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Comparison of *PD-L1* mRNA Expression Measured with the CheckPoint Typer® Assay with PD-L1 Protein Expression Assessed with Immunohistochemistry in Non-small Cell Lung Cancer

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Abstract. *Background: Immunohistochemical (IHC) assessment of programmed death-ligand 1 (PD-L1) in non-small cell lung cancer (NSCLC) has become important since the development of anti-PD-1/-PD-L1 directed drugs. Various PD-L1 antibodies and cut-offs have been used in different trials to predict response to these drugs, thus comparison of those studies is difficult. We compared PD-L1 mRNA expression measured by RT-qPCR with PD-L1 protein expression evaluated by IHC. Moreover, we investigated the impact of different tumour tissue acquisition methods on the reliability of PD-L1 measurement techniques. Materials and Methods: NSCLC cases (N=22), including n=9 mediastinal lymph node biopsies acquired by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and n=5 metastases, were evaluated prospectively for PD-L1 protein on tumor cells (TC) and immune cells (IC) using E1L3N and 28-8 antibodies and PD-L1 mRNA using the CheckPoint TYPER® assay. Results: In primary NSCLC tissues, agreement between PD-L1 mRNA and TC staining using the 28-8 antibody was excellent (κ=0.85, p=0.0002). Comparing both PD-L1 antibodies against each other showed a kappa value of 0.58 (p=0.0106). In EBUS-TBNA, PD-L1 mRNA correlated perfectly with the 28-8 antibody (κ=1.0, p=0.0023). PD-L1 mRNA levels significantly differed when comparing 28-8 TC staining of tumours >49% with 1-49% and 0% (p=0.0040; p=0.0081, respectively). In metastatic lesions, differences between PD-L1 mRNA and IHC became apparent (κ=0.2, p=0.2525). Conclusion: Testing of PD-L1 mRNA and 28-8 IHC showed an excellent agreement in NSCLC samples including mediastinal lymph node biopsies. Since PD-L1 expression in >50% TC detected by 28-8 IHC can be reliably detected by RT-qPCR, quantitative PD-L1 mRNA determination should be considered as an alternative to IHC as there is no interobserver variability in RNA results.*

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Investigation of cancer development and the immune system is one of most relevant research topics in oncology in the last decade: both the tumour-promoting role of chronic inflammation and the tumour escape from immune destruction are regarded now as "hallmarks of cancer" by Hanahan and Weinberg (1). During tumour evolution and spread, several mechanisms of immunoediting can be found: immunogenic tumour cells are recognised by the immune cells and are either partially (equilibrium) or totally

eliminated by the adaptive immune system or they evade immune response. Furthermore, some tumours do not show immunogenicity from the beginning. (2) Besides further immune regulatory mechanisms, such as regulation of tumour-associated macrophages and regulatory T-cells, tumour escape can be mediated by influencing the interaction of T-cell inhibitory receptors. T-cells express many co-stimulatory and co-inhibitory molecules on their surface that could be drug-targeted. Understanding the mechanism of immunosuppressive and immunoactive signaling as well as the interaction between IC and TC enabled to find new targets for antitumour therapy (3).

