Diplomarbeit

HUMAN VS. MACHINE: PD-L1 IMMUNOHISTOCHEMISTRY EVALUATION IN NON-SMALL CELL LUNG CARCINOMA AND PLEURAL MESOTHELIOMA

eingereicht von

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zur Erlangung des akademischen Grades

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Diagnostik- & Forschungsinstitut für Pathologie

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Graz, am 15.09.2021

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Danksagung

An dieser Stelle möchte ich mich besonders bei meinem Betreuer Univ. FA Priv.-Doz. Dr.med.univ. Dr.rer.nat. Luka Brcic bedanken, der mich während der gesamten Zeit kompetent unterstützt hat und immer erreichbar war.

Außerdem möchte ich mich bei Dipl-Ing. BSc Markus Plass bedanken, der mir während des Arbeitsprozesses tatkräftig zur Seite stand und mir bei technischen/statistischen Fragestellungen stets behilflich war.

Des Weiteren möchte ich mich ganz herzlich bei meiner Freundin Elisabeth bedanken, die mir auch in stressigen Phasen des Studiums immer zur Seite stand und stets Rücksicht auf mich genommen hat.

Zum Abschluss möchte ich mich noch bei allen Freunden und Familienmitgliedern bedanken, die mich mein ganzes Studium lang begleitet haben und mich immer unterstützt haben.

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Abbreviations

AC	Adenocarcinoma
AI	Artificial intelligence
ALK	Anaplastic lymphoma kinase
ASC	Adenosquamous carcinoma
BAP1	BRCA associated protein 1
CDKN2A	Cyclin-dependent kinase inhibitor 2A
EGFR	Epidermal growth factor receptor
FISH	Fluorescence in-situ hybridization
IHC	Immunohistochemistry
IVD	In vitro diagnostic
LCC	Large cell carcinoma
LCNEC	Large cell neuroendocrine carcinoma
ML	Machine learning
NSCLC	Non-small cell lung carcinoma
PD-1	Programmed death-1
PD-L1	Programmed death ligand 1
ROI	Region of interest
SCC	Squamous cell carcinoma
SCLC	Small cell lung carcinoma
SIADH	Syndrome of inappropriate antidiuretic hormone secretion
TNM	Tumor-nodes-metastases
TTF-1	Thyroid transcription factor 1

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Zusammenfassung

Einleitung: Digitalisierung, künstliche Intelligenz (AI) und maschinelles Lernen (ML) werden zunehmend als Unterstützung in der Diagnostik von Patholog*innen verwendet. Diese Technologien zielen darauf ab, schnell objektivere und genauere Ergebnisse als Menschen zu liefern. In diversen Richtungen der Pathologie werden Technologien wie AI/ML bereits zur Unterstützung der alltäglichen Arbeit verwendet und zeigen vielversprechende Ergebnisse. Die Auswertung von PD-L1 in Lungentumoren ist derzeit noch sehr subjektiv und ist von Patholog*in zu Patholog*in unterschiedlich. Dennoch ist die immunhistochemische Auswertung essentiell, weshalb das Ziel dieser Studie ist, die menschliche PD-L1 Auswertung mit jener von AI zu vergleichen.

Methoden: In dieser retrospektiven Studie wurden 51 Proben mit NSCLC, die 2020 am Institut für Diagnostik und Forschung der Pathologie an der Medizinischen Universität Graz diagnostiziert wurden, und 24 Proben mit pleuralen Mesotheliomen, die zwischen 2018 und 2020 diagnostiziert wurden, eingeschlossen. Alle 75 Proben wurden auf drei Gruppen aufgeteilt: kleine Biospieproben (NSCLC), resezierte Tumorproben (NSCLC) und Mesotheliome. Für die automatisierte Analyse wurde das Bildanalyseprogramm uPath PD-L1 (SP263) von Ventana (Roche) verwendet. Die Resultate wurden anschließend mit den Ergebnissen eines erfahrenen Thoraxpathologen und eines Studenten verglichen, deren Auswertung unter dem Mikroskop erfolgte.

Ergebnisse: Die Übereinstimmung der Ergebnisse, die die Software bzw. der Pathologe/Student erzielten, fiel in den einzelnen Gruppen unterschiedlich aus. In der Gruppe mit den kleinen Biopsieproben ergab die Berechnung des Cohen's kappa eine geringe Übereinstimmung. In den beiden anderen Gruppen waren Übereinstimmungen kaum feststellbar.

Fazit: In der Studie war die Übereinstimmung zwischen der maschinellen bzw. der menschlichen Auswertung der NSCLC und Mesotheliome nicht so hoch wie erwartet. Dennoch weist diese Studie einige Limitationen auf und es müssen weitere Studien durchgeführt werden, um unsere vorläufigen Ergebnisse zu verifizieren oder falsifizieren.

Abstract

Introduction: Digitalization, the use of artificial intelligence (AI), and machine learning (ML) are increasingly used to support pathologists in diagnostic procedures. Such technologies aim to achieve faster, more objective, and more accurate results compared to human evaluation. In many areas of pathology, AI/ML is already used as a supporting tool in routine work and provides promising results. PD-L1 scoring in lung carcinomas is very subjective and varies among pathologists. However, it is very important for the decision about immunotherapy. This study aimed to compare the PD-L1 scoring of lung carcinomas and mesotheliomas between AI and humans.

Methods: In this retrospective study, 51 samples of NSCLC, diagnosed in 2020 at the Diagnostic and Research Institute of Pathology at the Medical University of Graz, and 24 samples of pleural mesothelioma, diagnosed between 2018 and 2020, were included. All 75 samples were divided into three groups: small biopsy samples (NSCLC), resected tumors (NSCLC), and mesothelioma samples. uPath PD-L1 (SP263) image analysis by Ventana (Roche) was used for automated scoring. Those results were then compared to the evaluated scores of an experienced thoracic pathologist and a student who analyzed the samples under the microscope.

Results: Concordance between the AI scores and the pathologist/student scores varied between the three groups. In the small biopsy group, the calculated Cohen's kappa score showed a slight agreement. The results for both resected samples and mesothelioma samples were classified as poor agreement.

Conclusion: This study shows that the concordance between AI scoring and human scoring among NSCLC and mesotheliomas is not as high as expected. However, our study has several limitations, and further studies are needed to confirm or dispute our preliminary results.

1. Introduction

Globally, lung cancer is the leading cause of cancer-related deaths in men. In women, it is the second leading cause after breast cancer (1). Nevertheless, the number of deaths has been constantly decreasing over the past decades. One of the main reasons for this reduction is the development of therapy according to the histologic types and the development of immunohistochemistry. This is essential for the proper histologic classification of tumors (2). The differentiation between the histological subtypes and the tumor staging is indispensable for assigning the most appropriate therapy.

In the past years, the importance of PD-L1, a predictive biomarker, has been growing due to the positive outcome of immunotherapy compared to conventional chemotherapy (3). However, evaluation of the PD-L1 positivity is not always easy and straightforward, so the results among pathologists can vary. As a solution, several companies are developing automated detection systems to standardize the PD-L1 scoring using digitalized slides. Because of that, we aimed to compare the results of currently only IVD-approved software for PD-L1-evaluation in non-small cell lung carcinoma, uPath PD-L1 image analysis (Ventana, Roche), with the evaluation of a pathologist. Two tumor types were selected- non-small cell lung carcinoma (NSCLC) (for which this software is IVD-approved) and pleural mesothelioma (for which recently the first immunotherapy was approved).

2. General information about NSCLC

2.1 Epidemiology of lung cancer

2.1.1 Incidence

In 2020, Globocan registered about 2.2 million new cases of lung cancer worldwide. This accounts for 11.4% of all cancer cases (1). In Austria, the incidence of lung cancer patients has been increasing steadily over the past years. In 2017, about 2800 men and 1937 women were diagnosed with lung carcinoma (4). While the number of new cases has been increasing in Austria, it has been declining in the

United States in both males and females, from 2006 to 2015 (men) and 2008 to 2015 (women) (2).

More than half of all new cases in 2020 worldwide were registered in Asia (59.6%), followed by Europe (21.6%) and North America (11.5%). Africa constitutes only 2% and South America, including the Caribbean, 4.4%. But here, one must consider that, for example, Brazil is one of the few countries in South America which have a register for lung cancer. Within Asia, China has the highest incidence, with around half a million new cases every year. However, there is a remarkably higher occurrence of lung cancer in the east of China, especially in the cities, where the lifestyle is more similar to the Western world (5).

This difference exists not only between countries and continents but also in regards to gender. Globally, lung cancer is the leading carcinoma diagnosed in men of all ages. When it comes to women, the most common cancer is located in the breast, followed by the colorectum. Lung cancer is placed third and makes up approximately 8.4% of all neoplasms among women.

This gap between the sexes is very large in some countries, like in Russia. While lung cancer is the most frequent within the male population in the Russian Federation, females tend to have a much lower risk of developing lung carcinoma (1). The male to female ratio is around 4:1. The leading cause for this enormous difference could be smoking, as it is the most important risk factor for developing lung carcinoma. In Russia, smoking is still not as popular with women as it is with men (5).

2.1.2 Mortality

The number of deaths associated with lung cancer had a peak in 1991. This constant increase of both cancer incidence and mortality was basically linked with the rising tobacco consumption in the 20th century, which is the main etiologic factor for the development of lung carcinoma. Since 1991, mortality has dropped every year due to improved and earlier detection and a decline in smoking (2).

According to the Statistik Austria (2020) (4), it is still the leading cancer-related death cause in Austria. Especially in Vienna, the most deaths have been reported between 2013 and 2015, while the lowest incidence and mortality was observed in Upper Austria. In the years between 2014 and 2017, almost 4000 patients died annually with the diagnosis of lung cancer. However, the relative 1-year survival rate has increased from 42% to 52% (4).

Globally, lung cancer was still one of the top causes of cancer-related deaths in absolute numbers in 2020. Particularly in the industrialized countries and continents like Australia, North America, Europe, and China, the mortality was much higher than other types of cancer, as illustrated in Figure 1. All blue areas represent the countries where lung cancer was the leading cause of death due to cancer.

