# MLH1 IHC pharmDx (Dako Omnis)
# Rx Only
# Code GE079

Primary antibody for use with MMR IHC Panel pharmDx (Dako Omnis)
60 tests for use with Dako Omnis

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## 1. Intended 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).

## 2. Summary and Explanation

The MMR pathway is used by normal proliferating cells to repair mutations that may occur during DNA replication. Loss of function of any of the following four MMR proteins, MLH1, PMS2, MSH2, MSH6, results in MMR deficiency (dMMR) and can lead to an increased mutation rate, promotion of tumorigenesis, and generation of neoantigens. dMMR tumors may be more responsive to immunotherapies than tumors with functioning MMR pathways due to the increased presence of neoantigens and immune cell recruitment.[1,2] MLH1 IHC pharmDx (Dako Omnis) is part of MMR IHC Panel pharmDx (Dako Omnis), which is an IHC panel that is used to detect loss of function of any of the four MMR proteins.

Bristol-Myers Squibb sponsored trial, CHECKMATE-8HW (CA2098HW), investigated the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in identifying MMR deficient metastatic CRC patients who may respond to treatment with OPDIVO alone or in combination with YERVOY.[3,4]

*OPDIVO and YERVOY are trademarks owned by Bristol-Myers Squibb Company.*

## 3. Principle of Procedure

MLH1 IHC pharmDx (Dako Omnis) is an optimized antibody reagent with the protocol required to complete an IHC staining procedure of FFPE specimens using the Dako Omnis instrument. Following incubation with the primary monoclonal antibody to MLH1, the specimen is sequentially incubated with peroxidase block, two 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 chromogen results in precipitation of a visible reaction product at the site of antigen. The specimen may then be counterstained and coverslipped. Results are interpreted using a bright field microscope. MMR Negative Control Reagent, Mouse (GE101) slide should be run alongside the MLH1 IHC pharmDx (Dako Omnis) slide. Please consult Dako Omnis User Guide for detailed instructions on loading and unloading of slides, reagents, bulk fluids and waste.

## 4. Materials Provided

The product includes 12 mL of primary antibody to MLH1 protein (approximately 3.4 µg/mL) sufficient for 60 tests. The product has been optimized for use with the Dako Omnis instrument. Please refer to the Dako Omnis User Guide for further information.

| *Quantity* | *Description* |
|---|---|
| 1 x 12 mL | **MLH1 IHC pharmDx (Dako Omnis)** |

**MLH1 IHC pharmDx**
**(Dako Omnis)**

Monoclonal mouse anti-human MLH1, clone ES05, in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.

## 5. Materials Required, but Not Supplied

Dako Omnis (Code GI100)
MSH2 IHC pharmDx (Dako Omnis) (Code GE085)
MSH6 IHC pharmDx (Dako Omnis) (Code GE086)
PMS2 IHC pharmDx (Dako Omnis) (Code GE087)
MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101)
MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102)
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 (see 'Quality Control', Section 11)
pH meter

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

*__NOTE__: *Use Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) for MLH1 IHC pharmDx (Dako Omnis) (Code GE079) testing. Do not use EnVision FLEX Target Retrieval Solution, High pH (50x) from EnVision FLEX, High pH (Dako Omnis), Code GV800 or GV823.*

**__NOTE__: *Not all sources of distilled or de-ionized water may be of sufficient quality for IHC reagent preparation. Agilent recommends reagent-grade distilled or de-ionized water (corresponding to Clinical Laboratory Reagent Water [CLRW] standard as specified by CLSI), or water similar in quality to be used for reagent preparation.*[5]

## 6. Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, $NaN_3$ may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.[6]
4. MLH1 IHC pharmDx (Dako Omnis) contains material of animal origin. As with any product derived from biological sources, proper handling procedures should be used in accordance with local requirements.
5. Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection, and disposed of with proper precautions.[7]
6. Incubation times, temperatures, or methods other than those specified may give erroneous results.
7. Reagents have been optimally diluted. Further dilution may result in loss of visible MLH1 immunoreactivity.
8. Paraffin residue may lead to false negative results.
9. Use of reagent volumes other than recommended may result in loss of visible MLH1 immunoreactivity.
10. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
11. Unused solution should be disposed of in accordance with all local, regional, national and international regulations.
12. Safety Data Sheets are available on www.agilent.com or on request.
13. Lack of adherence to the maintenance schedule for the Dako Omnis instrument may give erroneous results. Refer to Dako Omnis User Guides for additional information and for additional instrument-related precautions.
14. Contact Agilent Pathology Support via www.agilent.com to report any unusual staining.

## 7. Storage

Store MLH1 IHC pharmDx (Dako Omnis) in the original product packaging at 2–8 °C when not in use on Dako Omnis. During storage, the flip top vial cap should be closed.

**Do not use the reagent after the expiration date printed on the reagent vial label.** If the reagents are stored under any conditions other than those specified in the instructions for use, they must be validated by the user. The expiry date on the label is valid for unopened vials as well as opened (in-use) vials when handled according to instructions.

Onboard reagent stability for MLH1 IHC pharmDx (Dako Omnis) has been validated to 375 hours. After staining completion, the reagents should be removed from Dako Omnis and stored in the original product packaging at 2–8 °C with flip top vial caps closed securely on the vials. For onboard stability of all ancillary components including diluted working solutions of Wash Buffer and Target Retrieval Solution, pH 9, refer to respective instructions for use. Onboard time of reagents is tracked by the Dako Omnis software; refer to Dako Omnis User Guide for details.

**NOTE**: There are no obvious visual signs to indicate incorrect product storage or handling of this product during the product's shelf life. Positive and negative controls should be run simultaneously with patient specimens, preferably on the same slide, to monitor product performance during the product's shelf life. If a problem is suspected with the antibody during the shelf life that cannot be explained by incorrect product storage or handling, or other variations in laboratory procedures, contact Agilent Pathology Support. Refer to 'Quality Control', Section 11 and 'Troubleshooting', Section 16 for more information.

## 8. Specimen Preparation

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

### 8.1. Paraffin-embedded tissue

FFPE tissues are suitable for use with the primary antibody to the MLH1 protein. 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 [such as 10% unbuffered formalin, Bouin's fixative, and acetic formalin alcohol (AFA)] have not been validated and may give erroneous results. 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.

### 8.2. 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.

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.

**NOTE**: The tissue specimens must be positioned on the glass within the defined slide staining area. Please consult the Dako Omnis User Guide for dimensions of slide staining area.

## 9. Reagent Preparation

The user should adhere to appropriate personal protective equipment requirements and become familiar with all components prior to use (see 'Precautions', Section 6).

Target Retrieval Solution, pH 9 (50x) (Code GC309) and Wash Buffer (20x) (Code GC807) must be prepared according to their respective instructions for use. Refer to the GC309 and GC807 instructions for use 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.

## 10. Staining Procedure

**Procedural Notes**

The user should read these instructions carefully and become familiar with all the components and the instrumentation prior to use (see 'Precautions', Section 6).

The automated staining procedure for MLH1 IHC pharmDx (Dako Omnis) on Dako Omnis includes deparaffinization of tissue sections, target retrieval, and staining. The slides are unloaded in the Unloading Station. All protocol steps are preprogrammed into the Dako Link Omnis Workstation software. The "MMR MLH1 IHC pDx GE079**"** protocol is used for MLH1 IHC pharmDx (Dako Omnis). If the appropriate MLH1 IHC pharmDx (Dako Omnis) protocol is not on your server, please contact your local Technical Service Representative or Agilent Pathology Support to obtain the protocols. Refer to the Dako Omnis User Guide for further information on how to operate and maintain the instruments.

**NOTE:** The MLH1 reagent and instructions supplied with this product have been designed for optimal performance. Further dilution of the antibody or alteration of staining protocol may give erroneous or discordant results. Differences in tissue processing and technical procedures in the user's laboratory may invalidate the assay results.

**NOTE**: Laboratories located at high elevations should determine the best method of maintaining the required temperature (97 °C) during heat-induced epitope retrieval. Any adjustments required to address elevation concerns must be validated by the user. Refer to the Dako Omnis User Guide for additional information.

**Prestaining procedure**

1. Choose the MMR MLH1 IHC pDx GE079 protocol to be applied for each slide from the Dako Link Omnis Workstation software.
2. Ensure the Dako Link Omnis Workstation software is configured to print slide labels with the protocol name displayed.
3. Print slide labels and attach them to the glass slides.
4. Place the slides in the Slide Rack. A Slide Rack can hold from one to five slides.
5. Ensure that the bulk bottles with fluids are onboard and registered by the Dako Omnis instrument. Bulk bottle fluids:
    a. Clearify™ clearing agent (Code GC810)
    b. Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) **diluted to 1x working concentration with distilled or de-ionized water.**
    c. Wash buffer (Code GC807) **diluted to 1x working concentration with distilled or de-ionized water.**
6. Ensure that all flip top vial caps are open and locked in place before loading all required reagents in the Reagent Storage Module:
    a. MLH1 IHC pharmDx (Dako Omnis) (Code GE079)
    b. EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis) (Code GV800)
    c. EnVision FLEX Visualization Reagent (Dako Omnis) (Code GV800)
    d. EnVision FLEX Substrate Buffer (Dako Omnis) (Code GV800)
    e. EnVision FLEX DAB+ Chromogen (Dako Omnis) (Code GV800)
    f. EnVision FLEX+ Mouse LINKER (Dako Omnis) (Code GV821)
    g. EnVision FLEX+ Rabbit LINKER (Dako Omnis) (Code GV809)
    h. Optional: Hematoxylin (Dako Omnis) (Code GC808) or equivalent
    i. Sulfuric Acid (Code GC203)
7. Load the Slide Rack onto Dako Omnis.
8. Follow the instructions on the Touch Screen and tap "Done" to initiate the staining procedure.
9. Ensure the slide Unloading Station is filled with distilled or de-ionized water to prevent slides from drying.

**NOTE:** When using the overnight staining feature (delayed start) slides must be removed from the Unloading Station the morning the staining has been completed.

**NOTE:** The MLH1 IHC pharmDx (Dako Omnis) protocol on the Dako Omnis instrument can be monitored on the Dako Link Omnis Workstation.

**Dako Omnis Staining Protocol**

When processing slides for staining with the MMR IHC Panel pharmDx (Dako Omnis) assay, the Dako Omnis automated platform executes the protocol for MMR MLH1 IHC pDx GE079 as stated in Table 1. Refer to MMR Negative Control Reagent, Mouse (GE101) IFU for details on the GE101 staining protocol. The MMR MLH1 IHC pDx GE079 protocol has been designed for optimal performance. Any changes to the staining protocol may alter the performance of the device and must be validated by the user. Unless otherwise noted, each step of the staining procedure is executed at the instrument's fixed temperature of 32 ºC. The instrument's fixed temperature is not an editable parameter.

**Table 1. MMR MLH1 IHC pDx GE079 Staining Protocol**

| Protocol Step | Reagent | Setting |
|---|---|---|
| **Dewax** | Clearify Clearing Agent | 25 °C, 10 s incubation top, 1 min incubation bottom, 1 cycle |
| | DI Water | 5 s incubation, 1 cycle |
| **Target retrieval** | TRS, pH 9* | 97 °C*, 30 min incubation* |
| | Cooling fluid DI Water | N/A |
| **Staining** | Wash Buffer | 2:40 min incubation, 2 cycles |
| | Primary antibody (MLH1*) | 25 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Peroxidase-Blocking Reagent | 3 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Mouse LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Rabbit LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX/HRP | 20 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Substrate Working Solution | 5 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| **Counterstaining** | Hematoxylin* | 3 min incubation* |
| | DI Water* | 2 min incubation, 10 cycles |
| | Wash Buffer* | 2 min incubation, 10 cycles |

*Parameter is editable by the end user when creating a copy. Any changes to the staining protocol may alter the performance of the device and must be validated by the user.

**Counterstain**

Slides may be counterstained with Hematoxylin (Dako Omnis) (Code GC808). The "MMR MLH1 IHC pDx GE079" protocol on Dako Omnis includes a counterstaining step that is set as editable for the user. If a counterstain other than the recommended Hematoxylin (Dako Omnis) (Code GC808) is used, the alternative counterstain must be validated by the user. See the Dako Omnis User Guide for further information on editing protocols.

Preprogrammed:

Slides are counterstained for 3 minutes with Hematoxylin (Dako Omnis) (Code GC808). The Hematoxylin incubation time is preprogrammed in the protocol. Slides are ready for mounting when removed from the Unloading Station.

User Defined:

If the selected protocol does not include an automated counterstaining process, it is the responsibility of the user to counterstain the specimen(s) per internally validated procedure prior to mounting.

**Mounting**

After staining onboard Dako Omnis, the sections must be dehydrated, cleared, and mounted using nonaqueous, permanent mounting methods.

**NOTE:** Some fading of stained slides may occur, depending on several factors including, but not limited to, counterstaining, mounting materials and methods, and slide storage conditions. To minimize fading, store stained slides in the dark at room temperature (20–25 °C).

## 11. Quality Control

MLH1 IHC pharmDx (Dako Omnis) has been quality controlled for IHC using the required reagents and staining procedures outlined in 'Reagent Preparation', Section 9 and 'Staining Procedure', Section 10. Deviations from the recommended procedures may lead to significant variability in results. Consult the quality control guidelines of the College of American Pathologists (CAP) Accreditation Program for Immunohistochemistry. See also Agilent's Education Guide: Immunohistochemical Staining Methods and CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[8,9]

### 11.1. 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 must be used, where possible, as internal positive controls instead of a separate positive control tissue. In very rare cases, nonmalignant elements may have loss of biomarker expression, in which case nonmalignant elements of the negative control tissue may be used to qualify the staining procedure.

Refer to Table 4 for further information on positive and negative control tissues.

### 11.2. Negative control reagent

MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101) should be used in place of the 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 negative control reagent (NCR). Refer to the MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101) instructions for use for details.

### 11.3. Assay verification

Prior to initial use of a staining system in a diagnostic procedure, the user should verify the assay's performance by testing it on a series of lab-supplied tissues with known IHC performance characteristics representing known positive and negative tissues. Refer to the quality control procedures outlined in 'Quality Control', Section 11, as well as to the quality control requirements of the CAP Certification Program for Immunohistochemistry and/or CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[9] These quality control procedures should be repeated for each new primary antibody lot. Troubleshooting options for potential problems, their causes and suggested corrective actions are outlined in Table 16.

## 12. Staining Interpretation

A Hematoxylin and Eosin (H&E) stained section is used to determine if a specimen is acceptable for IHC. MMR IHC Panel pharmDx (Dako Omnis) and H&E staining should be performed on serial sections from the same FFPE block of the specimen to confirm:

1. The histologic diagnosis of CRC.
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.

Each specimen should be evaluated using 4x–20x magnification. The specific staining pattern 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.

The entire tissue section should be considered, avoiding edge effects, noninvasive components, necrotic areas, and areas with obvious fixation artifacts. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.

Nonspecific cytoplasmic staining may be present in some tissues. 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, epithelia cells
2. Edge effects
3. Necrotic areas
4. Areas with noninvasive components (normal epithelium, adenoma)
5. Areas with obvious fixation artifacts should not be scored or scored with caution

Protein status of Intact or Loss is determined for MLH1 using the guidelines described in Table 2.

**Table 2. Determination of MLH1 Intact or Loss status.**

| | |
|---|---|
| Intact | Nuclear staining in viable malignant cells must be unequivocal, with at least the same overall staining intensity as in adjacent internal positive controls.<br>If focal staining is present, the tissue is considered intact if:<br>1) continuous in multiple glands/nests<br>and<br>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.<br>If focal staining is present, the tissue is considered loss if:<br>1) continuous in only a single gland/nest,<br>2) discontinuous in multiple glands/nests,<br>or<br>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.

