Multi-Site External Reproducibility of PD-L1 IHC 22C3 pharmDx Performance on Dako Omnis and Comparison to Autostainer Link 48 for Head and Neck Squamous Cell Carcinoma using the Combined Positive Score

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Introduction

PD-L1 IHC 22C3 pharmDx is a diagnostic assay for use on the Autostainer Link 48 (ASL48) instrument that is intended for use in the detection of PD-L1 protein in multiple cancer types. However, to date PD-L1 IHC 22C3 pharmDx on the Dako Omnis platform has only been validated for non-small cell lung cancer. This study sought to demonstrate external reproducibility of PD-L1 IHC 22C3 pharmDx for head and neck squamous cell carcinoma (HNSCC) using the Dako Omnis platform (Investigational Use Only/Performance Evaluation Only)#. This study also investigated the concordance of PD-L1 IHC 22C3 pharmDx on HNSCC on both ASL48 and Dako Omnis staining platforms at external sites.

Methods

Study Design

This study was a three-site, blinded, randomized study of PD-L1 IHC 22C3 pharmDx (Investigational Use Only/Performance Evaluation Only) on FFPE human HNSCC specimens. The study was divided into two parts: inter-site/intra-site reproducibility and an assay performance comparison study between PD-L1 IHC 22C3 pharmDx (Code GE006) performed on Dako Omnis platform versus PD-L1 IHC 22C3 pharmDx (Code SK006) performed on Autostainer Link 48 within each of the three sites. The slide sets were evaluated by site pathologists trained on the Combined Positive Score (CPS) scoring algorithm and evaluated at two cutoffs (CPS ≥ 1 and CPS ≥ 20). Forty of the specimens were predetermined to be analyzed for CPS ≥ 1 and 40 specimens were predetermined to be analyzed for CPS ≥ 20.

Inter- and Intra-site Reproducibility:

Specimen sets used for analyses were stained and evaluated (for inter-site reproducibility across three sites and 5 rounds/ intra-site reproducibility across 5 rounds within three sites). A round is considered an iteration of staining and scoring using PD-L1 IHC 22C3 pharmDx (Code GE006). All staining was performed using automated staining runs of tissue sections on Dako Omnis. All slides were re-randomized for evaluation order between rounds and interpreted by the pathologists blinded from specimen identity as well as the previous evaluations.

Inter-platform Performance Comparison:

Three sets of slides from HNSCC FFPE specimens were stained by Agilent Technologies, Inc. using PD-L1 IHC 22C3 pharmDx (Code SK006) on the Autostainer Link 48 platform and provided to the three sites. The specimens stained with SK006 were prepared from the same cases as those that were stained by GE006 at each site. The same pathologist who performed GE006 scoring for each cutoff for the inter- and intra-site reproducibility portion evaluated the one set of SK006 stained slides.

Scoring

PD-L1 protein expression in HNSCC is determined by using the Combined Positive Score (CPS), which is the number of PD-L1 staining cells at any intensity (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100. Although the CPS can exceed 100, the maximum score is defined as CPS 100. CPS is defined as follows:

$$\text{CPS} = \frac{\text{\# PD-L1 staining cells (tumor cells, lymphocytes, macrophages)}}{\text{Total \# viable tumor cells}} \times 100$$

By definition, PD-L1 staining cells are:

- Tumor cells with convincing partial or complete membrane staining
- Mononuclear inflammatory cells directly associated with tumor response demonstrating membrane and/or cytoplasmic staining

Statistical Analysis

The analytical agreements of the diagnostic outcome (positive/negative) were estimated by evaluating negative percent agreement (NPA), positive percent agreement (PPA), and overall agreement (OA) calculations by making comparisons in PD-L1 status (positive/negative) between individual observations to the consensus PD-L1 status (most frequent PD-L1 status occurrence in the specimen) at the CPS ≥ 1 and CPS ≥ 20 cutoffs separately.

Two-sided 95% confidence intervals (CI) were then calculated for NPA, PPA, and OA using a non-parametric percentile bootstrap method. The Wilson score method was used to calculate CIs for agreement parameters with 100% point estimates. Acceptance criteria (AC) were that the lower bound value of CI for NPA, PPA, and OA must meet or exceed 85.0%.

#Note: the head and neck squamous cell carcinoma (HNSCC) indication is not approved by regulatory authorities for PD-L1 IHC 22C3 pharmDx (Code GE006) on the Dako Omnis platform.

