/3	Invasive mucinous adenocarcinoma

8254/3	Mixed invasive mucinous and nonmucinous adenocarcinoma
8480/3	Colloid adenocarcinoma
8333/3	Fetal adenocarcinoma
8144/3	Enteric adenocarcinoma
	Minimally invasive adenocarcinoma
8256/3	Nonmucinous
8257/3	Mucinous
	Preinvasive lesions
8250/0	Atypical adenomatous hyperplasia
	Adenocarcinoma in situ
8250/2	Nonmucinous adenocarcinoma in situ
8253/2	Mucinous adenocarcinoma in situ
8070/3	Squamous cell carcinoma
8071/3	Keratinizing squamous cell carcinoma
8072/3	Nonkeratinizing squamous cell carcinoma
8083/3	Basaloid squamous cell carcinoma
	Preinvasive lesion
8070/2	Squamous cell carcinoma in situ
	Neuroendocrine tumors
8041/3	Small cell carcinoma
8045/3	Combined small cell carcinoma
8013/3	Large cell neuroendocrine carcinoma
8013/3	Combined large cell neuroendocrine carcinoma
	Carcinoid tumors
8240/3	Typical carcinoid tumor
8249/3	Atypical carcinoid tumor
	Preinvasive lesion
8040/0	Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
8012/3	Large cell carcinoma
8560/3	Adenosquamous carcinoma
	Sarcomatoid carcinomas
8022/3	Pleomorphic carcinoma
8032/3	Spindle cell carcinoma
8031/3	Giant cell carcinoma
8980/3	Carcinosarcoma

8972/3	Pulmonary blastoma
	Other and Unclassified carcinomas
8082/3	Lymphoepithelioma-like carcinoma
8023/3	NUT carcinoma
	Salivary gland-type tumors
8430/3	Mucoepidermoid carcinoma
8200/3	Adenoid cystic carcinoma
8562/3	Epithelial-myoepithelial carcinoma
8940/0	Pleomorphic adenoma
	Papillomas
8052/0	Squamous cell papilloma
8052/0	Exophytic
8053/0	Inverted
8260/0	Glandular papilloma
8560/0	Mixed squamous and glandular papilloma
	Adenomas
8832/0	Sclerosing pneumocytoma
8251/0	Alveolar adenoma
8260/0	Papillary adenoma
8470/0	Mucinous cystadenoma
8480/0	Mucous gland adenoma
	Mesenchymal tumors
8992/0	Pulmonary hamartoma
9220/0	Chondroma
	PEComatous tumors
9174/1	Lymphangioleiomyomatosis
8714/0	PEComa, benign
8005/0	Clear cell tumor
8714/3	PEComa, malignant
8827/1	Congenital peribronchial myofibroblastic tumor, Diffuse pulmonary lymphangiomatosis
8825/1	Inflammatory myofibroblastic tumor
9133/3	Epithelioid hemangioendothelioma
8973/3	Pleuropulmonary blastoma
9040/3	Synovial sarcoma

9137/3	Pulmonary artery intimal sarcoma
8842/3	Pulmonary myxoid sarcoma with EWSR1–CREB1 translocation
	Myoepithelial tumors
8982/0	Myoepithelioma
8982/3	Myoepithelial carcinoma
	Lymphohistiocytic tumors
9699/3	Extranodal marginal zone lymphomas of mucosa-associated
9680/3	Lymphoid tissue (MALT lymphoma) Diffuse large cell lymphoma
9766/1	Lymphomatoid granulomatosis
9712/3	Intravascular large B cell lymphoma
9751/1	Pulmonary Langerhans cell histiocytosis
9750/1	Erdheim–Chester disease
	Tumors of ectopic origin
	Germ cell tumors
9080/0	Teratoma, mature
9080/1	Teratoma, immature
8580/3	Intrapulmonary thymoma
8270/3	Melanoma
9530/0	Meningioma, NOS
	Metastatic tumors

Table 3 Explanation of the morphological axis of the WHO/ICDO classification system

ICD-O morphological key	Tumor behavior
/0	Benign tumor
/1	Uncertain neoplasm (benign or malignant)
/2	Tumor in situ
/3	Primary infiltrative malignant neoplasm
/6	Secondary malignant tumor
/9	Malignant neoplasm, uncertain if primary or secondary

5.2 *Adenocarcinoma*

Adenocarcinomas account for about 50% of all lung cancer cases, making it the most frequent pulmonary tumor (Janssen-Heijnen et al., 2001). The analysis of these tumors is carried out with a pathologic assessment of the tissue specimens as well as molecular tests to detect possible driver mutations, which might be an indicator for the tumor's susceptibility to targeted therapy options. Therefore, neoplastic gland formation, pneumocyte marker expression, or intracytoplasmic mucin are investigated. The major subtypes of the neoplastic gland formation comprise acinar, papillary, micropapillary, lepidic, or a solid growth pattern, while cribriform, colloid, enteric, and fetal growth patterns are less frequently encountered. The growth pattern, as well as the presence or absence of pneumocyte marker expression or intracytoplasmic mucin, have a significant influence on the tumor's prognosis. Adenocarcinomas usually stain positive for thyroid transcription factor, mucin, napsin-A, surf-A, and surf-B in immunohistochemistry (IHC) (Travis et al., 2015).

Adenosquamous carcinoma is defined as lung cancer with a cell population that comprises more than 10% percent malignant glandular and squamous components. Therefore, this tumor shows a mixed histology, IHC and heterogeneity and its incidence rate ranges from 0.4-4% of all pulmonary tumors (Travis et al., 2015). Since this tumor is rather aggressive it shows a worse prognosis compared to adenocarcinoma and squamous cell carcinoma (Cooke et al., 2010). A molecular genetic analysis of mutant kinases, such as EGFR, KRAS, and KIF5B-RET mutations, as well as ALK fusions, is highly recommended for this tumor (Wang et al., 2014).

Until the 1980s the squamous cell carcinoma was the most frequently diagnosed tumor of the pulmonary system, but like large and small cell carcinoma its incidence numbers decreased, and nowadays it was overtaken by adenocarcinoma, especially in women, and accounts for only about 20% of all NSCLCs (Meza et al., 2015). The characteristic feature of this tumor is the presence of keratin production in the tumor cells or the intercellular desmosomes as well as a specific staining pattern in the IHC, namely the expression of p40, p63, CK5/CK6, CK7, and desmoglein (Ma et al., 2015). Since the 2015 edition of the WHO classification squamous cell carcinomas are categorized as non-keratinizing, keratinizing, and basaloid subtypes and a cytologic feature, which is the clear cell change. The first subtype shows an absence of keratinization, while the second shows its presence and the last subtype shows basaloid features in more than 50% of the tumor. The IHC analysis of the keratinization feature allows the distinction of squamous carcinoma, solid type adenocarcinoma, and large cell carcinoma with a null phenotype. About 60-80% of all squamous cell carcinomas arise in the proximal

tracheobronchial tree, but squamous metaplasia-dysplasia-carcinoma have a higher tendency to arise in the lung's periphery, but both, central and peripheral lesions tend to show distinct central necrosis. Some subtypes present themselves with an exophytic, endobronchial, or papillary lesion character (Funai et al., 2003). Classic clinical symptoms of squamous cell carcinomas are a persistent cough, recurrent hemoptysis, and relapsing pulmonary infections. Usually, at the time of the diagnosis, this tumor has a low stage and hence show a good prognosis with a 5-year OSR of over 60% (Dulmet-Brender et al., 1986, Travis et al., 2015).

Large cell carcinomas (LCC) are malignant epithelial tumors, which lack glandular and squamous differentiation as well as cytologic features. Most commonly these tumors present themselves as a large peripheral mass with distinctive necrosis and with sheets of round or sometimes polygonal cells, which contain noticeable nucleoli and weakly stained cytoplasm without prominent cell organelles. Therefore, LCCs are diagnosed by the absence of any of squamous or adenocarcinomatous immunohistochemical features and are often poorly differentiated NSCLCs (Travis et al., 2015). Electron microscopy, IHC, and molecular biological studies allowed the diagnosis of squamous, glandular, or neuroendocrine differentiation features in about 90% of these poorly differentiated cases. Therefore, only the remaining 10% are classified as LCCs (with a null phenotype) (Clinical Lung Cancer Genome Project and Network Genomic Medicine, 2013).

Sarcomatoid carcinomas are a heterogeneous group of non-small cancer cells containing sarcoma or sarcoma-like elements. Usually, this classification is used as an intermediate diagnosis until a more specific diagnosis is possible, therefore patients with sarcomatoid carcinomas are rare, accounting for less than 1% of all lung tumor cases (Travis et al., 2015, Yendamuri et al., 2012). For this tumor group, a molecular genetic testing for the PD-L1 expression level as well as mutations in TP53, NF1 RB1, KRAS and regarding microsatellite instability, such as the MutL homolog mismatch repair genes, is highly recommended (Nakagomi et al., 2018).

Pleomorphic carcinomas are NSCLCs comprising at least 10% giant or spindle cells. Spindle cell carcinomas are composed of malignant spindle cells, which is diagnosed with an IHC of keratin stains in some tumor cells. Giant cell carcinomas are at least 40 µm tumor cells containing multiple nuclei, cytoplasmic polymorphonuclear leukocytes without histological features of squamous cell and adenocarcinomas and have a poor prognosis. Carcinosarcomas are characterized by squamous or adenocarcinoma cells which contain sarcomatous elements such as rhabdomyosarcoma, osteosarcoma, and chondrosarcoma. Pulmonary blastomas are biphasic malignant neoplasms with an adenocarcinoma and a primitive mesenchymal stroma.

At the time of the diagnosis, the cells are usually large and have a poor prognosis (Travis et al., 2015).

5.3 *Neuroendocrine tumors*

Neuroendocrine tumors include small cell neuroendocrine carcinoma, large cell neuroendocrine carcinoma, typical carcinoid, atypical carcinoid, and diffuse idiopathic pulmonary neuroendocrine cell hyperplasia. Because small and large cell neuroendocrine carcinomas have an aggressive growth pattern with early metastatic development (and therefore a worse prognosis), they can be differentiated from pulmonary carcinoid by a high Ki-67 score, which indicates a high number of mitoses (Travis et al., 2015).

SCLC is the most frequent pulmonary tumor of the neuroendocrine group, accounting for approximately 15% of all malignant lung neoplasms and shows a significant correlation with cigarette smoking. The limit for SCLC cells is the size of three resting lymphocyte nuclei, while their pattern is either round, oval, or angulated with little cytoplasm, hyperchromatic nuclei. Compared to other lung cancers SCLCs show a high frequency of genetic mutations, especially in the chromosome 3p and a good response to chemotherapeutic therapy, but usually relapses with a chemotherapy-resistant disease in less than one year. Heterogeneity and association with isolated larger cells with a similar cytology are quite common for this tumor, while in about 5% they combine with squamous carcinoma, adenocarcinoma, or large cell neuroendocrine carcinoma (Travis et al., 2015).

Large cell neuroendocrine carcinomas are usually found in the lung's periphery, show an organoid, trabecular, or palisading growth pattern, a distinctive necrosis and little eosinophilic cytoplasm. Like SCLCs they stain with chromogranin and synaptophysin in IHC and are high-grade tumors with a poor prognosis. Large cell neuroendocrine carcinoma cells are considerably larger than three resting lymphocytes (Travis et al., 2015).

Carcinoid tumors are lower-grade lesions compared to small and large cell neuroendocrine carcinomas. They are differentiated into typical or atypical carcinoid tumors (Travis et al., 2015). The first type shows a distinct organoid, trabecular, or insular growth pattern and is composed of polygonal cells with round to oval nuclei, small nucleoli with finely dispersed chromatin, whereas the latter shows a carcinoid morphology, necrosis and a higher mitosis frequency (el-Naggar et al., 1991).