After a protein status of Intact or Loss is assigned to each biomarker (MLH1, PMS2, MSH2, and MSH6) for a given specimen, a diagnostic status of MMR proficient or MMR deficient is given using the definitions in Table 3.

**Table 3. Definitions of MMR proficient and MMR deficient.**

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

Some cases may be more challenging to interpret due to particular staining patterns, morphology, nonspecific staining, and/or tissue or staining artifacts. For additional guidance on MMR staining interpretation and examples of challenging cases, refer to the MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual for details.

### 13. Tissue Evaluation

The following table provides the order of slide evaluation for interpretation of MLH1 IHC pharmDx (Dako Omnis). Per the intended use and MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual, evaluation must be performed in conjunction with other required biomarkers.

**Table 4. Recommended order of tissue evaluation.**

| Tissue | Rationale | Requirements |
|---|---|---|
| 1. Patient tissue stained with H&E | An H&E stain of the patient tissue is evaluated first to assess tissue histology and preservation quality.<br>Note: The H&E may be reviewed again in the context of the patient tissue slides stained with the NCR and primary antibody (Steps 5 and 6) | The H&E and MMR IHC Panel pharmDx (Dako Omnis) stains should be performed on serial sections from the same FFPE block of the specimen.<br>Tissue specimens should be well preserved, confirm the diagnosis of CRC, and include at least 50 viable malignant tumor cells. |
| 2. Positive control stained with primary antibody to the MLH1 protein (GE079) | The positive control tissue stained with primary antibody to the MLH1 protein should be examined next.<br>**Patient CRC tissues** contain positive nonmalignant elements that serve as an internal positive control. This internal positive control eliminates the need for a separate control tissue.<br>In rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In cases that demonstrate complete loss of biomarker expression in nonmalignant elements, internal positive controls within the negative control tissue should be reviewed to qualify the staining procedure.<br>Positive tissue controls should be used for monitoring the performance of processed tissues and test reagents. | Nuclear staining of benign or normal epithelium, lymphocytes, and stromal cells present in the patient tissue must demonstrate moderate to strong staining intensity.<br>If patient tissue demonstrates focal loss of biomarker expression in nonmalignant elements, the remaining areas with nuclear staining in the internal positive control elements may still be used to qualify the staining procedure.<br>If patient tissue demonstrates complete loss of biomarker expression in both malignant and nonmalignant elements, use internal positive control within the negative control tissue.<br>If the internal positive controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid. |
| 3. Negative control tissue stained with primary antibody to the MLH1 protein (GE079)<br>(Lab-supplied) | The negative control tissue stained with primary antibody to the MLH1 protein should be examined next to verify labeling specificity of the target antigen by the primary antibody. | Negative control tissue should be prescreened CRC tissues with loss of MLH1 expression.<br>At least one negative control tissue section should be included in each staining procedure, either as an on-slide control or as a separate negative control slide. On-slide tissue controls are recommended and eliminate the need for a separate control slide.<br>Slides stained with primary antibody to the MLH1 protein should exhibit no nuclear staining or focal weak nuclear staining in malignant tumor cells in the presence of moderate to strong staining in internal positive controls in the tumor area. Internal positive control elements include nuclear staining in normal epithelium, lymphocytes, or stroma.<br>If the negative tissue controls fail to demonstrate appropriate staining, results with the test specimens should be considered invalid. |

| Tissue | Rationale | Requirements |
|---|---|---|
| 4. Optional:<br>Negative control tissue stained with MMR Negative Control Reagent, Mouse (GE101)<br>(Lab-supplied) | NCR may be used to stain the negative control tissue specimen if needed for troubleshooting purposes. | NCR slides must exhibit no or weak staining in malignant tumor cells. |
| 5. Patient tissue stained with MMR Negative Control Reagent, Mouse (GE101) | Examine patient specimens stained with NCR. NCR is used in place of the primary antibody and aids in interpretation of specific staining at the antigen site. | NCR slides must exhibit no or weak staining in malignant tumor cells. If weak staining is present in tumor nuclei, it should be used as a baseline to evaluate the MLH1 slide. Staining at the same intensity or lower that may occur in the MLH1 slide should be disregarded upon interpretation.<br>NCR slides with moderate or strong staining in malignant tumor cells are invalid and the corresponding MLH1 slide is considered nonevaluable. The patient tissue must be retested. |
| 6. Patient tissue stained with primary antibody to the MLH1 protein (GE079) | Examine the patient specimen stained with the primary antibody to the MLH1 protein last to assess MLH1 protein status. | All malignant tumor cells should be evaluated for the MLH1 protein expression and included in the MLH1 protein scoring assessment. Positive staining intensity should be assessed within the context of any nonspecific staining observed on the patients NCR slide. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.<br>In very rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In such cases, the patient tissue may still be evaluated if the staining procedure is qualified using the internal positive controls within the negative control tissue.<br>As with any IHC test, absence of staining means that the antigen was not detected, not necessarily that the antigen was absent in the cells/tissue assayed.<br>For staining interpretation guidelines, refer to 'Staining Interpretation', Section 12. |

## 14. Limitations

### 14.1. General limitations

1. For prescription use only (Rx only).
2. IHC is a multistep process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
3. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any staining or its absence must be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls. It is the responsibility of a qualified pathologist, who is familiar with the antibodies, reagents and methods used, to interpret the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
6. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[10]
7. Reagents may demonstrate unexpected reactions in previously untested tissue types. The possibility of unexpected reactions even in tested tissue types cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Agilent Pathology Support with documented unexpected reactions.
8. False-positive results may be seen due to nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes) and endogenous peroxidase activity (cytochrome C).[8]

9. The reagents and instructions supplied for this assay have been designed for optimal performance. Further dilution of the reagents or alteration of incubation times or temperatures may give erroneous or discordant results.
10. Slides flagged in the slide log on the Dako Omnis Workstation should be investigated by qualified personnel. Refer to the Dako Omnis User Guide for further details.
11. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date. Improper storage and use of reagents may lead to erroneous results.
12. Canceled slides indicate a significant issue occurred during staining and should not be used. The specimen will require restaining. Refer to the Dako Omnis User Guide for further details.
13. This device is not intended to be used to identify patients with Lynch syndrome or to differentiate between sporadic CRC and Lynch syndrome.

### 14.2. Product-specific limitations

1. False-negative results could be caused by degradation of the antigen in the tissues over time. Once mounted on slides, specimens should be stored in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C. Tissue sections should be stained within 2 months of sectioning.
2. Use of MLH1 IHC pharmDx (Dako Omnis) on specimens fixed in fixatives other than NBF has not been validated.
3. Use of MLH1 IHC pharmDx (Dako Omnis) on decalcified tissues has not been validated.
4. Reduced staining was observed with 10% unbuffered formalin, Bouin's fixative, and AFA, so they are not acceptable for use with this assay.
5. This product has undergone a transport simulation study to account for anticipated temperature variations during ambient condition shipping. However, it is possible that this product, when shipped under ambient conditions, may be exposed to shipping conditions outside of tested ranges (-20 ºC to 37 ºC). Therefore, it is essential to use controls, as specified in this IFU, to confirm expected performance of this product.

## 15. Performance Evaluation

### 15.1. Analytical performance evaluation: normal and neoplastic tissues

Table 5 summarizes monoclonal mouse anti-MLH1, clone ES05, immunoreactivity on the recommended panel of normal tissues. Table 6 summarizes monoclonal mouse anti-MLH1, clone ES05, immunoreactivity on a panel of neoplastic tissues. All tissues were FFPE and stained with MLH1 IHC pharmDx (Dako Omnis) according to the instructions in this package insert. Nuclear staining was observed in a majority of tissue types tested. In some cases, cytoplasmic and/or extracellular staining was observed.

**Table 5. Summary of MLH1 IHC pharmDx (Dako Omnis) normal tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|---|---|
| Adrenal (3) | 2/3 | Lung (3) | 3/3 | Salivary gland (3) | 3/3 |
| Bladder (3) | 3/3 | Mesothelial cells (3) | 3/3 | Skin (3) | 3/3 |
| Bone marrow (3) | 2/3* | Muscle, cardiac (3) | 3/3 | Small intestine (3) | 3/3 |
| Breast (3) | 2/3 | Muscle, skeletal (3) | 3/3 | Spleen (3) | 3/3 |
| Cerebellum (3) | 3/3 | Nerve, peripheral (3) | 3/3 | Stomach (3) | 3/3 |
| Cerebrum (3) | 2/3 | Ovary (3) | 3/3 | Testis (3) | 3/3 |
| Cervix (3) | 3/3 | Pancreas (3) | 3/3 | Thymus (3) | 3/3** |
| Colon (3) | 3/3 | Parathyroid (3) | 3/3 | Thyroid (3) | 3/3 |
| Esophagus (3) | 3/3 | Pituitary (3) | 3/3 | Tonsil (3) | 3/3 |
| Kidney (3) | 3/3 | Prostate (3) | 3/3 | Uterus (3) | 3/3 |
| Liver (3) | 2/3 | | | | |

*cytoplasmic staining pattern for at least one case
**cytoplasmic and extracellular staining pattern for at least one case

**Table 6. Summary of MLH1 IHC pharmDx (Dako Omnis) neoplastic tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|
| Bladder carcinoma (2) | 2/2 | Ovarian dysgerminoma (1) | 1/1 |
| Breast carcinoma (5) | 5/5 | Ovarian granulosa cell tumor (1) | 1/1 |
| Cholangiocarcinoma (1) | 1/1 | Pleomorphic rhabdomyosarcoma (1) | 1/1 |
| Colon adenocarcinoma (1) | 1/1 | PNET scrotum (1) | 1/1 |
| Endometrial sarcoma (1) | 1/1 | Prostate adenocarcinoma (1) | 1/1 |
| Ewing's sarcoma (1) | 1/1 | Prostate benign prostatic hyperplasia (1) | 1/1 |
| Gastric adenocarcinoma (2) | 2/2 | Renal cell carcinoma (1) | 1/1 |
| Kidney transitional cell carcinoma (1) | 1/1 | Squamous carcinoma of ear (1) | 1/1 |
| Lung carcinoma (3) | 3/3 | Testicular embryonal carcinoma (1) | 1/1 |
| Lymphoma of cecum (1) | 1/1 | Testicular yolk sac tumor (1) | 1/1 |
| Melanoma (3) | 3/3 | Thymoma (1) | 1/1 |
| Merkel cell tumor (1) | 1/1 | Thyroid carcinoma (1) | 1/1 |
| Ovarian carcinoma (2) | 2/2 | Uterine adenomatoid tumor (1) | 1/1 |

### 15.2. Analytical performance evaluation: CRC

#### 15.2.1. Analytical sensitivity

Analytical sensitivity of MLH1 IHC pharmDx (Dako Omnis) was evaluated across 171 unique specimens of FFPE CRC tissues. The prevalence of loss of MLH1 expression observed was 8.2% (14/171). Assessment of MMR IHC Panel pharmDx (Dako Omnis) staining in these 171 unique specimens demonstrated a dMMR prevalence of 8.8% (15/171).

#### 15.2.2. Precision

The precision of MLH1 IHC pharmDx (Dako Omnis) was evaluated. Diagnostic status was recorded as 'Intact' or 'Loss'. The inter-observer analysis was conducted to evaluate the scoring precision of MLH1 IHC pharmDx (Dako Omnis) across multiple observers at a single site. Percent agreement of loss (LPA), percent agreement of intact (IPA), and overall percent agreement (OA) 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 100.0%.

**Table 7. Precision of MLH1 IHC pharmDx (Dako Omnis) tested at one site.**

| 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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (92.6, 100.0)<br>(92.6, 100.0)<br>(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<br>IPA<br>OA | 97.9<br>100.0<br>98.9 | (93.8, 100.0)<br>(92.4, 100.0)<br>(96.8, 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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (94.9, 100.0)<br>(94.9, 100.0)<br>(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<br>IPA<br>OA | 98.3<br>100.0<br>99.2 | (95.0, 100.0)<br>(94.0, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (94.4, 100.0)<br>(95.3, 100.0)<br>(97.4, 100.0) |
| Inter-Observer | 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<br>IPA<br>OA | 94.0<br>97.7<br>95.9 | (89.3, 98.8)<br>(94.2, 100.0)<br>(93.0, 98.3) |

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

Additionally, the precision of MMR IHC Panel pharmDx (Dako Omnis) scoring across multiple observers at a single site was evaluated. Diagnostic status was recorded as 'pMMR' or 'dMMR'. dMMR percent agreement (dMPA), proficient MMR percent agreement (pMPA) and OA were computed with corresponding two-sided 95% percentile bootstrap CIs.

**Table 8. Inter-Observer precision of MMR IHC Panel pharmDx (Dako Omnis) tested at one site.**

| Precision Study | Study Design | % Agreement (95% CI) | | |
|---|---|---|---|---|
| Inter-Observer | One set of 58 CRC 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<br>pMPA<br>OA | 95.7<br>98.8<br>97.1 | (91.3, 98.9)<br>(96.2, 100.0)<br>(94.7, 99.4) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

#### 15.2.3. External reproducibility

The reproducibility of MLH1 IHC pharmDx (Dako Omnis) was evaluated at three external testing sites. Diagnostic status was recorded as 'Intact' or 'Loss'. LPA, IPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 9. Reproducibility of MLH1 IHC pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>IPA<br>OA | 98.6<br>99.5<br>99.3 | (95.8, 100.0)<br>(98.6, 100.0)<br>(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<br>IPA<br>OA | 98.6<br>99.5<br>99.3 | (95.8, 100.0)<br>(98.6, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (97.7, 100.0)<br>(99.0, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (97.7, 100.0)<br>(99.0, 100.0)<br>(99.3, 100.0) |

LPA = Percent Agreement of Loss; PPA = Percent Agreement of Intact; OA = Overall Percent Agreement

In the same study, the reproducibility of MMR IHC pharmDx (Dako Omnis) was analyzed. MMR diagnostic status was recorded as 'Proficient' (pMMR) or 'Deficient' (dMMR). dMPA, pMPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 10. Reproducibility of MMR IHC Panel pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>pMPA<br>OA | 98.6<br>99.3<br>99.0 | (95.7, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 100.0<br>99.3<br>99.7 | (97.3, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |
| 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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

### 15.3. Clinical performance evaluation: colorectal cancer (OPDIVO [nivolumab] alone and OPDIVO [nivolumab] in combination with YERVOY [ipilimumab])

CHECKMATE-8HW was a phase 3, randomized, open-label, multi-center, three-arm clinical trial of nivolumab monotherapy, nivolumab plus ipilimumab combination therapy, or standard chemotherapy in recurrent or metastatic dMMR/microsatellite instability high (MSI-H) CRC across lines of therapy. CHECKMATE-8HW had dual primary objectives: comparing clinical efficacy as evaluated through progression-free survival (PFS) per blinded independent central review (BICR) of nivolumab plus ipilimumab vs chemotherapy in first-line treatment (1L) and comparing clinical efficacy as evaluated through PFS per BICR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of therapy.

#### 15.3.1. Clinical Study Overview

CHECKMATE-8HW was a randomized, 3-arm, open-label trial in immunotherapy-naive patients across all lines of therapy with unresectable or metastatic CRC with known tumor MSI-H or dMMR (MSI-H/dMMR) status as determined in accordance with local standard of practice.

Eligible patients were ≥ 18 years of age, with recurrent or metastatic dMMR or MSI-H CRC not amenable to surgery. Enrollment was based on confirmation of dMMR/MSI-H status by local standard of practice, referred to here as the Clinical Trial Assay (CTA). Modalities for the CTA included: IHC, polymerase chain reaction (PCR), or next generation sequencing (NGS). Patients were considered CTA-positive and eligible for enrollment if they were identified as dMMR and/or MSI-H by at least one of the CTA modalities. Patients were randomized to OPDIVO (nivolumab) monotherapy, OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy, or investigator's choice chemotherapy in a 2:2:1 ratio. Patients who progressed after 2 prior lines of therapy were randomized in a 1:1 ratio to OPDIVO (nivolumab) monotherapy or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. Randomization was stratified by tumor location (right vs left) and by prior lines of therapy (0, 1, 2L+).

