CURRENT ONCOLOGY
A Canadian Cancer Research Journal

REVIEW ARTICLE

Harmonization of PD-L1 testing in oncology: a Canadian pathology perspective

D.N. Ionescu MD,* M.R. Downes MB BCh BAO MD,[†‡] A. Christofides MSc RD,[§] and M.S. Tsao MD[‡||]

ABSTRACT

Checkpoint inhibitors targeting the programmed cell death 1 protein (PD-1) and programmed cell death ligand 1 (PD-L1) are demonstrating promising efficacy and appear to be well tolerated in a number of tumour types. In non-small-cell lung cancer, head-and-neck squamous cell carcinoma, and urothelial carcinoma, outcomes appear particularly favourable in patients with high PD-L1 expression. However, assays for PD-L1 have been developed for individual agents, and they use different antibody clones, immunohistochemistry staining protocols, scoring algorithms, and cut-offs. Given that laboratories are unlikely to use multiple testing platforms, use of one PD-L1 assay in conjunction with a specific therapy will become impractical and could compromise treatment options. Methods to harmonize testing methods are therefore crucial to ensuring appropriate treatment selection. This paper focuses on lung, bladder, and head-and-neck cancer. It reviews and compares available PD-L1 testing methodologies, summarizes the literature about comparability studies to date, discusses future directions in personalized diagnostics, and provides a pathologist's perspective on PD-L1 testing in the Canadian laboratory setting.

Key Words Immunotherapy, lung cancer, bladder cancer, PD-L1 assays, immunohistochemistry

Curr Oncol. **2018 June;25(3):e209-e216** **www.current-oncology.com**

INTRODUCTION

The programmed cell death 1 protein (PD-1) is a checkpoint co-inhibitory receptor on the surface of immune cells that, under normal conditions, represents a negative feedback mechanism to switch T cell activation off, thereby protecting tissues from damage during immune system stimulation[1]. When activated through binding with one of its ligands, PD-L1 or PD-L2, PD-1 inhibits kinases involved in T cell activation through the phosphatase SHP2. Expression of PD-1 is high in $CD4^+Foxp3^+$ regulatory T cells, which are present in tumours in large numbers. In contrast, PD-L1 expression has been found in human tumour-associated antigen-presenting cells such as dendritic cells, macrophages, fibroblasts, and T cells[2].

The induction of PD-L1 expression is controlled by cytokines such as interferon γ (IFNγ)[2]. Effector T cell IFNγ is thought to be responsible for high levels of PD-L1 expression in the tumour microenvironment, which occurs as a resistance mechanism after immune challenge. Other mechanisms of PD-L1 upregulation involve gene amplification and other cancer signalling pathways. Amplification of chromosome 9p24.1, which is responsible for PD-L1 and PD-L2, was observed in Hodgkin lymphoma and was subsequently detected in a subgroup of patients with gastric carcinoma, colon carcinomas, triple-negative breast cancers, and glioblastomas. In addition, signalling pathways thought to influence PD-L1 expression include *PTEN* deletions, *PI3K* or *AKT* mutations (or both), *EGFR* mutations, *MYC* overexpression, cyclin-dependent kinase 5 disruption, and an increase in PD-L1 transcripts[3]. A large proportion of tumour-infiltrating lymphocytes show PD-1 expression. By expressing PD-L1 to activate the PD-1 signalling cascade, cancer cells might evade immune-mediated destruction by tumour-infiltrating lymphocytes[1]. Novel checkpoint inhibitors that block the PD-1 pathway are therefore being developed to enhance antitumour immune functions in the treatment of cancer.

Several of the immune checkpoint inhibitors that have been developed have demonstrated remarkable clinical activity[4]. For the sake of brevity, the discussion here is limited to non-small-cell lung cancer (NSCLC), head-and-neck squamous cell carcinoma (HNSCC), and urothelial carcinoma (UC)—tumour types in which PD-L1 testing is currently necessary or provides important information to select patients who are most likely to benefit from checkpoint inhibitor therapies. Immune checkpoint therapy has, however, been used successfully in other

Correspondence to: Diana Ionescu, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia V6T 2B5. E-mail: dionescu@bccancer.bc.ca ■ **DOI:** https://doi.org/10.3747/co.25.4031
Supplemental material available at www.current-oncology.com.

cancer types such as melanoma[5,6], Merkel cell carcinoma[7], Hodgkin lymphoma[8], and solid tumours with mismatch repair deficiency[9]—uses that are not addressed further in this review.

To date, the PD-1 inhibitors nivolumab and pembrolizumab and the PD-L1 inhibitors atezolizumab, durvalumab, and avelumab have demonstrated promising efficacy and tolerability in the tumour types to be discussed here[10–13] (supplementary Appendix A). Nivolumab and pembrolizumab have both been approved by Health Canada, conditional upon confirmatory results of further studies to verify clinical benefit in patients with incurable advanced-stage NSCLC who experience disease progression during or after platinum-containing chemotherapy[14,15]. Nivolumab is also approved for the treatment of adult patients with recurrent or metastatic HNSCC[15]. In addition, atezolizumab and durvalumab have been given marketing authorization, conditional on confirmatory results of further studies to verify clinical benefit for the treatment of locally advanced metastatic UC in patients with disease progression during or after platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy[16,17]. Of the foregoing agents, pembrolizumab for therapy of NSCLC requires that patients be PD-L1 positive, as determined using a validated test at a designated cut-off point for PD-L1 expression[14].

