SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Immunohistochemistry test, DNA mismatch repair (MMR) protein assay

Device Trade Name: MMR IHC Panel pharmDx (Dako Omnis)

Device Procode: QNH

Applicant's Name and Address: Agilent Technologies, Inc.
6392 Via Real
Carpinteria, CA 93013 USA

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P250004

Date of FDA Notice of Approval: August 15, 2025

II. INDICATIONS FOR USE

For In Vitro Diagnostic Use.

MMR IHC Panel pharmDx (Dako Omnis) is a qualitative immunohistochemical (IHC) assay intended for use in the assessment of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, and MSH6) in formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) tissue using EnVision FLEX visualization system on Dako Omnis automated staining instrument. MMR IHC Panel pharmDx (Dako Omnis) consists of MLH1 IHC pharmDx (Dako Omnis), PMS2 IHC pharmDx (Dako Omnis), MSH2 IHC pharmDx (Dako Omnis), and MSH6 IHC pharmDx (Dako Omnis), which must be used together to identify MMR deficient CRC patients.

MMR IHC Panel pharmDx (Dako Omnis) is indicated as an aid to identify MMR deficient CRC patients eligible for treatment with OPDIVO® (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY® (ipilimumab).

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the MMR IHC Panel pharmDx (Dako Omnis) labeling:

a. MLH1 IHC pharmDx (Dako Omnis (Code GE079)
b. MSH2 IHC pharmDx (Dako Omnis) (Code GE085)
c. PMS2 IHC pharmDx (Dako Omnis) (Code GE087)
d. MSH6 IHC pharmDx (Dako Omnis) (Code GE086)
e. MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101)
f. MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102).

V. DEVICE DESCRIPTION

MMR IHC Panel pharmDx (Dako Omnis) is designed to be run on the Dako Omnis automated staining system with Dako Omnis Solution software. The MMR panel primary antibodies and negative control reagents (NCRs) are sold separately, each with adequate reagent for 60 tests. Each primary monoclonal antibody is provided in a ready-to-use 12 mL volume, in liquid form, in a buffer containing stabilizing protein and 0.015 mol/L sodium azide. MMR panel antibodies are listed in Table 1.

Table 1: MMR Panel Primary Antibodies

Product Name	Clone	Isotype
MLH1 IHC pharmDx (Dako Omnis)	ES05	Mouse IgG_1 kappa
MSH2 IHC pharmDx (Dako Omnis)	FE11	Mouse IgG_1 kappa
MSH6 IHC pharmDx (Dako Omnis)	EP49	Rabbit IgG
PMS2 IHC pharmDx (Dako Omnis)	EP51	Rabbit IgG

The isotype monoclonal mouse and rabbit NCRs are provided in a ready-to-use 12 mL volume, in liquid form, containing stabilizing protein and 0.015 mol/L sodium azide. The isotype NCRs are not directed against any known human antigen. They are used to detect nonspecific staining in CRC tissues stained with MMR IHC Panel pharmDx (Dako Omnis) rabbit or mouse antibodies. MMR NCRs are listed in Table 2.

Table 2: Negative Control Reagents

Description	Clone	Isotype
MMR Negative Control Reagent, Mouse (Dako Omnis)	NA	Mouse IgG_1 kappa
MMR Negative Control Reagent, Rabbit (Dako Omnis)	NA	Rabbit IgG

Reagents and equipment required for performing testing, but not supplied as part of MMR IHC Panel pharmDx (Dako Omnis), are listed below:
Dako Omnis (Code GI100)
Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309)
EnVision FLEX, High pH (Dako Omnis) (Code GV800 or GV823), containing:
- EnVision FLEX DAB+ Chromogen (Dako Omnis)
- EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis)
- EnVision FLEX Substrate Buffer (Dako Omnis)
- EnVision FLEX Visualization Reagent (Dako Omnis)

EnVision FLEX+ Mouse LINKER (Dako Omnis) (Code GV821)
EnVision FLEX+ Rabbit LINKER (Dako Omnis) (Code GV809)
Wash Buffer (20x) (Dako Omnis) (Code GC807)
Sulfuric Acid, 0.3 M (Code GC203)
Hematoxylin (Dako Omnis) (Code GC808) or equivalent
Clearify™ clearing agent (Code GC810)
Distilled or de-ionized water (reagent-grade water)
Drying oven, capable of maintaining 60 °C or less
Ethanol, absolute and 95%
Xylene, or xylene substitute
Bright field microscope (4–20x objective magnification)
Coverslips
Nonaqueous, permanent mounting medium and ancillary reagents required for mounting coverslips
Microscope slides: FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus slides
Tissues to use as process controls
pH meter

All instrumentation should be maintained and calibrated per manufacturer's recommendation.

Device Instrument and Software

MMR IHC Panel pharmDx (Dako Omnis) is designed to be run on the Dako Omnis automated staining system with Dako Omnis Instrument Software (OIS) and the Dako Link Omnis Workstation and Server software (WSS). The Dako Omnis system is designed to process slides on a continuous basis and can run different staining protocols for individual slides at the same time to optimize capacity utilization and patient case management.

Specimen Preparation

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

Paraffin-embedded tissue

Formalin-Fixed Paraffin-Embedded (FFPE) tissues are suitable for use with MMR IHC Panel pharmDx (Dako Omnis). Recommended handling and processing conditions are: ≤ 1 hour ischemia time prior to immersion in fixative, and 6 to 48 hours fixation time in 10% neutral buffered formalin (NBF). Alternative fixatives have not been validated and may give erroneous results. Reduced staining was observed with 10% unbuffered formalin, Bouin's fixative, and acetic formalin alcohol (AFA), so they are not acceptable for use with this assay. Specimens should be blocked into a thickness of 3 or 4 mm, fixed in 10% NBF, 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. Handling and processing outside of the recommended conditions should be validated by the user.

Tissue sections

FFPE tissue specimens should be cut into sections of 4 µm. After sectioning, tissues should be mounted on FLEX IHC Microscope Slides (Code K8020) or Superfrost Plus microscope slides and then placed in a 58 ± 2 °C calibrated oven for 1 hour. The tissue specimens must be positioned on the glass within the defined slide staining area per the Dako Omnis User Guide.

To preserve antigenicity, tissue sections mounted on slides should be stained within 2 months of sectioning when held in the dark at 2–8 °C (preferred), or at room temperature up to 25 °C. Slide storage and handling conditions should not exceed 25 °C at any point after mounting to ensure tissue integrity and antigenicity.

Reagent Preparation

Target Retrieval Solution, pH 9 (50x) (Code GC309) and Wash Buffer (20x) (Code GC807) must be prepared according to their respective instructions for use (IFU). Refer to the GC309 and GC807 IFU for proper reagent preparation and storage information. Note the color of the Target Retrieval Solution, pH 9 (50x) is blue.

Reagents do not need to be equilibrated to room temperature before loading into the instrument. However, they should be loaded into the instrument before starting the staining procedure, which allows sufficient time for equilibration.

Test Controls

System level controls

System-level controls are intended to ensure the validity of the staining procedure, including reagents, tissue processing and instrument performance. If controls are not fixed in the same way as the test specimen, then the control tissue may only be used as a staining control.

Negative control tissue (lab-supplied) with known expression should be run for each staining procedure. The negative control should be prescreened CRC tissue with loss of biomarker expression in malignant cells compared to moderate to strong nuclear staining in adjacent internal positive controls. It is recommended that negative control tissue is stained on the same slide as the patient tissue.

The positive control should be tissue with positive biomarker expression. Positive nonmalignant elements (lymphocytes, stromal cells, and normal epithelium) present in the patient tissue should be used, where possible, as internal positive controls instead of a separate positive control tissue. In rare cases where nonmalignant elements may have loss of biomarker expression, nonmalignant elements of the negative control tissue may be used to qualify the staining procedure.

Negative control reagent

MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101) and MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102) should each be used in place of the respective species-matched primary antibody with a section of each patient specimen to evaluate nonspecific staining and allow correct interpretation of specific staining at the antigen site. Use the Dako Omnis protocol "MMR NCR Mo GE101" for slides stained with the mouse negative control reagent (NCR) and "MMR NCR Rb GE102" for slides stained with the rabbit NCR. Refer to the MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101) and MMR Negative Control Reagent, Rabbit (Dako Omnis) (GE102) instructions for use for details.

Principle of Operation

MMR IHC Panel pharmDx (Dako Omnis) primary antibodies and NCRs are used in combination with detection reagents and ancillary reagents (sold separately) to complete an IHC staining procedure on the Dako Omnis automated staining system. Vials are labeled with a bar code that can be recognized by Dako Omnis Solution software. For each antibody, an optimized staining protocol is provided in the software.

Following incubation with one of the four primary antibodies or either of the NCRs, specimens are sequentially incubated with peroxidase block, sequential linker antibodies, and a visualization reagent consisting of secondary antibody molecules and horseradish peroxidase (HRP) molecules coupled to a dextran polymer backbone. The enzymatic conversion of the subsequently added diaminobenzidine (DAB) chromogen results in precipitation of a visible reaction product at the antigen site. The specimen may then be counterstained and coverslipped.

Deparaffinization, rehydration, target retrieval, staining and counterstaining procedures are automatically performed on the Dako Omnis system. Coverslipping can be manual or automated, so capabilities for this are also required, but not supplied.

The presence or absence of target proteins is determined by visual examination of the specimen slide under a light microscope by a qualified pathologist.

Staining Protocol

When processing slides for staining with the MMR IHC Panel pharmDx (Dako Omnis) assay, the Dako Omnis automated platform executes the following protocols:

Table 3: Dako Omnis Staining Protocols for MMR IHC Panel pharmDx (Dako Omnis)

Protocol Parameter	Reagent	MLH1 (GE079)* or msNCR (GE101)*	MSH2 (GE085)*	PMS2 (GE087)* or MSH6 (GE086)* or rbNCR (GE102)*
		Temperature and incubation times/cycles		
Dewax	Clarify Cleaning Agent	25 °C, 10 s incubation top, 1 min incubation bottom, 1 cycle	25 °C, 10 s incubation top, 1 min incubation bottom, 1 cycle	25 °C, 10 s incubation top, 1 min incubation bottom, 1 cycle
	DI water	5 s incubation, 1 cycle	5 s incubation, 1 cycle	5 s incubation, 1 cycle
Target Retrieval	TRS, pH 9	97 °C, 30 min incubation	97 °C, 30 min incubation	97 °C, 30 min incubation
	DI water	N/A, cooling fluid	N/A, cooling fluid	N/A, cooling fluid
Staining	Wash buffer	2:40 min incubation, 2 cycles	2:40 min incubation, 2 cycles	2:40 min incubation, 2 cycles
	Primary Antibody	25 min incubation (GE079 or GE101)	20 min incubation (GE085)	20 min incubation (GE087, GE086, or GE102)
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	EnV FLEX Peroxidase-Blocking Reagent	3 min incubation	3 min incubation	3 min incubation
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	EnV FLEX+ LINKER (Rabbit or Mouse)	10 min incubation (Mouse)	10 min incubation (Mouse)	10 min incubation (Rabbit)
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	EnV FLEX+ LINKER	10 min incubation (Rabbit)	10 min incubation (Rabbit)	10 min incubation (Mouse)

Protocol Parameter	Reagent	MLH1 (GE079)* or msNCR (GE101)*	MSH2 (GE085)*	PMS2 (GE087)* or MSH6 (GE086)* or rbNCR (GE102)*
		Temperature and incubation times/cycles		
	(Rabbit or Mouse)			
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	EnV FLEX/HRP	20 min incubation	20 min incubation	20 min incubation
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	DI water	31 s incubation, 1 cycle	31 s incubation, 1 cycle	31 s incubation, 1 cycle
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	EnV FLEX Substrate Working Solution**	5 min incubation	5 min incubation	5 min incubation
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	DI water	31 s incubation, 1 cycle	31 s incubation, 1 cycle	31 s incubation, 1 cycle
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
Counterstaining	Hematoxylin	3 min incubation	3 min incubation	3 min incubation
	DI Water	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles
	Wash Buffer	2 min incubation, 10 cycles	2 min incubation, 10 cycles	2 min incubation, 10 cycles

*The staining protocol names for each of the MMR products are as follows: MMR MLH1 IHC pDx GE079, MMR MSH2 IHC pDx GE085, MMR MSH6 IHC pDx GE086, MMR PMS2 IHC pDx GE087, MMR NCR Mouse GE101, and MMR NCR Rabbit GE102.
**EnV FLEX Substrate Working Solution consists of two components: EnVision FLEX DAB+ Chromogen and EnVision FLEX Substrate Buffer. Timely mixing of these two components is automatically performed onboard Dako Omnis.

Interpretation of MMR IHC Panel pharmDx (Dako Omnis)

Results of the MMR IHC Panel pharmDx (Dako Omnis) should be interpreted by a qualified pathologist within the context of clinical presentation, morphology, and other histopathological criteria and complemented by proper controls.

A Hematoxylin and Eosin (H&E) stain of the patient tissue is evaluated first to assess tissue histology and preservation quality. MMR IHC Panel pharmDx (Dako Omnis) and the H&E staining should be performed on serial sections from the same paraffin block of the specimen to confirm:

1. The histological diagnosis of colorectal cancer.
2. The specimen contains a minimum of 50 viable malignant cells.
3. The specimen has been properly fixed and prepared for IHC analysis. Only well-preserved and well-stained areas of the specimen should be used to make a diagnostic status determination.

The specific staining pattern of MMR IHC Panel pharmDx (Dako Omnis) is nuclear and is evaluated using the following rules:

1. Only nuclear staining is considered; cytoplasmic staining should be ignored.
2. Brown DAB signal must be unequivocal.
3. The staining must cover the entire nucleus.

NCR slides must exhibit no or weak staining in malignant cells. If weak staining is present in tumor nuclei it should be used as a baseline to evaluate the species-matched primary antibody slides. Staining at the same intensity or lower that may occur in a species-matched primary antibody slide should be disregarded upon interpretation. NCR slides with moderate or strong staining in malignant cells are invalid and the species-matched antibody slides are considered nonevaluable and must be retested. For example, if the rabbit NCR slide is valid and the mouse NCR slide is invalid, only the mouse NCR and two mouse antibodies, MLH1 and MSH2, must be retested.

System level controls are intended to ensure the validity of the staining procedure, including reagents, tissue processing and instrument performance. If controls are not fixed in the same way as the test specimen then the tissue may only be used as a staining control. Nonspecific cytoplasmic staining may be present in some tissues stained with MMR IHC Panel pharmDx (Dako Omnis). As long as cytoplasmic staining does not interfere with the evaluation of biomarker status, then the slide is considered acceptable. If cytoplasmic staining does interfere with the evaluation of biomarker status, then repeat staining for the affected test.

Components of tumor areas that frequently demonstrate positive staining with MMR proteins, but are excluded from scoring are:

1. Normal cells such as lymphocytes, stromal cells, epithelial cells
2. Edge effects
3. Necrotic areas
4. Areas with adenoma component
5. Areas with obvious fixation artifacts should not be scored or scored with caution

Protein status of Intact or Loss is determined for MLH1, PMS2, MSH2, and MSH6, separately, using the following guidelines:

Table 4. Guidelines for Determining Protein Status of Intact or Loss

Intact	Nuclear staining in viable malignant cells must be unequivocal, with at least the same overall staining intensity as in adjacent internal positive controls. If focal staining is present, the tissue is considered intact if: 1) continuous in multiple glands/nests and 2) equal or stronger in intensity than internal positive controls.
Loss	No or equivocal nuclear staining in viable malignant cells compared to moderate or strong nuclear staining in adjacent internal positive controls. If focal staining is present, the tissue is considered loss if: 1) continuous in only a single gland/nest, 2) discontinuous in multiple glands/nests, or 3) weaker in intensity than internal positive controls.

Only unequivocal brown DAB staining that covers the entire nucleus of tumor cells and exhibits at least the same overall staining intensity as in adjacent internal positive controls should be considered intact MMR biomarker expression. Punctate nuclear staining of tumor cells, along with other incomplete nuclear staining patterns, should be considered loss of MMR biomarker expression.

Internal positive control elements must also be assessed when evaluating for MMR biomarker status. Cells with intact nuclear staining must have at least the same overall staining intensity as in adjacent internal positive controls. Cells with loss of nuclear

staining must have no or equivocal staining compared to adjacent internal positive controls. If the specimen demonstrates equivocal internal positive control staining and a protein status for the biomarker cannot be determined, it is recommended to first evaluate all biomarkers together. If the MMR status cannot be determined using all biomarkers, retesting of equivocal staining should be performed.

Specimen qualities that may make a case difficult or challenging to interpret include: focal staining in loss of expression tissue, focal staining in intact tissue, heterogeneous staining; necrosis, stromal cell staining, signet ring cell adenocarcinoma, mucinous colorectal cancer, areas without IPC staining, nonspecific/background staining tissue/staining artifacts (e.g., edge effect, tissue folding, etc.), and punctate staining.

After a protein status of Intact or Loss is assigned to each biomarker for a given specimen, a diagnostic status of MMR proficient or MMR deficient is given using the following definitions:

Table 5. Definition for MMR Diagnostic Status

MMR Proficient (pMMR)	MMR Deficient (dMMR)
Intact for all four biomarkers	Loss of one or more biomarkers

For additional guidance on MMR staining interpretation, refer to the MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or approved alternative class III immunohistochemistry assays available for detection of MMR in formalin-fixed, paraffin-embedded (FFPE) colorectal cancer (CRC) specimens to aid to identify MMR deficient CRC patients eligible for treatment with OPDIVO (nivolumab) alone or in combination with YERVOY (ipilimumab).

