SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	In vitro diagnostic immunohistochemistry (IHC) for detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) human tissue sections
Device Trade Name:	PD-L1 IHC 22C3 pharmDx
Device Procode:	PLS
Applicant's Name and Address:	Dako North America, Inc. 6392 Via Real Carpinteria, CA 93013
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P150013/S014
Date of FDA Notice of Approval:	June 10, 2019

The original PMA (P150013) for PD-L1 IHC 22C3 pharmDx intended to detect PD-L1 protein in non-small cell lung cancer (NSCLC) was approved on October 2, 2015. Subsequently, four additional indications were approved for gastric and gastroesophageal junction adenocarcinomas (S006) on September 22, 2017, cervical cancer (S009) on June 12, 2018, and urothelial cancer (S011) on August 16, 2019. The SSED for these indications are available on the CDRH website and is incorporated by reference here. The current supplement was submitted to expand the indication for the PD-L1 IHC 22C3 pharmDx to include head and neck squamous cell carcinoma (referred to as HNSCC throughout this SSED).

II. INDICATIONS FOR USE

PD-L1 IHC 22C3 pharmDx is a qualitative immunohistochemical assay using Monoclonal Mouse Anti-PD-L1, Clone 22C3 intended for use in the detection of PD-L1 protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung cancer (NSCLC) and gastric or gastroesophageal junction (GEJ) adenocarcinoma, cervical cancer, urothelial carcinoma and head and neck squamous cell carcinoma (HNSCC) tissues using EnVision FLEX visualization system on Autostainer Link 48.
PD-L1 protein expression in NSCLC is determined by using Tumor Proportion Score (TPS), which is the percentage of viable tumor cells showing partial or complete membrane staining at any intensity.

PD-L1 protein expression in gastric or GEJ adenocarcinoma, cervical cancer, urothelial carcinoma and HNSCC is determined by using Combined Positive Score (CPS), which is the number of PDL1 staining cells (tumor cells, lymphocytes, macrophages) divided by the total number of viable tumor cells, multiplied by 100.

Companion Diagnostic Indications

Tumor Indication*	PD-L1 Expression Level	Intended Use
NSCLC	TPS ≥ 1%,	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying NSCLC patients for treatment with KEYTRUDA® (pembrolizumab). *
Gastric or GEJ adenocarcinoma	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying gastric or GEJ adenocarcinoma patients for treatment with KEYTRUDA® (pembrolizumab).
Cervical Cancer	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying cervical cancer patients for treatment with KEYTRUDA® (pembrolizumab).
Urothelial Carcinoma	CPS ≥ 10	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying urothelial carcinoma patients for treatment with KEYTRUDA® (pembrolizumab). *
HNSCC	CPS ≥ 1	PD-L1 IHC 22C3 pharmDx is indicated as an aid in identifying head and neck squamous cell carcinoma patients for treatment with KEYTRUDA® (pembrolizumab). *

**See the KEYTRUDA® product label for specific clinical circumstances guiding PD-L1 testing.

III. CONTRAINDICATIONS

There are no known contraindications for the use of this test.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the PD-L1 IHC 22C3 pharmDx product labeling.

V. DEVICE DESCRIPTION

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an immunohistochemical staining procedure for formalin-fixed and paraffin-embedded (FFPE) specimens using the Dako Autostainer Link 48 automated staining and the EnVision FLEX visualization system. The principle component of the kit is the mouse monoclonal anti PD-L1 clone 22C3 antibody that binds to PD-L1 protein expressed on FFPE tissue. Each kit includes 19.5 mL of PD-L1 primary antibody (approximately 3µg/mL protein concentration) and reagents shown in Table 1 necessary to perform 50 tests in up to 15 individual runs. Wash buffer and hematoxylin are required for the assay but not included in the kit. PT Link Pre-Treatment Module is required for deparaffinization, rehydration and target retrieval of the tissues. Cover-slipping is required but can be performed by either manual or automated methods.

Table 1. Overview of PD-L1 IHC 22C3 pharmDx Components

Reagent	Description
Peroxidase Blocking Reagent	Buffered solution containing hydrogen peroxide, detergent and 0.015mol/L sodium azide.
Monoclonal Mouse anti–PD-L1, Clone 22C3	Monoclonal mouse anti-PD-L1 antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.
Negative Control Reagent	Monoclonal mouse control IgG antibody in a buffered solution, containing stabilizing protein, and 0.015mol/L sodium azide.
Linker, Anti-Mouse	Rabbit secondary antibody against mouse immunoglobulins in a buffered solution containing stabilizing protein and 0.015mol/L sodium azide.
Visualization Reagent-HRP	Dextran coupled with peroxidase molecules and goat secondary antibody molecules against rabbit and mouse immunoglobulins in a buffered solution containing stabilizing protein and an antimicrobial agent.
DAB+ Buffered Substrate	Buffered solution, containing hydrogen peroxide and an antimicrobial agent.
DAB+ Chromogen	3,3'-diaminobenzidine tetrahydrochloride in an organic solvent.
DAB Enhancer	Cupric sulfate in water.
Target Retrieval Solution Low pH (50X)	Buffered solution, pH 6.1, containing detergent and an antimicrobial agent.
Cell Line Control Slides	Each slide contains sections of two pelleted, formalin-fixed paraffin-embedded cell lines: NCI-H226 with moderate PD-L1 protein expression and MCF-7 with negative PD-L1 protein expression.

Device Instrumentation and Software

PD-L1 IHC 22C3 pharmDx assay is performed on the Dako Autostainer Link 48 automated staining system using the DakoLink software 4.0.3 or later. The Autostainer system is designed to mimic the staining steps performed manually by a lab technician. The PD-L1 IHC 22C3 pharmDx protocol is assay specific. The DakoLink software has been designed to recognize and group PD-L1 IHC 22C3 pharmDx reagents, requiring that all system reagents are used together. Deparaffinization, rehydration and target retrieval (3-in-1) procedures are performed in the PT Link Pre-treatment module (PT100/200 modules).

Specimen Preparation

HNSCC specimens must be handled appropriately to preserve the tissue for IHC staining. Standard methods of tissue processing should be used for all specimens.

Formalin-fixed, paraffin-embedded tissues are suitable for use. Alternative fixatives have not been validated and may give erroneous results. Fixation time for 12-72 hours in 10% neutral buffered formalin (NBF) is recommended. Fixation times of ≤3 hours may result in variable PD-L1 detection. Specimens should be blocked into a thickness of 3 or 4 mm, fixed in formalin and dehydrated and cleared in a series of alcohols and xylene, followed by infiltration with melted paraffin. The paraffin temperature should not exceed 60 °C.

Tissue specimens should be cut into sections of 4-5µm, mounted on charged microscope slides, and then placed in a 58 ± °C oven for 1 hour. To preserve antigenicity, tissue sections, once mounted on slides, should be held in the dark at 2-8 °C (preferred), or at room temperature up to 25°C, and stained within 1 months of sectioning. Slide storage and handling conditions should not exceed 25°C at any point post-mounting to ensure tissue integrity and antigenicity.

