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Review

Cytology for PD-L1 testing: A systematic review

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ABSTRACT

Evaluation of tumoral programmed cell death ligand-1 (PD-L1) expression is standard practice for patients with advanced non-small-cell lung cancer (NSCLC) who may be candidates for treatment targeting the programmed cell death-1 (PD-1)/PD-L1 pathway. Currently, all of the commercially available immunohistochemistry assays have been validated for use with histology specimens although, in routine clinical practice, approximately 30–40 % of patients with advanced NSCLC have only cytology specimens available for diagnosis, staging, and biomarker analysis. This systematic review evaluated the success rate, concordance, and clinical utility of using cytology specimens to assess tumor PD-L1 expression levels compared with histology specimens from patients with advanced NSCLC. EMBASE and PubMed database searches identified 142 unique, relevant publications, of which 15 met the inclusion criteria for at least one analysis. In 709 specimens, across seven publications, the proportion of cytology specimens evaluable for PD-L1 testing was 92.0 %. Among nine studies eligible for concordance analysis between cytology and histology specimens at a PD-L1 tumor cell expression cutoff of ≥50 %, overall percentage agreement was 89.7 % (n = 428), 72.0 % for positive percentage agreement (n = 218), and 95.0 % for negative percentage agreement (n = 258); results using a tumor PD-L1 expression cutoff of ≥1 % were similar. Our analyses suggest that using cytology specimens to assess PD-L1 expression is feasible, with good levels of concordance between cytology and histology specimens using PD-L1 tumor cell expression cutoffs of ≥1 % and ≥50 %. In conclusion, there is no convincing evidence that cytology specimens are inadequate or inferior to histology specimens for assessing PD-L1 expression in patients with NSCLC.

1. Introduction

Antibodies targeting the programmed cell death-1 (PD-1)/programmed cell death ligand-1 (PD-L1) pathway have improved outcomes for patients with advanced non-small-cell lung cancer (NSCLC), and are now approved in several countries [1–7]. As improved clinical outcomes with these antibodies are often associated with higher tumor PD-L1 expression levels in NSCLC [1,3,7,8], different PD-L1 QJ/>expression cutoffs have been investigated in clinical trials and as part of the co-development of immunohistochemistry (IHC) assays. This has led to a number of approvals which restrict the use of PD-(L)1-targeting antibodies to patients whose tumors express PD-L1 above prespecified thresholds. For example, pembrolizumab is approved by both the United States Food and Drug Administration and the European Medicines Agency for the first-line treatment of patients with metastatic NSCLC whose tumors have high PD-L1 expression (tumor proportion score ≥50 %) and no *EGFR* mutations or *ALK* rearrangements, with a recent extension in the United States and Japan to all patients whose tumors express PD-L1 (tumor proportion score ≥1 %) [3,8–11]. Alongside molecular testing for genomic aberrations, PD-L1 testing is therefore mandated or recommended to help guide treatment decisions [12,13].

The validity of testing for PD-L1 expression in cytology specimens, which are characterized by the dispersed nature of the tumor cells (TCs) within them, represents a practical problem for pathologists and oncologists. To reiterate, only histology specimens, in which TCs are maintained within their structural and architectural context, were used in clinical trials of antibodies targeting the PD-1/PD-L1 pathway and during the co-development of IHC-based assays. As a result, a range of IHC assays for PD-L1 testing are now approved and commercially available as either companion or complementary diagnostics. These include the VENTANA PD-L1 (SP142) Assay [14]; the VENTANA PD-L1 (SP263) Assay [15]; the PD-L1 IHC 22C3 pharmDx assay [16]; and the PD-L1 IHC 28-8 pharmDx assay [17].