The inhibitory receptor programmed death 1 (PD-1), expressed on T-cells, is activated by the ligand PD-L1 expressed on antigen presenting cells and tumour cells. This PD-1/PD-L1 axis and interaction with further immune co-receptors/ligands was investigated by Freeman *et al*. (4 PD-1/PD-L1 interaction leads to down-regulation of the cytotoxic T-cell activity against the tumour and thus, can lead to immune escape. Blocking the interaction of the PD-1/PD-L1 axis became a major goal in development of the so called immune checkpoint inhibitory drugs. Treatment with the anti-PD-1 drugs, nivolumab and pembrolizumab, and the anti-PD-L1 antibody, atezolizumab, showed good response rates in a variety of tumour entities, *e.g*. melanoma, renal cell, bladder, and lung cancer (5). Nivolumab improved overall survival in advanced NSCLC in several phase III trials in contrast to classical chemotherapy (6 (7) and hence, was approved by FDA. Additionally, a phase II/III trial, showed an outcome benefit with the PD-1 inhibitory substance pembrolizumab compared to docetaxel in advanced NSCLC when at least 50% of tumour cells were PD-L1 positive (IHC staining) (8). Hence, PD-L1 protein expression is discussed to be a predictive biomarker and recently, pembrolizumab was approved for first-line application in metastatic NSCLC with tumours expressing >50% of PD-L1 (9). However, PD-L1 IHC expression evaluation lacks uniform standardisation. In a variety of the phase II/III trials for each anti-PD-L1/PD-1 immune checkpoint inhibitory substance, PD-L1 protein detection by IHC was performed using a different antibody, and PD-L1 positivity was defined using different cut-offs for each antibody. Because of this complex variety of PD-L1 IHC testing and treatment options, Scheel *et al*. compared assessment of four PD-L1 antibodies regarding the comparability of the antibody per se and regarding the interobserver concordance in a round-robin study (nine pathologists). They found SP263 to stain most tumour cells, 22C3 and 28-8 to stain the same proportions of positive tumour cells, and SP142 to stain less tumour cells than the other antibodies. Depending on using a 6-step-score system or dichotomous cut-offs, interobserver agreement for PD-L1 positive tumour cells were moderate (Light's kappa=0.47-0.50) and good (κ=0.12-0.25), respectively (10). Smith *et al*. and Coqswell *et al*. compared SP263 and 28-8, respectively, with another antibody (E3L1N) in NSCLC and reported SP263 and 28-8, to be superior (11) (12).

Another issue in evaluation of PD-L1 status in NSCLC is whether PD-L1 staining depends on the modality of tissue acquisition and origin of tumour tissue, respectively. In daily clinical routine, tumour tissue is not always extracted directly from the primary lung tumour but often from mediastinal lymph nodes via endobronchial ultrasound as transbronchial fine-needle aspiration (EBUS-TBNA). Occasionally, biopsy material originates from liver, adrenal, bone or brain metastases.

In this study, we addressed two issues: First, we analysed whether measurement of PD-L1 mRNA expression is comparable to the evaluation of PD-L1 protein expression, since comparison of all the different immune checkpoint inhibitor studies, PD-L1 antibodies, and cut-offs is complicated, and moreover IHC can be subject to analytical errors, *e.g*. interobserver variability. Secondly, we also investigated whether the measurements differ depending on the origin of the tumour sample, derived by primary lung, mediastinal lymph node or distant metastasis.

Materials and Methods

Study design and cases. Prospectively, a total number of n=22 NSCLC, diagnosed during routine diagnostics at the Institute of Pathology Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg (including n=9 probes obtained by EBUS-TBNA and n=5 cases of distant metastatic tissue) was collected in 2015. Approval of the local academic ethics committee of the University of Erlangen was obtained. After review of all cases regarding adequate tumour tissue on haematoxylin and eosin (H&E) slides and marking of tumour region, IHC and RNA extraction were performed in the Institute of Pathology Erlangen. In 2015, the PD-L1 antibody routinely used in the Institute of Pathology Erlangen was the clone E1L3N (Cell Signaling, USA). At the end of 2015, the Institute of Pathology Erlangen established 28-8 (Abcam, UK) for routine diagnostics, according to numerous clinical trials using 28-8. Therefore, all cases were reevaluated for PD-L1 protein expression on tumour cells (TC) and separately on immune cells (IC).

Tissue processing and IHC. Immunohistochemical staining was conducted on 1 μm thick sections of formalin-fixed paraffin embedded (FFPE) tumour blocks according to manufacturer's protocol on Ventana Benchmark Ultra (Ventana Medical Systems, Inc. Tucson, AZ, USA).

PD-L1 IHC scoring. Positivity of PD-L1 was diagnosed when at least 1 % of TC and IC, respectively, was stained. Intensity of staining was classified as weak, intermediate, and strong. Additionally, IHC subgroups were divided (0 %, 1-49 % and ≥50% positive stained cells). E1L3N staining was conducted in daily routine (*i.e*. in different batches) and assessed routinely by one of two experienced pathologists (A.H., R.R). 28-8 IHC was stained in one batch and evaluated retrospectively by two experienced pathologists (A.H., R.E.) blinded to the E1L3N results.