Test Controls and Calibrators

Run controls are included in each staining run to establish the validity of the test results. In the device labeling, Dako recommends the following controls to be run with the assay:

1) Control cell line slides provided as part of the kit should be used to verify the staining procedure. One Control Slide should be stained with the primary antibody to PD-L1 in each staining run. Each slide contains sections of 2 pelleted, FFPE cell lines: one with moderate PD-L1 protein expression and one that is negative for PD-L1 expression. The evaluation of the Control Slide cell lines supplied in the kit indicates the validity of the staining run. The Control Slides should not be used as an aid in interpretation of patient results.

2) Run controls are to be provided by the end-user laboratory. Positive and negative run controls should be fresh biopsy/surgical specimens of the same tumor indication as the patient specimen, fixed, processed and embedded as soon as possible in the same manner as the patient sample(s). The positive control tissue should include weak staining for PD-L1 to detect subtle changes in assay sensitivity. Negative control tissue

is required to detect unintended antibody cross reactivity to tissue and is expected to be negative for PD-L1 expression.

3) The Kit includes a Negative Control Reagent that is used in parallel with the PD-L1 Clone 22C3 primary antibody on patient tissue. The matched negative control aids the reader in differentiating a true signal from tissue-specific background staining that occurs from reaction with detection chemistry and not the anti PD-L1 primary antibody.

Additional information about the use of controls is available in the product labeling.

Principle of Operation

PD-L1 IHC 22C3 pharmDx contains optimized reagents required to complete an IHC staining procedure on FFPE specimens using the Autostainer Link 48. Following deparaffinization of the tissue sections, rehydration and target retrieval, the slides are incubated with the primary monoclonal antibody to PD-L1 (Clone 22C3) or the Negative Control Reagent. The slides are then incubated with an anti-mouse Linker antibody, which is specific to the host species of the primary antibody. Following this, the slides are incubated with a ready-to-use Visualization Reagent consisting of secondary antibody molecules and horseradish peroxidase molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added DAB+ Chromogen results in precipitation of a visible reaction product at the antigen sites. The color of the chromogenic reaction is modified by a chromogen enhancement reagent, DAB Enhancer. The specimen may then be counterstained with hematoxylin and cover-slipped.

Staining Procedure:

The PD-L1 IHC 22C3 pharmDx is designed to be run on the Autostainer Link 48 with DakoLink software.

The staining protocol on the Autostainer Link 48 is as follows:

- Peroxidase-Blocking Reagent (2 drop zones x150µL): 5 minutes (± 1 minute)
- Rinse in buffer
- Monoclonal Mouse anti-PD-L1 (or Negative Control Reagent) (2 drop zones
- x150µL): 30 minutes (± 1 minute)
- Rinse in buffer
- Linker, anti-Mouse Ig (2 drop zones x150µL): 30 minutes (± 1 minute)
- Rinse in buffer
- Visualization Reagent (2 drop zones x150µL): 30 minutes (± 1 minute)
- Rinse in buffer: 5 minutes
- DAB+ solution (2 drop zones x150µL): 2 x 5 minutes (± 1 minute)
- Rinse in buffer
- DAB+ Enhancer (2 drop zones x150µL): 5 minutes (± 1 minute)
- Rinse in buffer
- Hematoxylin (2x150µL): 5 minutes (± 1 minute)
- Rinse in deionized water

- Rinse in buffer: 5 minutes
- Rinse in deionized water
- Remove slides from autostainer and place in bath of reagent water

Interpretation of PD-L1 Staining

The labeling instructs that all viable tumor cells on the entire tissue must be evaluated and included in PD-L1 scoring assessment. A minimum of 100 viable tumor cells must be present in the PD-L1 stained slide for the specimen to be considered adequate for PD-L1 evaluation. If patient specimens include more than one biopsy (i.e., 3-5 biopsies) on a slide, all tissues on the slide need to be evaluated to generate a single CPS for determining the PD-L1 expression level. Each biopsy should not be reported independently.

The labeling instructs that slide evaluation must be performed by a pathologist using a light microscope. For determination of PD-L1 expression, an objective of 20x magnification is required.

Assessment of PD-L1 expression in HNSCC includes:

- Any partial or complete linear membrane staining (at any intensity) of tumor cells that is perceived distinct from cytoplasmic staining
- Any membrane and/or cytoplasmic staining (at any intensity) of tumor associated lymphocytes and macrophages (mononuclear inflammatory cells, MICs) within the tumor nests and/or adjacent supporting stroma.

Tumor PD-L1 expression in HNSCC specimens is determined by Combined Positive Score (CPS), which is the number of PD-L1 staining cells (tumor cells, macrophages, lymphocytes) divided by the total number of all viable tumor cells, multiplied by 100. Distinction of viable tumor cells, lymphocytes, and macrophages is essential for accurate denominator estimation. Although the result of the calculation can exceed 100, the maximum score is defined as CPS 100. CPS is defined as follows:

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

The table below provides details about which tissue elements are included in and excluded from the CPS numerator in HNSCC.

Table 2. Tissue Elements in Determining the Combined Positive Score

Tissue Elements	Included in the Numerator	Excluded from the Numerator
Tumor Cells	Convincing partial or complete linear membrane staining (at any intensity) of viable tumor cells including:	• Non-staining tumor cells • Tumor cells with only cytoplasmic staining

Immune Cells	Membrane and/or cytoplasmic* staining (at any intensity) of mononuclear inflammatory cells (MICs) within tumor nests and adjacent supporting stroma.** • Lymphocytes (including lymphocyte aggregates) • Macrophages*** Only MICs directly associated with the response to the tumor are scored.	• Non-staining MICs • MICs (including lymphoid aggregates) associated with ulcers, or other inflammatory process • MICs associated with carcinoma in situ • MICs associated with benign structures • Neutrophils, eosinophils and plasma Cells
Other Cells	Not included	• Carcinoma in situ • Benign Cells • Stromal cells (including fibroblasts) • Necrotic cells and/or cellular debris

In **MICs membrane and cytoplasmic staining are often indistinguishable due to high nuclear to cytoplasmic ratio. Therefore, membrane and/or cytoplasmic staining of MICs is included in the score.*

*****Adjacent MICs** are defined as being within the same 20x field as the tumor. However, MICs that are NOT directly associated with the response to the tumour should be excluded.*

****Macrophages and histiocytes are considered the same cells.*

For each staining run, slides should be examined in the order recommended in the product labeling. The labeling instructs users to examine patient specimens stained with PD-L1 and the Negative Control Reagent from PD-L1 IHC 22C3 pharmDx when evaluating PD-L1 expression. Specimens stained with NCR must have 0 specific staining and ≤ 1+ nonspecific staining.

HNSCC specimens are evaluated for PD-L1 expression at CPS ≥1 and CPS ≥20 cut offs.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There is currently no alternative FDA-cleared or approved immunohistochemistry assay available for use as an aid in identifying patients with HNSCC for treatment with KEYTRUDA (pembrolizumab).

