IMMUNO-ONCOLOGY AND THE ROLE OF BIOMARKERS, COMPANION AND COMPLEMENTARY DIAGNOSTICS

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The recent evolution of immunotherapies and their potential applications have greatly impacted patients' options for effective oncology treatments. These advances have been powered in part by the use of a variety of biomarkers, companion diagnostics (CDx) and complementary diagnostics.

Having supported immuno-oncology drug development from preclinical studies to clinical trials, and post-market introduction of the therapy and associated diagnostics, global contract research organizations (CROs) like Covance have a unique perspective on the novel developments in this field. This whitepaper shares some of the highlights in personalized medicine Covance has been involved with during the last few years as new immuno-oncology therapies have emerged. You'll learn about:

- Programmed death-ligand 1 (PD-L1) expression patterns, assay formats and method comparison studies
- The current use of companion and complementary diagnostics
- Commercial implications and trends
- Advances and applications for genomic biomarker assays

The Pivotal Role of PD-L1 Expression in Immuno-Oncology

With a series of recently approved companion and complementary diagnostics, such as Dako's PD-L1 IHC 28-8 and 22C3 pharmDx immunohistochemical assays (IHC), and the Ventana PD-L1 (SP142) IHC assay, it's important to understand how PD-L1 expression correlates with potential therapeutic response rates. Many treatments, such as nivolumab, pembrolizumab and atezolizumab, target PD-1 or PD-L1 and use either companion and/or complementary diagnostics to evaluate the likely safety and effectiveness of the treatment. Regardless of the assay or indication, a higher level of PD-L1 expression generally indicates a greater likelihood of response to the therapy. (see example in Figure 1)

The PD-L1 assays are different than other recently approved CDx assays such as the BRAF mutation assays for the determination of eligibility for Zelboraf® (vemurafenib) treatment, in which response can be thought of as a binary function. If a patient has the specific V600 mutation, he/she will potentially respond, and therefore be considered for this therapeutic option—and if not, the likelihood of a response is almost zero.

However, with PD-L1, it's been shown that some patients who don't express PD-L1 can still respond to treatment, highlighting that this assay may not fit the traditional type of companion diagnostic used to stratify patients. This is one reason that the terminology has evolved to include both companion diagnostics, which are necessary for the safe and effective use of a specific therapy and complementary diagnostic, which while informative of the potential response are not required for use of the specific therapy.

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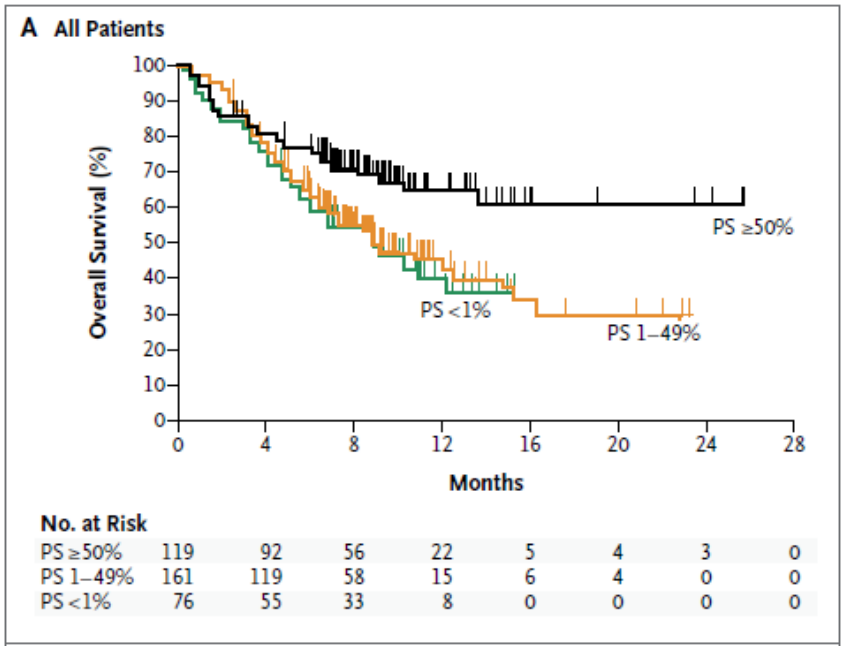
FIGURE 1 - Expression Levels of PD-L1 and Response to Pembrolizumab



Source: Garon et al, NEJM 372: 2018, 2015

Understanding PD-L1 Assays: Different Formats, Multiple Variations

From the perspective of the laboratory and the clinician end-user, the fact that there are different PD-L1 assays creates a variety of challenges. For example, the approved immunotherapies targeting either PD-1 or PD-L1– each have a different assay format that is associated with that specific therapy consideration. (see Table 1)