The diagnosis of neuroendocrine lung tumors is usually reached with light microscopy. For difficult cases, IHC for neuropeptides such as CD56, chromogranin, synaptophysin provides additional information (Travis et al., 2015).

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is a preinvasive tumor and is associated with numerous lung diseases such as bronchiectasis, fibrosis and obliterative bronchiolitis (Davies et al., 2007, Nassar et al., 2011). DIPNECH might be a potential for pulmonary neuroendocrine tumors, but with only a low incidence risk (Travis et al., 2015).

5.4 *Metastatic tumors*

Besides primary lung cancer metastatic tumors that show a similar morphology may arise. When their histology, which corresponds to the primary lesion's, is considered, these tumors are not counted among tumors of the pulmonary system but spread tumor cells, which infiltrated lung tissue cells. For the differential diagnosis of primary and secondary pulmonary tumors, IHC is commonly applied, especially in patients with a known primary tumor. The evaluated antibodies include CK7 (primary lung tumor), CK 20 (primary colon carcinoma) and CDX2 (primary gastrointestinal tumor) (Travis et al., 2015).

6 Staging of Tumors of the Pulmonary System

While the ICDO system is used for the differentiation of lung cancers, the Union for International Cancer Control tumor/node/metastasis (TNM) system is used for an objective staging of the tumor (especially in NSCLCs), correlating the disease's anatomic (instead of molecular characterizations) extent with its prognosis and OSR. Therefore, especially in regard to the rapid advancements in immunotherapeutic substances, such as checkpoint inhibitors, molecular tests should be conducted as well to determine the best possible treatment options. The eighth and latest edition of the TNM system for lung cancers, which was developed using a database of approximately 95,000 lung tumor patients, who were diagnosed and underwent multimodality treatment between 1999 and 2010, was published in late 2016 and was enacted in 2017. The three letters indicate the staging of the primary tumor (T), presented in Table 4, the regional lymph nodes (N), presented in Table 5, and the distant metastases (M), presented in Table 6 (American Joint Committee on Cancer, 2017, Detterbeck et al., 2017, Goldstraw et al., 2016).

Table 4 Staging of the primary tumor (T) of the TNM system (edited after (Goldstraw et al., 2016))

Stage		Description
Ty		Primary tumor not assessable or visualizable, but malignant cells present in the sputum or bronchial washings.
T0		No evidence for neoplastic development.
Tis		Carcinoma in situ
T1	T1a(mi)	Minimally invasive adenocarcinoma
	T1a	The tumor's greatest extent measures ≤1 cm.
	T1b	The tumor's greatest extent measures >1 cm but ≤2 cm.
	T1c	The tumor's greatest extent measures >2 cm but ≤3 cm.
T2		The tumor's greatest extent measures >3 cm but ≤5 cm and has at least one of the following features: ➢ Involvement of the main bronchus, but not the carina. ➢ Invasion of the visceral pleura. ➢ Association with atelectasis or obstructive pneumonitis extending to the hilar region, involving at least part of the lung.
	T2a	The tumor's greatest extent measures >3 cm but ≤4 cm.

	T2b	The tumor's greatest extent measures >4 cm but ≤5 cm.
T3		The tumor's greatest extent measures >5 cm but ≤7 cm or is associated with separate tumor nodule(s) in the primary tumor's lobe or shows signs of a direct invasion of the chest wall, including the parietal pleura and superior sulcus tumors, the phrenic nerve or parietal pericardium.
T4		The tumor's greatest extent measures >7 cm or is associated with separate tumor nodule(s) in a different ipsilateral lobe in regard to the tumor location or shows signs of a direct invasion of the diaphragm, the mediastinum, the heart, the great vessels, the trachea, recurrent laryngeal nerve, esophagus, vertebral body, or carina.

Table 5 Staging of regional lymph nodes (N) of the TNM system (edited after (Goldstraw et al., 2016))

Stage	Description
Nx	The regional lymph nodes are not assessable.
N0	No regional lymph node metastasis.
N1	Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, possible involvement due to direct extension.
N2	Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3	Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene, or supraclavicular lymph node(s)

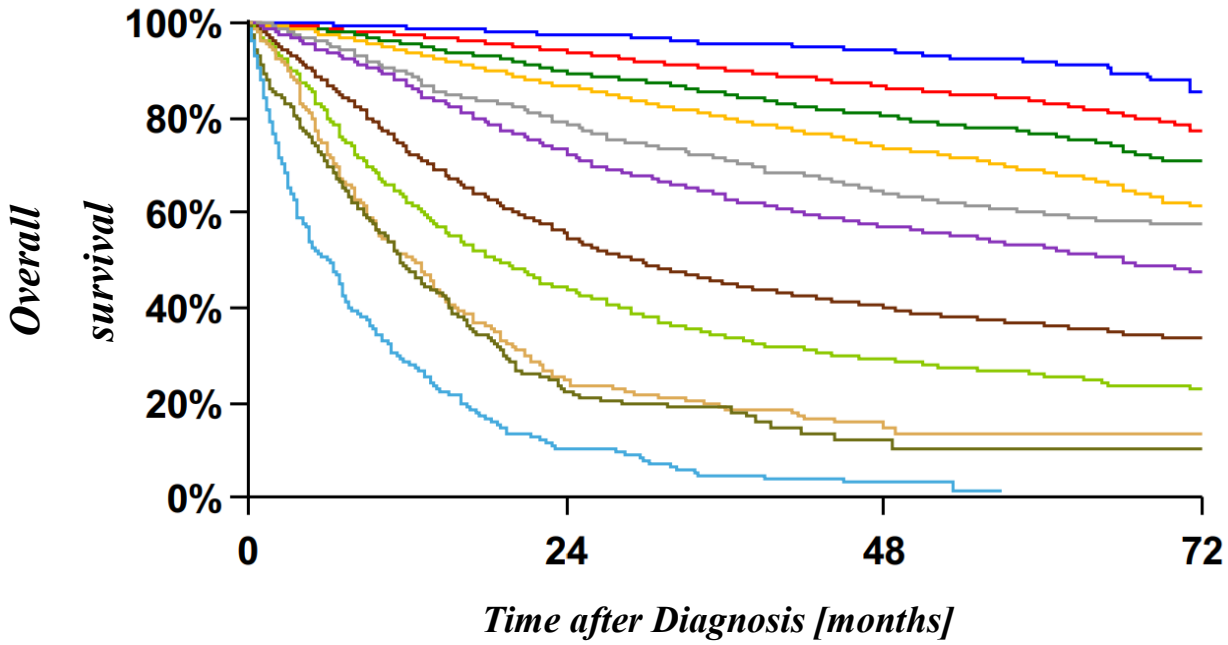
Table 6 Staging of distant metastasis (M) of the TNM system (edited after (Goldstraw et al., 2016))

Stage	Description
M0	No distant metastasis.
M1a	Sseparate tumor nodule(s) in a contralateral lobe, pleural or pericardial nodule(s) or malignant pleural or pericardial effusion.
M1b	Single extrathoracic metastasis.
M1c	Multiple extrathoracic metastases in one or more organs**.**

The combination of all three staging aspects (T, N, M) results in the American Joint Committee on Cancer (AJCC) classification (currently in its eighth edition), which allows the estimation of the tumor's prognosis, therapy options, and OSR based on the TNM staging results (Goldstraw et al., 2016). The stages are presented in Table 7, and the corresponding OSRs are shown in Figure 7.

Table 7 AJCC classification system (edited after (Goldstraw et al., 2016))

TNM stage	Primary tumor stage	Regional lymph node stage	Metastasis stage
Stage 0	Tis	N0	M0
Stage IA1	T1a(mi)	N0	M0
	T1a	N0	M0
Stage IA2	T1b	N0	M0
Stage IA3	T1c	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T2b	N0	M0
Stage IIB	T1a–c	N1	M0
	T2a	N1	M0
	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1a–c	N2	M0
	T2a–b	N2	M0
	T3	N1	M0
	T4	N0	M0
	T4	N1	M0
Stage IIIB	T1a–c	N3	M0
	T2a–b	N3	M0
	T3	N2	M0
	T4	N2	M0
Stage IIIC	T3	N3	M0
	T4	N3	M0
Stage IVA	Any T	Any N	M1a
	Any T	Any N	M1b
Stage IVB	Any T	Any N	M1c



Stage	5-year survival rate	Stage	5-year survival rate
Stage IA1	92%	Stage IIIA	36%
Stage IA2	83%	Stage IIIB	26%
Stage IA3	77%	Stage IIIC	13%
Stage IB	68%	Stage IVA	10%
Stage IIA	60%	Stage IVB	0%
Stage IIB	53%		

Figure 7 AJCC stages and corresponding OSR. The graph shows the OSR changes for the first 3 years, while the table provides the 5-year OSR (edited after (Goldstraw et al., 2016))

7 *Diagnosis of Tumors of The Pulmonary System*

7.1 *Radiographic imaging*

The first line for the clinical staging of suspected pulmonary lesions, especially in NSCLC, is the application of a radiographic imaging method, such as a contrast-enhanced computed tomography (CT) or positron emission tomography (PET) scans to define the anatomic location of an intrapulmonary tumor or metastatic lesions. Although these imaging tests cannot provide a confirmed diagnosis, they can provide the initial TNM assessment and allow the guidance of precise tissue sampling (Ost et al., 2013, Silvestri et al., 2013).

The CT is a computer-assisted evaluation, which registers X-ray images from multiple directions and is, therefore, able to obtain a three-dimensional image of the concerned region and tissue. This procedure enables statements about the volume structure of a transilluminated body (Buzug, 2002). The major disadvantage of computed tomography is the radiation exposure, which can be up to 1000 times higher than in x-ray examinations, which should be taken into consideration for the chosen diagnostic method (Kalender, 2006).

In pulmonary tumor patients, the differentiation between the mediastinal invasion of the primary tumor or metastatic lymph nodes from vascular structures is not always possible with native CT examinations, hence the application of an intravenous contrast enhancement agent is recommended, as well as an imaging of the liver and adrenal glands (Lusic and Grinstaff, 2013). This CT scan delivers information about the TNM/AJCC status of the pulmonary tumor and possible metastases in the pleura, liver, and adrenal glands, allowing subsequent guided biopsies of suspicious tissues to confirm the diagnosis. Especially T3 and T4 lesions, biopsy targets in N1-N3 lymph nodes, as well as additional lung diseases such as emphysema, are accurately visualized. The threshold for a metastatic lymph node is a short axis diameter >1 cm. Therefore, a differentiation in four different groups according to their CT findings has been suggested to improve the selection of targets for tissue biopsies and their subsequent treatment options. These groups comprise patients with the following radiographic findings/implications.

- Bulky tumors and an invasion of mediastinal structures, with no possible distinction between lymph nodes and the primary tumor
- Discrete lymph node enlargement >1 cm, allowing the distinction of the isolated lymph nodes and the primary tumor
- High risk for N2/3 involvement
- Low risk for N2/3 involvement or distant metastases

Because of the CT's low specificity and sensitivity, as well as other pathologies that might deliver similar radiographic results like tuberculosis or histoplasmosis, NSCLC staging requires a histological assessment besides the imaging (Silvestri et al., 2013).

The PET analyses metabolic rates of cells and due to a tumor's elevated metabolic turnover rate compared to healthy cells, this is reflected in an increased need for glucose and amino acids. Therefore, radioactive tracers, for instance [18F]-Fluorodeoxyglucose as a modified sugar and [11C]-Methionine as a modified amino acid, are incorporated by cells instead of the endogenous metabolites, but they cannot be metabolized and remain within the cells. Therefore, they allow the visualization of cells and tissues with increased metabolic activity, allowing a differentiation of malignant and healthy cells (after an incubation time of about an hour) (Herzog, 2007, Krause et al., 2007).