RNA Isolation and RT-qPCR. For RNA extraction from FFPE tissue, a single 10 μm curl was processed according to a commercially available bead-based extraction method (XTRACT kit; STRATIFYER Molecular Pathology GmbH, Cologne, Germany). RNA was eluted with 100 μl elution buffer and RNA eluates were then stored at –80˚C until use. The mRNA expression levels of PD-1 and PD-L1 as well as reference gene CALM2, were determined by RT-qPCR, which involves reverse transcription of RNA and subsequent amplification of cDNA executed successively as a 1-step reaction using Taqman Primer/Probes. Each patient sample or control was analysed with each assay mix in triplicates. The experiments were run on a Versant kPCR system (Siemens, Erlangen, Germany) according to the following protocol: 5 min at 50˚C, 20 sec at 95˚C followed by 40 cycles of 15 sec at 95˚C and 60 sec at 60˚C. Forty amplification cycles were applied and the cycle quantification threshold (Cq) values of three markers and one reference gene for each sample (S) were estimated as the median of the triplicate measurements. The final values were generated by using ΔCT from the total number of cycles to ensure that normalised gene expression obtained by the test is proportional to the corresponding mRNA expression levels. Measurements took place continuously in the routine setting of the Institute of Pathology Erlangen and were done by one experienced investigator (S.H.).

Statistical analysis. Statistical analysis assessing agreement between the two protein-based IHC and the RNA based RT-qPCR technology was performed by kappa statistics using the JMP SAS 9.0.0 and group-wise comparison by scatter plots and non-parametric Mann-Whitney testing was done using Graph Pad PRISM 5.04 software (R.W.).

Results

We evaluated PD-L1 protein expression by IHC separately in TC and IC, and also PD-L1/PD-1 mRNA levels in formalin-fixed paraffin embedded tumour tissue of NSCLC (total NSCLC cohort, n=22). Tissue was acquired either from primary lung tumour by biopsy (n=8), from mediastinal lymph node by EBUS-TBNA (n=9) or from distant metastasis (liver or bone) (n=5) (Figure 1). In n=17 cases (entire data set) IHC staining was performed with both antibodies, and 72.7% and 73.3% were stained positive for TC with 28-8 and E1L3N, respectively.

There was a kappa value of 0.58 (p=0.0106) when both PD-L1 antibodies were compared with each other. Figure 2 shows NSCLC cases with positive, negative and discordant PD-L1 staining, respectively, using both the 28-8 and the E1L3N antibody. PD-L1 mRNA expression was significantly associated with tumour cell positivity determined by 28-8 IHC (Spearman rho 0.8975; p<0.001). Agreement between PD-L1 mRNA and 28-8 TC staining was excellent (κ=0.85, p=0.0002). Furthermore, there was a significant positive association between PD-1 mRNA expression and 28-8 TC staining (Spearman rho 0.8075; p=0.0002) and between PD-1 mRNA and PD-L1 mRNA, respectively (Spearman rho 0.8212; p<0.001). Both PD-1 mRNA and PD-L1 mRNA showed no significant association with the PD-L1 positive immune cell infiltrate (28-8 PD-L1 IC, Figure 3, Table I). In our common data set (5 cases excluded from all data set due to missing data of IHC or qRT-PCR), agreement between PD-L1 mRNA and 28-8 TC staining was even perfect (κ=1.0, p=0.0003) whereas agreement was only moderate when comparing 28-8 with E1L3N staining (κ=0.57, p=0.0142) (Table II).

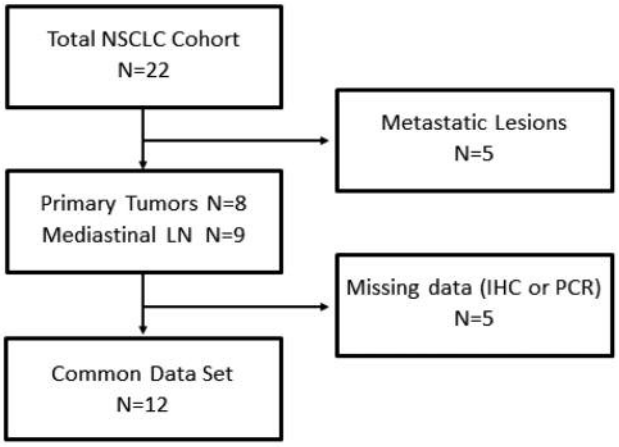

Figure 1. *Cohort diagram. NSCLC, non-small cell lung cancer; IHC, immunohistochemistry; LN, lymph nodes; PCR, polymerase chain reaction.*

In mediastinal lymph node material (n=8) PD-L1 mRNA expression levels showed perfect agreement with the 28-8 antibody (κ=1.0, p=0.0023) whereas the PD-L1 TC staining with both antibodies showed only a trend to moderate agreement (Table III).

