Do We Need PD-L1 as a Biomarker for Thyroid Cytologic and Histologic Specimens?

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The link between cancer and inflammation was first suggested about 170 years ago with the observation by Rudolf Virchow (1821-1902), a German pathologist, that tumors in different organs often arose in sites of chronic inflammation and that inflammatory cells were present within and around tumors. In the thyroid gland, an association between chronic lymphocytic (Hashimoto) thyroiditis and papillary thyroid carcinoma (PTC) has been reported in several studies since the 1950s. Although chronic inflammation is a well known risk factor for tumor initiation and promotion, the ability of cancer cells to avoid the immune damage by disabling components of the host immune system is now considered a hallmark of cancer. In cancer, the immune response is turned off by various mechanisms, including: 1) inactivation of cytotoxic T lymphocytes and natural killer cells by secreting immunosuppressive factors; 2) recruitment of immunosuppressive cells (eg, myeloid-derived suppressor cells, regulatory T cells); and 3) expression of inhibitory ligands for the immune checkpoint receptors, including CTLA-4 (cytotoxic T-lymphocyte associated protein 4) and PD-L1 (programmed death-ligand 1). Therefore, the complex interactions between immune cells and tumor cells play a major role in tumor development, including tumor growth, invasion, and metastasis, and subsequent patient outcomes.

The development of immunotherapies for cancer, and immune checkpoint inhibitors (ICIs) in particular (eg, anti–CTLA-4 and anti–PD-1), has revolutionized treatment for many malignancies. As a result, the 2018 Nobel Prize in Physiology or Medicine was awarded jointly to James Allison and Tasuku Honjo for their pioneering work on CTLA-4 and PD-1/PD-L1. According to Allison, immunotherapy is quickly becoming the “fourth pillar” of cancer therapy, joining long-established treatments of surgery, radiation, and chemotherapy, and, although immunotherapy will not replace these other 3 treatments, it can be combined with them to offer patients the best chance of a cure. The clinical benefit observed with ICIs varies with both the drug and the type of cancer. The best responses are generally observed in patients who have high tumor expression of PD-L1 and high numbers of tumor-infiltrating immune cells, particularly CD8-positive T cells. Several antibodies targeting the PD-1/PD-L1 pathway have been approved by the US Food and Drug Administration to treat certain types and stages of cancers including, melanoma; lung, kidney, bladder, gastric, liver, cervical, colorectal, head and neck, and Merkel cell carcinomas; and Hodgkin lymphoma. Clinical trials are underway in many other cancer types, including thyroid carcinoma.

PTC, the most common thyroid malignancy (80%-90%), is usually associated with an excellent prognosis. However, from 10% to 30% of patients with PTC who have undergone primary thyroidectomy and radiiodine treatment develop recurrences and metastases, largely in regional lymph nodes, which are usually sensitive to further radiiodine treatment. In contrast, although it is rare, anaplastic thyroid carcinoma (ATC) has a miserable prognosis because patients are rarely candidates for surgery, and their tumors typically are
resistant to conventional treatments. Therefore, there is a need for new treatment modalities, including targeted therapies and/or immunotherapies, mostly for patients who have advanced and/or poorly differentiated/undifferentiated cancers. More treatment options are available since the approval of kinase inhibitors; however, despite being effective, the duration of response to kinase inhibitors is limited, and disease ultimately progresses. To date, fine-needle aspiration (FNA) cytology (FNAC) represents the main diagnostic tool for the evaluation of thyroid nodules. However, from 20% to 30% of thyroid FNACs are cytologically indeterminate. Different molecular tests (ie, Afirma [Veracyte Inc], ThyroSeqV3 [University of Pittsburgh Medical Center/CBLPath-Sonic], ThyGenX/ThyraMIR [Interpace Diagnostics, LLC], and RosettaGX Reveal [Rosetta Genomics, Ltd]) can be used successfully to overcome this inherent limitation of FNAC and to refine preoperative diagnosis and risk stratification, with increasingly diagnostic performances. Large multigene panels like ThyroSeqV3 can also identify a small subset of aggressive thyroid cancers, especially those with \( \text{BRAF} \) V600E, \( \text{TP53} \), and/or \( \text{TERT} \) promoter mutations, which is very helpful to determine the extent and timing of surgery. This novel approach is promising, although these tests are still expensive, not widely available, and still need extensive validation in large series and integration with clinical and histologic data.