Given the likely future availability of additional PD-L1 inhibitors, PD-L1 assays will become increasingly important in identifying patients most likely to benefit from treatment. However, the PD-L1 assays for use with durvalumab, pembrolizumab, nivolumab, and atezolizumab have all been developed independently, and they use different antibody clones, immunohistochemistry (IHC) staining protocols, scoring algorithms, and cut-offs for determining PD-L1 status[18]. Questions have therefore been raised about whether the tests can be used interchangeably to inform treatment decisions for the various PD-1 and PD-L1 inhibitors. Given the variability in PD-L1 testing paradigms and the lack of available resources to perform multiple tests within laboratories, harmonization of methods is crucial. In this paper, we review and compare available PD-L1 testing methods, summarize the literature about comparability studies to date, discuss future directions in personalized diagnostics, and provide a pathologist's perspective on PD-L1 testing in the Canadian laboratory setting.

COMPARING PD-L1 TESTING METHODS

The PD-L1 assay is an IHC procedure in which the primary antibody recognizes and binds to a protein called the epitope, which is present in the tissue section[19]. Several PD-L1 assays have been developed, each with a specific antibody that is designed to bind to a different epitope of PD-L1 and that was created to be used with a specific checkpoint inhibitor (Table I). The methods differ in the choice of antibody clone, staining protocol, platform, and cut-off point for what is considered to be PD-L1–positive or –negative expression in NSCLC, HNSCC, and UC.

In addition to tests developed commercially (for example, PharmDx: Dako North America, Carpinteria, CA,

TABLE I PD-L1 testing methods

PD-L1 antibody	Platform	Detection system	Agent	Cut-off		
				NSCLC	HNSCC	UC
SP263[20]	Ventana BenchMark Ultra[a]	OptiView DAB IHC Detection Kit[a]	Durvalumab[b]	TC: ≥25%	TC: ≥25%	TC or IC: ≥25%
22C3[21]	Dako Autostainer Link 48[c]	EnVision FLEX visualization system[c]	Pembrolizumab[d]	TC: ≥1%, ≥50% TPS	TC or IC: >1%, >50%	TC and IC: ≥10% TPS
28-8[22]	Dako Autostainer Link 48	EnVision FLEX visualization system	Nivolumab[e]	TC: >1%, >5%	>1%, >5%	TC: >1%, >5%
SP142[23]	Ventana BenchMark Ultra	OptiView DAB IHC Detection Kit and OptiView Amplification Kit[a]	Atezolizumab[f]	TC: ≥10%, IC: ≥50%	TC: ≥5%, IC: ≥5%	IC: ≥5%
73-10[24]	Dako Autostainer Link 48	EnVision FLEX visualization system	Avelumab[d,g]	TC: ≥1%, 50%, 80%	NA	TC: ≥5%

[a] Ventana Medical Systems, Tucson, AZ, U.S.A.
[b] Agilent Technologies, Mississauga, ON.
[c] AstraZeneca, Cambridge, U.K.
[d] Merck, Kenilworth, NJ, U.S.A.
[e] Bristol–Myers Squibb, New York, NY, U.S.A.
[f] Roche, Basel, Switzerland.
[g] Pfizer, New York, NY, U.S.A.

NSCLC = non-small-cell lung cancer; HNSCC = head-and-neck squamous cell carcinoma; UC = urothelial carcinoma; IHC = immunohistochemistry; TC = tumour cell; IC = immune cell; TPS = tumour proportion score (PD-L1–expressing TCs and infiltrating ICs relative to the total number of TCs); NA = not applicable.

U.S.A.), some diagnostic centres use laboratory-developed tests (LDTs) to reduce testing costs and to harmonize assays across disease sites. A LDT is any test that differs from the commercial assays, no matter how small the modification to one or more of its components or testing procedures. The LDTs use commercially available PD-L1 clones[25]. Unfortunately, development of LDTs can be limited by an inability to standardize many of their components. As a result, they are likely to be less robust than the commercial tests and will introduce additional variability to results. Also, given that commercial tests have been examined within the context of clinical trials for specific checkpoint inhibitors, the clinical validity of LDT assays in regard to their ability to predict patient response is unclear. With the likely development of LDTs, external quality assessment (EQA) schemes for assurance of test quality in the clinical setting are an ongoing need.

Given variances between testing methods, PD-L1 assays are likely to differ in their classification of patients as PD-L1–positive or –negative. When different assays are used, patients might therefore be misclassified based on the method and scoring technique validated for a specific checkpoint inhibitor. As a consequence, patients might be inappropriately selected for or excluded from a specific treatment, potentially leading to unnecessary toxicities or exclusion from treatments that could improve outcomes. This misclassification might be further affected by the use of LDTs in which the variability of results is likely greater than that with commercial assays.