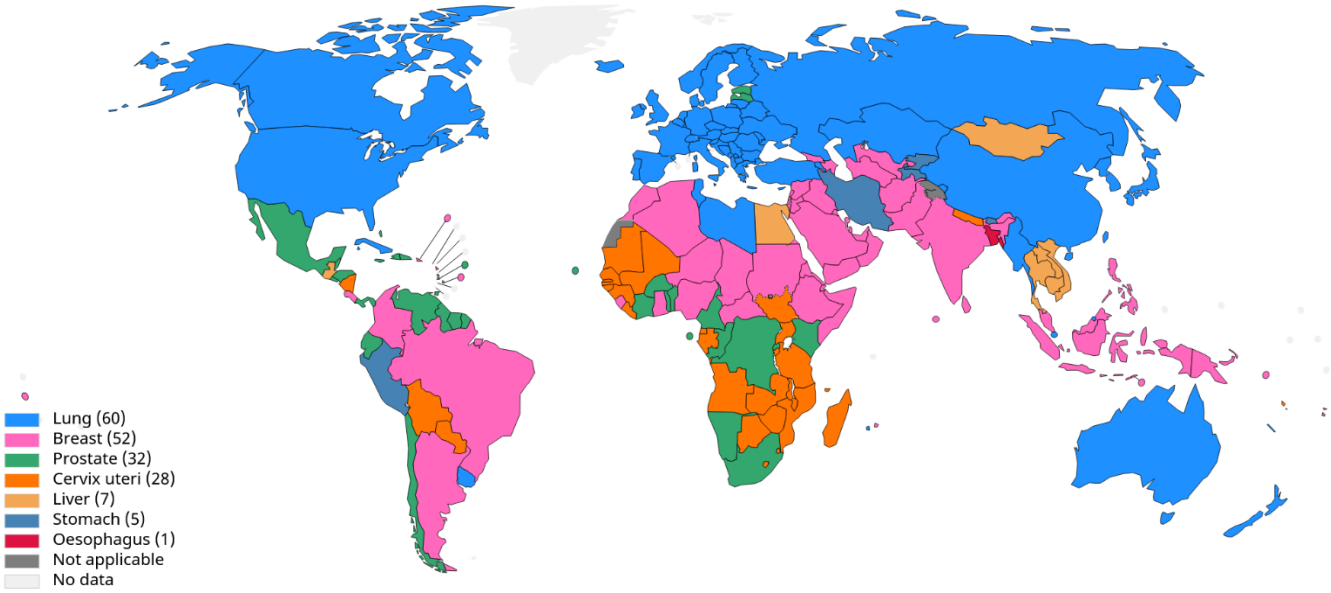

Figure 1: Top cancer mortality per country in 2020, both sexes, all ages, and age-standardized mortality rate (1)

With 35.9 per 100.000 inhabitants, Turkey registered more deaths related to lung cancer than any other country in the world. Another country with remarkably high mortality is China, with 30.2 per 100.000 inhabitants.

In comparison, Austria has a mortality rate of 20.4. In Central Africa, this rate is notably low. For instance, in the Democratic Republic of the Congo, it is 2.1 (1).

What needs to be mentioned in this context is the fact that there are substantial disparities between the sexes and ethnic groups within the different countries. In the US, for example, black men have mortality twice as high as Asian Americans (6, 7).

The difference between the sexes in Russia has already been described above. This disparity is also striking in Turkey, where the mortality rate is almost seven times higher in men than in women (67.5 vs. 10.7) (1).

2.2 Causes

2.2.1 Risk factors

Lung cancer has various risk factors ranging from intrinsic factors like genetics to extrinsic factors like tobacco smoking or rare causes such as asbestos exposition. Numerous studies have shown a connection between a confirmed case of lung cancer in the family history and a higher incidence of early-onset lung cancer (8). Furthermore, it is associated with genetic diseases like the Li-Fraumeni syndrome, which expresses multiple neoplasms throughout the body due to a mutation of the p53 tumor-suppressor gene (9).

However, by far the most prominent risk factor is the consumption of tobacco. Cigarettes are the common form of tobacco consumption in the Western world, which is linked to the high incidence of lung carcinoma. There is also a correlation between less popular forms like cigars, cigarillos, and pipes and the occurrence of neoplasms in the lung (10).

Besides, to increase the risk of developing lung carcinoma, it is not even necessary to smoke actively. Second-hand smokers have a higher chance of suffering from lung cancer than people without this exposure. In addition, it has to be mentioned that patients who are second-hand smokers are most commonly women. Only approximately one out of five of those are men (11).

Another important risk factor that has a carcinogenic impact on the lung is the radioactive gas radon. This gas appears in the soil and was especially important in the 20th century among mine workers (12). Moreover, there is a connection between radon exposure and tumor mutations (13). The risk for those who have radon exposure and are active smokers is 25 times higher than for the non-smoking population (14).

As mine-workers often get in touch with radon, other occupational groups are frequently exposed to different substances that can lead to lung cancer. Among these substances are asbestos (15) or pesticides. Other occupations with a higher risk are waiters and waitresses or bartenders due to second-hand smoking (16).

Even though in Europe solid fuel is barely used in households for cooking and heating, in countries with a low income, this is almost standard (17, 18). Especially in parts of Asia, it is common to utilize coal to heat houses or stoves. During the combustion of the domestic fuel develops smoke, which is, according to the IARC

(17), carcinogenic even for people who do not smoke, and it pollutes the air inside the house (19).

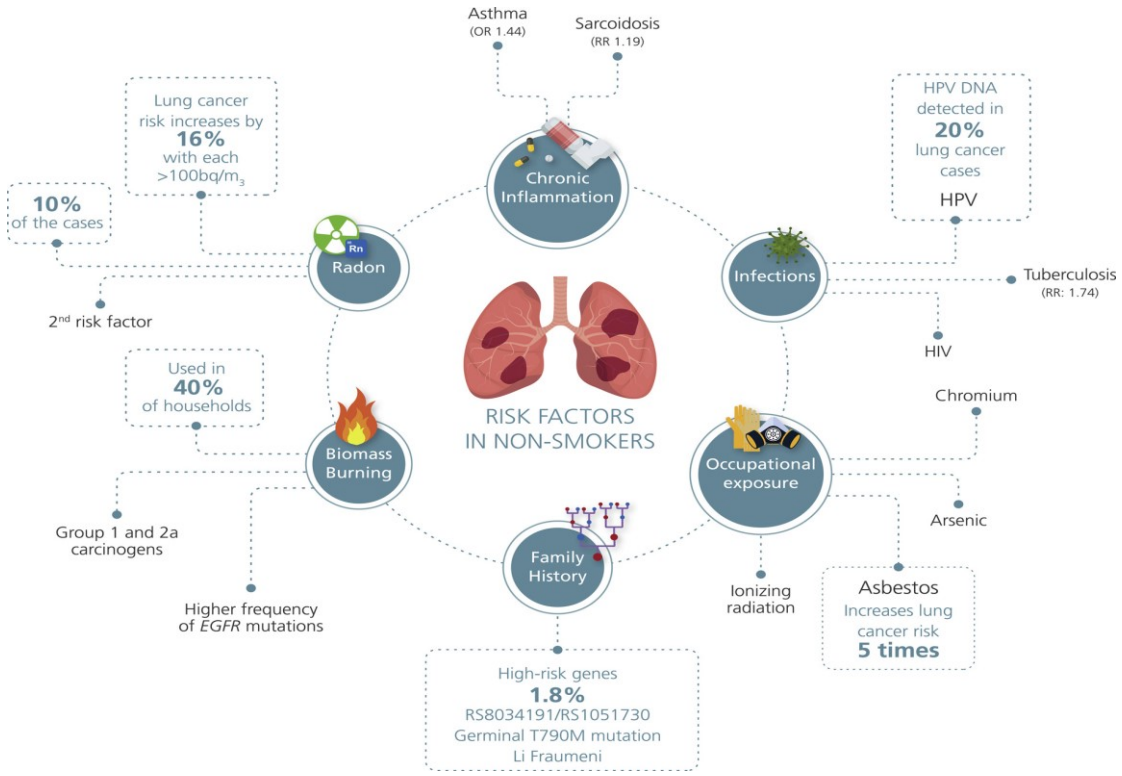

Figure 2: Possible causes for lung cancer in non-smokers (20)

As presented in Figure 2 (20), many other factors can cause lung cancer besides smoking. Apart from the risks mentioned before, like radon exposure, biomass burning, family history, or occupational exposures, some more need to be addressed. Radiation exposure, for example, is considered as another risk factor for developing a neoplasm in the lung, especially in patients with a previous cancer diagnosis in the chest area and a subsequent radiation therapy (21-23). It is also known that chronic inflammation like asthma and sarcoidosis (24) or infections such as pulmonary tuberculosis, HIV, or HPV can have a role in the development of lung carcinoma, as shown in Figure 2 (20).

When it comes to HIV, lung cancer is the most death-related malignancy in this group of patients (25-27). The incidence of NSCLC is higher in infected patients than among uninfected (28-31).

To conclude, even though tobacco smoking is by far the most important cause of lung cancer, there are various other reasons, even for non-smokers, as presented in Figure 2 (20).

2.3 Symptoms

First of all, it has to be mentioned that the symptoms must not occur in every patient, they occur late in the course of the disease, and they can vary from person to person. A major symptom is a newly emerged cough. This applies to roughly half of the patients, who are smokers or former smokers.

Patients who already suffer from chronic obstructive pulmonary disease often show an aggravation in the course of their disease. Decisive for those exacerbations can also be numerous recurring lung infections (32). Another very frequent symptom is dyspnoea. However, some symptoms are not as frequent as dyspnoea or a cough, such as huskiness or chest pain due to local tumor invasion. While coughing, there can also appear a bloody secretion but is not severe in most cases (33).

Patients can also have intra- and extrathoracic symptoms through the spread of cancer. Extrathoracic symptoms do not have to be very specific. Loss of weight, for instance, can be the consequence of lung cancer but also of various other cancers or diseases. Inside the thorax, symptoms like the superior vena cava syndrome, dysphagia, or pain in the arm or shoulder can occur (32). Furthermore, metastases are possible in the bone or the brain, and they can cause paraneoplastic syndromes. The paraneoplastic syndrome integrates neurologic syndromes, SIADH, or hypercalcemia (34).

2.4 Histologic Classification

In 2021, the World Health Organization published the most recent classification of lung tumors, including benign and malignant forms. This classification distinguishes between over fifty different entities, while most of them belong to malignant lung carcinomas (35).

Generally, we can divide lung carcinomas into two main groups: non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC). NSCLC accounts for

85% of all lung cancer types, while SCLC makes up the remaining 15%. It is not enough to differentiate between those two groups for today's therapy decision, but the correct histological diagnosis is of great importance (36).

2.4.1 Non-small cell lung carcinoma (NSCLC)

NSCLC includes many different subtypes. The main ones are adenocarcinoma and squamous cell carcinoma. These two histologic subtypes make up already more than two-thirds of all lung cancer cases reported worldwide.

Rare forms include large-cell carcinoma, adenosquamous carcinoma, and pleomorphic carcinoma (37).

2.4.1.1 Adenocarcinoma (AC)

A histologic examination reveals an adenocarcinoma (AC) in over 40% of patients diagnosed with lung cancer. Furthermore, it is not only occurring among adult men and women but is also present in relatively young people and never-smokers (38). In addition, a steady growth of the relative frequency of AC has been registered in the past decades (39).

AC is, per definition, a malignant epithelial neoplasm, which either shows a glandular differentiation or a production of mucus. Commonly, this tumor type expresses markers that are usual for pneumocytes. These are the thyroid transcription factor (TTF-1) and the Napsin A. Those two markers are detectable in more than 85% of the diagnosed ACs (40-42)

Histologically, five different predominant growth patterns are distinguished. These are lepidic, acinar, papillary, micropapillary, and solid. They differ in the level of cytological atypia. Figure 3 presents a histologic presentation of a typical AC of the lung with predominant acinar pattern.

Besides these growth patterns, another four variants are invasive mucinous, colloid, fetal, and enteric AC. These variants can be assigned to the other subtypes of the lung AC described above (38).

