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Prediction of patients' response to immune checkpoint inhibitors in the treatment of advanced NSCLC

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List of abbreviations

ADC	adenocarcinoma
ALK	anaplastic lymphoma kinase
APC	antigen-presenting cells
ASC	adenosquamous carcinoma
ANOVA	analysis of variance
BSC	best supportive care
CD	cluster of differentiation
CDC	complement-mediated cytotoxicity
CI	confidence interval
CML	chronic myeloid leukemia
CRP	c-reactive protein
CT	computer tomography
CTL	cytotoxic T cells
CTLA-4	cytotoxic T lymphocyte antigen-4
CTLs	cytotoxic lymphocytes
CV	cross-validation
DC	dendritic cell
DHFR	dihydrofolate reductase
EBUS TBNA	endobronchial ultrasound transbronchial needle aspiration
ECOG	eastern cooperative oncology group
ED	early death
EGFR	epidermal growth factor receptor
FcγR	fragment crystallizable region gamma receptor
FDA	food and drug administration

GL-Index:	granulocytes lymphocytes index
GM-CSF	granulocyte macrophages' colonies stimulating factor
HBV	hepatitis B virus
HIV	human immunodeficiency viruses
HPV	human papilloma virus
IASLC	international association for study of lung cancer
IC	immune cells
ICI	immune checkpoint inhibitor
IFN	interferon
Ig	immunoglobulin
IL	interleukin
KRAS	Kirsten rat sarcoma virus
LAG-3	lymphocyte activation gene-3
LDH	lactate dehydrogenase
LKI	Lungenfachklinik Immenhausen
mAbs	monoclonal antibodies
MAPK	mitogen-activated protein kinase
MET:	mesenchymal-epithelial transition factor
MHC 1	major histocompatibility complex class I molecules
MRI	magnetic resonance imaging
NHL	non-Hodgkin lymphoma
NK	natural killers
NKG2D	transmembrane receptor natural killer group 2 member D
NOS	not otherwise specified
NSCLC	non-small-cell lung cancers

OR	objective response
ORR	objective response rate
OS	overall survival
PD-1	programmed cell death receptor
PD-L1	programmed cell death ligand 1
PET-CT	positron emission tomography–computed tomography
PFS	progression-free survival
PsPr	pseudo-progressive disease
RCTs	randomized clinical trials
RECIST	response evaluation criteria in solid tumors
ROC	receiver operating characteristic
ROS	proto-oncogene 1, receptor tyrosine kinase
RPD	real progressive disease
RT	radiotherapy
SCC	squamous cell carcinoma
SCLC	small cell lung cancer
SD	stable disease
SLE	systemic lupus erythematosus
TAAs	tumor-associated antigens
TCR	T-cell receptor
TDLNs	tumor-draining lymph nodes
TGF-β	transforming growth factor-β
TIM-3 T	cell immunoglobulin and mucin protein-3
TNM	tumor, lymph node and metastasis
TPS	tumor proportion score

TRAIL	tumor necrosis factor-related apoptosis-inducing ligand
TS	thymidylate synthase
UICC	Union for International Cancer Control
VATS	video-assisted thoracoscopic surgery
VEGF	vascular endothelial growth factor
WHO	World Health Organization

1 Introduction

1.1 Lung cancer

1.1.1 Overview

Lung cancer is the leading cause of cancer deaths worldwide with a European age-standardized mean five-year survival of 13% (Angelis et al. 2014). Histological classification of lung cancer is the cornerstone for diagnosis, treatment and prognosis. The vast majority of lung cancers are non-small-cell lung cancers (NSCLC) which account for approximately 80%, with the remainder being mostly small cell lung cancers (SCLC) (Oser et al. 2015). The emerging use of immunohistochemistry techniques and the integration of molecular testing have played a significant role in the new World Health Organization (WHO) classification of lung cancer in 2015. This has helped with pathological subtyping of NSCLC depending on certain histological and molecular features, and started a new era of disease-specific therapy (Travis et al. 2015).

NSCLC is further classified into adenocarcinoma, which is the most common type of lung cancer (about 40%), and squamous cell carcinoma (SCC), which arises from epithelial cells lining the airways as well as alveolar cells type II. The last and least common type of NSCLC is undifferentiated large cell carcinoma, which lacks the morphological and immunohistochemical features of the other types. (Zappa and Mousa 2016)

Small cell lung cancer (SCLC) accounts for nearly 15% of lung cancer cases. It is characterized by a rapid progressive course and typically affects older men with a heavy smoking history. Among lung cancers it is the most common cause of paraneoplastic syndrome, even though it appears to be very sensitive to cytotoxic chemotherapy (van Meerbeeck et al. 2011).

Due to the lack of effective screening, most lung cancer patients are diagnosed in late disease stages. Lung cancer-suspected patients are usually symptomatic patients with risk factors. Patients usually present with nonspecific symptoms of cough and shortness of breath. Symptoms can also be triggered by the local spread of the primary tumor, metastatic lesions or paraneoplastic syndrome (Latimer 2018).

1.1.2 Epidemiology

Lung cancer is the most common type of cancer worldwide, contributing to 12.4% of all newly diagnosed cancer cases, and is by far the first leading cause (17.6%) of cancer deaths. The incidence in developing countries has been increasing in the last two decades and has almost

equalized its incidence in the world's developed countries. Due to lifestyle changes and the increase of tobacco consumption among women, since 1985, the incidence has increased by 76% in women versus 44% in men with an ongoing male predominance, and with about a 1.5:1 male to female incidence ratio (Dela Cruz et al. 2011).

In Germany, lung cancer is the second most diagnosed cancer in men and the third in women and still the most common cause of cancer deaths in men (24.4% of all cancer deaths), and the second most common cause of cancer deaths in women (15.4%) (Robert Koch-Institute).

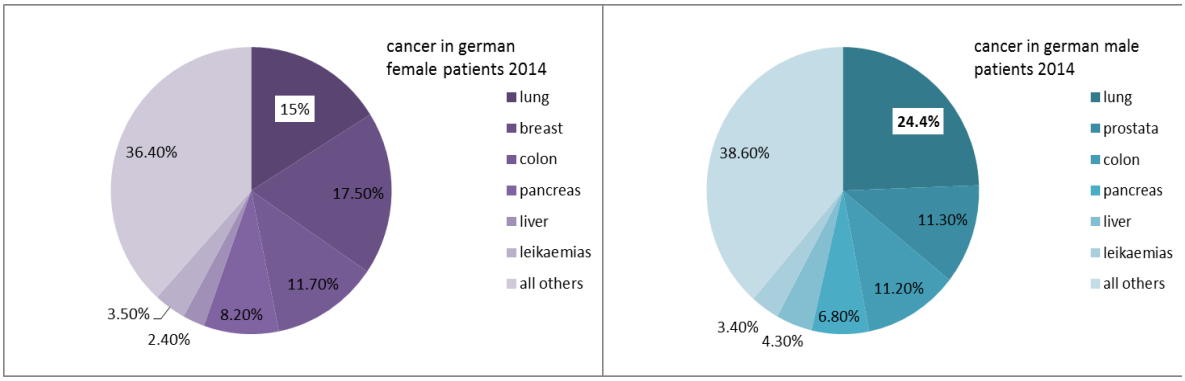

Figure 1:Pie charts presenting the numbers of cancer cases in Germany as a percentage based on tumor site and gender according to the German cancer registry 2014 (Robert Koch-Institute).

Unlike in men, since 1998, the incidence of lung cancer and its related mortality have been rising continuously in German women. This can be explained by the change of smoking habits in German society. According to the German cancer registry (2014), the lung cancer five-year survival was 20% in women versus 15% in men (Robert Koch-Institute).

1.1.3 Risk factors

More than 90% of lung cancer cases can be attributed to tobacco smoke (Dela Cruz et al. 2011). Smoking causes about 5.4 million deaths per annum around the globe. Notwithstanding, it remains a preventable cause of death (World Health Organization, 2008). The chance of developing lung cancer within a lifetime due to smoking is 20-fold higher than in those who have never smoked. Cigarette smoke contains numerous carcinogens and mutagens (Dela Cruz et al. 2011). Lung cancer in never smokers has been an interesting topic in the last three decades. Several large cohort studies with patients from different ethnicities concluded that the majority of never-smoker lung cancer patients were females with adenocarcinoma (Sun et al. 2007).

The trend of using low tar or filtered cigarettes did not appear to lower the harm caused by smoking. On the contrary, it has been connected to increased rates of lung adenocarcinoma by increasing the volume of inhaled smoke and consequently increasing toxin distribution through the lungs. This was concluded in 2014 in the Surgeon General's Report in the Health Consequences of Smoking (Song et al. 2017).

Occupational lung cancer was recognized in the eighteenth century. Schneeberg lung disease was the first name given for radon-induced lung cancer. In 1860, two German physicians, Härting and Hesse, described the incidence of a fatal lung disease in miners who were working in Schneeberg Mountains in the so called kingdom of Saxony. They succeeded in performing autopsies on some dead workers and they sent these to the pathological institute of Leipzig University. The autopsies recognized this as a malignant disease. Härting and Hesse were the first who described occupational lung cancer (Schüttmann 1993). Radon is a noble radioactive gas that results from the normal decaying chain of Uranium. It is a human carcinogen with a dose-dependent effect. Nowadays, it is recognized to be the second leading cause of lung cancer. Radon exposure and smoking have a synergetic effect. The mortality rates of cancer patients were higher in smokers who had a confirmed exposure to radon (Lantz et al. 2013).

Other risk factors include air pollution. An analysis of 17 cohort studies from European countries concluded a significant association between exposure to particle matters in the air and the incidence of lung cancer in Europe (Raaschou-Nielsen et al. 2013).

Some studies suggest that asbestos fibers can generate up-regulated signaling pathways which are responsible for cancer development and therapy resistance. This effect is thought to be mediated by the direct interaction between asbestos fibers and cell surface receptors or by asbestos-induced reactive oxygen species (Heintz et al. 2010).

A pooled analysis on 24,607 cases and 81,829 controls demonstrated a higher risk of lung cancer in patients with previous lung disease (chronic bronchitis, pneumonia, emphysema and tuberculosis). Inflammation plays a significant role in lung cancer development by increasing genetic mutations, anti-apoptotic signaling and angiogenesis (Brenner et al. 2012).

1.1.4 Approach and Staging

Patients with lung cancer typically present with non-specific symptoms at the time of diagnosis. Less frequently, patients are referred for further investigation of a suspicious accidental finding. Diagnosis of lung cancer is a comprehensive work up that includes conventional test-

ing and invasive approaches that aim to establish a definitive diagnosis and to evaluate a patient's functional status and relevant comorbidities. The initial work up involves clinical history and examination, reviewing of possible risk factors, and conventional imaging (Hammerschmidt and Wirtz 2009). A plain chest radiograph is a simple accessible informative test with a negligible radiation exposure. It is a sensible primary test for patients with non-specific respiratory symptoms (Rogers 2010).

Other diagnostic modalities could include contrast-enhanced or native computer tomography (CT) or magnetic resonance imaging (MRI), ultrasonography, bone scintigraphy and positron emission tomography–computed tomography (PET-CT). A histological confirmation is fundamental for the therapy and should be conducted in all patients if possible. Bronchoscopy provides a sensitivity of 88% and 78% for central and peripheral tumors respectively (Rivera and Mehta 2007). An ultrasound or CT-guided lung biopsy, mediastinoscopy, endobronchial and esophageal ultrasonography with transbronchial needle aspiration (TBNA) of regional lymph nodes can also be performed (Hammerschmidt and Wirtz 2009).

PET-CT is a widely used diagnostic modality which plays a crucial role in diagnosis, staging, restaging and therapy planning. Cancer cells have a better capability to uptake the tracer (mostly used is Fludeoxyglucose 18F) providing a precise demarcation of the primary tumor mass and allowing the detection of involved lymph nodes and distant metastasis. Because of the brain cells' natural high glucose uptake activity, PET-CT is considered to be a low sensitive modality for diagnosing brain metastasis. Other limitations include false negative findings of small lesions or tumors with low metabolic activity as well as a high cost. Despite the PET-CT significance in the process of lung cancer diagnosis and treatment, it did not appear to improve the five-year survival of lung cancer patients (Chao and Zhang 2012).

The evaluation of a patient's functional performance is a part of the diagnostic process and is important to ensure the most appropriate therapy. This includes the evaluation of cardiovascular comorbidities, liver or renal impairment, and the assessment of functional operability (Hammerschmidt and Wirtz 2009).

As with other solid tumors, lung cancer has been integrated to the Union for International Cancer Control (UICC) tumor, lymph node and metastasis (TNM) staging system since 1966. The first lung cancer TNM-staging review came to light in 1973 and was done by Mountain et al. The International Association for Study of Lung Cancer (IASLC) commenced a staging

project depending on a multi-centric, international database with a large pool of patients collected over 12 years. The (IASLC) revision of lung cancer staging was published in the seventh edition of the "UICC- TNM classification of malignant tumors" in 2010. The new TNM classification is applicable for both NSCLC and SCLC (Mirsadraee et al. 2012).

The TNM Staging system is considered to be most reliable prognostic factor in lung cancer recurrence and survival rate (Woodard et al. 2016). In the TNM staging system T stands for the size of the primary tumor in the long axis. In the 7th classification T1 and T2 tumors are further divided into subgroups. A major modification is the downgrading of other nodules found on the same lung lobe to T3 (was T4) and the consideration of other ipsilateral nodules outside the primary lobe, which are histologically similar to the primary tumor T4 instead of M1 (Carson and Finley 2011).

N defines nodal involvement. Since the new data didn't show a difference in nodal staging related survival, there were no changes made to nodal staging in the new 7th edition. There are many approaches to determine the level of nodal involvement. Studies show that the combined use of endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) and esophageal endoscopic ultrasound needle aspiration (EUS-TBNA) has a higher sensitivity (93%) compared to mediastinoscopy (80-90%). Other techniques include computer tomography (CT) and PET-CT with a sensitivity of 60% and 84% respectively. Anyway, the collection of the database used in the 7th classification preceded the prevalent use of PET-CT (Mirsadraee et al. 2012).

M staging describes extranodal metastasis. In the majority of cases bronchial carcinomas are metastatic (stage IV) at time of diagnosis. Lung cancer is able to metastasize through blood and lymphatic vessels, ideally to the contralateral lung, liver, adrenal glands, bone and brain (Popper 2016). In the 7th edition of TNM staging, pleural or pericardial carcinosis is upgraded from T4 to a new category of M1a. To this category belongs contralateral lung metastasis. Extrathorasic metastasis has been classified to M1b (Mirsadraee et al. 2012).

In January 2017 the 8th TNM stage classification of lung cancer was released and carried out some major changes. The T1 and T2 tumor sizes were subdivided in 1 cm additions up to 5 cm, where T3 was defined by tumors >5 but ≤7 cm, and T4 for tumors larger 7 cm or tumors involving the diaphragm. Tumors with extrathorasic metastasis were subdivided to M1b when it has one single metastasis and M1c for tumors with multiple extrathoracic metastases

(Detterbeck 2018). A summary of the 8th TNM classification (Goldstraw et al. 2016; Detterbeck 2018) is given in table 1 and table 2.

Table 1: The 8th TNM clinical staging system for NSCLC

T (primary tumor)		
0	No primary tumor	
Tis	Carcinoma in situ	
T1	Tumor ≤3 cm	
	T1mi	Minimally invasive adenocarcinoma
	T1a	Superficial spreading tumor in central airways**
	T1a	Tumor ≤1 cm
	T1b	Tumor >1 but ≤2 cm
	T1c	Tumor >2 but ≤3 cm
T2	Tumor >3 but ≤5 cm or involving visceral pleura or main bronchus	
	T2a	Tumor >3 but ≤4 cm
	T2b	Tumor >4 but ≤5 cm
T3	Tumor >5 but ≤7 cm or invading chest wall, pericardium, phrenic nerve; or separated tumor nodules in the same lobe	
T4	Tumor >7 cm or tumor invading: mediastinum, diaphragm, heart, great vessels, recurrent laryngeal nerve, carina, trachea, esophagus, spine; or tumor nodule(s) in different ipsilateral lobes	
N (regional lymph nodes)		
N0	No nodal metastasis	
N1	Metastasis in ipsilateral pulmonary or hilar nodes	
N2	Metastasis in ipsilateral mediastinal or subcarinal nodes	
N3	Metastasis in contralateral mediastinal, hilar, or supraclavicular nodes	

M (distant metastasis)
M0 No distant metastasis
M1a Malignant pleural or pericardial effusion or pleural or pericardial nodules or separate tumor nodule(s) in a contralateral lobe
M1b Single extrathoracic metastasis
M1c Multiple extrathoracic metastases in one or more than one organ
∗Superficial spreading tumor of any size but confined to the tracheal or bronchial wall. Tumors causing atelectasis or bronchial obstruction are classified as T2a if >3 and ≤4 cm, T2b if >4 and ≤5 cm.