VII. MARKETING HISTORY

PD-L1 IHC 22C3 pharmDx has been marketed in the United States since approval of P150013 on October 2, 2015. PD-L1 IHC 22C3 pharmDx has also been marketed in Albania, Algeria, Argentina, Australia, Austria, Bahrain, Belgium, Bosnia and Herzegovina, Brazil, Canada, Chile, Colombia, Costa Rica, Denmark, Ecuador, Egypt, Finland, France, Germany, Hong Kong, India, Indonesia, Hungary, Iceland, Ireland, Iraq, Israel, Italy, Japan, Jordan, South Korea, Kazakhstan, Kosovo, Kuwait, Lebanon, Lichtenstein, Macau, Macedonia, Malaysia, Montenegro, Morocco, Netherlands,

New Zealand, Norway, Oman, Panama, Peru, Philippines, Poland, Qatar, Russia, Saudi Arabia, Singapore, Slovakia, Serbia, South Africa, Spain, Sweden, Switzerland, Taiwan, Thailand, Turkey, Ukraine, United Arab Emirates, Uruguay and Vietnam.

This device has not been withdrawn from marketing for any reason related to safety and effectiveness."

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect PD-L1 test results and subsequently improper assignment of treatment with KEYTRUDA®. Patients with a false negative assay result may not be considered for treatment with KEYTRUDA (pembrolizumab). Patients with a false positive assay result may receive treatment with KEYTRUDA (pembrolizumab) for which there is no expectation of benefit and exposure to potential toxicity. There is also a risk of delayed results, which may lead to delay in treatment.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Preclinical studies were performed using the PD-L1 IHC 22C3 pharmDx to establish analytical performance of the device. The scoring algorithm used in these studies included a clinical score (i.e., PD-L1 positive or negative) and/or analytical score (CPS 0-100). Binary outcomes were assessed for all studies with the scoring algorithm developed for clinical interpretation of the PD-L1 22C3 IHC Assay. Continuous scores were reported for some studies to ensure assay performance in borderline cases. Antibody characterization studies for clone 22C3, including specificity and tour of body/ tour of tumor, control cell line validation, kit stability and preanalytical variables were submitted and reviewed in the original PMA (P150013) for this device. Study designs and results are available in the Summary of Safety and Effectiveness Data: https://www.accessdata.fda.gov/cdrh_docs/pdf15/P150013B.pdf. Results from studies performed to support the HNSCC indication are summarized in the sections below.

1. Analytical Specificity

Assessment of analytical specificity of the monoclonal mouse anti-human PD-L1 clone 22C3 antibody was provided and reviewed under P150013 and included Western blot and immunoreactivity in human tissues, both normal and tumor. Refer to the SSED associated with the original PMA for study design and results.

2. Analytical Sensitivity

Analytical sensitivity of PD-L1 IHC 22C3 pharmDx was tested on 112 unique HNSCC FFPE tissue specimens using one lot of the device. The specimens were chosen at random and represented the full range of PD-L1 expression and staining intensity (i.e., CPS range 0-100). One specimen was not evaluable due to high background staining. A total of 72% (n=81) of cases were PD-L1 positive at CPS expression level ≥1 and 45% (n=50) expressed PD-L1 at CPS ≥20..

3. Repeatability

The objective of this study was to demonstrate that PD-L1 IHC 22C3 pharmDx would produce consistent staining in normal day-to-day testing of HNSCC specimens with multiple lots of test kit.

Precision was assessed in 3 separate studies: intra-run, combined precision (inter-instrument/operator/day/lot) and reader precision. Intra-day/run and combined precision studies were performed with HNSCC specimens spanning the range of PD-L1 expression, and at least 25% of these represented specimens around the CPS ≥1 or CPS ≥20 cut off. Near cut-off specimens was defined as specimens with CPS score ≥ 1 and <10 for CPS≥1 and CPS≥ 10 and <30 for CPS ≥20. Precision was evaluated at CPS≥1 and CPS≥20 in separate studies, with overlapping specimens for each cut-offs but unique around cut off specimens enrolled into individual studies.

The intra-run and combined precision studies were performed with 34 HNSCC specimens, and reader precision studies included 24 unique HNSCC specimens for CPS≥1 and 48 specimens for CPS≥20. Study specimens along with control slides were stained, then blinded and randomized prior to evaluation of PD-L1 expression status. Specimens were assessed for qualitative PD-L1 status as PD-L1 positive or PD-L1 negative for CPS≥1 and CPS≥20 cut offs. Statistical analysis using pair-wise analysis was used to calculate average negative agreement (NPA), positive agreement (PPA), and overall agreement (OA) were computed with two-sided 95% confidence intervals using the bootstrap method for the CPS ≥ 1 cutoff as shown in Table 3 and the CPS ≥ 20 in Table 4. The results met the pre-specified acceptance criteria (i.e., 95% lower bound of the two-sided CI computed on % agreement must be ≥ 85%) with the exception of the reader precision specimen. This was due to the high number of specimens around the cut-off. Additionally, the reproducibility study included a more robust assessment of reader reproducibility and met the acceptance criteria.

Table 3. Summary of Repeatability in HNSCC for CPS≥ 1

Precision Endpoint	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision (Inter-Operator, Inter-Instrument,	CPS ≥ 1	Each of 34 head and neck squamous cell carcinoma specimens (12 PD-L1-negative and 22 PD-L1-positive) with a range of PD-L1 IHC expression was	NPA 100%* (94.0-100%) PPA 99.1% (97.3-100%) OA 99.4% (98.2-100%)

Precision Endpoint	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-Day, and Inter-Lot as combined variables)		tested using five operators, on five Autostainer Link 48 instruments, using five reagent lots, over five days.	
Intra-run* (Repeatability)	CPS ≥ 1	Each of 34 HNSCC specimens (16 PD-L1-negative and 18 PD-L1-positive) with a range of PD-L1 IHC expression was tested with five replicates within a run on the Autostainer Link 48 instrument.	NPA 98.8% (96.2-100%) PPA 97.8% (94.4-100%) OA 98.2% (95.9-100%)
Inter-observer precision	CPS ≥ 1	24 HNSCC specimens (11 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by three pathologists over three non-consecutive days with a 2-week washout between reads.	NPA 88.9% (78.8-98.0%) PPA 99.1% (97.4-100%) OA 94.4% (89.8-98.6%)
Intra-observer precision	CPS ≥ 1	24 HNSCC specimens (11 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by three pathologists over three non-consecutive days.	NPA 98.8% (96.3-100%) PPA 95.4% (92.3-98.4%) OA 96.7% (94.0-99.1%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Percent Agreement; CPS=Combined Positive Score

*The percentile bootstrap method cannot compute confidence intervals if 100% agreement is observed, therefore the Wilson Score method was used to compute confidence intervals for Intra-run precision NPA agreement. Note that the Wilson Score method has limitations as it assumes independence of data. Since all data is compared to a consensus outcome, the data are not independent.