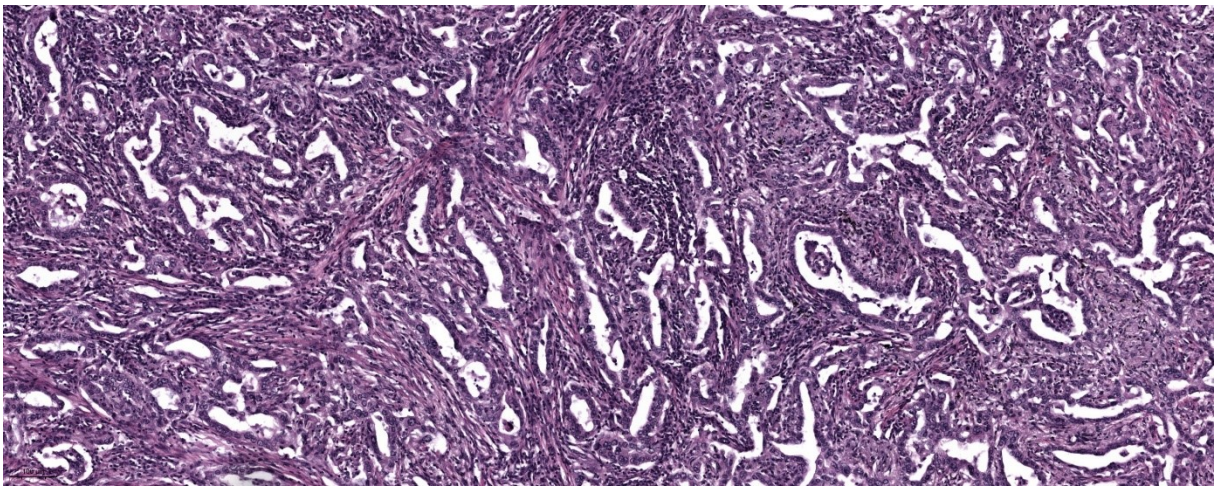

Figure 3: Histologic presentation of typical adenocarcinoma of the lung, here with predominant acinar pattern.

2.4.1.2 Squamous cell carcinoma (SCC)

The squamous cell carcinoma (SCC) is a malignant epithelial tumor demonstrating squamous differentiation, with or without keratinization (37). Compared to the other forms of NSCLC, SCC has the by far highest association to cigarette smoking and second-hand smoking (43). In the last years, the relative incidence of this lung cancer type has decreased, but it still accounts for approximately 25-30% of all diagnosed lung carcinomas (36).

Histologically, there are three different variants of squamous cell carcinoma according to the 2021 World Health Organization classification (35). These variants are the keratinizing SCC, the nonkeratinizing SCC, and the basaloid SCC. In cases where the squamous differentiation is not found or where it is ambiguous immunohistochemical markers like p40 (or p63) are used for the diagnosis. The SCC also presents with more extensive areas of necrosis in comparison to AC, which can lead to a pseudo-glandular appearance (36, 37).

In Figure 4, the histologic presentation of SCC is shown.

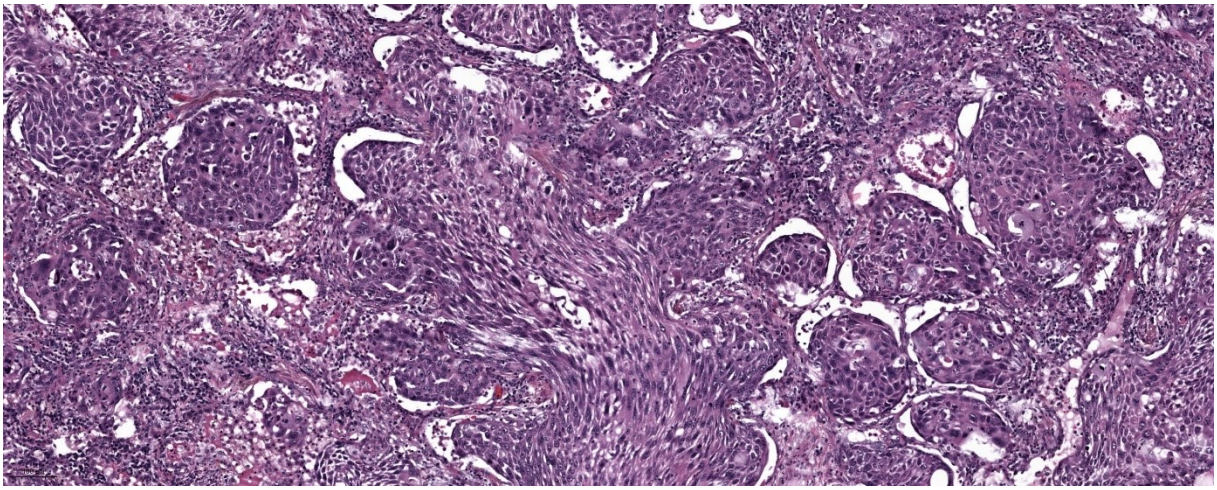

Figure 4: Histologic presentation of squamous cell carcinoma of the lung, with areas of inflammation, and without obvious keratinization.

2.4.1.3 Adenosquamous Carcinoma (ASC)

Another subtype of the NSCLC is adenosquamous carcinoma (ASC). ASC only accounts for a maximum of 5% of all lung cancers. As the name already reveals, this carcinoma consists of parts of the adenocarcinoma and parts of the squamous cell carcinoma of the lung, each represented in at least 20% of the whole tumor mass (44).

On the molecular level, the case is more complex. This type of tumor can express oncogenic mutations like EGFR or KRAS typical for AC (36). According to the survey of Kang et al. (45), EGFR-mutations are predominant in women and never-smokers.

The diagnosis can be problematic, as the biopsy or cytology might only show one component of this cancer type. If this is, for example, the SCC fraction, it will be diagnosed as SCC. Because of the unrepresentativeness of the small biopsies/cytologic samples, according to the international recommendations, the ASC diagnosis should be made only on resected specimens (37).

2.4.1.4 Large cell carcinoma (LCC)

A large cell carcinoma (LCC), like previously mentioned ASC, can be diagnosed only on resected material (37). LCC makes up only a small percentage of the lung cancers diagnosed. Today, tumors are only diagnosed as LCC when there is a lack of AC, SCC, or neuroendocrine carcinoma differentiation in morphology and/or immunohistochemistry.

Histologically, the cancer cells are large and polygonal. Furthermore, the nucleus has a vesicular shape, but the morphology can vary. These cells form either solid sheets or nests and do not have any specific pattern. (46, 47).

2.4.2 Neuroendocrine tumors

In the current 2021 WHO classification, small-cell lung carcinoma (SCLC) and large-cell neuroendocrine carcinoma (LCNEC), as well as carcinoid tumors, are listed among neuroendocrine tumors (35). Nevertheless, there are strong differences between those carcinomas concerning epidemiology, histology, and clinical presentation. SCLC has the highest incidence among neuroendocrine tumors and is, as well as LCNEC, associated with heavy smoking exposure. On the other hand, the carcinoid tumor is more present among younger patients, and the etiological correlation is not associated with smoking as strongly. Histologically, both SCLC and LCNEC show a higher mitotic rate and larger necrotic areas than the carcinoids. In SCLC, one will find hyperchromatic nuclei, without nucleoli and cytoplasms are scant. On the other hand, LCNEC has more vesicular nuclei, with prominent nucleoli and cytoplasms are bigger. Furthermore, different lung cancer types like ACs or SCCs can be present simultaneously with SCLC and LCNEC, and they are diagnosed as combined carcinomas (48). Furthermore, small-cell lung carcinomas and large-cell neuroendocrine carcinoma are associated with genetic abnormalities which are generally different from the ones found in carcinoid tumors (37, 49, 50). Figures 5 and 6 present the histologic images of SCLC and LCNEC in the hematoxylin-eosin staining.

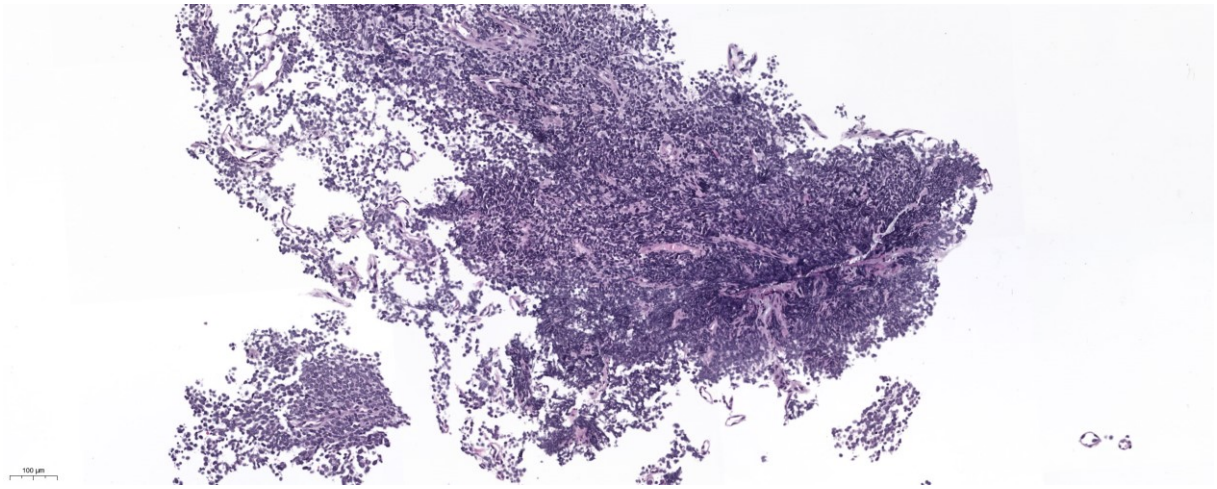

Figure 5: Histologic presentation of small cell lung cancer, with tumor cells with scant cytoplasm and in the middle of the image pronounced „crushing“ artifact.

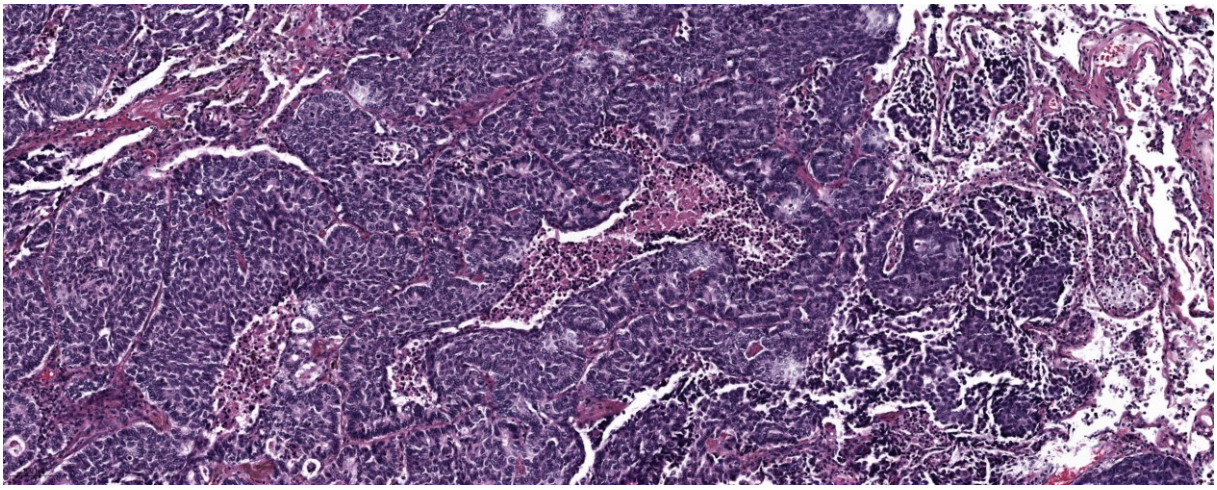

Figure 6: Histologic presentation of large cell neuroendocrine carcinoma in the lung, with areas of necrosis, and tumor cells with more abundant cytoplasm.