With the availability of checkpoint inhibitors that target the PD-1/PD-L1 pathway steadily increasing, robust methods that can identify the cohort of patients likely to respond to these agents is crucial. However, given that PD-L1 diagnostic assays have been developed independently for each agent, it is possible that several tests will become available in Canada. The use of multiple assays within individual laboratories is impractical given resource, platform [for example, Ventana (Ventana Medical Systems, Tucson, AZ, U.S.A.) vs. Dako (Dako North America, Carpinteria, CA, U.S.A.)], and tissue availability. To reduce the need for multiple tests and to provide testing options that are compatible with available platforms, an examination of the compatibility of testing assays is therefore important to determine whether those assays can be used interchangeably. That examination of compatibility is crucial to providing accurate and reliable tools that will optimize treatment decisions for patients.

PD-L1 ASSAY COMPARABILITY STUDIES

Comparability Studies in NSCLC

A number of studies have looked at the comparability of PD-L1 assays in NSCLC (Table II). Of nine studies examining the 28-8, 22C3, and SP263 assays, all showed high levels of agreement in the ability of the assays to detect PD-L1 in tumour cells (TCs)[26–33]. However, the level of agreement might depend on the cut-off points used[31]. Of seven studies[26,28–30,32–34] that included the SP142 assay, six showed lower agreement in comparisons with other antibodies such as 28-8, 22C3, and SP263. In addition, preliminary results from phase 2 of the Blueprint study, which also included the recently developed 73-10 assay, showed that more TCs were stained positive by the 73-10 assay than by the SP263, 28-8, and 22C3 assays, and confirmed that SP142 detected fewer positive TCs[24]. The analytic similarity shown between the SP263, 28-8, and 22C3 assays suggests that those tests could be used interchangeably at appropriate cut-off points. However, the variability in both immune cell (IC) and TC staining with the SP142 assay suggests that it is not comparable to other assays.

Thus far, three studies have examined the interchangeability of LDTs with commercial assays[27,32,34]. A study by Scheel *et al.*[32] showed that staining in 6 of 11 LDT protocols was similar to that in the 22C3 and 28-8 assays. In addition, results of an ongoing retrospective study by Velcheti *et al.*[34], which included a total of 1728 test results (323 from LDTs), demonstrated no difference in the measurement of PD-L1 expression using LDTs compared with other assays. Moreover, results of an ongoing study by Adam *et al.*[27] showed that LDTs have various levels of agreement when compared with 3 commercial assays. Notably, LDTs using the SP263 clone had the greatest agreement across all platforms for both IC and TC staining, whereas some LDTs with clones 28-8, 22C3, and E1L3N, but not SP142, showed good correlation with the 3 commercial assays for TCs only. Interestingly, NordiQC, a leading European organization managing EQA for IHC, has used the 22C3, 28-8, and SP263 PharmDx assays, as well as LDTs, to assess 68 laboratories[25]. Results of a pilot survey demonstrated a pass rate of 80% for the 3 PharmDx assays; however, the pass rate for LDTs was only 20%[25]. Those results suggest that LDTs might be more variable than PharmDx assays and must be carefully validated, with adequate training and EQA schemes.

An important finding of studies into the interpretation of PD-L1 tests in NSCLC is that the variability of staining appears to be higher for ICs than for TCs[27,29,30,35]. A study by Rehman *et al.*[36] confirmed that finding, demonstrating 94% agreement between pathologists for TC staining, but only 27% agreement for stromal or IC assessment. However, the biologic implication of IC staining does not seem to be as important in NSCLC as in other tumours; it might depend on the assay and the specific checkpoint inhibitor in question. Therefore, it is not necessary that assays for NSCLC measure PD-L1 expression in ICs.

Comparability Studies in HNSCC and UC

In HNSCC, one comparability study is ongoing in 501 archival clinical HNSCC tumour samples, with interim results presented at the 2016 European Society for Medical Oncology meeting[37]. As in NSCLC, preliminary results in UC showed good agreement between the 22C3, 28-8, and SP263 assays, with much lower agreement for SP142. Also, as happened for NSCLC, increased variability between pathologists was observed in the assessment of ICs. However, as in NSCLC, the biologic implications of IC staining do not seem to be as important in HNSCC as in other tumour types.

Analyses on UC tissue, using SP142 on the Ventana platform, have shown that PD-L1 is expressed more commonly on ICs than TCs, suggesting that IC PD-L1 expression could be more relevant in UC than in NSCLC[38]. With the exception of the 28-8 assay, commercial assays tend to use ICs as components of their algorithms (Table I).