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Cytology specimens are therefore not formally validated as a substrate for the assessment of PD-L1 expression using IHC assays. However, approximately 30–40 % of patients with advanced NSCLC have only cytology specimens available for diagnosis and analysis of predictive or prognostic biomarkers [18,19]. These cytology specimens include smears or cell blocks obtained from pleural or pericardial effusions or by fine-needle aspiration (FNA) of the primary tumor or its metastases, including endoscopic bronchial ultrasound (EBUS)-guided aspirates of intrathoracic lymph nodes. FNAs are not only less invasive than tissue biopsies, but facilitate sampling multiple zones within the same tumor and its metastases during a single procedure [20]. Furthermore, there is an additional rationale to evaluate this approach for PD-L1 assessment as current guidelines already accept the use of cytology specimens for detecting *EGFR* mutations and *ALK* or *ROS1* gene rearrangements [21]. With EBUS-guided aspiration of intrathoracic lymph nodes now the preferred approach in most thoracic oncology centers for the diagnosis, staging, and profiling of NSCLC [22], exclusion of such specimens from PD-L1 testing will potentially deny many patients appropriate and effective treatment. Understanding the feasibility and validity of using cytology specimens for assessing PD-L1 expression in patients with NSCLC is therefore highly pertinent.

Here, we report the results of a systematic review of PD-L1 testing in cytology specimens from patients with advanced NSCLC. We evaluated the success rate of using cytology specimens to assess PD-L1 expression levels and concordance with histology specimens, and reviewed any available data comparing efficacy in patients who had PD-L1 expression assessed using cytology or histology specimens.

2. Methods

We searched the EMBASE and PubMed databases (search date February 11–12, 2019) using the terms 'PD-L1 and NSCLC and (cytolog* OR FNA OR fine-needle aspirate OR cell smear OR cell block OR endobronchial ultrasound [EBUS] OR transbronchial needle aspiration [TBNA])' present in any field. The asterisk (*) serves as a truncation symbol. Additional references were identified using bibliographies. Abstracts and papers were manually reviewed for relevance. Articles that were not written in English and studies that did not report PD-L1 assessed by IHC were excluded.

For the analysis of success rate of PD-L1 testing, specimens had to have ≥100 TCs. Success was defined as the proportion of submitted specimens that was evaluable for PD-L1 testing. For the concordance analysis, prespecified inclusion criteria were: negative, overall, and positive percentage agreement (NPA, OPA, and PPA, respectively) reported or that were derivable from raw data; analysis of paired (matched patient) cytology and histology specimens; reported cutoff for PD-L1 TC expression (defined as the percentage of TCs with any membranous PD-L1 expression); and NSCLC. Data from studies using either cell blocks or smears were included in the concordance analysis. Where data from both were presented within the same paper, results from cell blocks were used. Both success rate and concordance analyses used only studies reported in peer-reviewed journals. However, the review of clinical efficacy data included abstracts due to limited data availability. If multiple abstracts or manuscripts referred to the same dataset, the most recent manuscript was used, or the most recent abstract where only abstracts were available.

For the concordance analysis, upper and lower 95 % confidence intervals (CIs) were calculated using the Clopper–Pearson method [23].

3. Results

Our searches of the EMBASE and PubMed databases identified a total of 141 unique publications and abstracts, with one additional publication identified through bibliography searches (Fig. 1). In total, 13 peer-reviewed publications were included in at least one analysis. Seven peer-reviewed publications were included in the analysis of success rate, and nine were included in the concordance analysis; one manuscript and two abstracts reporting clinical efficacy data were also identified. Due to the scarcity of data and the small sample numbers analyzed in these publications, efficacy data are described in the Discussion section.

3.1. Success rate analysis

Based on 709 specimens tested across seven studies reported in peer-reviewed publications [24–30], the success rate for PD-L1 testing