2.5 TNM-Classification of lung carcinoma

The TNM-Classification is an important tool to classify tumors based on their size or depth of infiltration (T) and taking into account lymph node- and distant metastases (N and M, respectively). TNM is used both by pathologists and clinicians as the basis

for the communication between them and as a basis for communication with patients. This classification is used to decide on the most suitable treatment, and it has prognostic significance (51).

Table 1 features the latest TNM-Classification of lung carcinoma according to the 8th edition of the American Joint Committee on Cancer (AJCC) (52)

TNM-Classification of lung cancer		
Category	**Staging**	**Description**
T (Primary Tumor)	TX	Primary tumor not evaluable, or malignant cells cannot be visualized by imaging or bronchoscopy
	T0	No evidence of primary tumor
	Tis	Carcinoma in situ
	T1	Tumor ≤3cm, surrounded by lung or visceral pleura, more proximal than the lobar bronchus
	T1mi	Minimally invasive adenocarcinoma
	T1a	Tumor ≤1cm
	T1b	Tumor >1cm and <2cm
	T1c	Tumor >2cm and <3cm
	T2	Tumor >3cm and ≤5cm or involving the main bronchus without the carina; infiltrating visceral pleura; atelectasis or obstructive pneumonitis up to the hilus
	T2a	Tumor >3cm and ≤4cm
	T2b	Tumor >4cm and ≤5cm
	T3	Tumor >5cm and ≤7cm or separate tumor nodules in the equal lobe as a primary tumor; invasion into mediastinum, heart, diaphragm, trachea, esophagus, carina, great vessels
	T4	Tumor >7cm or separate tumor nodules

		in different lobe than primary tumor; Invasion into mediastinum, heart, diaphragm, trachea, esophagus, carina, great vessels
N (Regional lymph node involvement)	NX	Regional lymph nodes not evaluable
	N0	No existent lymph node metastasis
	N1	Lymph node metastasis ipsilateral/peribronchial or ipsilateral hilar
	N2	Lymph node metastasis ipsilateral/ mediastinal or subcarinal
	N3	Lymph node metastasis contralateral mediastinal, ipsilateral/contralateral scalene, contralateral hilar, supraclavicular
M (Distant metastasis)	M0	Distant metastases not existent
	M1	Presence of a distant metastasis
	M1a	Tumor nodules additionally in the contralateral lobe or pleural/pericardial
	M1b	Single extrathoracic metastasis
	M1c	Multiple metastases in at least one organ extrathoracic

Table 1: TNM-Classification of lung carcinoma (52)

As this table shows, the T stage corresponds to the primary tumor size in the lung. However, it is important to bear in mind that the tumor size depends on the time of the measurement. For example, the study of Park et al. (53) showed that fixation in formalin could change the extent of the tumor up to 5%. As Table 1 demonstrates, this primary tumor category is divided into six main groups (TX, T0, Tis, T1, T2, T3, T4).

The N category describes whether regional lymph nodes are infiltrated with the tumor cells or not. These guidelines recommend resection of at least six lymph nodes for an exact staging. However, a pathologist can make N classification, even if the recommended number of lymph nodes is not present (51). In staging, it is crucial to

distinguish between N1 and N2 because the subsequent therapy varies substantially. By appropriate T stage, with N0 and N1, surgical resection with a possible adjuvant therapy afterward is recommended. The patients with the same T stage and N2 receive only adjuvant chemotherapy (54).

M category considers the presence of distant metastases and whether they are single or multiple. Even though there is no significant difference in the survival rate between M1a and M1b, the local treatment is more aggressive in patients with a tumor in stage M1b (55).

2.6 Immunohistochemistry

Previously, terms like NSCLC and SCLC were mainly used because this differentiation was the only important one for the therapy decision. A further classification in its morphology was therefore not required. However, with the development of therapies, and their different effect based on the histologic subtype, it has become mandatory to consider histological differentiation before deciding about treatment.

Nowadays, there are more than fifty subtypes reported that differ in their histomorphological differentiation (37, 56, 57). Sometimes, especially in the small samples, this differentiation is not possible without the application of immunohistochemistry (IHC) (58).

In IHC, we use antibodies to identify cells through their markers expressed on their surface (59). Based on many different studies, the best combination of markers to use in the daily routine to differentiate between AC and SCC are TTF-1, P40, and Napsin A.

Napsin A and TTF-1 are markers of glandular differentiation (AC). P40 is a marker of squamous differentiation (SCC) (42, 60-63). In small biopsies, if there is no obvious morphologic differentiation, TTF-1 and p40 are negative, and morphology is not of SCLC, tumors should be classified as non-small cell carcinoma - not otherwise specified (NSCC-NOS) (37).

2.7 Therapy

The treatment in non-small cell lung carcinomas varies, depending on the clinical staging and presence or absence of predictive markers (like PD-L1 and targetable mutations).

2.7.1 Surgical resection

In early tumor stages, I to IIIa, surgical resection is recommended. Among the different surgical resection techniques, lobectomy is the method of choice (64). An advantage of the lobectomy is a higher survival rate compared to a limited resection of the tumor (65, 66). If surgery is not indicated, patients will receive some other form or combination of different below-mentioned therapies.

2.7.2 Chemotherapy

Patients with a clinical-stage II or IIa should receive postsurgical (adjuvant) chemotherapy, usually cisplatin-based (64). Chemotherapy can also be applied prior to surgery (neoadjuvant chemotherapy) to reduce the tumor as much as possible to conduct a complete resection. This approach has fewer side effects than adjuvant chemotherapy (64). However, no increased chance of survival has been reported, even though numerous studies have been performed (67, 68). In contrast to neoadjuvant chemotherapy, the adjuvant approach has a proven positive impact on the 5-year survival rate in patients with this treatment (69).

2.7.3 Targeted Therapies

There are several mutations used as predictive biomarkers in lung cancer patients for different targeted therapies. EGFR mutation, for instance, is found in around 15% of all advanced NSCLC diagnoses in the Western population (70). If an activated EGFR mutation is confirmed, the therapy consists of EGFR-TKI. Those TKIs include erlotinib, osimertinib, afatinib, and dacomitinib, to mention just a few of them. The advantage of these targeted therapies compared to the platinum-based chemotherapy is a better progression-free survival and easier application (71-73).

Other predictive biomarkers in this group are, for example, ALK, ROS-1, and BRAF mutations. Each of them occurs in less than 5% of patients with NSCLC, mostly AC. ALK protein overexpression is the result of gene fusion. Like for the treatment of EGFR mutated carcinomas, a TKI is used here. In this case, one option can be

crizotinib (74), or ALK-TKIs of new generations like alectinib or ceritinib. These are newer TKIs, more potent than crizotinib (75, 76). Some ALK-TKIs also impact tumors with detected ROS1 rearrangements (77).

Within the last years, the development of targeted therapies for lung carcinoma has exploded and improved survival in this group of patients. Furthermore, it caused the necessity of using parallel multigene testing (also known as next-generation sequencing) as the best option when only a limited amount of tumor tissue is available. Interestingly, the distribution of these mutations varies significantly based on geography, gender, and sometimes smoking status. Shi et al. (78) showed that activating EGFR mutations are expressed in up to 50% of the patients in the Asian cohort, while only in less than 15% in the Caucasian cohorts. Even more, if all targetable mutations are combined, only a small number of lung carcinoma patients (ca. 25%) will be eligible for this type of targeted therapy (79).

2.7.4 Immunotherapy

It is known that the immune system, with its capability to detect and destroy tumor cells, is important for carcinogenesis (80). To control that non-malignant cells are not destroyed, the immune system manages activating and inhibitory pathways (81). If there is an imbalance between those pathways, or malignant cells are “disguised” as non-malignant cells, the neoplastic process will progress.

Programmed death-1 (PD-1), for example, is a transmembrane receptor expressed in many different tissues (82). The ligand (PD-L1) can be found on T cells and tumor cells. If ligand and receptor interact, the T cell gets inactivated, which is one mechanism of how T cells evade the destruction of normal tissue. However, if T-cells recognize malignant cells as normal cells, they will thrive (83). The basic principle of immunotherapy is to aim those PD-1/PD-L1 interactions with antibodies and block them (82).

Many immune checkpoint inhibitors have been developed and approved for different solid tumors in the last years due to excellent results regarding progression-free and/or overall survival. Likewise, immunotherapy in lung carcinoma patients was another breakthrough in therapy.

2.8 PD-L1

As already mentioned above, PD-L1 is located on T cells of the immune system and tumor cells (83). This predictive biomarker is associated with an improved survival rate after the application of immunotherapy because the chances of responding to it are higher in patients with higher PD-L1 expression (3). Although PD-L1 evaluation is not needed for all immune checkpoint inhibitors in NSCLC, the assessment of this PD-L1 expression is a common practice. For the subsequent therapy, it is essential to assess the PD-L1 expression correctly. The main problem is that different clones of PD-L1 antibodies are designed for various clinical studies of different pharmaceutical companies.

Currently, four clones are mostly used routinely in NSCLC: 22C3, 28-8, SP263, and SP142. All those antibodies bind to different epitopes and evaluate the positivity on tumor cells in a similar but different way. Studies have shown that three antibodies are very similar in their expression, but none is actually interchangeable (84). For most pathological laboratories, it is not possible to have four antibodies (and adequate staining platforms) available for routine diagnostics. Another issue with PD-L1 evaluation is that different cut-offs are used for different antibodies and different indications. So, the assays cannot be replaced among each other unlimitedly (85).

Furthermore, studies have shown that correlation of evaluation varies between pathologists and is sometimes rather time-consuming (86-89). Because of the previously mentioned points, the idea of having reliable software performing PD-L1 evaluation is very attractive. Today, when many pathologies are on their way to include digital pathology in their workflow, it could be easily integrated into this “digitalization” process, providing pathologists with already scored slides, and therefore saving time.

3. Pleural mesothelioma

3.1 Epidemiology and etiology

Epidemiologically, pleural mesothelioma (PM) is a rare but very aggressive neoplasm with a poor prognosis. Overall, PM makes up less than 0.3% of all malignancies (90). Etiologically, it is associated with the exposure of asbestos. The latency period between the asbestos exposure and the actual manifestation is approximately 40 years (91). Even though asbestos was banned decades ago, there is an incidence peak expected in the following years due to the long latency period (92).

3.2 Histologic classification

In the 2021 World Health Organization classification, histological subtypes are divided into three major groups: epithelioid, sarcomatoid, and biphasic (35, 90).