Although PET is commonly used as a routine staging tool in (pulmonary) tumors with better accuracy compared to the CT it should be applied after considering its limited anatomic resolution (Maziak et al., 2009). On the upside, it does provide information on the primary tumor's metabolic activity, potential mediastinal involvement, and distant metastases, although there is no standardized threshold for a positive PET result or the standardized uptake value (Paesmans et al., 2015, Pak et al., 2015). However, lymph nodes with a higher [18F]-Fluorodeoxyglucose uptake compared to the mediastinal blood pool suggest the presence of a metastatic disease (Gould et al., 2003). The main advantage of the PET compared to the contrast-enhanced CT is the full body scan modality, which allows the detection of liver, adrenal, bone, and pleural metastases. Furthermore, it was shown, that enlarged lymph nodes (in CT) also lead to an increased PET sensitivity. Some studies suggested that the main advantage for PET lies in patients with potentially resectable NSCLCs stages I-IIIA and a reduced number of thoracotomies, but these cases often undergo surgery without a prior application of PET (Fischer et al., 2009, Kozower et al., 2008). Frequently encountered issues in the PET are benign FDG-avid lesions (false positives) and non-enlarged lymph nodes or microscopic metastasis foci (false negatives) (Gupta et al., 2000, Silvestri et al., 2013, Yamamoto et al., 2008).

The combination of the two mentioned methods is the so-called integrated PET/CT. The indications, consequences (tissue sampling) and advantages for PET/CT remain unchanged compared to PET, but the addition of the coregistered CT provides additional anatomic information (De Wever et al., 2007, Fischer et al., 2009).

The imaging of metastases should be applied symptom-focused or CT-directed. Therefore, patients with focal symptoms, anomalies in the cardiovascular ultrasound or

electrocardiogram as well as suspicious laboratory parameters such as deviating blood cell counts, liver enzymes, bone parameters or a decreased renal function, should receive PET imaging. For stage III or IV patients an increased risk for intracranial metastases was found, therefore these patients should receive additional brain imaging, either with gadolinium-enhanced magnetic resonance imaging or PET/CT (Silvestri et al., 2013). Some tumor cases might require repetition of the imaging due to the occurrence of new symptoms or therapy (initiation) delay. The detection of unsuspected metastases in NSCLC patients leads to the adjustment of the therapy in about 20% of all patients, although an improved OSR due to these adjustments has yet to be proven (Heo et al., 2010, Morgensztern et al., 2008).

Other imaging methods for NSCLC metastases include a combination of CT, ultrasound, and radionuclide imaging (as working alternative to PET) for the detection of liver lesions (usually benign cysts or hemangiomas) (Kagohashi et al., 2003), bone scintigraphy for bony metastases (as inferior alternative to PET) (Hsia et al., 2002), and a combination of PET, CT, ultrasound, and magnetic resonance imaging to detect pleural metastases (Gupta et al., 2002, Marom et al., 1999).

Recent studies performed by the National Lung Screening Trial researched the outcome of patients who underwent potential screening tests for the detection of pulmonary cancer, including sputum cytology, chest radiography, and low-dose CT. Out of the tested modalities, the low-dose CT showed the best results, demonstrating a 20% mortality reduction. Therefore, the current US Preventive Services Task Force guidelines propose an annual low-dose CT for high-risk populations to allow the early detection of lung tumors and facilitate curative treatment. In most European countries such an early detection method is not implemented yet, because of controverse discussions regarding the potential benefit and the risks resulting from overdiagnosis and high false positive rates, resulting in invasive follow-up diagnosis (Midthun, 2016).

7.2 *Biopsy and histological examination*

As discussed before, a tissue biopsy and the subsequent histological examination is essential for the patient's diagnosis and staging, which should be based on 2015 WHO classification and the eighth edition of the TNM and AJCC system. Commonly, a higher stage requires a less invasive initial biopsy method to avoid possible tissue spread. Other factors that should be considered are the localization and expansion of a target lesion and the patient's preferences. Therefore, difficult lesions are often biopsied with the aid of a fiberoptic or endobronchial ultrasound-directed bronchoscopy, due to the higher accuracy and shorter

intervention time (Navani et al., 2015). The diagnosis can be made on cytopathologic or histological tissue samples, further testing includes IHC and genetic analysis and allows the differentiation of metastatic patterns, recurrences and the OSR among histological subtypes. The distinction among the different NSCLC subtypes as well as SCLC and benign lesions is getting more important, because it guides the subsequent specific mutation testing and the treatment choice, because some subtypes are chemotherapy-resistant, while some mutations can be treated with targeted therapies or checkpoint inhibitors. Examples of mutations, which allow a personalized treatment plan are the epidermal growth factor receptor (EGFR) and the anaplastic lymphoma kinase (ALK). Other mutations which can be identified with next-generation sequencing are the receptor tyrosine kinase 1, (KIF5B)-ret proto-oncogene, and human epidermal growth factor receptor 2. Furthermore, NSCLC and SCLC can usually be distinguished according to their clinical features. SCLC shows a much faster development of lung cancer symptoms such as cough, dyspnea, hemoptysis, and chest pain as well as a rapidly growing lesion in the CT, the Pancoast's syndrome, which results from the compression or involvement of cervical sympathetic nerves and the brachial plexus, is commonly encountered in NSCLC patients, while paraneoplastic syndromes are classical indicators for SCLC. Other pathologies, like the superior vena cava syndrome and metastases, are found in both tumor types (Travis et al., 2015). Detailed differentiations, as well as the IHC of the NSCLC subtypes, are presented in the classification chapter of this thesis.

8 Treatment of Tumors of the Pulmonary System

This chapter presents the current treatment modalities of pulmonary lesions, especially of NSCLC, which are recommended by the European Society for Medical Oncology (ESMO) (Novello et al., 2017, Postmus et al., 2017). Since most immunotherapeutic substances are not components of the currently recommended treatment plans, they will be discussed only shortly in this chapter but will be explained in further detail in the corresponding chapter.

Before a treatment plan is designed the patient's smoking habits should be surveyed, because smoking was found to interact with systemic therapies (Baser et al., 2006) as well as reduce the bioavailability of tyrosine kinase inhibitors (TKI) such as erlotinib (Hughes et al., 2009).

Most important in the determination of a therapy plan is the tumor's pathology according to the WHO/ICDO classification as well as the TNM and AJCC system. Therefore, a histological assessment before the therapy is essential for any curative treatment (except for patients, who have a high risk for a malignant lesion according to the published criteria), the tissue samples usually derive from a bronchoscopy or a CT-guided biopsy. An important indicator for the regional therapy's treatment plan is the patient's pre-therapy and the predicted post-treatment status, especially regarding the reduction of the pulmonary and vascular reserve capacity due to surgical resection or radiotherapy (RT). As objective values for this determination, the forced expiratory volume in the first second (FEV1) and the diffusing capacity of the lung for carbon monoxide (DLCO) are commonly used. The critical values for interventional procedures are FEV1 and DLCO of $> 40\%$, but the number of resected bronchopulmonary segments and the regional ventilation and perfusion should be considered as well. Furthermore, the evaluation of postoperative morbidity and mortality is possible by the application of risk-specific models, but those have yet to be validated in tumor patients (Nakamura et al., 2015).

An exception for the recommended treatment guidelines are elderly patients because it was found that they are more susceptible for toxicity compared to younger patients and therefore, the recommended therapy for patients ≥ 70 years consist of a single-agent therapy such as docetaxel, vinorelbine or gemcitabine (Kudoh et al., 2006). But more recent studies suggest, that, despite the increased toxicity, the combination therapy with two chemotherapeutic substances, one being a platinum-based agent, showed a significant survival advantage (Des Guetz et al., 2012, Socinski et al., 2013). Although comprehensive geriatric assessment predicts morbidity and mortality accurately, there was no association with improved OSR (Corre et al., 2016).

8.1 *Treatment of stage I and II diseases*

8.1.1 *Surgical resection*

First, the treatment of the stages I and II shall be discussed. The gold standard of therapeutic options is the surgical intervention to resect the tumor (Rosen et al., 2016). In case of non-compliance of the patient or in certain high-risk lesions, the alternative treatment method is curative RT, which can be either stereotactic radiotherapy (SABR) or fractionated (high-dosage) RT. But with absent nodal metastases (N0) in the imaging, surgical resection is highly recommended (Blasberg et al., 2010). The currently used surgical intervention for small lesions (T1) is lobectomy, especially in squamous cell carcinoma lobectomy was proven as superior to segmentectomy or wedge resection, while in adenocarcinoma (T1 and cT1a) lobectomy was superior to wedge resection but showed similar outcomes compared to segmentectomy (Koike et al., 2016, Veluswamy et al., 2015). Therefore, the treatment of *in situ* lesions with only minimal invasion might be possible with limited resection methods (Liu et al., 2016, Yu et al., 2016). This topic is currently investigated in two phase III studies (Blasberg et al., 2010, Nakamura et al., 2010).

Whether open thoracotomy or video-assisted thoracoscopic surgery should be considered, it was shown that both procedures had similar results for tumor clearance and nodal dissection, but video-assisted thoracoscopic surgery showed a reduced postoperative morbidity and mortality, making it the better approach in stage I tumors (Bendixen et al., 2016, Petrella and Spaggiari, 2016).

The goal of the surgery is always the R0 resection (resection in healthy tissue), therefore, evaluation of six or more lymph nodes, at least three mediastinal, is necessary for stage I tumors. The lymph node assessment method was proven to have no significant impact on OSR, local recurrence and distant metastasis rate, while the approach of systematic nodal dissection in stages II and IIIA is highly recommended (Huang et al., 2014).

In patients with multifocal lung tumors (curative) complete resection is recommended, but a combination of surgical resection and SABR delivers effective results as well (Chang et al., 2013, Griffioen et al., 2013).

8.1.2 *Systemic therapies*

For patients with a nodal involvement (N1 or N2) and subsequently higher tumor stages (II, III), a systemic therapy approach is taken. Therefore, the patient receives an adjuvant chemotherapy, which results in a 4-5% improved 5-year OSR (Artal Cortes et al., 2015).

The most commonly applied chemotherapeutic substances for NSCLC treatment, according to the American Cancer Society are cisplatin, carboplatin, paclitaxel (Taxol®), albumin-bound paclitaxel (Abraxane®), docetaxel (Taxotere®), gemcitabine (Gemzar®), vinorelbine (Navelbine®), irinotecan (Camptosar®), etoposide (VP-16®), vinblastine and pemetrexed (Alimta®) (American Cancer Society, 2018b). These agents are usually divided into two groups, the chemically and biologically active substances, which inhibit the tumor cell division.

Cisplatin and related platinum complexes such as carboplatin are assigned to the alkylating cytostatic agents, although they do not contain alkyl groups. Yet their mechanism is similar to the bifunctional alkylating agents due to the release of platinum ions to the environment, forming ammonium salts and light cisplatin, which link the DNA strands. The two chlorine atoms of cisplatin interact with nucleophilic groups of nucleic acids and proteins, thus the compound remains inactive in chloride-containing solutions or bio-fluids, such as blood. Due to the lower cytoplasmatic concentration of chloride ions, the conversion to an electrophilic aquo complex (bioactive form, interacts with nucleophilic centers) occurs. Although all DNA bases may be attacked, platinum complexes show a preferred binding to the guanidine's N(7), which results in a cross-linking of GpG sequences within the same DNA strand and therefore faulty transcription, cell cycle arrest, and apoptosis. Platinum agents are parenterally applied and their cellular uptake depends on various factors such as pH, sodium and potassium concentrations as well as the presence of reducing agents, such as glutathione (Bundschuh, 2016, Steinhilber et al., 2010).