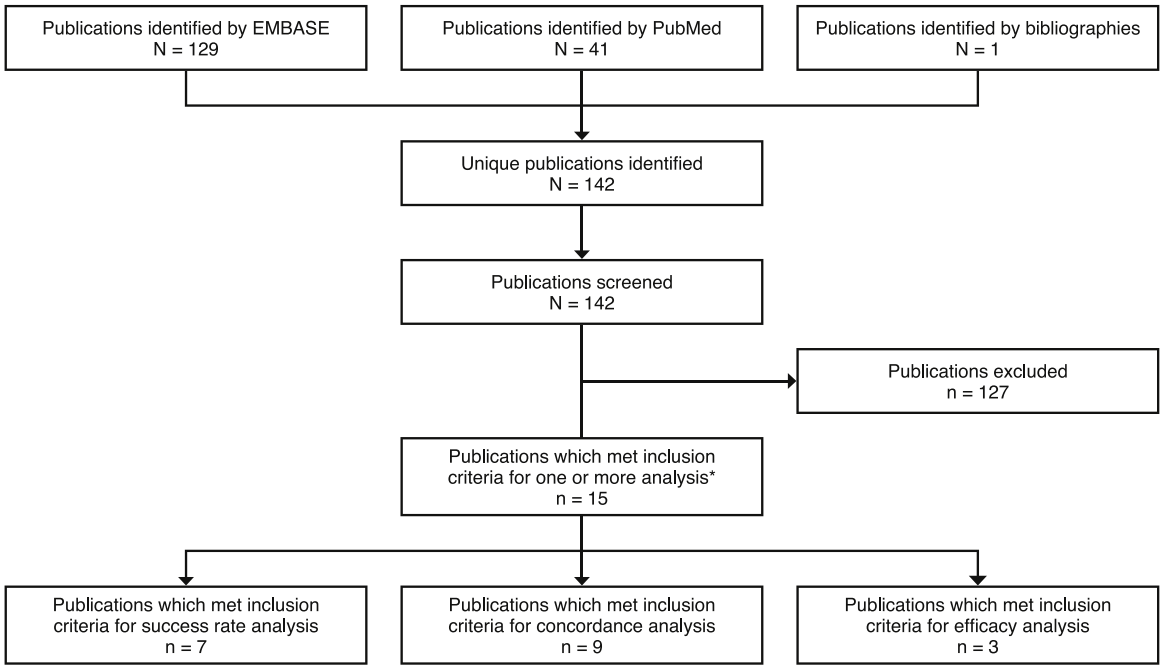

Fig. 1. Flow diagram of study inclusion.
*Publications which met inclusion for success rate, concordance or efficacy analysis; publications may have been included in more than one analysis.

Table 1
Success[a] rate of PD-L1 testing using cytology specimens[b] from patients with NSCLC in eligible publications identified by systematic review.

Reference	Sample type	Fixation method	Antibody clone	N	Success rate (%)
Capizzi et al. (2018) [24]	Smear	MicroFix spray (Diapath SpA, Italy)	Ventana SP263	50	98
Noll et al. (2018) [25]	Smear	Not specified	22C3 pharmDx	41	90
	Cell block	Formalin fixation	22C3 pharmDx	41	93
Wang et al. (2018) [26]	Cell block	Cytolyt (Hologic Inc., Marlborough, MA, USA) or alcohol, followed by 10 % NBF; 10 % NBF alone; TissuFix (Chaptec Inc, Montreal, Canada); or Cytolyt or 50 % alcohol alone[c]	22C3 pharmDx	371	92
Torous et al. (2018) [27]	Cell block	Formalin fixation[d]	22C3 pharmDx	94	94
Heymann et al. (2017) [28]	Cell block	NBF or Cytolyt	22C3 pharmDx	40	90
Biswas et al. (2018) [29]	Cell block	Alcohol fixation	22C3 pharmDx	50	86
Stoy et al. (2018) [30]	Cell block	Not specified	Abcam 28.8	22	91

NBF, neutral-buffered formalin; TC, tumor cell.
[a] Success defined as the proportion of submitted specimens that were evaluable for PD-L1 testing.
[b] All cytology specimens used to determine the success rate had ≥100 TCs.
[c] The fixation method was known for 261 (76 %) cytology specimens.
[d] Specimens were collected into Cytolyt fixative solution, before further formalin fixation.

Table 2
Concordance in PD-L1 staining levels between matched histology and cytology specimens[a] from patients with NSCLC in eligible studies identified by systematic review.