The clinical efficacy of the OPDIVO (nivolumab) and OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination was evaluated in the randomized patient population with centrally confirmed MSI-H/dMMR status. Central assessment of MSI-H status using PCR (Idylla MSI) test and dMMR status using MMR IHC Panel pharmDx (Dako Omnis) was conducted retrospectively on patient tumor specimens used for local MSI-H/dMMR status determination. Patients with confirmed MSI-H/dMMR status by either central test comprised the primary drug efficacy population.

The evaluation of the drug efficacy relied on the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) versus chemotherapy in the first-line (1L) setting and the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines setting. The major efficacy outcome measure was BICR-assessed PFS per RECIST 1.1.

#### 15.3.2. IVD Bridging Study

Specimens from CHECKMATE-8HW were analyzed in an IVD bridging study to establish the clinical performance of the companion diagnostic MMR IHC Panel pharmDx (Dako Omnis) for detection of dMMR in patients with unresectable or metastatic CRC (mCRC) who would benefit from OPDIVO (nivolumab) alone or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. The IVD bridging study was designed to bridge the clinical efficacy from the CTA-positive population (dMMR and/or MSI-H by at least one of the local modalities, including IHC, PCR, and/or NGS) to the intended use population of dMMR by MMR IHC Panel pharmDx (Dako Omnis), which will be demonstrated by the clinical utility analysis through concordance (positive percent agreement [PPA] and negative percent agreement [NPA]) of MMR IHC Panel pharmDx (Dako Omnis) against CTA.

The endpoints to demonstrate the clinical utility of the companion diagnostic are:

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects with mCRC.

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab)

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines randomized subjects with mCRC.

Analyses were performed to demonstrate comparable efficacy based on dMMR by MMR IHC Panel pharmDx (Dako Omnis). These analyses support the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population.

#### 15.3.3. Concordance Analysis

For the concordance analysis, PPA between CTA-positive+ and dMMR by MMR IHC Panel pharmDx (Dako Omnis) was estimated using CHECKMATE-8HW clinical samples. 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, NPA was assessed using commercially procured samples that were predetermined as pMMR and/or MSS using test methods representative of the CTA.

The PPA and the two-sided 95% confidence interval (CI) were calculated between CTA-positive and dMMR by MMR IHC Panel pharmDx (Dako Omnis) using clinical specimens and CTA-positive status as the reference. The NPA and the two-sided 95% CI were calculated between procured samples with pMMR/MSS status (CTA-negative) and pMMR by MMR IHC Panel pharmDx (Dako Omnis) with CTA-negative as the reference. There are no formal acceptance criteria for PPA and NPA. The success criteria for the IVD bridging study depends on the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) dMMR for its intended use.

The clinical efficacy of OPDIVO (nivolumab) plus YERVOY (ipilimumab) from CHECKMATE-8HW was based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the randomized patients as well as the PFS comparisons of OPDIVO (nivolumab) plus ipilimumab vs OPDIVO (nivolumab) alone in all lines of the randomized patients. Each PFS comparison was estimated by hazard ratio (HR) and the 95% CI in a stratified Cox proportional hazards model using the randomized arm as a single covariate; line of therapy (for all lines comparison) and tumor sidedness as the stratification factors. PFS curves were estimated and presented using Kaplan-Meier product-limit methodology from the patients with the concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1). Median PFS with two-sided 95% CI using the Brookmeyer and Crowley method (with log-log transformation) was computed. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 2.

Additionally, to assess the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population to identify dMMR patients with mCRC treated with OPDIVO (nivolumab) plus YERVOY (ipilimumab), a tipping point analysis was conducted to consider the missing patients who were not enrolled due to their local pMMR/MSS status, which were potentially misclassified by the CTA and may be dMMR by MMR IHC Panel pharmDx (Dako Omnis). Tipping point analysis was conducted by assuming that the PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of these patients ranged from the best scenario (i.e., HR equal to that estimable from the enrolled patients with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis)), to the worst scenario (i.e. HR equal to 1). A full range of the tipping point analysis results were assessed for the clinical utility of dMMR status by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population. The tipping point analysis is shown graphically in Figure 3.

The clinical utility for MMR IHC Panel pharmDx (Dako Omnis) to identify dMMR patients in the intended use population for treatment with OPDIVO (nivolumab) plus YERVOY (ipilimumab) was also based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines of the randomized patients and of the centrally confirmed dMMR patients by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4) per the same methods outlined above. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 5. Tipping point analysis was also conducted for this clinical endpoint per the same methods outlined above. The tipping point analysis is shown graphically in Figure 6.

A total of 839 subjects were randomized in CHECKMATE-8HW, and of those, 837 were CTA-positive. Of the 837 CTA-positive randomized subjects, 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). To avoid introducing bias to the concordance and the clinical performance evaluation, imputations and sensitivity analyses were conducted after these analyses were done with the available data and are reflected in the clinical performance results presented below.

#### 15.3.3.1. Concordance results

Concordance between the CTA and MMR IHC Panel pharmDx (Dako Omnis) was assessed via PPA using CHECKMATE-8HW clinical samples and NPA using commercially-procured samples (Tables 11 and 12). The conservative estimate for MMR IHC Panel pharmDx (Dako Omnis) positivity rate is 79.1% (662/837), assuming all 60 excluded samples would have resulted in a negative status by MMR IHC Panel pharmDx (Dako Omnis). Point estimates for positive percent agreement (PPA) and negative percent agreement (NPA) were 85.2% and 97.5%, respectively. The level of agreement achieved between the CTA and MMR IHC Panel pharmDx (Dako Omnis) is shown in Table 12.

**Table 11. Specimen distribution of comparison between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| | | CTA | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| MMR IHC Panel PharmDx (Dako Omnis) | Positive | 662 | 5 | 667 |
| | Negative | 115 | 199 | 314 |
| Total | | 777 | 204 | 981 |

**Table 12. Analytical concordance between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| Performance Criteria | Point Estimate of Percent Agreement (95% CI, Wilson Score) |
|---|---|
| PPA | 85.2 (82.5, 87.5) |
| NPA | 97.5 (94.4, 98.9) |

CI, confidence interval by Wilson score method; NPA, negative percent agreement; PPA, positive percent agreement.

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 utility analysis 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.

### 15.3.4. Clinical Efficacy Results

#### 15.3.4.1. OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects

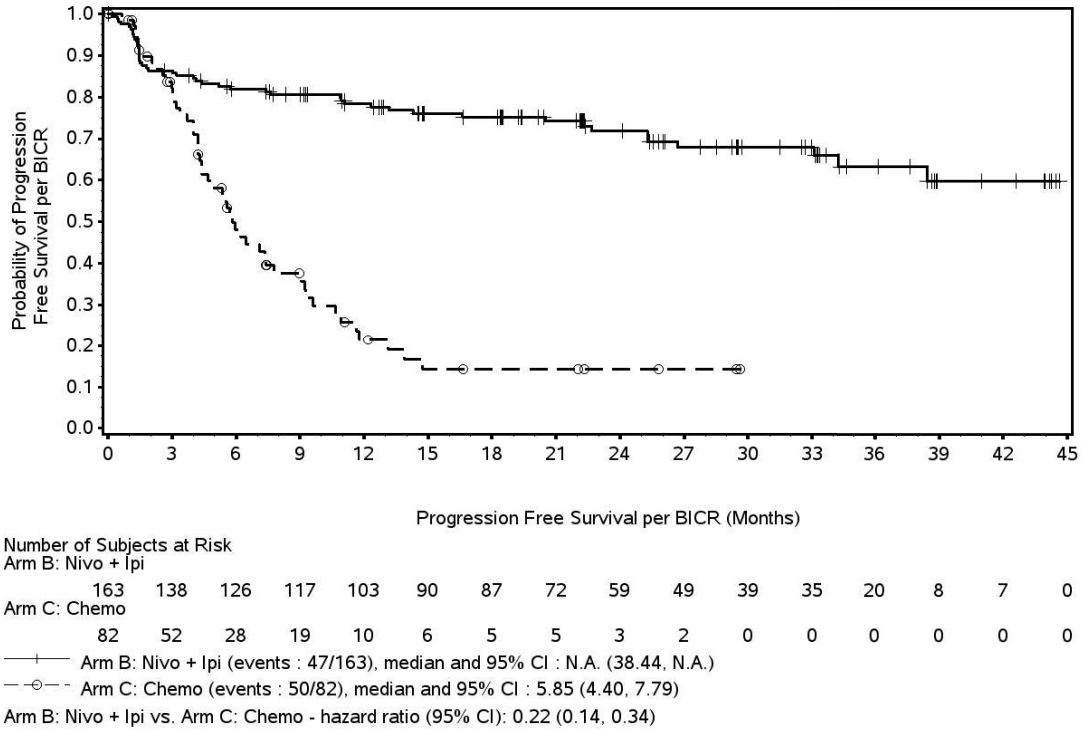
A total of 301 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 200) or chemotherapy (n = 101). The study included 88 sites in 22 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, 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. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the chemotherapy group was 65 years. Most subjects were white (n=259, 86%), 32 (10.6%) were Asian, 4 (1.3%) were black. The ethnicity of subjects was 32 (10.6%) Hispanic,150 (49.8%) non-Hispanic, and 119 (39.5%) not reported. The number of male and female subjects was 139 (46.2%) and 162 (53.8%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR (median PFS not reached) over chemotherapy (PFS 6.21 months) in 1L randomized subjects with dMMR/MSI-H status determined by the CTA (HR 0.32) (Table 12). The PFS benefit observed in 1L randomized subjects with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CDx-positive) is consistent with that in 1L CTA-positive randomized subjects (HR 0.22) (Table 12, Figure 1). A similar improvement in PFS is not observed in the CTA-positive/CDx-negative population, which showed PFS worsening when comparing OPDIVO (nivolumab) plus YERVOY (ipilimumab) (median PFS 1.81 months) with chemotherapy (median PFS 11.53 months).

**Table 13: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

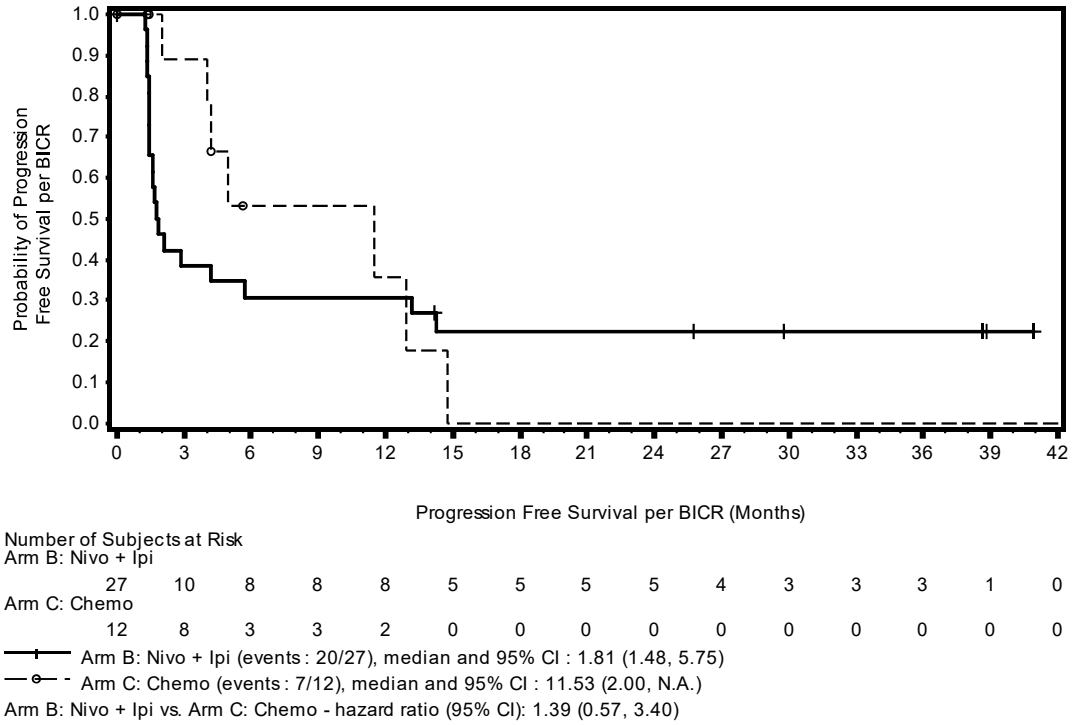
| | CTA-positive/CDx-positive 1L randomized subjects | | CTA-positive/CDx-negative 1L randomized subjects | | 1L Randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N=163 | Chemotherapy N=82 | Nivolumab plus ipilimumab N=27 | Chemotherapy N=12 | Nivolumab plus ipilimumab N=200 | Chemotherapy 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), mo[a] | NR (38.44, NR) | 5.85 (4.40, 7.79) | 1.81 (1.48, 5.75) | 11.53 (2.00, NR) | NR (34.30, NR) | 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. PFS 95% CI upper-bound values of NR 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) 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NR, not reached; PFS, progression-free survival. Subject number totals for 1L randomized CTA-positive (n=301) and 1L randomized subjects with CDx results (n=284) are not equal due to insufficient tissue availability or invalid test status of some subjects 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 1. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**



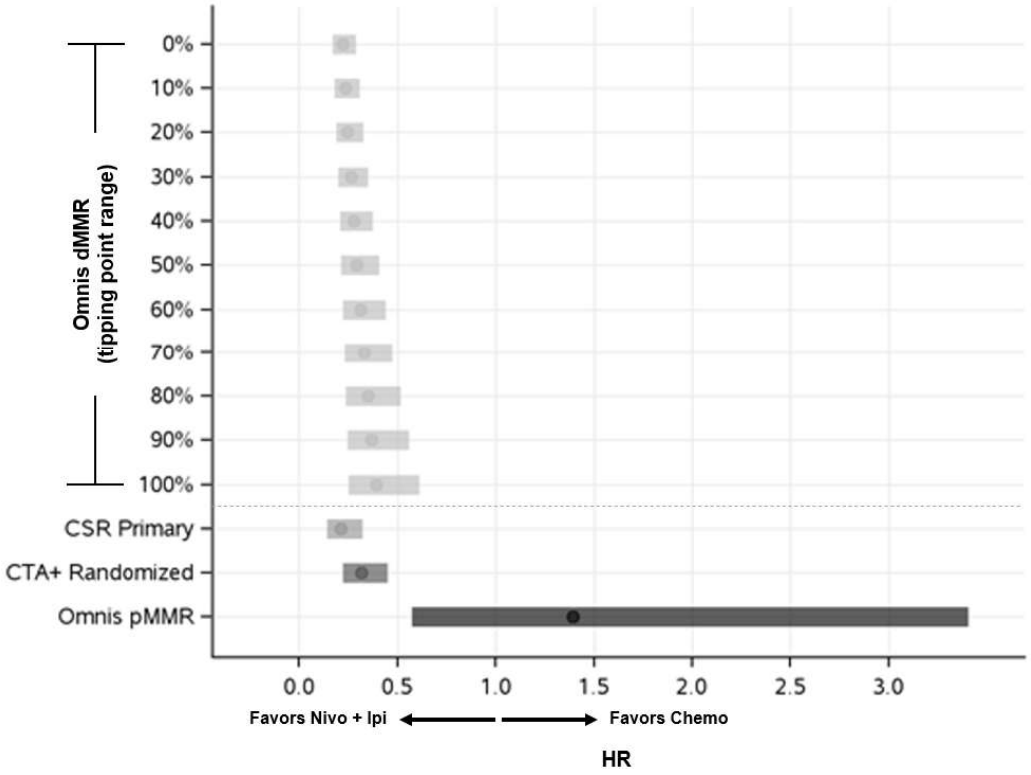

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date.
1L, first-line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

**Figure 2. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2023Oct12 when only the 1L subjects in OPDIVO (nivolumab) plus YERVOY (ipilimumab) and chemotherapy arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for HR of the PFS in these subjects (CTA-negative/CDx-positive, HR = 1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.22). The tipping point PFS HR CI values ranged from a minimum of 0.16 (0% tipping point range CI lower-bound) to a maximum of 0.61 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.16-0.30, and the 100% tipping point 95% CI range at 0.25-0.61.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in the 1L CTA-positive randomized patients and in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 255). **CTA+ Randomized**: Subjects randomized in CHECKMATE-8HW with locally confirmed MSI-H/dMMR status (N = 301). **Omnis pMMR**: Subjects randomized in CHECKMATE-8HW with centrally tested pMMR status and locally confirmed MSI-H/dMMR status (N = 39). chemo, chemotherapy; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab;.