Gemcitabine is a chemically active cytidine-nucleoside analog with a modified ribose component, which inhibits the enzymes of DNA and RNA synthesis or DNA replication. The modifications are found at the C(2') and an incorrect position of the hydroxyl group feigning the existence of a 2'-deoxy structure. The triphosphate is the gemcitabine's active form and competitively inhibits the DNA polymerase, whose usual substrate is deoxycytidine triphosphate (Steinhilber et al., 2010).

Folic acid analogs, such as pemetrexed, mainly inhibit the dihydrofolate reductase. Furthermore, they also inhibit the thymidylate synthase, glycinamide ribonucleotide formyltransferase, and the 5-aminoimidazole-4carboxamid ribonucleotide transformylase. By competitive inhibition of the dihydrofolate reductase's binding site, these compounds interfere with DNA synthesis. Hence, cells with increased DNA synthesis rates are affected at higher levels. Their mechanism is based on a replacement of the carbonyl oxygen in position 4, which leads to a change in the hydrogen bond donor and acceptor properties of amino groups,

resulting in a different binding mechanism of pemetrexed. Due to its increased binding affinity, it pseudo-irreversibly inhibits to the dihydrofolate reductase's carrier system and leads to the agent's rapid intracellular accumulation (Bundschuh, 2016, Steinhilber et al., 2010).

Topoisomerase inhibitors prevent the uncoiling of DNA and comprise of topoisomerase I and II inhibitors. Topoisomerase I inhibitors, such as irinotecan, are commonly natural cytotoxic agents, which interfere with the supercoiled DNA's relaxation, which is an essential step in transcription, replication and chromatin remodeling. Hence, topoisomerases I act as reversible nucleases, which covalently bind to a DNA phosphate group, cleave the phosphate ester groups and relink them after the DNA's rotation. The accumulation of these DNA strand breaks is prevented by a faster relinking compared to the opening. This relinking process of the covalent intermediate of the DNA double helix strands is targeted by topoisomerase I inhibitors. Because the presence of single-strand breaks (and subsequently double-strand breaks) are essential for the formation of a ternary DNA-topoisomerase-inhibitor complex, the effect of topoisomerase inhibitors is limited to the cell cycle's S phase by preventing the replication and the transcription of DNA. Topoisomerase II inhibitors, such as etoposide, derive from podophyllotoxin and their mechanism is based on the formation of a ternary DNA-topoisomerase II complex. But these substances target the DNA's relinking rather than the topoisomerase reaction, which results in an inhibited cleavage of covalent complexes and induces permanent double-strand breaks. Etoposide is a β-*D*-glucopyranoside and an acetal, whose saccharide residue interacts with the DNA, while its A, B and C rings, as well as the 4'-hydroxyl group and two methoxy groups, inhibit the relinking of DNA (Bundschuh, 2016, Steinhilber et al., 2010).

Tubulin modulators, such as paclitaxel, docetaxel, vinorelbine, and vinblastine, block the mitosis and disrupt the construction or dismantling of the spindle apparatus/microtubules. These microtubules consist of long, filamentous protein polymers, composed of α- and ß-tubulin heterodimers (6 different α- and 7 β-tubulins with different expression patterns and cell types are distinguished). The heterodimers of α- and β-tubulin form a small-sized microtubulus-nucleus, which is extended at both ends by the addition of other tubulin dimers. Due to their dynamical instability, the microtubules are characterized by constant construction and dismantling, with a more pronounced assembly and disassembly at the (+)- compared to the (-)-pole. This turnover is essential for the cell division, because of the microtubules' chromosome separation function during the mitosis. Colchicine, vinca alkaloids and their semisynthetic analogs disrupt the microtubule formation process and microtubule dynamics.

Vinca alkaloids derive from *Catharanthus roseus* and are commonly administered with other cytotoxic drugs as combination therapy. Their cytotoxicity results from their high binding affinity to the β-subunit of tubulin (vinca binding site). This site is usually located at the (+)-pole of the microtubules and its reversible binding slows down the microtubule formation and increases the time amount without microtubule conversion, which results in a blocked mitosis during the metaphase (due to the inability of chromosome separation). Vinca alkaloids can be administered in low(er) doses because they accumulate intracellularly (10-100-fold) compared to extracellular space (Bundschuh, 2016, Steinhilber et al., 2010).

For a more detailed overview of the mechanism of action and the pharmacology of these cytotoxic substances see 'Characteristics, diagnosis, and therapy of tumours of the central nervous system with emphasis on chemotherapeutical options', chapter chemotherapeutics (Bundschuh, 2016).

The recommended curative systemic therapy consists of a platinum-based chemotherapeutic agent in combination with a second cytotoxic substance because it was shown that platinum-based combinations had a 22% higher 1-year OSR (74% higher probability) compared to non-platinum combinations (Bronte et al., 2015, Pujol et al., 2006). A comparison of cisplatin and carboplatin showed a slightly higher OSR for cisplatin (Ardizzoni et al., 2007). Furthermore, it was proven that six or more chemotherapy cycles as well as a combination of three chemotherapeutic substances showed no improved OSR, but had a higher toxicity rate, hence 4 cycles of chemotherapy with two combined substances are recommended (Rossi et al., 2014). Another study found that albumin-bound paclitaxel/carboplatin had a higher OSR and less neurotoxicity compared to paclitaxel/carboplatin, while pemetrexed-based combinations showed a significantly improved OSR compared with gemcitabine- or docetaxel-based combinations, but should be restricted to advanced disease treatment (Ciuleanu et al., 2009, Li et al., 2012, Scagliotti et al., 2009). Furthermore, bevacizumab combined with paclitaxel/carboplatin was proven to result in an improved OSR (Soria et al., 2013, Zhou et al., 2015)

According to the recent guidelines, adjuvant chemotherapy should be conducted in stage II and III patients after the surgical resection of the NSCLC, but also in patients with stage IB and a primary tumor with a diameter of >4cm (Wakelee et al., 2016). The commonly applied chemotherapy is a combination of two agents. Cisplatin with a dosage of >300 mg/m^2 and 3-4 cycles is the preferred choice and is often administered in combination with vinorelbine (Arriagada et al., 2010).

In low stages studies, which investigated a potential benefit of adjuvant chemotherapy showed mixed results, with stage IA having an even worse outcome for postoperative chemotherapy, while stage IB showed a benefit, especially in patients with primary tumors >4cm (Artal Cortes et al., 2015, Salazar et al., 2017, Strauss et al., 2008).
The application of neoadjuvant chemotherapy was proven to deliver similar OSR compared to adjuvant chemotherapy (Lim et al., 2009) as well as the potential benefit of the tumor's preoperative downstaging and hence a less extensive resection (Gilligan et al., 2007, NSCLC Meta-analysis Collaborative Group, 2014).

8.1.3 *Primary radiotherapy*

Radiotherapy uses high-energy radiation, which interferes with the cell division process by damaging the genetic material of tumor cells. For stage I patients, who are inoperable due to their comorbidities or other reasons, the SABR or stereotactic body therapy, with a biologically equivalent tumor dose of at least 100 Gy, pose the best treatment options, delivering a 5-year OSR of 90% (Lindberg et al., 2015, Louie et al., 2015, Verstegen et al., 2015). SABR is usually based on 4-dimensional CTs, multiple radiation beams or arcs to minimize the organ toxicity risk, especially in high-risk patients with interstitial lung fibrosis and COPD (Bahig et al., 2016, Chen et al., 2017). In elderly patients with stage I lesions, the application of SABR showed a significantly improved OSR (Haasbeek et al., 2012).
Although the analysis of recent studies (STARS and ROSEL) suggested a similar recurrence-free 3-year OSR for surgical resection and SABR, other studies found that SABR showed inferior results compared to surgical resection (Allibhai et al., 2012, Chang et al., 2015b, Chen et al., 2010, Dickhoff et al., 2016, Hamaji et al., 2015, Hamamoto et al., 2012, Neri et al., 2010, Taira et al., 2014, Verstegen et al., 2016).
Therefore, new trial studies will compare the toxicity and life quality of surgery and SABR and their dependency on the patient's operative risk (Cherny et al., 2015, Siva and Ball, 2016).
In comparison, central tumors, which are located within 2 cm any important mediastinal structure such as the bronchial tree, oesophagus, heart, brachial plexus, major vessels, spinal cord, phrenic nerve and recurrent laryngeal nerve as well as tumors of the hilar regions showed high response and limited toxicity rates for SABR, while the surgical resection of these tumors is associated with high-risk operations (Chang et al., 2015a, Senthi et al., 2013). Until the definite study results are published, the application of hypofractionated RT is

recommended by the guidelines (Cheung et al., 2014) (hyperfractionated RT is associated with a higher OSR, but also higher toxicity rates (Mauguen et al., 2012)).
While SABR produces good results for central lesions, ultracentral lesions, whose radiotherapeutic target volume overlaps the trachea or main bronchi, show an increased toxicity, hence RT is not a suitable treatment option for these tumors (Tekatli et al., 2016).
For the few patients who have contraindications for surgery and SABR as well, radiofrequency ablation is the most commonly applied treatment modality, although its effectiveness is based only on observational studies (Ambrogi et al., 2015).

8.1.4 *Postoperative radiotherapy*

While the indications for RT as a primary treatment are well supported by published data, postoperative RT for R1 resection (surgical tumor resection, but not in healthy tissue), seems feasible but lacks sufficient data, hence the guidelines suggest the addition of a chemotherapy in stage IB-III patients. while RT as an adjuvant treatment of intrasurgical detection of nodular involvement (N2) is currently researched in a large clinical trial (Le Pechoux, 2011).

8.2 *Treatment in stage III*

Within 4 weeks before the treatment's start of stage III lesions, the patient should be evaluated with a contrast-enhanced CT and a PET scan of the chest and upper abdomen as well as contrast-enhanced brain imaging to rule out mediastinal lymph node involvement and distant metastases. The most common treatment plan for resectable as well as unresectable tumors involves a platinum-based chemotherapy combination (Postmus et al., 2017).
The resection of stage III NSCLCs is possible for single station N2 lesions (the surgery should be followed by an adjuvant chemotherapy), T4N0 tumors (R0 resection is feasible) and downstaged nodal metastases. Therefore, patients with multistation N2 or N3 tumors should receive a neoadjuvant RT or chemotherapy. Surgical resection with lobectomy was proven to have a superior OSR compared to RT, while less extensive resection showed no advantage compared to RT (van Meerbeeck et al., 2007). More recent studies showed comparable results of surgical resection after induction therapy with RT and/or chemotherapy (Pless et al., 2015, Tsitsias et al., 2014) and definitive chemotherapy/RT, although the results suggest a certain minimal radiation level of 45 Gy and a boost in the final RT week (Eberhardt et al., 2015).
For unresectable lesions, whose complete surgical resection (R0), even after induction therapy, is not achievable, concurrent chemoradiotherapy (sequential chemotherapy followed by definitive RT with 60-66 Gy distributed to 30-33 daily fractions) is the recommended

treatment (Brunelli et al., 2009). Phase III trials investigating concurrent chemoradiotherapy found that the early mortality rate is about 10% (Warner et al., 2016). Furthermore, an increased irradiance (74 Gy) showed a worse outcome (Bradley et al., 2015), whereas accelerated RT treatment resulted in a 2,5% benefit in the 5-year OSR (Mauguen et al., 2012). Therefore, accelerated RT schedules after chemotherapy are the preferred treatment modality. For patients with contraindications, the sequential chemotherapy (cisplatin and etoposide, cisplatin and vinorelbine) is the recommended treatment option (Auperin et al., 2010).