Table 4: Summary of Repeatability (CPS ≥ 20)

Precision Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Combined Precision* (Inter-Operator, Inter-Instrument, Inter-Day, and Inter-Lot as combined variables)	CPS ≥ 20	Each of 34 HNSCC specimens (17 PD-L1- negative and 17 PD-L1-positive) with a range of PD-L1 IHC expression was tested using five operators, on five Autostainer Link 48 instruments, over five days, using five reagent lots.	NPA 100.0% (95.7-100.0%) * PPA 96.5% (90.6-100.0%) OA 98.2% (95.3-100.0%)

Intra-run precision (Repeatability)	CPS ≥ 20	Each of 34 HNSCC specimens (18 PD-L1- negative and 16 PD-L1-positive) with a range of PD-L1 IHC expression was tested with five replicates within a run on the Autostainer Link 48 instrument.	NPA 97.7% (92.9-100.0%) PPA 98.7% (96.2-100.0%) OA 98.2% (95.2-100.0%)
Inter-observer precision	CPS ≥ 20	48 HNSCC specimens (27 PD-L1-negative and 21 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by three pathologists over three non-consecutive days with a minimum two- week washout period in between each read.	NPA 96.3% (91.8-100.0%) PPA 93.1% (87.3-97.9%) OA 94.9% (91.4-97.9%)
Intra-observer precision	CPS ≥ 20	48 HNSCC specimens (27 PD-L1-negative and 21 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, were scored by three pathologists over three non-consecutive days with a minimum two- week washout period in between each read.	NPA 98.0% (95.9-99.6%) PPA 96.8% (94.4-98.9%) OA 97.5% (95.8-98.8%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Percent Agreement; CPS=Combined Positive Score

*The percentile bootstrap method cannot compute confidence intervals if 100% agreement is observed, therefore the Wilson Score method was used to compute confidence intervals for combined precision NPA agreement. Note that the Wilson Score method has limitations as it assumes independence of data. Since one specimen contributes more son to majority call, the data are not independent.

4. External Reproducibility

Reproducibility studies for HNSCC were designed to evaluate the performance of PD-L1 IHC 22C3 pharmDx for PD-L1 detection across laboratories on the Dako Autostainer Link 48. The study included specimens that were pre-qualified at Dako to represent full PD-L1 expression range and a minimum of 25% of the specimens were around cut offs. The specimen set was randomized and blinded prior to testing at 3 external reproducibility sites and assessed for performance with regard to site-to-site and day-to-day reproducibility or inter and intra-site reproducibility. Reproducibility for CPS≥ 1 and CPS≥ 20 cut off was assessed in a single study. A total of 46 specimens were enrolled in the study, of which overlapping sets of 38 specimens each were pre-assigned for evaluation at the CPS ≥ 1 and CPS≥ 20 cut offs. Therefore, reproducibility was assessed with 38 specimens (19 positive and 19 negatives including 14 around cut off) being tested across 3 sites over 5 non-consecutive days.

Reproducibility studies also included assessment of observer-to-observer variability with specimens that spanned the expression range of PD-L1. These specimens were stained at

the Dako facility and shipped to the 3 external reproducibility sites for assessment of PD-L1 expression by pathologists for both inter-observer and intra-observer reproducibility. Seventy-six (76) pre-stained specimens (with a minimum of 25% near the CPS ≥ 1 and CPS ≥20 cut off) were enrolled into the study. From the 76 specimens enrolled in the study, a set of 62 specimens were pre-designated for evaluation of reproducibility at each of the two cut offs. The specimens were assessed 3 times by 3 readers with a two-week washout between reads. All IHC tests were interpreted by certified clinical pathologists to determine the positive/negative PD-L1 status based on the CPS ≥1 and CPS ≥ 20 cut off at three external sites. NPA, PPA and OA and 95% CI was calculated by pairwise comparison to the majority call as reference. The results of the reproducibility studies are included in Table 5 and Table 6..

Table 5. Three Site Reproducibility in HNSCC

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 1	Each of 38 HNSCC specimens (19 PD-L1 negative and 19 PD-L1 positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 570 comparisons to majority call.	NPA 96.8% (92.6-100.0%) PPA 93.3% (86.7-98.6%) OA 95.1% (91.2-98.2%)
Intra-site	CPS ≥ 1	Each of 38 HNSCC specimens (19 PD-L1 negative and 19 PD-L1 positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three sites on a total of 570 comparisons to majority call.	NPA 95.7% (91.3-99.0%) PPA 97.0% (94.5-98.9%) OA 96.3% (93.5-98.6%)
Inter-observer	CPS ≥ 1	Scoring of 62 HNSCC specimens (30 PD-L1-negative and 32 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non- consecutive days. Inter-observer analysis was performed between three sites on a total of 556 comparisons to majority call.	NPA 94.0% (89.3-97.8%) PPA 97.2% (94.4-99.3%) OA 95.7% (93.0-98.0%)

Intra-observer	CPS ≥ 1	Scoring of 62 HNSCC specimens (30 PD-L1-negative and 32 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non-consecutive days. Intra-observer analysis was performed for three sites on a total of 555 comparisons to majority call.	NPA 97.3% (95.4-98.9%) PPA 98.3% (96.8-99.7%) OA 97.8% (96.8-98.9%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Percent Agreement; CPS=Combined Positive Score

Table 6. Three Site Reproducibility in HNSCC (CPS ≥ 20)

Reproducibility Study	**Diagnostic Cutoff**	**Study Design**	**% Agreement (95% CI)**
Inter-site	CPS ≥ 20	Each of 38 HNSCC specimens (25 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 570 comparisons to majority call.	NPA 95.5% (92.0-98.4%) PPA 81.0% (71.3-90.3%) OA 90.5% (86.5-94.4%)
Intra-site	CPS ≥ 20	Each of 38 HNSCC specimens (25 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three sites on a total of 570 comparisons to majority call.	NPA 96.9% (94.6-98.8%) PPA 90.6% (86.3-94.9%) OA 94.9% (92.8-96.8%)
Inter-observer	CPS ≥ 20	Scoring of 62 HNSCC specimens (31 PD-L1-negative and 31 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non- consecutive days. Inter-observer analysis was performed between three sites on a total of 556 comparisons to majority call.	NPA 93.1% (87.2-97.8%) PPA 91.0% (85.7-95.7%) OA 92.1% (88.2-95.5%)

Intra-observer	CPS ≥ 20	Scoring of 62 HNSCC specimens (31 PD-L1-negative and 31 PD-L1-positive) with a range of PD-L1 IHC expression, stained with PD-L1 IHC 22C3 pharmDx, was performed by three pathologists, one at each of three study sites, on three non- consecutive days. Intra-observer analysis was performed for three sites on a total of 555 comparisons to majority call.	NPA 96.8% (94.5-98.7%) PPA 97.8% (96.0-99.3%) OA 97.3% (95.9-98.6%)

NPA= Negative Percent Agreement; PPA= Positive Percent Agreement; OA=Overall Percent Agreement; CPS=Combined Positive Score

The results met the pre-specified acceptance criteria (i.e., 95% lower bound of the two-sided CI computed on % agreement must be ≥ 85%) for all study end points for the CPS ≥ 1 cut off and met all study end points for CPS ≥ 20 except for PPA for inter- site reproducibility. The root cause analysis indicated that study have failed to meet acceptance criteria due to imbalance in PD-L1 positive and negative specimens at the CPS>20 cut off as well as including a large number of around cut off specimens in the analysis.

A second inter site reproducibility study was conducted address failure of assay to meet the acceptance criteria for PPA for CPS >20. The repeat study included 30 specimens that were pre-qualified at Dako with 13 positive and 17 negatives including 10 around cut off being tested across 3 sites over 5 non-consecutive days. The specimen set was randomized and blinded prior to testing at 3 external reproducibility sites and assessed for performance with regard to site-to-site and day-to-day reproducibility or inter and intra-site reproducibility.