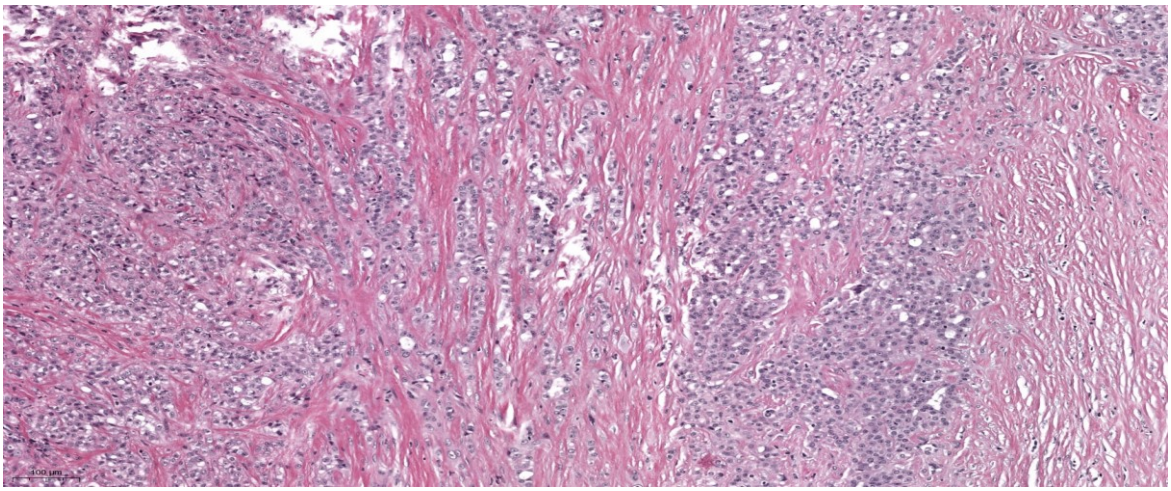

Figure 7: Histologic presentation of epithelioid pleural mesothelioma with pronounced desmoplastic stroma.

Epithelioid pleural mesotheliomas (Figure 7) have a better prognosis compared to the biphasic and sarcomatoid. For the correct diagnosis, architectural patterns, cytology, and stromal features should be reported. Histologically, mesotheliomas with at least 10% of each epithelioid and sarcomatoid pattern are diagnosed as biphasic mesotheliomas (35). However, the cut-off level was chosen arbitrarily. For example, the study of Galateau et al. (93) showed that the cut-offs can be set at different levels and still afford a better prognosis stratification.

As it is sometimes difficult to clearly identify sarcomatoid mesothelioma only by histology, immunohistochemistry improves the correct diagnosis. Per definition, sarcomatoid mesotheliomas have spindle, atypical cells within the fibrous or desmoplastic stroma (93).

3.3 Diagnostic and predictive IHC and molecular findings

Immunohistochemistry is crucial for diagnosing mesotheliomas because it is difficult to differentiate between mesothelial lesions and other tumors (metastases to pleura and sarcoma) or sometimes even reactive lesions.

Overall, at least four IHC markers should be used. Two markers positive in mesothelial cells, and two markers negative in mesothelial cells (carcinoma markers). However, the exact number of markers depends on the histological presentation and differential diagnosis (94).

Differentiation between benign and malignant mesothelial proliferation is currently performed with BRCA associated protein 1 (BAP1) as a marker. If BAP1 is not present as a nuclear staining, mesothelial proliferation is assumed as malignant. Nevertheless, BAP1 loss is not necessarily specific for mesotheliomas, as it can occur in other malignant tumors (95). Another helpful method for confirming malignancy is the deletion of *CDKN2A*, which is analyzed by fluorescence in-situ hybridization (FISH) (96). A combination of both BAP-1 IHC and *CDKN2A* FISH can provide a proper assignment to either benign or malignant cells in many cases (90).

Very recently, immunotherapy also showed potentially promising results in overall survival in patients with pleural mesothelioma. Therefore, the importance of PD-L1 evaluation is increasing, even though it is only used for prognostic reasons so far (97-100).

4. Aims

Our study aimed to compare the scoring performed by the only IVD-approved software for the PD-L1 testing in lung cancer with a manual, microscope-based evaluation by pathologists. Furthermore, we aimed to assess the applicability of this software in the PD-L1 assessment in pleural mesotheliomas.

5. Material and Methods

5.1 Study design

This retrospective study included 51 consecutive samples of NSCLC (adenocarcinoma and squamous cell carcinoma), diagnosed in 2020 at the Diagnostic and Research Institute of Pathology, Medical University of Graz. 26 were small biopsy samples (small), and 25 samples were obtained after surgery as resection samples (resect). Furthermore, 24 samples of pleural mesotheliomas (meso), diagnosed between 2018 and 2020. were also included. All samples were formalin-fixed (24 to 48 hours) paraffin-embedded samples. The inclusion criteria for samples was the availability of enough tumor tissue in paraffin blocks for new PD-L1 staining.

This retrospective study conformed to the principles outlined in the Declaration of Helsinki (as revised in 2013). It was approved by the Ethics Committee of the Medical University of Graz (30-105 ex 17/18).

5.2 PD-L1 immunohistochemistry

For the PD-L1-staining, new 4µm-thick sections were prepared. PD-L1 clone SP263 (Ventana, Roche) was used as a ready-to-use assay on the BenchMark Ultra (Ventana, Roche). Afterward, the corresponding hematoxylin-eosin-stained slides and the PD-L1-stained slides were digitalized. For the digitalization, the DP 200 scanner (Ventana, Roche) was used with x20 magnification. The scans were then stored on the server of the Medical University of Graz.

Representative images of PD-L1 staining in NSCLC and mesothelioma are presented in Figures 8-12.

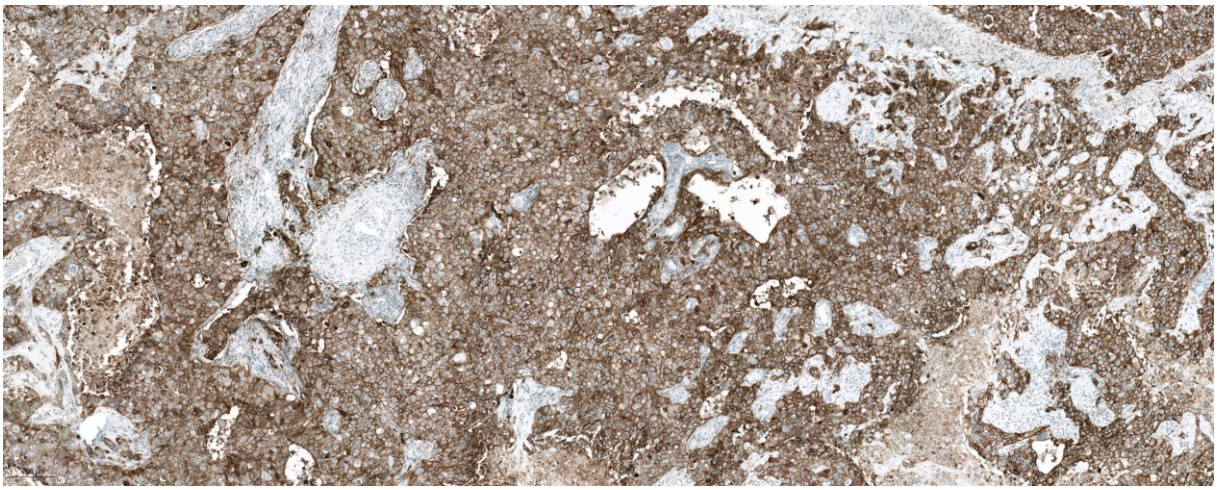

Figure 8: Strong positive PD-L1 reaction in all tumor cells, in a resection material of lung squamous cell carcinoma.

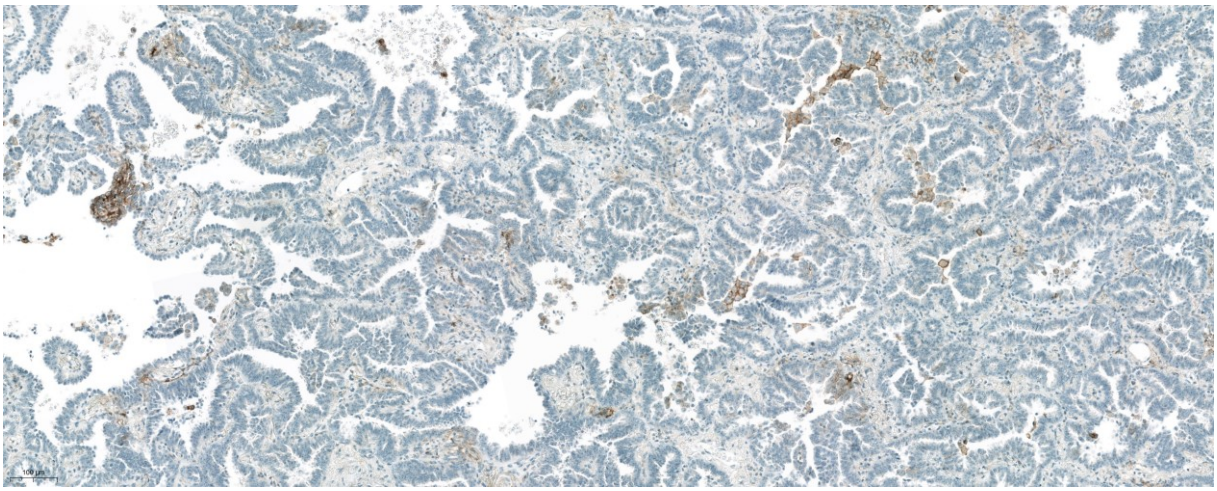

Figure 9: Resection material with negative PD-L1 reaction in tumor cells of lung adenocarcinoma, with positive reaction in macrophages.

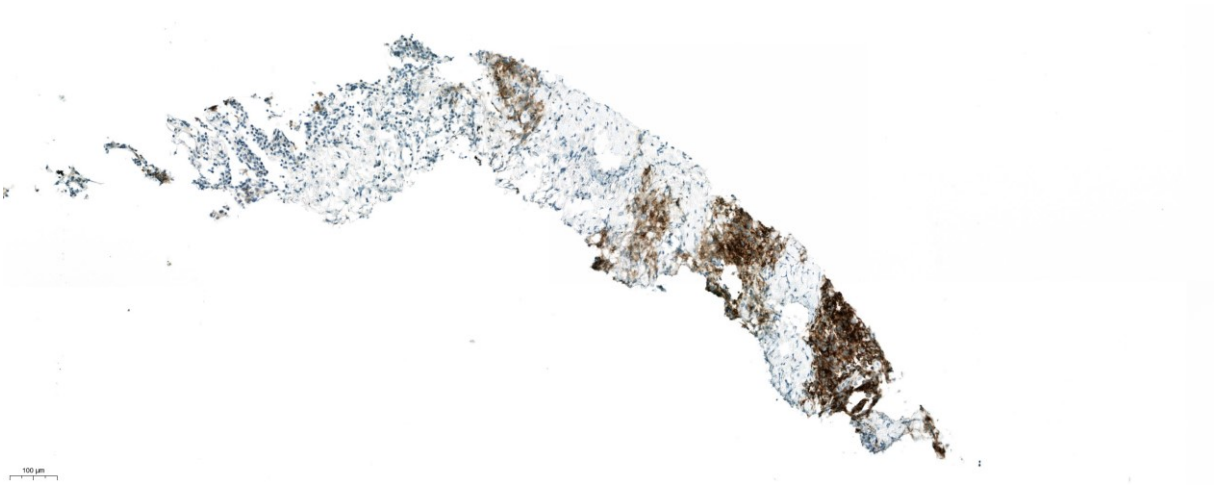

Figure 10: CT-guided needle biopsy of lung carcinoma with strongly positive PD-L1 reaction.

Figure 11: Completely negative reaction with PD-L1 in a CT-guided needle biopsy of lung carcinoma.