**Figure 3. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus ipilimumab) vs chemotherapy in 1L subjects (CHECKMATE-8HW)**

#### 15.3.4.2. OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects

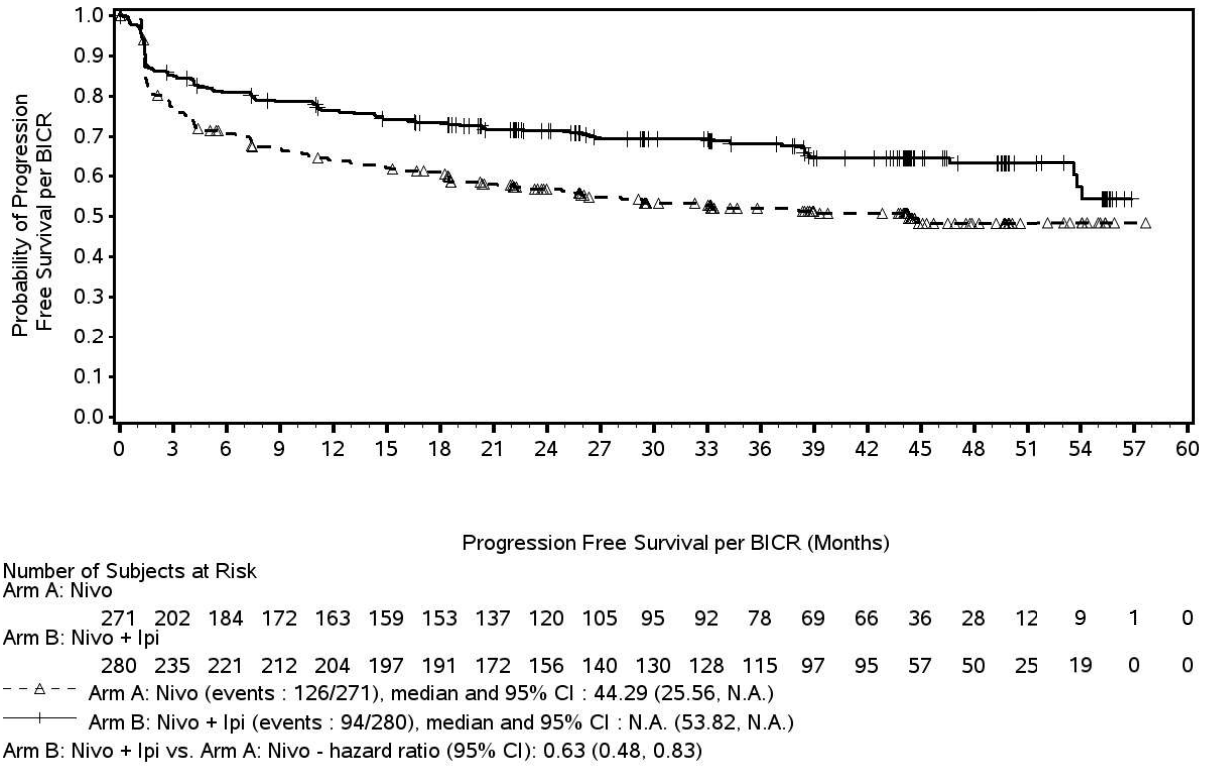
A total of 705 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 352) or OPDIVO (nivolumab) (n= 353). The study included 88 sites in 23 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, Denmark, France, Germany, Greece, Ireland, Italy, Japan, Netherlands, Norway, Romania, Spain, Turkey, UK, and US). Most subjects were from US/Canada/European Union (n = 495, 70.2%), 59 (8.4%) were from Asia, and 151 (21.4%) were from the rest of the world. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the OPDIVO (nivolumab) group was 63 years. Most subjects were white (n=614, 87.1%), 63 (8.9%) were Asian, 11 (1.6%) were black. The ethnicity of subjects was 66 (9.4%) Hispanic, 353 (50.0%) non-Hispanic, and 286 (40.6%) not reported. The number of male and female subjects was 351 (49.8%) and 354 (50.2%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA (HR 0.63). PFS benefit observed in all lines of therapy with concordant CTA-positive/CDx-positive population is consistent with that in all lines CTA-positive randomized subjects (HR 0.63) (Table 14, Figure 4). No clinically meaningful PFS benefit was observed in all lines subjects with the CTA-positive/CDx-negative status, shown in median PFS < 2.5 months in both OPDIVO (nivolumab) plus YERVOY (ipilimumab) and OPDIVO (nivolumab) monotherapy.

**Table 14: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[d]**

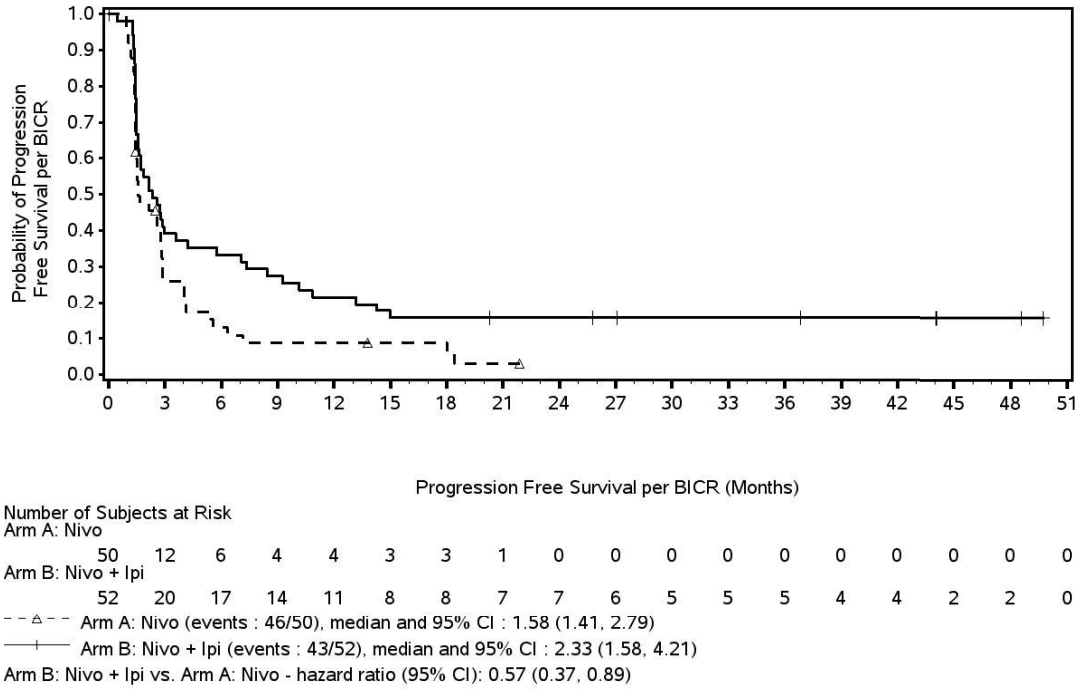
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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), mo[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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NA, not available; NR, not reached; PFS, progression-free survival. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.




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 4. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW)**



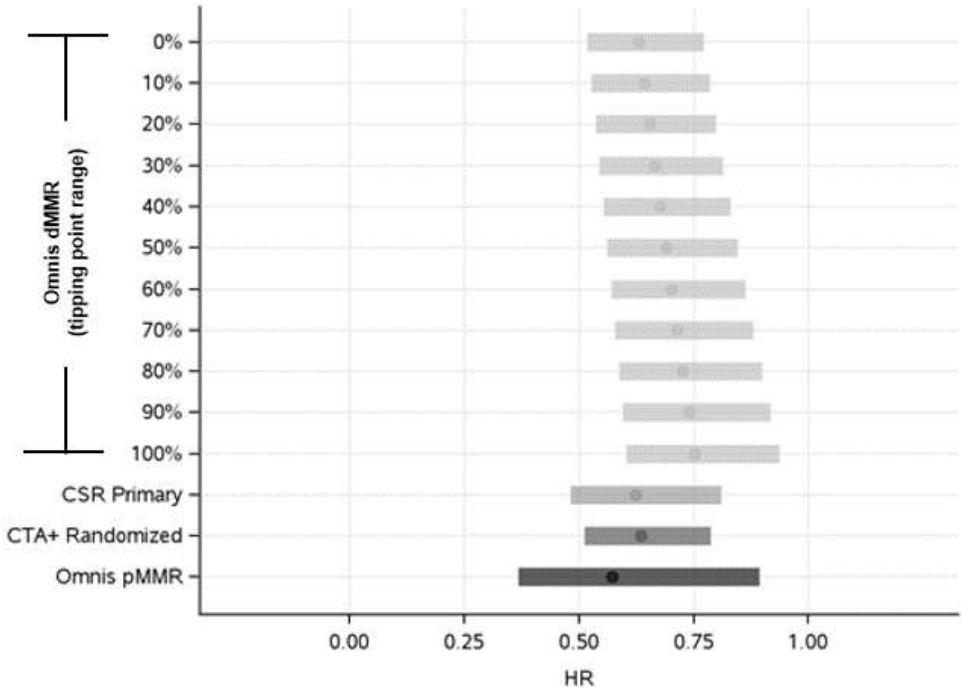

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) and prior lines of therapy (0, 1, ≥2) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first 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 OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW).**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, 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 HR of PFS of the subjects not enrolled (CTA-negative/CDx-positive, HR=1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.63). The tipping point PFS HR CI values ranged from a minimum of 0.52 (0% tipping point range CI lower-bound) to a maximum of 0.94 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.52-0.77, and the 100% tipping point 95% CI range at 0.60-0.94.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 582). **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). 3L+, all lines treatment; BICR, blinded independent central review; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; MSI-H, microsatellite instability high; Nivo, nivolumab; pMMR, mismatch repair proficient.

**Figure 6. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CHECKMATE-8HW).**

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in Objective Response Rate (ORR) over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA, with ORR 63.6% (95% CI: 58.4, 68.7) vs. 49.3% (95% CI: 44.0, 54.6), respectively. ORR benefit observed in 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) vs. 58.3% (95% CI: 52.2, 64.2), respectively (Table 15). No clinically meaningful ORR benefit was observed in all lines subjects with the CTA-positive/CDx-negative status (p=0.0568).

**Table 15: ORR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[c]**

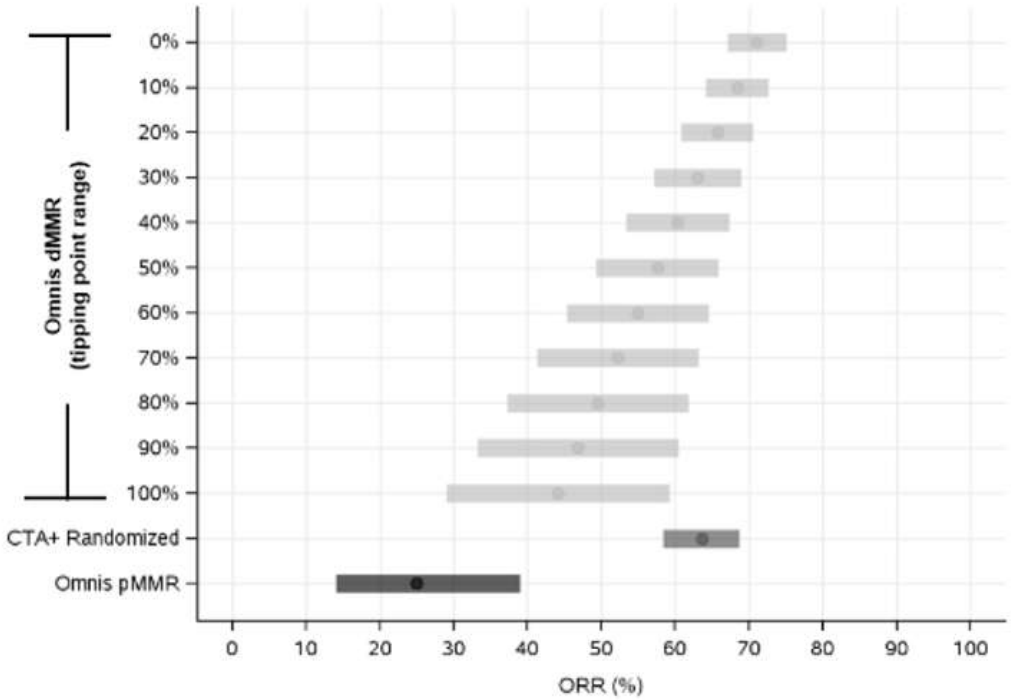
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed dMMR/MSI-H status using clinical trial assay modalities; CDx-positive: deficient mismatch repair (dMMR); CDx-negative: proficient mismatch repair (pMMR); ORR, overall response rate. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.

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. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

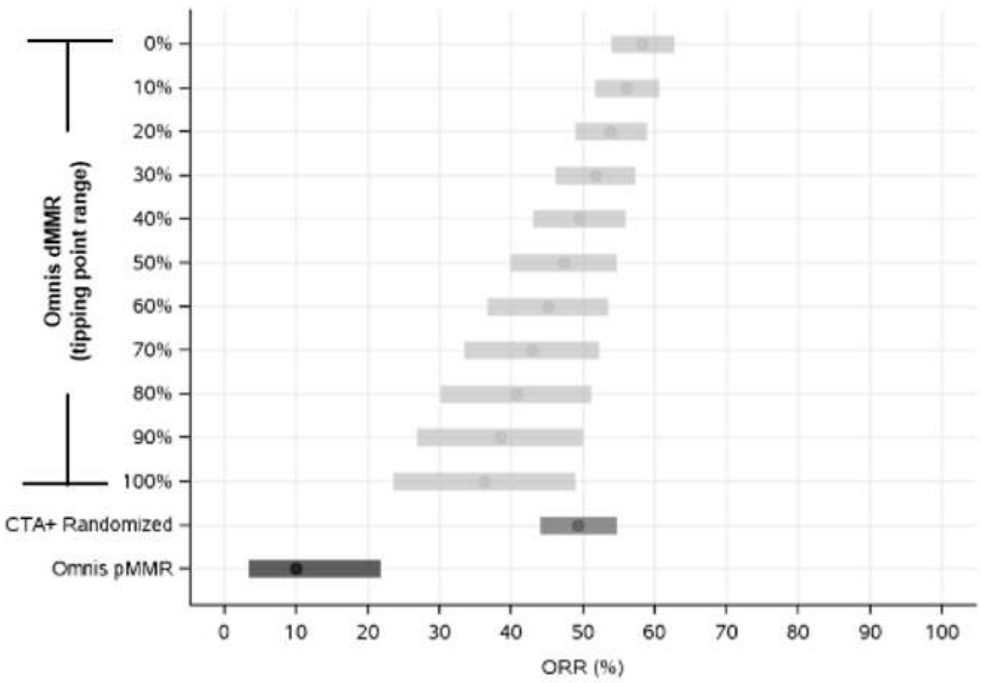
Zero to 100% of the tipping point range was assumed for the missing ORR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of these patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for the ORR of the subjects not enrolled (ORR = 0 for CTA-negative/CDx-positive). The tipping point range of 0% assumes a best-case scenario where the ORR for these subjects is equal to the observed ORR for enrolled subjects (ORR = 0.711 for nivolumab plus ipilimumab in CTA-positive/CDx-positive subjects; ORR = 0.583 for nivolumab monotherapy in CTA-positive/CDx-positive subjects). For nivolumab plus ipilimumab, the tipping point ORR point estimate values ranged from 0.438 to 0.7107 and CI values ranged from a minimum of 0.2881 (100% tipping point range CI lower-bound) to a maximum of 0.7501 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2881-0.5878, and the 0% tipping point 95% CI range at 0.6714-0.7501. For nivolumab monotherapy, the tipping point ORR point estimates ranged from 0.3593 to 0.5830 and CI values ranged from a minimum of 0.233 (100% tipping point range CI lower-bound) to a maximum of 0.6265 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2330-0.4856, and the 0% tipping point 95% CI range at 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).