However, the SP142 assay assesses only ICs[23], whereas the SP-263, 22C3, and 73-10 assays include both ICs and TCs[20,21]. Another key difference with the SP142 assay is that it calculates PD-L1–expressing ICs in the tumour area only; other assays use the IC area as a whole. It is therefore likely that the SP142 and 28-8 assays could not be used interchangeably with the other assays in UC. In addition to the variability in testing compartments, commercial assays also differ in their scoring algorithms in UC. For example, the 22C3 assay uses a combined positive score,

TABLE II PD-L1 comparability studies in non-small-cell lung cancer

Study	Assays	Samples (*n*)	Findings
Gaule *et al.,* 2016[26]	28-8 22C3 SP263 SP142 E1L3N 9A11	30	■ When using chromogenic staining in isogenic cell lines, high levels of agreement were observed for all antibodies (R^2=0.76–0.99).
Adam *et al.,* 2017[27] (French study)	28-8 22C3 SP263 LDTs	41	■ Assays 22C3, 28-8, SP263 performed in several centres were highly concordant. ■ Using a 4-category scale with 1%, 5%, and 10% thresholds, immune cell staining agreement was low. ■ Laboratory-developed tests demonstrated variable levels of agreement, with SP263 being most concordant.
Brunnstrom *et al.,* 2017[28] (Swedish study)	28-8 22C3 SP263 SP142	55	■ The highest values for comparisons were seen between 22C3 and 28-8; the lowest were seen between SP142 and the other assays. ■ Agreement was fairly good for 28-8, 22C3, and SP263, but not as good for SP142.
Hirsch *et al.,* 2017[29] (Blueprint 1)	28-8 22C3 SP263 SP142	39	■ The percentage of PD-L1–stained tumour cells was comparable with 22C3, 28-8, and SP263, but the SP142 assay showed fewer stained tumour cells. ■ Variability of staining was higher for immune cells than for tumour cells.
Rimm *et al.,* 2017[30] (Bristol–Myers Squibb, NCCN)	28-8 22C3 SP142 E1L3N	90	■ SP142 was an outlier, detecting significantly less PD-L1 expression in tumour cells and immune cells. ■ Compared with either 28-8 or E1L3N, 22C3 showed slightly, yet statistically significantly, lower staining. ■ The immune cell score showed poor agreement with any antibody.
Saito *et al.,* 2017[31]	22C3 28-8	420	■ Percentage agreement between assays was fair. ■ Positive agreement was suboptimal when the cut-off was ≥25% and ≥50%.
Scheel *et al.,* 2017[32]	28-8 22C3 SP263 SP142 LDTs	21	■ The SP142 staining pattern was distinct. ■ Staining with 22C3, 28-8, SP263 was similar. ■ Of 11 laboratory-developed tests, 6 showed staining similar to that with 22C3 and 28-8.
Scott *et al.,* 2017[33], Ratcliffe *et al.,* 2017[18] (AstraZeneca study)	28-8 22C3 SP263 SP142	493	■ SP263, 22C3, and 28-8 showed strong agreement in immune cell scoring. ■ Immune cell agreement and PD-L1 staining or agreement were both less with SP142 than with SP263.
Tsao *et al.,* 2017[24] (Blueprint 2, abstract)	28-8 22C3 SP263 SP142 73-10	81 (from routine practice)	■ Results of Blueprint phase 1 were affirmed. ■ The 22C3, 28-8, and SP263 assays are comparable; SP142 detects fewer cells, and 73-10 stains more PD-L1–positive tumour cells. ■ Variability of staining was higher for immune cells than for tumour cells.
Velcheti *et al.,* 2017[34]	28-8 22C3 SP142 LDTs	1728 (retrospective database)	■ No differences in PD-L1 expression using 22C3, 28-8, and laboratory-developed tests. ■ SP142 was an outlier.

NCCN = U.S. National Comprehensive Cancer Network.

which does not capture PD-L1 expression in ICs alone[21]. Thus far, no published data from comparability studies in UC are available. However, interim data from an ongoing study, sponsored by AstraZeneca and presented at the Society for Immunotherapy of Cancer 2017 annual meeting, showed a high level of agreement between the SP263, 22C3, and 28-8 assays for both TC and IC staining[39]. The SP142 assay showed good agreement with respect to IC staining, but significant differences for TC staining. In addition, ongoing work by author MRD and colleagues has shown substantial inter-observer agreement in the interpretation of IC scoring in bladder using both SP263 and SP142 (data on file).

FUTURE DIRECTIONS

The use of PD-L1 expression as a biomarker of response is important in identifying patients who could obtain a clinically meaningful benefit from treatment with a checkpoint inhibitor. It is clear from clinical trials in NSCLC, HNSCC, and UC that responses to those inhibitors are improved in patients having PD-L1–positive tumours compared with patients having PD-L1–negative tumours[40] (supplementary Appendix A). However, when checkpoint inhibitors are used in combination with agents involving non-redundant pathways, good responses have also been demonstrated in patients with low PD-L1 expression[41,42]. Monotherapy might therefore be optimal for PD-L1–positive patients, with the use of immunotherapy combinations or alternative therapies being appropriate for those with low PD-L1 expression. The benefit of improved efficacy with combination therapies in PD-L1–negative patients will have to be weighed against an increased risk of toxicity. Those outcomes suggest that PD-L1 testing could aid in identifying patients who are most likely to respond to monotherapy with checkpoint inhibitors, thus aiding in the selection of the most appropriate therapy, optimizing resource utilization and fiscal expenditure, and minimizing treatment toxicities. In addition, the role of PD-L1 testing will likely extend beyond NSCLC, HNSCC, and UC, with the U.S. Food and Drug Administration having recently approved pembrolizumab for previously treated patients with recurrent locally advanced or metastatic gastric or gastroesophageal junction cancers whose tumours express PD-L1 as determined by the 22C3 PharmDx assay[43].