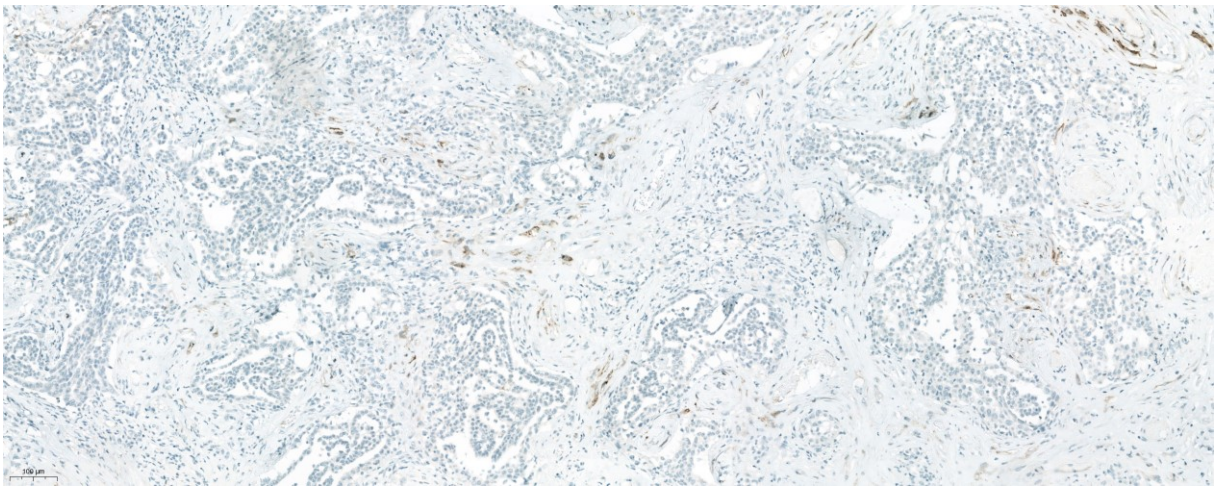

Figure 12: PD-L1 staining in epithelioid pleural mesothelioma. Tumor cells are completely negative, while some stromal/inflammatory cells demonstrate positive reaction.

5.3 PD-L1 scoring

Scoring under the microscope was performed by an experienced thoracic pathologist (LB) and a student (CM). Results of the evaluation were expressed as the percentage of positive tumor cells of all tumor cells. Staining of any intensity, incomplete, or complete membranous staining was regarded as positive. Both pathologist and student have performed evaluation alone. Results were compared, discrepancies discussed, and consensus (final percentage) was reached and used for comparison. Scores were registered using the following cut-offs: <1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%.

For the automated scoring, uPath PD-L1 (SP263) image analysis for non-small cell lung carcinomas was used (Ventana, Roche). Slides were analyzed by software without prior selection of the region of interest. In other words, the whole slides were analyzed. The percentage of positive tumor cells was automatically calculated based on the total number of counted tumor cells.
Both scores (obtained from humans and machine) were then registered in an Excel table.

5.4 Statistical analysis

The statistical analysis is based on the Cohen's kappa coefficient to calculate the Interrater-Observability.

For the calculation the obtained scores were grouped to 5 different, and clinically relevant categories. The total range from 0% to 100% was divided in the following categories: 0=0-0.99%; 1=1-9.99%; 2=10-24.99%; 3=25-49.99%; 4=50-100% (Table 2), named 0,1,2,3 and 4.

Scores in %	Categories
0-0.99	0
1-9.99	1
10-24.99	2
25-49.99	3
50-100	4

Table 2: Categories for PD-L1 scoring

Each investigated group, namely small biopsies, resection materials, and mesotheliomas were separately analyzed for each of the raters (Pathologist and Software).

With the help of Data-Matrix the Cohen's kappa coefficient was according to the following formula was calculated:

$$\mathrm{K} = \frac{p_0 - p_e}{1 - p_e}$$

Resulting in a number between -1 and 1, afterwards the Cohen's kappa coefficient was interpreted after Landis et al. (101), see Table 3.

Values	Interpretation
Smaller than 0.00	Poor Agreement
0.00 to 0.20	Slight Agreement
0.21 to 0.40	Fair Agreement
0.41 to 0.60	Moderate Agreement
0.61 to 0.80	Substantial Agreement
0.81 to 1.00	Almost Perfect Agreement

Table 3: Kappa-score values (101)

6. RESULTS

Obtained values of PD-L1 scoring are presented with actual percentages obtained from humans and software in Table 3. Scoring results after categorization are shown in Table 4.

Obtained results are presented as a data matrix (Table 5-7).

Mesothelioma	Humans	Humans Score	Artificial Intelligence	Artificial Intelligence Score
01_MESO	5	0	0,9	0
02_MESO	20	0	1,9	0
03_MESO	10	0	7,6	0
04_MESO	10	0	0,8	0
05_MESO	40	0	0	0
06_MESO	0	0	4,6	1
07_MESO	0	0	0,3	1
08_MESO	5	0	6,3	1
09_MESO	0	0	4,3	1
10_MESO	20	0	6,5	1
11_MESO	0	0	1,1	1
12_MESO	0	0	0,6	1
13_MESO	10	1	0,5	0
14_MESO	1	1	2,5	0
15_MESO	0	1	1,8	0
16_MESO	0	1	1,3	1
17_MESO	0	1	0,5	1

18_MESO	0	1	0,4	1
19_MESO	1	1	0,5	2
20_MESO	5	2	1	0
21_MESO	1	2	0,6	0
22_MESO	5	2	16,8	1
23_MESO	0	2	3,8	1
24_MESO	0	2	0,2	1
Resection samples-NSCLC	**Humans**	**Humans Score**	**Artificial Intelligence**	**Artificial Intelligence Score**
01_RESECT	0	0	2,1	1
02_RESECT	70	0	16,3	1
03_RESECT	0	0	2	2
04_RESECT	100	0	86,3	1
05_RESECT	30	0	15,1	1
06_RESECT	20	0	35,1	1
07_RESECT	0	0	15,8	1
08_RESECT	20	1	1,8	3
09_RESECT	90	1	17,2	2
10_RESECT	0	1	5,5	2
11_RESECT	20	1	10,7	1
12_RESECT	30	1	17	1
13_RESECT	0	2	4,3	3
14_RESECT	5	2	30	1
15_RESECT	5	2	12	2
16_RESECT	20	2	9,1	1
17_RESECT	10	2	3,8	1
18_RESECT	60	2	8,8	2
19_RESECT	100	3	51,2	2
20_RESECT	5	3	11,7	2
21_RESECT	0	4	5	2
22_RESECT	1	4	2,5	4
23_RESECT	0	4	7,1	2
24_RESECT	20	4	14,8	1
25_RESECT	1	4	2,5	4
Small biopsies-NSCLC	**Humans**	**Humans Score**	**Artificial Intelligence**	**Artificial Intelligence Score**
01_Small	100	0	12,9	0
02_Small	0	0	0	0
03_Small	1	0	0,1	0

04_Small	0	0	0	1
05_Small	0	0	0,3	1
06_Small	5	0	1,1	0
07_Small	0	0	1	1
08_Small	0	0	2,4	0
09_Small	0	0	0,2	0
10_Small	0	0	1,6	0
11_Small	100	0	45,6	0
12_Small	70	1	42,8	0
13_Small	60	1	10,7	1
14_Small	40	1	2,9	1
15_Small	80	1	6,3	2
16_Small	100	2	11	1
17_Small	90	3	13,6	1
18_Small	10	3	1,7	1
19_Small	0	4	0	2
20_Small	80	4	0,1	3
21_Small	30	4	3,6	3
22_Small	5	4	1,8	2
23_Small	0	4	0,6	1
24_Small	0	4	0,3	2
25_Small	0	4	0,1	2
26_Small	1	4	10,4	0

Table 4: Comparison of PD-L1 evaluation between humans and software

6.1 NSCLC small biopsies

Small							
Software							
Pathologist	**Category**	**0**	**1**	**2**	**3**	**4**	**Total**
	0	8	3	-	-	-	11
	1	1	2	1	-	-	4
	2	-	1	-	-	-	1
	3	-	2	-	-	-	2
	4	1	1	4	2	-	8

	Total	10	9	5	2	0

Table 5: Data matrix small biopsy group

The data matrix in Table 5 presents the differences and concordances between the scoring of the humans and the software in tumors of the small biopsy group. As previously mentioned, in this group, a total of 26 samples were evaluated. Humans and the software had the best concordance in 8 samples assigned to category 0. In 3 further samples, humans gave category 0 and software 1. What is very discrepant is the distribution of categories 3 and 4. Humans assigned category 4 to 8 samples, and software did not find any sample with more than 50% of positive tumor cells. Even more, in 6 out of these 8 cases, the software categorized them as category 0 (1 case), 1 (1 case), and category 2 (4 cases). In category 3, humans found two samples, and software classified them as category 2. The calculated Cohen`s kappa score for this group was 0.204 which indicates a slight agreement, as presented in Table 3.

6.2 NSCLC resection samples

Resect							
Software							
Pathologist	**Category**	**0**	**1**	**2**	**3**	**4**	**Total**
	0	-	6	1	-	-	7
	1	-	2	2	1	-	5
	2	-	3	2	1	-	6
	3	-	-	2	-	-	2
	4	-	1	2	-	2	5
	Total	0	12	9	2	2	

Table 6: Data matrix lung resection group

In this group, 25 samples were examined. The Data matrix is presented in Table 6. Interestingly, for categories 0 and 3, no concordance in the evaluation of the software and humans was found. Humans have 7 cases categorized in category 0, while these

were recognized as category 1 (6 cases) and category 2 (1 case) by software. The remaining categories 1, 2, and 4 had a concordance in two samples each. This accounts for a total number of 6 concordant samples out of 25 investigated samples.

As is clearly showed in Table 6, the software had more than twice as many scores in category 1 (12 cases) than humans (5 cases). Even more, out of 12 cases assigned by software to category 1, humans had only 2 cases in the same category. Other cases were in category 0 (6 cases), category 2 (3 cases), and even category 4 (1 case). Humans had also assigned a total of 5 samples to category 4. Two of them matched with the results of the software. The other three cases were allocated in category 1 (1 case) and 2 (2 cases) by the software.

In this group, a kappa score of 0.0433 was achieved representing poor agreement.