Table 7. Repeat Three Site Reproducibility in HNSCC (CPS ≥ 20)

Reproducibility Study	Diagnostic Cutoff	Study Design	% Agreement (95% CI)
Inter-site	CPS ≥ 20	Each of 30 HNSCC specimens (17 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days. Inter-site analysis was performed between three sites on a total of 450 comparisons to majority call.	NPA 93.7% (86.3-100%) PPA 82.6% (73.3-91.3%) OA 88.9% (83.3-94.0%)
Intra-site	CPS ≥ 20	Each of 38 HNSCC specimens (25 PD-L1-negative and 13 PD-L1-positive) with a range of PD-L1 IHC expression was tested on five non-consecutive days at each of three study sites. Intra-site analysis was performed for three sites on a total of 450 comparisons to majority call.	NPA 98.5% (97.0-99.6%) PPA 96.1% (92.7-98.9%) OA 97.6% (95.8-99.1%)

The repeat study failed to meet prespecified acceptance criteria for PPA and OA for inter site reproducibility. The sponsor attributed the failure to poor reader performance at one of

the three sites. Based on the failure to meet pre-specified acceptance criteria in two inter site reproducibility stud the following limitation statement was included under Section 15.2 of. the device label:

Clinicians should use caution when interpreting test results at the CPS ≥ 20 cutoff, because PD-L1 IHC 22C3 pharmDx failed to meet pre-specified acceptance criteria for positive percent agreement in two independent inter-site reproducibility studies and overall percent agreement in one inter-site reproducibility study conducted on HNSCC specimens at the CPS ≥ 20 cutoff. All pre-specified acceptance criteria were met in the independent inter-site reproducibility study conducted on HNSCC specimens at the CPS ≥ 1 cutoff.

5. Robustness Studies:

Robustness of the staining performance of PD-L1 IHC 22C3 pharmDx in HNSCC was evaluated by testing the performance of the assay when varying the following conditions as described below. On one lot of reagents was assessed.

- Tissue sections cut at three thicknesses:
  - 3 µm
  - 4 µm
  - 5 µm
- Target Retrieval Time at three incubation times
  - 18 minutes
  - 20 minutes-standard
  - 22 minutes
- Target Retrieval Temperature at three incubation temperatures
  - 95°C
  - 97°C -standard
  - 99°C
- Target Retrieval Solution pH at three pH levels
  - pH 5.9
  - pH 6.1-standard
  - pH 6.3
- Target Retrieval Solution after first use and third use

The CPS≥ 1 and CPS ≥ 20 were evaluated in separate studies. Specimens for the CPS≥ 20 evaluation was generated by staining specimens with CPS≥ 10 and <20 to represent samples around the cut-off and combined with a subset of specimens used for evaluation of robustness at CPS≥ 1. Samples were combined, blinded and randomized prior to evaluation at the CPS ≥ 20 cut off.

Tissue thickness included at least 24 HNSCC specimens spanning the range of PD-L1 expression and included a minimum of 20% of specimens around the cut off. Target retrieval studies were performed on PT100 and PT200 modules with a minimum of 35

HNSCC specimens and included a minimum of 20% specimens around the cut off. Staining performance was evaluated for both CPS score and intensity of staining. NPA, PPA and OA for pairwise comparison against the reference condition and the 95% CI calculated with Bootstrap method. Acceptance criteria for the study specified that lower bound of the 95% CI would meet or exceed 85% for each condition tested. The study passed acceptance criteria and no significant difference in results was observed for any of the recommended experimental conditions above.

6. Impact of Intra-Case Heterogeneity

The objective of these studies was to investigate whether tumor heterogeneity affects PD-L1 IHC staining results with PD-L1 IHC 22C3 pharmDx.

a. Primary vs. Metastatic Tumor Tissues

Matched primary versus metastatic blocks were obtained from 18 subjects and evaluated by PD-L1 IHC 22C3 pharmDx. Two (2) HNSCC intra-case specimen pairs were PD-L1 negative (CPS <1), Fourteen (14) pairs were PD-L1 positive (CPS ≥1), and 2 pairs had a discordant PD-L1 status when assessed at the CPS ≥ 1 cut off. One discordant case was positive for the metastatic tumor but negative for primary.

Assessment of the same specimens at CPS ≥ 20 yielded seven (7) concordant PD-L1 negative pairs and seven (7) PD-L1 positive concordant pairs and four (4) discordant pairs. Of the four discordant pairs two were positive for PD-L1 expression in primary but negative for metastatic and two were negative for primary tumor and positive for metastatic tumor. Results may not be representative of all HNSCC specimens, as tumor heterogeneity is unique for each specimen.

b. Multiple FFPE Blocks from the Same Subjects (Variability in PD-L1 expression between anatomic sites within patients)

Multiple blocks (at least 2) of 20 HNSCC subjects obtained from the same tumor were evaluated to demonstrate within-patient concordance for PD-L1 status. At CPS ≥ 1 assessment all 20 sets (100%) of HNSCC intra-subject specimens were concordant across sister tissue blocks At the CPS ≥ 20 assessment 18 pairs were concordant, and two cases were discordant. Four of the evaluable 20 cases were near cut-off pairs (10-30%) for CPS ≥ 20 and 3 cases were around cut off (≥ 0-10) for CPS ≥ 1. Results may not be representative of all HNSCC specimens, as tumor heterogeneity is unique for each specimen.

c. Intra Block Heterogeneity

Heterogeneity within one HNSCC FFPE blocks was assessed. The 1st and 40th cut sections from 33 unique FFPE blocks were stained with PD-L1 IHC 22C3 pharmDx and assessed for PD-L1 expression. Of the 36 specimens, 12 were evaluated at CPS≥1 cut off and 22 cases included in analysis for CPS≥ 20 cut off. One case assessed for CPS ≥20 was excluded from analysis due to high background. Cases were selected to span the dynamic range of PD-L1 expression and at least 25% of specimens were around the cut off. Equal numbers of positive and negative

specimens were enrolled into the study. At the CPS ≥ 1 cut-off, 12 blocks pairs demonstrated agreement in PD-L1 expression (100% overall agreement) among the two cut sections. High overall agreement in PD-L1 expression within individual HNSCC cancer blocks was observed to at least 150µm.
Of the twenty-two (22) cases assessed at CPS ≥20, twenty (20) cases were concordant while two (2) were discordant at for the front and back sections of the 150µM block. Results may not be representative of all HNSCC cancer specimens, as tumor heterogeneity is unique for each specimen.

7. Stability testing

a. PD-L1 IHC 22C3 pharmDx Stability

The Real time reagent stability testing was previously performed on PD-L1 IHC 22C3 pharmDx for NSCLC and reviewed in P150013. Based on data provided in P150013 the stability of the device is established and approved at 9 months for storage at 2-8 ^{0}C.

b. FFPE Cut Section Stability

A real-time stability study was designed to evaluate the shelf life of cut tissue sections of HNSCC FFPE blocks using PD-L1 IHC 22C3 pharmDx when stored in the dark at 2-8 °C or 25 °C. The study included 6 HNSCC specimens. Based on these studies, stability dating for cut slides in HNSCC is 6months for storage at 2-8 °C and 4 months for storage at 25 °C.