TABLE 1 – Assay Features and Uses

	Pembrolizumab	Nivolumab	Durvalumab	Atezolizumab
Drug Developer	Merck	BMS	Medimmune/ AstraZeneca	Genentech/Roche
MAb	Humanized IgG4	Humanized IgG4	Human Fc-modified IgG1	Human Fc-modified IgG1
Target	PD-1	PD-1	PD-L1	PD-L1
Approved Indications	Melanoma, NSCLC	Melanoma, NSCLC	NA	Bladder and renal cancers
IHC Developer	Dako	Dako	Ventana	Ventana
AB Clone	22C3, mouse	28-8, rabbit	SP263, rabbit	SP142, rabbit
Expression	Tumor cells (membrane) and stroma	Tumor cells (membrane)	Tumor cells (membrane)	Tumor cells, TICs
Scoring Cut-Off	>1% TCs in NSCLC	>1% TCs in melanoma and NSCLC	25% in NSCLC, SCCH&N	Intensity (2+, 3+) and % in TCs and TICs

NSCLC = non-small cell lung cancer
SCCH&N= squamous cell carcinomas of the head and neck
TIC=Tumor associated immune cells in the table above
TC=Tumor Cell

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In addition to variations in the platforms and reagents, each assay has a different scoring cutoff and interpretation characteristics. With the example of the current commercially available assays and associated therapies, clinicians face a complex situation with the use of the different diagnostics. Furthermore, because PD-L1 is heterogeneously and dynamically expressed, an assessment of a single sample prior to treatment may not provide a complete picture of PD-L1 status for the patient. As a result of these variations in assays, there has been an effort to harmonize, or at least compare, the methodologies.

Comparison of Assay Formats

To better understand and compare the analytical performance of the various companion and complementary diagnostics assays, our team at Covance looked at a cohort of non-small cell lung cancer (NSCLC) samples, which are known to have a wide range of PD-L1 expression. We stained these samples with two commercially-available assays for NSCLC: the Dako 28-8 pharmDx assay and the Ventana SP263 assay. Looking at the number of positive samples and range of expression, we noted some differences, which were mainly related to the different cutoffs for the intended use.

When examining the percentage of negative samples, there is a high agreement between the two assays; whereas, the positive agreement is significantly lower. The lower concordance for positive samples is mainly due to the different assay cutoffs, since each shows a similar dynamic range of expression. Therefore, biology isn't necessarily driving all the differences in detection rate—instead one must focus on how the assays are used and interpreted.

Evaluating the Current Use of Companion and Complementary Diagnostics

At Covance, our relationship with LabCorp gives us a unique capability to perform end-to-end services to support trials, starting as early as preclinical work and extending through late-stage pivotal clinical trials. Also in our current environment, as these assays become available, we can help sponsors commercialize them and offer for clinical use.

This distinct advantage gives us insights regarding both the analytical performance and usage of companion versus complementary diagnostics, including differences in how the assays perform, the number of positive cases along with the range of expression and how often an indeterminate result is obtained.

For example, in Table 2 we compared how the two Dako IHC assays used for NSCLC patients analytically perform, evaluating the number of positive samples as well as the distribution of staining results. As demonstrated in this analysis, while the distribution of staining intensities for the clinical samples evaluated is very similar, the percentage of positive samples indicated difference. This difference is mainly related to the assay specific cutoffs used (>1% versus >50%) rather than other performance features of the assays.

TABLE 2 - Comparison of Two Commercially Available PD-L1 Assays for NSCLC - Analytical Performance of Assays, Positive Samples and Spectrum of IHC Staining

	% Positive Samples	% Negative Samples
22C3 pharmDx IHC	28.9	71.1
28-8 pharmDx IHC	55.6	44.4

	<1%	1-10%	11-25%	26-49%	50-75%	>76%
22C3 pharmDx IHC	35.5	20.1	8.2	7.3	14.6	14.3
28-8 pharmDx IHC	44.4	20.3	5.3	3.3	12.8	13.9

Source: "Companion and Complementary Diagnostics for PD-L1 Expression Assessment in Non-Small Cell Lung Cancer," Steven M. Anderson, PhD, et al., 2016

In addition, we can evaluate the commercial ordering patterns of clinicians to better understand the use of a companion diagnostic versus a complementary diagnostic. Based on the number of specimens that have been submitted to LabCorp for commercial testing over a six month period after both assays received regulatory approval, it showed that the vast majority of the requests (a 5:1 ratio) have been for the companion diagnostic. Given that a CDx assay is required to prescribe the drug and not just informative of potential response, this difference in utilization is not surprising. Because the two different assays use the same CPT®[1] procedural code there is no specific way to differentiate the usage by payors and health systems.