VII. MARKETING HISTORY

MMR IHC Panel pharmDx (Dako Omnis) has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

MMR IHC Panel pharmDx (Dako Omnis) is intended for in vitro diagnostic (IVD) use only. As with any IVD test, the potential risks are associated with an incorrect test result or incorrect interpretation of results. Failure of the device to perform as expected or failure to correctly interpret test results may lead to improper patient management decisions.

For the specific adverse events that occurred in the OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) clinical studies, please see the FDA approved package inserts, which are available at Drugs@FDA.

IX. SUMMARY OF NON-CLINICAL STUDIES

Non-clinical studies were performed using the MMR IHC Panel pharmDx (Dako Omnis) to establish analytical performance of the device in CRC patients. These studies were conducted to characterize the MMR IHC Panel pharmDx (Dako Omnis), demonstrate the impact of pre-analytical variables on assay performance, evaluate assay precision and robustness, and establish assay stability. The study results detailed below establish sensitivity, specificity, precision, robustness, stability, external reproducibility, and other performance characteristics of the device.

A. Laboratory Studies

1. Analytical Sensitivity

A set of 171 unique CRC specimens that were not preselected by mismatch repair (MMR) status was tested to assess if the MMR IHC Panel pharmDx (Dako Omnis) antibodies can detect the presence or absence of the target proteins (MLH1, MSH2, MSH6, and PMS2). The prevalence of loss of staining at the individual MMR biomarker level and the prevalence of dMMR status at the MMR panel level were calculated. The prevalence of dMMR panel status was 8.8%. The prevalence of loss of staining for individual MMR biomarkers was between 0% and 8.8%. Given the observed 0% MSH2 loss status in the 171 specimens included in the analytical sensitivity study, a supplemental MSH2 sensitivity study was initiated to assess further the prevalence of loss of MSH2 biomarker expression in a set of intended use samples that were not preselected based on MMR diagnostic status. An additional set of 98 unique cases was combined with the previously tested 171 cases for a total of 269 unique cases stained with MMR IHC MSH2 pharmDx (Dako Omnis). The prevalence of loss of staining of MSH2 was 2.6%. Table 6 summarizes the results for all biomarkers.

Table 6. MMR Panel dMMR/pMMR and MMR Biomarker Intact/Loss Results

Biomarker	N Specimens Total	Result	N Specimens (% of Total)
MLH1	171	Intact	157 (91.8%)
		Loss	14 (8.2%)
PMS2	171	Intact	156 (91.2%)

Biomarker	N Specimens Total	Result	N Specimens (% of Total)
		Loss	15 (8.8%)
MSH2	269	Intact	262 (97.4%)
		Loss	7 (2.6%)
MSH6	171	Intact	169 (98.8%)
		Loss	2 (1.2%)
MMR Panel*	171	pMMR	156 (91.2%)
		dMMR	15 (8.8%)

*MMR panel results do not include the additional cases tested for MSH2, as only MSH2 was supplemented.

2. Analytical Specificity

Specificity testing was conducted to demonstrate that MMR IHC Panel pharmDx (Dako Omnis) will detect the target antigens in the appropriate tissue elements and cellular compartment. Tests included testing of immunoreactivity on normal tissues and neoplastic tissues (tour of tumor); Western blot; and peptide inhibition.

i. Western Blot

Western blots analyses were conducted to demonstrate that the antibodies specifically detect the proteins of predicted molecular weight for each of the 4 MMR pharmDx antibodies using cell lines with known MMR loss or intact status. The MLH1, MSH2, PMS2, and MSH6 antibodies demonstrated the ability to detect the presence or absence of their target antigens in cell lines known to have positive or negative expression, respectively. The Western blot study supports the conclusion that each of the MMR antibodies is specific for their respective antigens. Additionally, IHC testing confirmed alignment between Western blot and IHC results in the tested cell lines. Further studies investigated the specificity using RNA-sequencing and BLAST to evaluate the expression of the target proteins and potential cross-reacting proteins in the cell lines used for Western blot testing. The results confirmed that the MMR antibodies are specific for their respective antigens.

ii. Epitope Mapping and Peptide Inhibition

Epitope mapping was conducted to elucidate the epitope of each antibody, and this data was used to synthesize epitope-containing peptides for use in the inhibition study. The UniProt BLAST and ALIGN tools were used to identify the proposed epitope sequence within the context of each of the target proteins and to identify potential cross-reactivity with any non-target proteins.

A peptide inhibition study was conducted to confirm the specificity of the MMR IHC Panel pharmDx (Dako Omnis) antibodies (MLH1, MSH2, MSH6 and PMS2) for their respective protein targets as identified in the epitope mapping study. For each biomarker, sections from five CRC specimens (four intact and one loss) were stained with one of the MMR antibodies in the presence or absence of a peptide containing the corresponding epitope. Solutions of various peptide-to-antibody (peptide:antibody) molar ratios (0.5:1 [MSH6 only], 2:1 [MSH6 only], 4:1, 10:1, 50:1, 100:1, 200:1 [PMS2 only], and 300:1 [PMS2 only]) were prepared for use in place of the uninhibited Ready-to-use (RTU) antibody in the primary antibody step of the staining protocol. Slides were also stained with standard RTU antibody as the reference condition. To verify specificity of inhibition, specimens were also stained with the MMR antibody mixed with a negative control peptide and tested at the highest peptide:antibody molar ratio. Each negative control peptide contained the same amino acids as the corresponding epitope-containing peptide in a scrambled order. For MSH2, the peptides (test and negative control) were not soluble in water and had to be reconstituted in 3% ammonium hydroxide, so an ammonium hydroxide diluent in RTU antibody as a control was also included.

IHC staining by the MMR antibodies was inhibited in the presence of epitope-containing peptides, confirming specificity to the target antigen.

iii. Immunoreactivity in Normal Tissues

Immunohistochemistry based specificity testing was conducted to demonstrate that MMR IHC Panel pharmDx (Dako Omnis) will detect the target substance in the appropriate tissue elements and cellular compartment in a variety of normal tissues. The expectation of which structures should be positive and negative was established using peer-reviewed scientific literature. There was no non-specific staining observed in tissue types tested. All tissues were FFPE and stained with MMR IHC Panel pharmDx (Dako Omnis) according to the instructions in the package insert.

MMR IHC Panel pharmDx (Dako Omnis) detected expression of the MMR proteins (MLH1, MSH2, MSH6 and PMS2) in all 31 normal tissue types tested. Because the MMR proteins are required for normal DNA

replication, it is expected that they were expressed in all normal cells. There were no unexpected results observed in cell types or tissue types tested. The observed staining was consistent with the reported literature for MMR expression in normal tissues. Table 7 summarizes MMR immunoreactivity on the recommended panel of normal tissues.

Table 7: Specificity of MMR IHC Panel pharmDx (Dako Omnis) in Normal Tissue

Reactivity Tissue	# positive / total cases			
	MLH1	PMS2	MSH2	MSH6
Salivary Gland	3/3	3/3	3/3	3/3
Bone Marrow	2/3[a]	2/3 [a]	3/3 [a]	2/3 [a]
Pituitary Gland	3/3	3/3	3/3	1/3
Cerebrum	2/3	3/3	1/3	1/3
Adrenal Gland	2/3	2/3	3/3	2/3
Cerebellum	3/3	3/3	3/3	1/3
Uterus	3/3	3/3	3/3	3/3
Ovary	3/3	3/3	3/3	3/3
Cervix	3/3	3/3	3/3	3/3
Breast	2/3	2/3	2/3	2/3
Nerve Peripheral	3/3	3/3	3/3	1/3
Prostate	3/3	3/3	3/3	3/3
Skin	3/3	3/3	3/3	3/3
Testis	3/3	3/3	3/3	3/3
Mesothelial Cells	3/3	3/3	3/3	3/3
Skeletal Muscle	3/3	3/3	3/3	3/3
Lung	3/3	3/3	3/3	3/3
Heart	3/3	3/3 [a]	3/3 [a]	2/3
Stomach	3/3	3/3	3/3	2/3
Small Intestine	3/3	3/3	3/3	3/3

Reactivity Tissue	# positive / total cases			
	MLH1	PMS2	MSH2	MSH6
Esophagus	3/3	3/3	3/3	3/3
Colon	3/3	3/3	3/3	3/3
Kidney	3/3	3/3	3/3	3/3
Liver	2/3	3/3 [a]	3/3 [a]	3/3 [a]
Pancreas	3/3	3/3	3/3	3/3
Spleen	3/3	3/3	3/3	2/3
Tonsil	3/3	3/3	3/3	3/3
Thyroid	3/3	3/3	3/3	2/3
Thymus	3/3[b]	3/3	3/3 [b]	3/3 [b]
Parathyroid	3/3	3/3	3/3	3/3
Bladder	3/3	3/3	3/3	3/3

[a] cytoplasmic staining pattern for at least one case

[b] cytoplasmic and extracellular staining pattern for at least one case

iv. Immunoreactivity in Neoplastic Tissues

Neoplastic tissue specimens were evaluated using various neoplastic tumors in cores from a tissue microarray. The expected staining pattern was established through review of peer-reviewed scientific literature. MMR IHC Panel pharmDx (Dako Omnis) identifies MMR expression in multiple cancer types, and findings of no expression of one or more biomarker in breast and colon samples are consistent with reported dMMR prevalence in the literature. All tissues were FFPE and stained with MMR IHC Panel pharmDx (Dako Omnis) according to the instructions in the package insert.

Expression of one or more of the MMR proteins was identified in 42 tumor types. There were no unexpected results observed in the tumor specimens tested. The observed staining was consistent with the reported literature for MMR expression in neoplastic tissues. Table 8 summarizes MMR immunoreactivity on a panel of neoplastic tissues.

Table 8: Specificity of MMR IHC Panel pharmDx (Dako Omnis) in Neoplastic Tissue

Tissue Type (# tested)	# positive/total cases evaluated [a]			
	MLH1	PMS2	MSH2	MSH6
Bladder Carcinoma (2)	2/2	2/2	2/2	2/2
Breast Carcinoma (6)	5/5	3/4	5/5	4/5
Cholangiocarcinoma (1)	1/1	1/1	1/1	1/1
Colon Adenocarcinoma (1)	1/1	0/1	1/1	1/1
Endometrial Sarcoma (1)	1/1	1/1	1/1	1/1
Ewing's Sarcoma (1)	1/1	1/1	1/1	1/1
Gastric Adenocarcinoma (2)	2/2	2/2	2/2	2/2
Hepatoma (1)	NE	NE	1/1	NE
Islet cell tumor of pancreas (1)	NE	NE	1/1	NE
Kidney Transitional Cell Carcinoma (1)	1/1	1/1	1/1	NE
Liver cell adenoma (1)	NE	NE	1/1	NE
Lung Carcinoma (4)	3/3	2/2	4/4	3/3
Lymphoma of Cecum (1)	1/1	1/1	1/1	1/1
Melanoma (3)	3/3	3/3	3/3	3/3
Merkel Cell Tumor (1)	1/1	1/1	1/1	1/1
Ovarian Carcinoma (2)	2/2	2/2	2/2	2/2
Ovarian Dysgerminoma (1)	1/1	1/1	1/1	1/1
Ovarian Granulosa Cell Tumor (1)	1/1	1/1	1/1	1/1
Pancreatic adenocarcinoma (1)	NE	NE	NE	1/1
Pancreatic glucagonoma (1)	NE	NE	1/1	1/1
Papillary Serous carcinoma (1)	NE	NE	1/1	NE
Pleomorphic Rhabdomyosarcoma (1)	1/1	1/1	1/1	1/1
PNET Scrotum (1)	1/1	NE	1/1	1/1
Prostate Adenocarcinoma (2)	1/1	1/1	2/2	1/1
Prostate Benign Prostatic Hyperplasia (1)	1/1	1/1	1/1	1/1
Renal Cell Carcinoma (1)	1/1	1/1	1/1	NE
Squamous Carcinoma of Ear (1)	1/1	1/1	1/1	1/1
Testicular Embryonal Carcinoma (1)	1/1	1/1	1/1	1/1
Testicular Yolk Sac Tumor (1)	1/1	1/1	1/1	1/1
Thymic carcinoid tumor (1)	NE	NE	1/1	1/1
Thymoma (1)	1/1	1/1	1/1	1/1
Thyroid Carcinoma (2)	1/1	1/1	2/2	2/2

Tissue Type (# tested)	# positive/total cases evaluated [a]			
	MLH1	PMS2	MSH2	MSH6
Uterine Adenomatoid Tumor (1)	1/1	1/1	1/1	1/1

[a] Number evaluated may be less than total number tested due to non-evaluable (NE) internal positive controls for a single biomarker
NE = Non-evaluable

3. Precision

The precision of MLH1 IHC pharmDx (Dako Omnis), MSH2 IHC pharmDx (Dako Omnis), MSH6 IHC pharmDx (Dako Omnis), and PMS2 IHC pharmDx (Dako Omnis) was evaluated at one (1) internal laboratory. Mismatch Repair NCRs (mouse and rabbit) were used in the generation of the precision study and did not show staining on any of the tissues.

Diagnostic status was recorded as 'Intact' or 'Loss' for biomarker-level analysis and was recorded as 'pMMR' or 'dMMR' for panel-level analysis. Percent agreement of loss (LPA), percent agreement of intact (IPA) and overall percent agreement (OPA), using comparisons to the consensus diagnostic status as reference, were computed with corresponding two-sided 95% percentile bootstrap confidence intervals (CIs). The Wilson score limits were used to calculate confidence intervals for agreement parameters with point estimates equal to 100%. The sample sets varied between each biomarker, so only biomarker results (intact/loss) were determined. The sample sets included 18 challenging cases for MLH1, 5 challenging cases for PMS2, 18 challenging cases for MSH2, and 13 challenging cases for MSH6.

i. Intermediate Precision and Repeatability

Table 9. Intermediate Precision of MLH1

Precision Study	Study Design	% Agreement (95% CI)
Intra-rack	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument within the same rack/staining module. Intra-rack analysis was performed between 4 replicates stained within the same rack/staining module on a total of 96 comparisons to consensus.	LPA 100.0 (92.6, 100.0) IPA 100.0 (92.6, 100.0) OPA 100.0 (96.2, 100.0)
Inter-rack	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument on different racks/staining modules. Inter-rack analysis was performed between 4 racks/staining modules on a total of 95 comparisons to consensus.	LPA 97.9 (93.8, 100.0) IPA 100.0 (92.4, 100.0) OPA 98.9 (96.8, 100.0)
Inter-instrument	Each of 24 CRC specimens (12 loss, 12 intact) was tested across 3 different	LPA 100.0 (94.9, 100.0) IPA 100.0 (94.9, 100.0)

Precision Study	Study Design	% Agreement (95% CI)
	Dako Omnis instruments. Inter-instrument analysis was performed between 3 different Dako Omnis instruments on a total of 144 comparisons to consensus.	OPA 100.0 (97.4, 100.0)
Inter-day	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument over 5 nonconsecutive days. Inter-day analysis was performed between 5 nonconsecutive days on a total of 120 comparisons to consensus.	LPA 98.3 (95.0, 100.0) IPA 100.0 (94.0, 100.0) OPA 99.2 (97.5, 100.0)
Inter-lot	Each of 24 CRC specimens (11 loss, 13 intact) was tested on a single Dako Omnis instrument using 3 unique lots of reagents. Inter-lot analysis was performed between 3 unique lots of reagents on a total of 143 comparisons to consensus.	LPA 100.0 (94.4, 100.0) IPA 100.0 (95.3, 100.0) OPA 100.0 (97.4, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 10. Percent Loss Results Per Sample for Intermediate Precision of MLH1

Case ID	Inter-Day Percent Loss Results, % (n/N)	Inter-Instrument Percent Loss Results, % (n/N)	Inter-Lot Percent Loss Results, % (n/N)	Inter-Rack Percent Loss Results, % (n/N)	Intra-Rack Percent Loss Results, % (n/N)
1	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
2	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
3	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
4	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
5	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
6	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
7	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/3)	0% (0/4)
8	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
9	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
10	0% (0/5)	0% (0/6)	0% (0/6)	N/A	N/A
11	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
12	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
13	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)

Case ID	Inter-Day Percent Loss Results, % (n/N)	Inter-Instrument Percent Loss Results, % (n/N)	Inter-Lot Percent Loss Results, % (n/N)	Inter-Rack Percent Loss Results, % (n/N)	Intra-Rack Percent Loss Results, % (n/N)
14	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
15	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
16	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
17	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
18	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
19	100% (5/5)	100% (6/6)	0% (0/6)	75% (3/4)	100% (4/4)
20	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
21	100% (5/5)	100% (6/6)	100% (5/5)	100% (4/4)	100% (4/4)
22	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
23	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
24	80% (4/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
25	N/A	N/A	N/A	0% (0/4)	0% (0/4)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 11. Intermediate Precision of MSH2