B. Animal Studies

None

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

A. Study Design

The clinical performance of PD-L1 IHC 22C3 pharmDx was evaluated in the study Keynote-048 (KN048/ NCT02358031) which is an ongoing, Phase 3, randomized, multicenter, active-controlled, open-label clinical study in patients with recurrent, metastatic (R/M) HNSCC considered incurable by local therapies. The three treatment arms in the study are KEYTRUDA plus chemotherapy (platinum plus 5-FU), KEYTRUDA as single agent and standard treatment (the regimen consists of cetuximab plus platinum plus 5-FU and is referred to as the EXTREME regimen).

Note: While Keynote-048 was a three-arm study, clinical validation of the PD-L1 IHC 22C3 device was based only on results from the KEYTRUDA as single agent and cetuximab in combination with chemotherapy.

The study randomized a total of 882 patients: 301 patients to the KEYTRUDA as single agent arm, 281 patients to KEYTRUDA plus chemotherapy arm, and 200 patients to cetuximab in combination with chemotherapy arm. Enrollment was open to all participants regardless of PD-L1 tumor expression.

Participants were stratified for randomization according to HPV status (positive versus negative), ECOG PS (0 versus 1), and PD-L1 expression by TPS (≥50% versus not ≥50%). Assessment of FFPE tumor sample sections for PD-L1 expression was performed centrally. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical study is presented below.

1. Clinical Inclusion and Exclusion Criteria (abbreviated list)
   Enrollment in the KN052 study was limited to patients who met the following inclusion criteria:
   - Male/female at least 18 years of age.
   - Presence of histologically or cytologically-confirmed diagnosis of recurrent or metastatic HNSCC that is considered incurable by local therapies.
   - Patients may not have had prior systemic therapy administered in the recurrent or metastatic setting. Systemic therapy which was completed more than 6 months prior to signing consent if given as part of multimodal treatment for locally advanced disease was allowed.
   - The eligible primary tumor locations were oropharynx, oral cavity, hypopharynx, and larynx.
   - Have provided tissue for biomarker analysis from a newly obtained core or excisional biopsy of a tumor. Adequacy of the biopsy specimen for PD-L1 biomarker analysis must be confirmed by the central laboratory. If obtained for a patient with recurrent disease for locally advanced disease, then it must have been obtained after completion of the previous initial management with no other treatment from the time of biopsy until the start of study treatment.
   - Note: Patients for whom newly obtained samples could not be obtained (e.g. inaccessible or patient safety concern) were allowed to submit an archived specimen only upon agreement from the Sponsor. Newly obtained tissues were required to be obtained up to 90 days prior to treatment initiation.
   - Have measurable disease based on RECIST 1.1 as determined by central review. Tumor lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.
   - Have a performance status of 0 or 1 on the ECOG Performance Scale.

   Patients were not permitted to enroll in the KN052 study if they met any of the following exclusion criteria:

   - Has disease that is suitable for local therapy administered with curative intent.
   - Current or prior receipt of study therapy or prior participation in a study of an investigational agent and received study therapy or used an investigational device within 4 weeks of the first dose of treatment.

- Patients with known active central nervous system (CNS) metastases and/or carcinomatous meningitis.
- Any known additional malignancy that requires active treatment
- Treatment within 4 weeks prior to study Day 1 or who has not recovered (i.e., ≤ Grade 1 or at baseline) from adverse events due to agents administered more than 4 weeks earlier.
- Active autoimmune disease that required systemic treatment in the prior 2 years
- HIV positive patients.
- Any prior therapy with an anti-PD-L1, anti-PD-L2 agent, or any agent directed to another co-inhibitory T-cell receptor.

2. Follow-up Schedule

Patients received KEYTRUDA 200 mg every 3 weeks either as single agent or in combination with chemotherapy. Treatment with KEYTRUDA continued until RECIST v1.1 (modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ) defined progression of disease as determined by the investigator, unacceptable toxicity, or a maximum of 24 months. Administration of KEYTRUDA was permitted beyond RECIST defined disease progression if the patient was clinically stable and considered to be deriving clinical benefit by the investigator. Patients without disease progression could be treated for up to 24 months. Assessment of tumor status was performed at Week 9 and then every 6 weeks for the first year, followed by every 9 weeks through 24 months. The major efficacy outcome measures were progression free survival (PFS) and overall survival (OS).

3. Clinical Endpoints

Based on training date set comprising of HNSCC patients enrolled in Keynote-012 and Keynote-055, the clinical cut off for evaluation of efficacy in the KEYNOTE-048 was amended to prespecify PD-L1 expression status at CPS ≥1 and CPS≥20 as determined by PD-L1 IHC 22C3 pharmDx. PD-L1 status was evaluated at two clinical cut offs of CPS ≥ 1 and CPS≥ 20 in two independent retrospective reevaluations of patient specimens stained and assessed for TPS≥ 50 as stratification factor for randomization.

The main efficacy outcome measures were OS and PFS as assessed by blinded independent central review (BICR) according to RECIST v1.1 sequentially tested in the subgroup of patients with CPS ≥20, the subgroup of patients with CPS ≥1, and the overall population.

Major secondary endpoints were PFS at 6 months or 12 months per RECIST 1.1 by BICR, objective response rate (ORR) ORR was defined as the proportion of the patients who have a confirmed complete response (CR) or partial response (PR) in ITT population and PD-L1 subgroups CPS ≥ 1, CPS≥ 20.

B. Accountability of PMA Cohort

At the time of database lock, June 2018, In KEYNOTE-048, of 882 total participants, 601 were randomized to either the KEYTRUDA as single agent (301 participants) or the standard treatment group (300 participants). All treated subjects had a tumor tissue sample. Primary efficacy analysis was based on hierarchical analysis of data from subjects whose tumors expressed PD-L1 at CPS ≥1, CPS≥ 20 and the intent to treat (ITT) population. Of the 601 enrolled subjects in KN048, 89 subjects had tumors with PD-L1 CPS <1 expression and 512 subjects had tumors with PD-L1 CPS ≥ 1 expression. Independent evaluation at CPS ≥20 cut off identified 255 subjects whose PD-L1 expression was CPS<20 and 342 subjects with CPS ≥20. A description of the specimen characteristics is shown in Table 8. Refer to Table 9 for the PD-L1 distribution by specimen type and Table 10 for PD-L1 distribution in the treatment arms at the CPS ≥ 1 and Table 11 for distribution at the CPS≥20 cut off.

Table 8. Accountability of PMA Cohort in KN048

Number of Study subjects n (%)		
PD-L1 Cut off	**CPS ≥ 1**	**CPS ≥ 20**
Enrolled	601	601
Quantifiable PD-L1 expression	601	597
Unevaluable PD-L1 expression	0	4
Site of Collection:		
Primary Site	367(61.1%)	366(61.3%)
Metastatic Site	234(38.1%)	231(38.7%)
Specimen type		
Biopsy	520(86.5%)	520(86.5%)
Resection	78(13.0%)	78(13.0%)
Unknown	3(0.5%)	3(0.5%)

Table 9: Tumor PD-L1 by Specimen Type

Tumor Tissue	**Number (%) with CPS ≥ 1**	**Number (%) with CPS < 1**	**Number (%) with CPS ≥ 20**
Overall study n=601	512 (85)	89 (15)	255 (43)**
Archival Tissue*	132 (83)	27 (17)	69 (44)**

n=159			
Newly Obtained Tissue* n= 442	380 (86)	62 (14)	186 (42)**

* In the context of clinical trial KEYNOTE-048, newly obtained tissue biopsy was defined as the biopsy collected within 90 days of initiation of treatment with pembrolizumab. Specimens that were > 90 days were classified as archival.