(Goldstraw et al. 2016; Detterbeck 2018)

Table 2: Overall NSCLC stages based on T, N and M criteria

Stage	**Primary Tumor**	**Lymphnode**	**Metastases**
0	Tis	N0	M0
IA1	T1a (mi)	N0	M0
	T1a	N0	M0
IA2	T1b	N0	M0
IA3	T1c	N0	M0
IB	T2a	N0	M0
IIA	T2b	N0	M0
IIB	T1a-c	N1	M0
	T2a	N1	M0
	T2b	N1	M0

Stage	Primary Tumor	Lymphnode	Metastases
	T3	N0	M0
IIIA	T1a-c	N2	M0
	T2a-b	N2	M0
	T3	N1	M0
	T4	N0	M0
	T4	N1	M0
IIIB	T1a-b	N3	M0
	T2a-b	N3	M0
	T3	N2	M0
	T4	N2	M0
IIIC	T3	N3	M0
	T4	N3	M0
IVA	any T	any N	M1a
	any T	any N	M1b
IVB	any T	any N	M1c

(Goldstraw et al. 2016; Detterbeck 2018)

1.1.5 Treatment

The treatment choice depends mainly on the initial stage of the disease, as well as the patient's functional performance and comorbidities. Accordingly, the first step is to determine the purpose of the therapy, considering the patient's life expectancy and quality. Decision-making through a multidisciplinary team results in better outcomes.

About 25% of all cases present at early disease stages (stage I and II), where a curative therapy should be conducted. In operable patients, surgery, either lobectomy or pneumonectomy, is the treatment of choice. Most lung cancer patients are current or former smokers with concurrent pulmonary restrictions and cardiovascular comorbidities. Pulmonary function tests and

cardiovascular assessment are mandatory for all local therapy candidates. Patients may need to get cardiopulmonary function tests (Spiroergometry) or lung perfusion (Scintigraphy). Procedures below lobectomy (wedge resection or segmentectomy) may be performed in primarily operable tumors in patients with functional restrictions. Studies show a high rate of recurrence and a decrease in the five-year survival in patients of sublober surgery when the tumor size exceeds 3 cm. The introduction of Video-assisted thoracoscopic surgery (VATS) provides a better post-operative course with fewer complications and minimizes the post-operative hospital stay period (van Schil et al. 2017; Gadgeel et al. 2012).

Other curative treatment modalities for non-operable patients include conventional radiotherapy (RT). Radiation pneumonitis is a major complication for this type of local therapy. Stereotactic radiosurgery is an ingenious type of radiotherapy using a proton beam to deliver a high power of radiation to a small body area. This type of radiation shows high successful rates of local control with a five-year survival of more than 50%. The application of adjuvant chemotherapy in stage II and III in patients who have undergone curative surgery shows an increase of the five-year survival of about 5%. This should be considered in patients with a non-complicated post-operative course with a good functional status. Typically four cycles of platinum-based dual chemotherapy are given. The aim of the adjuvant therapy is to reduce the recurrence through undetectable micro metastasis (Gadgeel et al. 2012).

As stated in the 8th UICC classification of lung cancer, stage III disease is subdivided into three groups: IIIA, IIIB and IIIC. Patients with T3N1M0 (Stage IIIA) disease undergo surgery with every effort to obtain disease-free margins (R0); in the case of a primary tumor mass more than 5 cm or ipsilateral lymph node metastasis, an adjuvant chemotherapy should be conducted. In case of proven microscopic marginal residuals (R1), the choice of post-operative radiotherapy could be reviewed. N2 status disease is considered be a big controversy in thoracic oncology. Many clinical trials with different treatment modalities and different studies have failed to conclude the superiority of one single treatment modality in this case. Studies show a discrepancy in the disease free year survival, depending on the N2 status. Mediastinal nodal involvement (N2) with a concomitant ipsilateral node (N1) or a multi-stational N2 has proven to have a worse prognosis than a single nodal N2 disease. Treatment approaches vary between initial surgical treatment followed by adjuvant chemotherapy and starting with induction therapy followed by restaging and eventually surgical resection. All patients with suspected N2 status should undergo an invasive diagnosis (e.g. EBUS-TBNA) before surgical resection. The varia-

tion of N2 status: single, multiple or bulky, and the assessment of collateral N1 status have implications on the treatment approach as well as free year survival rates (Gadgeel et al. 2012; Rocco et al. 2016; van Schil et al. 2017). The following table shows a subtyping of N staging criterion according to the 8th UICC classification:

Table 3: Subdivisions of the N staging criterion according to the 8th TNM classification system

Nodal subdivision	**Definition**
N1a	Single N1 station
N1b	Multiple N1 stations
N2a1	Single N2 station
N2a2	Single N2 station (with N1 involvement)
N2b	Multiple N2 stations
N3	Contralateral hilar or mediastinal lymph node stations or scalene or supraclavicular lymph nodes

(van Schil et al. 2017)

For (T4 with N0-1) stage III diseases, the size and the tumor invasion of the surrounding structure make a free marginal resection difficult to achieve. An induction therapy might be helpful before surgery (Gadgeel et al. 2012; Rocco et al. 2016; van Schil et al. 2017).

More than 40% of NSCLC patients present with advanced disease at the time of diagnosis. For patients with advanced stages (stage IIIB and IV), studies have proven the superiority of systemic therapy over best supportive care (BSC) in survival rates and life quality. In 2016, pembrolizumab was the first immune checkpoint inhibitor (ICI) to be approved as first-line monotherapy in advanced NSCLC. Pembrolizumab was approved as first-line treatment when at least 50% of tumor cells express PD-L1 in the absence of therapy-relevant mutations (Reck et al. 2019). Otherwise a combination of platinum-based (cisplatin or carboplatin) medications with third-generation cytotoxic agents are considered to be the choice of first-line treatment for advanced NSCLC. Patient performance (Eastern Cooperative Oncology Group, ECOG) scale 0–1 should be assessed before initiation of the therapy. In patients with reduced perfor-

mance (ECOG 2) a monotherapy might be more sensible. Patients with poor performance (ECOG 3-4) should be offered the best supportive care (Bareschino et al. 2011; Zarogoulidis et al. 2013; Gadgeel et al. 2012).

Table 4: Performance status according to the Eastern Cooperative Oncology Group

Grade	**Performance status**
0	Active with no pre-disease performance limitations
1	Limited hard physical activity with no restrictions in light activity
2	Able to be self-caring, up and working for more than 50% of waking hours
3	Limited self-care; sit or lie more than 50% of waking hours
4	Disabled even in self-care
5	Dead

(Oken et al. 1982).

The histological subgrouping of NSCLC has recently been playing a crucial role in therapy choice. Studies data shows a significant difference in the response of lung squamous cell carcinoma (SCC) to cisplatin and gemcitabine versus cisplatin and pemetrexed. Non-squamous cell carcinomas also show a better response to cisplatin and pemetrexed than cisplatin and gemcitabine, which might be due to an increased expression of thymidylate synthase , dihydrofolate reductase (DHFR) in SSC, which reduces the response to therapy with the third-generation cytotoxic antifolate agent pemetrexed. A combined therapy of a platinum-based agent and pemetrexed is considered the first-line therapy for non-squamous NSCLC (Bareschino et al. 2011).The first-line therapy in advanced NSCLC is typically administrated for four cycles. In the case of an objective response or stable disease a maintenance therapy has appeared to delay the disease progress and increase the year survival (Gadgeel et al. 2012).

The detection of particular molecular properties in different carcinomas has brought the new generation of targeted therapy to light. This type of selective therapy depends on precise immunohistochemical features to interfere with the process of cell division, angiogenesis and apoptosis. The application of the targeted therapy in combination with the traditional chemotherapy as first-line treatment, as maintenance, or in second-line treatment, has been shown to improve the progression free time as well as overall year survival in patients with advanced NSCLC. The use of the novel monoclonal anti-angiogenic endothelial growth factor (VEGF) antibody bevacizumab in combination with a platinum-based agent and pemetrexed in permitted patients of non-squamous NSCLC prolongs the time to progression and improves the objective response (OR) (Reck and Rabe 2017; Mayekar and Bivona 2017; Assoun et al. 2017). Examples of commonly used targeted therapy agents are listed in the following table (Reck and Rabe 2017):

Table 5: Examples of targeted therapies used in the treatment of NSCLC

Drug	**Target**	**Indication**
Erlotinib	EGFR	EGFR-mutated metastatic NSCLC
Osimertinib	EGFR, T790M mutation	EGFR-mutated NSCLC with T790M mutation
Crizotinib	ALK, ROS1	ALK-positive or ROS1-positive metastatic NSCLC
Bevacizumab	VEGF	Advanced non-squamous NSCLC combined with platinum-based chemotherapy as first-line therapy.

ALK: anaplastic lymphoma kinase; EGFR epidermal growth factor receptor; VEGF: vascular endothelial growth factor, ROS: proto-oncogene 1, receptor tyrosine kinase.

1.2 Cancer Immunotherapy

1.2.1 Cancer Immunity and Immunoediting

The relationship between our immune system and cancer has been always a point of interest. Several preclinical and clinical observations revealed the crucial role of the immune system in cancer development. Earlier, preclinical studies compared the development of cancer in immunocompetent and immunocompromised mice. Other studies focused on cancer incidence in patients with congenital or acquired immunodeficiencies and the increased risk of cancer in elderly people (Finn 2018).

The fact that cancer cells release specific antigens is pivotal in understanding cancer immunity (Rosenberg 2004). Neo-antigens resulting from mutant cancer cells are responsible for stimulating clinical immune response (Farkona et al. 2016). If an alteration in cell function or structure occurs, the immune system is going to raise alarms using different mechanisms. These include: cytokines, cells lysis products, expression of stress ligands and, eventually, tumor-specific antigens (Schreiber et al. 2011). The concept of cancer immunotherapy is to support our own immune system to recognize cancer antigens and to trigger an appropriate immune response (Marin-Acevedo et al. 2018). Immunosurveillance is the process by which cells of adaptive immunity recognize neoplastic transformed cells and eliminate them. Immunosurveillance is crucial in immune system-cancer interaction and is the first step in immunoediting (Teng et al. 2013).

Immunoediting describes the immune system behavior toward cancer cells. Immunoediting is a process of three phases: *Elimination, Equilibrium and Escape*. Antigen-presenting cells (APC) cells are responsible for detecting and presenting cancer-specific antigens. Elimination begins in the pre-clinical phase. This means before the tumor becomes visible. When intrinsic tumor suppressors like P53 fail to prevent cells from malignant transformation, transformed cells produce stress ligands and antigens expressed by the major histocompatibility complex class I molecules (MHC 1) or the transmembrane receptor Natural killer group 2 member D (NKG2D), which will be recognized by cells of both innate and adoptive immunity like Natural killers (NK) and cluster of differentiation 8 (CD8) T cells. This will eventually initiate sequential reactions of tumor-antigens presentation by dendritic cells (DCs) at most, release gamma interferon (IFN-γ) and cytokines which recruit more immune cells to the tumor's microenvironment. IFN-γ has anti-proliferative anti-angiogenic activities against cancer cells and

activates macrophages to produce reactive oxygen species (ROS). NK and CD8 T cells induce apoptosis through activation of the so-called 'death receptors' Fas (CD95/APO-1) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) on cancer cells. DCs migrate to the tumor-draining lymph nodes (TDLNs) and activate CD4 T helper cells, which maintain the production of tumor-specific CD8 T cells and increase their activity by producing Interleukin 2 (IL-2). These apoptotic tumoricidal activities will eliminate the transformed cancer cells. A complete tumor eradication means the end of immunoediting (Dunn et al. 2004; Germenis and Karanikas 2007; Schreiber et al. 2011; Mittal et al. 2014).

Immune self-limitation through negative feedback inhibitors at the site of the inflammation works to restrain the anti-tumor immune activity. APCs release of inhibitory cytokines like Interleukin 10 (IL-10) and transforming growth factor-β (TGF-β) may bring the tumor cells to a state of dormancy (Kim et al. 2007).

The state of dormancy or *Equilibrium* describes the phase by which the cells of adaptive immunity are not able to eradicate tumor cells completely but contain them. This state of latency could explain the long period between cancer cells' transformation and the time of clinical disease recognition. It can also explain cancer recurrence either locally or as metastatic disease. Pre-clinical modules suggest that (IFN-γ) released by tumor antigen-specific T cells play a role in inhibiting disseminate micrometastasis (Vesely et al. 2011). In time, sustained sculpting of cancer cells results in eliminating immunogenic cancer cells, which consequently results in the selection of low immunogenic cancer cells. This indirect selection of low immunogenic cancer cells is the core of immunoediting (Kim et al. 2007).

Cancer *Escape* represents the failure of the immune system to maintain the state of equilibrium. Tumor cells go through genetic and epigenetic changes and overcome the state of balance in favor of cancer outgrowth. The immune system contributes to this by immunselections of resistant cancer variants and by the inhibition of the tumoricidal immune activity (Vesely et al. 2011). Tumor cells are mainly responsible for escape by reducing their own exposure to the immune system through reduction in tumor recognition by presenting low immunogenic antigens or by alternating gene presentation of MHC class I and its co-molecules or increasing the production of anti-apoptotic molecules like Bcl2. In addition tumor cells express immunosuppressors like VEGF, PD-1 and PD-L1 at the tumor-immune cells' interaction surface (Mittal et al. 2014).

1.2.2 Classes of cancer immunotherapy

The introduction of interferons (IFN-α,-β,-γ) in treating some types of malignancies like chronic myeloid leukemia (CML) and non-Hodgkin lymphoma (NHL) represents the first generation of immunotherapy. Interferons are glycoproteins with a dose-dependent immunomodulatory effect (Murphy 2010; Stanculeanu et al. 2016). Interferon has an antitumor immune stimulatory effect through activation of natural killers, increasing cancer antigen presentation by increasing MHC expression and provoking the differentiation of Th1 cells. On the other hand, interferons offer immunosuppressing effects by stimulating IL-10 production and enhancing the adaptive immunity to T-cells by up-regulating PD- L1 on APCs and tumor cells. This dual functionality maintains the homeostatic role of interferons during an extensive inflammatory state as a self-protection mechanism (Minn 2015).

Cancer vaccine is an approach that mainly increases immune system recognition of cancer cells by exploiting high immunogenic tumor-associated antigens (TAAs) and increasing their presentation. *Peptide-based vaccines* utilize the tumor-associated antigens' epitopes presented on MHC in the tumor microenvironment to escalate CD8 T cells' activity against these antigen-carrying cells. They can be administrated with cytokines like granulocyte macrophages' colonies stimulating factor (GM-CSF) to increase their exposure on DCs. These epitopes are peptides of short amino acid chains that are cost effective and simple to produce. However, obtaining immunogenic epitopes is challenging and restricted to their expression on MHC. *APCs-based vaccine* is a safe therapy which is proven to induce a significant tumor regression. Patients' APCs, mainly the most effective DCs, could be extracted and cultured in vitro with TAAs and APCs stimulating factors and then retransferred to patients with the aim of inducing CD8-specific cancer cells. A landmark in *APCs-based vaccine* is the FDA-approved autologous sipuleucel-T for hormone refractory prostate cancer. *Cancer cells-based vaccine* is another type of cancer vaccine based on delivering autologous or allogeneic cancer cells to possibly expose the immune system to numerous MHC nondependent antigens. M-Vax is an autologous tumor cells-based vaccine which showed clinical efficacy treating metastasized melanoma. *Oncolytic viruses* or virus-based vaccine is one of the first-used cancer vaccines. They are used like conventional vaccines in term of cancer prevention. For example, HBV vaccine reduced the incidence of hepatocellular carcinoma and the Human papilloma virus (HPV) vaccine against cervical carcinoma (Butterfield 2015; Raval et al. 2014; Ventola 2017).

Monoclonal antibodies (mAbs) are a novel class of cancer immunotherapy that has been proving its clinical efficacy. The development of new biotechnological techniques like hybridoma technology revolutionized this type of immunotherapy. Monoclonal antibodies' production requires a precise understanding of cancer biology, the distribution of the targeted antigens in malignant and in normal cells, and the role that these targeted antigens play in cellular proliferation and programmed cell death (Scott et al. 2012).

Monoclonal antibodies have different immunomodulatory pathways (Adams and Weiner 2005; Simpson and Caballero 2014):

1- Antibody-dependent cytotoxicity: When an antibody binds to an antigen on a target cell and the fragment crystallizable (Fc) of the antibody engages the Fc gamma receptor (FcgR) on the effector cells, which are, in this case, natural killers and macrophages. This leads to cell destruction and increases tumor debris exposure to APCs. Consequently, this will trigger production of tumor-specific cytotoxic lymphocytes (CTLs). This pathway is FcgR-dependent. Some preclinical data supports the importance of this pathway. For example, an inferior antitumor activity of the monoclonal antibodies (rituximab) on FcgR-deficient mice compared to wild mice. There is little clinical data on this type of immunomodulation.
2- Complement-mediated cytotoxicity (CDC): The antibody antigen complex is able to initiate the naturally occurring complements-dependent cytotoxicity. The most effective antibodies to stimulate the complements pathway are IgM. However, their lack of the extravasation capability makes the IgG subclasses more frequently used in clinical practice. CDC is one of the rituximab mechanisms of action.
3- Antibody-tumoricidal conjugate: the concept of this approach is to deliver cytotoxic agents or radioisotopes to targeted tumor cells through tumor-specific antibodies with the aim to reduce systemic toxicity. The FDA-approved Anti-CD30 antibody-drug conjugate (brentuximab vedotin-monomethyl auristatin E) is a successful example used in treating CD30-expressing lymphomas (Yi et al. 2017).