Currently, the process of validating and standardizing LDTs is difficult and laborious. However, standardization arrays using cell lines could allow laboratories to check assays to ensure that they are able to correctly quantify PD-L1 based on known limits of detection, quantification, and saturation. In addition, a framework being developed by the Canadian Partnership Against Cancer for molecular biomarker testing will recommend that testing laboratories undergo accreditation, participate in EQA schemes, and produce standard operating procedures for all tests. The Canadian Cancer Research Alliance is also developing a national network of molecular diagnosis and pathology groups that could be used to support translational research and personalized medicine.

In the future, combining PD-L1 IHC with T cell activation measures might boost the ability to predict patient response to checkpoint inhibitors. New testing paradigms that include multiplex gene expression markers, including PD-L1, are being developed by NanoString Technologies (Seattle, WA, U.S.A.) and might provide additional testing options[44]. However, whether these newer paradigms will be superior to currently available IHC assays is unclear. Finally, microsatellite instability, mismatch repair deficiency, and tumour mutational burden are being targeted as genomic features in certain cancers that could be responsive to checkpoint inhibitors. Assays that identify patients with those genomic features might replace or complement PD-L1 immuno-expression. To date, PD-L1 testing is the most evaluated and accepted methodology used in selecting patients for checkpoint inhibitors in NSCLC, HNSCC, and UC.

CANADIAN PERSPECTIVE ON PD-L1 TESTING

The SP142 assay developed by Ventana, used according to its published protocol, differs significantly from others, given its distinct staining pattern showing both membranous and granular cytoplasmic staining in TCs[35]. Studies to date in both NSCLC and HNSCC have demonstrated good comparability between the 22C3, 28-8, and SP263 assays, with the SP142 assay representing a clear outlier. In addition, preliminary results of the Blueprint 2 study demonstrated that the 73-10 assay shows greater staining of TCs in NSCLC[24]. Because the goal is to provide interchangeable PD-L1 assays, SP142 should not, given its low agreement, be used in place of other methods, with the exception of use for atezolizumab. Results of comparability studies, at least in NSCLC and HNSCC, suggest that the 22C3, 28-8, and SP263 assays can be used interchangeably based on their relevant cut-offs. The choice of assay should therefore be based on the platform and the availability of pathologists who are trained in interpreting the tests. The reliability of assay interpretation should also be considered when adopting a standard method.

As for any biomarker, a greater volume of samples allows laboratories to gain experience, becoming proficient in testing methods. Consequently, it is important that testing be conducted at sites that will receive a reasonable volume of samples. Given the high prevalence of lung cancer, it is anticipated that testing centres will receive a large volume of samples and that the use of PD-L1 testing will expand beyond its current indication. In addition, given differences in the testing methods by disease site, it is important that assays are interpreted by specialized pathologists with training in PD-L1 interpretation. At least initially, testing should be conducted centrally so that experience can be gained through the analysis of a large volume of samples. Centralized testing would also allow for standardization in recording PD-L1 results. One concern with NSCLC samples is that, because of the heterogeneity of PD-L1 expression in a tumour and the minimal requirement for the presence of only 50 TCs (SP142) or 100 TCs (22C3 and 28-8) for the test[19], PD-L1 results from small biopsy and resection specimens might be discordant, with the concordance between them varying in the 52%–92% range[3,45]. However, most NSCLC patients are diagnosed at an advanced stage and are not treated with surgery; only biopsy or cytology specimens are available for ancillary testing, including PD-L1. Moreover, in clinical trials,

approximately 10% of NSCLC patients reported as PD-L1–negative responded to immunotherapy[19] (supplementary Appendix A). It is therefore likely that a fraction of patients are miscategorized as PD-L1–negative and might otherwise have responded to immunotherapy. To optimally treat such patients, testing methods that are more sensitive are therefore needed to identify PD-L1 expression from smaller samples.

Although oncologists might prefer to have access to the exact percentage of tumour cells expressing PD-L1, that result is likely less accurate than results reported within a broader category. As a compromise, pathologists might consider reporting the tumour proportion score within the comments section. It is also important for oncologists to specify the checkpoint inhibitor that they would like to choose for their patient, given that testing labs might not know which drug will be prescribed.