Results

Representative images of FFPE HNSCC specimens stained with GE006 and SK006

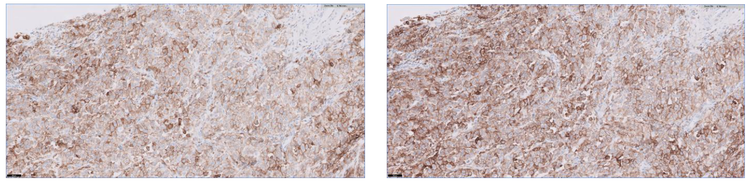

Figure 1. HNSCC specimens stained with PD-L1 IHC 22C3 pharmDx on Dako Omnis (Code GE006) (20X; left image) and on Autostainer Link 48 (Code SK006) (20x; right image) (Scale bar = 50 µm)

Results for Inter- and Intra-site Reproducibility Endpoints

Table 1 Inter-site Reproducibility Analysis, CPS ≥ 1 Cutoff

Inter-Site Reproducibility Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
NPA	95.6%	90.5%	100.0%
PPA	100.0%	98.7%**	100.0%**
OA	97.7%	95.0%	100.0%

Table 2 Intra-Site Reproducibility Analysis, CPS ≥ 1 Cutoff

Intra-Site Reproducibility Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
NPA	99.7%	98.9%	100.0%
PPA	99.3%	98.0%	100.0%
OA	99.5%	98.5%	100.0%

Table 3 Inter-Site Reproducibility Analysis, CPS ≥ 20 Cutoff

Inter-Site Reproducibility Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
NPA	94.0%	87.7%	98.6%
PPA	95.6%	91.1%	99.4%
OA	94.8%	91.2%	98.0%

Table 4 Intra-Site Reproducibility Analysis, CPS ≥ 20 Cutoff

Intra-Site Reproducibility Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
NPA	96.5%	92.7%	99.3%
PPA	97.8%	95.8%	99.4%
OA	97.2%	95.0%	99.0%

Results for Inter-platform Performance Comparison Endpoints

Table 5 Inter-platform Performance Comparison Analysis, CPS ≥ 1 Cutoff

Inter-Platform Performance Comparison Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
NPA	98.7%	96.0%	100.0%
PPA	100.0%	98.7%**	100.0%**
OA	99.3%	98.0%	100.0%

Table 6 Inter-platform Performance Comparison Analysis, CPS ≥ 20 Cutoff

Inter-Platform Performance Comparison Agreement Summary			
Performance Criteria	Point Estimate	95% Confidence Interval (Bootstrap)	
		Lower-bound: 2.5%	Upper-bound: 97.5%
NPA	95.4%	90.6%	98.7%
PPA	96.8%	94.2%	99.0%
OA	96.2%	93.3%	98.7%

**The percentile bootstrap method cannot compute confidence bounds if 100% agreement is observed. The Wilson score limits are used to calculate confidence intervals for agreement parameters with point estimates equal to 100%.

Results (continued)

Summary Representation of the Diagnostic Estimate of External Reproducibility Study

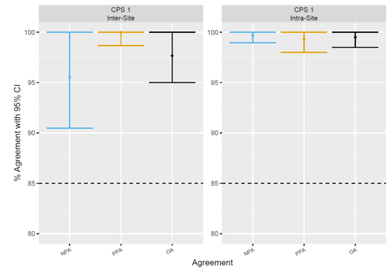

Figure 2. Summary of % Agreement for CPS ≥ 1 Cutoff

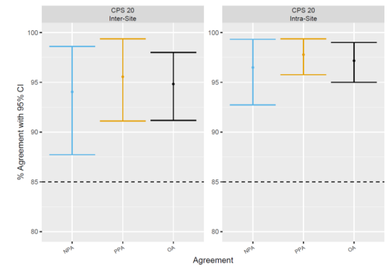

Figure 3. Summary of % Agreement for CPS ≥ 20 Cutoff

Summary Representation of the Diagnostic Estimate of Inter-platform Performance Comparison

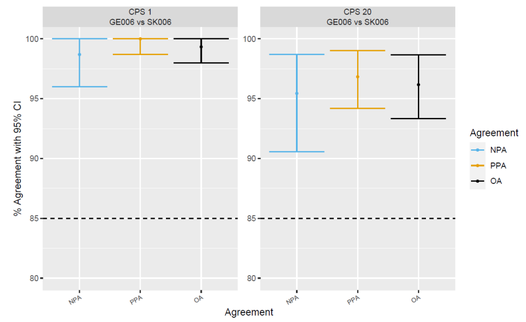

Figure 4. Summary of Results for CPS ≥ 1 and ≥ 20 Cutoffs

Conclusions

At the CPS ≥ 1 cutoff, inter-and intra-site NPA/PPA/OA met AC with point estimates (PE) ≥ 95.6% and confidence interval (CI) lower bounds ≥ 90.5%. At the CPS ≥ 20 cutoff, inter-and intra-site NPA/PPA/OA met AC, with PE ≥ 94.0% and CI lower bounds ≥ 87.7%.

At the CPS ≥ 1 cutoff, inter-platform performance between PD-L1 IHC 22C3 pharmDx performed on Dako Omnis (Code GE006) versus Autostainer Link 48 (Code SK006) met AC with PE ≥ 98.7% and CI lower bounds ≥ 96.0%. At the CPS ≥ 20 cutoff, inter-platform comparison met AC with PE ≥ 95.4% and CI lower bounds ≥ 90.6%.

To summarize, the results of these studies demonstrate external inter- and intra-laboratory reproducibility of PD-L1 IHC 22C3 pharmDx (Code GE006) and equivalence to PD-L1 IHC 22C3 pharmDx (Code SK006) with respect to expression determination in HNSCC at the CPS ≥ 1 and CPS ≥ 20 cutoffs.

Ethics Approval

The external reproducibility study was approved by WCG IRB; study numbers 1363131, 1362724, and 1364731.

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