Precision Study	Study Design	% Agreement (95% CI)
Intra-rack	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument within the same rack/staining module. Intra-rack analysis was performed between 4 replicates stained within the same rack/staining module on a total of 96 comparisons to consensus.	LPA 100.0 (92.6, 100.0) IPA 100.0 (92.6, 100.0) OPA 100.0 (96.2, 100.0)
Inter-rack	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument on different racks/staining modules. Inter-rack analysis was performed between 4 racks/staining modules on a total of 96 comparisons to consensus.	LPA 100.0 (92.6, 100.0) IPA 100.0 (92.6, 100.0) OPA 100.0 (96.2, 100.0)
Inter-instrument	Each of 24 CRC specimens (12 loss, 12 intact) was tested across 3 different Dako Omnis instruments. Inter-instrument analysis was performed between 3 different	LPA 100.0 (94.9, 100.0) IPA 100.0 (94.9, 100.0) OPA 100.0 (97.4, 100.0)

Precision Study	Study Design	% Agreement (95% CI)
	Dako Omnis instruments on a total of 144 comparisons to consensus.	
Inter-day	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument over 5 nonconsecutive days. Inter-day analysis was performed between 5 nonconsecutive days on a total of 120 comparisons to consensus.	LPA 100.0 (94.0, 100.0) IPA 100.0 (94.0, 100.0) OPA 100.0 (96.9, 100.0)
Inter-lot	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument using 3 unique lots of reagents. Inter-lot analysis was performed between 3 unique lots of reagents on a total of 144 comparisons to consensus.	LPA 98.6 (95.8, 100.0) IPA 98.6 (95.8, 100.0) OPA 98.6 (96.5, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 12. Percent Loss Results Per Sample in Intermediate Precision of MSH2

Case ID	Inter-Day Percent Loss Results, % (n/N)	Inter-Instrument Percent Loss Results, % (n/N)	Inter-Lot Percent Loss Results, % (n/N)	Inter-Rack Percent Loss Results, % (n/N)	Intra-Rack Percent Loss Results, % (n/N)
1	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
2	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
3	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
4	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
5	0% (0/5)	0% (0/6)	16.7% (1/6)	0% (0/4)	0% (0/4)
6	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
7	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
8	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
9	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
10	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
11	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
12	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
13	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
14	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
15	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
16	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
17	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
18	100% (5/5)	100% (6/6)	83.3% (5/6)	100% (4/4)	100% (4/4)
19	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
20	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
21	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
22	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
23	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
24	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 13. Intermediate Precision of MSH6

Precision Study	Study Design	% Agreement (95% CI)
Intra-rack	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument within the same rack/staining module. Intra-rack analysis was performed between 4 replicates stained within the same rack/staining module on a total of 96 comparisons to consensus.	LPA 100.0 (92.6, 100.0) IPA 100.0 (92.6, 100.0) OPA 100.0 (96.2, 100.0)

Precision Study	Study Design	% Agreement (95% CI)
Inter-rack	Each of 23 CRC specimens (11 loss, 12 intact) was tested on a single Dako Omnis instrument on different racks/staining modules. Inter-rack analysis was performed between 4 racks/staining modules on a total of 92 comparisons to consensus.	LPA 100.0 (92.0, 100.0) IPA 100.0 (92.6, 100.0) OPA 100.0 (96.0, 100.0)
Inter-instrument	Each of 24 CRC specimens (12 loss, 12 intact) was tested across 3 different Dako Omnis instruments. Inter-instrument analysis was performed between 3 different Dako Omnis instruments on a total of 144 comparisons to consensus.	LPA 100.0 (94.9, 100.0) IPA 100.0 (94.9, 100.0) OPA 100.0 (97.4, 100.0)
Inter-day	Each of 24 CRC specimens (10 loss, 14 intact) was tested on a single Dako Omnis instrument over 5 nonconsecutive days. Inter-day analysis was performed between 5 nonconsecutive days on a total of 120 comparisons to consensus.	LPA 100.0 (92.9, 100.0) IPA 98.6 (95.7, 100.0) OPA 99.2 (97.5, 100.0)
Inter-lot	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument using 3 unique lots of reagents. Inter-lot analysis was performed between 3 unique lots of reagents on a total of 144 comparisons to consensus.	LPA 100.0 (94.9, 100.0) IPA 100.0 (94.9, 100.0) OPA 100.0 (97.4, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 14. Percent Loss Results Per Sample in Intermediate Precision of MSH6

Case ID	Inter-Day Percent Loss Results, % (n/N)	Inter-Instrument Percent Loss Results, % (n/N)	Inter-Lot Percent Loss Results, % (n/N)	Inter-Rack Percent Loss Results, % (n/N)	Intra-Rack Percent Loss Results, % (n/N)
1	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
2	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
3	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
4	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
5	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
6	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
7	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
8	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
9	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
10	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
11	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
12	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
13	0% (0/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
14	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
15	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
16	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
17	100% (5/5)	100% (6/6)	100% (6/6)	N/A	N/A
18	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
19	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
20	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
21	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
22	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
23	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
24	20% (1/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
25	N/A	N/A	N/A	N/A	100% (4/4)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 15. Intermediate Precision of PMS2

Precision Study	Study Design	% Agreement (95% CI)
Intra-rack	Each of 24 CRC specimens (13 loss, 11 intact) was tested on a single Dako Omnis instrument within the same rack/staining module. Intra-rack analysis was performed between 4 replicates stained within the same rack/staining module on a total of 96 comparisons to consensus.	LPA 100.0 (93.1, 100.0) IPA 100.0 (92.0, 100.0) OPA 100.0 (96.2, 100.0)
Inter-rack	Each of 23 CRC specimens (13 loss, 11 positive) was tested on a single Dako Omnis instrument on different racks/staining modules. Inter-rack analysis was performed between 4 racks/staining modules on a total of 96 comparisons to consensus.	LPA 100.0 (93.1, 100.0) IPA 100.0 (92.0, 100.0) OPA 100.0 (96.2, 100.0)
Inter-instrument	Each of 24 CRC specimens (12 loss, 12 intact) was tested across 3 different Dako Omnis instruments. Inter-instrument analysis was performed between 3 different Dako Omnis instruments on a total of 144 comparisons to consensus.	LPA 100.0 (94.9, 100.0) IPA 98.6 (95.8, 100.0) OPA 99.3 (97.9, 100.0)
Inter-day	Each of 24 CRC specimens (12 loss, 12 intact) was tested on a single Dako Omnis instrument over 5 nonconsecutive days. Inter-day analysis was performed between 5 nonconsecutive days on a total of 120 comparisons to consensus.	LPA 100.0 (94.0, 100.0) IPA 100.0 (94.0, 100.0) OPA 100.0 (96.9, 100.0)
Inter-lot	Each of 24 CRC specimens (14 loss, 10 intact) was tested on a single Dako Omnis instrument using 3 unique lots of reagents. Inter-lot analysis was performed between 3 unique lots of reagents on a total of 144 comparisons to consensus.	LPA 100.0 (95.6, 100.0) IPA 100.0 (94.0, 100.0) OPA 100.0 (97.4, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 16. Percent Loss Results Per Sample in Intermediate Precision of PMS2

Case ID	Inter-Day Percent Loss Results, % (n/N)	Inter-Instrument Percent Loss Results, % (n/N)	Inter-Lot Percent Loss Results, % (n/N)	Inter-Rack Percent Loss Results, % (n/N)	Intra-Rack Percent Loss Results, % (n/N)
1	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
2	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
3	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
4	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
5	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
6	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
7	0% (0/5)	16.7% (1/6)	0% (0/6)	0% (0/4)	0% (0/4)
8	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
9*	0% (0/5)	0% (0/6)	100% (6/6)	100% (4/4)	100% (4/4)
10	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
11	0% (0/5)	0% (0/6)	0% (0/6)	0% (0/4)	0% (0/4)
12	0% (0/5)	0% (0/6)	100% (6/6)	0% (0/4)	0% (0/4)
13	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
14	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
15	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
16	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
17	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
18	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
19	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
20	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
21	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
22	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
23	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)
24	100% (5/5)	100% (6/6)	100% (6/6)	100% (4/4)	100% (4/4)

*Inter-observer variability observed in biomarker status for this case across the different sub-studies was attributed to the challenging quality of the case (i.e., focal staining). The variability had no impact on the intermediate precision results, as the intra-observer scoring was consistent, and the sub studies are analyzed separately.

Note: n/N denotes the number of loss replicates/the total number of replicates.

ii. Internal Inter-Reader Precision

The between-reader precision of MLH1 IHC pharmDx (Dako Omnis), MSH2 IHC pharmDx (Dako Omnis), MSH6 IHC pharmDx (Dako Omnis), and PMS2 IHC pharmDx (Dako Omnis) was evaluated at one (1) internal laboratory. The sample set included 12 challenging cases. Mismatch Repair NCRs (mouse and rabbit) were used in the generation of the precision study and did not show staining on any of the tissues.

Diagnostic status was recorded as 'Loss' or 'Intact' for biomarker-level analysis and was recorded as 'dMMR' or 'pMMR' for panel-level analysis. Loss percent agreement (LPA), intact percent agreement (IPA) and overall percent agreement (OPA), using comparisons to the consensus diagnostic status as reference, were computed with corresponding two-sided 95% percentile bootstrap confidence intervals (CIs) for the biomarker-level analysis. dMMR percent agreement (dMPA), pMMR percent agreement (pMPA), and OPA were computed with corresponding two-sided 95% percentile bootstrap CIs for the panel-level analysis. The Wilson score limits were used to calculate confidence intervals for agreement parameters with point estimates equal to 100%.

Table 17. Inter-Observer Precision at one site

Biomarker	Study Design	% Agreement (95% CI)
Panel	One set of 58 stained specimens (31 dMMR, 27 pMMR) was evaluated in turn by each of 3 observers at a single site. Inter-observer analysis was performed between 3 observers on a total of 172 comparisons to consensus.	dMPA 95.7 (91.3, 98.9) pMPA 98.8 (96.2, 100.0) OPA 97.1 (94.7, 99.4)
MLH1	One set of 58 CRC stained specimens (28 loss, 30 intact) was evaluated in turn by each of 3 observers at a single site. Inter-observer analysis was performed between 3 observers on a total of 172 comparisons to consensus.	LPA 94.0 (89.3, 98.8) IPA 97.7 (94.2, 100.0) OPA 95.9 (93.0, 98.3)
MSH2	One set of 58 CRC stained specimens (4 loss, 54 intact) was evaluated in turn by each of 3 observers at a single site. Inter-observer analysis was performed between 3 observers on a total of 172 comparisons to consensus.	LPA 90.9 (75.0, 100.0) IPA 98.8 (96.9, 100.0) OPA 98.3 (96.0, 100.0)
MSH6	One set of 58 CRC stained specimens (6 loss, 52 intact) was evaluated in turn by each of 3 observers at a single site. Inter-observer analysis was performed between 3 observers on a total of 172 comparisons to consensus.	LPA 82.4 (70.6, 94.4) IPA 99.4 (98.1, 100.0) OPA 97.7 (95.9, 99.4)
PMS2	One set of 58 CRC stained specimens (28 loss, 30 intact) was evaluated in turn by each of 3 observers at a single site. Inter-observer analysis was performed between 3 observers on a total of 172 comparisons to consensus.	LPA 95.2 (90.5, 98.8) IPA 97.7 (94.2, 100.0) OPA 96.5 (93.6, 98.8)

dMPA=dMMR Percent Agreement; pMPA=pMMR Percent Agreement; OPA=Overall Percent Agreement; LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact

Table 18. Biomarker-level and Panel-level Percent Loss Results Per Sample in Inter-Observer Precision at One Site

Case ID	MLH1 Percent Loss Results, % (n/N)	MSH2 Percent Loss Results, % (n/N)	PMS2 Percent Loss Results, % (n/N)	MSH6 Percent Loss Results, % (n/N)	Panel Percent dMMR, % (n/N)
1	0% (0/2)	0% (0/2)	0% (0/2)	0% (0/2)	0% (0/2)
2	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
3	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
4	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
5	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
6	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
7	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
8	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
9	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
10	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
19	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
22	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
23	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
24	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
25	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
27	50% (1/2)	100% (2/2)	50% (1/2)	100% (2/2)	100% (2/2)
28	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)	100% (3/3)
29	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)	100% (3/3)
30	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
31	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
32	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)

Case ID	MLH1 Percent Loss Results, % (n/N)	MSH2 Percent Loss Results, % (n/N)	PMS2 Percent Loss Results, % (n/N)	MSH6 Percent Loss Results, % (n/N)	Panel Percent dMMR, % (n/N)
33	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
34	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
35	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
36	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
37	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
38	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
39	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
40	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
41	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
42	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
43	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
44	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
45	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
46	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
47	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
48	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
49	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
50	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
51	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
52	100% (3/3)	0% (0/3)	100% (3/3)	33.3% (1/3)	100% (3/3)
53	66.7% (2/3)	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
54	33.3% (1/3)	0% (0/3)	33.3% (1/3)	0% (0/3)	33.3% (1/3)
55	66.7% (2/3)	0% (0/3)	66.7% (2/3)	0% (0/3)	66.7% (2/3)
56	66.7% (2/3)	33.3% (1/3)	66.7% (2/3)	66.7% (2/3)	66.7% (2/3)
57	66.7% (2/3)	33.3% (1/3)	66.7% (2/3)	66.7% (2/3)	66.7% (2/3)
58	66.7% (2/3)	66.7% (2/3)	66.7% (2/3)	66.7% (2/3)	66.7% (2/3)

Note: n/N denotes the number of loss or dMMR replicates/the total number of replicates.

iii. Inter-Laboratory (External Reproducibility) Study

The reproducibility of the panel (MMR IHC pharmDx Panel (Dako Omnis)) was evaluated at three external testing sites. The inter-/intra-site sample set included 2 challenging cases. MMR diagnostic status was recorded as 'Proficient' or 'Deficient'. dMMR percent agreement (dMPA),

pMMR percent agreement (pMPA), and overall percent agreement (OPA) were computed with corresponding two-sided 95% percentile bootstrap confidence intervals (Table 19).

In the same study, the reproducibility of the individual biomarkers MLH1 IHC pharmDx (Dako Omnis), MSH2 IHC pharmDx (Dako Omnis), MSH6 IHC pharmDx (Dako Omnis), and PMS2 IHC pharmDx (Dako Omnis) were analyzed. Individual MMR antibody status was recorded as 'Intact' or 'Loss'. Mismatch Repair NCRs (mouse and rabbit) were used in the generation of the reproducibility study and did not show staining on any of the tissues.

LPA, IPA, and OPA were computed with corresponding two-sided 95% percentile bootstrap confidence intervals for each of the individual MMR antibodies. No acceptance criteria were applied to the statistical analysis of each individual MMR antibody.

The Wilson score limits were used to calculate confidence intervals for agreement parameters with point estimates equal to 100%.

Table 19. External Reproducibility of MMR IHC Panel pharmDx (Dako Omnis)

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-site	Each of 32 CRC specimens (16 dMMR, 16 pMMR) was tested on 5 nonconsecutive days at each of 3 study sites. Inter-site analysis was performed between 3 sites on a total of 286 comparisons to consensus.	dMPA 98.6 (95.7, 100.0) pMPA 99.3 (97.9, 100.0) OPA 99.0 (97.2, 100.0)
Intra-site	Each of 32 CRC specimens (16 dMMR, 16 pMMR) was tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 286 comparisons to consensus.	dMPA 100.0 (97.3, 100.0) pMPA 99.3 (97.9, 100.0) OPA 99.7 (98.9, 100.0)
Inter-observer	One set of 60 stained specimens (30 dMMR, 30 pMMR) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to consensus.	dMPA 99.6 (98.9, 100.0) pMPA 100.0 (98.6, 100.0) OPA 99.8 (99.4, 100.0)

Reproducibility Study	Study Design	% Agreement (95% CI)
Intra-observer	One set of 60 stained specimens (30 dMMR, 30 pMMR) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to consensus.	dMPA 99.6 (98.9, 100.0) pMPA 100.0 (98.6, 100.0) OPA 99.8 (99.4, 100.0)

dMPA=dMMR Percent Agreement; pMPA=pMMR Percent Agreement; OPA=Overall Percent Agreement

Table 20. Panel-level Per Site Percent dMMR Results Per Sample in External Reproducibility of MMR IHC Panel pharmDx (Dako Omnis)

Case ID	All Sites Percent dMMR Results, % (n/N)	Site 1 Percent dMMR Results, % (n/N)	Site 2 Percent dMMR Results, % (n/N)	Site 3 Percent dMMR Results, % (n/N)
1	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
2	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
3	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
4	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
5	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
6	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
7	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
10	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
20	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
21	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
22	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
23	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
24	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)

Case ID	All Sites Percent dMMR Results, % (n/N)	Site 1 Percent dMMR Results, % (n/N)	Site 2 Percent dMMR Results, % (n/N)	Site 3 Percent dMMR Results, % (n/N)
25	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
27	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
28	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	77.8% (7/9)	100% (3/3)	33.3% (1/3)	100% (3/3)
31	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
32	11.1% (1/9)	0% (0/3)	33.3% (1/3)	0% (0/3)

Note: n/N denotes the number of dMMR replicates/the total number of replicates.