In metastatic lesions (n=5) differences between predefined PD-L1 mRNA NSCLC categorisation and PD-L1 protein expressed on TC became apparent (κ=0.2, p=0.2525). Comparison between both 28-8 and E1L3N TC staining results in metastases was invalid due to the small number of cases (Table IV).

Furthermore, we compared PD-L1 mRNA levels between 28-8 TC IHC groups according to current standards (0% *vs.* 1-49% *vs.* >49%) in the common data set. There was a nice and significant association between expression by mRNA and IHC, respectively. Here, PD-L1 mRNA expression measured by RT-qPCR showed a direct correlation to 28-8 IHC staining defined by 0%, 1-49% and >49% positively stained TC, respectively. (p=0.0040, p=0.0081, respectively) (Figure 4).

Discussion

Our results indicate that prospective testing of PD-L1 mRNA by CheckPoint TYPER® in clinical routine setting and central reevaluation of 28-8 protein staining proved to have

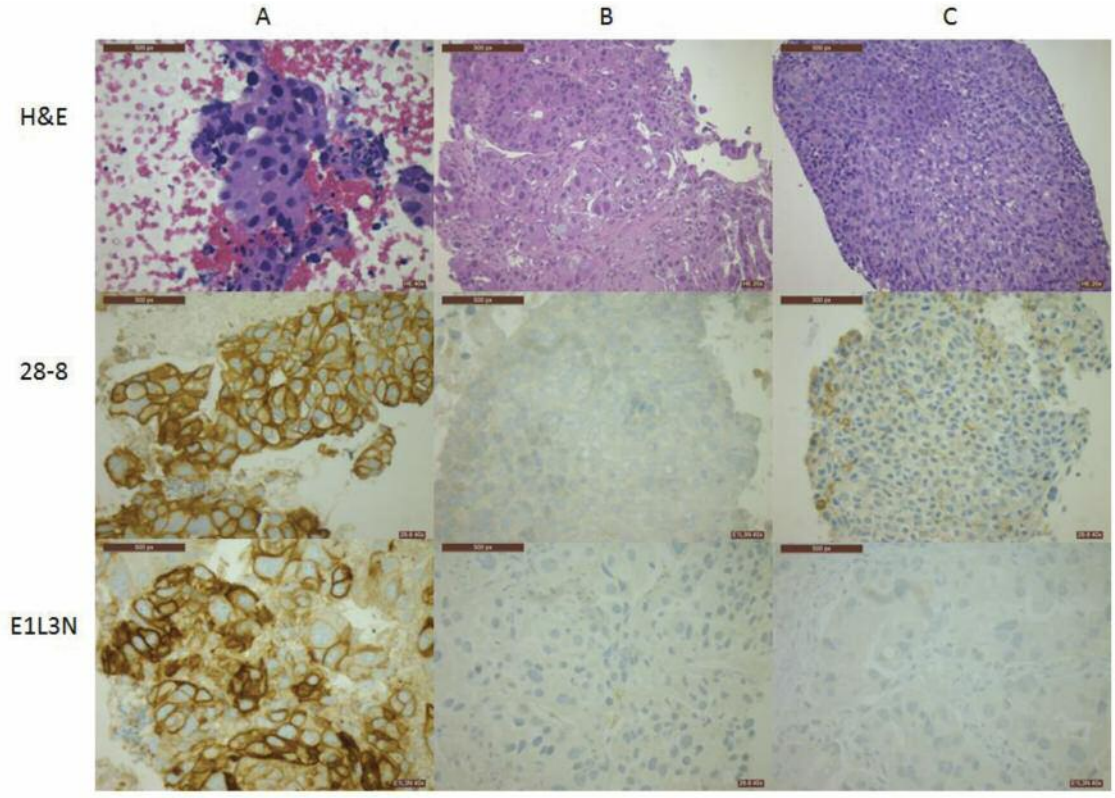

Figure 2. *Staining of three NSCLC cases with H&E (case A with 400×, cases B and C with 200× magnification, respectively), PD-L1 IHC using 28-8 antibody (400× magnification), and PD-L1 IHC using antibody E1L3N (400× magnification) A) positive case; B) negative case; C) discrepant case. NSCLC, Non-small cell lung cancer; PD-L1, programmed death-ligand 1; IHC, immunohistochemistry; H&E, haematoxylin and eosin; 28-8 and E1L3N, anti-PD-L1 antibodies.*