Over the past decade, numerous studies have expanded our understanding of PD-L1 as part of ICIs as a critical predictive biomarker (and potentially also as a diagnostic and prognostic marker) for many cancers, mostly lung. In contrast, PD-L1 evaluation for thyroid cancer represents a newer area of research. A recent PubMed search using the keywords PD-L1 and thyroid retrieved only 103 publications, with 88% published over the last 3 years, whereas there were 2351 hits with the keywords PD-L1 and lung.

The study by Dell’Aquila and colleagues published in this issue of Cancer Cytopathology is a timely article that examines for the first time the performance of PD-L1 immunostaining in liquid-based cytology (LBC) from thyroid FNAs to determine whether this could be a biomarker of malignancy or aggressive disease. In total, 236 thyroid lesions that were diagnosed by FNAC as “benign,” “indeterminate,” “suspicious for malignancy,” or “malignant” were enrolled, and PD-L1 immunostaining was performed both on LBC slides and on corresponding histology samples. Increased membranous and cytoplasmic PD-L1 expression was found in 79 cases (38.5%), including 61 PTCs (conventional and variants). Negative PD-L1 expression was found in noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and benign lesions. \( \text{BRAF} \) V600E was mutated in 15% of PD-L1–positive malignancies. The authors suggest that PD-L1 expression in the thyroid gland and thyroid LBC might represent a marker of malignancy that correlates with PTC, but not with NIFTP. A correlation between PD-L1 expression and PTCs with aggressive features and/or \( \text{BRAF} \) V600E mutation was also found, suggesting that they are associated with a more aggressive behavior. Finally, their study demonstrates that the quantification of PD-L1 immunostaining in LBC slides is feasible and appears to correlate with corresponding histology.

Although these data are encouraging, as they suggest that PD-L1 can be performed on LBC specimens, because all benign cases are negative for PD-L1, the article by Dell’Aquila et al raises many open questions on the potential utility of PD-L1 for thyroid nodules and cancers in the preoperative and postoperative setting. Do we actually need PD-L1 as a potential diagnostic, prognostic, or predictive marker for thyroid specimens, especially on cytology? Although further research is anticipated to answer these questions, some insight is given below.