8.3 *Treatment in stage IV*

The current standard therapeutic approach for stage IV and EGFR- and ALK-negative tumors is the palliative systemic therapy with platinum-based chemotherapeutic agents in combination with a second cytotoxic substance to alleviate the symptoms caused by the primary tumor and/or the metastases (Ahn et al., 2015). Studies researching the effects of pemetrexed-cisplatin in stage IV patients showed no improvements aside from less hematological toxicity compared to cisplatin-etoposide (Senan et al., 2016). Similar results were found for the effects of subsequent chemotherapy after chemoradiotherapy (Socinski et al., 2012).

Radiotherapy is commonly used to address radiating pain due to infiltrations of the chest wall, a superior vena cava syndrome, or in cases of soft tissue or neural invasion, hemoptysis, symptomatic airway obstruction. Some of these diseases, such as the superior vena cava compression can also be addressed with a surgical treatment approach, the vascular stenting. Other interventions address abscesses, hemoptysis, spinal cord compressions or pathological bone fractures (Postmus et al., 2017). Furthermore, pleurodesis, which is done to control the recurrent pleural effusions, and endoscopy, which is used to treat major airway obstructions or postobstructive infections, are commonly applied (Shaw and Agarwal, 2004). In cases where bronchial obstruction or trapped lungs prevent pleurodesis, the application of indwelling subcutaneous pleural catheters provides an alternative treatment method. (Davies et al., 2012). Although palliative care is a very important oncological field, studies and statistical records are hard to find. But a randomized trial determined that the palliative care of patients diagnosed with stage IV disease led to a significantly higher quality of life, treatment reduction and higher OSR (Temel et al., 2010).

Newer approaches research the effects of immunotherapeutic agents such as durvalumab, an anti-programmed cell death protein 1 ligand (PD-L1) antibody as a treatment modality for NSCLC tumor patients. The results of this clinical trial showed significant improved

progression-free OSR (16.8 months vs. 5.6 months), longer response times (ongoing response 18 months after initial treatment: 72.8% vs. 46.8%) as well as higher response rates (28.4% vs. 16.0%) and longer progression-free intervals (18 months after initial treatment: 44.2% vs. 27.0%) for durvalumab compared to a placebo (Antonia et al., 2017). Other researched immunotherapeutic and targeted therapeutic substances, which were shown to have a beneficial effect in advanced stage NSCLC patients, are other PD-L1, such as atezolizumab and BMS-936559, and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) checkpoint inhibitors, such as ipilimumab (Gettinger, 2018), as well as EGFR TKI and ALK inhibitors (Huang et al., 2016).

Furthermore, an increasing number of recent case reports published the possibility to get stage IV tumor patients on a curative treatment plan by the combination of chemotherapy, immunotherapy, and surgery, for instance by an application of capecitabine, bevacizumab and surgical resection in colorectal mucinous adenocarcinoma (Vernmark et al., 2015).

8.4 *Treatment of metastases*

8.4.1 *Brain metastases*

The treatment options and the patient's prognosis for brain metastases mainly depend on the presence or absence of driver mutations in the primary tumor, the number of brain metastases, additional extracranial metastases, and Karnofsky performance status scale (Sperduto et al., 2012). The Karnofsky performance status scale provides an objective assessment tool for functional impairment and hence it is used to compare the effectiveness of different therapies and to assess the prognosis in individual patients (a lower Karnofsky score indicates a decreased survival rate) (Karnofsky and Burchenal, 1949). For cases with absent driver mutations, the estimated prognosis is calculated based on the Radiation Therapy Oncology Group recursive partitioning analysis, which differentiates three classes.

- Class I comprises patients below 65 years of age and with a Karnofsky performance score ≥70% (no functional impairment, only mild disease symptoms), which indicates the patient can lead an autonomous life, no extracranial metastases, and a controlled primary tumor
- Class III comprises patients with a Karnofsky performance score <70% (functional impairment, considerable disease symptoms)
- Class II represents the remaining patients.

Because class III patients have a median survival time of fewer than two months, they are usually only treated with the best supportive care. For patients with only a single metastasis,

resection or stereotactic radiosurgery (SRS) is a feasible option, whereas the presence of multiple metastases is only treated in class I and II by the application of SRS. A recent study suggested that whole brain RT (WBRT) before surgery or SRS had no effect on the OSR (Patil et al., 2017), while other studies found that SRS delivered similar or even better results compared to WBRT (Soon et al., 2014, Yamamoto et al., 2014), especially in patients below 50 years of age (Sahgal et al., 2015).

The recommended treatment of three or more brain metastases is the WBRT in class I and II patients, although the benefit of WBRT compared with supportive care has yet to be studied in randomized trials (Novello et al., 2017). To address symptoms and/or edema caused by brain metastases, dexamethasone or another corticosteroid is the common treatment modality, but should be tapered after the end of the RT (Vecht et al., 1994).

Patients with driver mutations, such as EGFR and ALK in the primary tumor, have a risk of 44-60% to develop brain metastases. For asymptomatic brain metastases, the application of TKI might lead to a delay of the cranial RT, symptomatic brain metastases are treated like the driver mutation-absent metastases (Costa et al., 2011, Sahgal et al., 2015). Recent studies proved that afatinib treatment of brain metastases with EGFR mutations had a similar Karnofsky performance status scale compared to non-EGFR mutated metastases and even better results in comparison to chemotherapy (Schuler et al., 2016), therefore the effects of osimertinib are currently researched. ALK-positive patients often show progression even under TKI therapy, while a recent phase I study showed a clinical benefit for patients treated with ceritinib compared to crizotinib (Kim et al., 2016).

8.4.2 *Bone metastases*

Because bone metastases show a high development rate (although lower compared to brain metastases) of 30-40% in NSCLC patients, an evaluation of the potential presence of bone metastasis at the time of the diagnosis of the primary pulmonary tumor is highly recommended. In the prevention of metastasis-accompanying diseases, mainly skeletal-related events, the application of zoledronic acid and/or denosumab was proven to be an effective method, especially in stage IV patients with bone metastases (Henry et al., 2011, Rosen et al., 2004). Denosumab showed improved OSR in metastatic NSCLC patients in a phase III trial (Scagliotti et al., 2012).

8.4.3 *Oligometastatic NSCLC*

Oligometastases refer to hematogenous metastases arising in a limited number, but the definitions differ among three and five metastases or a limitation regarding the number of affected organs. Another differentiation is between synchronous (diagnosed within one month of the primary tumor's identification) and metachronous metastases (appear after the tumor's treatment is finished). These metastases might differ regarding their tumor biology, therapy, and prognosis (Kozower et al., 2013, Novello et al., 2017). The usual therapy for stage IV patients with synchronous oligometastases is systemic therapy combined with high-dose SABR and/or surgery, while metachronous metastases are treated with radical SABR or surgery (Tonnies et al., 2014). A retrospective study researched the outcome of synchronous and metachronous NSCLC metastases and found that the best 5-year OSR of 48% was for metachronous metastatic patients, while synchronous metastatic and N0 patients showed a 36% 5-year OSR and synchronous metastatic and intrathoracic N1/N2 patients had an OSR of only 14% (Ashworth et al., 2014). Other studies researched the histology and the outcome of metastases and found that most secondary tumors have the same histology as the original lesion and that the treatment with SABR and surgery showed comparable 5-year OSR of the treated patients (Chang et al., 2013, Griffioen et al., 2013, Palma et al., 2014).

A study investigating the surgical resection of brain metastases and the NSCLC primary tumor showed a 1-year and 2-year OSR of 62% and 24%, respectively (Cheufou et al., 2014). Although this study reported that nodal involvement did not change the OSR, lymphatic metastases are commonly considered contraindications for surgical resection (Kozower et al., 2013, Novello et al., 2017, Spratt et al., 2016).

8.5 *Maintenance therapy*

Maintenance therapy is an important aspect to preserve a certain level cytotoxicity after the (platinum-based) chemotherapy. Therefore, clinical trials researched the effects of pemetrexed as well as erlotinib and found an improved OSR as well as longer progression-free intervals for NSCLC patients (Cappuzzo et al., 2010, Ciuleanu et al., 2009, Paz-Ares et al., 2013), while a study researching the effects of combined pemetrexed-bevacizumab found only a trend for an improved OSR (Barlesi et al., 2014). Furthermore, it was found that continuative therapy with necitumumab, gemcitabine, and cisplatin showed survival benefits, although they are technically not maintenance therapy (Thatcher et al., 2015)

8.6 *Follow-up and response evaluation of NSCLC patients*

Especially within the first 30 days after the surgical resection patients show a high complication and readmission rate of 12.8%. They showed a 6-fold mortality rate compared to complication-free patients (Hu et al., 2014), which is reflected by the 3-month mortality rate (Pezzi et al., 2014). The recorded complications were mainly respiratory insufficiency, pneumonia, pneumothorax and cardiac complications (Novello et al., 2017). Risk factors for post-surgical complications were already discussed in the surgery subchapter.

Various follow-up studies revealed that the recurrence range is 6-10% per year for the first four years (year 1 and 2: primary recurrence, year 3 and 4: distant metastases), and decreased after this time period (Demicheli et al., 2012, Lou et al., 2013). This is true for non-smokers as well as smokers, but cessation was associated with better treatment responses and outcomes (Ripley et al., 2014). Because curative treatment of recurring primary tumors is often impossible, this group only has a 5-year OSR of 15%, while secondary tumors range from 25-60%. Therefore, a follow-up with physical examination and chest CT every 6 months for the first two years and an annual control thereafter is recommended, but might be adjusted to the patient's individual risk situation. Especially stage III patients who underwent chemotherapy and RT showed a high rate of local and metastatic tumor progression (Dickhoff et al., 2016, Hamaji et al., 2013, Hamaji et al., 2015, Hung et al., 2009, Rosengart et al., 1991, Taira et al., 2014, Verstegen et al., 2016).

The response evaluation includes a radiographic investigation, preferably the initial diagnostic imaging method, and is conducted after 6-9 weeks of first-line chemotherapy and/or immunotherapy, dependent on the individual treatment modality. In patients with a good response rate to the first-line therapy, a close-meshed radiological follow-up is recommended to allow an early initiation of a second-line therapy (Novello et al., 2017).

8.7 *Second-line therapy*

When maintenance therapy fails to prevent tumor progression, either local recurrences or distant metastases, the patients are offered second-line therapies. Compared to first-line chemotherapy, the administration of combination chemotherapy shows no benefit over monotherapy treatment plans, although both improve tumor-related symptoms as well as the OSR (Di Maio et al., 2009). Frequently applied substances include pemetrexed and docetaxel, which show a similar outcome profile, although pemetrexed has a better toxicity profile (Hanna et al., 2004). Furthermore, erlotinib is used as second- or third-line therapy for patients, who are not eligible for further chemotherapy and shows similar OSRs, but shorter

progression-free intervals compared to pemetrexed and docetaxel (Karampeazis et al., 2013, Kawaguchi et al., 2014, Shepherd et al., 2005, Zhao et al., 2014).

A combination of docetaxel and nintedanib, a TKI and angiokinase inhibitor, showed a similar quality of life, significant longer progression-free interval, as well as a higher OSR in adenocarcinoma patients, compared to docetaxel alone, making it a feasible second-line therapy especially for patients showing progression within 9 months of the first-line chemotherapy's start (Novello et al., 2015). Furthermore, a combination of paclitaxel-bevacizumab was compared with docetaxel and showed no significant difference in the OSR, although the combination's progression-free interval was longer (Cortot et al., 2016), whereas nivolumab was proven to show a favorable OSR, response duration and toleration and as well as a similar progression-free interval compared to docetaxel, especially in PD-L1 tumors (Borghaei et al., 2015, Reck et al., 2014).