1.2.3 Checkpoint pathway inhibitors

To understand the pivotal role of the checkpoint pathways in cancer immunity we summarize the cancer immune cycle as follows: cancer-specific antigens will be presented on the MHC I of the APCs or MHC II of the T regulatory cells respectively. This will activate cytotoxic T

cells (CD8) which in turn migrate to the tumor site and initiate an antitumor reaction (Dine et al. 2017). The interaction between cytotoxic T cells and APCs or cancer cells depends mainly on the T cell receptor (TCR) which binds to antigen peptides on the effector cells (APC, cancer cells) as a first signal. However, a second signal between a group of transmembrane proteins expressed on the T cells membrane, and their ligands on the effector cells, is necessary to complete priming of native T cells. These transmembrane proteins belong to a superfamily of immunoglobulin that regulates T cells' activity. The first-known co-stimulatory receptor expressed on native T cells is the CD28 receptor. When it binds to its ligands (B7-1 and B7-2) on the APC, intracellular pathways will be activated to produce cytokines such as IL-2 which promote further T cell activity. Checkpoint receptors also belong to this superfamily of transmembrane proteins and are responsible for the inhibitory signaling which will eventually down regulate TCRs and T cells' anticancer activity. Inhibitory checkpoint receptors like CTLA-4 (Cytotoxic T Lymphocyte Antigen-4), PD-1 (Programmed Death-1), LAG-3 (Lymphocyte Activation Gene-3), TIM-3 (T cell Immunoglobulin and Mucin protein-3) act in the context of an immune system self-protection mechanism to avoid an overt immunoreaction and to regulate its response toward self-proteins (Nirschl and Drake 2013).

The longstanding exposure of cytotoxic T cells (CTL) to specific antigens during chronic viral infections or persistent tumor antigen stimulation could result in T cells' shut down. This phenomenon is called 'T cell exhaustion' and it describes a gradable loss of cytotoxic T cell functions including: the ability to secrete cytokines, the capacity of proliferation and cytotoxicity, as well as degranulation and memory cells' generation. This state of T cells dysfunction is connected to up-regulation of the checkpoint co-inhibitory receptors on lymphocytes of peripheral blood obtained from cancer patients or patients with chronic viral infections like HIV, and Hepatitis B and C. T cell exhaustion depends on the duration and degree of T cells' exposure to an antigen. Preclinical studies show an increase of exhausted T cells in the tumor microenvironment compared to those in peripheral blood. PD-1 up-regulation in the tumor microenvironment is considered to be a hallmark of CTL exhaustion (Okoye et al. 2017; Granier et al. 2017).

Cytotoxic T Lymphocyte Antigen-4, also called (CD152), is a co-inhibitory homolog of the transmembrane co-stimulatory protein CD28 with a higher binding affinity to their mutual ligands B7-1 and B7-2 presented on APCs. CTLA-4 is a central T cells' regulator, it acts primarily on nodal T cells, comparing with the programmed cell death PD1 checkpoint receptor

which appears to be predominant in peripheral T lymphocytes (Buchbinder and Desai 2016). Mutations in the CTLA-4 coding gene may lead to loss of its down regulatory function and may be seen in insulin-dependent diabetes mellitus and many other autoimmune disorders like systemic lupus erythematosus (SLE) (Fagerberg et al. 2014). CTLA-4 is an intracellular glycoprotein that exists almost only in T lymphocytes. T cell second signal activation through CD28:B7-1 and B7-2 conjugation is an activation signal for CTLA-4. After CTLA-4 activation it will be translocated to the immune synapse to complete CD28 in binding to the B7 ligands, which in turn down regulate T cell activity through an intracellular cascade that leads to inhibition of TCR and decreases cytokines secretion (Intlekofer and Thompson 2013). CTLA-4 is also involved in the modulation of the T regulator cells (Tregs) (Callahan et al. 2010; Buchbinder and Desai 2016).

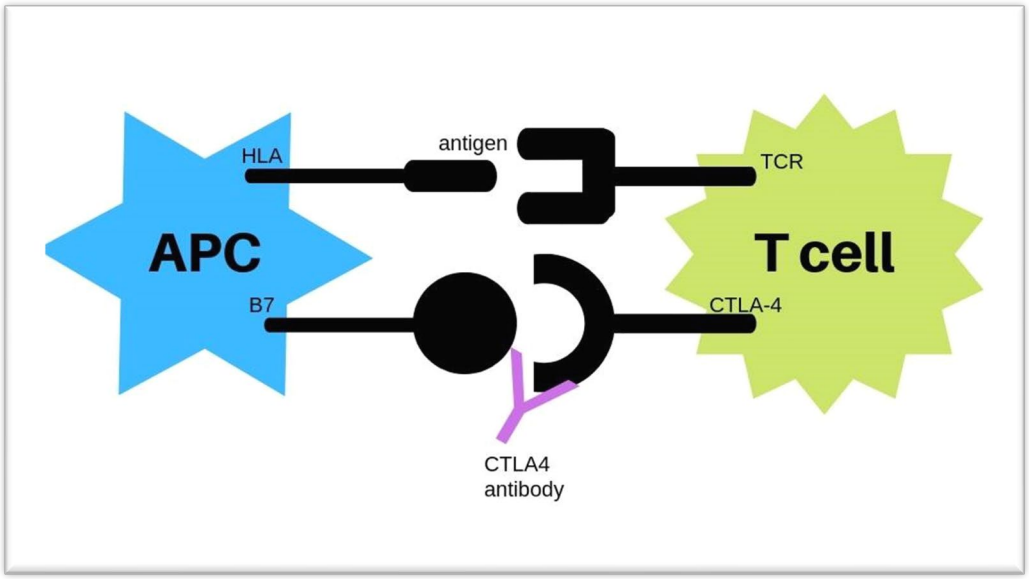

Figure 2: The mechanism of CTLA-4 pathway inhibition: The CTLA4 antibody interferes with the down-regulatory effect of the peripheral membrane protein (B7) by blocking its binding site at the CTLA4 checkpoint receptor. APC: antigen presenting cell, CTLA: cytotoxic T lymphocyte antigen-4, HLA: human leukocyte antigen, TCR: T cell receptor

Ipilimumab is a CTLA-4 inhibitor. A recombinant human immunoglobulin (Ig) G1 monoclonal antibody binds to the CTLA-4 receptor and detains its inhibitory action. Ipilimumab is an approved anti-cancer monoclonal antibody and it is a successful treatment for metastatic melanoma (Della Vittoria Scarpati et al. 2014).

Programmed cell death 1 (PD 1 or CD279) is also a transmembrane checkpoint protein of the globulin superfamily with a well-defined function in immune down regulation. It is a 288 amino acid with an extracellular domain and cytoplasmic tail (Dong et al. 2016). It was thought to be an apoptosis-regulator protein; it was tested in PD1-deficient mice and recognized in 1999 as a cell-mediated immunity down-regulator by Tasuku Honjo and colleagues at Kyoto University. In preclinical studies PD 1 deficiency elicited a spontaneous autoimmune disease pattern. PD 1 is expressed on CD4 and CD8-activated cells, APCs (DC and monocytes) as well as T regulatory cells (Bardhan et al. 2016). PD 1 is highly expressed on the T cells in a tumor microenvironment due to chronic antigen exposure, and its expression is considered a sign of T cells' exhaustion (Dong et al. 2016). PD 1 expression is also up-regulated through γ -chain cytokines such as IL-2, IL-7, IL-15, and IL-21 (Bardhan et al. 2016). PD 1 engagement with its ligand PD-L1 (B7-H1) and PD-L2 (B7-DC) triggers termination of T cells' activity. PD-L1 is expressed by cancer cells and APCs, and has a higher affinity to PD -L1 than PD-L2 (Alsaab et al. 2017). The PD 1 PD-L1 engagement on antigen-presenting cells hinders specific T cell activation and encourages the differentiation of Treg. This engagement in the tumor microenvironment through the PD-L1 expressed on tumor cells inhibits T cells' antitumor activity. The PD 1 pathway activation causes alteration in the intracellular pathways to decrease T cell cytotoxic activity and tumor-antigen recognition by TCR modulation (Bardhan et al. 2016).

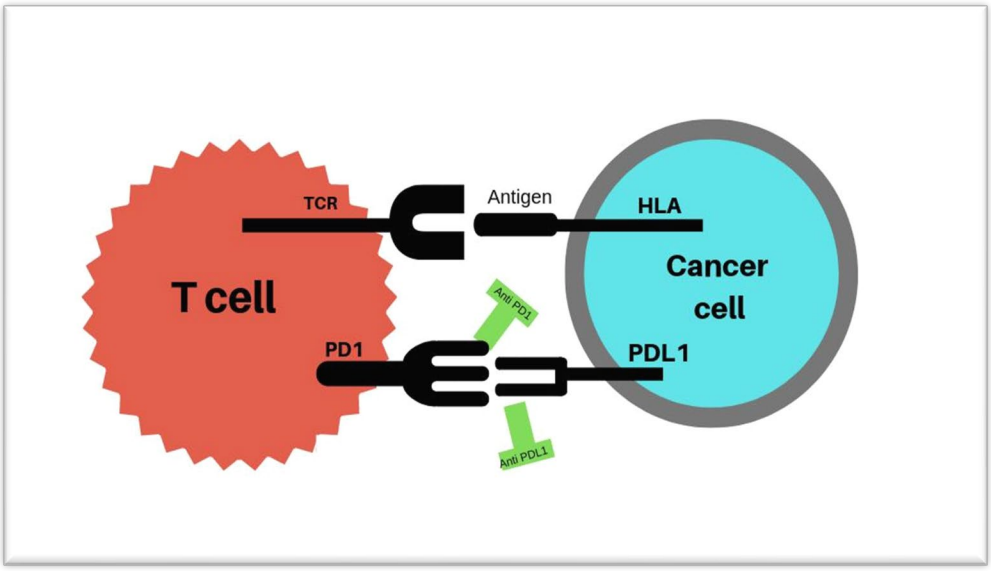

Figure 3: The mechanism of PD1/PD-L1 pathway inhibition: The PD1 or PD-L1 antibodies bind to either the PD1 checkpoint receptor or to PD1 ligand to interfere with the down-regulatory effect of the PD/PD-L1 pathway on T-cell activity. HLA: human leukocyte antigen, PDL: programmed cell death ligand, PD: programmed cell death receptor, TCR: T cell receptor.

The introduction of PD-1/PDL-1 inhibitors is a big step forward in treating different advanced malignancies. Several clinical trials have demonstrated the efficacy of programmed cell death inhibitors and resulted in the approval of many new anti-cancer medications (Sunshine and Taube 2015).

Pembrolizumab (KEYTRUDA®; Merck) is a highly selective humanized IgG4 monoclonal PD 1 receptor inhibitor approved as a first-line therapy for metastatic NSCLC with a PD-L1 tumor proportion score (TPS) ≥ 50% in the absence of ALK and EGFR mutations (Lim et al. 2016), as well as a second-line therapy for patients with a TPS ≥ 1% who failed to respond to conventional platinum-based chemotherapy (Rihawi et al. 2017) pembrolizumab therapy shows efficacy treating PD-L1 positive malignancies such as NSCLC, head and neck cancer, gastric carcinoma and urothelial cancer as well as hematological malignancies like PD-L1-positive Hodgkin lymphoma and mesothelioma (Khoja et al. 2015).

1.3 Objectives

The main aim of this work is to validate a weighted score of various laboratory values in a population of patients with metastatic NSCLC treated in the first line with pembrolizumab. The mentioned score has been described earlier in a pretreated population of patients with advanced NSCLC who received treatment with nivolumab. As it was possible that the score had to be slightly adapted in first-line treatment, other clinical, radiological and pathological characteristics were registered as well and correlated with patient response to treatment with pembrolizumab. As there is no indisputable test to predict patient response to treatment with immune-checkpoint-inhibitors (ICI), a score could be a helpful tool in monitoring patients under treatment with ICI, especially as during treatment with ICI a so-called pseudoprogression is hard to distinguish from a really progressive disease.

2 Material and methods

2.1 Overview

We retrospectively analyzed 66 consecutive patients who received therapy with pembrolizumab at the LKI Lungenfachklinik Immenhausen (Medical director Prof. Dr. med. S. Andreas) who commenced treatment between 16th of November 2016 to 30th of April 2018. Pembrolizumab is approved in Germany as a first-line monotherapy in patients with metastatic non-small cell lung cancer with PD-L1-expressing tumors (Tumor Proportion Score [TPS] ≥ 50%) without targetable EGFR or ALK driver-mutations.

All patients were treated with pembrolizumab at a flat dose of 200 mg as an intravenous infusion over one hour every three weeks, equivalent to one cycle. The majority of patients received their therapy in the oncological outpatient clinic. Patients with poor clinical status were admitted to an inpatient ward if necessary. On each day of the treatment, after taking a brief clinical history and a clinical examination, patients received a chest X-ray, and blood tests of full blood count, liver, kidney functions tests and C-reactive protein. Side effects and weight were documented on every visit. The therapy was continued for up to six cycles if no intolerable adverse events occurred. After each three cycles, a chest CT was performed and evaluated according to RECIST 1.1.

2.2 Documentation

The documentation of patients' data was carried out with the spreadsheet program Microsoft® Excel 2016, Redmond. For a retrospective data collection we utilized patients' information and laboratory parameters from the patient information software Medico ® Idstein and medical records at the LKI Lungenfachklinik Immenhausen. This includes discharge or transfer reports and documents of a local tumor board. The data were anonymized by giving each patient an identification number. The collected data are summarized in (Table 6).

Table 6: Overview of the extracted data form patients' medical records

Extracted data	**Clinical variables, range and measuring units**
Date of birth	Age in years
Gender	Male/female
Smoking status	Current, former or never smoker
Smoking quantity	Pack years
Tumor main histological type	Adenocarcinoma (ADC), squamous cell carcinoma (SCC), adenosquamous carcinoma (ASC), sarcomatoid carcinoma or not otherwise specified (NOS)
KRAS, MET and TP53 Mutation	Positive: mutated Negative: wild-type
PD-L1 Status	TPS score, range: 0.5-1.0 IC score, range 0.0-1.0
Date of first diagnosis	Survival time in days
Date of death (if available)	
Date of last contact with the patient	
GL-Index	10^3 µl/ 10^3 µl
LDH	U/L
CRP	mg/L
Weight	Kg
Target lesion size in millimeter at 1st day of the therapy and after every 3 cycles. Target lymph nodes' and secondary lesions' size in millimeters at 1st day of the therapy and after every 3 cycles.	To define the treatment response according to revised RECIST1.1 criteria (table 7)

Pack years: (number of packs of cigarettes smoked per day x the number of years the person has smoked), KRAS: Kirsten rat sarcoma virus, MET: mesenchymal-epithelial transition factor p53: tumor suppressor. TPS: tumor proportion score, IC: PD-L1 infiltration in immune cells. GL-Index: granulocytes (neutrophils)/lymphocytes [103 x microliter/ 103 x microliter]. LDH: lactate dehydrogenase units/liter. CRP: c reactive protein milligram/liter. Kg: kilogram.

2.3 Laboratory diagnostics and weight

In our analysis, we studied biomarkers that might have a potential prognostic value in patients with NSCLC. We included measures of lactate dehydrogenase (LDH) (Petrelli et al. 2015), c-reactive protein (CRP) (Koch et al. 2009) and the granulocyte to lymphocyte index (Gu et al. 2015) at baseline and at every therapy cycle. The longitudinal changes in the laboratory parameters were calculated at days 43 and 106 of the treatment and correlated to changes in tumor size obtained from CT- imaging. Likewise, the longitudinal changes in body weight were correlated to treatment response as well. Further, values of laboratory parameters and weight were integrated to a score that was proposed to predict treatment response to ICIs.

2.4 Histopathological Examination

The histological and molecular pathological examinations were carried out for the main tumor histology and possible mutations in the KRAS protein (Kirsten rat sarcoma virus), mesenchymal-epithelial transition factor (MET) and tumor suppressor p53. PD-L1 expression was evaluated in diagnostic biopsies using an immunohistochemical staining with PD-L1 antibodies 22C3 pharmDx (Dako, Inc.). PD-L1 status was expressed as a percentage of PD-L1 positive tumor cells by a tumor proportion score (PD-L1 TPS), as well as the PD-L1 expression in immune cells (PD-L1 I.C). These features were inserted into our data and stratified in each of the different response groups. The specimens were examined in Pathology Institute, Nordhessen.

2.5 Evaluation of patients' response according to the RECIST 1.1

As already stated, a restaging computer tomography study of the chest was done after every three cycles of the therapy. The new results including the size of the primary lesion, secondary lesions or lymph nodes and newly detected lesions were measured, evaluated and compared to the baseline chest CT at day one of the therapy. After ruling out patients with early death (who died before the first CT-scan) the rest of the patients were classified to: stable disease (SD), real progressive disease (RPD) and objective response (OR). The restaging was repeated after six cycles when a pseudo-progressive disease (PsPr) was added to the response groups. The evaluation was done in the Lungenfachklinik Immenhausen based on new response evaluation criteria in solid tumors, revised RECIST 1.1 guidelines (Eisenhauer et al. 2009), summarized in table 7.

Table 7: Treatment response according to revised RECIST 1.1 criteria

Response group	**Definition**
Complete response	Disappearance of all target lesions and reduction of pathological lymph node diameter for < 10 mm in the short axis.
Partial response	Persistence of target lesions of >30% reduction in the sum of the longest diameter.
Progressive disease	Increase of the sum of the largest diameter of target lesions by >20% compared with the longest sum diameter before treatment
Stable disease	None of the above

2.6 Statistical Analysis

For each response group, patients' features and clinical variables were presented by mean±sd (continuously scaled) or by absolute and relative frequencies (nominally scaled). Each clinical variable was tested and compared in all response groups by an appropriate test, ex.: the ANOVA analysis and the Fisher's exact test (Du Prel et al. 2010). The change from baseline value (Delta) for each potential variable (weight, LDH, CRP, GL-Index and the tumor size) was calculated at predetermined points of time (day 43 and 106) to correlate the values with tumor response according to CT scans. The development over time was visualized using median boxplots overlaid with line plots.