** Based on patients with known PD-L1 expression; 4 patients had unknown PD-L1 expression status (one specimen was archival tissue and three specimens were newly obtained tissue).

Table 10: Subject PD-L1 Distribution (CPS ≥ 1 cut off)

PD-L1 Status	KEYTRUDA Arm	Cetuximab Platinum 5FU Arm
PD-L1 CPS < 1	44	45
PD-L1 CPS ≥ 1	257	255
Unknown	0	0
Total	301	300

Table 11: Subject PD-L1 Distribution (CPS ≥ 20cut off)

PD-L1 Status	KEYTRUDA Arm	Cetuximab Platinum 5FU Arm
PD-L1 CPS < 20	167	175
PD-L1 CPS ≥ 20	133	122
Unknown	1	3
Total	301	300

C. Study Population Demographics and Baseline Parameters

A total of 601 patients were randomized to the KEYTRUDA as single agent and cetuximab in combination with chemotherapy arms; 301 patients to the KEYTRUDA as single agent arm, and 300 patients to the cetuximab in combination with chemotherapy arm. The study population characteristics were: median age of 61 years (range: 22 to 94); 36% age 65 or older; 85% male; 74% White and 19% Asian; 61% ECOG PS of 1; and 79% were former/current smokers. Disease characteristics were: 22% HPV positive, 85%, 42%, and 22% had PD-L1 expression defined as CPS ≥1, CPS ≥20, and TPS ≥50%, respectively, and 96% had Stage IV disease (Stage IVa 20%, Stage IVb 6%, and Stage IVc 70%). The two treatment arms were generally balanced for all baseline characteristics.

D. Safety and Effectiveness Results

1. Safety Results:

Patients with HNSCC whose specimens have PD-L1 expression with CPS <1 are not eligible for Keytruda. Safety of the device for this cut-off was demonstrated in the analytical validation studies described above. Patients whose specimens have CPS ≥ 20 have an increased benefit than patients whose tissues express less at this cut-off. There are no safety issues related to the use of this score. Safety with respect to treatment with KEYTRUDA at the different PDL1 expression levels is addressed in the review of the associated therapeutic application and summarized below. As compared to the overall study population, no meaningful differences in adverse events from treatment with KEYTRUDA® was observed based on PD-L1 expression level.

2. Effectiveness Results

Clinical performance of PD-L1 IHC 22C3 pharmDx was assessed in the KN048 trial. 601 subjects treated in the KEYTRUDA as single agent and cetuximab in combination with chemotherapy arms of the trial had tumor specimens tested with the PD-L1 IHC 22C3 pharmDx assay. PD-L1 status was evaluated at two clinical cut offs of CPS ≥ 1 and CPS≥ 20 in two independent retrospective evaluations of patient specimens stained and assessed for TPS≥ 50 as stratification factor for randomization. PD-L1 status was evaluable for all subjects at the CPS ≥1 cut off. Of 601 subjects 512 (85%) had PD-L1 expression of CPS ≥1 and 352 (57%) with CPS ≥20 and 4 (0.5%) subjects' tumors were not evaluable for PD-L1 expression at the CPS ≥20 cut off.

Efficacy in PD-L1 sub groups CPS≥1 and CPS≥20

The KN048 trial demonstrated a statistically significant improvement in OS for the subgroup of patients with PD-L1 CPS ≥1 randomized to KEYTRUDA as a single agent compared to chemotherapy and cetuximab. The median OS for PD-L1 CPS≥ 1 subgroup in months was 12.3 (95% CI:10.8, 14.9) in KEYTRUDA as single agent arm and 10.3 (95% CI: 9.0-11.5) in the cetuximab in combination with chemotherapy arm with a hazard ratio (HR) of 0.78 (95% CI: 0.64,0.96) and p value of 0.0171.The median OS for PD-L1 CPS ≥ 20 sub-group in months was 14.9 (95% CI:11.6-21.5) in KEYTRUDA as single agent arm and 10.7 (95% CI: 8.8-12.8) in the cetuximab in combination with chemotherapy arm with a hazard ratio (HR) of 0.61 (95% CI: 0.45,0.83). In an exploratory subgroup analysis for HNSCC patients with CPS 1-19, the median OS was 10.8 months (95% CI: 9.0, 12.6) for KEYTRUDA as a single agent and 10.1 months (95% CI: 8.7, 12.1) for cetuximab in combination with chemotherapy, with an HR of 0.90 (95% CI: 0.68, 1.18).The hazard ratio for OS in the PD-L1 CPS<1 sub group for KEYTRUDA as single agent vs cetuximab in combination with chemotherapy was 1.44(95% CI: 089, 2.34) and the interaction term* of regression coefficient for PD-L1 status by CPS ≥1 and treatment effect on OS was determined to be 0.64 with p-value 0.017.

* Based on multiple Cox PH model with treatment, PD-L1 expression level, treatment by PD-L1 status interaction, baseline tumor size, HPV, ECOG, sex, prior systemic platinum therapy, as the covariates

Table 12: Efficacy Results for KEYTRUDA as a single agent in KEYNOTE-048 (CPS ≥1 and CPS ≥20