Reference	Antibody clone	Sample collection	N	PD-L1 cutoff[b]	OPA	PPA	NPA
Arriola et al. (2018) [31]	22C3 pharmDx	Core needle biopsy	15	TC ≥ 1 %	80.0	84.6	50.0
				TC ≥ 50 %	100	100	100
Hernandez et al. (2019) [32]	22C3 pharmDx	FNA, pleural effusion or bronchial brushes	52	TC ≥ 1 %	76.9	71.1	92.9
				TC ≥ 50 %	82.7	69.6	93.1
Heymann et al. (2017) [28]	22C3 pharmDx	EBUS-FNA, FNA, thoracentesis or pericardiocentesis	4	TC ≥ 1 %	75.0	75.0	N/A
				TC ≥ 50 %	100	100	100
Ilie et al. (2018) [33]	22C3 (laboratory developed test)	Pleural effusion or bronchial washes	70	TC ≥ 1 %	97.1	95.8	97.8
				TC ≥ 50 %	95.7	94.7	96.1
Noll et al. (2018) [25]	22C3 pharmDx	EBUS-FNA, ultrasound-guided FNA, or CT-guided FNA	38	TC ≥ 1 %	97.4	100	90
				TC ≥ 50 %	92.1	81.3	95.4
Sakata et al. (2018) [34]	22C3 pharmDx	EBUS-TBNA	61	TC ≥ 1 %	86.9	72.4	100
				TC ≥ 50 %	82.0	46.7	93.5
Skov et al. (2017) [35]	22C3 pharmDx	CT-guided FNA, FNA, EBUS-/EUS-FNA, or pleural effusion	86	TC ≥ 1 %	84.9	80.0	89.1
				TC ≥ 50 %	94.2	100	93.3
Capizzi et al. (2018) [c] [24]	Ventana SP263	FNA	49	TC ≥ 1 %	69.4	61.8	86.7
				TC ≥ 50 %	83.7	64.2	94.3
Munari et al. (2019) [c] [36]	Ventana SP263	FNA	53	TC ≥ 1 %	81.1	84.4	76.2
				TC ≥ 50 %	90.6	66.7	100

CT, computed tomography; EBUS-FNA, endobronchial ultrasound-guided fine-needle aspiration; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; EUS-FNA, endoscopic ultrasound-guided fine-needle aspiration; FNA, fine-needle aspiration; N/A, not applicable or calculable (no negative histology specimens based on the TC ≥1 % cutoff level); NPA, negative percentage agreement; OPA, overall percentage agreement; PPA, positive percentage agreement; TC, tumor cell; TPS, tumor proportion score.
[a] All data presented were based on analysis of cell blocks, except as indicated.
[b] PD-L1 expression level based on proportion of TC staining or TPS.
[c] Comparison based on cytological smear.

using cytology specimens was 92.0 % (Table 1). This was consistent with the success rate based on data published additionally in 13 abstracts (93.2 %; N = 1331; data not shown).

3.2. Concordance analysis

Nine studies were eligible for analysis of concordance between matched cytology and histology specimens with PD-L1 expression data based on staining on TCs (Tables 2 and 3) [24,25,28,31–36]. Across the nine studies identified, the OPA for cytology and histology specimens (n = 428) was 88.3 % (95 % CI: 86.9–91.2) and 89.7 % (95 % CI: 86.5–92.4) for specimens with ≥1 % and ≥50 % of TCs expressing PD-L1, respectively. The PPA for cytology and histology specimens with ≥1 % of TCs expressing PD-L1 (n = 218) was 78.0 % (95 % CI:

Table 3
Overall concordance in PD-L1 staining levels between matched cytology and histology specimens across nine studies identified by systematic review.