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 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 = 0.583).**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

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

### 15.3.5. Clinical Performance Summary

In summary, successful bridging between CTA-positive and dMMR status by MMR IHC Panel pharmDx (Dako Omnis) was achieved based on similar clinical utility between patients with dMMR/MSI-H status by CTA and those with dMMR status by MMR IHC Panel pharmDx (Dako Omnis).

A clinically meaningful improvement in PFS per BICR was demonstrated with:

- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to chemotherapy in 1L treatment of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).
- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to OPDIVO (nivolumab) monotherapy in all lines of therapy of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).

Re-estimation of efficacy results using imputations and sensitivity analyses by considering the missing assessments of MMR IHC Panel pharmDx (Dako Omnis) supported these findings.

These results support the clinical performance of MMR IHC Panel pharmDx (Dako Omnis) by demonstrating the assay's clinical utility in identifying patients with dMMR CRC who may benefit from treatment with OPDIVO (nivolumab) alone or in combination with YERVOY (ipilimumab) in accordance with their approved US package inserts.

## 16. Troubleshooting

Refer to the Troubleshooting section in Agilent's Education Guide for remedial action or contact Agilent Pathology Support to report unusual staining.[8]

Dako Omnis is an automated system designed to alert the user if anything in the run has been outside of specifications. Please refer to the Dako Omnis User Guide for details on what conditions are flagged and how. Table 16 is a troubleshooting guide for results and conditions that are not easily identified through the Dako Omnis warning and alert system.

The user should always ensure adherence to the maintenance schedule for the Dako Omnis instrument. Always ensure to use the appropriate controls as described in 'Quality Control', Section 11.

**Table 16. Troubleshooting.**

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| 7. No or weak staining of slides. | 1a. Excessive heating of mounted tissue sections prior to loading on Dako Omnis may lead to loss of visible MLH1 immunoreactivity and/or tissue morphology. | 1a. Dry the tissue sections at 58 ± 2 °C for a maximum of 1 hour, using a calibrated oven with uniform heat distribution.[10] |
| | 1b. Wrong storage conditions used for reagents. | 1b. Check that reagents have been stored correctly according to listed storage conditions. |
| | 1c. Inappropriate fixation method used. | 1c. Ensure that patient tissue is not fixed for too short or too long a time period, that ischemia time has been minimized, and that the correct fixative (10% NBF) was used. |
| | 1d. Reagent is used past its expiration date. | 1d. Check Dako Link Omnis Workstation software to determine if slides were flagged suspicious. Ensure reagent is not used past its expiration date. |
| | 1e. Reagent is used past its onboard stability. | 1e. Check Dako Link Omnis Workstation software to determine if slides were flagged as suspicious. Ensure reagent is not used past its onboard stability. |
| | 1f. Incorrect placement of dynamic gap lids in staining modules. | 1f. Check placement of dynamic gap lids. |
| | 1g. Damaged dynamic gap lids. | 1g. Check integrity of dynamic gap lids. |
| | 1h. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution (50x) concentrate. | 1h. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 1i. Incorrect Target Retrieval Solution is used. | 1i. Ensure that correct Target Retrieval Solution specified in 'Materials Required, but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |
| | 1j. 1x Target Retrieval Solution does not meet pH specifications. | 1j. Check pH of 1x Target Retrieval Solution. If pH is outside acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. Refer to Problem #6 for additional troubleshooting. |
| 8. Excessive nonspecific staining of slides. | 2a. Starch additives used in mounting sections to slides. | 2a. Avoid using starch additives for adhering sections to glass slides. Many additives are immunoreactive. |
| | 2b. Sections dried after staining procedure/prior to coverslipping. | 2b. Verify that the Unloading Station is filled with sufficient water. |
| | | 2b. Avoid stained slides drying out after staining completion (e.g., between removal from Dako Omnis and coverslipping). |
| | 2d. Inappropriate fixation method used. | 2d. Ensure that approved fixative was used. Alternative fixative may cause excessive nonspecific staining. |
| | 2e. Paraffin incompletely removed. | 2e. Check appearance of solvent coupling. Refer to Dako Omnis User Guide for details. |

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| | 2f. Nonspecific binding of reagents to tissue. | 2f. Ensure that correct fixation method of the specimen is used and avoid large areas of necrosis. |
| | 2g. Re-use of mixing strip. | 2g. Ensure that new mixing strips are used. |
| 9. Excessively strong specific staining. | 3. Inappropriate fixation method used. | 3. Ensure that only approved fixatives and fixation methods are used. |
| 10. Tissue detaches from slides. | 4. Use of incorrect slides. | 4. Use FLEX IHC Microscope Slides (Code K8020), or SuperFrost Plus slides. |
| 11. Slide is flagged as suspicious. | 5a. Reagent is used beyond its expiration date. | 5. Slides flagged as suspicious should be evaluated by qualified personnel, contact an Agilent Technologies representative if further action is needed. |
| | 5b. Reagent is stored onboard Dako Omnis beyond its validated onboard stability. | |
| | 5c. Maintenance overdue or other factors. | |
| 12. 1x Target Retrieval Solution does not meet pH specifications | 6a. pH meter is not calibrated correctly. | 6a. Ensure pH meter is calibrated per manufacturer's recommendations. After recalibration, retest pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If pH is outside of the acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. |
| | 6b. Target Retrieval Solution pH is measured at incorrect temperature. | 6b. Ensure that 1x Target Retrieval Solution pH is measured at ambient temperature. |
| | 6c. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution concentrate. | 6c. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 6d. Incorrect Target Retrieval Solution is used. | 6d. Ensure that the correct Target Retrieval Solution specified in 'Materials Required but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |

**NOTE:** If the problem cannot be attributed to any of the causes in Table 16, or if the suggested corrective action fails to resolve the problem, please contact Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found in Agilent's Education Guide (available from www.agilent.com), Atlas of Immunohistology, and Immunoperoxidase Techniques. A Practical Approach to Tumor Diagnosis.[8,12,13]

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**Explanation of symbols**

| | | | | | |
|---|---|---|---|---|---|
| REF | Catalogue number | | Temperature limitation | IVD | In vitro diagnostic medical device |
| | Manufacturer | LOT | Batch code | Σ | Contains sufficient for <n> tests |
| | Use by | | Consult instructions for use | BIO | Contains biological material of animal origin |
| | Caution | EC REP | Authorized representative in the European Community | | |

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Revision 2025.08

# MSH2 IHC pharmDx (Dako Omnis)
# Rx Only
# Code GE085

Primary antibody for use with MMR IHC Panel pharmDx (Dako Omnis)
60 tests for use with Dako Omnis

**Table of Contents**

## 1. Intended 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).

## 2. Summary and Explanation

The MMR pathway is used by normal proliferating cells to repair mutations that may occur during DNA replication. Loss of function of any of the following four MMR proteins, MLH1, PMS2, MSH2, MSH6, results in MMR deficiency (dMMR) and can lead to an increased mutation rate, promotion of tumorigenesis, and generation of neoantigens. dMMR tumors may be more responsive to immunotherapies than tumors with functioning MMR pathways due to the increased presence of neoantigens and immune cell recruitment.[1,2] MSH2 IHC pharmDx (Dako Omnis) is part of MMR IHC Panel pharmDx (Dako Omnis), which is an IHC panel that is used to detect loss of function of any of the four MMR proteins.

Bristol-Myers Squibb sponsored trial, CHECKMATE-8HW (CA2098HW), investigated the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in identifying MMR deficient metastatic CRC patients who may respond to treatment with OPDIVO alone or in combination with YERVOY.[3,4]

*OPDIVO and YERVOY are trademarks owned by Bristol-Myers Squibb Company.*

## 3. Principle of Procedure

MSH2 IHC pharmDx (Dako Omnis) is an optimized antibody reagent with the protocol required to complete an IHC staining procedure of FFPE specimens using the Dako Omnis instrument. Following incubation with the primary monoclonal antibody to MSH2, the specimen is sequentially incubated with peroxidase block, two 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 chromogen results in precipitation of a visible reaction product at the site of antigen. The specimen may then be counterstained and coverslipped. Results are interpreted using a bright field microscope. MMR Negative Control Reagent, Mouse (GE101) slide should be run alongside the MSH2 IHC pharmDx (Dako Omnis) slide. Please consult Dako Omnis User Guide for detailed instructions on loading and unloading of slides, reagents, bulk fluids and waste.

## 4. Materials Provided

The product includes 12 mL of primary antibody to MSH2 protein (approximately 0.75µg/mL) sufficient for 60 tests. The product has been optimized for use with the Dako Omnis instrument. Please refer to the Dako Omnis User Guide for further information.

| *Quantity* | *Description* |
|---|---|
| 1 x 12 mL | **MSH2 IHC pharmDx (Dako Omnis)** |

**MSH2 IHC pharmDx**

**(Dako Omnis)**

Monoclonal mouse anti-human MSH2, clone FE11, in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.

## 5. Materials Required, but Not Supplied

Dako Omnis (Code GI100)
MLH1 IHC pharmDx (Dako Omnis) (Code GE079)
MSH6 IHC pharmDx (Dako Omnis) (Code GE086)
PMS2 IHC pharmDx (Dako Omnis) (Code GE087)
MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101)
MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102)
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 (see 'Quality Control', Section 11)
pH meter

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

*__NOTE__: *Use Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) for MSH2 IHC pharmDx (Dako Omnis) (Code GE085) testing. Do not use EnVision FLEX Target Retrieval Solution, High pH (50x) from EnVision FLEX, High pH (Dako Omnis), Code GV800 or GV823.*

**__NOTE__: *Not all sources of distilled or de-ionized water may be of sufficient quality for IHC reagent preparation. Agilent recommends reagent-grade distilled or de-ionized water (corresponding to Clinical Laboratory Reagent Water [CLRW] standard as specified by CLSI), or water similar in quality to be used for reagent preparation.*[5]

## 6. Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, $NaN_3$ may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.[6]
4. MSH2 IHC pharmDx (Dako Omnis) contains material of animal origin. As with any product derived from biological sources, proper handling procedures should be used in accordance with local requirements.
5. Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection, and disposed of with proper precautions.[7]
6. Incubation times, temperatures, or methods other than those specified may give erroneous results.
7. Reagents have been optimally diluted. Further dilution may result in loss of visible MSH2 immunoreactivity.
8. Paraffin residue may lead to false negative results.
9. Use of reagent volumes other than recommended may result in loss of visible MSH2 immunoreactivity.
10. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
11. Unused solution should be disposed of in accordance with all local, regional, national and international regulations.
12. Safety Data Sheets are available on www.agilent.com or on request.
13. Lack of adherence to the maintenance schedule for the Dako Omnis instrument may give erroneous results. Refer to Dako Omnis User Guides for additional information and for additional instrument-related precautions.
14. Contact Agilent Pathology Support via www.agilent.com to report any unusual staining.

## 7. Storage

Store MSH2 IHC pharmDx (Dako Omnis) in the original product packaging at 2–8 °C when not in use on Dako Omnis. During storage, the flip top vial cap should be closed.

**Do not use the reagent after the expiration date printed on the reagent vial label.** If the reagents are stored under any conditions other than those specified in the instructions for use, they must be validated by the user. The expiry date on the label is valid for unopened vials as well as opened (in-use) vials when handled according to instructions.

Onboard reagent stability for MSH2 IHC pharmDx (Dako Omnis) has been validated to 375 hours. After staining completion, the reagents should be removed from Dako Omnis and stored in the original product packaging at 2–8 °C with flip top vial caps closed securely on the vials. For onboard stability of all ancillary components including diluted working solutions of Wash Buffer and Target Retrieval Solution, pH 9, refer to respective instructions for use. Onboard time of reagents is tracked by the Dako Omnis software; refer to Dako Omnis User Guide for details.

**NOTE**: There are no obvious visual signs to indicate incorrect product storage or handling of this product during the product's shelf life. Positive and negative controls should be run simultaneously with patient specimens, preferably on the same slide, to monitor product performance during the product's shelf life. If a problem is suspected with the antibody during the shelf life that cannot be explained by incorrect product storage or handling, or other variations in laboratory procedures, contact Agilent Pathology Support. Refer to 'Quality Control', Section 11 and 'Troubleshooting', Section 16 for more information.

## 8. Specimen Preparation

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

### 8.1 Paraffin-embedded tissue

FFPE tissues are suitable for use with the primary antibody to the MSH2 protein. 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 [such as 10% unbuffered formalin, Bouin's fixative, and acetic formalin alcohol (AFA)] have not been validated and may give erroneous results. 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.

### 8.2 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.

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.

**NOTE**: The tissue specimens must be positioned on the glass within the defined slide staining area. Please consult the Dako Omnis User Guide for dimensions of slide staining area.

## 9. Reagent Preparation

The user should adhere to appropriate personal protective equipment requirements and become familiar with all components prior to use (see 'Precautions', Section 6).

Target Retrieval Solution, pH 9 (50x) (Code GC309) and Wash Buffer (20x) (Code GC807) must be prepared according to their respective instructions for use. Refer to the GC309 and GC807 instructions for use 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.

## 10. Staining Procedure

**Procedural Notes**

The user should read these instructions carefully and become familiar with all the components and the instrumentation prior to use (see 'Precautions', Section 6).

The automated staining procedure for MSH2 IHC pharmDx (Dako Omnis) on Dako Omnis includes deparaffinization of tissue sections, target retrieval, and staining. The slides are unloaded in the Unloading Station. All protocol steps are preprogrammed into the Dako Link Omnis Workstation software. The "MMR MSH2 IHC pDx GE085**"** protocol is used for MSH2 IHC pharmDx (Dako Omnis). If the appropriate MSH2 IHC pharmDx (Dako Omnis) protocol is not on your server, please contact your local Technical Service Representative or Agilent Pathology Support to obtain the protocols. Refer to the Dako Omnis User Guide for further information on how to operate and maintain the instruments.

**NOTE:** The MSH2 reagent and instructions supplied with this product have been designed for optimal performance. Further dilution of the antibody or alteration of staining protocol may give erroneous or discordant results. Differences in tissue processing and technical procedures in the user's laboratory may invalidate the assay results.

**NOTE**: Laboratories located at high elevations should determine the best method of maintaining the required temperature (97 °C) during heat-induced epitope retrieval. Any adjustments required to address elevation concerns must be validated by the user. Refer to the Dako Omnis User Guide for additional information.