Genomic Biomarkers in Immuno-oncology

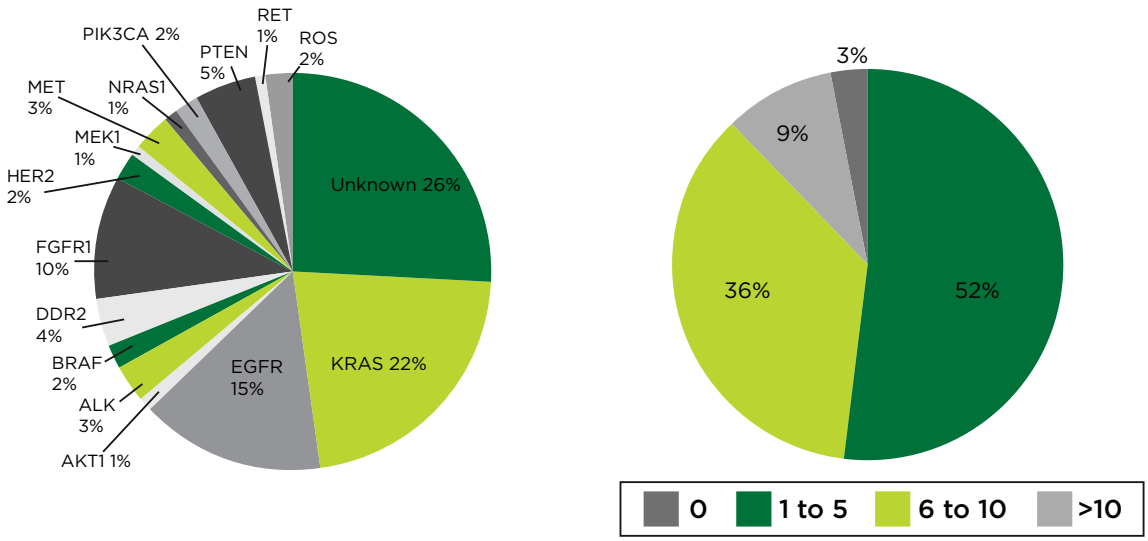
A variety of other assays, besides PD-L1 status, also have potential value in helping determine which patients may respond best to a given immunotherapy. For example, mutational burden/content analysis can be helpful in a variety of cancers including those in which there is a significant potential environmental mutagenic component, such as lung cancer tumors and tobacco use and melanomas and UV exposure. In these cancers it is not only the number of mutations that is important, but whether a particular mutation produces a novel protein or neo-antigen, which the immune system may find highly immunogenic.

Figure 2 displays both the spectrum of mutations and mutational burden demonstrated in lung adenocarcinomas. An increase in tumor burden may be surrogate marker of the likelihood of the production of neo-antigens and thereby an indication of response to immunotherapy agents.

[1]CPT® is a registered trademark of the American Medical Association.

FIGURE 2 - Mutational Spectrum and Burden in Non-Small Cell Lung Cancer

Genomics of Lung Adenocarcinoma-Targeted DNA Sequence Analysis



Source: "Companion and Complementary Diagnostics for PD-L1 Expression Assessment in Non-Small Cell Lung Cancer," Steven M. Anderson, PhD, et al., 2016

In colorectal cancer, genomic instability—as defined by deficiencies in the mismatch repair genes—is potentially a strong predictor of response to checkpoint inhibition, with microsatellite unstable tumors responding to treatment and microsatellite–stable essentially demonstrating little or no response. Mismatch repair deficiencies occur in about 20 percent of colon cancer samples, and are also common in other tumor types, indicating that this is a potential marker of response in other cancers as well.

Gene expression profiling provides another tool that can be used to sub-classify tumor types beyond standard pathology, and in NSCLC helps us better understand which tumor sub-types may be primed to respond to an immunotherapy. In addition, the gene expression analysis of a broad spectrum of genes associated with the tumor and the infiltrating lymphocytes (TILs) can be helpful in identifying potential biomarkers of response to immunotherapy. For example, immune function genes (i.e., T-effector and T-regulator genes), inflammatory markers (cytokines, chemokines), and/or HLA expression appear to provide potentially predictive information in some tumor types. Lastly, the T-cell repertoire of the TILs associated with the tumor sample can also be evaluated, as studies have shown that the diversity of the T-cells infiltrating a tumor is also relevant to responsiveness to immunotherapies.

Future Considerations

Immuno-oncology drug development is inherently complex and requires special considerations across the entire spectrum of activities, from preclinical studies to commercialization of the therapy and relevant diagnostic assays. A variety of biomarker assays can be used to assess the potential efficacy of a specific therapeutic approach. Many proteomic and genomic approaches are being evaluated in a variety of tumor types and with various immunotherapeutic agents, emphasizing that PD-L1 expression, while important, is not the only potential biomarker that can provide clinical value in this rapidly evolving therapeutic area.

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