In order to obtain a prediction score that works robustly independent of the actual day, values of potential variables from six points time (days: 1- baseline, 22, 43, 64, 85, and 106 of the treatment) were included in data testing. Additionally, gender, age, histological type, PD-L1 TPS, PD-L1 I.C, and smoking status were part of the potential predictors.

The tested predictor is composed of a two-stage hierarchically structured receiver operating characteristic (ROC) analysis (Hajian-Tilaki 2013) for each variable of the tested data. In its first stage, the response group RPD was separated from the other response groups. In its second stage, the response group OR was separated from the remaining groups SD and PsPr. In both ROC analyses a cutoff corresponding to the Youden index was used. For each variable, samples classified as RPD get zero points, samples classified as SD or PsPr get 1 point, and samples classified as OR get 2 points in the predictor. The sum of these points constitutes the overall prediction score.

The classification performance has been evaluated in a 10-times repeated 10-fold cross validation (CV) (Simon et al. 2011) . The scores of the patients in the test set have been calculated at day 43. The difference of the assigned scores between the groups has been assessed using a linear mixed effect model including the results across all 10 repeats of the CV.

A classification into RPD vs. the other response groups (non-RPD) was achieved via a cutoff on this score. The cutoff was chosen at the Youden index in the ROC analysis.

The performance of this predictor was assessed by means of a 10-times repeated stratified 10-fold cross validation. The evaluation was performed using data from the baseline and day 43. The accuracy, area under the curve (AUC), and the achieved sensitivity at a specificity of 50 % at best setting were reported.

The overall survival was analyzed via two separate Cox regressions (Stel et al. 2011) , the first to assess the effect of all response groups on the survival time and the second to assess the effect of the derived predicted scores at day 43 on survival. Kaplan-Meier curves have been created to display these effects.

The significance level was set to alpha = 50% for all statistical tests. The statistical analysis was carried out by Dr. Andreas Leha (Head of central service unit - Department of Medical Statistics at University medical center, Gottingen). All analyses were performed with the statistics software R (version 3.5.1; R Core Team 2018).

2.7 Ethics

This study was approved by the Ethics Committee of the University Medical Center, Göttingen (application number 11/4/18). The principles of data protection complied with the law of data protection of Lower Saxony.

3 Results

3.1 Patients' main characteristics

Patients' characteristics were analyzed at the baseline for the whole population and then for each of the five response groups.

Table 8: Baseline demographic and clinical characteristics

Parameter	**Level**	**Value**
Total number		66
Gender		
	Female	24 (36.4%)
	Male	42 (63.6%)
Age in years		
	mean ± sd	70 ± 9.2
	median (min; max)	71 (50; 87)
Smoking status		
	Current smoker	33 (50.0%)
	Former smoker	27 (40.9%)
	Never smoker	5 (7.6%)
	Passive smoker	1 (1.5%)
Smoking quantity (pack years)		
	mean ± sd	42 ± 29
	median (min; max)	40 (0; 140)
	Missing	3

About 63% of the patients were males. The median age at time of diagnosis was 71 years and the youngest patient was diagnosed at the age of 50. About 91 % of the patients have had a known tobacco consumption with a median quantity of 40 pack years. One patient was a passive smoker and the rest 7.6% (n=5) were never smokers.

According to patient response the patient population (n=66) was further divided into the following response groups based on the RECIST 1.1 on days 43 and 106:

Table 9: Classification of patients according to objective response rate

Objective response rate (ORR)	**Number of patients**
ED	9 (13.85%)
OR	27 (41.54%)
PsPr	6 (9.23%)
RPD	14 (21.53%)
SD	9 (13.85%)
NA	1

ED: early death, OR: objective response, PsPr: pseudoprogression, RPD: real progressive disease, SD: stable disease, NA: not available.

Patients with early death (n=9) are those who died before completing the first three cycles of the treatment, i.e., in the first 43 days and didn't receive a follow-up chest CT. "Not Available" represents one patient who had his therapy with pembrolizumab ended after one cycle due to suspected pembrolizumab-induced pneumonitis. Diagnosing patients with pseudoprogression was carried out at the second follow up after day 106.

In order to study each group separately, we analyzed the baseline clinical characteristics for each group separately as presented in table 10:

Table 10: Baseline demographics and clinical characteristics according to response group

Parameter	**Level**	**ED**	**OR**	**PsPr**	**RPD**	**SD**	**P-value**	**Test**
n		9	27	6	14	9		
Gender							0.13	Fisher's Exact Test for Count Data
	F	5 (55.6%)	11 (40.7%)	1 (16.7%)	2 (14.3%)	5 (55.6%)		
	M	4 (44.4%)	16 (59.3%)	5 (83.3%)	12 (85.7%)	4 (44.4%)		
Age in years							0.46	Analysis of Variance
	mean ± sd	72 ± 7.1	69 ± 7.8	68 ± 9.1	74 ± 12	72 ± 8		
	median (min; max)	71 (60; 82)	69 (55; 87)	70 (57; 81)	80 (50; 87)	75 (57; 84)		
Smoking status							0.70	Fisher's Exact Test for Count Data
	Current smoker	3 (33.3%)	15 (55.6%)	4 (66.7%)	5 (35.7%)	5 (55.6%)		
	Former smoker	5	10	2	7	3		

Parameter	Level	ED	OR	PsPr	RPD	SD	P-value	Test
		(55.6%)	(37.0%)	(33.3%)	(50.0%)	(33.3%)		
	Never smoker	1 (11.1%)	2 (7.4%)	0 (0.0%)	2 (14.3%)	0 (0.0%)		
	Passive smoker	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (%11.1)		
Smoking quanti-ty (pack years)							0.24	Kruskal-Wallis rank sum test
	mean ± sd	33 ± 23	44 ± 29	45 ± 17	37 ± 38	54 ± 28		
	median (min; max)	35 (0; 80)	40 (0; 120)	50 (20; 60)	30 (0; 140)	48 (30;12)		
	missing	0	1	0	1	1		

Almost two-thirds of the patients responded well to pembrolizumab treatment. About 35% had a progressive disease or died early. The table shows variations in patients' baseline features in all response groups. The medians of age for objective response and real progressive disease were 69 and 80 respectively. About 86% of patients with progressive disease were males and only one female patient had a PsPr (n=6). There were five never smokers, one died early, two had OR and the other two patients had RPD.

3.2 Pathological characteristics

In addition to the pathological subtypes of the tumor, patients were tested for programmed cell death ligand (PD-L1) expression in tumor cells represented by Tumor Proportion Score (TPS) as well as for PD-L1 infiltration in immune cells at the tumor site (I.C.). Moreover, patients with non-squamous cell cancer (n=46) were investigated for mutations in the oncogenes: Kirsten Rat Sarcoma virus (KRAS), tyrosine-protein kinase (Met) and Tumor Protein 53 (TP53).

Table 11: Baseline tumor histology, molecular pathology and immunohistochemistry

Parameter	**Level**	**Value**
N		66
Histology		
	ADC	43 (65.2%)
	NOS	1 (1.5%)
	SCC	20 (30.3%)
	Sarcomatoid carcinoma	1 (1.5%)
	ASC	1 (1.5%)
PD-L1 TPS		
	mean ± sd	0.78 ± 0.17
	median (min; max)	0.8 (0.5; 1)
PD-L1 I.c		

Parameter	Level	Value
	mean ± sd	0.21 ± 0.26
	median (min; max)	0.1 (0; 0.9)
	missing	4
KRAS mutation		
	neg.	27 (57.4%)
	pos.	20 (42.6%)
	missing	19
MET mutation		
	neg.	41 (87.2%)
	pos.	6 (12.8%)
	missing	19
TP53 mutation		
	neg.	28 (59.6%)
	pos.	19 (40.4%)
	missing	19

neg.: means wild type non-mutated gene. pos.: mutated genes. The 19 missing come from patients with SCC who were not tested for mutations. ADC: adenocarcinoma. NOS: not otherwise specified. SCC: squamous cell carcinoma. ASC: adenosquamous carcinoma. sd.: standard deviation, PD: programmed cell death, TPS: tumor proportion score, I.c.: immune cells, KRAS: Kirsten rat sarcoma, MET: mesenchymal-epithelial transition factor p53: tumor suppressor.

The majority of patients had adenocarcinoma (65%) followed by squamous cell carcinoma (30%). As the least accepted TPS was 50%, we had a TPS median of 80%. The PD-L1 I.c is not one of the prerequisites to initiate a monotherapy with pembrolizumab and shows a wide variation (0-90%). KRAS mutation was prevailing (42.6%) followed by TP53 mutation in 40% of the tested patients.

Table 12: Baseline tumor histology, molecular pathology and immunohistochemistry according to response group

Parameter	**Level**	**ED**	**OR**	**PsPr**	**RPD**	**SD**	**P-value**	**Test**
n		9	27	6	14	9		
Histology							0.76	Fisher's Exact Test for Count Data
	ADC	8 (88.9%)	18 (66.7%)	4 (66.7%)	7 (50.0%)	5 (55.6%)		
	NOS	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.1%)	0 (0.0%)		
	PEC	1 (11.1%)	7 (25.9%)	2 (33.3%)	6 (42.9%)	4 (44.4%)		
	Sarcomatoid	0 (0.0%)	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
	SqAD	0 (0.0%)	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
PD-L1 TPS							0.60	Analysis of Variance
	mean ± sd	0.82 ± 0.2	0.78 ± 0.18	0.74 ± 0.14	0.73 ± 0.18	0.83 ± 0.13		
	median (min; max)	0.9 (0.5; 1)	0.8 (0.5; 1)	0.7 (0.6; 1)	0.8 (0.5; 1)	0.8 (0.6; 1)		

Parameter	Level	ED	OR	PsPr	RPD	SD	P-value	Test
PD-L1 I.c							0.53	Analysis of Variance
	mean ± sd	0.18 ± 0.22	0.23 ± 0.28	0.055 ± 0.097	0.25 ± 0.29	0.28 ± 0.25		
	median (min; max)	0.05 (0; 0.6)	0.1 (0; 0.9)	0.015 (0; 0.25)	0.15 (0; 0.7)	0.25 (0; 0.7)		
	missing	0	1	0	2	1		
KRAS mutation							0.39	Fisher's Exact Test for Count Data
	neg.	5 (62.5%)	10 (50.0%)	4 (100.0%)	6 (66.7%)	2 (40.0%)		
	pos.	3 (37.5%)	10 (50.0%)	0 (0.0%)	3 (33.3%)	3 (60.0%)		
	missing	1	7	2	5	4		

Parameter	Level	ED	OR	PsPr	RPD	SD	P-value	Test
MET mutation							0.62	Fisher's Exact Test for Count Data
	neg.	6 (75.0%)	18 (90.0%)	4 (100.0%)	7 (77.8%)	5 (100.0%)		
	pos.	2 (25.0%)	2 (10.0%)	0 (0.0%)	2 (22.2%)	0 (0.0%)		
	missing	1	7	2	5	4		
TP53 mutation							0.64	Fisher's Exact Test for Count Data
	neg.	3 (37.5%)	12 (60.0%)	3 (75.0%)	5 (55.6%)	4 (80.0%)		
	pos.	5 (62.5%)	8 (40.0%)	1 (25.0%)	4 (44.4%)	1 (20.0%)		
	missing	1	7	2	5	4		

neg.: means wild type non-mutated gene. pos.: mutated genes. The 19 missing come from patients with SCC who were not tested for mutations. ADC: adenocarcinoma. NOS: not otherwise specified. SCC: squamous cell carcinoma. ASC: adenosquamous carcinoma. sd.: standard deviation, PD: programmed cell death, TPS: tumor proportion score, I.c.: immune cells, KRAS: Kirsten rat sarcoma, MET: mesenchymal-epithelial transition factor p53: tumor suppressor.

Table 12 shows the histological features in all response groups. About 34% of patients with SSC (n=20) had ED or RPD. The numbers are similar in patients with ADC (n=42), 35% had ED or RPD and about two-thirds of both histologies responded well to the treatment. There was no statistical significance of the histological type in the different response group. The range TPS Score median was 70% to 90% in all groups (p=0.6). The PD-L1 infiltration in immune cells was markedly lower in patients with PsPr (< 0.6%) compared to other response groups (22-29%). In addition, there was only one oncogenic mutation in patients with PsPr.

3.3 Laboratory parameters and weight

Laboratory parameters and weight were documented and the median was calculated at baseline for each response group:

Table 13: Baseline weight, laboratory parameters and tumor size according to response group

Parameter	**Level**	**ED**	**OR**	**PsPr**	**RPD**	**SD**	**P-value**	**Test**
N		9	27	6	14	9		
Weight (kg)							0.70	Analysis of Vari-ance
	mean ± sd	71 ± 6.8	74 ± 17	77 ± 21	83 ± 18	74 ± 19		
	median (min; max)	70 (65; 78)	73 (36;120)	73 (53;104)	78 (51;112)	72 (58;120)		
	Missing	6	4	0	3	0		
CRP (mg/l)							< 0.01	Analysis of Vari-ance
	mean ± sd	114 ± 73	26 ± 36	17 ± 22	79 ± 55	45 ± 66		
	median (min; max)	136 (12; 190)	7.5 (3.3;148)	5.7 (3.3; 59)	65 (11;197)	4.2 (3.1;196)		
	missing	3	1	0	0	0		

Parameter	Level	ED	OR	PsPr	RPD	SD	P-value	Test
LDH (U/l)							< 0.01	Analysis of Variance
	mean ± sd	365 ± 195	207 ±65	189 ± 16	216 ± 55	275 ±106		
	median (min; max)	325 (181; 695)	190 (99; 357)	183 (176;213)	224 (142;295)	268 (146;523)		
	missing	4	1	0	0	0		
GL index							0.17	Analysis of Variance
	mean ± sd	14 ± 19	5.4 ±3.3	2.7 ± 2	9.1 ± 14	6.1 ± 3.8		
	median (min; max)	7.1 (4.1; 60)	4.1 (1.7; 14)	2.1 (0.6; 6.3)	4.1 (1.2; 54)	4.7 (2.4; 13)		
	missing	1	1	0	1	0		
Tumor size (mm)							0.26	Analysis of Variance

Parameter	Level	ED	OR	PsPr	RPD	SD	P-value	Test
	missing	9	0	0	2	0		
	mean ± sd	NA	62 ± 27	51 ± 21	78 ± 38	72 ± 35		
	Median (min; max)	NA	61 (18;124)	44 (27; 80)	75 (19; 168)	55 (38; 138)		

CRP: c reactive protein milligram/liter. Kg: kilogram, GL-index: granulocytes (neutrophils)/lymphocytes, LDH: lactate dehydrogenase units/liter.

There were six missing weight values in the ED group (n=9), so the calculated median is not representative. Median weight for patients with RPD (83) was higher than the other groups (74-77). The baseline CRP was significantly higher in patients with ED and RPD, with a median of 136 and 65 respectively compared to other well response groups, median (4.2-7.5) P-value (< 0.01) according to Analysis of Variance. LDH median was higher in patients with ED (325) than in other response groups, the lowest medians were in patients with PsPr and OR (183 and 190) P value <0.01 adjusted to (0.03). Regarding G/L index, patients with ED showed the highest median (7.1). G/L index differences were not significant among other groups (P value= 1). Excluding patients with ED, the highest baseline tumor size median was in patients with RPD (75 mm).

In order to define possible associations between changes in the laboratory parameters and patient response, we observed variations of different parameter from the baseline value in each response group. The variations for each parameter (weight, CRP, LDH and the G/L-Index) were calculated according to the Delta formula at days 43 and 106. Thereafter, we integrated the RECIST curves into the diagram. RECIST curves describe the change of the tumor size measured by CT scan at the same points of time.