Non parametric Spearman Correlation

Variable	Covariable	Spearman ρ	p-value
CPT - PDL1 (40-DCT)	CPT - PD1 (40-DCT)	0,8212	<,0001*
28-8 - PDL1 - IC (%)	28-8 - PDL1 - TC (%)	0,2332	0,3678
CPT - PD1 (40-DCT)	28-8 - PDL1 - TC (%)	0,8075	0,0002*
CPT - PDL1 (40-DCT)	28-8 - PDL1 - TC (%)	0,8975	<,0001*
CPT - PD1 (40-DCT)	28-8 - PDL1 - IC (%)	0,2249	0,4024
CPT - PDL1 (40-DCT)	28-8 - PDL1 - IC (%)	0,4559	0,0759

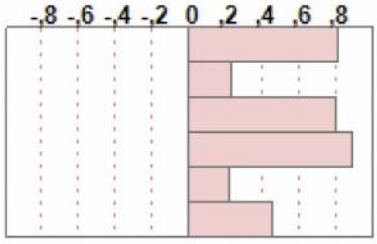

Figure 3. *Non parametric Spearman correlation between PD-L1 protein expression determined by 28-8 IHC as well as PD-L1 mRNA and PD-1 mRNA determined by RT-qPCR. PD-L1, Programmed death-ligand 1; PD-1, programmed death 1; mRNA, messenger ribonucleic acid, PD-1 NSCLC, non-small cell lung cancer; EBUS-TBNA, endobronchial ultrasound - transbronchial fine-needle aspiration; IHC, immunohistochemistry; 28-8, anti-PD-L1 antibody; TC, tumor cells; RT-qPCR, real time quantitative polymerase chain reaction; CPT, CheckpointTyper; DCT, delta CT (PCR cycle).*

an excellent correlation in primary NSCLC tumour samples including lymph node biopsies. In contrast, agreement of PD-L1 mRNA expression and protein determination in metastatic lesions requires cut-off adoption according to tissue type. However, PD-L1 mRNA expression can be reliably detected by RT-qPCR in non-macrodissected primary NSCLC tumour samples that have >50% PD-L1 positivity confirmed by 28-8 antibody labelling. Therefore, quantitative PD-L1 mRNA determination seems to be a reliable alternative to protein estimation by IHC.

Non-existing standardisation of an IHC protocol for the detection of PD-L1 protein expression is an issue of major concern. Out of many commercial available anti-PD-L1 clones like E1L3N (Cell Signaling), the antibodies used in the clinical trials are 28-8, 22C3 (both Dako), SP142 and SP263 (both Ventana) with the recommended cut-offs of positively stained TC ≥1%, ≥50%, ≥1% and ≥25%, respectively. Scheel *et al*. conducted a German-wide harmonisation study analysing 15 large pulmonary resection specimens (adenocarcinoma n=11, squamous cell carcinoma n=4) that were centrally stained for PD-L1 with two laboratory developed assays using E1L3N (Cell Signaling Technology, Cambridge, UK) and SP142 (Spring Bioscience Corporation, Pleasanton, CA, USA) antibodies and thereafter, decentral assessed by nine independent pathologists to enable better comparability of future companion diagnostic and trial results (17). However, previous phase II/III trials that showed clinical impact of immune checkpoint inhibitors were performed with different antibodies and cut-offs and hence, comparison is difficult.