Table 21. Panel-level Per Reader Percent dMMR Results Per Sample in External Reproducibility of MMR IHC Panel pharmDx (Dako Omnis)

Case ID	All Readers Percent dMMR Results, % (n/N)	Reader 1 Percent dMMR Results, % (n/N)	Reader 2 Percent dMMR Results, % (n/N)	Reader 3 Percent dMMR Results, % (n/N)
1	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
2	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
3	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
4	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
5	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
6	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
7	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
10	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent dMMR Results, % (n/N)	Reader 1 Percent dMMR Results, % (n/N)	Reader 2 Percent dMMR Results, % (n/N)	Reader 3 Percent dMMR Results, % (n/N)
19	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
22	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
23	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
24	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
25	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
26	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
27	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
31	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
32	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
33	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
34	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
35	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
36	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
37	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
38	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
39	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
40	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
41	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
42	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
43	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
44	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
45	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
46	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
47	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
48	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
49	88.9% (8/9)	66.7% (2/3)	100% (3/3)	100% (3/3)
50	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
51	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
52	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
53	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent dMMR Results, % (n/N)	Reader 1 Percent dMMR Results, % (n/N)	Reader 2 Percent dMMR Results, % (n/N)	Reader 3 Percent dMMR Results, % (n/N)
54	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
55	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
56	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
57	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
58	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
59	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
60	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 22. External Reproducibility of MLH1

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-site	Each of 32 CRC specimens (8 loss, 24 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Inter-site analysis was performed between 3 sites on a total of 286 comparisons to consensus.	LPA 98.6 (95.8, 100.0) IPA 99.5 (98.6, 100.0) OPA 99.3 (98.3, 100.0)
Intra-site	Each of 32 CRC specimens (8 loss, 24 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 286 comparisons to consensus.	LPA 98.6 (95.8, 100.0) IPA 99.5 (98.6, 100.0) OPA 99.3 (98.3, 100.0)
Inter-observer	One set of 60 stained specimens (18 loss, 42 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to consensus.	LPA 100.0 (97.7, 100.0) IPA 100.0 (99.0, 100.0) OPA 100.0 (99.3, 100.0)
Intra-observer	One set of 60 stained specimens (18 loss, 42 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to consensus.	LPA 100.0 (97.7, 100.0) IPA 100.0 (99.0, 100.0) OPA 100.0 (99.3, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 23. Biomarker-level Per Site Percent Loss Results Per Sample in External Reproducibility of MLH1

Case ID	All Sites Percent Loss Results, % (n/N)	Site 1 Percent Loss Results, % (n/N)	Site 2 Percent Loss Results, % (n/N)	Site 3 Percent Loss Results, % (n/N)
1	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
2	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
3	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
4	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
5	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
6	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
7	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	88.9% (8/9)	100% (3/3)	100% (3/3)	66.7% (2/3)
10	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
22	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
23	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
24	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
25	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
27	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
31	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
32	11.1% (1/9)	0% (0/3)	33.3% (1/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 24. Biomarker-level Per Reader Percent Loss Results Per Sample in External Reproducibility of MLH1

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
1	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
2	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
3	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
4	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
5	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
6	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
7	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
10	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
22	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
23	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
24	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
25	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
26	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
27	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
31	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
32	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
33	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
34	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
35	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
36	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
37	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
38	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
39	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
40	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
41	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
42	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
43	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
44	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
45	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
46	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
47	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
48	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
49	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
50	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
51	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
52	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
53	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
54	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
55	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
56	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
57	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
58	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
59	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
60	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 25. External Reproducibility of MSH2

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-site	Each of 32 CRC specimens (7 loss, 25 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Inter-site analysis was performed between 3 sites on a total of 286 comparisons to consensus.	LPA 96.8 (90.0, 100.0) IPA 99.1 (97.8, 100.0) OPA 98.6 (96.8, 100.0)
Intra-site	Each of 32 CRC specimens (7 loss, 25 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 286 comparisons to consensus.	LPA 100.0 (94.0, 100.0) IPA 99.1 (97.8, 100.0) OPA 99.3 (98.3, 100.0)
Inter-observer	One set of 60 stained specimens (10 loss, 50 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to consensus.	LPA 100.0 (95.9, 100.0) IPA 100.0 (99.2, 100.0) OPA 100.0 (99.3, 100.0)
Intra-observer	One set of 60 stained specimens (10 loss, 50 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to consensus.	LPA 100.0 (95.9, 100.0) IPA 100.0 (99.2, 100.0) OPA 100.0 (99.3, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 26. Biomarker-level Per Site Percent Loss Results Per Sample in External Reproducibility of MSH2

Case ID	All Sites Percent Loss Results, % (n/N)	Site 1 Percent Loss Results, % (n/N)	Site 2 Percent Loss Results, % (n/N)	Site 3 Percent Loss Results, % (n/N)
1	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
2	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
3	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
4	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
5	11.1% (1/9)	0% (0/3)	0% (0/3)	33.3% (1/3)
6	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
7	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	11.1% (1/9)	0% (0/3)	0% (0/3)	33.3% (1/3)
10	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
20	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
21	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
22	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
23	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
24	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
25	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
27	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	77.8% (7/9)	100% (3/3)	33.3% (1/3)	100% (3/3)
31	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
32	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 27. Biomarker-level Per Reader Percent Loss Results Per Sample in External Reproducibility of MSH2

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
1	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
2	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
3	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
4	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
5	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
6	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
7	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
10	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
22	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
23	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
24	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
25	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
27	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
31	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
32	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
33	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
34	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
35	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
36	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
37	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
38	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
39	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
40	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
41	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
42	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
43	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
44	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
45	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
46	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
47	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
48	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
49	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
50	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
51	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
52	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
53	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
54	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
55	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
56	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
57	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
58	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
59	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
60	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 28. External Reproducibility of MSH6

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-site	Each of 32 CRC specimens (8 loss, 24 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Inter-site analysis was performed between 3 sites on a total of 286 comparisons to consensus.	LPA 97.2 (91.3, 100.0) IPA 99.1 (97.7, 100.0) OPA 98.6 (96.8, 100.0)
Intra-site	Each of 32 CRC specimens (8 loss, 24 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Intra-site analysis was performed for 3 sites on a total of 286 comparisons to consensus.	LPA 100.0 (94.7, 100.0) IPA 99.1 (97.7, 100.0) OPA 99.3 (98.3, 100.0)
Inter-observer	One set of 60 stained specimens (12 loss, 48 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to consensus.	LPA 98.1 (95.4, 100.0) IPA 100.0 (99.1, 100.0) OPA 99.6 (99.1, 100.0)
Intra-observer	One set of 60 stained specimens (12 loss, 48 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to consensus.	LPA 98.1 (95.4, 100.0) IPA 100.0 (99.1, 100.0) OPA 99.6 (99.1, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 29. Biomarker-level Per Site Percent Loss Results Per Sample in External Reproducibility of MSH6

Case ID	All Sites Percent Loss Results, % (n/N)	Site 1 Percent Loss Results, % (n/N)	Site 2 Percent Loss Results, % (n/N)	Site 3 Percent Loss Results, % (n/N)
1	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
2	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
3	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
4	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
5	11.1% (1/9)	0% (0/3)	0% (0/3)	33.3% (1/3)
6	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Sites Percent Loss Results, % (n/N)	Site 1 Percent Loss Results, % (n/N)	Site 2 Percent Loss Results, % (n/N)	Site 3 Percent Loss Results, % (n/N)
7	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	11.1% (1/9)	0% (0/3)	0% (0/3)	33.3% (1/3)
10	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
20	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
21	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
22	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
23	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
24	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
25	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
27	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
28	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	77.8% (7/9)	100% (3/3)	33.3% (1/3)	100% (3/3)
31	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
32	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 30. Biomarker-level Per Reader Percent Loss Results Per Sample in External Reproducibility of MSH6

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
1	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
2	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
3	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
4	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
5	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
6	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
7	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
10	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
22	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
23	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
24	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
25	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
27	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
31	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
32	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
33	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
34	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
35	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
36	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
37	88.9 (8/9)	100% (3/3)	66.7% (2/3)	100% (3/3)
38	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
39	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
40	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
41	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
42	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
43	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
44	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
45	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
46	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
47	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
48	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
49	88.9% (8/9)	66.7% (2/3)	100% (3/3)	100% (3/3)
50	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
51	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
52	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
53	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
54	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
55	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
56	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
57	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
58	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
59	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
60	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 31. External Reproducibility of PMS2

Reproducibility Study	Study Design	% Agreement (95% CI)
Inter-site	Each of 32 CRC specimens (8 loss, 24 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Inter-site analysis was performed between 3 sites on a total of 286 comparisons to consensus.	LPA 98.6 (95.8, 100.0) IPA 99.5 (98.6, 100.0) OPA 99.3 (98.3, 100.0)
Intra-site	Each of 32 CRC specimens (8 loss, 24 intact) was tested on 5 nonconsecutive days at each of 3 study sites. Intra-site	LPA 98.6 (95.8, 100.0) IPA 99.5 (98.6, 100.0) OPA 99.3 (98.3, 100.0)

Reproducibility Study	Study Design	% Agreement (95% CI)
	analysis was performed for 3 sites on a total of 286 comparisons to consensus.	
Inter-observer	One set of 60 stained specimens (18 loss, 42 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Inter-observer analysis was performed between 3 sites on a total of 540 comparisons to consensus.	LPA 100.0 (97.7, 100.0) IPA 100.0 (99.0, 100.0) OPA 100.0 (99.3, 100.0)
Intra-observer	One set of 60 stained specimens (18 loss, 42 intact) was rotated across 3 sites and evaluated 3 times by the same pathologist at each site. Intra-observer analysis was performed for 3 sites on a total of 540 comparisons to consensus.	LPA 100.0 (97.7, 100.0) IPA 100.0 (99.0, 100.0) OPA 100.0 (99.3, 100.0)

LPA=Percent Agreement of Loss; IPA=Percent Agreement of Intact; OPA=Overall Percent Agreement

Table 32. Biomarker-level Per Site Percent Loss Results Per Sample in External Reproducibility of PMS2

Case ID	All Sites Percent Loss Results, % (n/N)	Site 1 Percent Loss Results, % (n/N)	Site 2 Percent Loss Results, % (n/N)	Site 3 Percent Loss Results, % (n/N)
1	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
2	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
3	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
4	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
5	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
6	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
7	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	88.9% (8/9)	100% (3/3)	100% (3/3)	66.7% (2/3)
10	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Sites Percent Loss Results, % (n/N)	Site 1 Percent Loss Results, % (n/N)	Site 2 Percent Loss Results, % (n/N)	Site 3 Percent Loss Results, % (n/N)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
22	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
23	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
24	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
25	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
26	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
27	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
31	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
32	11.1% (1/9)	0% (0/3)	33.3% (1/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

Table 33. Biomarker-level Per Reader Percent Loss Results Per Sample in External Reproducibility of PMS2

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
1	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
2	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
3	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
4	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
5	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
6	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
7	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
8	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
9	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
10	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
11	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
12	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
13	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
14	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
15	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
16	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
17	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
18	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
19	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
20	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
21	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
22	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
23	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
24	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
25	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
26	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
27	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
28	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
29	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
30	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
31	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
32	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
33	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
34	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
35	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
36	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
37	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
38	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
39	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
40	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
41	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
42	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
43	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
44	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
45	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
46	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
47	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Case ID	All Readers Percent Loss Results, % (n/N)	Reader 1 Percent Loss Results, % (n/N)	Reader 2 Percent Loss Results, % (n/N)	Reader 3 Percent Loss Results, % (n/N)
48	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
49	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
50	100% (9/9)	100% (3/3)	100% (3/3)	100% (3/3)
51	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
52	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
53	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
54	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
55	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
56	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
57	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
58	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
59	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)
60	0% (0/9)	0% (0/3)	0% (0/3)	0% (0/3)

Note: n/N denotes the number of loss replicates/the total number of replicates.

4. Robustness

Robustness testing was conducted to evaluate the staining performance of MMR IHC Panel pharmDx (Dako Omnis) under various laboratory conditions.

For robustness testing, 24 FFPE CRC specimens were tested per biomarker for each robustness sub-study. Colorectal cancer stages I, II, III and IV-4, primary and metastatic tumor, and surgical resection and tissue biopsy specimens were included. Replicate sections of CRC specimens were tested per biomarker according to the settings summarized below. Study slides were scored blinded and randomized for diagnostic status (intact or loss) by a certified observer.

- Target Retrieval Temperature
  - Replicates incubated in TRS at 97 °C (optimal) and 95 °C at the optimal 20 minutes incubation time.
- Target Retrieval Solution pH
  - Replicates incubated in TRS pH of pH 9.1 (optimal), pH 8.8, and pH 9.4.
- Tissue Section Thickness
  - Replicates with tissue section cut at 3 µm, 4 µm (standard), and 5 µm thicknesses.
- Slide Type
  - Replicates with tissue section placed on Agilent FLEX IHC slides (standard) versus Superfrost Plus charged glass slides.

Percent agreement of loss (LPA), percent agreement of intact (IPA), and overall percent agreement (OPA) were calculated for each robustness study using comparisons in diagnostic status between each observation under varied condition and the standard or optimal condition. Corresponding two-sided 95% confidence intervals using the percentile bootstrap method were calculated for each agreement parameter. Confidence intervals were calculated using the Wilson score method when 100% agreement was observed, as the percentile bootstrap method cannot be used in these cases.

Acceptance criteria required that the lower bound of the two-sided 95% CI computed on each percent agreement must meet or exceed 85% for all studies. These criteria were applied to each biomarker separately and to the confidence intervals calculated for LPA, IPA, and OPA for each study.

All robustness results met the acceptance criteria of LPA/IPA/OPA ≥ 85% at the lower bound of the 95% CI.

Overall, the robustness studies demonstrate that use of MMR IHC Panel pharmDx (Dako Omnis) on CRC specimens produces consistent results under the following conditions when scored according to diagnostic status (biomarker intact or loss):

- TRS temperature from 95 °C to 97 °C
- TRS pH from 8.8 to 9.4
- Tissue section thickness from 3 µm to 5 µm
- Slide type for FLEX IHC and Superfrost Plus microscope slides

5. Stability

i. Real-Time Stability

The purpose of the real-time stability study was to establish the shelf life of MMR IHC Panel pharmDx (Dako Omnis).

For each MMR IHC Panel pharmDx (Dako Omnis) product, a minimum of two intact and one loss CRC specimens were included in testing of each biomarker. All three lots were evaluated on the Dako Omnis instrument using one replicate per block per lot. Testing was conducted at timepoints T0 – T8, spanning 26 months total. Test lots were stored at 2−8 °C before and after transport (where applicable) and after each in-use/on-board cycle or timepoint. The final shelf-life at 2−8 °C was assigned based on the second-to-last passing result for all three test lots. All timepoints tested passed for all MMR IHC Panel pharmDx (Dako Omnis) products. Based on these results, the shelf life was calculated for each antibody.

Based on real time results shelf life from date of T0 testing for the MMR IHC Panel pharmDx (Dako Omnis) primary antibodies and NCRs is 22 months at 2-8 °C.

ii. In-Use /On-Board Stability Testing

On-board and in-use stability was tested by placing one test lot in the Dako Omnis reagent drawers for a total 80 hours prior to 6 months stability testing. Additionally, between 14 months and 16 months, the same test lots were placed in the Dako Omnis reagent drawers for enough time to accumulate at least a total of 375 hours on-board. Results are summarized in Table 34.

Table 34. Summary of In-Use/On-Board Stability Testing Results

Reagent	In-Use/On-Board Stability
MLH1	24 cycles from 2−8 °C to 18 °C for at least 2.5 hours and back to 2−8 °C Total hours: 392.5 hours at 18 °C
MSH2	22 cycles from 2−8 °C to 18 °C for at least 2.5 hours and back to 2−8 °C Total hours: 392 hours at 18 °C
MSH6	22 cycles from 2−8 °C to 18 °C for at least 2.5 hours and back to 2−8 °C Total hours: 393.5 hours at 18 °C
PMS2	22 cycles from 2−8 °C to 18 °C for at least 2.5 hours and back to 2−8 °C Total hours: 389.5 hours at 18 °C
NCR Mouse	22 cycles from 2−8 °C to 18 °C for at least 2.5 hours and back to 2−8 °C Total hours: 392 hours at 18 °C
NCR Rabbit	22 cycles from 2−8 °C to 18 °C for at least 2.5 hours and back to 2−8 °C Total hours: 389.5 hours at 18 °C

iii. Transport Simulation

Transport simulation with temperature cycling (-20ºC to 37ºC) was conducted with one lot per product to account for anticipated temperature variations during ambient condition shipping. All MMR IHC Panel pharmDx (Dako Omnis) primary antibodies and NCRs passed transport simulation; however, since the product may be exposed to shipping conditions outside of tested ranges (-20ºC to 37ºC), it is essential to use controls, as specified in this IFU, to confirm expected performance of this product.

iv. Cut Section Stability

The stability of the MMR antigens in CRC cut sections was evaluated for cut sections stored at ambient temperature (approximately 25 °C) and 2−8 °C. Six (6) FFPE CRC specimens were selected to represent at least four diagnostic intact specimens and at least one diagnostic loss specimen for each antibody. For each biomarker, the two temperature arms used overlapping specimen sets with at least 1 specimen differing between the sets. Mouse NCR was tested alongside MLH1, and Rabbit NCR was tested alongside PMS2.

Each FFPE block was sectioned and mounted on glass slides at T0 and held at their designated storage condition (2–8 °C or 25 °C). For each timepoint (T0 – Tend), sections from each block were stained, and slides were evaluated for IHC intensity on a 0–3 scale. The IHC intensity score for each specimen and timepoint was compared to the IHC intensity score for the corresponding T0 (reference) slides. The stability was determined based on the final passing timepoint according to the acceptance criteria (Table 35).

