Sensitivity of PDL-1-analysis from pleural effusion in Non-small cell lung cancer

Sensitivität einer PDL-1 Analyse bei NSCLC mit Pleuraerguß

Study Protocol

Study Sites

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1. Background / Rationale

The programmed death receptor-1 (PD-1) physiologically acts as an immune checkpoint receptor, enabling self-tolerance by T-cells in normal tissue. Unbound PD-1 allows the normal immune response by T cells to occur. Binding of PD-1 to the ligands PD-L1 and PD-L2 suppresses the immune response. Abnormal PD-L1 expression on tumor cells leads to activation of PD-1 and suppression of cytotoxic T-cell activity. The T-cell tolerance allows the tumour cells to avoid recognition and elimination by the immune system (1). In September 2014 Pembrolizumab, a humanized monoclonal immunoglobulin G4 kappa antibody against PD-1, was approved in the United States of America for the treatment of incurable melanoma in patients with disease progression following first line treatment. Anti-PD-1 therapies may be a novel therapeutic option in the treatment strategy of advanced non-small cell lung cancer (NSCLC). Data from the KEYNOTE-001 study showed that NSCLC patients with detectable PD-L1 expression had a response under treatment with Pembrolizumab (2). Positive results could be demonstrated in treatment-naïve as well as in previously treated advanced NSCLC. There is probably a correlation between tumor PD-L1 expression and improved pembrolizumab antitumor activity (3). In March 2015 Nivolumab, a humanized monoclonal immunoglobulin G4 kappa antibody against PD-1, was approved in the United States of America for the treatment of incurable NSCLC. The approval was reached after the publication of the CHECKMATE-017 trial, where a benefit in overall survival of 3.2 months could be demonstrated in comparison to docetaxel in subjects after standard platinum-based chemotherapy with following tumor progression (4). In the CHECKMATE-063 trial under Nivolumab a response could be achieved in 15% of subjects after at
least two lines of standard chemotherapy (5). In May 2015 a positive CHMP opinion was published by the EMA.
Lung cancer is the second most common cancer and is the primary cause of cancer-related death in both men and women in the United States (6). Currently, 80% of patients with lung cancer are given a diagnosis of primary NSCLC. Malignant pleural effusion (MPE) is a common complication of advanced lung cancer. The presence of MPE indicates a poorer prognosis for patients with lung cancer and impairs their quality of life. Pleural effusion is a convenient clinical sample with important clinical diagnostic significance. It may be an alternative source supplying useful information about the neoplasm's biology in terms of moleculargenetic and immunopathologic profile.

2. Objectives

This is a prospective diagnostic pilot study to create hypotheses regarding immunocytochemistry (ICC) PD-L1 analysis of pleural effusions in NSCLC patients as compared to the reference standard of PD-L1 immunohistochemistry (IHC). This comparison will be done to assess sensitivity and specificity of PD-L1 detection by ICC in pleural effusions.

The main hypothesis is, that ICC analysis of PD-L1 from pleural effusion has a relevant diagnostic value with a specificity of ~90% as compared to the immunohistochemical reference standard.

3. Outcome

3.1. Primary Outcome Measures

- Number/prevalence of PD-L1-positive patients according to immunohistochemistry (IHC) of pleural biopsy
- Number/prevalence of PD-L1-positive patients according to immunocytochemistry (ICC) of pleural aspirate

3.2. Secondary Endpoints

- IHC results of different bronchoscopic samples

4. Study Population

The population for selection of potential study participants is comprised of clinical routine patients presenting with (suspected malignant) pleural effusion. These patients must have an indication for pleural puncture. The cause and nature of the pleural effusion (e.g. presence of non-small cell lung cancer, NSCLC) at this time point will be unknown in many cases. To establish this, further routine diagnostic procedures are required. While patients will be included before the planned routine examinations and thus before presence of NSCLC can be confirmed, only patients with confirmed diagnosis of non-small cell lung cancer will be included in the data analysis.

A patient in whom suspicion of pleural manifestation of lung cancer is not confirmed, will be considered a screening failure and thus excluded from the study.

4.1. Inclusion Criteria

- Age ≥ 18 years
- Presence of malignant pleural effusion with indication for pleural puncture and thoracoscopy
- Confirmed diagnosis of non-small cell lung cancer according to ERS guidelines (to be confirmed after study inclusion when results from routine clinical examinations are available)
- Written informed consent

4.2. Exclusion Criteria

- Age <18 years
- Pregnancy and/or lactation
- Acute and life-threatening illness (instable angina pectoris, acute pulmonary arterial embolism, myocardial infarction, etc.)

- Any contraindication to undergo thoracoscopy (e.g. anticoagulation therapy which cannot be discontinued for the procedure)
- Any medical, psychological or other condition impairing the patient's ability to provide informed consent
- Exclusion of non-small cell lung cancer as cause of pleural effusion after clinical routine examinations leads to study exclusion of patient

5. Study Plan and Assessments

5.1. Selection of Patients

Patients presenting within clinical routine with pleural effusion and suspected or known underlying lung cancer and who satisfy the in- and exclusion criteria as far as they can be assessed at that time, are considered for study participation.