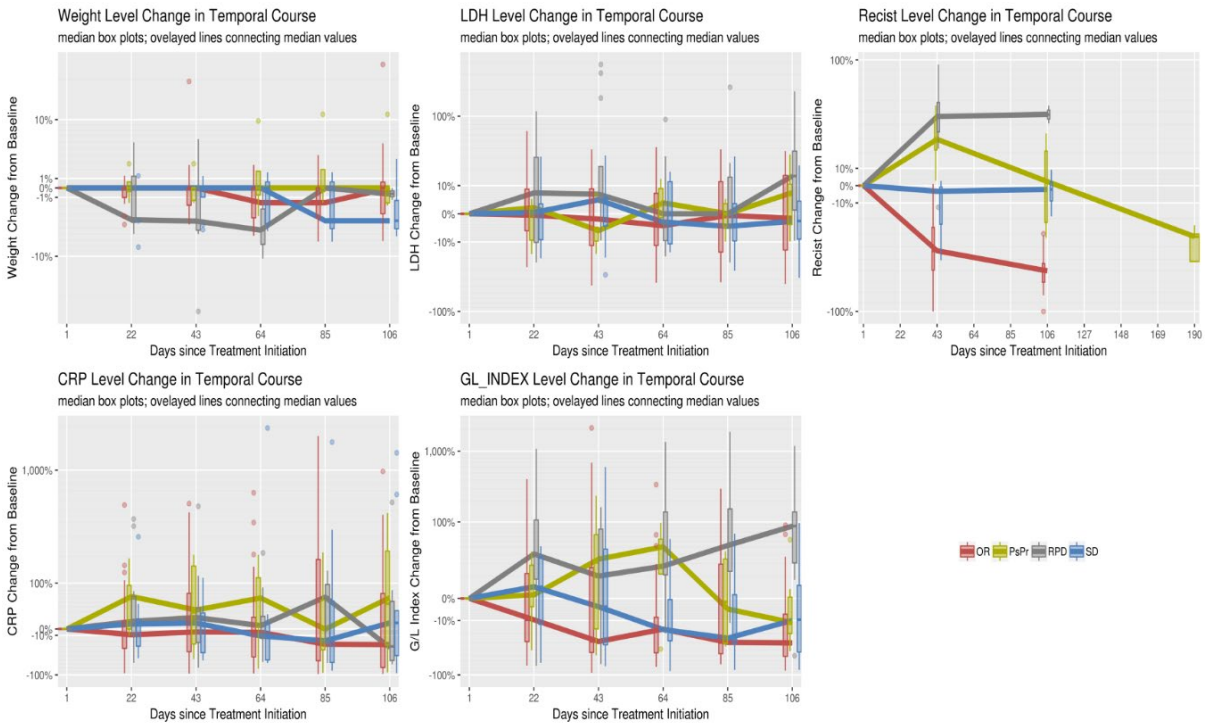

Figure 4: Visualization of variations of the potential biomarkers, tumor size and weight over time: The relative changes are shown on a log scale. The solid lines connect the median values at each time point. The boxplots represent the distribution at each day. From right to left: weight, LDH, tumor size (RECIST), CRP and G/L index. The change of tumor size for the group PsPr was extended to 190 days to show the decrease of tumor size beyond its baseline size.

As shown in the weight curves, patients with RPD had a weight drop as soon as the treatment was started until they reached more than 5% weight loss at day 64. Patients with SD had about 3% weight loss after day 64. There was a slight weight drop for patients with OR between days 43 and 106. Patients with PsPr disease always had a stable weight.

LDH values for patients with RPD increased after initiation of the therapy and ended up with an increase of more than 10% at day 106. For the SD group there was an LDH elevation till day 43, followed by a drop near the baseline value at day 106. In OR patients, there was a decline until day 43, followed by a stable course. Patients with PsPr showed alternating LDH measurements in the whole observation period.

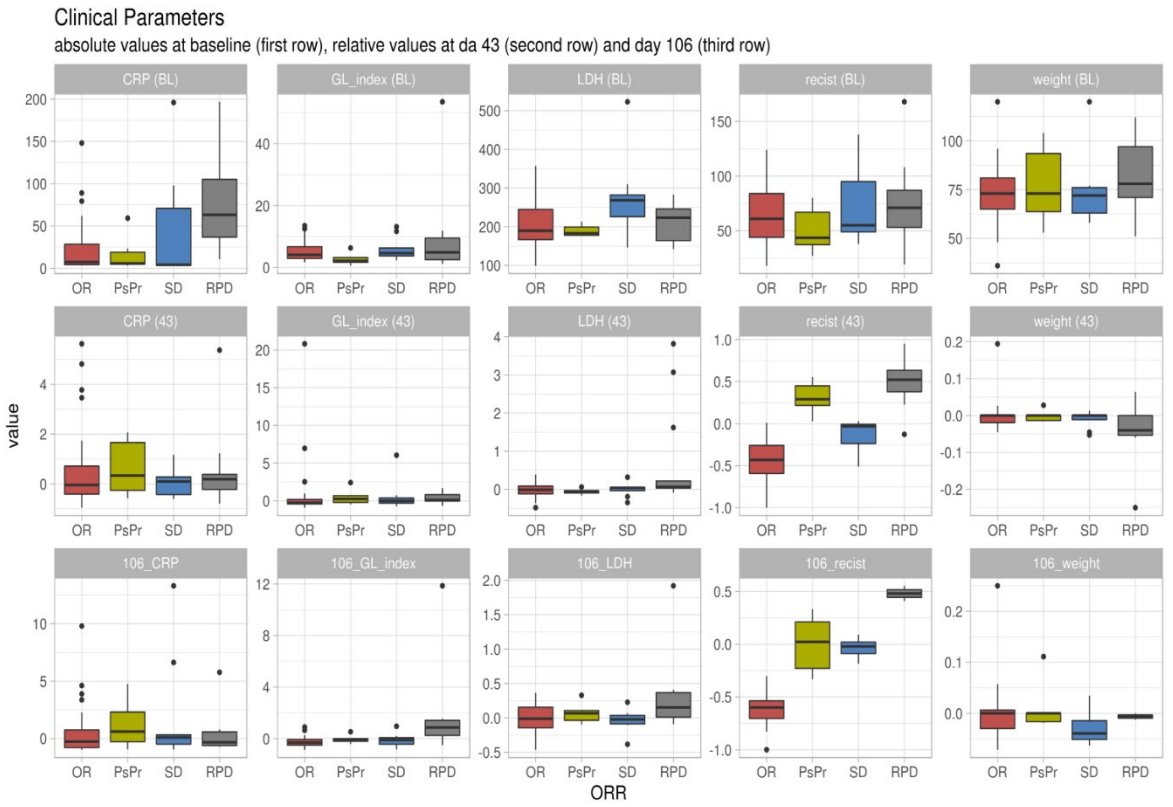

Figure 5: Boxplots of variations of the potential biomarkers, tumor size and body weight over time: response rate (ORR) on the (x-axis) at baseline (first row), at day 43 (second row) and at day 106 (third row). Absolute values are shown at baseline. The Y-axis shows the absolute value at baseline (first row) and then the changes relative to baseline (Δ) are shown for days 43 and 106 (second and third row).

The CRP in OR patients declined continuously and showed variable uneven changes in other response groups. G/L index declined in patients with OR and SD with a median almost always below the baseline. It rose until day 64 in the PsPr group and declined afterwards below the baseline value. Patients with RPD had a remarkable increase in the G/L index until 100% at day 106. RACIST curves showed the patients' response according to the change of tumor size at day 43 and 106. The curve was extended to day 190 for patients with PsPr disease to visualize the reduction of tumor size beneath its baseline value.

Based on the response group: patients with an objective response had a markedly continuous drop in the tumor size as well as in GL-Index values over time, and although there were some fluctuations in the GL-Index, the median has been always lower than the baseline value. The CRP and LDH declined as well. There was no significant change in weight. In patients with RPD, tumor size, LDH and GL-Index continued to increase. They also showed a significant decrease in their weight until day 63. Patients with SD didn't show a significant change in tu-

mor size. GL-Index medians didn't exceed the baseline value at any point of time. Likewise were the other parameters. Patients with pseudo-progressive disease were distinguished through the increase in the tumor size until day 43, followed by a continuous regression. GL-index showed a similar pattern with an increase until day 64 and a rash drop to below the baseline value afterwards.

3.4 Prediction of patient response

We developed a predictor using a two-stage hierarchically structured receiver operating characteristic (ROC) analysis for each variable of our data. In the first stage of the ROC analysis the response group RPD was separated from the others. In its second stage, the response group OR was separated from the remaining groups SD and PsPr. In both ROC analyses stages, a cutoff corresponding to the Youden index was used. For each of the variables, samples classified as RPD get zero points, samples classified as SD or PsPr get 1 point, and samples classified as OR get 2 points. The sum of these points constitutes the overall prediction score.

Table 14: Scoring scheme according to the two-stage ROC analysis

Parameter	**Score**		
	2	**1**	**0**
Age (years)	< 65.5	65.5 to 79.9	≥ 79.9
Gender	-	Female	Male
Histology	Non-squamous NSCLC	-	Squamous NSCLC or NOS
Smoking status	Current or former smoker		Never smoker
CRP-BL (mg/l)	< 23.4	23.4 to 29.2	≥ 29.2
CRP-43 (mg/l)	< 11.6	11.6 to 18.5	≥ 18.5
ΔCRP (mg/l)	< -0.7	-0.1 to -0.7	≥ -0.1
LDH-BL (U/l)	< 142	142 to 176	≥ 176
LDH-43 (U/l)	< 184	195 to 184	≥ 195

Parameter	Score		
	2	**1**	**0**
ΔLDH (U/l)	< -0.1	-0.1 to 0	≥ 0
GL index-BL	< 3.5	3.5 to 7.2	≥ 7.2
GL index -43	< 3.3	3.3 to 4.9	≥ 4.9
ΔGL index	< -0.4	-0.4 to 0	≥ 0
PD-L1 IC	= 0	0 to 0.7	≥ 0.7
PD-L1 TPS	= 0.5	0.5 to 0.6	≥ 0.6
Tumor size-BL (mm)	< 27	27 to 60	≥ 60
Tumor size-43 (mm)	< 27	27 to 89	≥ 89
Δ Tumor size (mm)	< -0.1	-0.1 to 0.2	≥ 0.2

This table shows how to assign scores according to the tested parameter. (Left to right) the first column shows the tested parameter. For each a score of 2 is assigned to the sample if the value is smaller than the one given in the second column, a score of 1 is assigned if the value falls between the cutoffs given in the third column, a score of 0 is assigned of the value is greater or equal to the cutoff given in the last column. 43: stands for day 43 (after 3 cycles). BL: baseline value. Δ stands for Delta= (X actual- X baseline). CRP: c reactive protein milligram/liter. Kg: kilogram, GL-index: granulocytes/lymphocytes, LDH: lactate dehydrogenase units/liter, TPS: tumor proportion score, IC: PD-L1 infiltration in immune cells.

The performance of this score was assessed by means of a 10-times repeated stratified 10-fold cross-validation (CV). The evaluation was performed using CRP, LDH and G/L index from baseline to day 106 (Figure 6). The difference in the assigned scores between the groups has been assessed using a linear mixed effects model including the results across all 10 repeats of the CV (Figure 7). A classification into RPD vs. the other response groups (non-RPD) was achieved via a cutoff on this score. The cutoff was chosen at the Youden index in an ROC analysis.

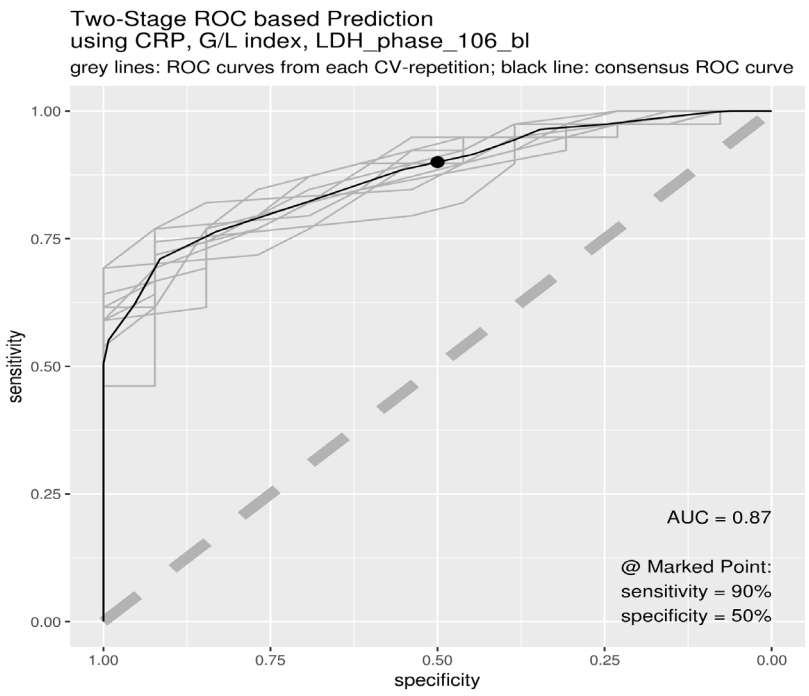

Figure 6: Two stages Receiver Operating Characteristic (ROC) based prediction using the laboratory parameters (CRP, LDH, and GL-index). Grey lines: ROC curves from each repeated cross validation. Black line: consensus ROC curve.

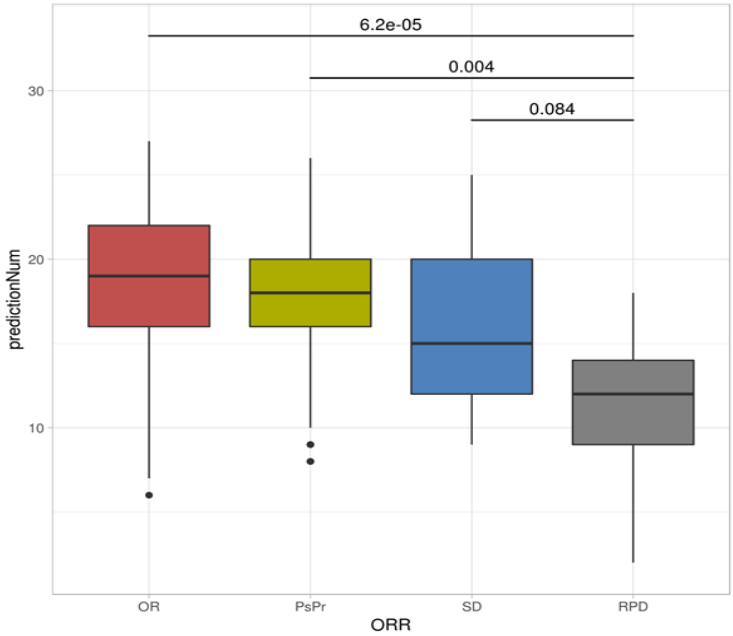

Figure 7: Boxplots demonstrate the distribution of the assigned scores in the treatment response groups. The mean of scores in patients with RPD was significantly lower than the score means in patients with OR and PsPr. The score difference between RPD and SD was statistically non-significant. P values are from a linear mixed effect regression, considering all the data from all repeats.

Scores of patients with OR at day 43 were significantly higher than scores for those who belong to RPD ($p=5x10^{-6}$). The p-values of score differences for patients with PsPr and SD compared to those with RPD were ($p=0.004$) and ($p=0.084$) respectively. The scores of the patients in the test set have been calculated at day 43 (Figure 8).

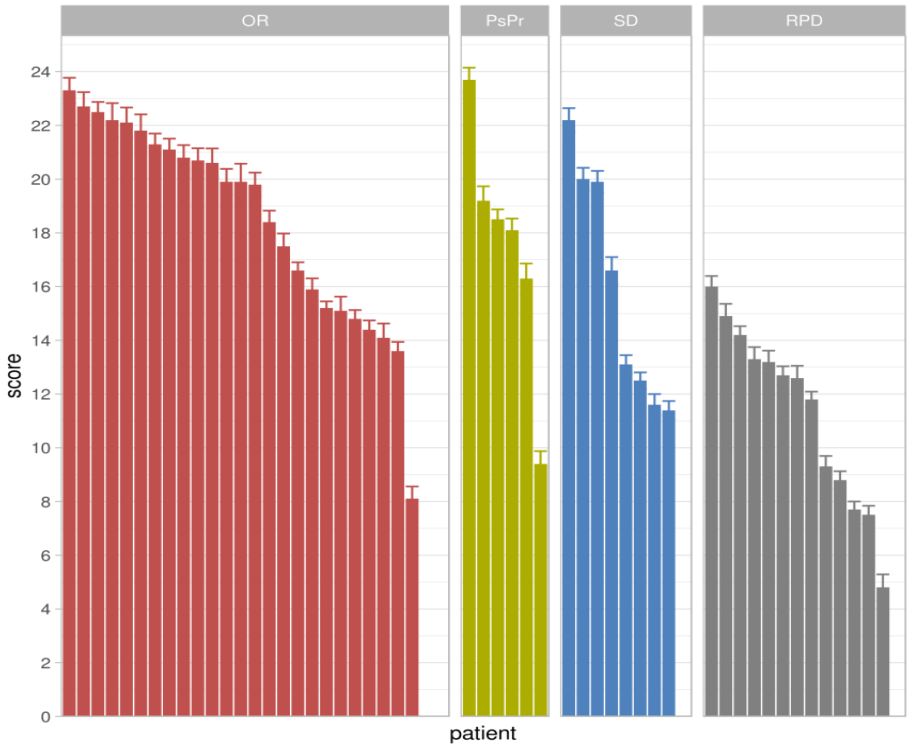

Figure 8: Predicted scores for each patient (x axis). The assigned score (y axis) at day 43. The total score of prediction is represented for each patient individually.

The ROC curve showed a predictor sensitivity of 90% at the best setting. The sensitivity is defined as the ability of the predictor to sort out patients who are going to benefit from the treatment, i.e., non RPD (= OR, PsPr and SD) from patients with RPD. The following parameters (CRP, LDH and G-Index) have shown the best achieved sensitivity compared to other score parameters at the same predetermined specificity of 50%.

The chosen best sensitivity parameters were also included in a previously suggested score (table 15) to predict patient response in a pretreated population of patients with metastatic NSCLC who received a second-line treatment with nivolumab. We tried to validate this score by applying it to our patient population who received a first-line treatment with pembrolizumab (figure 9).

Table 15: A previously described score to predict treatment response to nivolumab based on laboratory parameters and tumor size

Parameter	**Score**		
	2	**1**	**0**
Baseline CRP (mg/l)	< 5	5 to 60.4	≥ 60.4
Baseline LDH (U/l)	< 150	150 to 207	≥ 207
Baseline tumor size (mm)	< 32	32 to 61	≥ 61
ΔCRP (mg/l)	< -0.6	-0.6 to -0.2	≥ -0.2
Δ LDH (U/l)	< -0.1	-0.1 to 0	≥ 0
Δ GL index	< -0.3	-0.3 to 0.1	≥ 0.1
Δ Tumor size (mm)	< -0.1	-0.1 to 0	≥ 0

(Schiwitza et al. 2019). Δ demonstrates the difference in value between baseline and after 3 therapy cycles. CRP: c reactive protein, GL-index: granulocytes/lymphocytes, LDH: lactate dehydrogenase.