Table 35. Cut Section Stability Dating

Reagent	2−8 °C storage	25 °C storage
MLH1	10 months	10 months
PMS2	10 months	10 months
MSH2	12 months	12 months
MSH6	16 months	16 months
NCR Mouse	10 months	10 months
NCR Rabbit	10 months	10 months

Mouse and Rabbit NCRs were tested only with MLH1 and PMS2, respectively. Therefore, stability for the NCRs was determined based on the final passing timepoint of the associated antibody.

B. Animal Studies

None

C. Additional Studies

1. Tissue Heterogeneity

i. Within-Block

The objective of this study was to assess heterogeneity within a single block (intra-block) in a total of 20 unique FFPE CRC cases stained with MMR IHC Panel pharmDx (Dako Omnis). Concordance of diagnostic status between anterior and posterior cut sections was assessed in two ways. The first used the anterior section as reference for calculating percent agreement of loss (LPA), percent agreement of intact (IPA) and

overall percent agreement (OPA) for the biomarker-level analysis and dMMR percent agreement (dMPA), proficient MMR percent agreement (pMPA) and OPA for the panel-level analysis. The second analysis used the posterior section as reference for agreement calculations. Concordance analysis was performed on overall MMR panel IHC status and for each of the individual MMR biomarker statuses. The intra-block heterogeneity study results showed that some heterogeneity for MMR biomarkers MLH1 and PMS2 may be observed between the anterior and posterior cut sections of the same tumor block (90.0% overall percent agreement). For the other two MMR biomarkers, MSH2 and MSH6, 100% agreement was observed. Overall percent agreement at the MMR panel level was 90.0%.

ii. Within-Case

The objective of this study was to investigate MMR IHC Panel pharmDx (Dako Omnis) staining results on FFPE CRC specimens across different blocks from the same patient case (intra-case). Intra-case heterogeneity was evaluated between 16 unique CRC cases (consisting of 32 total blocks) using MMR IHC Panel pharmDx (Dako Omnis). Concordance analysis was performed on overall MMR panel IHC status and for each of the individual MMR biomarker statuses. The concordance point estimates were all 100%, with 16 of 16 cases demonstrated diagnostic agreement.

iii. Matched Primary versus Metastatic

The objective of this study was to investigate MMR IHC Panel pharmDx (Dako Omnis) staining results on FFPE CRC specimens across different tumor sites of the same patient case (primary vs metastatic). Cut sections from 26 unique paired primary and metastatic FFPE CRC cases (52 blocks) were stained with MMR IHC Panel pharmDx (Dako Omnis). Concordance between primary vs metastatic CRC cases was 100% when evaluating the MMR panel diagnosis. Discordance was observed for MSH2 and MSH6 in one case, where the primary tumor was evaluated as Intact and the metastasized tumor was evaluated as Loss.

2. Pre-Analytical Variables

This study assessed the effect of pre-analytical variables (PAVs) on the MMR antigens when stained with the MMR IHC Panel pharmDx (Dako Omnis). The PAVs tested were:

- Evaluation of various fixation times: 6, 24, and 48 hours in 10% NBF on normal colon or normal tonsil
- Evaluation of various fixation types: 10% unbuffered formalin, Bouin's fixative, acetic formalin, or alcohol and processed into paraffin blocks of normal colon and normal tonsil
- Evaluation of ischemia times 0.5, 1, 4, 24 and 72 hours in normal tonsil

Based on the studies conducted to analyze pre-analytical conditions, an ischemia time of ≤ 1 hour and fixation time of 6 – 48 hours in 10% neutral buffered formalin (NBF) is recommended.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of the MMR IHC Panel pharmDx (Dako Omnis) as a companion diagnostic (CDx) device to aid in the identification of patients with CRC who are likely to benefit from treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) was established in the CHECKMATE-8HW clinical study and the associated clinical bridging study.

A. Clinical Study Design

CHECKMATE-8HW (CA209-8HW, NCT03143153) was a randomized, three-arm, open-label trial in immunotherapy-naive patients with unresectable or metastatic CRC with known tumor MSI-H or dMMR (MSI-H/dMMR) status. Part 1 of the study included patients regardless of prior lines of therapy (i.e., all lines), and Part 2 of the study included patients who had not received prior therapy for metastatic disease (i.e., First Line, or 1L). The three arms consisted of treatment with: nivolumab alone (Arm A), nivolumab in combination with ipilimumab (Arm B), and chemotherapy (Arm C). In this trial, 839 patients with unresectable or metastatic microsatellite instability high or mismatch repair-deficient colorectal cancer by local testing were randomly assigned to receive nivolumab plus ipilimumab (354 patients), nivolumab (353 patients), or chemotherapy (132 patients) across all lines of therapy. Two participant samples from each arm were later determined to be local negatives (both pMMR and MSI-L/MSS).

The clinical bridging study for the MMR IHC Panel pharmDx (Dako Omnis) included retesting the biomarker positive (MSI-H/dMMR) patients enrolled into the CHECKMATE-8HW clinical study and biomarker negative (pMMR) samples that were procured from outside the trial, to evaluate the positive and negative percent agreement between the assays used for local enrollment and the MMR IHC Panel pharmDx (Dako Omnis). Further details of the clinical bridging study are provided in the Effectiveness Results in Section D below.

1. Clinical Inclusion and Exclusion Criteria

Key Inclusion Criteria

Enrollment in the CHECKMATE-8HW study was limited to patients who met the following inclusion criteria:

i. Histologically confirmed recurrent or metastatic CRC:
   a. Part 1: irrespective of prior treatment history with chemotherapy and/or targeted agents not amenable to surgery.

b. Part 2: with no prior treatment history with chemotherapy and/or targeted agents for metastatic disease and not amenable to surgery. Participants treated with adjuvant chemotherapy are eligible if disease progression occurred later than 6 months (≥ 6 months) after completion of chemotherapy.

ii. Known tumor MSI-H or dMMR status per local standard of practice.

iii. All participants must have measurable disease by CT or MRI per RECIST 1.1 criteria.

iv. Adequate tumor tissue available. Tumor tissue specimens, either a FFPE tissue block (preferred) or unstained tumor tissue sections (minimum of 30 positively charged slides) from primary or metastatic site, must be submitted to the central laboratory. Central laboratory must provide IRT with confirmation of receipt of evaluable tumor tissue prior to randomization. Tumor tissue specimen must meet either of the criteria below:

a. Obtained within 3 months of enrollment with no intervening systemic anti-cancer treatment between time of acquisition and randomization AND this must be the same tissue sample as was used for local MMR/MSI testing;
OR

b. If above is not available, archival tissue can be accepted if the same tissue was used for MMR/MSI testing.

Biopsy should be excisional, incisional or core needle. Fine needle aspiration is unacceptable for submission. Biopsies of bone lesions that do not have a soft tissue component or decalcified bone samples are also unacceptable for submission.

Key Exclusion Criteria

Patients were not permitted to enroll in the CHECKMATE-8HW study if they met any of the following exclusion criteria:

i. Prior treatment with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or immune checkpoint pathways, including prior therapy with anti-tumor vaccines or other immuno-stimulatory antitumor agents.

ii. Any serious or uncontrolled medical disorder that, in the opinion of the investigator, may increase the risk associated with study participation or study drug administration, impair the ability of the participant to receive protocol therapy, or interfere with the interpretation of study results.

2. Follow-up Schedule

For the CHECKMATE-8HW clinical study, PFS and OS are critical endpoints. Participants continue to be followed for collection of outcome and/or survival follow-up data until death or the conclusion of the study. All participants must be followed for at least 100 days after last dose of study treatment with the exception of participants in the chemotherapy arm that experience progressive disease on treatment and who enter the Crossover Cohort. These participants are required to

complete at least Follow Up Visit 1 following end of treatment in the chemotherapy arm.

3. Clinical Endpoints

The dual primary endpoints were:

i. Blinded independent central review (BICR)-assessed PFS of All Lines participants with central dMMR/MSI-H mCRC who were randomized to:
   a. the nivolumab plus ipilimumab combination therapy arm, or
   b. the nivolumab monotherapy arm

ii. BICR-assessed PFS of First Line (1L) participants with central dMMR/MSI-H mCRC who had not received prior treatment for metastatic disease and who were randomized to:
   a. the nivolumab plus ipilimumab combination therapy arm, or
   b. the chemotherapy arm

One of the secondary endpoints was the BICR-assessed ORR of All Lines participants with central dMMR/MSI-H mCRC who were randomized to:

a. the nivolumab plus ipilimumab combination therapy arm or
b. the nivolumab monotherapy arm

The primary endpoints for the clinical efficacy of the MMR IHC Panel pharmDx (Dako Omnis) in the clinical bridging study for the intended use were the same as the clinical study endpoints for the therapeutics and also included:

i. PPA with a two-sided 95% confidence interval (CI) between the local CTA and the central CDx
ii. NPA with a two-sided 95% CI between the representative CTA and central CDx

B. Accountability of PMA Cohort

Of the overall 837 randomized CTA+ participants in CHECKMATE-8HW, 7.2% (60/837) had missing assessments by MMR IHC Panel pharmDx (Dako Omnis) due to insufficient tissue availability or invalid test status by MMR IHC Panel pharmDx (Dako Omnis).

Table 36. Accountability of PMA Cohort for CHECKMATE-8HW

Randomized CTA+ Subjects	MMR Status by Local CTA[a]			Total
	dMMR	pMMR	Unknown	
Evaluable CDx	642	11	124	777
Unknown CDx[b]	49	0	11	60
Total	691	11	135	837

[a] MMR status based on local IHC testing (not accounting for MSI status by local PCR and/or NGC testing). Patients were eligible for enrollment if they had dMMR and/or MSI-H results from at least one local IHC, PCR, and/or NGS test per local standard of practice; thus, the MMR status was not determined for all enrolled patients, and some patients with local pMMR test results were enrolled based on local MSI-H status via PCR and/or NGS results.
[b] CDx Unknown includes: Insufficient tissue availability (e.g., tissue exhausted, unacceptable tissue type, stability dating surpassed, sample not received) and invalid test status (e.g., internal positive control failure, negative control reagent failure).

C. Study Population Demographics and Baseline Parameters

The following demographic, baseline disease, and specimen characteristics (Table 37) were summarized for all CTA-positive randomized participants and stratified by evaluable status of MMR IHC Panel pharmDx (Dako Omnis) test results.

Table 37. Patient Demographics, Baseline Disease, and Specimen Characteristics Summary by Valid and Missing Test Result by MMR IHC Panel pharmDx (Dako Omnis)

Parameter	All Lines Randomized Subjects (CTA+)		
	CDx Evaluable N=777	CDx Unknown N=60	Total N=837
Age (Years)			
Mean	60.5	60.6	60.5
Median	63	62.5	63
Min, Max	20, 87	29, 84	20, 87
Q1, Q3	51.0, 71.0	51.0, 72.0	51.0, 71.0
SD	13.7	13.5	13.7
Age, n (%)			
0-49	175 (22.5)	14(23.3)	169 (22.6)
50-64	242 (31.1)	21(35.0)	263 (31.4)
65-79	317 (40.8)	21 (35.0)	338 (40.4)
≥80	43 (5.5)	4 (6.7)	47 (5.6)
Sex, n (%)			
Male	384 (49.4)	31 (51.7)	415 (49.6)
Female	393 (50.6)	29 (48.3)	422 (50.4)
Race, n (%)			
White	673 (86.6)	54 (90.0)	727 (86.9)
Black or African American	12 (1.5)	1 (1.7)	13 (1.6)
Asian	75 (9.7)	3 (5.0)	78 (9.3)
Other	17 (2.2)	2 (3.3)	19 (2.3)

Parameter	All Lines Randomized Subjects (CTA+)		
	CDx Evaluable N=777	CDx Unknown N=60	Total N=837
Ethnicity, n (%)			
Hispanic or Latino	73 (9.4)	4 (6.7)	77 (9.2)
Not Hispanic or Latino	385 (49.5)	35 (58.3)	420 (50.2)
Not Reported	319 (41.1)	21 (35.0)	340 (40.6)
Disease Stage at Initial Diagnosis, n (%)			
Stage 0	0	0	0
Stage I	7 (0.9)	0	7 (0.8)
Stage II	138 (17.8)	8 (13.3)	146 (17.4)
Stage III	296 (38.1)	20 (33.3)	316 (37.8)
Stage IV	334 (43.0)	31 (51.7)	365 (43.6)
Not Reported	2 (0.3)	1 (1.7)	3 (0.4)
Histological Grade, n (%)			
GX	103 (13.3)	21 (35.0)	124 (14.8)
G1	84 (10.8)	7 (11.7)	91 (10.9)
G2	308 (39.6)	19 (31.7)	327 (39.1)
G3	260 (33.5)	11 (18.3)	271 (32.4)
G4	19 (2.4)	1 (1.7)	20 (2.4)
Not Reported	3 (0.4)	1 (1.7)	4 (0.5)
Cell Type, n (%)			
Adenocarcinoma	740 (95.2)	56 (93.3)	796 (95.1)
Other Types	36 (4.6)	4 (6.7)	40 (4.8)
Not Reported	1 (0.1)	0	1 (0.1)
Tumor Location, n (%)			
Rectum/Rectosigmoid Junction	80 (10.3)	11 (18.3)	91 (10.9)
Cecum	96 (12.4)	8 (13.3)	104 (12.4)
Colon ascending/hepatic flexure	334 (43.0)	23 (38.3)	357 (42.7)
Colon descending/splenic flexure	73 (9.4)	5 (8.3)	78 (9.3)
Colon sigmoid	83 (10.7)	11 (18.3)	94 (11.2)
Colon transverse	106 (13.6)	1 (1.7)	107 (12.8)
Unknown	5 (0.6)	1 (1.7)	6 (0.7)
Tumor Sidedness CRF), n (%)			
Left	236 (30.4)	27 (45.0)	263 (31.4)
Right	541 (69.6)	33 (55.0)	574 (68.6)

Parameter	All Lines Randomized Subjects (CTA+)		
	CDx Evaluable N=777	CDx Unknown N=60	Total N=837
Number of Prior Lines of Therapy (CRF), n (%)			
0	443 (57.0)	30 (50.0)	473 (56.5)
1	187 (24.1)	15 (25.0)	202 (24.1)
≥ 2	145 (18.7)	15 (25.0)	160 (19.1)
Not Reported	2 (0.3)	0	2 (0.2)
Anatomic Location by Site of Collection of Specimen, n (%)			
Primary	663 (85.3)	42 (70.0)	705 (84.2)
Colon	468 (60.2)	24 (40.0)	492 (58.8)
Metastatic	107 (13.8)	11 918.3)	118 (14.1)
Cervix	1 (0.1)	0	1 (0.1)
Liver	36 (4.6)	5	41 (4.9)
Lung	7 (0.9)	1 (1.7)	8 (1.0)
Lymph Node	20 (2.6)	1 (1.7)	21 (2.5)
Ovary	6 (0.8)	0	6 (0.7)
Peritoneum	12 (1.5)	1 (1.7)	13 (1.6)
Skin	1 (0.1)	0	1 (0.1)
Soft tissue	0	1 (1.7)	1 (0.1))
Stomach	1 (0.1)	0	1 (0.1)
Unknown/Not Reported	23 (3.0)	2 (3.3)	25 (3.0)
Recurrent	7 (0.9)	1 (1.7)	8 (1.0)
Colon	4 (0.5)	1 (1.7)	5 (0.6)
Unknown/Not Reported	3 (0.4)	0	3 (0.4)
Unknown/Not Reported	0	6 (10.0)	6 (0.7)

The results of this study are deemed to be generalizable to the US population (as per the CDER drug approval for these indications).

Procured Negative Samples

A total of 210 commercially procured samples were used in the NPA estimation for the concordance analysis between the CTAs and MMR IHC Panel pharmDx (Dako Omnis). The 157 single negatives had pMMR or MSS test results from IHC or PCR tests, respectively that were representative of the local CTA tests. The 53 double

negatives were randomly selected from 250 double negatives and had both pMMR and MSS test results from IHC and PCR tests, respectively. Of the 410 negative samples considered for inclusion, patients aged 70-79 years had the greatest representation (30.2%), followed by patients aged 60-69 years. The sex distribution was 43.5% female, 46.9% male, and 9.6% unknown. The race distribution was predominantly white (74.4%) and also included Asian (0.2%), black or African American (4.7%, and unknown (20.6%). The tissue types included colon (79.4%), rectum (13.8%), and cecum (6.9%). The 210 samples selected for the NPA estimation were represented from Stage I – IV of the disease, with Stage IV alone contributing 36.2%.

D. Safety and Effectiveness Results

1. Safety Results

No adverse events were reported for the use of MMR IHC Panel pharmDx (Dako Omnis) in connection with the bridging study used to support this PMA.

Data regarding the safety of OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) are summarized in the drug label(s). Refer to Drugs@FDA for complete safety information for the OPDIVO (nivolumab) and YERVOY (ipilimumab) United States Prescribing Information (USPI) for more information.