Furthermore, NSCLC biopsy material acquired in clinical routine can be of minor quality as surgical specimens due to crush artefact, less tumour cell content or contamination with non-tumourous cells. All those factors may influence validity of PD-L1 IHC results. Moreover, implementation of IHC assays at local sites might contribute to additional variances when compared to central staining by IHC test producers. Cree *et al*. discussed implementation challenges and developed guidance for PD-L1 assessment in the UK (13). They addressed further research topics including the relevance of origin of tissue and reproducibility of methods. Hence, they support the requirement of a standardised and valid method for assessment of PD-L1.

In conclusion, both, the use of several different antibodies as a predictor and IHC being subject to pre- and analytical errors (*e.g*. different methods of tissue acquiring, a variety of diagnostic antibodies, kits and platforms as well as cut-offs, interobserver variability), must be argued. In line with those criticisms of robustness and validity of PD-L1 IHC, McLaughlin *et al*. have described heterogeneity and discordance between different PD-L1 assays (IHC, clones E1L3N and SP142, and QIF) for NSCLC (14). This could be confirmed by the present study, where 28-8 and E1L3N TC IHC showed only a moderate correlation. In contrast, PD-L1 mRNA levels revealed a significant and excellent correlation with PD-L1 positive TC stained with the 28-8 PD-L1 antibody and thus, qRT-PCR can be discussed as a valid and robust alternative. This became even more apparent, when we divided 28-8 staining of TC in three subgroups defined by the cut-offs >1% and >49%, which comes up to clinical practice, at least for pembrolizumab pretreatment decision.

Standardised evaluation of PD-L1 status in NSCLC in daily routine is a great challenge not only concerning the reliability of the assessment method but also for its efficacy in different

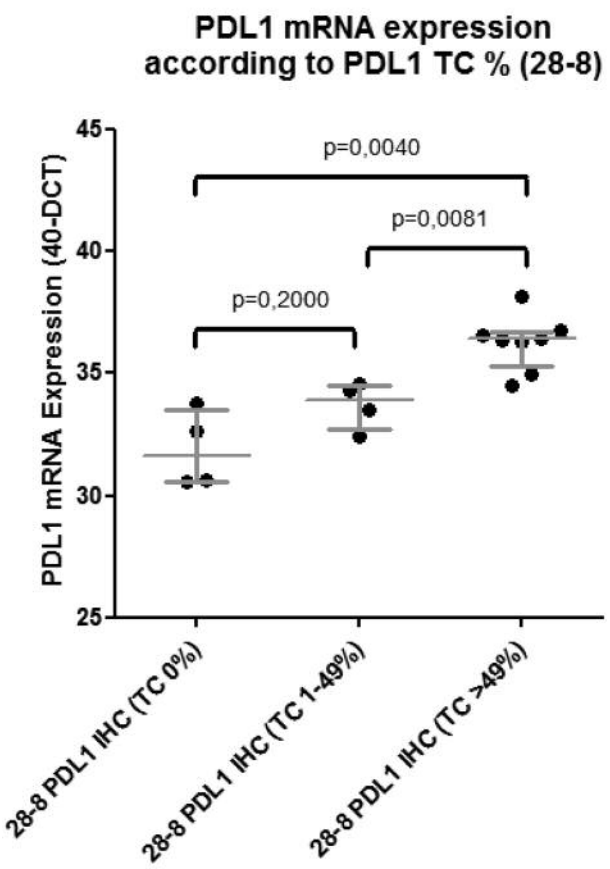

Figure 4. Scatter plot and non parametric Mann Whitney test of PD-L1 determination in primary NSCLC tumors by prospective CheckPoint Typer testing in comparison with central reevaluation of DAKO 28-8 TC staining. PD-L1, Programmed death-ligand 1; mRNA, messenger ribonucleic acid, PD-1 NSCLC, non-small cell lung cancer; EBUS-TBNA, endobronchial ultrasound - transbronchial fine-needle aspiration; IHC, immunohistochemistry; 28-8, anti-PD-L1 antibody; TC, tumor cells; DCT, delta CT (PCR cycle).