**Prestaining procedure**

1. Choose the MMR MSH2 IHC pDx GE085 protocol to be applied for each slide from the Dako Link Omnis Workstation software.
2. Ensure the Dako Link Omnis Workstation software is configured to print slide labels with the protocol name displayed.
3. Print slide labels and attach them to the glass slides.
4. Place the slides in the Slide Rack. A Slide Rack can hold from one to five slides.
5. Ensure that the bulk bottles with fluids are onboard and registered by the Dako Omnis instrument. Bulk bottle fluids:
    a. Clearify™ clearing agent (Code GC810)
    b. Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) **diluted to 1x working concentration with distilled or de-ionized water.**
    c. Wash buffer (Code GC807) **diluted to 1x working concentration with distilled or de-ionized water.**
6. Ensure that all flip top vial caps are open and locked in place before loading all required reagents in the Reagent Storage Module:
    a. MSH2 IHC pharmDx (Dako Omnis) (Code GE085)
    b. EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis) (Code GV800)
    c. EnVision FLEX Visualization Reagent (Dako Omnis) (Code GV800)
    d. EnVision FLEX Substrate Buffer (Dako Omnis) (Code GV800)
    e. EnVision FLEX DAB+ Chromogen (Dako Omnis) (Code GV800)
    f. EnVision FLEX+ Mouse LINKER (Dako Omnis) (Code GV821)
    g. EnVision FLEX+ Rabbit LINKER (Dako Omnis) (Code GV809)
    h. Optional: Hematoxylin (Dako Omnis) (Code GC808) or equivalent
    i. Sulfuric Acid (Code GC203)
7. Load the Slide Rack onto Dako Omnis.
8. Follow the instructions on the Touch Screen and tap "Done" to initiate the staining procedure.
9. Ensure the slide Unloading Station is filled with distilled or de-ionized water to prevent slides from drying.

**NOTE:** When using the overnight staining feature (delayed start) slides must be removed from the Unloading Station the morning the staining has been completed.

**NOTE:** The MSH2 IHC pharmDx (Dako Omnis) protocol on the Dako Omnis instrument can be monitored on the Dako Link Omnis Workstation.

**Dako Omnis Staining Protocol**

When processing slides for staining with the MMR IHC Panel pharmDx (Dako Omnis) assay, the Dako Omnis automated platform executes the protocols for MMR MSH2 IHC pDx GE085 as stated in Table 1. Refer to MMR Negative Control Reagent, Mouse (GE101) IFU for details on the GE101 staining protocol. The MMR MSH2 IHC pDx GE085 protocol has been designed for optimal performance. Any changes to the staining protocol may alter the performance of the device and must be validated by the user. Unless otherwise noted, each step of the staining procedure is executed at the instrument's fixed temperature of 32ºC. The instrument's fixed temperature is not an editable parameter.

**Table 1. MMR MSH2 IHC pDx GE085 Staining Protocol**

| Protocol Step | Reagent | Setting |
|---|---|---|
| **Dewax** | Clearify Clearing Agent | 25 °C, 10 s incubation top, 1 min incubation bottom, 1 cycle |
| | DI Water | 5 s incubation, 1 cycle |
| **Target retrieval** | TRS, pH 9* | 97 °C*, 30 min incubation* |
| | Cooling fluid DI Water | N/A |
| **Staining** | Wash Buffer | 2:40 min incubation, 2 cycles |
| | Primary antibody (MSH2*) | 20 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Peroxidase-Blocking Reagent | 3 min incubation |
| | Wash buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Mouse LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Rabbit LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX/HRP | 20 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Substrate Working Solution | 5 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| **Counterstaining** | Hematoxylin* | 3 min incubation* |
| | DI Water* | 2 min incubation, 10 cycles |
| | Wash Buffer* | 2 min incubation, 10 cycles |

***Parameter is editable by the end user when creating a copy. Any changes to the staining protocol may alter the performance of the device and must be validated by the user.

**Counterstain**

Slides may be counterstained with Hematoxylin (Dako Omnis) (Code GC808). The "MMR MSH2 IHC pDx GE085" protocol on Dako Omnis includes a counterstaining step that is set as editable for the user. If a counterstain other than the recommended Hematoxylin (Dako Omnis) (Code GC808) is used, the alternative counterstain must be validated by the user. See the Dako Omnis User Guide for further information on editing protocols.

Preprogrammed:

Slides are counterstained for 3 minutes with Hematoxylin (Dako Omnis) (Code GC808). The Hematoxylin incubation time is preprogrammed in the protocol. Slides are ready for mounting when removed from the Unloading Station.

User Defined:

If the selected protocol does not include an automated counterstaining process, it is the responsibility of the user to counterstain the specimen(s) per internally validated procedure prior to mounting.

**Mounting**

After staining onboard Dako Omnis, the sections must be dehydrated, cleared, and mounted using nonaqueous, permanent mounting methods.

**NOTE:** Some fading of stained slides may occur, depending on several factors including, but not limited to, counterstaining, mounting materials and methods, and slide storage conditions. To minimize fading, store stained slides in the dark at room temperature (20–25 °C).

## 11. Quality Control

MSH2 IHC pharmDx (Dako Omnis) has been quality controlled for IHC using the required reagents and staining procedures outlined in 'Reagent Preparation', Section 9 and 'Staining Procedure', Section 10. Deviations from the recommended procedures may lead to significant variability in results. Consult the quality control guidelines of the College of American Pathologists (CAP) Accreditation Program for Immunohistochemistry. See also Agilent's Education Guide: Immunohistochemical Staining Methods and CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[8,9]

### 11.1 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 must be used, where possible, as internal positive controls instead of a separate positive control tissue. In very rare cases, nonmalignant elements may have loss of biomarker expression, in which case nonmalignant elements of the negative control tissue may be used to qualify the staining procedure.

Refer to Table 4 for further information on positive and negative control tissues.

### 11.2 Negative control reagent

MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101) should be used in place of the 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 negative control reagent (NCR). Refer to the MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101) instructions for use for details.

### 11.3 Assay verification

Prior to initial use of a staining system in a diagnostic procedure, the user should verify the assay's performance by testing it on a series of lab-supplied tissues with known IHC performance characteristics representing known positive and negative tissues. Refer to the quality control procedures outlined in 'Quality Control', Section 11, as well as to the quality control requirements of the CAP Certification Program for Immunohistochemistry and/or CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[9] These quality control procedures should be repeated for each new primary antibody lot. Troubleshooting options for potential problems, their causes and suggested corrective actions are outlined in Table 16.

## 12. Staining Interpretation

A Hematoxylin and Eosin (H&E) stained section is used to determine if a specimen is acceptable for IHC. MMR IHC Panel pharmDx (Dako Omnis) and H&E staining should be performed on serial sections from the same FFPE block of the specimen to confirm:

1. The histologic diagnosis of CRC.
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.

Each specimen should be evaluated using 4x–20x magnification. The specific staining pattern 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.

The entire tissue section should be considered, avoiding edge effects, noninvasive components, necrotic areas, and areas with obvious fixation artifacts. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.

Nonspecific cytoplasmic staining may be present in some tissues. 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, epithelia cells
2. Edge effects
3. Necrotic areas
4. Areas with noninvasive components (normal epithelium, adenoma)
5. Areas with obvious fixation artifacts should not be scored or scored with caution

Protein status of Intact or Loss is determined for MSH2 using the guidelines described in Table 2.

**Table 2. Determination of MSH2 Intact or Loss status.**

| | |
|---|---|
| Intact | Nuclear staining in viable malignant cells must be unequivocal, with at least the same overall staining intensity as in adjacent internal positive controls.<br>If focal staining is present, the tissue is considered intact if:<br>1) continuous in multiple glands/nests<br>and<br>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.<br>If focal staining is present, the tissue is considered loss if:<br>1) continuous in only a single gland/nest,<br>2) discontinuous in multiple glands/nests,<br>or<br>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.

After a protein status of Intact or Loss is assigned to each biomarker (MLH1, PMS2, MSH2, and MSH6) for a given specimen, a diagnostic status of MMR proficient or MMR deficient is given using the definitions in Table 3.

**Table 3. Definitions of MMR proficient and MMR deficient.**

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

Some cases may be more challenging to interpret due to particular staining patterns, morphology, nonspecific staining, and/or tissue or staining artifacts. For additional guidance on MMR staining interpretation and examples of challenging cases, refer to the MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual for details.

### 13. Tissue Evaluation

The following table provides the order of slide evaluation for interpretation of MSH2 IHC pharmDx (Dako Omnis). Per the intended use and MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual, evaluation must be performed in conjunction with other required biomarkers.

**Table 4. Recommended order of tissue evaluation.**

| Tissue | Rationale | Requirements |
|---|---|---|
| 1. Patient tissue stained with H&E | An H&E stain of the patient tissue is evaluated first to assess tissue histology and preservation quality.<br>Note: The H&E may be reviewed again in the context of the patient tissue slides stained with the NCR and primary antibody (Steps 5 and 6) | The H&E and MMR IHC Panel pharmDx (Dako Omnis) stains should be performed on serial sections from the same FFPE block of the specimen.<br>Tissue specimens should be well preserved, confirm the diagnosis of CRC, and include at least 50 viable malignant tumor cells. |
| 2. Positive control stained with primary antibody to the MSH2 protein (GE085) | The positive control tissue stained with primary antibody to the MSH2 protein should be examined next.<br>**Patient CRC tissues** contain positive nonmalignant elements that serve as an internal positive control. This internal positive control eliminates the need for a separate control tissue.<br>In rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In cases that demonstrate complete loss of biomarker expression in nonmalignant elements, internal positive controls within the negative control tissue should be reviewed to qualify the staining procedure.<br>Positive tissue controls should be used for monitoring the performance of processed tissues and test reagents. | Nuclear staining of benign or normal epithelium, lymphocytes, and stromal cells present in the patient tissue must demonstrate moderate to strong staining intensity.<br>If patient tissue demonstrates focal loss of biomarker expression in nonmalignant elements, the remaining areas with nuclear staining in the internal positive control elements may still be used to qualify the staining procedure.<br>If patient tissue demonstrates complete loss of biomarker expression in both malignant and nonmalignant elements, use internal positive control within the negative control tissue.<br>If the internal positive controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid. |
| 3. Negative control tissue stained with primary antibody to the MSH2 protein (GE085)<br>(Lab-supplied) | The negative control tissue stained with primary antibody to the MSH2 protein should be examined next to verify labeling specificity of the target antigen by the primary antibody. | Negative control tissue should be prescreened CRC tissues with loss of MSH2 expression.<br>At least one negative control tissue section should be included in each staining procedure, either as an on-slide control or as a separate negative control slide. On-slide tissue controls are recommended and eliminate the need for a separate control slide.<br>Slides stained with primary antibody to the MSH2 protein should exhibit no nuclear staining or focal weak nuclear staining in malignant tumor cells in the presence of moderate to strong staining in internal positive controls in the tumor area. Internal positive control elements include nuclear staining in normal epithelium, lymphocytes, or stroma.<br>If the negative tissue controls fail to demonstrate appropriate staining, results with the test specimens should be considered invalid. |

| Tissue | Rationale | Requirements |
|---|---|---|
| 4. Optional:<br>Negative control tissue stained with MMR Negative Control Reagent, Mouse (GE101)<br>(Lab-supplied) | NCR may be used to stain the negative control tissue specimen if needed for troubleshooting purposes. | NCR slides must exhibit no or weak staining in malignant tumor cells. |
| 5. Patient tissue stained with MMR Negative Control Reagent, Mouse (GE101) | Examine patient specimens stained with NCR. NCR is used in place of the primary antibody and aids in interpretation of specific staining at the antigen site. | NCR slides must exhibit no or weak staining in malignant tumor cells. If weak staining is present in tumor nuclei, it should be used as a baseline to evaluate the MSH2 slide. Staining at the same intensity or lower that may occur in the MSH2 antibody slide should be disregarded upon interpretation.<br>NCR slides with moderate or strong staining in malignant tumor cells are invalid and the corresponding MSH2 slide is considered nonevaluable. The patient tissue must be retested. |
| 6. Patient tissue stained with primary antibody to the MSH2 protein (GE085) | Examine the patient specimen stained with the primary antibody to the MSH2 protein last to assess MSH2 protein status. | All malignant tumor cells should be evaluated for the MSH2 protein expression and included in the MSH2 protein scoring assessment. Positive staining intensity should be assessed within the context of any nonspecific staining observed on the patients NCR slide. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.<br>In very rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In such cases, the patient tissue may still be evaluated if the staining procedure is qualified using the internal positive controls within the negative control tissue.<br>As with any IHC test, absence of staining means that the antigen was not detected, not necessarily that the antigen was absent in the cells/tissue assayed.<br>For staining interpretation guidelines, refer to 'Staining Interpretation', Section 12. |

## 14. Limitations

### 14.1 General limitations

1. For prescription use only (Rx only).
2. IHC is a multistep process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
3. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any staining or its absence must be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls. It is the responsibility of a qualified pathologist, who is familiar with the antibodies, reagents and methods used, to interpret the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
6. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[10]
7. Reagents may demonstrate unexpected reactions in previously untested tissue types. The possibility of unexpected reactions even in tested tissue types cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Agilent Pathology Support with documented unexpected reactions.

8. False-positive results may be seen due to nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes) and endogenous peroxidase activity (cytochrome C).[10]
9. The reagents and instructions supplied for this assay have been designed for optimal performance. Further dilution of the reagents or alteration of incubation times or temperatures may give erroneous or discordant results.
10. Slides flagged in the slide log on the Dako Omnis Workstation should be investigated by qualified personnel. Refer to the Dako Omnis User Guide for further details.
11. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date. Improper storage and use of reagents may lead to erroneous results.
12. Canceled slides indicate a significant issue occurred during staining and should not be used. The specimen will require restaining. Refer to the Dako Omnis User Guide for further details.
13. This device is not intended to be used to identify patients with Lynch syndrome or to differentiate between sporadic CRC and Lynch syndrome.

### 14.2 Product-specific limitations

1. False-negative results could be caused by degradation of the antigen in the tissues over time. Once mounted on slides, specimens should be stored in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C. Tissue sections should be stained within 2 months of sectioning.
2. Use of MSH2 IHC pharmDx (Dako Omnis) on specimens fixed in fixatives other than NBF has not been validated.
3. Use of MSH2 IHC pharmDx (Dako Omnis) on decalcified tissues has not been validated.
4. Reduced staining was observed with 10% unbuffered formalin, Bouin's fixative, and AFA so they are not acceptable for use with this assay.
5. This product has undergone a transport simulation study to account for anticipated temperature variations during ambient condition shipping. However, it is possible that this product, when shipped under ambient conditions, may be exposed to shipping conditions outside of tested ranges (-20ºC to 37ºC). Therefore, it is essential to use controls, as specified in this IFU, to confirm expected performance of this product.

## 15. Performance Evaluation

### 15.1 Analytical performance evaluation: normal and neoplastic tissues

Table 5 summarizes monoclonal mouse anti-MSH2, clone FE11, immunoreactivity on the recommended panel of normal tissues. Table 6 summarizes monoclonal mouse anti-MSH2, clone FE11, immunoreactivity on a panel of neoplastic tissues. All tissues were FFPE and stained with MSH2 IHC pharmDx (Dako Omnis) according to the instructions in this package insert. Nuclear staining was observed in a majority of tissue types tested. In some cases, cytoplasmic and/or extracellular staining was observed.