2. Effectiveness Results

Concordance between CTA and MMR IHC Panel pharmDx (Dako Omnis)
For the concordance analysis, the PPA was estimated between the CHECKMATE-8HW subjects identified as CTA+ via local IHC, PCR, and/or NGS testing versus the results from central testing the same CTA+ samples with the MMR IHC Panel pharmDx (Dako Omnis). Of the total 837 CTA+ samples, there were 662 dMMR, 115 pMMR, and 60 non-evaluable results by MMR IHC Panel pharmDx (Dako Omnis). The NPA could not be estimated from CHECKMATE-8HW clinical samples since patients with pMMR and/or microsatellite stable (MSS) status by CTA were not enrolled in the trial, and there were no corresponding clinical samples able to be evaluated with MMR IHC Panel pharmDx (Dako Omnis). Therefore, the NPA was assessed using commercially procured samples that were predetermined as pMMR and/or MSS using test methods representative of the CTA. A total of 210 representative CTA- commercially procured samples, either pMMR by IHC and/or MSS by PCR, were tested by the MMR IHC Panel pharmDx (Dako Omnis), with 199 pMMR, 3 dMMR, and 6 non-evaluable results. Table 38 presents the concordance between the CTA and MMR IHC Panel pharmDx (Dako Omnis) test results, and Table 39 presents the PPA and NPA estimates with 95% CI.

Table 38. Concordance between CTA and MMR IHC Panel pharmDx (Dako Omnis)

MMR IHC Panel pharmDx (Dako Omnis)	CTA	
	CTA+[a]	CTA-[b]
dMMR	662	5
pMMR	115	199
Non-Evaluable	60	6
Total	837	210

[a]CTA+ specimens from CHECKMATE-8HW clinical study, as determined by local IHC, PCR, and/or NGC testing
[b]Commercially procured CTA- specimens, pMMR and/or MSS by IHC or PCR, respectively

Table 39. Agreement Rates for CTA and MMR IHC Panel pharmDx (Dako Omnis)

Performance Criteria	Sample Numbers	Point Estimate (% Agreement)	95% CI
PPA[a]	662/777	85.2	82.5 - 87.5
NPA[b]	199/204	97.5	94.4 - 98.9

[a]Analysis used CTA+ CHECKMATE-8HW clinical study specimens
[b]Analysis used commercially procured CTA- specimens
CI, confidence interval by Wilson score method; NPA, negative percent agreement; PPA, positive percent agreement.

The low PPA estimate observed in the bridging concordance study (85.2%, 95% CI 82.5-87.5%) combined with the observed 97.5% NPA (95% CI 94.4 - 98.9%) may be attributed to the improved ability of the MMR IHC Panel pharmDx (Dako Omnis) in identifying a subpopulation that would benefit from treatment, as implied by the better drug efficacy in the CDx+CTA+ populations versus the CDx-CTA+ populations (Tables 40 and 41 below).

Clinical Efficacy of the MMR IHC Panel pharmDx (Dako Omnis) for First Line (1L) Nivolumab in Combination with Ipilimumab

A total of 301 CHECKMATE-8HW subjects with dMMR and/or MSI-H status determined by CTA were randomized to receive nivolumab plus ipilimumab combination therapy (n = 200) or chemotherapy (n = 101). The study included 88 sites in 22 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, Denmark, France, Germany, Greece, Ireland, Italy, Japan, Netherlands, Romania, Spain, Turkey, UK, and US). Most subjects were from US/Canada/European Union (n = 202, 67.1%), 30 (10.0%) were from Asia, and 69 (22.9%) were from the rest of the world.

Nivolumab plus ipilimumab showed a clinically meaningful improvement in PFS per BICR over chemotherapy in the First Line (1L) randomized subjects with dMMR/MSI-H status determined by the CTA (HR 0.32) (Table 40, right panel). The median PFS for the 1L randomized subjects who received nivolumab plus ipilimumab was not reached (95% CI: 34.30 - N/A), while the 1L randomized subjects who received chemotherapy had a median PFS of 6.21 months (95% CI: 4.70 months - 9.00 months).

The specimens from these 1L randomized subjects were re-tested at a central laboratory. The PFS benefit observed in 1L randomized subjects with concordant CTA+ and central dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CDx+) is consistent with that in 1L CTA-positive randomized subjects (HR 0.22) (Table 40, left panel; Figure 1). The median PFS for the 1L CTA+/CDx+ subjects who received nivolumab plus ipilimumab was not reached (95% CI: 38.44, NA), while the 1L CTA+/CDx+ subjects who received chemotherapy had a median PFS of 5.85 months (95% CI: 4.40 months – 7.79 months). However, the median PFS for the 1L CTA+/CDx- subjects who received nivolumab plus ipilimumab was 1.81 months (95% CI: 1.48 months - 5.75 months) (Table 40, central panel; Figure 2), while the 1L CTA+/CDx- subjects who received chemotherapy had a median PFS of 11.53 months (95% CI: 2.00 months – N/A).

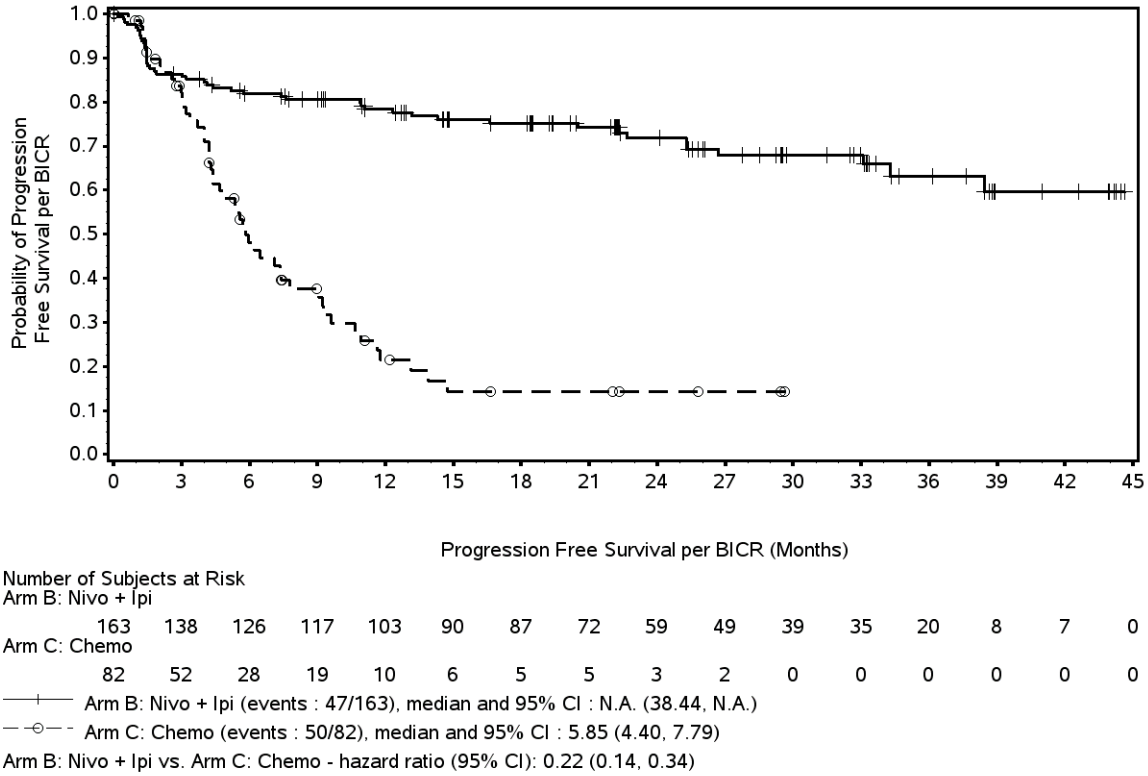
Table 40: PFS per BICR for Nivolumab plus Ipilimumab vs Chemotherapy in 1L Randomized CHECKMATE-8HW Subjects (CTA-positive) with and without Central CDx-positive and CDx-negative Results by MMR IHC Panel pharmDx (Dako Omnis)

	CTA-positive/CDx-positive 1L randomized subjects		**CTA-positive/CDx-negative 1L randomized subjects**		**1L Randomized subjects (CTA-positive)**	
	Nivolumab plus Ipilimumab N=163	**Chemo N=82**	**Nivolumab plus Ipilimumab N=27**	**Chemo N=12**	**Nivolumab plus Ipilimumab N=200**	**Chemo N=101**
PFS Events, n (%)	47 (28.8)	50 (61.0)	20 (74.1)	7 (58.3)	72 (36.0)	62 (61.4)
Median PFS (95% CI), months[a]	NR (38.44, NA)	5.85 (4.40, 7.79)	1.81 (1.48, 5.75)	11.53 (2.00, NA)	NR (34.30, NA)	6.21 (4.70, 9.00)
HR (95% CI)[b]	0.22 (0.14, 0.34)		1.39 (0.57, 3.40)		0.32 (0.22, 0.45)	
p-value[c]	<0.0001		0.4644		<0.0001	

[a]Based on Kaplan-Meier estimates.
[b]HR from a Cox proportional hazard model stratified by tumor sidedness (left vs right) per interactive response system.

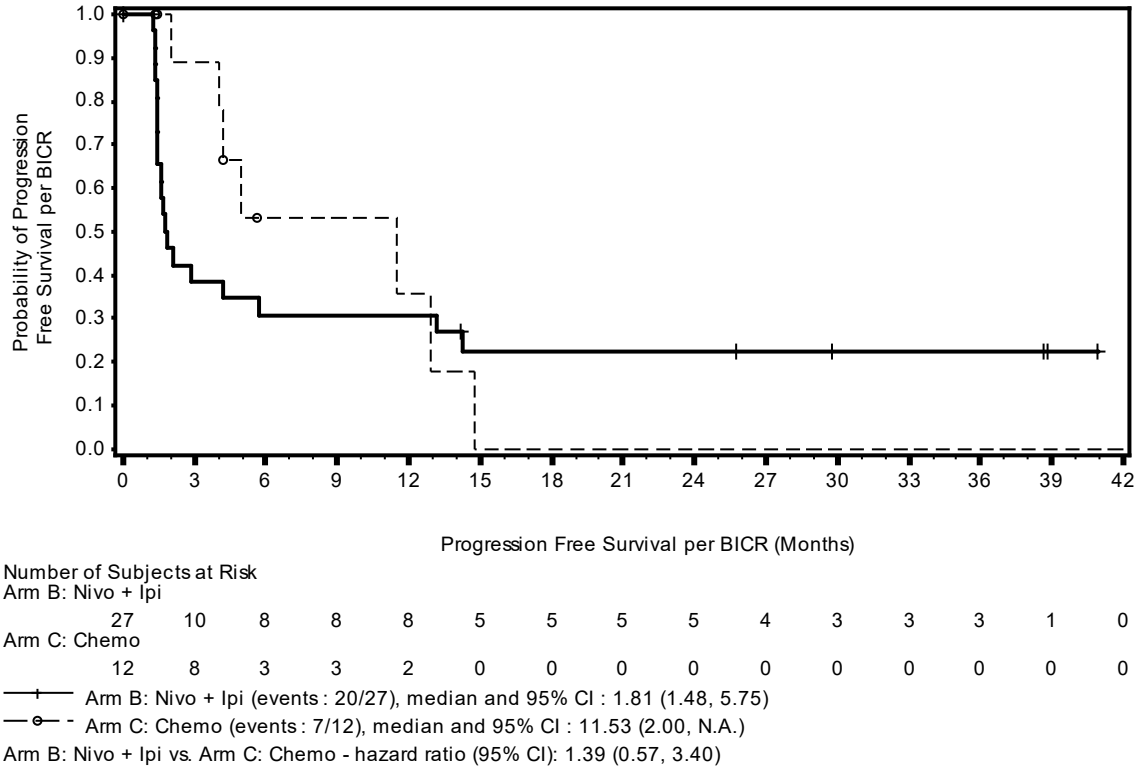
[c]Estimated from two-sided, log-rank test stratified by tumor sidedness (left vs. right) per IRT, and not evaluated for statistical significance.
Note: The clinical efficacy results presented in the drug labeling is based on the results from two different CDx tests (Idylla CDx MSI Test (PCR) and MMR IHC Panel pharmDx (Dako Omnis), but the CDx-positive and CDx-negative populations presented in this table include results from the MMR IHC Panel pharmDx (Dako Omnis) only.
1L, first-line treatment; BICR, blinded independent central review; Chemo, chemotherapy; CI, confidence interval; CTA-positive, subjects with local dMMR/MSI-H status using clinical trial assay modalities; CDx-positive:deficient mismatch repair (dMMR); CDx-negative: proficient mismatch repair (pMMR); HR., hazard ratio; mo, months; PFS, progression-free survival.

Figure 1. Kaplan-Meier curve of PFS per BICR for Nivolumab plus Ipilimumab vs Chemotherapy in 1L CTA+ Randomized CHECKMATE-8HW Subjects with Central dMMR Status by MMR IHC Panel pharmDx (Dako Omnis)



Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date. 1L, first-line treatment; BICR, blinded independent central review; HR, hazard ratio; PFS, progression-free survival.

Figure 2. Kaplan-Meier curve of PFS per BICR for Nivolumab plus Ipilimumab vs Chemotherapy in 1L CTA+ randomized CHECKMATE-8HW subjects with central pMMR status by MMR IHC Panel pharmDx (Dako Omnis)

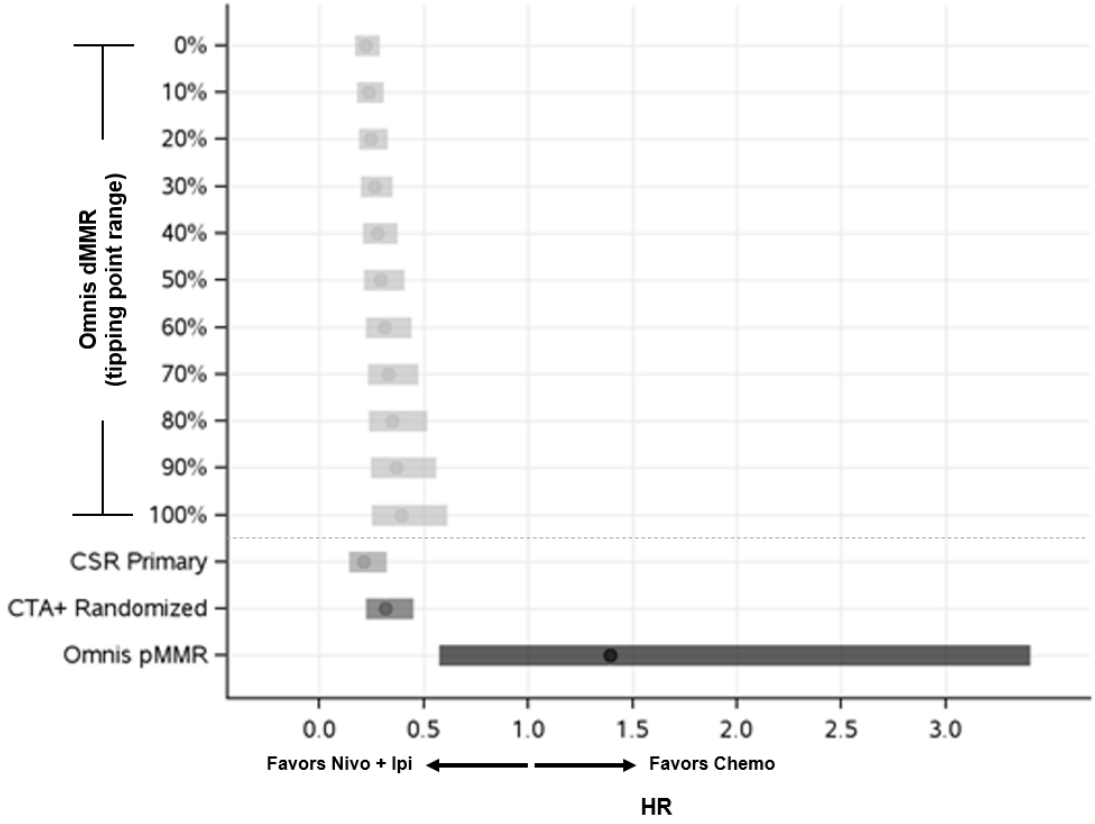


Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date. 1L, first-line treatment; BICR, blinded independent central review; HR, hazard ratio; PFS, progression-free survival.

To account for the patients that were not enrolled in CHECKMATE-8HW due to their local pMMR/MSS status that may have been misclassified by the CTA but may be dMMR by MMR IHC Panel pharmDx (Dako Omnis), a tipping point analysis was conducted (Figure 3). It was assumed the best-case scenario of the missing HR would be equal to the HR in the concordant population with CTA+ and MMR IHC Panel pharmDx (Dako Omnis) dMMR status (i.e., HR = 0.22) and the worst-case scenario would be HR=1. Zero to 100% of the tipping point range was assumed for the missing PFS comparison, with 1000% assuming a worst-case scenario for the PFS of the subjects not enrolled (HR = 1) and 0% assuming that the PFS for the subjects not enrolled is equal to the observed HR for enrolled subjects (i.e., HR = 0.22). The assumed HR values for subjects not enrolled (CTA-negative) were then used with the observed HR values for enrolled (CTA-positive) subjects to estimate the PFS of all 1L subjects. The overall PFS HR estimated for nivolumab plus ipilimumab versus chemotherapy in 1L randomized subjects from the tipping point analysis ranged from 0.22

(95% CI: 0.16 - 0.30) to 0.39 (95% CI: 0.25 - 0.61). The results showed that, across the tipping point range, the clinical efficacy for the intended use population with dMMR status per MMR IHC Panel pharmDx (Dako Omnis) (Figure 3, "Omnis dMMR" population) is similar to the efficacy observed from the CTA+ randomized population (Figure 3, "CTA+ Randomized" population), and different from the patients enrolled per their local CTA+ status but identified as dMMR based on central testing with MMR IHC Panel pharmDx (Dako Omnis) (Figure 3, "Omnis pMMR" population). These results further support the clinical efficacy of the MMR IHC Panel pharmDx (Dako Omnis) to identify patients in the intended use population that may benefit from treatment with nivolumab plus ipilimumab combination therapy.