These patients will be informed about all routine clinical examinations and assessments before informed consent will be obtained. However, informed consent for study participation will be obtained before (routine) pleural puncture to ensure that patients agree with the study-specific additional diagnostic analysis of the collected pleural effusion material.

All invasive procedures described herein are part of the patient's clinical routine treatment to achieve a clinical diagnosis. The routine examinations and study-associated data collection for each patient are outlined below. The individual patient's study participation will be concluded, once all of these steps are completed.

5.2. Anthropometry, Vital Signs, Medical History, Medication

The following data will be collected for analysis within the study:

1. Anthropometric data (age, gender, height, weight, BMI)
2. Office blood pressure according to ESH/ESC guidelines
3. Current medication
4. Medical history incl. any oncological pre-medication
5. Smoking history

5.3. **Pleural puncture** (non-study specific routine examination)

The pleural effusion will be treated and diagnosed by puncturing of the pleural cavity at the location of effusion. During this process the excess pleural fluid will be removed/collected and used for later analysis by an external pathology department.

5.4. **Thoracoscopic pleural biopsy** (non-study specific routine examination)

After pleural puncture, histological pleura samples will be obtained by pleural biopsy, primarily applying internistic thoracoscopy. If internistic thoracoscopy is not feasible (e.g. due to distinct intergrowth), the patient will undergo surgical thoracoscopy. In either case, at least five biopsies will be taken.

5.5. **Optional bronchoscopic lung biopsy** (non-study specific routine examination)

In some cases, suspicious tumor masses within the lung and/or intrathoracic lymph nodes are detected by preceding radiologic assessment and routinely sampled for further histological analyses. This applies in particular when no histological samples can be obtained from the pleura. In these cases, the corresponding results will also be considered for study-associated analysis.

5.6. Immunocytochemistry (ICC) and Immunohistochemistry (IHC)

The pleural fluid obtained by pleural puncture will be in part used for standard clinical routine analysis and another part will be subject to study-specific PD-L1 analysis. The same applies to the histological samples obtained by thoracoscopy and potential bronchoscopy. These analyses will reveal nature and cause of the pleural effusion. In case the corresponding results confirm an NSCLC diagnosis, all results from ICC and IHC will be collected for analysis within the study. Should the results rule out NSCLC, the patient will be considered a screening failure and excluded from the study.

For PD-L1 analysis in both ICC and IHC, the PD-L1 IHC 22C3 pharmDx Kit (Dako) will be used.

5.7. Documentation of Adverse Events

All adverse events occurring during each patient's actual study participation will be assessed for diagnostic procedure relatedness. Procedure-related adverse events will be documented for later study-specific analysis.

6. Statistical Analysis

6.1. Primary Analysis

The main hypothesis is, that ICC analysis of PD-L1 from pleural effusion has a relevant diagnostic value with a specificity of ~90% as compared to the immunohistochemical reference standard. To test this hypothesis, the number of PD-L1 positive and negative detections for each sample type will be documented and used for calculation of sensitivity and specificity. Furthermore, the frequency with 95% confidence interval of PD-L1 positive patients according to ICC of pleural fluid will be calculated.

6.2. Secondary Analyses

Secondary analyses comprise intra-individual comparative descriptive analyses of all pathological results to assess correlation between ICC and IHC results.

7. Sample Size Calculation

According to previous experience concerning the value of ICC in lung cancer diagnosis, sensitivity/specificity of this method is expected to be lower as compared to IHC. While exact figures for PD-L1 detection (ICC and IHC) are not available we anticipate a specificity of approx.. 90% of PD-L1 detection via ICC as compared to IHC. Previous application of the PD-L1 antibody used for detection in histological samples revealed a prevalence of approx. 25% in PD-L1 positive NSCLC patients (Garon et al. 2015). Buderer (1996) described a method for sample size calculation for sensitivity and specificity incorporating the prevalence of disease. We used this method and specified the required parameters as followed:

Sample size (n) was calculated according to the formula:

$$n = \frac{{Z_{\alpha/2}}^2 \frac{SP(1-SP)}{W^2}}{(1-P)}$$

where

$Z_{\alpha/2}$	z value from standard normal distribution with α=0.05
SP	specified specificity (90%)
W	width of 95% confidence interval (10%)
P	prevalence of disease (25%)

This resulted in a number of 46 patients, which was amended by a drop-out rate of approx. 10% to give a total patient number of

n=50 patients

which should at least allow for a valuable qualitative assessment of the feasibility of PD-L1 diagnostic based on ICC and provide a basis for possible future studies.

8. References

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9. Abbreviations

ICC	Immunocytochemistry
IHC	Immunohistochemistry
PD-L1	Programmed death-ligand 1
ERS	European Respiratory Society
ESH	European Society of Hypertension
ESC	European Society of Cardiology
BMI	Body-Mass Index
NSCLC	Non-small cell lung cancer