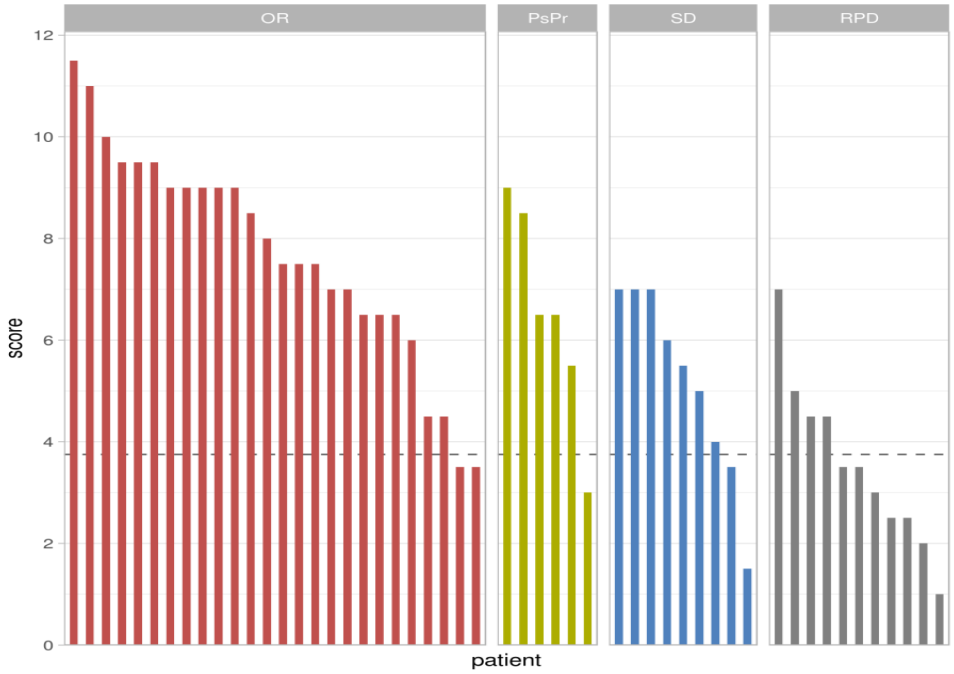

Figure 9: Predicted scores according to Schiwitza Score. For each patient (x axis) the assigned score (y axis) at day 43 presented as a bar. Different color codes for different response groups. Four patients (one patient with OR and three patients with RPD) were excluded due to missing parameters.

Applying the Schiwitza score with a modified cutoff of 3.75 points showed a sensitivity of almost 88% with a specificity of 63.6% (figure 10). Only two patients with OR (n=26), one patient with PsPr (n=6) and two patients with SD (n=9) had false negative predictions with a total of five false negatives out of 41. The score had a lower specificity with four false positives out of (RPD=11).

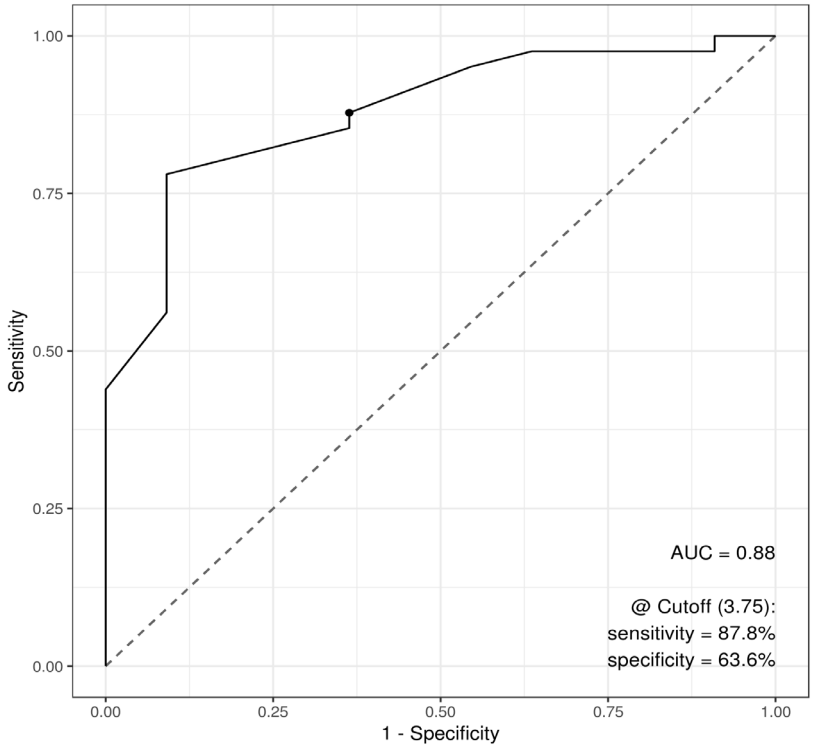

Figure 10: Receiver Operating Characteristic (ROC) shows the capacity of the pre-trained Schiwitza score in predicting treatment response to pembrolizumab. At a cutoff score of 3.75 an area under the curve of 88% could be achieved.

3.5 Overall survival

The overall survival was analyzed via two separate Cox regressions, the first to assess the effect of all response groups on the survival time (figure 11) and the second to demonstrate the effect of the derived predicted scores at day 43 from the cross-validation on patients' survival (figure 12). Kaplan-Meier curves have been created to display these effects.

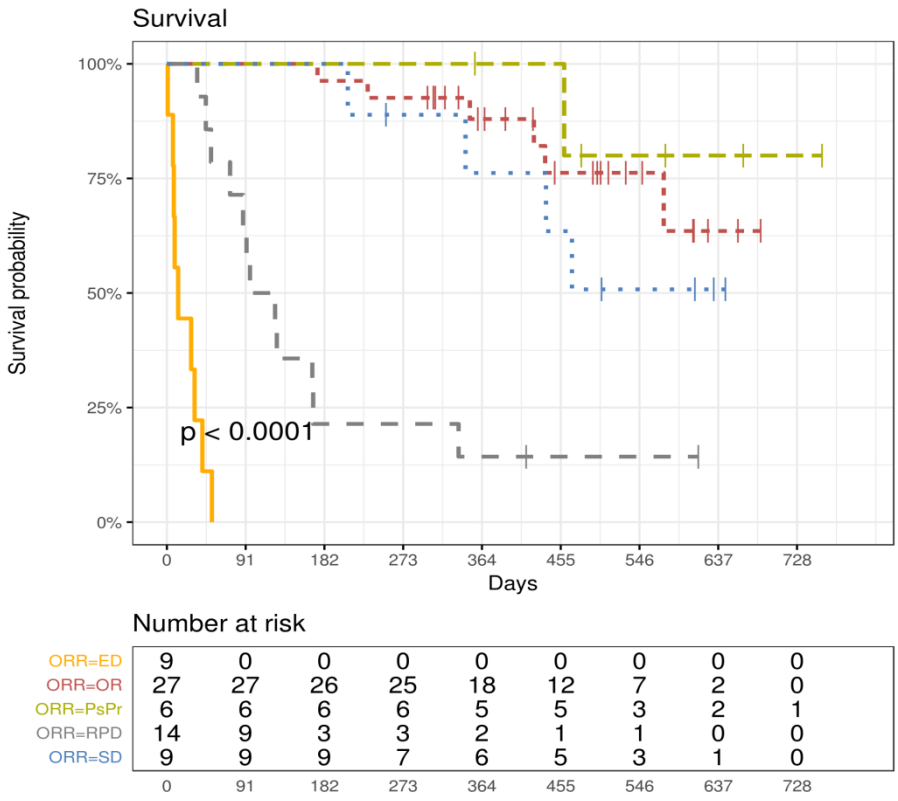

Figure 11: Kaplan-Meier curves show the differences in overall survival in the response groups classified according to RECIST 1.1 criteria after six therapy cycles : The response groups show significantly different survival times.

The overall survival times differed significantly according to treatment response based on the RECIST 1.1 criteria applied after six therapy cycles. 50% of patients with RPD died after 13 weeks of the treatment and only two patients survived after one year (14%) while after one year, 66% of patients with OR were still living. The one-year survival probabilities for patients with PsPr and SD were 83% and 66% respectively.

Based on a cutoff point at Youden Index, patients of cross validation were classified into predicted non-RPD (n=34) and predicted RPD (n=18). The predicted patient groups showed significantly different overall survival times in nine of the 10 CV repetitions and a tendency for a difference ($0.05 < p < 0.1$) in one repetition. Figure 12 shows a typical example. Further, the difference in overall survival between patients with non-RPD and patients with RPD classified according to the Schiwitza score is demonstrated in figure 13.

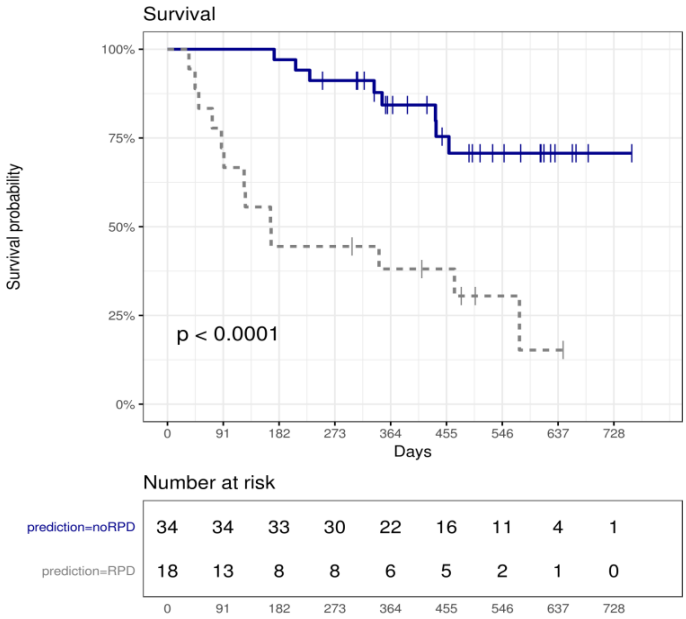

Figure 12: Kaplan-Meier curves show the differences in overall survival between predicted treatment response groups classified during the cross validation. This figure shows the results from one repetition of the CV. Patients with non-RPD according to the classifier showed a significantly higher survival probability than patients with RPD. The results of all repetition were similar across all folds.

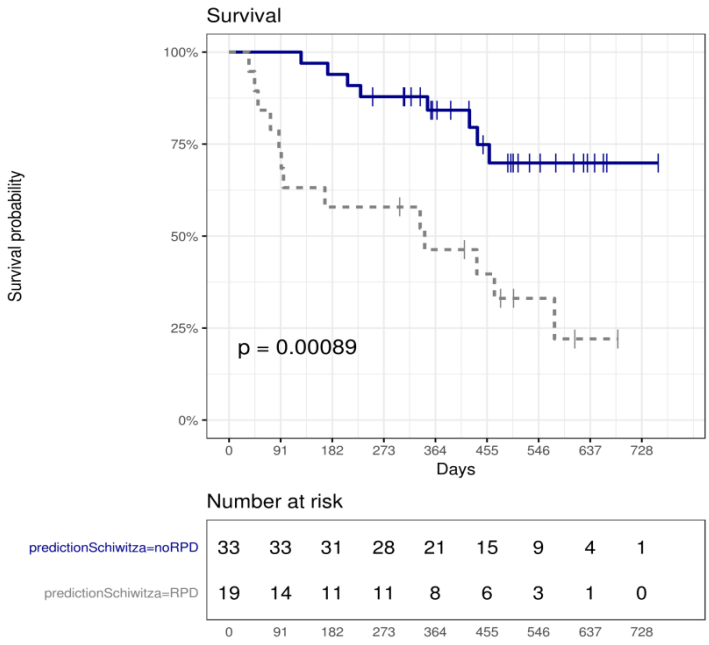

Figure 13: Kaplan-Meier curves show the differences in overall survival between the predicted treatment response groups based on the Schiwitza score.

4 Discussion

In this retrospective analysis, we correlated patients' and disease features to the response to pembrolizumab treatment, and we were able to validate a weighted score of response prediction. The mentioned score has been described earlier in a pretreated population of patients with advanced NSCLC who received treatment with nivolumab (Schiwitza et al. 2019).

There are several published studies investigating the effect of patient characteristics or histological features on patient response to treatment with ICIs in different malignancies. The importance of some factors in the prediction of patients' outcome, like response rate and overall survival, has been reviewed as well. In the following discussion, we are going to compare the results of the analysis of our small dataset with the available published data and we will try to highlight the significance of the investigated factors in our study.

4.1 Influence of patients' characteristics on treatment outcome

Since lung cancer is a disease of the elderly, a key point is to know if an immunosenescence (age-related decline in the immune function) would reduce the efficacy of ICI in elderly patients. Moreover, it is important to know to what extent older patients tolerate this treatment modality, if the incidence of adverse events is higher, and if it consequently results in treatment limitation in this group of patients. In our small dataset, the difference between medians of age in all response groups was not significant (adjusted p=1) and age didn't affect the response rate. The median of age for patients with RPD was the highest among all groups (80 years). Yet, the median for patients with early death (71 years) was not notably higher than the medians for other good response groups, OR and PsPr (69, 70 years). Of 38% of our patients (n=25) aged over 75 years, three died early, eight had RPD and the rest (n=14) (56%) responded well to the treatment. Less than half of patients (n=4) with ED (n=9) and eight patients (56%) with RPD (n=14) were older than 75 years.

In a study level meta-analysis with a total of 5265 patients (ICIs: 2925; controls: 2340) from nine randomized clinical trials (RCTs), (Nishijima et al. 2016) compared the difference in overall survival (OS) and progression-free survival (PFS) between younger and older patients who were treated with ICIs. Subgroups were formed according to ICIs (CTLA-4 mAbs or PD-1 mAbs) and tumor type, mostly melanoma, but patients with advanced NSCLC were also included. No significant difference in improvement of PFS was observed in both younger and

older patients in all subgroups at a cutoff of 65 years (p=0.23). The analysis showed survival benefits in younger (HR, 0.75; 95% CI, 0.68-0.82) and older (HR, 0.73; 95% CI, 0.62-0.87) patients as well, except for a subgroup of patients aged over 75 years and treated with PD-1 antibodies (patients from four trials). The study group contributed the last finding to the heterogeneity of the included nine trials in this analysis and to a possible role of immunosenescence in this subgroup. One of the four trials was CheckMate 017. In this study, patients with previously treated advanced squamous NSCLC underwent a treatment with the PD-1 mAbs (nivolumab) and docetaxel (as control group). The ORR under nivolumab was 20% versus 9% under docetaxel (p=0.008). Overall, survival was significantly higher under nivolumab (9.2 months) versus 6.0 months with docetaxel (95% CI, 5.1 to 7.3) except for a subgroup of patients aged over 75 years. It is important to mention that there was also a small subgroup in which docetaxel patients had a better performance status than the nivolumab group (Brahmer et al. 2015). We should take into consideration that in real clinical practice, older patients with marginal performance status get a therapy attempt with ICI maybe more often than with a conventional chemotherapy. CheckMate 153 is a community-based safety study of nivolumab where 1308 patients were subdivided with an age cutoff of ≥70. In this study, patients aged over 70 (40%) had a similar six months' survival (63%; 95% CI, 58–67%) compared to patients aged under 70 years (63%; 95% CI, 59–67%). Both groups showed a comparable accepted safety profile (Casaluce et al. 2018). In a cohort outside the clinical trial, (Bagley et al. 2017) investigated 175 patients with advanced NCSLC, with (25%) of the patients aged over 75. Elderly patients were not inferior in response rates or overall survival compared to the younger group. Poor outcome was associated with bad performance status regardless of age. ECOG PS ≥ 2 was associated with lower ORR (7.1% vs. 23.3%; OR 0.25, 95% CI 0.07 e 0.88; p¼0.03), inferior PFS (median 1.8 vs. 2.3 months; HR 1.9, 95% CI 1.3 e 2.8; p¼0.001), and inferior OS (median 3.6 vs. 7.8 months; HR 2.6, 95%CI 1.6 e 4.1; p<0.001). In another group of 275 patients with advanced NCSLC, a subgroup (14%) aged over 75 with a median age of 81 years showed no inferiority in response rate or OS (12.7m vs 15.3m; p = 0.92) (Marrone et al. 2018).

Male and female patients have different immune responses to different triggers and show variations among their innate and adaptive immunity (Klein and Flanagan 2016). There is a remarkable prevalence of autoimmune disorders in females (up to 80%). Such a fact reflects the presence of gender-related immunity discrepancies. Factors like sex hormones, X chromosome, environmental factors and lifestyle could contribute to this variability (Moroni et al.