Figure 3. Forest Plot for Tipping Point Analysis of PFS per BICR of Nivolumab plus Ipilimumab versus Chemotherapy in 1L CHECKMATE-8HW subjects



Populations: **Omnis dMMR**: Subjects with dMMR status determined by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the tipping point analysis ranging from worst case scenario (100%) assuming HR of PFS of the subjects not enrolled (CTA-negative/CDx-positive) as 1 to best case scenario assuming HR of PFS for these subjects equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.22). **CSR Primary**: Subjects randomized in CHECKMATE-8HW (i.e., dMMR/MSI-H by CTA) with central dMMR/MSI-H status. **CTA+ Randomized**: Subjects randomized in CHECKMATE-8HW with local MSI-H/dMMR status. **Omnis pMMR**: Subjects randomized in CHECKMATE-8HW with local MSI-H/dMMR status and central pMMR status. chemo, chemotherapy;

CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab.

Clinical Efficacy of the MMR IHC Panel pharmDx (Dako Omnis) for All Lines for Nivolumab in Combination with Ipilimumab

A total of 705 CHECKMATE-8HW subjects with dMMR and/or MSI-H status determined by CTA were randomized irrespective of prior treatment history (i.e., All Lines) to receive nivolumab plus ipilimumab combination therapy (n = 352) or nivolumab alone (n = 353). Nivolumab plus ipilimumab showed a clinically meaningful improvement in PFS per BICR over nivolumab alone (HR 0.63) (Table 41, right panel). The median PFS for the subjects who received nivolumab plus ipilimumab was 54.08 months (95% CI: 46.62 months - NA), while the subjects who received nivolumab alone had a median PFS of 18.43 months (95% CI: 9.20 months – 28.16 months).

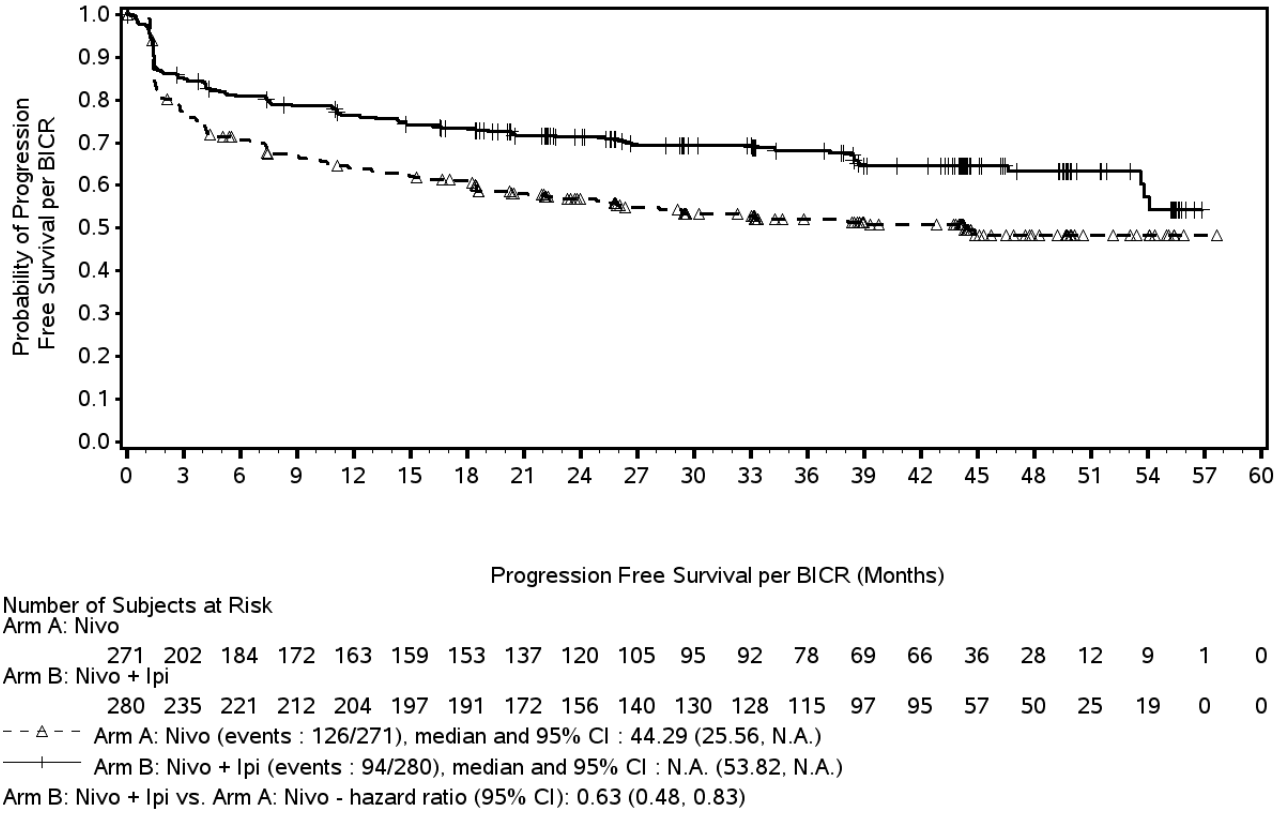
The specimens from these subjects were re-tested at a central laboratory with the MMR IHC Panel pharmDx (Dako Omnis). The PFS benefit observed in the subjects with concordant CTA+ and central dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CDx+) is consistent with that in the CTA-positive randomized subjects (HR 0.63) (Table 41, left and right panels; Figure 4). The median PFS for the CTA+/CDx+ subjects who received nivolumab plus ipilimumab was not reached (95% CI: 353.82, NA), while the CTA+/CDx+ subjects who received nivolumab alone had a median PFS of 44.29 months (95% CI: 25.56 months – NA). However, the median PFS for the CTA+/CDx- subjects who received nivolumab plus ipilimumab was 2.33 months (95% CI: 1.58 months – 4.21 months) (Table 41, central panel; Figure 5), while the CTA+/CDx- subjects who received chemotherapy had a median PFS of 1.58 months (95% CI: 1.41 months – 2.79).

Table 41: PFS per BICR for Nivolumab plus Ipilimumab) and Nivolumab) in All Lines Randomized Subjects (CTA-positive) With and Without Central CDx-positive and CDx-negative Results by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[d]

	CTA-positive/CDx-positive all lines randomized subjects		CTA-positive/CDx-negative all lines randomized subjects		All lines randomized subjects (CTA-positive)	
	Nivolumab plus Ipilimumab N = 280	Nivolumab N = 271	Nivolumab plus Ipilimumab N = 52	Nivolumab N = 50	Nivolumab plus Ipilimumab N = 352	Nivolumab N = 353
PFS Events, n (%)	94 (33.6)	126 (46.5)	43 (82.7)	46 (92.0)	147 (41.8)	196 (55.5)
Median PFS (95% CI), months[a]	NR (53.82, NA)	44.29 (25.56, NA)	2.33 (1.58, 4.21)	1.58 (1.41, 2.79)	54.08 (46.62, NA)	18.43 (9.20, 28.16)
HR (95% CI)[b]	0.63 (0.48, 0.83)		0.57 (0.37, 0.89)		0.63 (0.51, 0.79)	
p-value[c]	0.0007		0.0139		<0.0001	

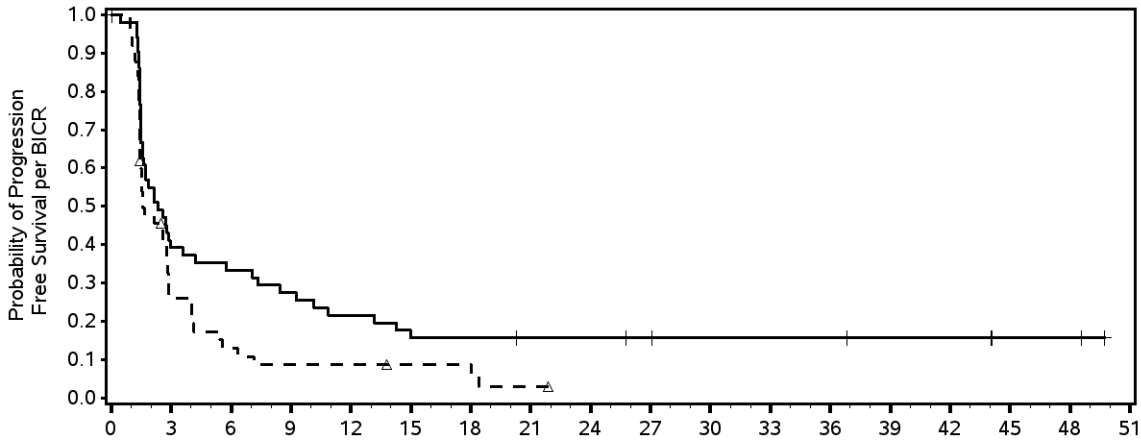
[a]Based on Kaplan-Meier estimates. PFS 95% CI upper-bound values of NA are due to not having a high enough occurrence of events to estimate an upper-bound for PFS for the duration of the clinical trial.
[b]HR from a Cox proportional hazard model stratified by tumor sidedness (left vs right) per interactive response system.
[c]Estimated from two-sided, log-rank test stratified by tumor sidedness (left vs. right) and prior lines of therapy (0, 1, >=2) per IRT, and not evaluated for statistical significance.
[d]The clinical efficacy results presented in the drug labeling are based on the results from at least one of the two different CDx tests (Idylla CDx MSI Test (PCR) and MMR IHC Panel pharmDx (Dako Omnis), but the CDx-positive and CDx-negative populations for the clinical efficacy data presented in the MMR IHC Panel pharmDx (Dako Omnis) labeling consider only the results from this single test.
Note: The clinical efficacy results presented in the drug labeling are based on the results from at least one of the two different CDx tests (Idylla CDx MSI Test (PCR) and MMR IHC Panel pharmDx (Dako Omnis), but the CDx-positive and CDx-negative populations presented in this table consider only the results from the MMR IHC Panel pharmDx (Dako Omnis). Subject number totals for all lines randomized CTA-positive (n=705) and all lines randomized subjects with CDx results (n=653) are not equal due to insufficient tissue availability or invalid test status of some subjects by MMR IHC Panel pharmDx (Dako Omnis).
1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with local dMMR/MSI-H status using clinical trial assay modalities; CDx-positive: deficient mismatch repair (dMMR); CDx-negative: proficient mismatch repair (pMMR); HR., hazard ratio;; NA, not available; NR, not reached; PFS, progression-free survival.

Figure 4. Kaplan-Meier curve of PFS per BICR for Nivolumab plus Ipilimumab vs Nivolumab Alone in ALL Lines CTA+ Randomized CHECKMATE-8HW Subjects with Central dMMR Status by MMR IHC Panel pharmDx (Dako Omnis)



Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) and prior lines of therapy (0, 1, ≥2) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date. BICR, blinded independent central review; HR, hazard ratio; PFS, progression-free survival.

Figure 5. Kaplan-Meier curve of PFS per BICR for Nivolumab plus Ipilimumab vs Nivolumab Alone in ALL Lines CTA+ randomized CHECKMATE-8HW subjects with central pMMR Status by MMR IHC Panel pharmDx (Dako Omnis)

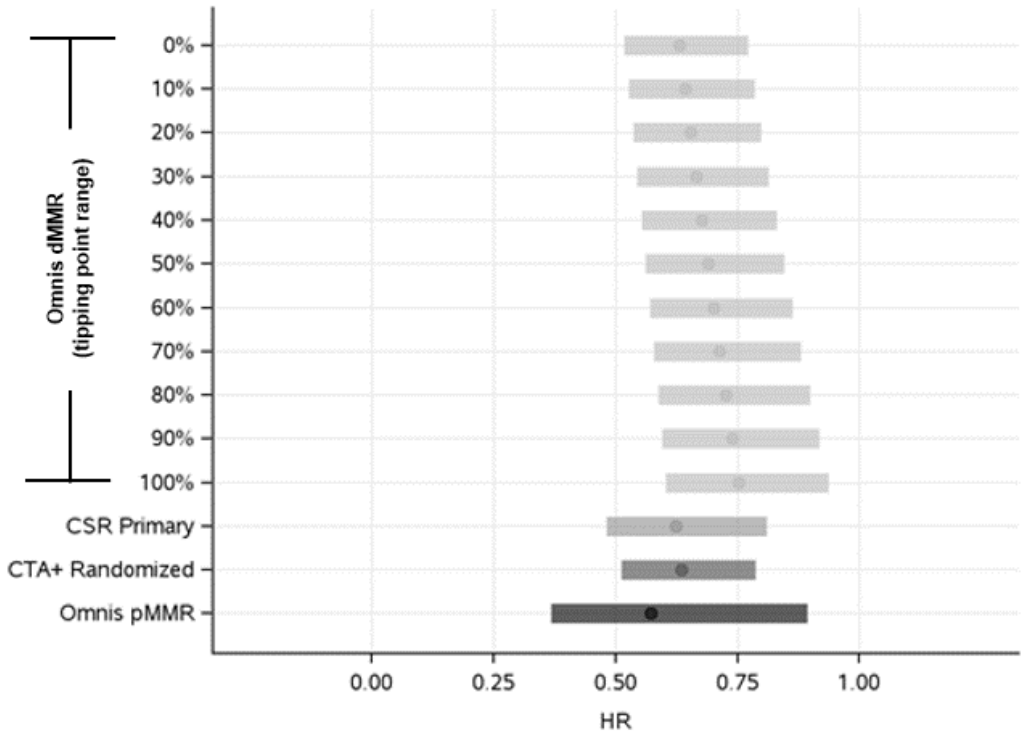


Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) and prior lines of therapy (0, 1, ≥2) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date. BICR, blinded independent central review; HR, hazard ratio; PFS, progression-free survival.

To account for the patients that were not enrolled in CHECKMATE-8HW due to their local pMMR/MSS status that may have been misclassified by the CTA but may be dMMR by MMR IHC Panel pharmDx (Dako Omnis), a tipping point analysis was conducted (Figure 6). It was assumed the best-case scenario of the missing HR would be equal to the HR in the concordant population with CTA+ and MMR IHC Panel pharmDx (Dako Omnis) dMMR status (i.e., HR = 0.63) and the worst-case scenario would be HR=1.

The overall PFS HR estimated for nivolumab plus ipilimumab versus nivolumab alone in All Lines randomized subjects from the tipping point analysis ranged from 0.63 (95% CI: 0.52 – 0.77) to 0.75 (95% CI: 0.60 - 0.94). The results showed that, across the tipping point range, the clinical efficacy for the intended use population with dMMR status per MMR IHC Panel pharmDx (Dako Omnis) (Figure 6, "Omnis dMMR" population) is similar to the efficacy observed from the CTA+ randomized population (Figure 6, "CTA+ Randomized" population).

Figure 6. Forest Plot for Tipping Point Analysis of PFS per BICR of Nivolumab plus Ipilimumab versus Nivolumab in All Lines CHECKMATE-8HW subjects



Populations: **Omnis dMMR**: Subjects with dMMR status determined by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the tipping point analysis ranging from worst case scenario (100%) assuming HR of PFS of the subjects not enrolled (CTA-negative/CDx-positive) as 1 to best case scenario assuming HR of PFS for these subjects equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.63). **CSR Primary**: Subjects randomized in CHECKMATE-8HW (i.e., dMMR/MSI-H by CTA) with central dMMR/MSI-H status. **CTA+ Randomized**: Subjects randomized in CHECKMATE-8HW with local MSI-H/dMMR status. **Omnis pMMR**: Subjects randomized in CHECKMATE-8HW with local MSI-H/dMMR status and central pMMR status. chemo, chemotherapy; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab.

A multiple imputation approach was performed to impute the missing assessments by MMR IHC Panel pharmDx (Dako Omnis) for the enrolled CTA-positive patients based on a set of baseline demographics, disease, and specimen characteristics collected with the study enrollment. A total of 500 different statuses were imputed for each patient with a missing assessment, which resulted in the PPAs ranging from 85.5% (Wilson score 95% CI: 83.0% - 87.8%) to 85.9% (Wilson score 95% CI: 83.4% - 88.1%). The missing assessments by MMR IHC Panel pharmDx (Dako Omnis) for the procured tissue specimens with CTA-negative status were imputed by considering the missing assessments to be concordant with CTA-negative in the probabilities from 0% to 100%, which resulted in the NPAs ranging from 94.8% (Wilson score 95% CI: 90.9% - 97.1%) to 98.1% (Wilson score 95% CI: 95.2% - 99.3%). After the imputations, the clinical performance was re-evaluated with the imputed statuses by MMR IHC

Panel pharmDx (Dako Omnis) and showed consistent results with those estimated from the evaluable assessments.