Another recently approved immunotherapeutic substance for second-line therapy of stage IV NSCLC is ramucirumab, a vascular endothelial growth factor receptor-2 inhibitor, which delivered superior OSR and progression-free intervals in combination with docetaxel after a platinum-based first-line therapy compared to a placebo (Garon et al., 2014).

8.8 *Targeted therapy*

EGFR and ALK receive high attention and are mutations that NSCLC patients should be simultaneously tested for (Kerr et al., 2014). The detection is done either with polymerase chain reaction (PCR), fluorescence in situ hybridization or IHC (Lindeman et al., 2013, Wu et al., 2013). Several studies demonstrated that the administration of EGFR TKIs such as gefitinib, erlotinib, and afatinib in EGFR-sensitized patients resulted in improved OSR, response rates, quality of life and prolonged progression-free intervals compared to platinum-based chemotherapy combinations, especially in patients with advanced non-squamous cell carcinoma (Lee et al., 2014, Mitsudomi et al., 2010, Rosell et al., 2012). The comparison of afatinib and erlotinib, as well as gefitinib as a first-line therapy, demonstrated that the outcome was significantly better under afatinib treatment in both studies (Park et al., 2016, Soria et al., 2015). In comparison to platinum-based chemotherapy a higher OSR of EGFR-sensitized patients under afatinib-therapy seems likely (Sequist et al., 2013, Wu et al., 2014, Yang et al., 2015).

If the presence of an EGFR-sensitizing mutation is diagnosed at a time when first-line platinum-based chemotherapy has already started, the completion of four cycles is recommended and the EGFR TKI is subsequently administered as maintenance or second-line

treatment (Douillard et al., 2014). A combination of bevacizumab-erlotinib showed significantly increased OSRs compared to erlotinib alone (Yoshida and Yamada, 2015). A huge problem of EGFR TKI is the progression of most patients within 9-12 months after the therapy and the accompanying resistance mechanisms acquired through mutations in the exons 18-21 such as the T790M mutation, a missense mutation within exon 20, increasing the ATP binding affinity, aggravating the competitive inhibition by gefitinib and erlotinib (Cortot and Janne, 2014, Stahel et al., 2015, Sueoka-Aragane et al., 2016). Osimertinib, a third-generation EGFR TKI, was specifically designed to target this T790M mutation and was already approved for this treatment application (Janne et al., 2015). Therefore, patients who received an EGFR TKI therapy should undergo either a tissue rebiopsy or liquid biopsy to determine the eventual presence of mutations such as T790M, L858R mutation of exon 21, or an exon 19 deletion, and the subsequent indicated administration of third-generation EGFR TKIs (Mitsudomi et al., 2015, Oxnard et al., 2016, Yu et al., 2013).

The first studies regarding the more frequent ALK-rearrangements (especially in non-smokers, younger adenocarcinoma patients) and the efficiency of ALK inhibitors showed a significantly improved OSR, progression-free intervals, symptomatology and quality of life for crizotinib compared to established treatment options such as pemetrexed or docetaxel (Camidge et al., 2012, Kwak et al., 2010, Shaw et al., 2013). Therefore, the administration of crizotinib in ALK-positive NSCLC as second-line therapy was recommended shortly after the publication of these results (Solomon et al., 2014). Another study investigated crizotinib as first-line therapy compared to cisplatin and pemetrexed and found a significantly longer progression-free interval, which resulted in the approval of crizotinib as a first-line therapy in ALK-positive NSCLC patients (Blackhall et al., 2014).

For patients with EGFR-mutated and ALK-positive cells, a combination of crizotinib and surgical resection or RT might be a feasible treatment approach (Weickhardt et al., 2012).

Since ALK-rearranged NSCLC show a high tendency to metastasize to the brain the main issue of crizotinib is its low penetration of the blood-brain barrier and hence its low concentrations in the cerebrospinal fluid and the formation of various (crizotinib) resistance mechanisms (Costa et al., 2011, Kim et al., 2016). In recent studies (conducted in Japan and Europe) ceritinib and alectinib, second-generation ALK inhibitors, were proven superior to crizotinib by showing a significant activity in resistant as well as non-resistant ALK-positive cells as well as in brain metastases and lower toxicity (Hida et al., 2017, Ou et al., 2016, Peters et al., 2017a, Shaw et al., 2016).

9 *Immunotherapy as Innovative and Targeted Treatment Option*

Nowadays, cancer immunotherapy is one of the most researched topics in life science and provides a promising alternative to the already well-established surgery, cytotoxic chemotherapy, radiation, and targeted treatment modalities, which often allows the treatment of patients, where all other measures have failed.

The research of immunotherapeutic substances and their effects on tumor regression dates back to the second half of the 19th century when in 1868 Busch found that infections were associated with tumor regression in a tumor patient with erysipelas (Busch, 1868) and in 1882 Fehleisen identified the pathogen as *Streptococcus pyogenes* (Fehleisen, 1882). The first targeted immunotherapy was conducted by William Coley, who found that the injection of Coley's toxin, an isolate of *Serratia marcescens* and *Streptococcus*, triggered a systemic inflammation in the patient, which led to tumor regression in numerous patients (Coley, 1906). Although this toxin provided the first immunotherapeutic cancer treatment it disappeared, due to the lacking reproducibility of the effect as well as the rise of RT and chemotherapy. Yet, the principles Coley proposed were proven in 1976, when Morales showed that Bacillus Calmette-Guérin (BCG) could be used for the treatment of bladder cancer (Morales et al., 1976). Another important discovery was the tumor necrosis factor, which was identified in 1975 (Carswell et al., 1975).

9.1 *The immune system as tumor defense system*

The immune system is comprised of an innate and an adaptive arm, although these arms show overlapping functions and a close relation. While the innate immune system includes dendritic cells, natural killer cells (NKs), macrophages, neutrophils, eosinophils, basophils and mast cells, the adaptive immune system includes B lymphocytes, $CD4^+$ helper T lymphocytes, and $CD8^+$ cytotoxic T lymphocytes. Another difference besides the involved cell types is the necessity for antigen-presenting cells for the adaptive immune system's activation, while the innate system functions independently of antigen stimulation (Brodin and Davis, 2017). The adaptive immune system generates antigen-specific T- and B-cell lymphocytes.

Usually, the immune system recognizes damaged and potentially malignant cells and attacks these cells. The recognition of malignant cells is based on the expression of tumor-associated antigens, such as mutated proto-oncogenes, tumor suppressor genes, over- or untypically expressed proteins, altered glycolipids, and glycoproteins, and cell type-specific differentiation antigens, which are presented on the cell's major histocompatibility complex

(MHC) molecules (Stoler et al., 1999). For the activation of the T lymphocytes, additional costimulatory signals are required, for instance, an interaction of the T lymphocyte's CD28 receptor with B7 ligands of the antigen-presenting cell. The formation of this costimulatory complex is also termed immunological synapse and triggers the T lymphocyte's proliferation. Many of the possible combinations between the antigen-presenting cell and T lymphocyte have an inhibitory function, such as the programmed cell death protein 1 receptor (PD-1)/programmed cell death protein 1 ligand (PD-L1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)/B7 complexes (Vesely et al., 2011, Wang and Wang, 2017).

But some malignancies possess the ability to evade the immune system by changing their microenvironment and cellular expression patterns. This ability is termed immunoediting, where the initial elimination response is followed by an equilibrium and evasion phase. The elimination is triggered by the presentation of tumor antigens and the subsequent formation of tumor-specific $CD4^+$ and $CD8^+$ T lymphocytes, that have the potential to eliminate malignant cells. In case of an incomplete tumor elimination, the equilibrium phase is next. In this phase, the remaining tumor cells are unable to progress but are not attacked by immune cells. Afterwards, in the escape phase, the malignant cells start to grow and migrate/metastasize to other organ systems (Dunn et al., 2002). Possible evasion patterns are a decreased MHC class I or an increased immune checkpoint molecule expression. The latter emulates nonmalignant cells and results in a negative feedback loop and prevents inflammation by T lymphocyte suppression (Sharma et al., 2017, Tsukahara et al., 2006). The first human tumor antigen, which was recognized by T lymphocytes was found in 1991 when van der Bruggen and his team successfully cloned the melanoma antigen-encoding gene and identified it as an immunotherapeutic target (van der Bruggen et al., 1991).

The formation of a tumor microenvironment is achieved by a recruiting of immune cells, which shield the tumor cells from the remaining immune system. This process includes the production of cyto- and chemokines, which ultimately results in an adaption of the recruited immune cells (McAllister and Weinberg, 2014).

9.2 *Targets for immunotherapeutic substances*

- $CD8^+$ lymphocytes, termed cytotoxic T cells, as well as $CD4^+$ lymphocytes, termed T-helper cells, are activated by MHC class 1 ($CD8^+$ cells) and MHC class 2 ($CD4^+$ cells) (van der Merwe and Dushek, 2011). After the activation by antigen recognition, they release cytolytic enzymes and cytokines, whose expression level are controlled by immune checkpoint molecules such as the CTLA-4 and PD-1 receptors, T lymphocyte

immunoglobulin and mucin domain 3 (TIM-3) receptor, and lymphocyte activation gene 3 (LAG3) receptor, as well as cytokine cascades and the expression of inhibitory and stimulating molecules (Wherry, 2011).

- Natural killer (NK) cells target cells with a low MHC class 1 expression and secret inhibitory molecules such as various killer immunoglobulin-like receptor subtypes (Gras Navarro et al., 2015).
- Macrophages release either interferon (IFN) and phagocyte or release cytokines such as IL-4 and IL-10, which leads to a transformation of growth factor beta and increases the inflammatory responses (Laoui et al., 2014).
- Other cells are the $FoxP3^+$, $CD25^+$, $CD4^+$ T-regulator and myeloid-derived suppressor cells, which inhibit the $CD8^+$ T lymphocytes cytotoxic activity (Marvel and Gabrilovich, 2015, Savage et al., 2014).

Tumor cells evade this adaptive immune responses by selection-biased resistance mechanism developments, such as the alteration of antigens, loss of MHC 1 expression required for T lymphocyte recognition, the alluring of immunosuppressive molecules (IL-6, IL-10 and the transformation of growth factor beta), and an increased expression of checkpoint inhibitor molecules/ligands for checkpoint inhibitors (e.g. PDL1), which also provide new approaches for targeted oncological therapies (Catalan et al., 2015, Donia et al., 2015, Guo et al., 2016, Matsushita et al., 2012, Rooney et al., 2015, Tumeh et al., 2014, Zaretsky et al., 2016).

Since pulmonary cancers in general and NSCLC in particular, were considered unsusceptible to immunotherapy such as cytokine therapies, the current guidelines contain only a few immunotherapeutic substances (approved for NSCLC therapy). But recent developments in the field of checkpoint inhibitors provide a promising and innovative approach for NSCLC immunotherapy. These immune checkpoints are pathways with an inhibitory function to allow the cell's self-tolerance and attenuate the immune responses in peripheral tissues. The checkpoints addressed in NSCLC research are mainly PD-L1, as well as PD-1 and CTLA-4 receptor.

PD-1 is a transmembrane protein expressed on T, B, and NK cells and its inhibition is controlled by the binding of PD-L1 and PD-L2. While PD-L1 is expressed on multiple tissue surfaces, including numerous tumor cells, NK cells, monocytes and hematopoietic cells, PD-L2 is commonly restricted to dendritic cells and macrophages (Amarnath et al., 2011). Their expression is regulated by cytokines such as IL-12 and IFN-γ, and leads to a suppressed immune response including the activity of $CD8^+$ lymphocytes and therefore decreased tumor cell killing (Francisco et al., 2009).

9.3 *Immunotherapeutic options*

9.3.1 *Cytokines and vaccines*

This group comprises mainly non-specific immunostimulatory cytokines such as IL-2 or IFN-γ.