2012). We tried to investigate if such a difference has implications on patient response to the therapy with ICIs. In our data set, there was no significant difference in gender distribution among response groups (p=13). The male dominance in response groups PsPr and RPD (83.3%, 85.7%) is not significant due to a low number of patients and can be explained by the male dominance in our cohort (63%). Conforti and colleagues carried out a meta-analysis on 11,351 patients from 20 clinical trials. Patients with different malignancies, mostly melanoma and NCSLC (3482) (31%), were treated with ICI including pembrolizumab and nivolumab. 7646 (66%) were males and the rest, 3705 (33%), were females. The pooled overall survival HR compared to control groups were 0,72 (95% CI 0,65–0·79), 0,86 (95% CI 0,79–0,93) in male and female patients, respectively (Conforti et al. 2018). This analysis shows that both sexes can benefit from ICI therapy, yet with significance for male patients. In this analysis, the authors did not review factors that may contribute to lower female benefits like high tumor mutational burdens or lower PDL1 expression (Ulrich and Guibert 2018). One of the included trials in the Conforti analysis was the KEYNOTE-024, in which 305 patients with advanced NSCLC were subdivided into two arms: pembrolizumab (154) and platinum-based chemotherapy (151). The subgroup analysis favored male patients (n=187) with a HR of death of 0.54 (0.36 to 0.79) over 0.95 (0.56 to 1.62) for female patients (n=118) (Reck et al. 2016b). Subgroup analyses in the KEYNOTE-024 included patients from both study arms. Part of the female subgroup received a platinum-based chemotherapy. The findings in Wu and colleagues' meta-analysis (n=6096) from 11 trials, CTLA-4 or PD-1 inhibitors (n=3584) versus chemotherapies or other therapies (n=2512), NSCLC (2192), are consistent with the Conforti analysis. A better OS was observed in males (HR, 0.62; 95% CI, 0.53-0.71; p<0.001) treated with ICI versus controls than females (HR, 0.74; 95% CI, 0.65-0.84; p<0.001). In this analysis, sex had more implication for the OS among melanoma patients more than in NSCLC group (Wu et al. 2018). The last and largest meta-analysis was from Grassadonia and colleagues: 21 trials (n=12,635). They found that ICIs reduced the risk of death for all patients, males and females (HR 0.73, p < 0.001 and HR 0.77, p < 0.001, respectively). The results were similar for anti-PD1 and anti-PDL-1 even when the NSCLC subgroup was considered separately (HR 0.73, 95% CI 0.63–0.84, p < 0.001 for men and HR 0.66, 95% CI 0.48–0.91, p = 0.011 for women). In contrast, the anti-CTLA-4 treatment was effective in men (HR 0.77, 95% CI 0.63–0.94, p = 0.012), but not in women (HR 0.89, 95% CI 0.76–1.05, p = 0.162). Anti-CTLA-4A showed benefits in both sexes only when it was restricted to melanoma (HR 0.67, 95% CI 0.50–0.90, p = 0.008 and HR 0.80, 95% CI 0.68–0.94, p = 0.006, respectively) (Grassadonia et al.).

To examine the implication of tobacco consumption on the outcome of ICIs treatment we stratified the data from smoking status to all response groups. The number of smokers was higher in all good response groups, (100%) in SD and PsPr, (92.6%) in OR, than patients with ED (88.9%) or RPD (85.7%). The highest number of current smokers was in PsPr patients (66.7%) followed by OR and SD (55.6% for each). Current smokers were (35.7%) of RPD and least in ED (33.3%). We defined current smoking as ongoing tobacco consumption until time of first diagnosis. In our predictor ever smokers get (2 points) versus (zero points) for never smokers, so smoking is a positive outcome predictor. After all, no conclusion can be drawn from this data as we didn't control the smoking status through the whole therapy period and due to the small data set we have, the data did not show a statistical significance between smokers and non-smokers (p= 0.70).

For the same purpose Bingjia Li and colleagues carried out a meta-analysis on 1981 patients with NSCLC from four phase III clinical trials (ICIs vs. chemotherapy). ICIs were exclusively PD-1/PD-L1 inhibitors: nivolumab (n=826), pembrolizumab (n=305), and atezolizumab (n = 850). Never smokers were (n=298), former or current smokers (1683). Programmed cell death inhibitors increased the OS (HR, 0.69; 95% CI, 0.60–0.78) and PFS (HR, 0.55; 95% CI, 0.43–0.67; p = 0.027) in smoking patients versus chemotherapy and no significant improvement in OS (HR, 0.8; 95% CI, 0.54–1.06; p > 0.05) or PFS (HR, 0.90; 95% CI, 0.11–7.59; p = 0.637) was observed in non-smoking patients with anti-PD-1 immunotherapy compared with chemotherapy. The authors contributed the results to possible gene mutations and molecular differences in smokers (Li et al. 2018). A review of nine clinical trials done by (Norum and Nieder 2018) showed that smoker NSCLC patients have higher PD-L1 TPS scores and better response rates than never smokers. Furthermore, one study revealed higher mutational burdens in smokers. Two of the reviewed studies showed no significant difference. Another meta-analysis (Kim et al. 2017b) included (2389) ever smokers and (413) never smokers with NSCLC treated with ICIs versus chemotherapy. The results are consistent with Bingjia Li analysis; ICIs significantly prolonged OS compared with chemotherapy in ever smokers (HR = 0.70 [95% CI, 0.63–0.79], p < 0.00001) but not for never smokers (HR = 0.79 [95% CI, 0.59–1.06], p = 0.12) and the authors concluded that smoking status may be a positive predictive factor for survival benefits under ICIs.

Desrichard and his study group analyzed tobacco-associated mutational burdens in DNA and RNA sequencing data sets from subjects with lung squamous cell carcinoma LUSC (n=205)

and head and neck squamous cell carcinoma HNSC (n=423). They described an increase in smoking-induced mutational burdens in the tumor microenvironment of both groups. The authors found that these mutations have direct immunosuppressive effects including lower levels of immune infiltration, cytolytic activity, and interferon-γ pathway signaling in the HNSC group, while the effect was completely the opposite with an increase of the inflammatory tone in the tumor microenvironment in the LUSC group (Desrichard et al. 2018). In this study there was no data about T cell load or PD-L1 expression in the tumor microenvironment in the specimens of the included subjects.

4.2 Importance of tumor histological features and mutational analysis

In our small cohort there were no preferences among response rate depending on the main histological type. About 65% of patients with squamous histology (n=13/20) and 64% of adenocarcinoma patients (n=27/42) responded well to the therapy. To the best of our knowledge there is no published clinical data that favors one of the NSCLC histological subtypes either in response rate or OS under treatment with ICI. In the pivotal trial (Checkmate 024) patients with squamous (18.8%) and non-squamous NSCLC (81.8%) benefitted from pembrolizumab treatment. The HR of disease progression or death in patients with squamous NSCLC under ICI versus chemotherapy 0.35 (95% CI, 0.17-0.71) was comparable to the adenocarcinoma subgroup 0.55 (95% CI 0.39 0.76) (Reck et al. 2016a). A similar result was also observed in the outcome of adding pembrolizumab to chemotherapy in both squamous and nonsquamous NSCLC. In the (KEYNOTE-189) study, a total of 616 patients with nonsquamous NSCLC were randomly assigned to the pembrolizumab/pemetrexed/carboplatin combination group (n= 410) and the placebo/same chemotherapy combination group (n=206). The median PFS was 8.8 months (95% CI, 7.6 to 9.2) in the pembrolizumab-combination group versus 4.9 months (95% CI, 4.7 to 5.5) in the placebo-combination group (hazard ratio for disease progression or death, 0.52 (95% CI, 0.43 to 0.64; p<0.001) (Gandhi et al. 2018). In another phase III clinical trial, a total of 559 patients with squamous NSCLC were randomized in a 1:1 ratio to pembrolizumab/paclitaxel/carboplatin and placebo/ same chemotherapy study arms. The median PFS was 6.4 months (95% CI, 6.2 to 8.3) in the pembrolizumab-combination group and 4.8 months (95% CI, 4.3 to 5.7) in the placebo-combination group (hazard ratio for disease progression or death, 0.56 (95% CI, 0.45 to 0.70; p<0.001) (Paz-Ares et al. 2018).

We hypothesized that the response rate correlates proportionally to the PD-L1 expression rate. In our data set, the medians of PD-L1 expression on tumor cells ranged between 70% and 90%. The same applies for PD-L1 expression on immune cells. The medians PD-L1 IC were between 18% and 28% except for PsPr (<1%). Due to the low patient numbers in each of the subgroups we could not observe a significant difference between the PD-L1 medians among all response groups (adjusted p=1). The effect of PD-L1 expression on patients' outcomes has been reviewed in many studies. The effect of TPS on patients' OS was very well demonstrated in the phase III clinical trial (KEYNOTE-042, published in April 2019). The median OS in patients with NSCLC (n=1274) treated with pembrolizumab monotherapy (n=637) versus platinum-based chemotherapy (n=637) with TPS ≥50% , ≥20 and ≥1%, were 20·0 months (95% CI 15·4–24·9) for pembrolizumab versus 12·2 months (10·4–14·2) for chemotherapy, 17·7 months (15·3–22·1) versus 13·0 months (11·6–15·3), and 16·7 months (13·9–19·7) versus 12·1 months (11·3–13·3), respectively (Mok et al. 2019). A retrospective data analysis of 112 patients with NSCLC under pembrolizumab treatment showed that patients with higher TPS experience better response rates, PFS and OS. The patient cohort was homogenous, i.e., no significant differences in age, sex, histology, smoking status or oncogenic mutations. The cohort was divided to two subgroups: patients with PD-L1 TPS of 50-74% (N = 44), and TPS of 75-100% (N = 68). Patients with TPS 75-100% had a significantly higher ORR (47.1% vs 13.6%, P < 0.01), significantly longer median PFS (5.1 months [95% CI: 1.8-4.5] vs 2.5 months [95% CI: 3.8-7.4], p = 0.02), and higher 12-month OS (76.4% vs 54.4%) compared to patients with TPS 50%-74% (Jimenez Aguilar et al. 2018). Corresponding results were also observed in many others trials under different ICI; a better response rate was demonstrated in patients with higher TPS in the treatment with nivolumab plus ipilimumab as the first line for advanced NSCLC (Hellmann et al. 2017) and under atezolizumab versus docetaxel in previously treated NSCLC (OAK trial) (Rittmeyer et al. 2017). The effect of the PD-L1 expression on immune cells was not always in the focus of many clinical trials. However, the increase of PD-L1IC showed ORR benefits in the OAK (Rittmeyer et al. 2017) and the phase 2 POPLAR trial for atezolizumab (Fehrenbacher et al. 2016).

The generated score from our small data set showed, according to the two-stage ROC analysis, a negative effect of the PD-L1 expression on the ORR. This heterogenic statistical finding is explained with the slightly elevated TPS in patients with ED (90%) and RPD (80%) and with the small patient numbers in all subgroups. This finding has shown no statistical significance

and was ignored as we validated the Schiwitza score on our cohort. Particularly, it was inconsistent with the available published data from different, large clinical trials.

KRAS mutation is the most common, known oncogenic alteration in NSCLC and occurs in 20%-40% of lung adenocarcinoma with a prevalence in Western vs Asian population (26% vs. 11%) and in smokers vs never smokers (30% vs. 10%). Some preclinical and clinical trials suggest that KRAS mutation may have implications on patient outcomes under ICI treatment, especially when it coexists with other oncogene mutations like TP53 (Adderley et al. 2019). We tested all patients with nonsquamous and one patient with squamous histology (n=46) for KRAS, TP53 and MET mutations. Nineteen patients (41%) proved to have at least one KRAS or TP53 mutation, and six patients (13%) had MET mutation. KRAS and TP53 mutations coexisted in nine patients: four OR, three ED, one RPD and one stable disease. For patients with OR mutated KRAS, the wild-type phenotype ratio was 1:1.

Ten patients with mTP53 responded well to the treatment versus nine patients with ED or RPD. In our data set there was no remarkable effect of oncogenic mutations on response rate. We compared our results to the available clinical data. A retrospective analysis of 282 patients with NSCLC (all histological subgroups) treated with 13 different ICIs (about 88% of the patients treated with nivolumab), in different treatment lines (about 53% 2nd line, 24% 1st line and 24% 3rd line), including 162 (57.4%) with KRAS mutation, 27 (9.6%) with other mutations, and 93 (33%) with a wild-type phenotype. KRAS mutation did not show any predictive efficacy in ORR, OS or PFS (Jeanson et al. 2019). In another study, 138 patients with a KRAS mutant NSCLC and 371 with a KRAS wild-type tumor from three clinical studies were included in a meta-analysis. All patients were treated with atezolizumab in the second or third line. Compared to chemotherapy with docetaxel, atezolizumab improved OS in patients with KRAS mutant NSCLC (hazard ratio = 0.64 [95% confidence interval, 0.43-0.96], p = 0.03). For patients with KRAS wild-type NSCLC, atezolizumab did not improve the OS over that with docetaxel (hazard ratio = 0.88 [95% confidence interval, 0.68-1.13], p = 0.30) (Kim et al. 2017a). The inconsistency between the available clinical data about the predictive role of oncogenic mutations under ICI treatment may be due to the presence of many other influencing factors in the study groups that contribute to the prediction of patients' outcomes. The predictive efficacy of mutant oncogene under ICI treatment stays controversial.

4.3 Efficacy of serum biomarkers as predictors of treatment outcome

C- reactive protein is an important tumor marker with a great prognostic value. A retrospective analysis for data of about 5000 patients with different solid tumors demonstrated that high CPR was associated with a statistically and clinically higher risk of death (Shrotriya et al. 2018). Despite the fact that CRP is a nonspecific inflammatory marker, it certainly proved to have an important prognostic value in solid tumors in several clinical trials (Shrotriya et al. 2015). A meta-analysis of eight clinical studies exclusively included 1668 NSCLC patients, and clarified that CRP is an independent prognostic factor for patients with NSCLC and that elevated CRP levels may predict a poor five-year overall survival (Jing et al. 2015). A retrospective data analysis of 124 NSCLC patients treated with nivolumab concluded that the ORR in patients with elevated CRP levels was significantly worse than those with normal CRP levels (cutoff= 1 mg/dl) (8.3 vs 23.4%, p = 0.0180). The same study demonstrated that elevated LDH levels were significantly associated with a shorter PFS and OS as well (Oya et al. 2017). Lactate dehydrogenase is an essential serological tumor marker as it correlates with tumor burdens (Buder-Bakhaya and Hassel 2018). LDH was included in the American joint committee on cancer as a staging criterion for melanoma and is still considered as an important survival predictor in melanoma patients (Gershenwald et al. 2017). In advanced NSCLC a clinical data analysis of a total of 394 patients suggested that the LDH level at baseline (time of patient's presentation) correlates proportionally with the extent of whole body metastases and is a modest, independent predictor of OS in stage IV NSCLC (Lee et al. 2016). In our data analysis, the medians of baseline CRP serum level were significantly higher in patients with ED (136 mg/L) and RPD (65 mg/L) versus (7.5, 5.7, 4.2 mg/L) in patients with OR, PsPr and SD respectively, (p= < 0.01). A baseline CRP value (>29.2 mg/L) was a negative predictor in our score according to the trained ROC analysis. A decline in the CRP level at day 43 for more than 10% associated with a better response versus a negative prediction value when the decline was less than 7%. The median LDH at baseline was significantly higher in ED (325u/l) and RPD (224u/l) compared to OR (190u/l), PsPr (183u/l) as well (p= < 0.01). A baseline LDH (>195u/l) or a constant increased LDH at day 43 were negative predictors. A decline of the LDH >10% at day 43 was associated with a better response. The prognostic importance of G/L index in solid tumors and NSCLC has been clarified in several clinical studies. In a clinical data analysis of 138 NSCLC patients at G/L index cutoff of (<3.24 or ≥3.24), the calculated median overall survival was 37.0 (95% CI 17.5-56.5) months in the group with a low G/L index versus 10.0

(95%CI 5.0-15.0) months in the group with a high NLR ($p<0.0001$) (Kos et al. 2015). In our data set a remarkable, elevated G/L index at baseline was noticed in ED (7.1) versus all other response groups (2.1-4.7), ($p=0.17$). Despite the statistical insignificance at baseline, a significant difference was noticed during the treatment, particularly at day 106 where the median of G/L index values in the RPD group was about 100% more than the baseline value, while the medians of all good response groups declined beyond the baseline line. A decline in the G/L index of more than 10% was associated with a better response in the ROC analysis.

To demonstrate the significance of these serum biomarkers we tested the previously mentioned "Schiwitza score", which combined all the three serum biomarkers, on our cohort. The score showed a prediction sensitivity of almost 88% with a specificity of about 64%. The score one-year survival prediction showed a statistical significance ($p= 0.00089$) in survival probability between the predicted groups. The one-year survival probability of predicted non-RPD was 64% compared to 69% in reality. The predicted survival probability in predicted RPD was 42% versus 14% in reality. The discrepancy in one-year survival between predicted RPD (42%) and real RPD (14%) is due to the score's low specificity (63%) as five patients were false negatively predicted as RPD.