It is clear that even if testing is conducted centrally, testing sites will not have access to both the Dako and Ventana platforms. It is therefore crucial that there be a choice of assays within each tumour type, allowing for testing on either platform. In Europe, the Ventana platform is the most widely used, which poses a challenge for PD-L1 testing related to pembrolizumab. The Conformité Européene designation has been awarded to SP263, which now allows the assay to be used in determining therapy with pembrolizumab and nivolumab in addition to durvalumab. To date, Health Canada has approved checkpoint inhibitors with the use of any validated assay. Consequently, there will be flexibility to use methods that are interchangeable across platforms. It is also clear that one specific assay and scoring algorithm will not be valid for multiple disease states. It will therefore be necessary to perform comparability studies in each tumour type to determine which assays can be used interchangeably. Ideally, the same antibodies could then be used across disease sites, thereby avoiding confusion for oncologists and pathologists.

Given the complexities of the testing methods and the need for EQA schemes to ensure the validity of LDTs, it is important to select commercial tests initially while centres gain experience. Over time, LDTs could be developed and validated, but further comparability studies are needed to ensure that they are interchangeable with the commercial tests. Academic laboratories with a large volume of samples might be able to research LDTs in their own populations treated with various checkpoint inhibitors to clinically validate those assays. In that context, it is important that LDTs be undertaken with caution, given their increased variability and lack of robustness. Ultimately, LDTs are useful only if they provide a more economical method of assessing PD-L1 status. It is possible that LDTs could prove cheaper than commercial assays, given their ability to test any volume of samples in one run. Larger runs might be of benefit to smaller laboratories; however, it is unclear whether smaller centres would receive sufficient samples to ensure the gain of sufficient technical and professional experience.

The reliability of PD-L1 scoring is also likely to be hindered by variability in interpretation between pathologists, which appears to be greater for IC than for TC staining. Increased variability is therefore probable for assays containing an IC component. For tumour types such as NSCLC and HNSCC, in which the biologic implications of the IC staining component is not as important, assays that focus on the TC component might produce less variability between pathologist readings. However, for UC, in which the IC component is more important, wider variability between pathologist readings is likely. Education and experience are therefore needed for improvement in the interpretation of IC staining and for ongoing assessment of intra- and inter-observer variability. Although PD-L1 tests are conducted using individual assays, sufficient comparability studies will have to be performed to permit the identification of a reasonable biomarker approach that can be implemented in a responsible and practical way across available platforms at the key testing sites.

SUMMARY

The number of PD-L1 inhibitors available for the treatment of NSCLC, HNSCC, and UC is growing, and indications are likely to expand to additional tumour types. However, PD-L1 assays have been developed independently for each agent and vary in their make-up. Use of a single PD-L1 assay in conjunction with a specific therapy will become impractical and could compromise treatment options for patients, driving the need for a reduction to the fewest possible tests. Test selection could then be based on the platform and the availability of pathologists trained in interpretation. Approaches to harmonizing testing methods are therefore crucial in ensuring appropriate treatment selection for Canadian patients.

Results of comparability studies, at least in NSCLC and HNSCC, suggest that the 22C3, 28-8, and SP263 assays can be used interchangeably at appropriate cut-offs. However, further studies are needed to determine the interchangeability of LDTs and to harmonize assays for other tumour types. A standardized plan has to be developed to allow for the rollout of new testing paradigms that considers the expanding need for PD-L1 testing. A reasonable approach could be to centralize testing using specialized pathologists. Commercial tests should be used until LDTs are validated with sufficient EQA. Eventually, the goal should be to develop a rational, cost-effective, and universal approach that can be applied in multiple indications to meet the needs of Canadian patients.

ACKNOWLEDGMENTS

The authors acknowledge financial and editorial support from AstraZeneca Canada, including medical writing support provided by Anna Christofides of IMPACT Medicom Inc.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare the following interests: DNI has received honoraria and served on advisory boards for Merck, Bristol–Myers Squibb, Pfizer, AstraZeneca, and Hoffmann–La Roche. MRD has received compensation from Hoffmann–La Roche and AstraZeneca for participating on advisory boards and has received honoraria from AstraZeneca. MST has received honoraria for advisory board meetings from Merck, AstraZeneca, and Bristol–Myers Squibb, and a research grant from Hoffmann–La Roche and Ventana.

AUTHOR AFFILIATIONS
*Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC; †Anatomic Pathology, Sunnybrook Health Sciences Centre, Toronto, ON; ‡Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON; §IMPACT Medicom Inc., Toronto, ON; ‖Department of Pathology, University Health Network, Princess Margaret Cancer Centre, Toronto, ON.