	PD-L1 expression cutoff			
	n	PD-L1 TC ≥ 1 %	n	PD-L1 TC ≥ 50 %
OPA, % (95 % CI)	428	88.3 (86.9–91.2)	428	89.7 (86.5–92.4)
PPA,[a] % (95 % CI)	218	78.0 (71.9–83.3)	100	72.0 (62.1–80.5)
NPA,[a] % (95 % CI)	140	89.3 (82.9–93.9)	258	95.0 (91.5–97.3)

CI, confidence interval; NPA, negative percentage agreement; OPA, overall percentage agreement; PD-L1, programmed cell death ligand-1; PPA, positive percentage agreement; TC, tumor cell.
[a] NPA and PPA data only derivable for a subset of publications.

71.9–83.3) and 72.0 % (95 % CI: 62.1–80.5) for specimens with ≥50 % of TCs expressing PD-L1 (n = 100), while the NPA was 89.3 % (95 % CI: 82.9–93.9) and 95.0 % (95 % CI: 91.5–97.3) for specimens with ≥1 % of TCs expressing PD-L1 (n = 140) and ≥50 % of TCs expressing PD-L1 (n = 258), respectively.

4. Discussion

To date, evaluation of tumoral PD-L1 expression as a predictive biomarker for response to immune checkpoint blockade in patients with NSCLC has been informed by the assessment of histology specimens with most data coming from pivotal, registration-directed trials. However, the reality is that, in many centers, cytology specimens are frequently used to evaluate PD-L1 expression levels and to inform treatment decisions for many patients with advanced NSCLC. A better understanding of the validity of using this sampling approach for PD-L1 analysis is therefore of considerable clinical relevance.

To our knowledge, this is the first systematic review comparing PD-L1 testing using cytology and histology specimens. Our analyses suggest that use of cytology specimens to assess PD-L1 expression is feasible, with over 90 % of PD-L1 tests using cytology specimens yielding an interpretable result. Additionally, we found good levels of concordance between cytology and histology specimens that had PD-L1 expression on ≥1 % or ≥50 % of TCs, with an OPA of 88.3 % and 89.7 %, respectively. Based on our results, which suggest high levels of OPA and NPA between cytology and histology specimens for scoring PD-L1 expression, false positive results are expected to be rare when using cytology specimens. However, because the PPA was lower than the OPA and NPA between cytology and histology specimens across both PD-L1 cutoffs, there is a risk that PD-L1 expression in cytology specimens might be underestimated in some cases. Despite this, these analyses support the use of cytology specimens for the current clinically relevant PD-L1 expression cutoffs, including the approvals for pembrolizumab in the first-line setting based on ≥1 % and ≥50 % cutoffs [3,8–11], and the approval of durvalumab in the European Union for patients with locally advanced, unresectable NSCLC who have not progressed following platinum-based chemoradiotherapy and whose tumors express PD-L1 (PD-L1 TC ≥ 1 %) [37].

Our systematic literature search identified three retrospective studies that compared clinical efficacy in patients with advanced NSCLC using either cytology or histology specimens to assess PD-L1 expression. In one study, 232 consecutive patients were assessed for PD-L1 expression levels; the majority of patients had advanced NSCLC and 81 patients had tumors with ≥50 % of TCs expressing PD-L1. Of these, 20 patients received first-line treatment with pembrolizumab (11 based on testing using cytology specimens and 9 based on histology specimens) and 19 were evaluable for efficacy analysis. The objective response rate was 20 % in the cytology group and 22 % in the histology group (p = 1.0). The disease control rate (DCR) at 6 weeks was 60 % and 56 % for the cytology and histology groups, respectively (p = 1.0) [27]. In another study, 65 patients with advanced NSCLC were treated with immunotherapy targeting the PD-1/PD-L1 pathway; 50 (77 %) patients received pembrolizumab, 10 (15 %) patients received nivolumab, and 5 (8 %) patients received other immunotherapies in randomized clinical trials. Twenty (31 %) patients were treated in the first-line setting, 36 (55 %) patients in the second-line setting, and the remaining patients were treated in the third-line setting. Thirty-eight of the 65 patients had tumors that expressed PD-L1 on ≥50 % of TCs and were available for efficacy analysis according to the type of specimen used for PD-L1 IHC staining. In these patients, there was no statistically significant difference between patients assessed for PD-L1 expression using cytology (n = 13; DCR 69 %) or small biopsy specimens (n = 25; DCR 68 %; p = 0.93) [38]. A third study compared clinical outcomes in patients seen by a medical oncologist for consideration of systemic therapy, including immune checkpoint blockade, between March 2014 and June 2017, and whose tumors had PD-L1 expression on ≥50 % of TCs based on either cytology or histology specimens. There was no difference in median overall survival between patients assessed using cytology (n = 32; 11.9 months) or histology specimens (n = 37; 8.0 months), irrespective of treatment received (adjusted hazard ratio 0.98; 95 % CI: 0.43–2.26). Of note, patients in the cytology group received more systemic treatment than the histology group (p = 0.009), while the histology group received more palliative radiotherapy (p = 0.044) [39]. Although the identified clinical data were limited by very small patient numbers, were of a retrospective nature and, in one publication identified, the compared patient groups received different amounts of systemic treatment and palliative radiotherapy, the results from these studies suggest that patients whose tumors have PD-L1 expression on ≥50 % of TCs assessed using cytology specimens may have similar clinical outcomes to those in whom it was assessed using histology specimens. However, these findings need to be confirmed in prospective, appropriately designed trials.