4.4 Molecular escape mechanisms under ICI

Patients respond differently to treatment with immune checkpoint inhibitors. They differ in the initial response to the treatment, some responding well from the beginning (responders), others failing to show any initial response (innate resistant) and some developing resistance after a temporal response (acquired resistance). Some patients show discrepancy in the response between two targeted lesions of the same disease (Jenkins et al. 2018). There are many extrinsic and intrinsic factors contributing to such diversity. Tumors could be classified into hot and cold tumors depending on the degree of antigen presentation by immune cells that infiltrate the tumor microenvironment. This has a crucial impact on a patient's response to ICI (Ramos et al. 2017). A primary innate resistance to ICI may result from extrinsic factors like insufficient tumor-specific antigen presentation, poor tumor immunogenicity or inadequate maturation of antigen-presenting cells, and inadequate T cells priming, or due to increased release of inhibitory cytokines in a tumor microenvironment (O'Donnell et al. 2016; Gide et al. 2018). Other intrinsic factors involve genetic alteration and intrinsic pathway modulation that affect specific antigen presentation or interaction with immune cells in a tumor microenviron-

ment. For example, a mitogen-activated protein kinase (MAPK) signaling pathway results in an increase of VEGF and IL-8 secretion and their down-regulatory effect on a T cell. Another important intrinsic resistance mechanism is the mutation of Janus kinase (JAK1/2) which is essential to the gamma interferon signaling pathways. This results in a decrease of IFN γ capacity in MHC up regulation and the recruitment of T cells (Sharma et al. 2017). Genetic mutations may also result in low specific antigens presentation, increased expression of PD-L1-like molecules (Jenkins et al. 2018). Sade Feldman and colleagues observed mutations in beta-2-microglobulin (B2M), an essential component of MHC class I antigen presentation in 29.4% of patients with progressive melanoma treated by checkpoints inhibitors (Sade-Feldman et al. 2017).

4.5 Pseudoprogression

This phenomenon has been observed in tumors under targeted therapies including immunotherapies and ICI. It describes an initial increase in tumor mass size due to inflammatory cell infiltration and edema associated with the desired immune response, which eventually resolve and uncover a responsive tumor mass. This early false finding as a specific response pattern under targeted therapies led to modifications in tumor response evaluation criteria under conventional therapy such as RECIST (Response Evaluation Criteria in Solid Tumors) (Chiou and Burotto 2015). Pseudoprogression is the increase of tumor burden or formation of new lesions after 12 weeks of treatment. In this case a radiological assessment is not enough to confirm a progressive disease depending on Immune-related Response Criteria (irRC) (Ozaki et al. 2017). A new histological examination is gold standard to confirm the diagnosis (Ranjan et al. 2018). In most cases a delayed good response (decrease of tumor size) that can be observed in later radiological assessments is enough to confirm the diagnosis. The patient clinical status during the treatment should be taken in consideration as well.

4.6 Limitations

This study has several limitations. First, it is a monocentric retrospective analysis which offers an inferior level of evidence compared to prospective studies. This limitation involves the retrospective evaluation of patients' response to the treatment with pembrolizumab and the subjective assessment by physicians concerning the time of therapy termination due to disease progression. Another study limitation is the small number of the included subjects, particularly, in patients with pseudoprogression; however, pseudoprogression is a relatively uncommon phenomenon to observe under treatment with ICIs. Finally, our study included only patients with previously untreated NSCLC who received a first-line treatment with the ICI pembrolizumab. Prospective larger cohort studies on patients with different ICI agents and subsequent therapy lines are required to confirm our results and to validate our proposed score to predict treatment response.

4.7 Conclusion

In a retrospective analysis, we studied the relationship between treatment response to a first-line therapy with pembrolizumab and several clinical factors like age, gender, smoking status and tumor histology features in patients with advanced NSCLC. Further, we were able to validate a previously suggested score to predict treatment response to ICIs after only three therapy cycles by combining laboratory parameters (LDH, CRP and the GL-index) and tumor size derived from CT-imaging. Furthermore, we studied the capacity of the suggested score in predicting the overall survival.

As there is no indisputable single clinical variable or biomarker to predict patient response to the treatment with ICIs, a weighted score entails the routinely collected laboratory parameters and tumor size might be a helpful tool in early predicting the long-term response to a treatment with pembrolizumab, especially, as during treatment with ICIs a so-called pseudoprogression is hard to distinguish from a real progressive disease.

In patients with advanced NSCLC, a score combining the routinely collected clinical variables might enable an early prediction of treatment response to ICIs. A prospective validation of our results in larger cohorts is necessary before firm conclusions affecting patient care can be drawn.

5 Abstract

Lung cancer is one of the most frequently occurring malignancies at the present time and still the leading cause of cancer deaths worldwide. Checkpoint pathway inhibitors are a family of cancer immunotherapy that has been shown to improve disease progression-free survival as well as overall survival in patients with advanced NSCLC.

In this retrospective study, we analyzed data of 66 consecutive patients with metastatic NSCLC who received pembrolizumab as a first-line monotherapy. We attempted to validate and improve the reliability of a previously described score of treatment response prediction. The score entails various laboratory values (LDH, CRP and granulocyte/lymphocyte ratio) as well as the change in tumor size derived by CT imaging. Further, we investigated other clinical, radiological and pathological characteristics and correlated them to patients' response to the treatment with pembrolizumab.

Aiming to examine the possibility of the prediction of patients' response, we applied the prediction score to our cohort of patients after three cycles of the treatment, and as a result, we classified them according to the score into four predicted response groups: stable disease, objective response, pseudoprogressive and real progressive disease. We examined the reliability of this prediction comparing the score results to patients' real response, which was defined based on the RECIST 1.1 criteria. The prediction score showed a prediction sensitivity of almost 88% with a specificity of about 64%. Sensitivity was defined as the ability of the predictor to sort out patients who are going to benefit from the treatment, i.e., non RPD (= OR, PsPr and SD), from patients with RPD. Furthermore, we validated the score by testing the possibility to predict patients' survival probability, the predicted one-year survival showed a statistical significance ($p= 0.00089$) in survival probability between the predicted groups. The one-year survival probability of predicted non-RPD was 64% compared to 69% in reality.

As there is no indisputable test to predict patient response to treatment with immune check point inhibitors (ICIs), a score could be a helpful tool in monitoring patients under treatment with ICIs, especially, as during treatment with ICIs a so-called pseudoprogression is hard to distinguish from a real progressive disease. A prospective validation of the concluded results and the score of prediction in a large cohort of patients would give a more reliable and applicable results.

6 Appendix

Table A1: Absolute values of C - reactive protein (mg/L)

Patient's Number	**Day 1**	**Day 22**	**Day 43**	**64**	**85**	**106**
1	10.7	56.3	13.4	3.8	18.5	4
2	137	124	189	116		
3	85.2	90.2	104.9			
4	36.9	33.9	33.1	43.7	55.6	66.3
5	196.8	84	40.6	207	65.2	178.3
6	5.4	2.9	8.4	5.4	15.3	4.3
7	61	4.3	3.4	4.4	3.6	3.3
8	3.2	3.7	3.5	4	14.3	3.6
9	11.9	23.2				
10	3.1	3.6	3.4	3.4	3.3	3.4
11	159	178	189			
12	3.9	3.9	17.4	3.5	0.3	4.5
13	118	3.3	3.3	16.1		
14	3.3	3.3	3.4	3.3	9.6	16.1
15	7.9	8.3	15.8	10.5	3.3	15.4
16	3.2	3.6				
17	5.9	4.8	4.8	4.4	4	2.7
18	98	53.7	55.9	39.7	36.4	49.7
19	31	44.4	69.2	30.6		
20	6	9.8	17.9	11.6	7.3	13.6
21	3.6	3.2	3	3.2	3.2	3.6

Patient's Number	Day 1	Day 22	Day 43	64	85	106
22	11.6	14.7	55.4	35.7	7	3.8
23	3.3	6.9	3.1	3.8	60.8	35.6
24	98.1	133.5				
25	26	38	10.2	8.6	18.7	34.3
26	4.8	3.3	3.7	3.6	3.6	3.5
27	196	138	111	73.9	51.1	57.8
28	3.3	3	3.2	3.3	11.4	14.4
29	5.4	15.7	9.1	6.8	4.2	3.5
30	15.8	41.3	43.3	78.8	60.5	51.7
31	154.5					
32	3.8	24.7	25.2	29.7	15.7	21.3
33	70.9	31.6	90.4	32.5	9	3.8
34	3.3	3.3	1.4			19
35	4.2	16.9	9.1	4.6		32
36	3.8	4.3	4.7	3.9	3.1	2.8
37	42	198.5	267.8			
38	19.6	3.9	3.8	3.8	5.8	8.7
39	3.8	4.1	6.1	79	63.8	54.3
40	4.6	4.1	4.5	14.1	14.6	16.8
41	5.6	3.7	9.9	3.7	4.1	16.1
42	39.2	57.9				
43	19.1	15.7	17.3	48.8	5.3	3.7
44	89	208.9	3.6	3.9	2.2	3.4

Patient's Number	Day 1	Day 22	Day 43	64	85	106
45	31.5	14	4.1	4.7	2.7	8.5
46	59.3	114.6	38.9	10.7	3.7	4.4
47	4.3	3.7	2.3	2.4	3.5	3.7
48	63.2	21	48.9	199.3	152.5	28.4
49	47.2	77.3	83.9	88.4	21.6	13.8
50						
51	105.1	186.1	117.5	127.2		
52	67.7	30.5	32.5	31.3	138.2	
53		164				
54	190					
55	173					
56						
57	62	61.3	6.2	9.4	26.6	11.3
58	79.4	4	4.5			6.7
59	23.4		71.8			22.1
60	148.1		110.5			4.2
61	15.5		90.2			4.4
62	7		5.7			7.8
63	4.5		4.5			4.7
64	45.8		46			4.2
65	29.2		16.9			197.5
66						

Table A2: Absolute values of lactate dehydrogenase (U/L)

Patient's number	**Day 1**	**Day 22**	**Day 43**	**Day 64**	**Day 85**	**Day 106**
1	185		192	191	185	184
2	142	290	372	272		
3	168	145	183	143		
4	149	159	157	149	186	153
5	239	190	217	195	184	317
6	179	179	193	193	207	207
7	165		185	168	165	161
8	282	257	296	261	296	301
9	278	411				
10	226	181	218	267	157	218
11	271	209	308			
12	289	211	232	210		
13	181					
14	134	223	186	184	186	174
15	246	278	239	232	245	273
16	652	298				
17	202	188	167	171	172	173
18	310	311	325	303	301	285
19	246	250	242	233	225	223
20	204	213	196	202	213	225
21	280	260	226	236	265	251
22	191	181	231	242	208	181

Patient's number	Day 1	Day 22	Day 43	Day 64	Day 85	Day 106
23	277	237	176	154	156	181
24	295					
25	257	336	271	272	246	264
26	151	144	130	120	131	131
27	523	594	689	466	368	323
28	177	168	173	168	178	171
29	176	160	169	200	172	195
30	171	177	174	168	179	186
31	325					
32	152	158	196	215	206	190
33	268	227	175	232	351	329
34	213	177	190			201
35	146	150	152	166		
36	203	195	203	197	189	246
37	223	477	1074			
38	324	388	271	292	245	277
39	183	186	184	205	187	182
40	176	179	165	174	180	234
41	171	167	165	172	143	169
42	695					
43	357	384	357	319	437	447
44	182	209	192	175	150	169
45	275	274	239	190	180	220

Patient's number	Day 1	Day 22	Day 43	Day 64	Day 85	Day 106
46	182	185	193	195	164	188
47	164	150	155	174	163	167
48	226	245	240	205	709	660
49	164	175	200	215	176	189
50						
51	155	274	631			
52	278	258	260	280	277	
53		346				
54	344					
55		271				
56						
57	99	108	108	118	137	120
58	104		113			4.2
59	184		153			166
60	288		149			155
61	212		202			156
62	188		187			201
63	241		221			200
64	230		275			266
65	283		292			399
66						

Table A3: Absolute values of granulocytes/ lymphocytes index

Patient's number	**Day 1**	**Day 22**	**Day 43**	**Day 64**	**Day 85**	**Day 106**
1	1.2096	2.4057	2.6818	1.3422	1.625	1.3059
2	11.879	139.906	12	171.339	93.384	
3	5.7692	7.4732	9.60975	6.7786	8.3252	
4	7.212121	7.814814	8.64367	8.35632	10.2876	10.21176
5	4.05673	3.6242	2.5079	5.21259	79.23	52.13
6	2.4647	2.2528	2.640522	2.1265	2.69753	3.1265
7	12.5327	3.5037	2.7723	3.0731	5.3603	2.2953
8	3.5126	4.6071	4.8085	4.7731	4.75	4.2277
9	4.0657	7.9573	3.781	2.994	3.7214	3.564
10	3.9506	5.5896	3.8333	3.1978	2.9948	3.7214
11	9.4188	20.8425	24.93			
12	5.3873	4.3627	5.4444	23.9516	3.7862	4.4607
13						
14	5.4898	2.2662	19	2.5026	1.7012	2.9025
15	3.3068	1.4866	3.8346	2.7716	3.1219	3.4973
16	4.3522	5.532				
17	2.8292	2.7622	23	2.1169	1.6348	1.482
18	13.1772	6.4418	9.3	10.3578	7.5648	6.8016
19	4	7.5806	3			
20	1.6796	1.6299	2	2.4201	2.0434	2.5669
21	3.7421	3.9075	3	3.4629	3.5873	3.8141

22	4.4347	8.5037	9.7	4.0397	3.8705	8.3984
23	8.031	5.8429	2.2527	2.1726	32.204	8.0078
24	3.914	5.006				
25	4.673	5.9354	3.0588	4.017	3.3458	4.017
26	2.6373	2.6272	1.6262	1.9509	1.4272	1.275
27	11.6956	4.0666	3.0685	1.5015	1.875	1.8965
28	3.7279	5.5664	2.4055	2.5449	3.3065	2.9602
29	0.5957	0.7949	1.0402	0.8153	0.7967	0.6157
30	11.6046	8.5641	6.2021	6.5402	16.75	9.1803
31	6.691	13.942				
32	5.4	3.7718	2.7486	2.9052	2.6859	3.0042
33	6.3115	5.5208	4.2421	2.8321	4.7142	3.6518
34	1.816	2.148	1.99			1.592
35	2.357	2.145	2.9067			
36	4.881	4.416	2.674	4.294	2.589	2.328
37	53.516	14.644	16.913			
38	7.077	4.879	3.637	6.438	3.623	5.034
39	5.228	5.736	9.091	8.078	8.693	10.229
40	3.485	2.062	1.764	2.132	2.456	2.68
41	3.502	6.3	4.22	4.177	2.043	3.367
42	59.7719					
43	1.683	1.385	1.637	2.748	3.039	1.146
44	3.755	19.189	7.276	3.484	5.506	1.928
45	5.759	5.841	8.341	1.952	3.34	1.777

46	6.33	6.406	21.541	12.467	4.117	3.666
47	3,296	4.34	3.835	4.685	4.415	5.431
48	2.317	5.585	2.418	13.779	5.915	5.303
49	9.827	8.6	11	7.878	8.678	4.88
50	6.84					
51	2.623	3.418	6.32	6.308	2.973	4.891
52	2.375	3.397	2.533	2.458	2.5	6.026
53	9.31	7.828				
54	6.17	5.81				
55	8.933	23.56				
56	7.35					
57	13.52		0.925			5.1
58	2.626		1.815			2.585
59	2.482		1.62			2.223
60	8.807		6.091			2.843
61	2.788		2.86			
62	2.343		1.408			0.326
63	3.674		3			2.889
64	8.187		4.771			4.023
65						
66						

Table A4: Absolute values of tumor size in millimeters

Patient's number	**Baseline**	**Day 43**	**Day 106**	**Day 190**
34	46	68	33	22
36	18	12	11	
57	66	35	23	
6	25	12	10	8
47	31	11	6	3
60	87	33	15	
38	95	42	30	22
12	57	40	25	21
14	124	82	65	57
22	70	51	42	39
23	57	32	26	
26	65	26	18	17
28	22	10	6	
41	61	38	25	24
45	75	31	29	41
44	47	13	8	
7	23	18	10	8
15	86	72	31	22
62	57	45	39	
17	82	66	34	
43	89	76	42	40
58	67	47	29	

Patient's number	Baseline	Day 43	Day 106	Day 190
32	89	67	62	61
30	60	24	19	18
61	44	25	17	
63	44	0	0	
64	104	105	61	
40	36	37	48	25
29	27	42	18	13
59	80	109	89	
46	74	90	69	50
20	41	50	51	31
2	168	206		
51	60	117		
4	65	89	101	
5	88	156		
11	45	69		
48	19	33		
1	71	62	100	
3	86	131		
37	46	70		
49	79	111		
52	108	150		
65				
8	55	27	60	62

Patient's number	Baseline	Day 43	Day 106	Day 190
10	38	29	31	
18	68	66	69	67
21	95	92	93	
25	49	36	42	
39	40	41	41	40
27	112	115	108	109
33	138	137		
35	52	48		
66	38	13	7	

Table A5: Survival in days and response

Patient's number	survival in days	Patient's response
40	459	PsPr
59	356	PsPr
46	479	PsPr
20	576	PsPr
29	757	PsPr
34	666	PsPr
1	337	RPD
2	92	RPD
3	73	RPD
24	125	RPD
37	45	RPD
65	168	RPD

Patient's number	**survival in days**	**Patient's response**
48	96	RPD
49	415	RPD
11	35	RPD
19	614	RPD
51	88	RPD
52	51	RPD
4	169	RPD
5	127	RPD
6	625	OR
7	660	OR
47	423	OR
60	337	OR
38	492	OR
12	549	OR
61	308	OR
14	530	OR
36	232	OR
15	574	OR
57	391	OR
62	321	OR
17	350	OR
63	310	OR
22	686	OR

Patient's number	**survival in days**	**Patient's response**
23	174	OR
64	301	OR
26	510	OR
28	608	OR
41	497	OR
30	501	OR
43	437	OR
32	424	OR
45	367	OR
58	359	OR
44	448	OR
66	609	OR
33	645	SD
25	209	SD
27	345	SD
8	610	SD
10	253	SD
39	502	SD
18	468	SD
21	632	SD
35	438	SD

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