REFERENCES

1. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012;12:252–64.
2. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: mechanisms, response biomarkers, and combinations. *Sci Transl Med* 2016;8:328rv4.
3. Bai J, Gao Z, Li X, Dong L, Han W, Nie J. Regulation of PD-1/PD-L1 pathway and resistance to PD-1/PD-L1 blockade. *Oncotarget* 2017;8:110693–707.
4. Wang X, Bao Z, Zhang X, *et al.* Effectiveness and safety of PD-1/PD-L1 inhibitors in the treatment of solid tumors: a systematic review and meta-analysis. *Oncotarget* 2017;8:59901–14.
5. Schachter J, Ribas A, Long GV, *et al.* Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). *Lancet* 2017;390:1853–62.
6. Homet Moreno B, Parisi G, Robert L, Ribas A. Anti–PD-1 therapy in melanoma. *Semin Oncol* 2015;42:466–73.
7. Colunga A, Pulliam T, Nghiem P. Merkel cell carcinoma in the age of immunotherapy: facts and hopes. *Clin Cancer Res* 2017;:[Epub ahead of print].
8. Merryman RW, Armand P, Wright KT, Rodig SJ. Checkpoint blockade in Hodgkin and non-Hodgkin lymphoma. *Blood Adv* 2017;1:2643–54.
9. Le DT, Uram JN, Wang H, *et al.* PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;372:2509–20.
10. Malhotra J, Jabbour SK, Aisner J. Current state of immunotherapy for non-small cell lung cancer. *Transl Lung Cancer Res* 2017;6:196–211. [Erratum in: *Transl Lung Cancer Res* 2017;6:612]
11. Popovic LS, Matovina-Brko G, Popovic M. Checkpoint inhibitors in the treatment of urological malignancies. *ESMO Open* 2017;2:e000165.
12. Ran X, Yang K. Inhibitors of the PD-1/PD-L1 axis for the treatment of head and neck cancer: current status and future perspectives. *Drug Des Devel Ther* 2017;11:2007–14.
13. Botta GP, Granowicz E, Costantini C. Advances on immunotherapy in genitourinary and renal cell carcinoma. *Transl Cancer Res* 2017;6:17–29.
14. Merck Canada. *Product Monograph, Including Patient Medication Information: Keytruda (Pembrolizumab)*. Kirkland, QC: Merck Canada; 2017.
15. Bristol–Myers Squibb Canada. *Product Monograph: Yervoy (Ipilimumab for Injection)*. Saint-Laurent, QC: Bristol–Myers Squibb Canada; 2017.
16. Hoffmann–La Roche. *Product Monograph, Including Patient Medication Information: Tecentriq (Atezolizumab)*. Mississauga, ON: Hoffmann–La Roche; 2017.
17. Cision. Health Canada Approves Imfinzi (durvalumab) for previously treated patients with advanced bladder cancer [news release]. Mississauga, ON: Cision; 2017. [Available online at: http://www.newswire.ca/news-releases/health-canada-approves-imfinzi-durvalumab-for-previously-treated-patients-with-advanced-bladder-cancer-655553073.html; cited 10 November 2017]
18. Ratcliffe MJ, Sharpe A, Midha A, *et al.* Agreement between programmed cell death ligand-1 diagnostic assays across multiple protein expression cutoffs in non-small cell lung cancer. *Clin Cancer Res* 2017;23:3585–91.
19. Tsao MS, Kerr KM, Dacic S, Yatabe Y, Hirsch FR, eds. *IASLC Atlas of PD-L1 Immunohistochemistry Testing in Lung Cancer*. Aurora, CO: International Association for the Study of Lung Cancer; 2017.
20. Ventana Medical Systems. Ventana PD-L1 (SP263) Assay [Web page]. Tucson, AZ: Ventana Medical Systems; n.d. [Available at: http://www.ventana.com/product/1880?type=2408; cited 15 August 2017]
21. Dako North America. *PD-L1 IHC 22C3 pharmDx*. Carpinteria, CA: Dako North America; 2015. [Available online at: http://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013c.pdf; cited 15 August 2017]
22. Dako North America. *PD-L1 IHC 28-8 pharmDx*. Carpinteria, CA: Dako North America; 2015. [Available online at: http://www.accessdata.fda.gov/cdrh_docs/pdf15/P150025c.pdf; cited 15 August 2017]
23. Ventana Medical Systems. *Ventana PD-L1 (SP142) Assay: Guiding Immunotherapy in NSCLC*. Tucson, AZ: Ventana Medical Systems; 2018. [Available online at: http://www.ventana.com/documents/PD-L1_SP142-NSCLC-Brochure.pdf; cited 15 August 2017]
24. Tsao MS, Kerr KM, Yatabe Y, Hirsch FR. Blueprint 2: PD-L1 immunohistochemistry comparability study in real-life, clinical samples [abstract PL 03.03]. *J Thorac Oncol* 2017;12(suppl 2):S1606. [Available online at: https://www.jto.org/article/S1556-0864(17)30835-3/fulltext; cited 26 April 2018]
25. Nordic Immunohistochemical Quality Control (NordicQC). *Assessment Run C1 2017 PD-L1*. Aalborg, Denmark: NordicQC; 2017. [Available online at: http://www.nordiqc.org/downloads/assessments/96_102.pdf; cited 19 September 2017]
26. Gaule P, Smithy JW, Toki M, *et al.* A quantitative comparison of antibodies to programmed cell death 1 ligand 1. *JAMA Oncol* 2016;:[Epub ahead of print].
27. Adam J, Rouquette I, Damotte D, *et al.* Multicentric French harmonization study for PD-L1 IHC testing in NSCLC [abstract PL04a.04]. *J Thorac Oncol* 2017;12(suppl):S11–12.
28. Brunnstrom H, Johansson A, Westbom-Fremer S, *et al.* PD-L1 immunohistochemistry in clinical diagnostics of lung cancer: inter-pathologist variability is higher than assay variability. *Mod Pathol* 2017;30:1411–21.
29. Hirsch FR, McElhinny A, Stanforth D, *et al.* PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project. *J Thorac Oncol* 2017;12:208–22.
30. Rimm DL, Han G, Taube JM, *et al.* A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. *JAMA Oncol* 2017;3:1051–8.
31. Saito T, Tsuta K, Ishida M, *et al.* Comparison study of PD-L1 immunohistochemistry assays with 22C3 and 28-8: analysis on surgical specimens of NSCLC [abstract MA 05] *J Thorac Oncol* 2017;12(suppl 2):S1816–17.
32. Scheel AH, Baenfer G, Baretton G, *et al.* Interlaboratory-concordance of PD-L1 immunohistochemistry for non-small cell lung cancer. *Histopathology* 2018;72:449–59.
33. Scott ML, Ratcliffe MJ, Sharpe A, *et al.* Concordance of tumor cell (TC) and immune cell (IC) staining with Ventana SP142, Ventana SP263, Dako 28-8 and Dako 22C3 PD-L1 IHC tests in NSCLC patient samples [abstract e14503]. *J Clin Oncol* 2017;35:. [Available online at: https://meetinglibrary.asco.org/record/145193/abstract; cited 16 April 2018]
34. Velcheti V, Cao X, Liu F, Patwardhan P. Distribution of PD-L1 tumor expression by assay type in patients with metastatic NSCLC (MNSCLC) [abstract OA 13.02]. *J Thorac Oncol* 2017;12(suppl 2):S1779–80.