**Table 5. Summary of MSH2 IHC pharmDx (Dako Omnis) normal tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|---|---|
| Adrenal (3) | 3/3 | Lung (3) | 3/3 | Salivary gland (3) | 3/3 |
| Bladder (3) | 3/3 | Mesothelial cells (3) | 3/3 | Skin (3) | 3/3 |
| Bone marrow (3) | 3/3* | Muscle, cardiac (3) | 3/3* | Small intestine (3) | 3/3 |
| Breast (3) | 2/3 | Muscle, skeletal (3) | 3/3 | Spleen (3) | 3/3 |
| Cerebellum (3) | 3/3 | Nerve, peripheral (3) | 3/3 | Stomach (3) | 3/3 |
| Cerebrum (3) | 1/3 | Ovary (3) | 3/3 | Testis (3) | 3/3 |
| Cervix (3) | 3/3 | Pancreas (3) | 3/3 | Thymus (3) | 3/3** |
| Colon (3) | 3/3 | Parathyroid (3) | 3/3 | Thyroid (3) | 3/3 |
| Esophagus (3) | 3/3 | Pituitary (3) | 3/3 | Tonsil (3) | 3/3 |
| Kidney (3) | 3/3 | Prostate (3) | 3/3 | Uterus (3) | 3/3 |
| Liver (3) | 3/3* | | | | |

*cytoplasmic staining pattern for at least one case
**cytoplasmic and extracellular staining pattern for at least one case

**Table 6. Summary of MSH2 IHC pharmDx (Dako Omnis) neoplastic tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|
| Bladder carcinoma (2) | 2/2 | Ovarian granulosa cell tumor (1) | 1/1 |
| Breast carcinoma (5) | 5/5 | Islet cell tumor of pancreas (1) | 1/1 |
| Cholangiocarcinoma (1) | 1/1 | Pancreatic glucagonoma (1) | 1/1 |
| Colon adenocarcinoma (1) | 1/1 | Papillary Serous Carcinoma (1) | 1/1 |
| Endometrial sarcoma (1) | 1/1 | Pleomorphic rhabdomyosarcoma (1) | 1/1 |
| Ewing's sarcoma (1) | 1/1 | PNET scrotum (1) | 1/1 |
| Gastric adenocarcinoma (2) | 2/2 | Prostate adenocarcinoma (2) | 2/2 |
| Hepatoma (1) | 1/1 | Prostate benign prostatic hyperplasia (1) | 1/1 |
| Kidney transitional cell carcinoma (1) | 1/1 | Renal cell carcinoma (1) | 1/1 |
| Liver cell adenoma (1) | 1/1 | Squamous carcinoma of ear (1) | 1/1 |
| Lung carcinoma (4) | 4/4 | Testicular embryonal carcinoma (1) | 1/1 |
| Lymphoma of cecum (1) | 1/1 | Testicular yolk sac tumor (1) | 1/1 |

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|
| Melanoma (3) | 3/3 | Thymic carcinoid tumor (1) | 1/1 |
| Merkel cell tumor (1) | 1/1 | Thymoma (1) | 1/1 |
| Ovarian carcinoma (2) | 2/2 | Thyroid carcinoma (2) | 2/2 |
| Ovarian dysgerminoma (1) | 1/1 | Uterine adenomatoid tumor (1) | 1/1 |

### 15.2 Analytical performance evaluation: CRC

#### 15.2.1 Analytical sensitivity

Analytical sensitivity of MSH2 IHC pharmDx (Dako Omnis) was evaluated across 269 unique specimens of FFPE CRC tissues. The prevalence of loss of MSH2 expression observed was 2.6% (7/269). Assessment of MMR IHC Panel pharmDx (Dako Omnis) staining in a subset of 171 unique specimens demonstrated a dMMR prevalence of 8.8% (15/171).

#### 15.2.2 Precision

The precision of MSH2 IHC pharmDx (Dako Omnis) was evaluated. Diagnostic status was recorded as 'Intact' or 'Loss'. The inter-observer analysis was conducted to evaluate the scoring precision of MSH2 IHC pharmDx (Dako Omnis) across multiple observers at a single site. The study was not designed with this supplemental analysis in mind which led to an imbalance in cases with MSH2 'Intact' and 'Loss' status; therefore, any results reliant on sample size in the 'Loss' population supplemental analysis will be underpowered. Percent agreement of loss (LPA), percent agreement of intact (IPA) and overall percent agreement (OA) 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 100.0%.

**Table 7. Precision of MSH2 IHC pharmDx (Dako Omnis) tested at one site.**

| 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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (92.6, 100.0)<br>(92.6, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (92.6, 100.0)<br>(92.6, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (94.9, 100.0)<br>(94.9, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (94.0, 100.0)<br>(94.0, 100.0)<br>(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<br>IPA<br>OA | 98.6<br>98.6<br>98.6 | (95.8, 100.0)<br>(95.8, 100.0)<br>(96.5, 100.0) |
| Inter-Observer | 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<br>IPA<br>OA | 90.9<br>98.8<br>98.3 | (75.0, 100.0)<br>(96.9, 100.0)<br>(96.0, 100.0) |

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

Additionally, the precision of MMR IHC Panel pharmDx (Dako Omnis) scoring across multiple observers at a single site was evaluated. Diagnostic status was recorded as 'pMMR' or 'dMMR'. dMPA, pMPA and OA were computed with corresponding two-sided 95% percentile bootstrap CIs.

**Table 8. Inter-Observer precision of MMR IHC Panel pharmDx (Dako Omnis) tested at one site.**

| Precision Study | Study Design | % Agreement (95% CI) | | |
|---|---|---|---|---|
| Inter-Observer | One set of 58 CRC stained specimens (31 dMMR, 27 positive) 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<br>pMPA<br>OA | 95.7<br>98.8<br>97.1 | (91.3, 98.9)<br>(96.2, 100.0)<br>(94.7, 99.4) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

### 15.2.3 External reproducibility

The reproducibility of MSH2 IHC pharmDx (Dako Omnis) was evaluated at three external testing sites. Diagnostic status was recorded as 'Intact' or 'Loss'. LPA, IPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 9. Reproducibility of MSH2 IHC pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>IPA<br>OA | 96.8<br>99.1<br>98.6 | (90.0, 100.0)<br>(97.8, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>99.1<br>99.3 | (94.0, 100.0)<br>(97.8, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (95.9, 100.0)<br>(99.2, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (95.9, 100.0)<br>(99.2, 100.0)<br>(99.3, 100.0) |

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

In the same study, the reproducibility of MMR IHC pharmDx (Dako Omnis) was analyzed. MMR diagnostic status was recorded as 'Proficient' (pMMR) or 'Deficient' (dMMR). dMPA, pMPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 10. Reproducibility of MMR IHC Panel pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>pMPA<br>OA | 98.6<br>99.3<br>99.0 | (95.7, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 100.0<br>99.3<br>99.7 | (97.3, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |
| 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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

## 15.3 Clinical performance evaluation: colorectal cancer (OPDIVO [nivolumab] alone and OPDIVO [nivolumab] in combination with YERVOY [ipilimumab])

CHECKMATE-8HW was a phase 3, randomized, open-label, multi-center, three-arm clinical trial of nivolumab monotherapy, nivolumab plus ipilimumab combination therapy, or standard chemotherapy in recurrent or metastatic dMMR/microsatellite instability high (MSI-H) CRC across lines of therapy. CHECKMATE-8HW had dual primary objectives: comparing clinical efficacy as evaluated through progression-free survival (PFS) per blinded independent central review (BICR) of nivolumab plus ipilimumab vs chemotherapy in first-line treatment (1L) and comparing clinical efficacy as evaluated through PFS per BICR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of therapy.

### 15.3.1 Clinical Study Overview

CHECKMATE-8HW was a randomized, 3-arm, open-label trial in immunotherapy-naive patients across all lines of therapy with unresectable or metastatic CRC with known tumor MSI-H or dMMR (MSI-H/dMMR) status as determined in accordance with local standard of practice.

Eligible patients were ≥ 18 years of age, with recurrent or metastatic dMMR or MSI-H CRC not amenable to surgery. Enrollment was based on confirmation of dMMR/MSI-H status by local standard of practice, referred to here as the Clinical Trial Assay (CTA).

Modalities for the CTA included: IHC, polymerase chain reaction (PCR), or next generation sequencing (NGS). Patients were considered CTA-positive and eligible for enrollment if they were identified as dMMR and/or MSI-H by at least one of the CTA modalities. Patients were randomized to OPDIVO (nivolumab) monotherapy, OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy, or investigator's choice chemotherapy in a 2:2:1 ratio. Patients who progressed after 2 prior lines of therapy were randomized in a 1:1 ratio to OPDIVO (nivolumab) monotherapy or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. Randomization was stratified by tumor location (right vs left) and by prior lines of therapy (0, 1, 2L+).

The clinical efficacy of the OPDIVO (nivolumab) and OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination was evaluated in the randomized patient population with centrally confirmed MSI-H/dMMR status. Central assessment of MSI-H status using PCR (Idylla MSI) test and dMMR status using MMR IHC Panel pharmDx (Dako Omnis) was conducted retrospectively on patient tumor specimens used for local MSI-H/dMMR status determination. Patients with confirmed MSI-H/dMMR status by either central test comprised the primary drug efficacy population.

The evaluation of the drug efficacy relied on the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) versus chemotherapy in the first-line (1L) setting and the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines setting. The major efficacy outcome measure was BICR-assessed PFS per RECIST 1.1.

#### 15.3.2 IVD Bridging Study

Specimens from CHECKMATE-8HW were analyzed in an IVD bridging study to establish the clinical performance of the companion diagnostic MMR IHC Panel pharmDx (Dako Omnis) for detection of dMMR in patients with unresectable or metastatic CRC (mCRC) who would benefit from OPDIVO (nivolumab) alone or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. The IVD bridging study was designed to bridge the clinical efficacy from the CTA-positive population (dMMR and/or MSI-H by at least one of the local modalities, including IHC, PCR, and/or NGS) to the intended use population of dMMR by MMR IHC Panel pharmDx (Dako Omnis), which will be demonstrated by the clinical utility analysis through concordance (positive percent agreement [PPA] and negative percent agreement [NPA]) of MMR IHC Panel pharmDx (Dako Omnis) against CTA.

The endpoints to demonstrate the clinical utility of the companion diagnostic are:

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects with mCRC.

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab)

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines randomized subjects with mCRC.

Analyses were performed to demonstrate comparable efficacy based on dMMR by MMR IHC Panel pharmDx (Dako Omnis). These analyses support the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population.

#### 15.3.3 Concordance Analysis

For the concordance analysis, PPA between CTA-positive+ and dMMR by MMR IHC Panel pharmDx (Dako Omnis) was estimated using CHECKMATE-8HW clinical samples. 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, NPA was assessed using commercially procured samples that were predetermined as pMMR and/or MSS using test methods representative of the CTA.

The PPA and the two-sided 95% confidence interval (CI) were calculated between CTA-positive and dMMR by MMR IHC Panel pharmDx (Dako Omnis) using clinical specimens and CTA-positive status as the reference. The NPA and the two-sided 95% CI were calculated between procured samples with pMMR/MSS status (CTA-negative) and pMMR by MMR IHC Panel pharmDx (Dako Omnis) with CTA-negative as the reference. There are no formal acceptance criteria for PPA and NPA. The success criteria for the IVD bridging study depends on the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) dMMR for its intended use.

The clinical efficacy of OPDIVO (nivolumab) plus YERVOY (ipilimumab) from CHECKMATE-8HW was based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the randomized patients as well as the PFS comparisons of OPDIVO (nivolumab) plus ipilimumab vs OPDIVO (nivolumab) alone in all lines of the randomized patients. Each PFS comparison was estimated by hazard ratio (HR) and the 95% CI in a stratified Cox proportional hazards model using the randomized arm as a single covariate; line of therapy (for all lines comparison) and tumor sidedness as the stratification factors. PFS curves were estimated and presented using Kaplan-Meier product-limit methodology from the patients with the concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1). Median PFS with two-sided 95% CI using the Brookmeyer and Crowley method (with log-log transformation) was computed. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 2.

Additionally, to assess the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population to identify dMMR patients with mCRC treated with OPDIVO (nivolumab) plus YERVOY (ipilimumab), a tipping point analysis was conducted to consider the missing patients who were not enrolled due to their local pMMR/MSS status, which were potentially misclassified by the CTA and may be dMMR by MMR IHC Panel pharmDx (Dako Omnis). Tipping point analysis was conducted by assuming that the PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of these patients ranged from the best scenario (i.e., HR equal to that estimable from the enrolled patients with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis)), to the worst scenario (i.e. HR equal to 1). A full range of the tipping point analysis results were assessed for the clinical utility of dMMR status by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population. The tipping point analysis is shown graphically in Figure 3.

The clinical utility for MMR IHC Panel pharmDx (Dako Omnis) to identify dMMR patients in the intended use population for treatment with OPDIVO (nivolumab) plus YERVOY (ipilimumab) was also based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines of the randomized patients and of the centrally confirmed dMMR patients by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4) per the same methods outlined above. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 5. Tipping point analysis was also conducted for this clinical endpoint per the same methods outlined above. The tipping point analysis is shown graphically in Figure 6.

A total of 839 subjects were randomized in CHECKMATE-8HW, and of those, 837 were CTA-positive. Of the 837 CTA-positive randomized subjects, 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). To avoid introducing bias to the concordance and the clinical performance evaluation, imputations and sensitivity analyses were conducted after these analyses were done with the available data and are reflected in the clinical performance results presented below.

#### 15.3.3.1 Concordance results

Concordance between the CTA and MMR IHC Panel pharmDx (Dako Omnis) was assessed via PPA using CHECKMATE-8HW clinical samples and NPA using commercially-procured samples (Tables 11 and 12). The conservative estimate for MMR IHC Panel pharmDx (Dako Omnis) positivity rate is 79.1% (662/837), assuming all 60 excluded samples would have resulted in a negative status by MMR IHC Panel pharmDx (Dako Omnis). Point estimates for positive percent agreement (PPA) and negative percent agreement (NPA) were 85.2% and 97.5%, respectively. The level of agreement achieved between the CTA and MMR IHC Panel pharmDx (Dako Omnis) is shown in Table 12.

**Table 11. Specimen distribution of comparison between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| | | CTA | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| MMR IHC Panel PharmDx (Dako Omnis) | Positive | 662 | 5 | 667 |
| | Negative | 115 | 199 | 314 |
| Total | | 777 | 204 | 981 |

**Table 12. Analytical concordance between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| Performance Criteria | Point Estimate of Percent Agreement (95% CI, Wilson Score) |
|---|---|
| PPA | 85.2 (82.5, 87.5) |
| NPA | 97.5 (94.4, 98.9) |

CI, confidence interval by Wilson score method; NPA, negative percent agreement; PPA, positive percent agreement.

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 utility analysis 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.