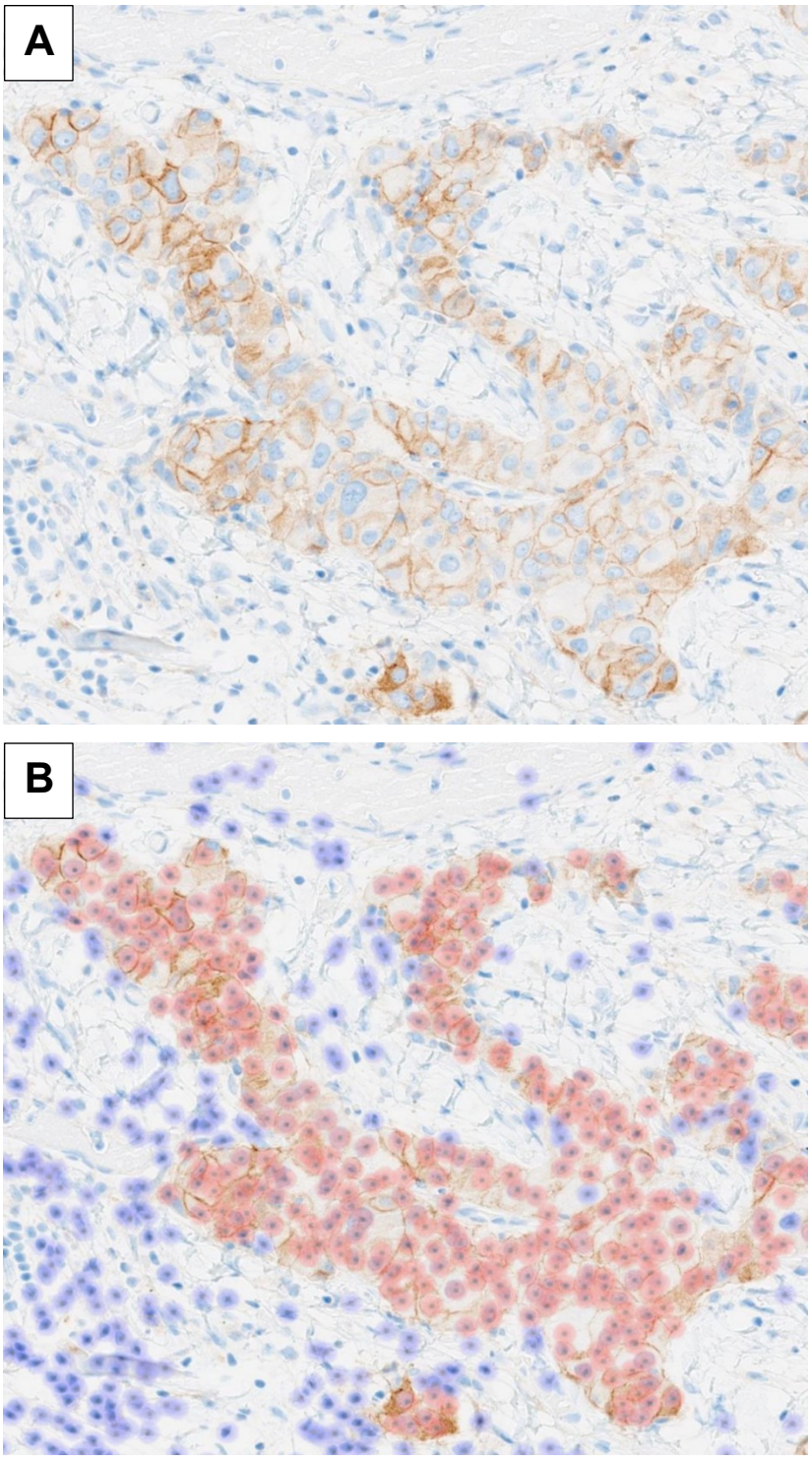

Figure 13: Two images representing slide with excellent concordance between pathologist and software regarding PD-L1 positivity in tumor cells. All optically positive tumor cells (stained brown in A) are also marked as positive tumor cells by software (red cells in B), while negative tumor cells are marked with blue (B).

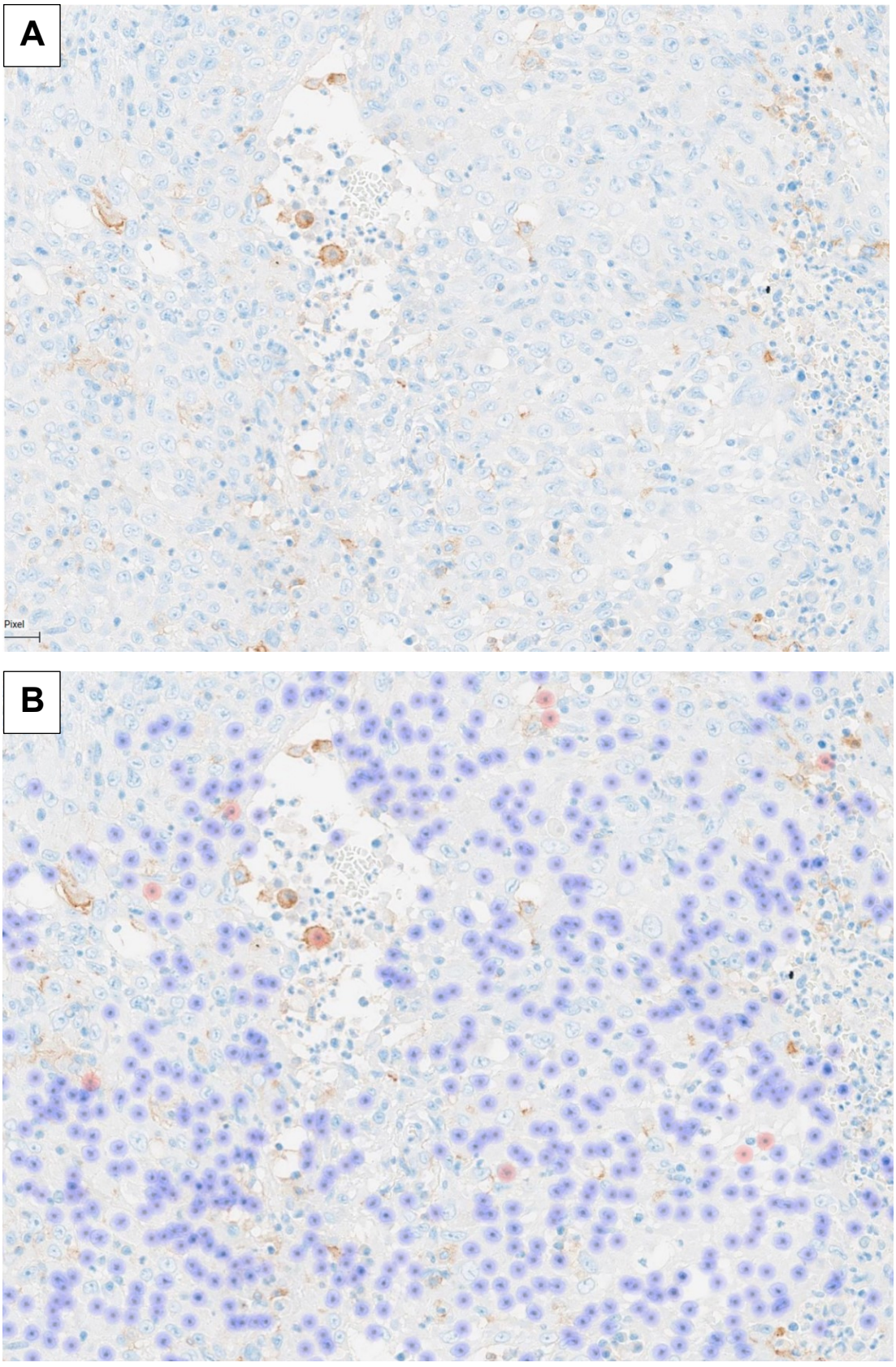

Figure 14: Two images representing slide with negative PD-L1 staining and adequate concordance between pathologist and software. All optically negative tumor cells (A) are also marked as negative tumor cells by software (blue cells in B). There are occasional false-positive tumor cells marked with red (B), corresponding to macrophages.

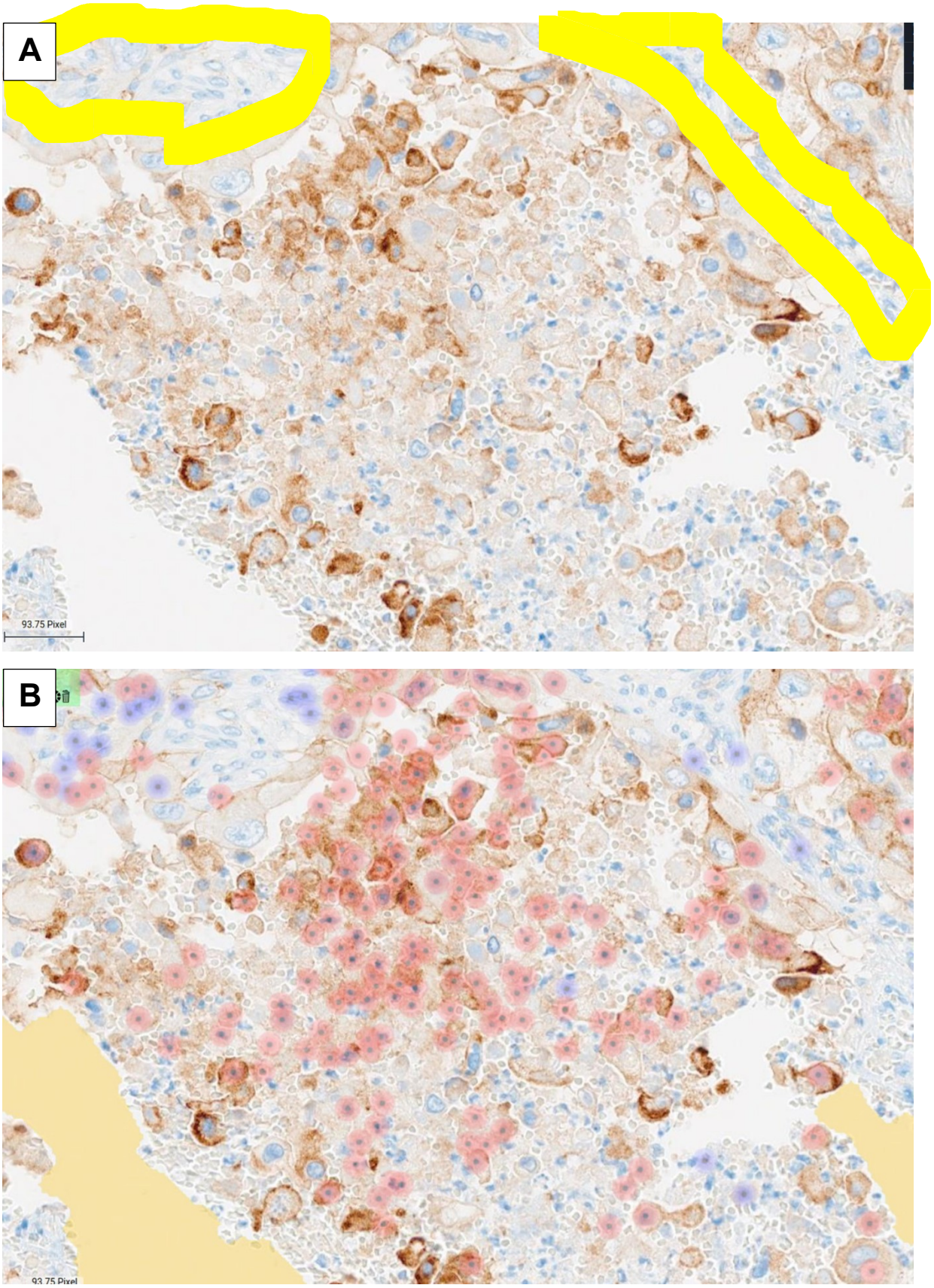

Figure 15: Two images representing slide with positive PD-L1 staining and not adequate concordance between pathologist and software. Software was not able to recognize negative tumor cells (marked with yellow line in A), while it performed evaluation on necrotic cells (red stained cells in B) and failed to recognize all vital and PD-L1 positive tumor cells.