**PD-L1 Expression and Thyroid Cancer Diagnosis**

Several recent studies have explored the role of the PD-1/PD-L1 axis in neoplastic and nonneoplastic thyroid diseases (for a review, see Ulisse et al). Most studies investigating PD-L1 in thyroid cancer have evaluated its prognostic relevance by correlating PD-L1 expression levels with clinicopathologic features, whereas some studies have evaluated the diagnostic value of PD-L1 in thyroid cancer. Ahn et al analyzed 401 thyroid carcinomas and found that tumoral PD-L1 was variably expressed in 6.1% of PTCs, 7.6% of follicular thyroid carcinomas, and 22.2% of ATCs. Cunha et al analyzed 293 PTCs/follicular thyroid carcinomas, 114 benign nodules, and 5 normal tissues and concluded that PD-L1 (B7-H1) immunohistochemistry did not have diagnostic utility. The study by Rosenbaum et al on poorly differentiated thyroid carcinoma (PDTC) also supports the concept that PD-L1 staining is often limited, focal, and heterogeneous,
limiting its use as a diagnostic marker.\textsuperscript{15} Fu et al analyzed the expression of PD-L1 in 52 NIFTPs, 45 invasive encapsulated follicular variants of PTC (EFVPTCs), and 40 benign nodules.\textsuperscript{16} They found no significant differences in PD-L1 cytoplasmic staining between NIFTPs and benign lesions, whereas there was a significant increase in invasive EFVPTCs. Because the majority of NIFTPs are diagnosed as indeterminate on cytology (ie, Bethesda categories III-V) and the distinction between NIFTP and invasive EFVPTC cannot be made on cytology, PD-L1 expression may contribute toward improving the diagnosis, although the initial treatment of both entities is similar (ie, diagnostic lobectomy). This being said, our opinion is that the currently available data does not justify considering or recommending the routine use of PD-L1 as a diagnostic marker for any type of thyroid tumor, especially on cytology, and there are many reasons for this. First, there are many technical and criteria/interpretational issues with PD-L1 immunochemistry that are outside the scope of this editorial but are nicely reviewed by Kim and Chung.\textsuperscript{5} Studies evaluating the expression of PD-L1 by immunohistochemistry in thyroid cancer and other organs have used different tissue preparations/fixations, processing procedures, detection antibodies, evaluated cells (tumor cells with or without immune cells), cutoff values, and control tissues (when available).\textsuperscript{4,5,17} Also, interpretations of results (ie, membranous and/or cytoplasmic staining of tumor cells) varies in different reports. In addition to these inherent preanalytical, analytical, and postanalytical issues, PD-L1 is a protein that is expressed with biologic continuity, akin to a microRNA, and often shows significant intratumoral heterogeneity (both spatial and temporal),\textsuperscript{5,14,15} unlike a genetic alteration such as a \textit{BRAF} V600E mutation, which is a binary system,\textsuperscript{9-11} or p16 immunohistochemistry for human papillomavirus-related squamous cell carcinoma (“block positivity”). Thus, it is even more crucial for PD-L1 immunohistochemistry to choose optimal cutoff levels to define positive and negative results in order for the test to have any predictive value. Although a strong staining may be informative and potentially relevant, a negative staining should be taken with caution, especially on cytology. Therefore, although immunocytochemistry for thyroid FNAs (eg, HBME-1, galectin-3, \textit{BRAF} V600E) is used by some laboratories, it is not widely accepted by most laboratories, and PD-L1 immunocytochemistry is even more questionable given the finding that its expression is often focal and heterogeneous.\textsuperscript{15,17} Also, an important pitfall (false-positive) for PD-L1 as a marker of neoplasia/malignancy for thyroid includes autoimmune thyroid diseases (AITDs) such as Graves and Hashimoto, in which reactive thyroid follicular cells (TFCs) are well recognized as mimics of PTC that may result in a false-positive diagnosis. It has been shown that PD-L1 is consistently expressed by TFCs in AITD glands and that this adaptive response (“don't kill me signal”) may restrain the autoimmune response and explain the chronicity of AITDs.\textsuperscript{18,19} Furthermore, byinterfering with the PD-1/PD-L1 axis in nontumoral TFCs, ICIIs can also exacerbate minimal/subclinical thyroiditis, resulting in clinically apparent AITD.\textsuperscript{19} Therefore, for indeterminate thyroid cytology, even a strong expression of PD-L1 should be taken with caution. PD-L1 expression can also be expressed in many cell types other than reactive TFCs, including histiocytes, dendritic cells, fibroblasts, as well as medullary thyroid carcinomas, parathyroid tumors, and metastases. Oncocytic lesions have rarely been tested with PD-L1; Dell’Aquila et al found that 79\% of their oncocytic neoplasms had PD-L1 expression.\textsuperscript{12} Given the notorious “stickiness” of oncocytes, it is likely that, as for other immunostains, these cells may also pick up nonspecific PD-L1 cytoplasmic staining.

**PD-L1 Expression and Thyroid Cancer Prognosis**

Interestingly, it has been demonstrated that the ERK-MAPK pathway regulates PD-L1 expression in different cancer types (Fig. 1).\textsuperscript{4} In thyroid carcinomas, there appears to be a correlation between \textit{BRAF} V600E mutation and the expression of PD-L1.\textsuperscript{20} A recent meta-analysis by Aghajani et al,\textsuperscript{17} which included 721 patients with nonmedullary thyroid carcinomas, showed a significant association between PD-L1 positivity, tumor recurrence, and poor survival, suggesting a possible role of PD-L1 as a prognostic marker (akin to \textit{BRAF} V600E). Conversely, although PD-L1 in PDTC was significantly associated with tumor size and multifocality, there was only a nonsignificant trend toward an association with prognostic factors.\textsuperscript{15} Thus, prospective clinical trials are needed to support these findings for different thyroid cancers.