There are several inherent challenges associated with assessing PD-L1 expression levels in cytology compared with histology specimens. It is clear, for example, that the dispersion of cells in cytology compared with histology specimens may make scoring PD-L1 expression particularly challenging at low expression levels and when applied to a cutoff of ≥1 % of TCs expressing PD-L1. Additionally, due to the fact that cell membranes in cell smears or cytospins are intact rather than cut, membranous expression of PD-L1 is less clear than in cell blocks or tissue sections [40]. These challenges may explain the observation that PPA was lower than the achieved values for OPA and NPA in our analysis. Most of the cytology PD-L1 expression data have, however, been derived from assessment of stained cut-sections from cell block preparations, an approach more likely to recapitulate the findings in a tissue biopsy sample.

There are also inherent challenges associated with the handling of cytology compared with histology specimens, including the use of fixatives. Compared with histology specimens, a much wider range of fixatives is used for cytology specimens and, in the case of cells blocks, there is also variability in the preservative solutions used before formalin fixation and paraffin embedding [40]. Additionally, it has been suggested that both the antigenicity and expression levels of PD-L1 might be affected by using alcohol-based fixatives, rather than neutral-buffered formalin as routinely used for histology specimens [18]. However, in one study, PD-L1 expression did not differ either qualitatively or quantitatively between cytology specimens fixed in formalin or alcohol [26]. Similarly, in a recent study comparing 50 matched pairs of EBUS-guided aspirates taken from the same tumor deposit or lymph node during the same procedure, there was no significant difference, qualitatively or quantitatively, between specimens fixed in either alcohol or formalin in terms of the pattern or extent of PD-L1 expression [41]. In addition, there was no difference according to whether the alcohol-fixed specimens were or were not post-fixed in formalin.

A minimum of 100 cells is generally accepted as a necessity to ensure reliable interpretation of PD-L1 expression using IHC [19,42]. Our analysis of the success rate of cytology specimens to assess PD-L1 expression was restricted to studies that specified that the specimens assessed had ≥100 % TCs. Across the nine studies included in the concordance analysis, six specified that specimens had to have ≥100 TCs and the remaining three studies had a majority of specimens with ≥100 TCs. Interestingly, in one study, agreement between PD-L1 testing of cytology specimens with fewer than 100 cells and the corresponding histology specimens was much lower than the agreement observed based on cytology specimens with ≥100 cells [32]. Another study, however, which included bronchial washings and cells from pleural effusions [33], described good concordance irrespective of the number of TCs in the specimen. However, in a further study, a minimum of 100 TCs was required in a single biopsy specimen to assess PD-L1 expression to predict responses to treatment with nivolumab in patients with non-squamous NSCLC [43].