L-MTP is a synthetically produced bacterial cell wall analog, which activates macrophages and monocytes, but is not Food and Drug Administration (FDA) approved (Kager et al., 2010). The FDA approved the vaccine for metastatic castrate-resistant prostate carcinoma is sipuleucel-T, which results in a prostatic acid phosphatase and granulocyte-macrophage colony-stimulating factor exposure of the dendritic cells (Gardner et al., 2012).

The common goal of vaccine treatments is the exposure to tumor antigens and triggering of the immune response with the formation of tumor-specific B and T lymphocytes. For instance, BCG leads to an increased tumor antigen expression following the internalization of the bacteria, which results in increased cytokine release, including IL-2, IL-12, INF-γ, tumor necrosis factor as well as IL-4, IL-5, IL-6 and IL-10 and subsequently an enhanced antitumor activity (Fuge et al., 2015).

9.3.2 *Oncolytic viruses*

Due to gene modifications, oncolytic viruses show a lack of virulence against nonmalignant cells, but invade and subsequently lyse cells without anti-viral cellular defenses such as tumor cells. After the cell's lysis, it is attacked by the immune system because of the released tumor antigens (Choi et al., 2016). The first FDA approved anti-tumor oncolytic virus (for melanoma) is T-VEC, which derives from a herpes simplex-1 virus and expresses granulocyte-macrophage colony-stimulating factor, thereby stimulating immune cell proliferation.

9.3.3 *Adoptive cell therapy*

This therapy is based on the isolation and the *in vitro* cultivation of tumor-specific T lymphocytes and the subsequent infusion into the patient and is mainly used in melanoma and tumors of the hematologic system. The techniques of adoptive cell therapy include the cultivation of tumor-infiltrating T lymphocytes – either isolated or genetically modified to attack the tumor, which are then termed as chimeric antigen receptor T lymphocytes. Nowadays, the most frequently used method for gene editing is the Clustered Regularly

Interspaced Short Palindromic Repeats technique, which emulates bacterial protection from invading nucleic acids of viruses and plasmids (Jinek et al., 2012).
The first FDA approved application for chimeric antigen receptor T lymphocytes is the treatment of relapsed and refractory B-cell acute lymphoblastic leukemia in children and young adults (FDA News Release, 2017a).
A reported problem of the adoptive cell therapy is the potential risk of a cytokine storm and cerebral edema, which even resulted in death (Offord, 2017).

9.3.4 *Immune checkpoint inhibitors*

The function of these immune checkpoints is the prevention of a permanent immune inflammatory response. Therefore, numerous checkpoints interact as ligands with T lymphocyte receptors, leading to the formation of immunological synapses and the regulation of the T lymphocytes' activity and proliferation. For the T lymphocyte's activation, the tumor antigen presented on the MHC has to be recognized by the T-cell receptor and a costimulatory T-cell receptor such as CD28 has to interact with the B7 ligand, which is suppressed by inhibitory immune checkpoints to prevent unwanted autoimmune processes (Sharma et al., 2017).
More than 20 checkpoint molecule complexes, inhibitory and stimulatory have been discovered, which are expressed on T lymphocytes as well as myeloid and lymphoid cells (Tsai and Hsu, 2017). Examples of such checkpoint receptors are CTLA-4, which is a structural homolog to CD28, but with a higher B7 ligand binding affinity, which results in an inhibited T lymphocyte proliferation and interleukin-2 (IL-2) secretion, PD-1, which leads to a decreased T lymphocyte activation by preventing the phosphorylation of key signaling intermediates, and LAG3, which is a structural homolog to CD4, but with a higher MHC class II binding affinity (Buchbinder and Desai, 2016, Krummel and Allison, 1995). PD-L1 and PD-L2 are members of the B7 ligand family and PD-L1 is expressed on naïve and activated T lymphocytes, epithelial, endothelial, and tumor cells, whereas PD-L2 is expressed on dendritic cells and monocytes, but its induction is possible on numerous immune and nonimmune cells (Yearley et al., 2017).
Due to the early immune reaction of the CTLA-4/B7 synapse, it is assigned a leading role among the checkpoint inhibitors, because it stops autoimmune T lymphocytes at early activation stages within the lymph nodes, while PD-1/PD-L1 reacts later, but plays an important role in the protection of cells from T lymphocyte attacks, which stays also true for

tumor cells and results in anti-tumor activity (Chen and Mellman, 2013, Fife and Bluestone, 2008)

9.3.5 *Immune checkpoint inhibitor therapy*

The first successful animal models with CTLA-4-blocking monoclonal antibodies were published in 1996 (Leach et al., 1996). These monoclonal antibodies were termed immune checkpoint inhibitors, although the systematically correct name would be anti-immune checkpoint inhibitors. The main advantages of these antibodies are their reduced toxicity compared to conventional tumor therapies and their ample application profile.

The current main issue for the indication of a checkpoint inhibitor such as anti-PD-L1 is the lack of an objective, standardized and sensitive assessment method, which can result in a difficult differentiation of PD-L1-positive tumor cells from the other PD-L1-positive cells in the tumor microenvironment (Mino-Kenudson, 2016).

Due to their reduced toxicity checkpoint inhibitors got FDA approval for six different, advanced-staged tumors, including lung cancer, melanoma, renal cell carcinoma, head and neck cancer, urothelial cancer, and Hodgkin's lymphoma (Burstein et al., 2017). Especially in smoking-related lung cancers and colorectal cancers higher mutation rates were found and therefore they show a higher response rate to immune checkpoint inhibitor therapy (Passardi et al., 2017, Rizvi et al., 2015).

Although the majority of immune checkpoint inhibitors are either receptors or ligands, they can be expressed as soluble substances, such as indoleamine 2,3-dioxygenase, which is usually produced by activated macrophages and overexpressed by numerous tumors. This enzyme leads to a tryptophan depletion in the (tumor) microenvironment and subsequently the production of kynurenine, which damages the cytotoxic T lymphocytes (Mbongue et al., 2015).

9.3.6 *FDA-approved and phase III immune checkpoint substances*

Nivolumab is an IgG4 monoclonal antagonist antibody against the PD-1 receptor and an FDA approved immunotherapeutic substance for advanced squamous NSCLC and non-squamous cell NSCLC patients with progression after platinum-based chemotherapy. Studies comparing nivolumab and docetaxel as second-line therapies in squamous as well as non-squamous NSCLC patients showed an improved OSR and response rate for nivolumab (Horn et al., 2017). Another study researched nivolumab vs. docetaxel as first-line treatment and the results indicated that both modalities have a similar outcome, except a prolonged response

duration under nivolumab treatment (Carbone et al., 2017). Another phase III trial comparing platinum-based chemotherapy and nivolumab with a combination of nivolumab combined with ipilimumab is currently recruiting.

Pembrolizumab is an IgG4 monoclonal antagonist antibody against the PD-1 receptor and an FDA approved immunotherapeutic substance for pretreated advanced NSCLC with a positive expression in ≥1% of all tumor cells. Furthermore, it was approved for treatment in metastatic tumors with ≥50 percent positive PD-L1 cells, without EGFR mutations or ALK rearrangements. A combination with pemetrexed and carboplatin is approved for treatment, even without positive PD-L1 expression (FDA News Release, 2017b). For second-line therapy following platinum-based chemotherapy the phase I study that led to FDA approval (KEYNOTE-001), showed a significantly improved OSR and response rate as well as only low toxicity grades in patients with a PD-L1 expression and pembrolizumab treatment (Garon et al., 2015). Subsequently, a phase II/III trial comparing pembrolizumab and docetaxel was conducted and showed a significantly higher OSR and progression-free interval in patients with PD-1 expression ≥50% of all tumor cells, whereas the overall population showed a significantly improved OSR and response rate as well as a comparable progression-free interval and less adverse effects (Herbst et al., 2016). Studies researching the first-line therapeutic effects of pembrolizumab showed an improved OSR and progression-free interval compared to platinum-based chemotherapy, while combined platinum-based chemotherapy, pemetrexed, and pembrolizumab showed a 26% longer response rate, a prolonged progression-free interval compared to carboplatin and pemetrexed alone (Langer et al., 2016, Reck et al., 2016). Two currently ongoing phase III trials compare pembrolizumab and platinum-based chemotherapy with chemotherapy alone.

Atezolizumab is an IgG1 antagonist antibody against PD-L1 and is FDA approved for the treatment of metastatic NSCLCs, which progress during or after platinum-based chemotherapy. NSCLC patients with EGFR mutations or ALK rearrangements should receive atezolizumab only under disease progression. Atezolizumab triggers an antibody-dependent cell-mediated cytotoxicity (ADCC) of activated T lymphocytes expressing PD-L1 (Peters et al., 2017b). In a phase III trial atezolizumab showed a significantly prolonged OSR and reduced numbers of adverse effects as well as a similar progression-free interval and response rate in squamous and non-squamous NSCLC compared to docetaxel (Rittmeyer et al., 2017). Currently, ongoing atezolizumab studies research the combination of platinum-based chemotherapy as well as erlotinib and ipilimumab.

Durvalumab is an IgG1 antagonist antibody against PD-L1 and is FDA approved for the treatment of unresectable stage III NSCLCs, which did not progress during or after platinum-based chemotherapy and RT. Durvalumab triggers an ADCC of activated T lymphocytes expressing PD-L1. For second-line therapy following platinum-based chemotherapy and concurrent RT, the phase I study that led to FDA approval (PACIFIC) showed a significantly improved OSR and prolonged progression-free interval compared to a placebo. Numerous currently ongoing studies researched the effects of durvalumab, for instance in advanced NSCLC following curative chemoradiation compared to chemotherapy-naive advanced NSCLC as well as in combination with tremelimumab and gefitinib (Antonia et al., 2017).

BMS-936559 is an IgG4 antagonist antibody against PD-L1, currently researched in a dose-escalation phase I trial in numerous cancers, including NSCLC. Currently, of the 49 NSCLC patients, 10% showed a partial response, whereas 12% showed a stable disease. Further results from this study have yet to be published (Brahmer et al., 2012).

The recommended treatment duration of PD-(L)1 inhibitors is until tumor progression or massive toxicity is diagnosed. This recommendation is based on the studies, which led to FDA approval, and suggests the continuation of the treatment until disease progression was found. The CheckMate-153 study, which researched nivolumab monotherapy, found that a continued treatment for more than a year showed a prolonged progression-free interval compared to the cohort, whose treatment was stopped after one year (Spigel et al., 2017).

Although increased PD-L1 expression suggests a beneficial correlation with PD-1 and PD-L1 inhibitor therapy, some issues should be addressed before PD-L1 presence becomes the exclusive criterion for PD-1 axis inhibitor treatment. These issues include the establishment of standardized PD-L1 IHC and of different PD-L1 positivity thresholds, as well as heterogeneity in small tumor tissue samples, and the influence of applied local and systemic therapies on the current PD-L1 status.

CTLA-4 was identified as a negative regulator of early T lymphocyte activity, due to its higher binding affinity to costimulatory receptors, including B7.1 and B7.2, compared to CD28 and hence it blocks the T lymphocyte activation (Tivol et al., 1995). Its expression level is controlled by the same cytokines as PD-1, which ultimately results in a feedback inhibition loop and a down-regulation of $CD4^+$ and $CD8^+$ T lymphocyte activation (Walker and Sansom, 2011). The first evidence for tumor regression activity of CTLA-4 was found in mouse models (Leach et al., 1996).