35. Scheel AH, Dietel M, Heukamp LC, *et al.* Harmonized PD-L1 immunohistochemistry for pulmonary squamous-cell and adenocarcinomas. *Mod Pathol* 2016;29:1165–72.
36. Rehman JA, Han G, Carvajal-Hausdorf DE, *et al.* Quantitative and pathologist-read comparison of the heterogeneity of programmed death-ligand 1 (PD-L1) expression in non-small cell lung cancer. *Mod Pathol* 2017;30:340–9.
37. Ratcliffe MJ, Sharpe A, Rebelatto MC, *et al.* A comparative study of PD-L1 diagnostic assays in squamous cell carcinoma of the head and neck (SCCHN) [abstract 955]. *Ann Oncol* 2016;27:328–50.
38. Powles T, Eder JP, Fine GD, *et al.* MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* 2014;515:558–62.
39. Zajac M, Scott M, Ratcliffe M, *et al.* A study of PD-L1 diagnostic assay concordance in urothelial carcinoma [abstract P104]. *J Immunother Cancer* 2017;5:. [Available online at: https://link.springer.com/article/10.1186%2Fs40425-017-0289-3; cited 27 April 2018]
40. Passiglia F, Bronte G, Bazan V, *et al.* PD-L1 expression as predictive biomarker in patients with NSCLC: a pooled analysis. *Oncotarget* 2016;7:19738–47.
41. Langer CJ, Gadgeel SM, Borghaei H, *et al.* on behalf of the KEYNOTE-021 investigators. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. *Lancet Oncol* 2016;17:1497–508.
42. Antonia S, Goldberg SB, Balmanoukian A, *et al.* Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. *Lancet Oncol* 2016;17:299–308.
43. Merck Sharp and Dohme. FDA Approves Merck's Keytruda (Pembrolizumab) for Previously Treated Patients with Recurrent Locally Advanced or Metastatic Gastric or Gastroesophageal Junction Cancer Whose Tumors Express PD-L1 (CPS Greater Than or Equal to 1) [news release]. Kenilworth, NJ: Merck Sharp and Dohme; 2017. [Available online at: http://investors.merck.com/news/press-release-details/2017/FDA-Approves-Mercks-KEYTRUDA-pembrolizumab-for-Previously-Treated-Patients-with-Recurrent-Locally-Advanced-or-Metastatic-Gastric-or-Gastroesophageal-Junction-Cancer-Whose-Tumors-Express-PD-L1-CPS-Greater-Than-or-Equal-to-1/default.aspx; cited 10 October 2017]
44. NanoString. Explore the complex relationship between the immune system and tumor biology to identify new therapeutic targets [Web page]. Seattle, WA: NanoString; 2017. [Available at: https://www.nanostring.com/scientific-content/immuno-oncology; cited 10 October 2017]
45. Kitazono S, Fujiwara Y, Tsuta K, *et al.* Reliability of small biopsy samples compared with resected specimens for the determination of programmed death-ligand 1 expression in non-small-cell lung cancer. *Clin Lung Cancer* 2015;16:385–90.