The Objective Response Rate (ORR) for OPDIVO (nivolumab) plus YERVOY (ipilimumab) in all lines of therapy with dMMR/MSI-H status determined by the CTA was 63.6% (95% CI: 58.4, 68.7) (Table 42, right panel). The ORR for OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA was 49.3% (95% CI: 44.0, 54.6). The ORR observed for both the combination therapy and monotherapy in the all lines of therapy with concordant CTA-positive/CDx-positive population is consistent with that in all lines CTA-positive randomized subjects, with ORR 71.1% (95% CI: 65.4, 76.3) for OPDIVO (nivolumab) plus YERVOY (ipilimumab) and 58.3% (95% CI: 52.2, 64.2) for OPDIVO (nivolumab) monotherapy (Table 42, left panel). No clinically meaningful ORR benefit was observed in all lines subjects with the CTA-positive/CDx-negative status (Table 42, center panel).

Table 42. ORR per BICR for Nivolumab plus Ipilimumab vs Nivolumab in All Lines Randomized Subjects (CTA-positive) With and Without Central CDx-positive and CDx-negative Results by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[c]

	CTA-positive/CDx-positive all lines randomized subjects		**CTA-positive/CDx-negative all lines randomized subjects**		**All lines randomized subjects (CTA-positive)**	
	Nivolumab plus Ipilimumab N = 280	**Nivolumab N = 271**	**Nivolumab plus Ipilimumab N = 52**	**Nivolumab N = 50**	**Nivolumab plus Ipilimumab N = 352**	**Nivolumab N = 353**
Response Rate, n (%) (95% CI)[a]	199 (71.1%) (65.4, 76.3)	158 (58.3%) (52.2, 64.2)	13 (25.0%) (14.0, 38.9)	5 (10.0%) (3.3, 21.8)	224 (63.6%) (58.4, 68.7)	174 (49.3%) (44.0, 54.6))
Complete Response Rate, n (%)	84 (30.0)	78 (28.8)	10 (19.2)	0 (0)	98 (27.8)	82 (23.2)
Partial Response Rate, n (%)	115 (41.1)	80 (29.5)	3 (5.8)	5 (10.0)	126 (35.8)	92 (26.1)
p-value[b]	0.0014		0.0568		0.0001	

[a]ORR (CR+PR), confidence interval based on the Clopper and Pearson method.

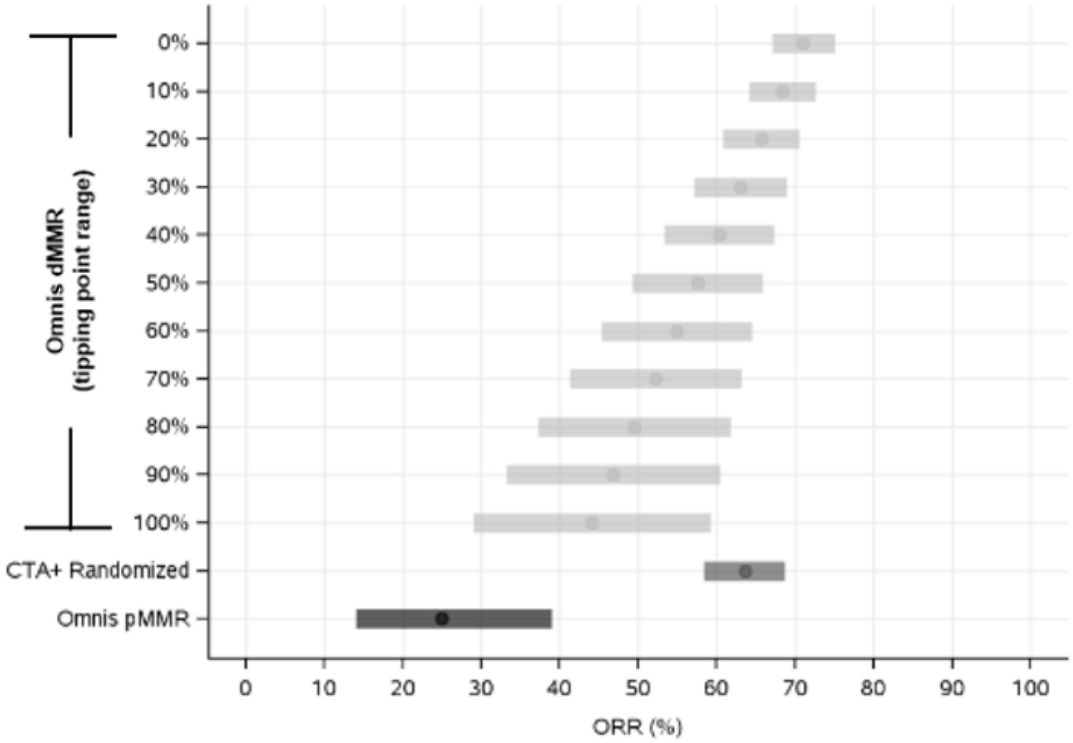
[b]Based on Cochran-Mantel-Haenszel test stratified by the same factors as used in the Cox proportional hazards model and not evaluated for statistical significance.
[c]The clinical efficacy results presented in the drug labeling are based on the results from at least one of the two different CDx tests (Idylla CDx MSI Test (PCR) and MMR IHC Panel pharmDx (Dako Omnis), but the CDx-positive and CDx-negative populations for the clinical efficacy data presented in the MMR IHC Panel pharmDx (Dako Omnis) labeling consider only the results from this single test.
Note: The clinical efficacy results presented in the drug labeling are based on the results from at least one of the two different CDx tests (Idylla CDx MSI Test (PCR) and MMR IHC Panel pharmDx (Dako Omnis), but the CDx-positive and CDx-negative populations presented in this table consider only the results from the MMR IHC Panel pharmDx (Dako Omnis). Subject number totals for all lines randomized CTA-positive (n=705) and all lines randomized subjects with CDx results (n=653) are not equal due to insufficient tissue availability or invalid test status of some subjects by MMR IHC Panel pharmDx (Dako Omnis).
1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with local dMMR/MSI-H status using clinical trial assay modalities; CDx-positive: deficient mismatch repair (dMMR); CDx-negative: proficient mismatch repair (pMMR); ORR, objective response rate.

To account for the patients that were not enrolled due to their local pMMR/MSS status that may have been misclassified by the CTA but may be dMMR by MMR IHC Panel pharmDx (Dako Omnis), a tipping point analysis was conducted. Tipping point analysis results range from the best to the worst scenario, where the best scenario represents objective response rate (ORR) equal to the estimated value from the data of the concordant population with CTA-positive and dMMR status by MMR IHC Panel pharmDx (Dako Omnis), and the worst scenario represents ORR equal to 0 for these patients.

Zero to 100% of the tipping point range was assumed for the missing ORR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of these patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for the ORR of the subjects not enrolled (CTA-negative/CDx-positive, ORR = 0). The tipping point range of 0% assumes a best-case scenario that the ORR for these subjects is equal to the observed ORR for enrolled subjects (CTA-positive/CDx-positive, ORR = 0.711 for nivolumab plus ipilimumab; ORR = 0.583 for nivolumab monotherapy). For nivolumab plus ipilimumab, the tipping point ORR ranged from a minimum of 0.438 (95%% CI, 0.2881, 0.5878) to a maximum of 0.7107 (95%% CI, 0.6714, 0.7501). For nivolumab monotherapy, the tipping point ORR ranged from a minimum of 0.3593 (95%% CI, 0.2330, 0.4856) to a maximum of 0.5830 (95%% CI, 0.5395, 0.6265).

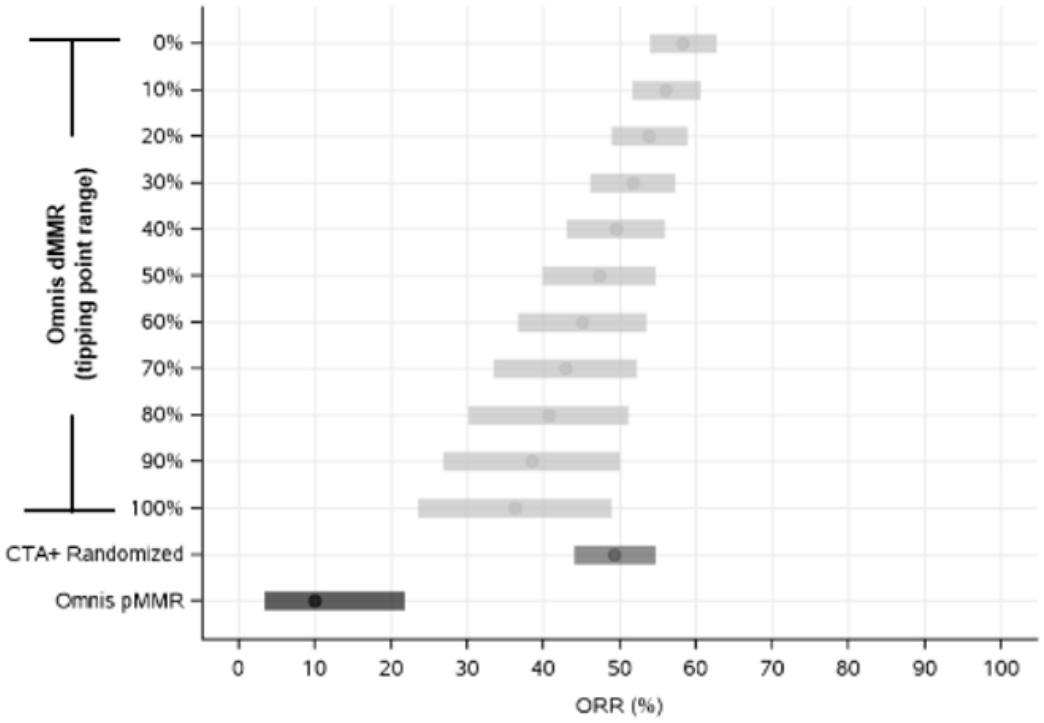
These results are also comparable with the ORR benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) and OPDIVO (nivolumab) alone in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figures 7 and 8).

Figure 7. Forest Plot of ORR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus YERVOY (ipilimumab) in all lines randomized subjects (CHECKMATE-8HW).



Populations: **Omnis dMMR**: Subjects with dMMR status determined by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the tipping point analysis ranging from worst case scenario (100%) assuming ORR of the subjects not enrolled (CTA-negative/CDx-positive) as 0 to best case scenario assuming HR of PFS for these subjects equal to the observed ORR for enrolled subjects (CTA-positive/CDx-positive, ORR = 0.711). **CSR Primary**: Subjects randomized in CHECKMATE-8HW (i.e., dMMR/MSI-H by CTA) with centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 551). **CTA+ Randomized**: Subjects randomized in CHECKMATE-8HW with locally confirmed MSI-H/dMMR status (N = 705). **Omnis pMMR**: Subjects randomized in CHECKMATE-8HW with centrally tested pMMR status and locally confirmed MSI-H/dMMR status (N = 102). chemo, chemotherapy; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab; ORR, overall response rate

Figure 8. Forest Plot of ORR for Omnis dMMR of the intended use OPDIVO (nivolumab) in all lines randomized subjects (CHECKMATE-8HW).



Populations: Omnis dMMR: Subjects with dMMR status determined by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the tipping point analysis ranging from worst case scenario (100%) assuming ORR of the subjects not enrolled (CTA-negative/CDx-positive) as 0 to best case scenario assuming HR of PFS for these subjects equal to the observed ORR for enrolled subjects (CTA-positive/CDx-positive, ORR = 0.583). CSR Primary: Subjects randomized in CHECKMATE-8HW (i.e., dMMR/MSI-H by CTA) with centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 551). CTA+ Randomized: Subjects randomized in CHECKMATE-8HW with locally confirmed MSI-H/dMMR status (N = 705). Omnis pMMR: Subjects randomized in CHECKMATE-8HW with centrally tested pMMR status and locally confirmed MSI-H/dMMR status (N = 102). chemo, chemotherapy; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab; ORR, overall response rate

3. Subgroup Analyses

   There was no subgroup analysis performed.

4. Pediatric Extrapolation

   In this premarket application, existing clinical data from CHECKMATE-8HW was leveraged to support the reasonable assurance of safety and effectiveness of the proposed device MMR IHC Panel pharmDx in the pediatric sub-population of adolescents (>12 years of age through 21 years of age) to identify MMR deficient CRC patients eligible for treatment with potential treatment with OPDIVO (nivolumab) alone and OPDIVO (nivolumab) in combination with YERVOY (ipilimumab). Two pediatric samples (from individuals ages 18 and 21) were enrolled in clinical validation study; both had concordant CTA and CDx results

(i.e., dMMR). Also, data from adult patients in this trial is considered generalizable to the pediatric population aged 12-21 years. Additionally, since there is no change in the specimen type (FFPE) to be used or assay workflow prescribed in the Instructions for Use based on the age of the patient, the validation data can be extrapolated to the pediatric population and can be relied upon to establish safety and effectiveness within the 12-21 years age group. MMR IHC Panel pharmDx (Dako Omnis) is not intended to detect dMMR status in CRC pediatric patients (<12 years of age) for potential treatment with OPDIVO (nivolumab) and OPDIVO (nivolumab) in combination with YERVOY (ipilimumab).

Please see the OPDIVO and YERVOY product labels for specific clinical circumstances guiding MMR IHC testing.

XI. 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 for the device included six 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.

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

In accordance with the provisions of section 515(c)(3) 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.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the MMR IHC Panel pharmDx (Dako Omnis) as a CDx device for identifying dMMR CRC patients who may be eligible for treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) is based on the clinical performance assessed in the CHECKMATE-8HW study. The clinical benefit of the MMR IHC Panel pharmDx (Dako Omnis) was demonstrated for patients enrolled in CHECKMATE-8HW clinical study, in which the MMR status was determined using CTAs. Overall, the observed clinical benefit in the ITT population selected by the CTAs was comparable to the subset of those patients verified as dMMR with the MMR IHC Panel pharmDx (Dako Omnis) (i.e., the BLA efficacy population). Additional sensitivity analyses combined with multiple imputation

approaches for missing CDx values supported the clinical benefit of the MMR IHC Panel pharmDx (Dako Omnis).

The performance of the MMR IHC Panel pharmDx (Dako Omnis) was also supported by the analytical validation studies. The results from analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of the MMR IHC Panel pharmDx (Dako Omnis) when used in accordance with the indications for use in identifying dMMR CRC patients who may benefit from treatment with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) in accordance with approved therapeutic product labeling.

B. Safety Conclusions

The risks of the device are based on data collected in the clinical study conducted to support PMA approval as described above.

The MMR IHC Panel pharmDx (Dako Omnis) is an in vitro diagnostic device, which tests FFPE tumor specimens collected from patients with CRC. The risks of the device are based on data collected in the clinical study, and no adverse events associated with the diagnostic testing procedure were reported during the CHECKMATE-8HW study. The process of testing on FFPE tumor specimens does not present additional significant safety concerns, as the required specimens are obtained using a medically routine sampling procedure that does not present a significant risk to the patient.

As the MMR IHC Panel pharmDx (Dako Omnis) is intended to identify CRC patients for treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab), failure of the device to perform as expected may lead to incorrect or false results and applicable CRC patients may not receive the proper treatment. Patients with false positive results may undergo treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) without much clinical benefit and may experience adverse reactions associated with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) therapy. Patients with false negative results may not be considered for treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab), and therefore, may receive other treatment options that do not offer the same clinical benefit.

C. Benefit-Risk Determination

The probable benefits of the device are based the analytical validation, clinical bridging study, and on the data collected in a clinical study conducted to support PMA approval as described above. For the intended use of identifying CRC patients with dMMR to be treated with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab), the probable

benefit of the MMR IHC Panel pharmDx (Dako Omnis) was demonstrated through a clinical efficacy analysis in the CHECKMATE-8HW trial. In the dMMR CRC, in the advanced or metastatic setting, the population defined by the MMR IHC Panel pharmDx (Dako Omnis), nivolumab + ipilimumab combination therapy demonstrated clinically meaningful benefit over chemotherapy in 1L. In all lines studied, for dMMR CRC as defined by the MMR IHC Panel pharmDx (Dako Omnis), nivolumab plus ipilimumab, or nivolumab alone (in the refractory setting) also had meaningful clinical efficacy.

The probable risks of the device are also based on data collected from the studies conducted to support PMA approval as described above. The risks associated with the use of this device are mainly due to 1) false negatives (false pMMR), false positives (false dMMR), and failure to provide a result and 2) incorrect interpretation of test results. Erroneous device results could adversely influence clinical interpretation and consultation for patients. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in treatment of patients with CRC. Patients with false positive (false dMMR) results may be treated with nivolumab alone or nivolumab in combination with ipilimumab without clinical benefit and may experience adverse reactions associated with the therapy. A false negative (false pMMR) result may prevent a patient from accessing appropriate treatment. There is also a risk of delayed results/failure to provide a result, which may lead to delay of treatment with indicated therapy. These risks are partially mitigated by the analytical and clinical performance of the device and device labeling; therefore, the risk of this device is considered clinically acceptable for the indication listed.

1. Patient Perspective

   This submission either did not include specific information on patient perspectives or the information did not serve as part of the basis of the decision to approve or deny the PMA for this device.

In conclusion, given the available information above, the data support that for CRC patients who are being considered for treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab), the probable benefits 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. The provided studies support use of MMR IHC Panel pharmDx (Dako Omnis) as an aid in identifying MMR deficient CRC patients eligible for treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab).

XIV. CDRH DECISION

CDRH issued an approval order on August 15, 2025.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

None