tissue sampling procedures. Since EBUS-TBNA is an often used, less invasive alternative to transbronchial biopsy (TBB) of the primary lung lesion, investigation whether primary lung specimen and mediastinal metastatic lymph nodes provide comparable information about PD-L1 status in NSCLC is mandatory. Addressing this topic, Sakakibara *et al*. compared EBUS-TBNA with TBB and surgical specimens and showed that EBUS-TBNA had less crush artefacts and more TC. Moreover, they found good agreement between PD-L1 expression in EBUS-TBNA samples and the corresponding primary tumors (n=6, r=0.75, p=0.086). (15) Sheffield *et al*. found high concordance when comparing the anti-PD-L1 clones 28-8, SP142, RBT-PDL1 and E1L3N with each other in 80 primary NSCLC (Cohen's Kappa=0.67) and 78% concordance of primary NSCLC with matched lymph nodes

Table I. *Cross tabulation of PD-L1 determination in primary NSCLC/EBUS-TBNA tumor samples by DAKO 28-8 IHC compared to prospective PD-L1 mRNA determination by RT-qPCR (upper section) and cell signaling E1L3N IHC (lower section) (all data sets without metastasis).*

PD-L1 status	28-8 TC negative	28-8 TC positive	
CPT-negative	4	1	5
CPT-positive	0	12	12
	4	13	17

Kappa value 0.85, p=0.0002

PD-L1 status	28-8 TC negative	28-8 TC positive	
E1L3N TC-negative	2	2	4
E1L3N TC-positive	0	9	9
	2	11	13

Kappa value 0.58, p=0.0106

NSCLC, Non-small cell lung cancer; EBUS-TBNA, endobronchial ultrasound - transbronchial fine-needle aspiration; PD-L1, programmed death-ligand 1; IHC, immunohistochemistry; 28-8 and E1L3N, anti-PD-L1 antibodies; TC, tumor cells; RT-qPCR, real time quantitative polymerase chain reaction; CPT, CheckpointTyper.

Table II. *Cross tabulation of PD-L1 determination by retrospective DAKO 28-8 IHC compared to both prospective PD-L1 mRNA determination by RT-qPCR (upper sectional) and cell signaling E1L3N IHC (lower section) (common data set).*

PD-L1 status	28-8 TC negative	28-8 TC positive	
CPT-negative	2	0	2
CPT-positive	0	10	10
	2	10	12

Kappa value 1.0, p=0.0003

PD-L1 status	28-8 TC negative	28-8 TC positive	
E1L3N TC-negative	2	2	4
E1L3N TC-positive	0	8	8
	2	10	12

Kappa value 0.57, p=0.0142

PD-L1, Programmed death-ligand 1; IHC, immunohistochemistry; 28-8 and E1L3N, anti-PD-L1 antibodies; TC, tumor cells; RT-qPCR, real time quantitative polymerase chain reaction; CPT, CheckpointTyper.

as well as a consistency between in situ hybridization (RNA) and IHC results. (16). Our results add to this information by providing an excellent correlation of PD-L1 mRNA levels with protein levels detected with 28-8 IHC in EBUS-TBNA obtained samples (TC), but only a trend to moderate agreement between PD-L1 protein levels detected with 28-8 and E1L3N IHC (TC). Moreover, concordance between primary tumour and NSCLC metastases regarding PD-L1 expression status needs further clarification. Pinato *et al*. described 12 % discordance between PD-L1 positive (IHC) NSCLC primary lesions (n=65) and the matched metastases (17). We did not investigate matched pairs of primary and metastatic lesions but NSCLC metastases per se as this reflects the routine clinical practice. Here, an association of PD-L1 mRNA with IHC could not be evaluated due to limited number of cases. In our study, 28-8 TC IHC was also significantly associated with PD-1 mRNA levels but impact on prognosis has to be investigated. Schmidt *et al*. found PD-1 positive tumour-infiltrating lymphocytes (TILs) assessed by IHC in 22% of a cohort of 321 NSCLC but no correlation with prognosis (18). In contrast, another study showed positive prognostic impact of PD-1 positive TILs (19). It remains unclear whether PD-L1 expression on TC or on IC has more impact on prognosis in NSCLC patients and on prediction of response to checkpoint inhibitors (20).