Ipilimumab is an IgG1 monoclonal antagonist antibody against the CTLA-4 receptor and is FDA approved for the treatment of metastatic melanoma and as combination therapy with

nivolumab for advanced renal cell carcinoma. A phase II trial regarding the effects of ipilimumab in NSCLC patients suggested a benefit for a combination of ipilimumab with carboplatin and paclitaxel (Lynch et al., 2012), while the subsequent phase III trial, which compared ipilimumab and chemotherapy with chemotherapy and placebo in chemotherapy-naïve metastatic squamous NSCLC, showed no improved OSR or progression-free interval (Govindan et al., 2017). Ipilimumab combined with erlotinib or crizotinib is currently researched in earlier-phase NSCLC trials, but results have yet to be published.

9.3.7 *Early-stage clinical trials*

Other potential checkpoint inhibitor targets are currently researched in early-stage clinical trials.

- TIM-3 is expressed by $CD8^+$ lymphocytes, T helper, and dendritic cells as well as monocytes. A binding of galectin-9 and the subsequent ligand's presence is an indicator of neoplastic activity and T helper cell death (Monney et al., 2002, Zhu et al., 2005). Therefore, an inhibition of TIM-3 results in T helper cell hyperproliferation and increased cytokine release (Anderson et al., 2007).
- LAG3 is expressed by B, T, NK cells, and tumor-infiltrating lymphocytes and enhances the activity of T regulatory cells by binding MHC class 2, which leads to a decreased T lymphocyte activity (Grosso et al., 2009, Kisielow et al., 2005). A combination of LAG3 and PD-1 showed improved outcomes in pre-clinical trials and a clinical trial with LAG3 and nivolumab showed activity in PD-1 resistant patients (Ascierto and McArthur, 2017 , Woo et al., 2012).
- B and T cell lymphocyte attenuator (BTLA) is a ligand of the herpes virus entry mediator found on B, T NK and antigen presenting cells and results in a decreased cytokine production and subsequently in a decreased $CD4^+$ lymphocyte activity (Murphy et al., 2006). The suppression of BTLA showed an enhanced $CD8^+$ T lymphocyte activity and had a positive impact on anti-PD-1 efficacy (Fourcade et al., 2012, Watanabe et al., 2003)
- V-domain Ig suppressor of T cell activation shares similarities with PD-L1 and functions as an inhibitory checkpoint ligand and is found in hematopoietic tissues and T cell-infiltrated structures (Lines et al., 2014). The downregulation of V-domain Ig suppressor of T cell activation in mouse models showed an increased T lymphocyte infiltration and regressive activity in tumor tissue (Le Mercier et al., 2014, Wang et al., 2011).

Aside from checkpoint inhibitors the costimulatory receptors, associated with tumor progression, are potential immunotherapy targets and are studied in either animal models or in early clinical phase development.

- 4-1BB is expressed on dendritic, activated T, T-regulatory, NK and NK T cells and promotes the activity of T lymphocytes, monocytes, neutrophils and dendritic cells (Vinay and Kwon, 2011). Anti-4-1BB antibodies show regressive effects on tumor tissue (Takeda et al., 2010, Youlin et al., 2013). Urelumab and PF-05082566 are currently researched in phase I studies as monotherapy and in combination with other substances (Hernandez-Chacon et al., 2011, Segal et al., 2014).
- OX40 is expressed on activated $CD4^+$ and $CD8^+$ T lymphocytes, as well as on neutrophils, T regulatory and dendritic cells and is controlled by the OX40 ligand, which is present on APCs and activated T lymphocytes (Weinberg et al., 2011). Preclinical studies showed a tumor-regressive efficacy of anti-OX-40 and its ligand as monotherapy as well as in combination with other substances (Gough et al., 2010 , Watanabe et al., 2010).
- Glucocorticoid-induced tumor necrosis factor-like receptor (GITR) is expressed on $CD4^+$ and $CD8^+$ T lymphocytes and its stimulation leads to increased T cell activity, activation-induced cell death, memory against further tumor rechallenge and counters T regulatory induced suppression (Ko et al., 2005, Nocentini et al., 2012, Schaer et al., 2012, Stephens et al., 2004).
- Inducible T cell co-stimulator is expressed on activated T lymphocytes and triggers the switch of isotypes, formation of germinal centers, and effector and regulatory $CD4^+$ T lymphocyte responses (Simpson et al., 2010). In preclinical studies, it was shown that a combination of its ligand and anti-CTLA-4 therapy resulted in an increased anti-tumor efficacy (Fan et al., 2014).

Although most of the discussed checkpoint inhibitors show tumor regression as monotherapy, many studies research the combination of various checkpoint inhibitors. The most researched combination is CTLA-4 and PD-1 inhibition. For instance, ipilimumab and nivolumab showed a significantly increased response rate, progression-free interval and OSR compared to ipilimumab, nivolumab or sunitinib monotherapy (Larkin et al., 2015, Wolchok et al., 2017). Further studies researching ipilimumab and nivolumab as well as durvalumab and tremelimumab are currently ongoing for multiple cancer types. An overview is provided in the outlook chapter, in Table 8.

9.4 *Immunotherapy response criteria*

For the evaluation of the effects of checkpoint inhibitors and immunotherapy immune-related response criteria have been published. First is the immune-related response criteria (irRC), which allows the characterization of response patterns seen under immunotherapy, such as checkpoint inhibitors (Wolchok et al., 2009).

- Immune-related complete response

  No measure- and nonmeasurable lesions can be found after the treatment.
- Immune-related partial response

  Compared to the pre-therapy the tumor presents a reduction of at least 50% percent of the overall tumor mass is found after the treatment. Therefore, a progression of individual lesions is possible.
- Immune-related stable disease

  Stopped tumor progression, but no reduction of the tumor mass.
- Immune-related progressive disease

  Tumor progression of at least 25% compared to the minimal tumor mass despite therapy.

For an objective assessment of all stages, a second consecutive assessment should be conducted four weeks after the therapy's end.

The importance of the irRC results from the delayed response from patients to the immunotherapy. Therefore, an assessment of the treatment efficacy with the Response Evaluation Criteria In Solid Tumors (RECIST) criteria might lead to a premature discontinuation of the immunotherapy in patients who might show a treatment response or a prolonged disease following the initial response delay; hence, the immunotherapy RECIST (iRECIST) was developed, based upon RECIST 1.1, for an objective evaluation of clinical trials, which researched the effects of immunotherapy (Seymour et al., 2017). These criteria are an extension of the irRC and include some modifications.

- The definitions of measurable and nonmeasurable lesions, the numbers, and sites of target diseases were adjusted to RECIST 1.1.
- Introduction of the immune unconfirmed progressive disease (iUPD), a progressive disease under therapy, and the immune confirmed progressive disease (iCPD), which indicates the presence of new lesions under therapy or increased size of new lesions. Therefore, the appearance of new lesions, when none were recorded, is also termed iCPD.

- Additional categories are immune complete response (iCR), immune partial response (iPR), and immune stable disease (iSD).

Other newly developed criteria are the immune-modified Response Evaluation Criteria In Solid Tumors (imRECIST), based upon the RECIST 1.1, which might provide a better assessment modality for the evaluation of a transient progression after initiation of immunotherapy (Hodi et al., 2018).

10 Outlook

The goal of immunotherapy in neoplastic diseases is an improved recognition of the tumor tissue as harmful and foreign and a subsequent increased immune response, which hampers the tumor progression and the accompanying suppression of the immune system. But since pulmonary tumors in general and NSCLC in particular, were considered unsusceptible to immunotherapy such as cytokine therapies, for a long time, an immunotherapeutic treatment approach in NSCLC patients was initially not promising.

However, the recent developments in the field of checkpoint inhibitors, such as PD-L1, as well as PD-1 and CTLA-4 receptors, provided new and effective approaches for NSCLC treatment. Other mutations and rearrangements, which enable the development of targeted therapies are the EGFR mutations and ALK rearrangements.

Due to the progress in immunotherapeutic research, numerous substances already got FDA approved as well as the recommendation by the ESMO for clinical NSCLC treatment. Even though many studies have shown the capability of immunotherapeutic substances as monotherapies, the best results could be achieved for combined platinum-based chemo- and immunotherapies.

Therefore, in the future, the standard treatment of NSCLC (resection, chemotherapy, RT) will become highly supported by immunotherapeutic substances for the treatment of localized and distant lesions. Especially in patients who have not received systemic therapy for advanced NSCLC and without contraindications to immunotherapy, PD-L1 inhibitors, such as pembrolizumab (in patients without EGFR mutations and ALK rearrangements), as well as a combined therapy with pembrolizumab, carboplatin, and pemetrexed (in nonsquamous NSCLC patients without a driver mutation), are already integral and recommended treatment components.

Besides the already approved and established checkpoint inhibitors, numerous other immunotherapeutic substances are in pre-clinical studies or already in clinical development. An overview of ongoing phase III trials testing the effects of PD-1 and PD-L1 inhibitors in NSCLC is presented in Table 8.

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Table 8 Ongoing phase III trials for PD-1 and PD-L1 inhibitors (edited after (UpToDate, 2018))

Substance	Trial number	Histology	Treatment modality
First-line therapy for advanced NSCLC			
Nivolumab (anti-PD-1)	CheckMate 026 NCT02041533	All	Nivolumab vs. chemotherapy
	CheckMate 227 NCT02477826	All	PD-L1-positive: chemotherapy vs. nivolumab vs. nivolumab + ipilimumab PD-L1-negative: chemotherapy vs. nivolumab + ipilimumab
Pembrolizumab (anti-PD-1)	Keynote 042 NCT02220894	All	Pembrolizumab vs. chemotherapy
	Keynote 024 NCT02142738	All	Pembrolizumab vs. chemotherapy
	Keynote 189 NCT02578680	Non-squamous	Chemotherapy vs. chemotherapy + pembrolizumab
	Keynote 407 NCT02775435	Squamous	Chemotherapy vs. chemotherapy + pembrolizumab
Atezolizumab (anti-PD-L1)	IMpower111 NCT02409355	Squamous	Atezolizumab vs. chemotherapy
	IMpower110 NCT02409342	Non-squamous	Atezolizumab vs. chemotherapy
	IMpower131 NCT02367794	Squamous	Atezolizumab + chemotherapy vs. chemotherapy
	IMpower130 NCT02367781	Non-squamous	Atezolizumab + chemotherapy vs. chemotherapy
	IMpower150 NCT02366143	Non-squamous	Atezolizumab + chemotherapy +/– bevacizumab vs. chemotherapy + bevacizumab
Durvalumab (anti-PD-L1)	Mystic NCT02453282	All	Durvalumab + tremelimumab vs. durvalumab vs. chemotherapy
Second-line therapy for advanced NSCLC after standard platinum doublet chemotherapy			
Nivolumab	CheckMate 017 NCT01673867	Squamous	Nivolumab vs. docetaxel
	CheckMate 057 NCT01642004	Non-squamous	Nivolumab vs. docetaxel
Pembrolizumab	Keynote 010 NCT01905657	All	Pembrolizumab vs. docetaxel
Atezolizumab	Oak NCT02008227	All	Atezolizumab vs. docetaxel
Avelumab (Anti-PD-L1)	Javelin Lung 200 NCT02395172	All	Avelumab vs. docetaxel
Third-line therapy for advanced NSCLC after two lines of chemotherapy			
Durvalumab	Arctic NCT02352948	All	PD-L1-positive: chemotherapy or erlotinib vs. durvalumab PD-L1-negative: durvalumab + tremelimumab vs. chemotherapy vs. durvalumab vs. tremelimumab
Second-line therapy for advanced EGFR mutant NSCLC after initial EGFR TKI therapy			
Durvalumab	Caural NCT02454933	T790M (EGFR)	AZD9291 vs. AZD9291 + durvalumab
Adjuvant therapy after curative NSCLC therapy			
Durvalumab	Pacific NCT02125461	All	Durvalumab vs. placebo
Durvalumab	NCT02273375	All	Durvalumab vs. placebo

11 List of Tables

12 List of Figures

13 Literature

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