**Anti–PD-1/PD-L–Directed Therapies and Thyroid Cancer**

The use of ICIIs in advanced thyroid cancer has not been well studied to date.\textsuperscript{1,21} Preclinical studies have
demonstrated the efficacy of PD-1/PD-L1 axis blockade in limiting the growth of ATC-derived cell lines injected into mice, but data from clinical trials with anti–PD-1/PD-L1 drugs for aggressive thyroid cancers have been limited and rather disappointing. In a retrospective study from The University of Texas MD Anderson Cancer Center, 12 patients with ATC received pembrolizumab in combination with kinase inhibitors at the time of disease progression. Of these patients, 5 had a partial response, 4 had stable disease, and 3 had progressive disease. A recent phase 1b study of pembrolizumab monotherapy in 22 patients with PD-L1–positive, advanced differentiated thyroid cancer showed that only 2 patients had confirmed partial responses lasting 8 and 20 months, respectively. Finally, the results from a phase 2 study of pembrolizumab combined with chemotherapy as initial treatment for ATC were published in October 2019. The 3 enrolled patients had favorable early tumor responses but died <6 months after therapy initiation, 1 from pulmonary metastases and 2 from unexpected fatal pulmonary complications subsequent to chemoradiotherapy, prompting study closure. Interestingly, there is no mention of PD-L1 immunohistochemistry testing in that study.

Overall, in our opinion, at the time of FNA, and especially on cytologic material, we doubt that there will be a significant role of PD-L1 immunohistochemistry for...
guiding therapeutics. The setting is very different from in the lung, in which patients often have advanced disease at diagnosis, and cytologic specimens are often the only material available for testing PD-L1, along with several other important predictive markers (eg, EGFR, ALK, ROS), and ICIs are well integrated into treatment options. In contrast, surgery remains the mainstay of treatment for the vast majority of thyroid cancers and generally offers better material for ancillary testing, if any. Cytologic materials also typically are excluded for PD-L1 assessment in clinical trials. A tissue biopsy, or conceivably a cell block, may be more suitable than LBC to assess PD-L1 as a predictive marker because the lack of tissue architecture in cytologic specimens precludes the ability to distinguish immune cells within the tumor area from those outside tumor boundaries, which are considered irrelevant for PD-L1 scoring. Other important predictive markers (eg, BRAF, DNA mismatch repair, PS6, NTRK) also can be assessed simultaneously.

**Future Perspectives**

Although PD-L1 has been assessed mostly by immunohistochemistry, a few studies have evaluated PD-L1 in tissues at the microRNA or the serum PD-L1 levels with interesting results. Rather than focusing just on a single marker, we anticipate that, in the future, the evaluation of PD-L1 for ICI therapy will be much more comprehensive, probably as part of a cancer “immunogram” (Fig. 2). In an era of personalized medicine, this is likely to be much more informative and relevant for prognostic/therapeutic purposes than a single marker like PD-L1 with so many flaws. Because of the multifactorial, variable, and complex nature of cancer-immune interactions, combinations of biomarker assays, by definition,
will be required.\textsuperscript{2,17} This was also the case for molecular markers, in which a comprehensive panel of gene alterations (ie, ThyroSeqV3) is now much more informative and relevant than a single-gene alteration such as \textit{BRAF} V600E or \textit{RAF}.\textsuperscript{9,10} Eventually, the integration of molecular data with immunogram data, ideally supplemented by artificial intelligence (which already has shown promising results in this field\textsuperscript{22}) is expected to guide treatment options in a more refined and personalized manner (precision immunoprofiling).

\textbf{Conclusions}

The limited retrospective data available to date suggest that PD-L1 could represent a prognostic marker for patients with thyroid cancer\textsuperscript{17} and that anti–PD-1/PD-L1 therapies could be an option for a very limited subset of patients who have aggressive thyroid cancers (PDTC or ATC) that are resistant to the currently available therapies.\textsuperscript{1,6-8,21} Provided cytologic samples are deemed adequate for PD-L1 testing, whether the information provided by this test will be clinically useful preoperatively is doubtful, and many issues remain to be addressed. It is also likely that a single test such as PD-L1 immunohistochemistry cannot be used as a reliable surrogate to predict the efficiency of immunotherapy and that taking into account other important components that affect complex and dynamic tumor-immune interactions will be required.

\textbf{FUNDING SUPPORT}

No specific funding was disclosed.

\textbf{CONFLICT OF INTEREST DISCLOSURES}

The authors made no disclosures.

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