The importance of assessing the reliability of cytology specimens for PD-L1 assessment across commercially available assays has been recognized by the Blueprint PD-L1 IHC Comparability Project [44]. In this study, 24 expert pulmonary pathologists from 15 different countries scored a set of 22 cytology specimens. Moderately good agreement was observed between pathologists for the scoring of TCs across five commercially available IHC assays (intraclass correlation coefficient [ICC] 0.78 and 0.85 for glass slide and digital readings, respectively), although the agreement was lower than that observed in histology specimens (ICC 0.89 and 0.93 for glass slide and digital readings, respectively). A further analysis from this study evaluated the concordance between FNAs, core biopsies, and large sections from the same resected tumor, and reaffirmed the comparability of cytology and histology specimens for the assessment of PD-L1 expression by IHC [45]. However, pathologists rated a greater proportion of specimens from FNA cell blocks compared with tissue biopsies as 'unevaluable' for determining PD-L1 expression level. A separate, prospective, observational study comparing PD-L1 expression in cytology and histology specimens from 184 patients with NSCLC of any stage (NCT03092739) has recently been completed, although data have yet to be published [46].

Limitations of our systematic review include the use of different assays for assessing PD-L1 expression in the studies reviewed. Additionally, none of the studies included in our review involved the assessment of PD-L1 expression on immune cells, which are likely to be more challenging to measure in cytology specimens. For example, reliable assessment of PD-L1 expression by immune cells cannot be made using aspirates of lymph nodes, because such cells might not be integral to the tumor. Various types of cytology specimen were analyzed and there was wide variation in sample handling as well as likely variability in the expertise of pathologists scoring the specimens. Some of the studies had only small sample numbers, particularly those comparing clinical outcomes based on specimen type used to evaluate PD-L1 expression.

The use of cytology specimens for assessing PD-L1 expression levels via IHC assays is rapidly becoming accepted clinical practice. Given the prevalence of patients who have only cytology specimens available for diagnosis, staging, and biomarker analysis, it is a real-world necessity that these specimens are used for the assessment of PD-L1 expression. Pragmatism is required, however, when considering the use of practices not formally validated in clinical trials to ensure that patients are not denied effective treatment. The exclusion of cytology specimens from clinical trials is unfortunate but, to some extent, understandable. Most of these trials incorporate supplementary studies for extensive translational biomarker research, although the variability of cytology specimens and the lack of adequate, usable tumor content in many of them would lead to their exclusion. Furthermore, companies developing PD-L1 biomarker assays will usually not have access to cytology specimens for assay validation. Consequently, validation of the practical approaches required in real-world clinical diagnostics is left to the clinical community. Comprehensive and robust data are essential to give this community confidence in using cytology specimens for PD-L1 testing and to reduce the use of more invasive procedures.

The available evidence from the studies identified and analyzed in this systematic review suggests that cytology specimens are an adequate substrate for assessing PD-L1 expression in patients with advanced NSCLC, with no convincing evidence that they are inferior to histology specimens. That said, there are two caveats: that assessment must be confined to TCs and that a minimum of 100 TCs must be available in the specimen for analysis. It might also be prudent to standardize across laboratories the procedures used in preparing cytology specimens for IHC, particularly with regard to routinely post-fixing in formalin specimens already fixed in alcohol. Until further evidence is provided, 10 % neutral buffered formalin should be considered the gold-standard fixative. Additionally, data summarized in this review support the use of cytology specimens for assessing PD-L1 expression at current clinically relevant cutoffs. Furthermore, although there will always be some inherent variability in the proficiency and experience among pathologists, there is nothing to suggest that cytology specimens cannot be used with confidence. Despite the degree of variation in pre-analytical factors and sample preparation with cytology specimens, compared with the relatively standard procedures used for histology specimens, we fully expect that experience in the use of cytology specimens will undoubtedly build knowledge and expertise in the different aspects of their assessment. As with all aspects of clinical practice, the evidence must be robust in order to provide confidence in utilizing a particular approach. In this instance, we may need to adopt a more pragmatic approach to assess the utility of cytology specimens which, for the reasons previously outlined, may not achieve validation through the usual clinical trial process.

Conflict of interests

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