Three different PD-L1 immunohistochemical (IHC) assays have recently been approved to help guide treatment decisions regarding anti-PD-1 [Keytruda® (Merck Sharp & Dohme Corp), Opdvo® (Bristol-Myers Squibb)] and anti-PD-L1 [Tecentriq® (Genentech USA, Inc.)] immuno-oncology based therapies. The three assays differ in the primary monoclonal antibodies used, the assay platform (Dako, Ventana), scoring and interpretation criteria, and intended use. Our clinical laboratories currently offer all 3 assays [pharmDx™ 22C3, pharmDx™ 28-8 (Agilent Technologies), and SP142] because they are associated with different intended uses, companion and complementary diagnostics for non-small-cell lung cancer (NSCLC), and complementary diagnostic for urothelial carcinomas (UC). In the NSCLC samples evaluated in the first year since the approval of the two pharmDx™ assays, we have noted a higher percentage of positive samples with the pharmDx™ 28-8 assay (54%) compared to the pharmDx™ 22C3 assay (32%). This difference is mainly due to the use of different assay cutoffs associated with the two assays, as there is good correlation between the overall staining patterns, including the distribution of the percentage of tumor cells exhibiting positive staining. For the SP142 assay tested on UC and NSCLC samples, 25% of the cases were considered positive. All 3 assays are robust, with a limited number of samples considered inconclusive (<10%), mainly due to an inadequate number of tumor cells. The companion diagnostic PD-L1 assay (pharmDx™ 22C3) has had the greatest utilization of the 3 assays, as it is required for the use of the Keytruda® in NSCLC samples. The performance features of the 3 different PD-L1 assays in the clinical laboratory setting has shown that all assays are robust and can be readily scored and interpreted by the pathology team. The utilization of the 3 assays correlates with respective intended use, tumor type and companion versus complementary diagnostic application, for each assay.

**Background**

PD-L1 expression has been an important biomarker used in the evaluation of patient samples for the consideration of treatment with checkpoint inhibitors such as Keytruda®, Opdvo® and Tecentriq®. While other biomarkers such as mutational burden, genetic alterations resulting in neo-antigens, genomic instability, and blood-based markers of immune function have also been investigated, PD-L1 expression continues to be a primary means of assessing for the potential patient response to treatment.1 Recently several assays have been approved for use as companion or complementary diagnostics for the specific therapy considerations. A companion diagnostic assay is required for the utilization of a specific therapy, whereas a complementary diagnostic provides useful information regarding the therapy and potential response, but is not required.2

**Methods**

Clinical FFPE samples submitted between October 2015 and March 2017 for PD-L1 immunohistochemistry were included in this evaluation. The PD-L1 immunohistochemistry (IHC) companion diagnostic for Keytruda® involves the use of a Mouse Monoclonal antibody (clone 22C3), run on the Dako Link48 platform with standard HRP-DAB detection. For this assay the tissue sample must contain at least 100 cells for evaluation. Partial to complete membrane staining (≥1+ intensity) is considered positive for PD-L1 staining. A Tumor Proportion Staining (TPS) score of >50% is considered as a positive result. The PD-L1 immunohistochemistry (IHC) companion diagnostic for Opdvo® involves the use of a Rabbit Monoclonal antibody (clone 28-8), run on the Dako Link48 platform with standard HRP-DAB detection. For this assay the tissue sample must contain at least 100 cells for evaluation. Partial to complete membrane staining (≥1+ intensity) is considered positive for PD-L1 staining. A sample is considered positive for PD-L1 staining if ≥1% of the tumor cells exhibit staining. The PD-L1 immunohistochemistry complementary diagnostic for Tecentriq® involves the use of a Rabbit Monoclonal Antibody (clone SP142) with the Optiview detection system. PD-L1 staining of tumor infiltrating immune cells and tumor cells are evaluated, with urothelial samples showing staining at any level in the infiltrating lymphocytes, covering ≥5% of the tumor area considered positive for PD-L1 expression. In NSCLC positive expression of PD-L1 is demonstrated by >10% of TICs or >50% of tumor cells staining positive.

For the purpose of this analysis expression levels were categorized as high, low and no expression based on specific assay scoring criteria.

**Results**

A comparison of the clinical use and analytical performance of two commercially available PD-L1 IHC assays is provided in the figures below.

**Discussion**

- Immunohistochemical (IHC) assays for PD-L1 expression are currently being implemented for routine clinical use
- In our reference lab setting the IHC assays with the companion diagnostic designation is more frequently ordered by clinicians than those with the complementary diagnostic indication
- The three assays evaluated in this comparison show very similar analytical performance features
- Differences in the frequency of samples indicated as positive for PD-L1 expression are more strongly associated with the different assay scoring algorithms and cutoffs rather than analytical performance differences of the assays

**References**

1. Hedge et al. (2016) CCR 22: 1865
2. Hanson et al. (2016) JAMA Oncology 2:15