We did not find any agreement between mRNA levels of both PD-1 and PD-L1 with 28-8 IHC in IC. Further investigation of the correlation between PD-L1 expression levels (imRNA and/or protein) and prognosis or response to anti-PD-1/PD-L1 therapy is of utmost importance. Velcheti *et al*. reported PD-L1 mRNA expression assessed by using the RNAscope method to be associated with tumour-infiltrating lymphocytes and better outcome (21). The most notable limitation of our study is the small number of cases. But nevertheless, the prospective measurements of PD-L1/PD-1 mRNA showed significant agreement with IHC. One can argue that 28-8 was evaluated retrospectively, however it was assessed by two independent pathologists, both blinded to former E1L3N IHC results. The fact that PD-L1 protein expression evaluated with the E1L3N clone was less well-associated with PD-L1 mRNA than 28-8, could be due to routine setting with changing batches. Nevertheless, if we define retrospective, blinded, and central assessed 28-8 TC IHC as gold standard and compare both prospectively and routinely performed CheckPoint TYPER® assay and E1L3N TC IHC with it, measurements of qRT-PCR performed by one technician on different days and in different batches seems to be more reliable than E1L3N TC IHC evaluated in daily routine. Additionally, based on our evidence we cannot make any statement in matters of clinical

Table III. *Cross tabulation of PD-L1 determination in EBUS-TBNA by retrospective DAKO 28-8 IHC compared to both prospective PD-L1 mRNA determination by RT-qPCR (upper sectional) and cell signaling E1L3N IHC (lower section) (common data set).*

PD-L1 status	28-8 TC negative	28-8 TC positive	
CPT-negative	2	0	2
CPT-positive	0	6	6
	2	6	8

Kappa value 1.0, p=0.0023

PD-L1 status	28-8 TC negative	28-8 TC positive	
E1L3N TC-negative	1	1	2
E1L3N TC-positive	0	6	6
	1	7	8

Kappa value 0.57, p=0.0607

Table IV. *Cross tabulation of PD-L1 determination in metastatic lesions (n=5) of the liver and bone by retrospective DAKO 28-8 IHC compared to prospective (upper sectional) PD-L1 mRNA determination by RT-qPCR and cell signaling E1L3 (lower section).*

PD-L1 status	28-8 TC negative	28-8 TC positive	
CPT-negative	1	2	3
CPT-positive	0	1	1
	1	3	4

Kappa value 0.2, p=0.2525

PD-L1 status	28-8 TC negative	28-8 TC positive	
E1L3N TC-negative	0	2	2
E1L3N TC-positive	0	2	2
	0	4	4

Kappa value not applicable (n.a.), p=n.a.

PD-L1, Programmed death-ligand 1; IHC, immunohistochemistry; 28-8 and E1L3N, anti-PD-L1 antibodies; TC, tumor cells; RT-qPCR, real time quantitative polymerase chain reaction; CPT, CheckpointTyper.

PD-L1, Programmed death-ligand 1; IHC, immunohistochemistry; 28-8 and E1L3N, anti-PD-L1 antibodies; TC, tumor cells; RT-qPCR, real time quantitative polymerase chain reaction; CPT, CheckpointTyper.

outcome. However, based on the results of this study we collect biopsies of NSCLC patients treated by immune checkpoint inhibitors and investigate the expression of PD-1 mRNA, PD-L1 mRNA, and PD-L1 protein as robust predictive markers. In conclusion, we could demonstrate that PD-L1 mRNA expression in NSCLC cells correlated significantly with PD-L1 protein expression, detected by 28-8 IHC assay. Moreover, this accordance applies also for the three cut off levels (<1%, 1-49%, >49%) used in clinical practice. Additionally, mediastinal lymph node samples obtained by EBUS TBNA are an excellent source of genetic material for PD-1 and PD-L1 mRNA expression evaluation. These data show that mRNA based expression of PD-L1 in FFPE material is at least an alternative to IHC, which is robust and not observer-dependent.

Novelty and Impact

Since assessment of PD-L1 status in non-small cell lung cancer is important but there is lack of a standardized evaluation of PD-L1 immunohistochemistry, we analysed whether measuring PD-L1 mRNA correlates with PD-L1 protein expression. Furthermore, we could show that evaluation of PD-L1 status in mediastinal lymph node biopsies (EBUS-TBNA) is as reliable as measuring in lung biopsies.

Conflicts of Interest

R.W. is founder of STRATIFYER Molecular Pathology GmbH. R.W. and E.V. are employees of STRATIFYER Molecular Pathology GmbH. F.F., J.H.F., A.H. and W.M.B. received honoraria from BMS, MSD, Novartis and Roche.

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