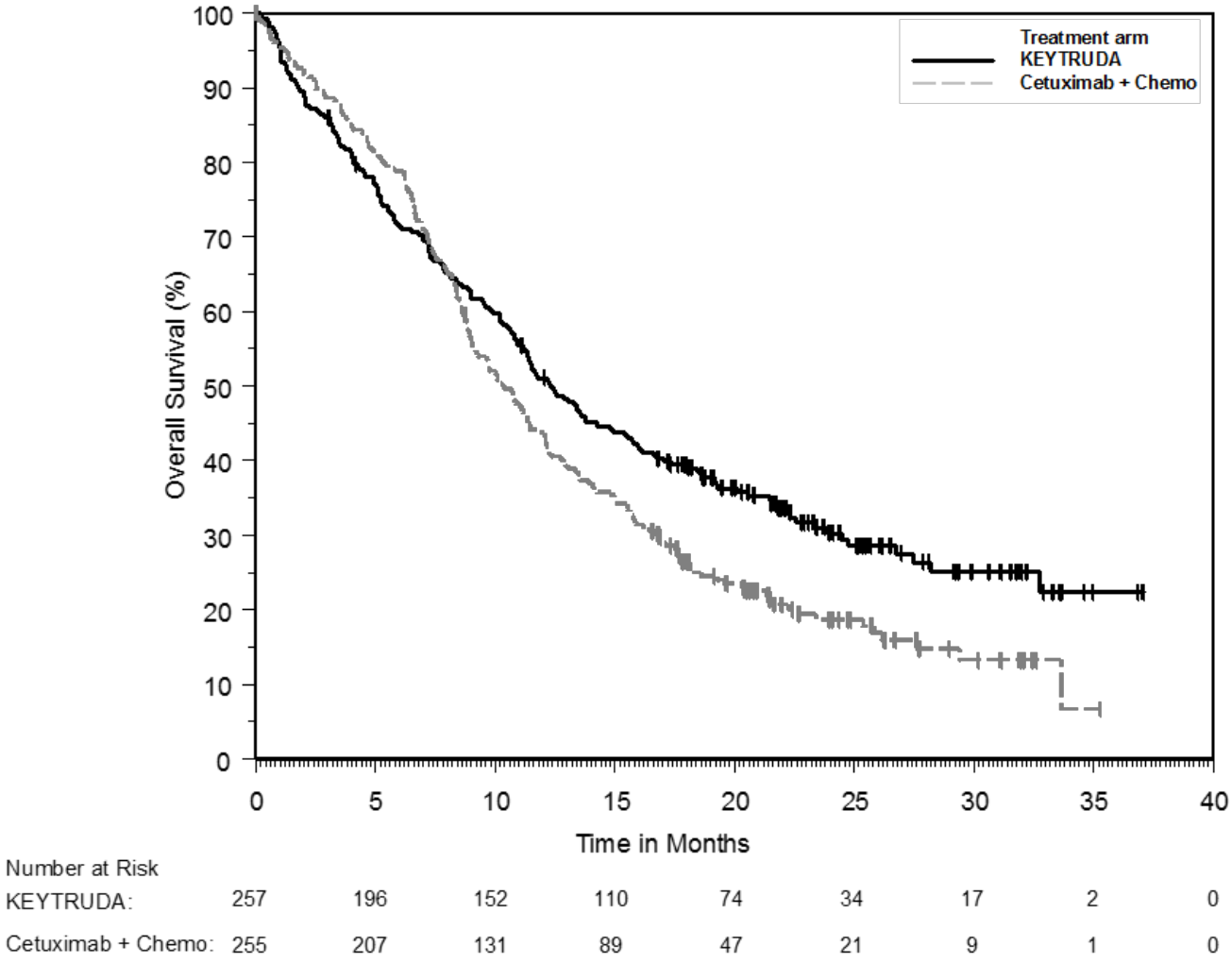
Endpoint	CPS ≥1		CPS ≥20	
	KEYTRUDA 200 mg every 3 weeks n=257	**Cetuximab Platinum FU n=255**	**KEYTRUDA 200 mg every 3 weeks n=133**	**Cetuximab Platinum FU n=122**
OS				
Number of events (%)	177 (69%)	206 (81%)	82 (62%)	95 (78%)
Median in months (95% CI)	12.3 (10.8, 14.9)	10.3 (9.0,11.5)	14.9 (11.6, 21.5)	10.7 (8.8, 12.8)
Hazard ratio* (95% CI)	0.78 (0.64, 0.96)		0.61 (0.45, 0.83)	
p-Value†	0.0171		0.0015	
PFS				
Number of events (%)	225 (88%)	231 (91%)	113 (85%)	111 (91%)
Median in months (95% CI)	3.2 (2.2, 3.4)	5.0 (4.8, 5.8)	3.4 (3.2, 3.8)	5.0 (4.8, 6.2)
Hazard ratio ‡ (95% CI)	1.15(0.95, 1.38)		0.99 (0.75, 1.29)	
Objective Response Rate				
ORR‡ (95% CI)	19% (14.5, 24.4)	35% (29.1, 41.1)	23% (16.4, 31.4)	36% (27.6, 45.3)
Complete response rate	5%	3%	8%	3%
Partial response rate	14%	32%	16%	33%
Duration of Response				
Median in months (range)	20.9 (1.5+, 34.8+)	4.5 (1.2+, 28.6+)	20.9 (2.7, 34.8+)	4.2 (1.2+, 22.3+)

*Based on the stratified Cox proportional hazard model

† Based on stratified log-rank test

‡ Response: Best objective response as confirmed complete response or partial response

Figure 1: Kaplan-Meier Curve for Overall Survival for KEYTRUDA as a Single Agent in KEYNOTE-048 (PD-L1 CPS ≥1)



3. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 3 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c) (2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Clinical benefit of PD-L1 IHC 22C3 pharmDx is based upon the results of the KEYNOTE-048 study which was conducted to evaluate the safety and the efficacy of KEYTRUDA (pembrolizumab) in patients with recurrent and metastatic HNSCC who had not previously received systemic therapy for recurrent or metastatic (R/M) disease and who were considered incurable by local therapies. In this study, PD-L1 IHC 22C3 pharmDx was used to determine PD-L1 expression status of patient tumors. While KN048 is a three-arm study that included evaluation of KEYTRUDA as single agent, KEYTRUDA in combination with chemotherapy vs. cetuximab in combination with chemotherapy, the clinical performance of PD-L1 IHC 22C3 pharmDx in HNSCC was established based on the KEYTRUDA as single agent vs. cetuximab in combination with chemotherapy results.

In the KEYTRUDA as single agent and Cetuximab in combination with chemotherapy arms, 89 patients (14.8%) had tumor PD-L1 expression of CPS <1, 512 patients (85.2%) had tumor PD-L1 expression of CPS ≥1.

In the population of participants whose tumors express PD-L1 CPS ≥1, KEYTRUDA as single agent demonstrated statistically significant and clinically meaningful OS benefit compared with standard treatment (HR 0.78 [0.64, 0.96], p=0.0171). Further, interaction coefficient for PD-L1 status by CPS ≥1 and treatment effect on OS was determined to be 0.64 with a p value of 0.017. Therefore, PD-L1 expression as detected by PD-L1 IHC 22C3 pharmDx in HNSCC was effective in identifying patients who would benefit from KEYTRUDA as single agent over cetuximab in combination with chemotherapy.

The performance of PD-L1 IHC 22C3 pharmDx was also supported by the analytical validation studies.

B. Safety Conclusions

Safety of the device for patient management is related to effectiveness (see effectiveness conclusions above). In general, risks of the PD-L1 IHC 22C3 pharmDx are associated with failure of the device to perform as expected or failure to correctly interpret test results (see Benefit-Risk Determination below). The process of testing

on FFPE tumor specimens does not present additional significant safety concerns, as these samples are routinely removed for HNSCC cancer diagnosis.

C. Benefit-Risk Determination

The probable benefits of this device are based on the data collected in the clinical study which demonstrated improved overall survival and duration of response to treatment with KEYTRUDA as a single agent in patients who were PD-L1 positive as determined by the device. The risks of the test are associated with false negative or false positive results which may lead to patients having no benefit from the treatment. The safety and efficacy of KEYTRUDA as a single agent in PD-L1 positive patients was determined to have clinical benefit when compared to the risks. The analytical validation conducted supports the test as a reliable method for detecting PD-L1 expression.

1. Patient Perspectives
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information detailed above, the data supports that, for the HNSCC carcinoma patients who are being considered for treatment with KEYTRUDA® (pembrolizumab), the probable benefits of PD-L1 IHC 22C3 pharmDx use outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use and product labeling. The provided studies support use of PD-L1 IHC 22C3 pharmDx as an aid in identifying patients with HNSCC for treatment with KEYTRUDA® (pembrolizumab).

XIII. CDRH DECISION

CDRH issued an approval order on June 10, 2019.

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality System (QS) regulation (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Warnings, and Precautions in the device labeling.

Post-approval Requirements and Restrictions: See approval order