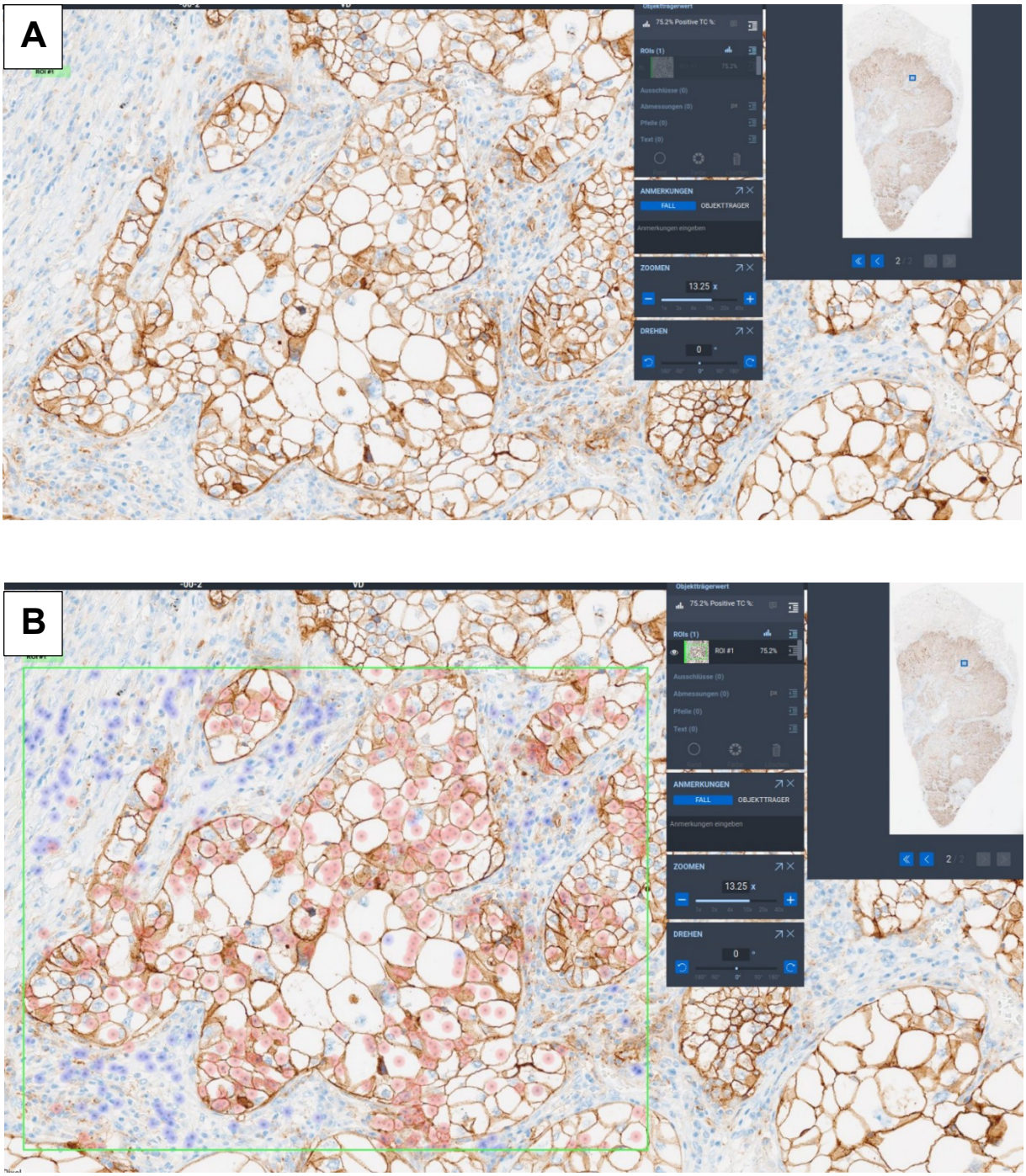

Figure 16: Two images representing slide with clearly positive PD-L1 staining in all tumor cells and not adequate concordance between pathologist and software. Software was not able to recognize that all tumor cells are positive (positive cells marked in red in B), while it recognized stroma cells as negative tumor cells (marked with blue in B).

6.3 Mesothelioma

MESO							
Software							
Pathologist	**Category**	**0**	**1**	**2**	**3**	**4**	**Total**
	0	5	7	-	-	-	12
	1	3	3	1	-	-	7
	2	2	3	-	-	-	5
	3	-	-	-	-	-	0
	4	-	-	-	-	-	0
	Total	10	13	1	0	0	

Table 7: Data matrix mesothelioma group

The data matrix of the mesothelioma group is presented in Table 7. In total, 24 mesotheliomas were evaluated. It is needed to be mentioned again that this software is not IVD-registered for the evaluation of PD-L1 in mesotheliomas. However, the results obtained are pretty similar to the results of the NSCLC evaluation.

Neither humans nor the software had a case with a score over 25% (no cases assigned to categories 3 and 4). In category 0, although the number of assigned cases is similar (12 by humans and 10 by software), there was an overall concordance of only 5 samples. In category 1, only three cases were assigned to the same category. Even though both humans and software categorized samples to category 2, none of them matched. The only case the software assigned to this class was evaluated as category 1 by humans. On the other hand, the 5 samples humans assigned to category 2 were rated as category 0 (2 cases) and 1 (3 cases) by software. Altogether, 8 out of 24 cases were concordant.

In this group, Cohen's kappa score calculation presented a value of -0.0667. Thus, the evaluation in the mesothelioma group showed a poor agreement.

7. Discussion

Even though lung cancer incidence has been decreasing over the past decades, it remains one of the most prevalent carcinomas in men and women. Furthermore, it is still the leading cause of cancer-related deaths (1). Since 1991, mortality in the USA has declined every year, which can be traced back to the reduction of tobacco smoking and the steady improvement in the early diagnosis and therapy of lung cancer (2).

In recent years there were two major breakthroughs in the therapy of NSCLC. The first one was the implementation of targeted therapies for lung carcinoma harboring activating mutations in EGFR (and since then, many other genes like ALK, ROS-1, BRAF…). The second one was the introduction of immune checkpoint inhibitors targeting PD-1/PD-L1 axis. Due to all these findings, today is reflex testing of lung adenocarcinoma for targetable mutations (by molecular methods), and all NSCLC for PD-L1 (by immunohistochemistry) a standard in diagnostic procedures of lung carcinoma. This is associated with careful management of small tissue/cytology samples to provide the correct and complete diagnosis with all necessary predictive markers. While molecular analysis is pretty much straightforward and not subject to pathologists´ subjective interpretation if a mutation is there or not, the PD-L1 evaluation is an entirely different story. First of all, there is more than one clone of PD-L1 antibody, which should be stained on different platforms. They are evaluated using different criteria (membranous vs. cytoplasmic vs. membranous/cytoplasmic staining) and different cut-offs for positivity (1%, 10%, 25%, 50%) depending on the potential drug and the line of therapy. Based on published data (84), these antibodies are pretty similar but not really interchangeable- they are not entirely concordant, and the results vary depending on the used clone. For example, clone SP142 will stain fewer tumor cells in comparison to SP263 or 22C3. If we set aside these issues concerning clones, platforms, and interpretation criteria, one variable which remains is a pathologist. It has been shown that pathologists among themselves are pretty concordant in evaluating PD-L1 (at least when dealing with tumor cells), but always with present differences, which persist even after training. Differences are not big, but even 10% means for certain patients a lot. If we think of therapy-relevant cut-offs, depending on the antibody and pathologist, one tumor sample can be scored as 40%, 50%, or 60%. Because of that, a fast method of evaluation that can be non-

subjective, with clear interpretation criteria, and algorithm of assessment, would be more than welcome.

Increasing digitalization and the use of Artificial intelligence (AI) and machine learning (ML) should support pathologists in finding the right diagnosis, with better prognostication and adequate evaluation of predictive markers. In many areas in pathology, it has already been demonstrated that there are certain advantages of these technologies compared to the evaluation of pathologists. Namely, the results are faster available, more objective, and more accurate (102). For example, breast cancer studies already showed an improvement of diagnostic accuracy using AI/ML as a supporting tool (103-105). Furthermore, evaluation of Gleason score in prostate carcinoma (106) is another excellent example of AI's possible application in the everyday, routine work of pathologists.

Based on these encouraging results and on the information that the first IVD software for PD-L1 evaluation has occurred, we decided to compare the results of evaluation made by humans to the one performed by software. On the one hand, we had data obtained by classical microscopic evaluation of PD-L1 stained slides compared with the results of digital scoring by uPath PD-L1 (SP263) image analysis for non-small cell lung carcinomas (Ventana, Roche).

Since this is ready-to-use software, we approached it in this way. Slides were simply scanned in the adequate scanner, and we run software on each slide without any annotations or selection of the region of interest (ROI). The concordance between the software results and the pathologist was not as high as expected. More precisely, the results were rather disappointing. The kappa score showed a slight agreement in the group with small biopsy samples and a poor agreement for the groups with resections and mesothelioma samples. We went back to the image analysis to see if we can detect the problem in this evaluation. While on some slides the recognition of tumor cells, stromal cells and PD-L1 positive cells was very good (Figures 13 and 14), it was not always the case. What was apparent is that the software was not able to recognize the tumor area by itself, so it practically evaluated the whole slides, regardless of the size or amount of tumor on it. The next issue was that it could not recognize the area of necrosis (which are often stained with secondary antibodies and therefore false positive) and macrophages, which are always nicely positive (Figures 15 and 16). Furthermore, when we looked closely at the cells which program identified as tumor cells, we have realized that it sometimes recognized the tumor

cells where there are not any tumor cells, and vice versa, it fails to recognize tumor cells (regardless of the reaction with PD-L1 immunohistochemistry) (Figures 15 and 16). Also, as expected, it did not interpret some positive reactions as positive, and therefore these cells were also not included in the final score.

At this point, we contacted Roche and discussed our obtained results. The software is IVD-approved for the detection of PD-L1 staining at the cut-off of 50% in NSCLC. However, it has not been tested/trained on enough small biopsy samples (due to availability). Furthermore, the prerequisite is that the pathologist should mark the ROI manually and, if possible, exclude areas of necrosis and/or macrophages. As we were aware, the software is not registered for mesothelioma evaluation. Still, we have decided to make this analysis in mesothelioma as well since there are some promising results in clinical studies with immunotherapy (97-100). After careful analysis of discrepancies from the company side, it was also noticed that in our PD-L1 staining protocol counterstaining with hematoxylin is 8 minutes. According to the protocol for software analysis, it should be 4 minutes. This can, of course, cause differences in interpretation because the contrast between positive and negative cells is smaller when our protocol was applied.

As this was a preliminary study for a bigger project, we have noticed important limitations. First of all, we have to adjust our PD-L1 staining protocol, which is not a problem. However, pathologists at our Institute prefer to have longer counterstaining because the tissue is better visualized in this way. Secondly, we have to mark manually on digital slides the ROI and exclude bigger areas of necrosis. This is also not a problem, but it is an additional time investment in routine work, making this automatic analysis less automatic. Even more, in the time needed for the annotations, an experienced pathologist might be finished with the PD-L1 scoring. And finally, the “real” slides should be evaluated by at least three different pathologists to obtain a value for software comparison. Only one on one, one human vs. software, it might be questionable whose score is correct.

To conclude, our study demonstrated huge discrepancies in the evaluation of PD-L1 on tumor cells in NSCLC and mesothelioma samples between humans and software. As mentioned in the previous paragraph, several points have to be addressed, and we expect the results to be much better (more concordant). However, even if those adjustments will be successful, the big question will probably remain- do we really

need software that cannot function autonomously, and pathologists must invest more time than required for the PD-L1 evaluation under a microscope?

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