### 15.3.4 Clinical Efficacy Results

#### 15.3.4.1 OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects

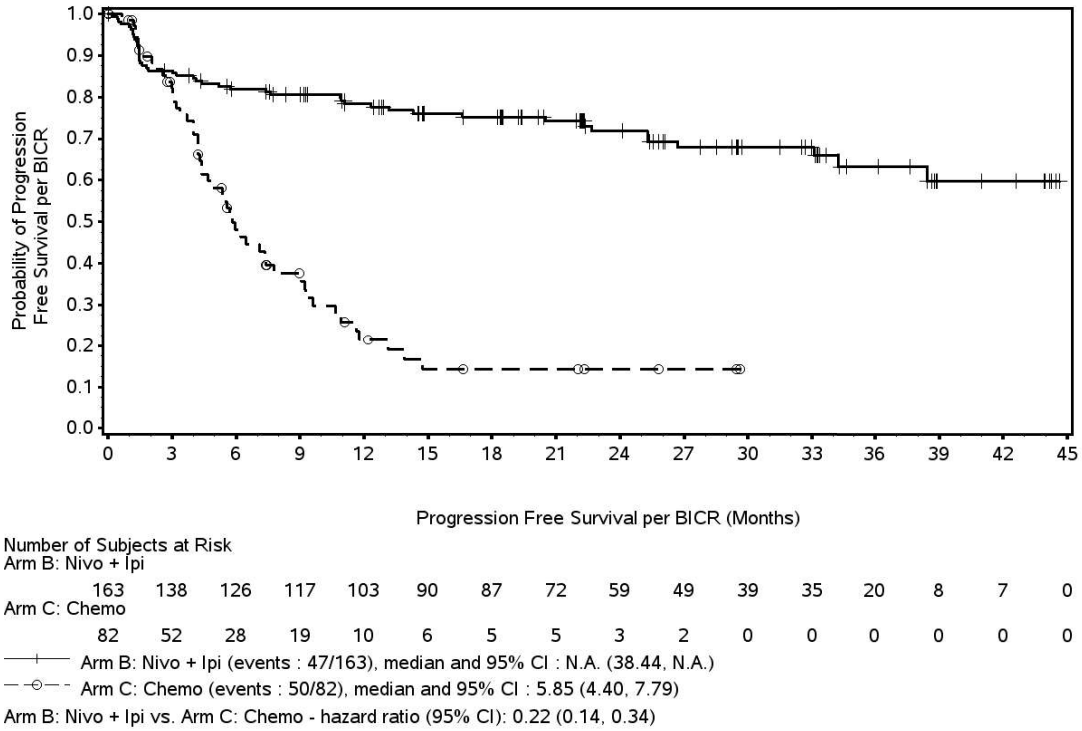
A total of 301 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 200) or chemotherapy (n = 101). The study included 88 sites in 22 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, 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. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the chemotherapy group was 65 years. Most subjects were white (n=259, 86%), 32 (10.6%) were Asian, 4 (1.3%) were black. The ethnicity of subjects was 32 (10.6%) Hispanic,150 (49.8%) non-Hispanic, and 119 (39.5%) not reported. The number of male and female subjects was 139 (46.2%) and 162 (53.8%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR (median PFS not reached) over chemotherapy (PFS 6.21 months) in 1L randomized subjects with dMMR/MSI-H status determined by the CTA (HR 0.32) (Table 12). The PFS benefit observed in 1L randomized subjects with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CDx-positive) is consistent with that in 1L CTA-positive randomized subjects (HR 0.22) (Table 12, Figure 1). A similar improvement in PFS is not observed in the CTA-positive/CDx-negative population, which showed PFS worsening when comparing OPDIVO (nivolumab) plus YERVOY (ipilimumab) (median PFS 1.81 months) with chemotherapy (median PFS 11.53 months).

**Table 13: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

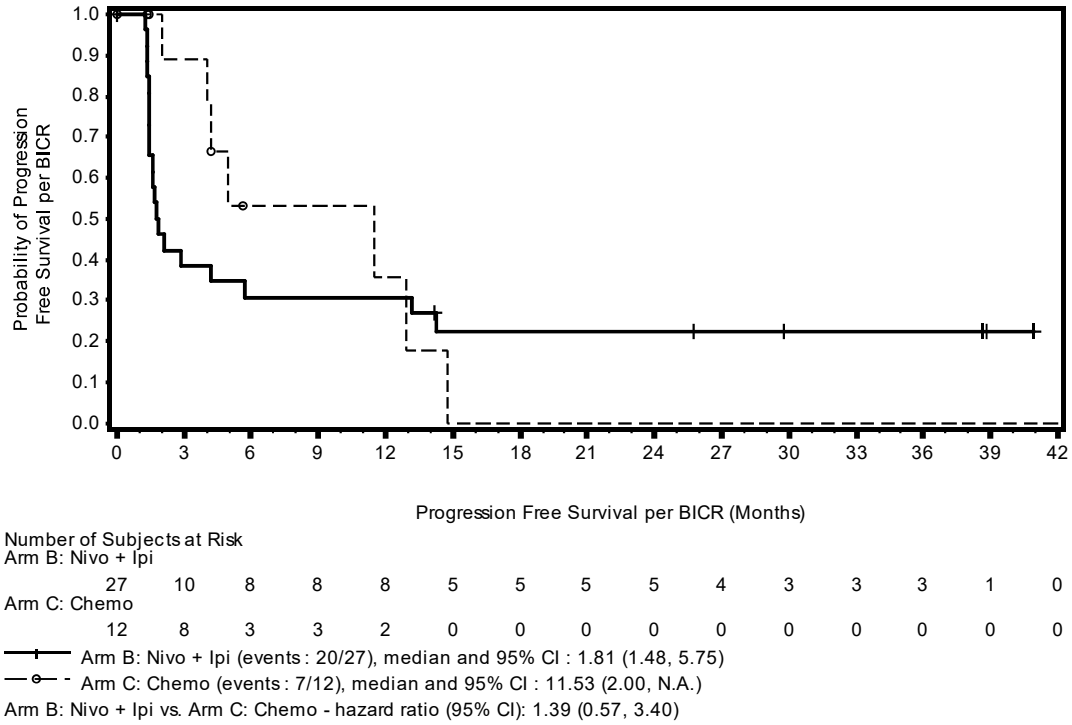
| | CTA-positive/CDx-positive 1L randomized subjects | | CTA-positive/CDx-negative 1L randomized subjects | | 1L Randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N=163 | Chemotherapy N=82 | Nivolumab plus ipilimumab N=27 | Chemotherapy N=12 | Nivolumab plus ipilimumab N=200 | Chemotherapy 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), mo[a] | NR (38.44, NR) | 5.85 (4.40, 7.79) | 1.81 (1.48, 5.75) | 11.53 (2.00, NR) | NR (34.30, NR) | 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. PFS 95% CI upper-bound values of NR 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) 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NR, not reached; PFS, progression-free survival. Subject number totals for 1L randomized CTA-positive (n=301) and 1L randomized subjects with CDx results (n=284) are not equal due to insufficient tissue availability or invalid test status of some subjects 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 1. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**



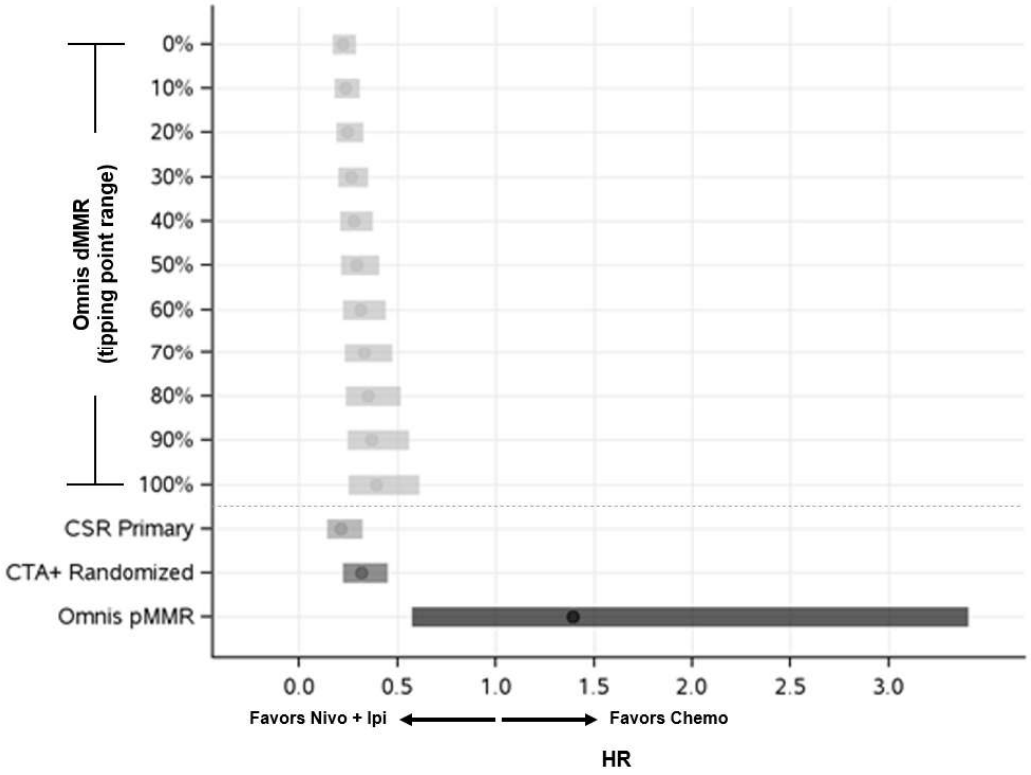

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date.
1L, first-line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

**Figure 2. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2023Oct12 when only the 1L subjects in OPDIVO (nivolumab) plus YERVOY (ipilimumab) and chemotherapy arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for HR of the PFS in these subjects (CTA-negative/CDx-positive, HR = 1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.22). The tipping point PFS HR CI values ranged from a minimum of 0.16 (0% tipping point range CI lower-bound) to a maximum of 0.61 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.16-0.30, and the 100% tipping point 95% CI range at 0.25-0.61.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in the 1L CTA-positive randomized patients and in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 255). **CTA+ Randomized**: Subjects randomized in CHECKMATE-8HW with locally confirmed MSI-H/dMMR status (N = 301). **Omnis pMMR**: Subjects randomized in CHECKMATE-8HW with centrally tested pMMR status and locally confirmed MSI-H/dMMR status (N = 39). chemo, chemotherapy; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab;.

**Figure 3. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus ipilimumab) vs chemotherapy in 1L subjects (CHECKMATE-8HW)**

#### 15.3.4.2 OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects

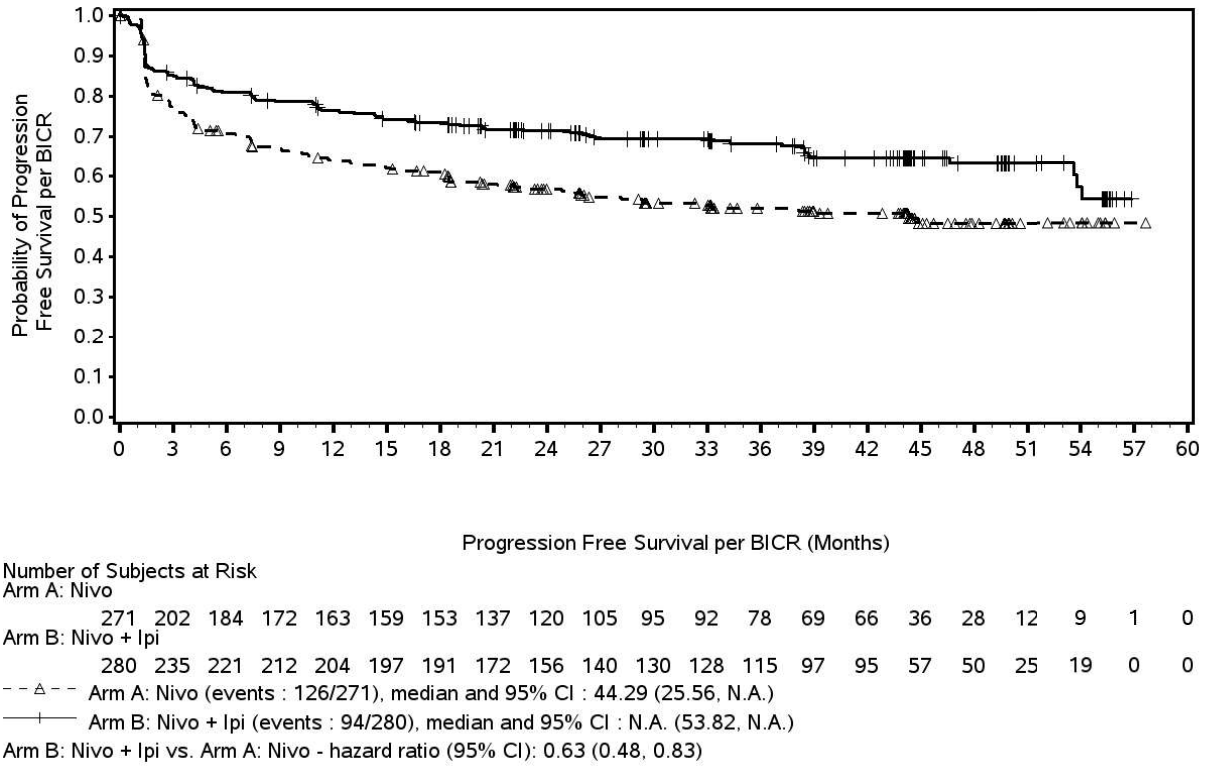
A total of 705 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 352) or OPDIVO (nivolumab) (n= 353). The study included 88 sites in 23 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, Denmark, France, Germany, Greece, Ireland, Italy, Japan, Netherlands, Norway, Romania, Spain, Turkey, UK, and US). Most subjects were from US/Canada/European Union (n = 495, 70.2%), 59 (8.4%) were from Asia, and 151 (21.4%) were from the rest of the world. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the OPDIVO (nivolumab) group was 63 years. Most subjects were white (n=614, 87.1%), 63 (8.9%) were Asian, 11 (1.6%) were black. The ethnicity of subjects was 66 (9.4%) Hispanic, 353 (50.0%) non-Hispanic, and 286 (40.6%) not reported. The number of male and female subjects was 351 (49.8%) and 354 (50.2%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA (HR 0.63). PFS benefit observed in all lines of therapy with concordant CTA-positive/CDx-positive population is consistent with that in all lines CTA-positive randomized subjects (HR 0.63) (Table 14, Figure 4). No clinically meaningful PFS benefit was observed in all lines subjects with the CTA-positive/CDx-negative status, shown in median PFS < 2.5 months in both OPDIVO (nivolumab) plus YERVOY (ipilimumab) and OPDIVO (nivolumab) monotherapy.

**Table 14: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[d]**

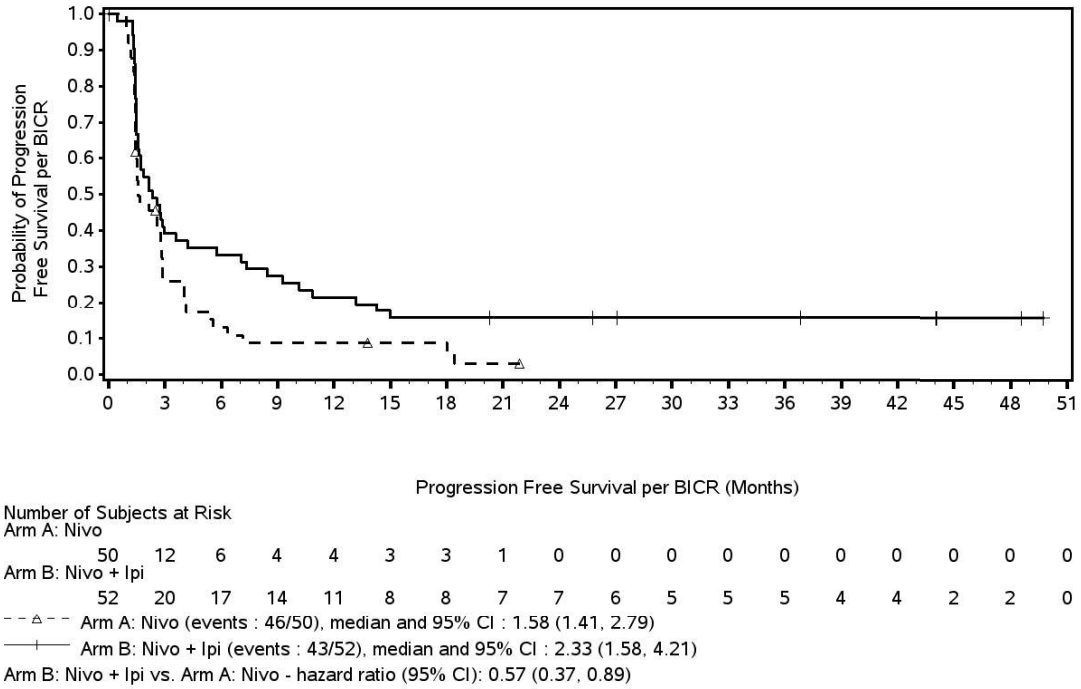
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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), mo[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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NA, not available; NR, not reached; PFS, progression-free survival. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.




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 4. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW)**



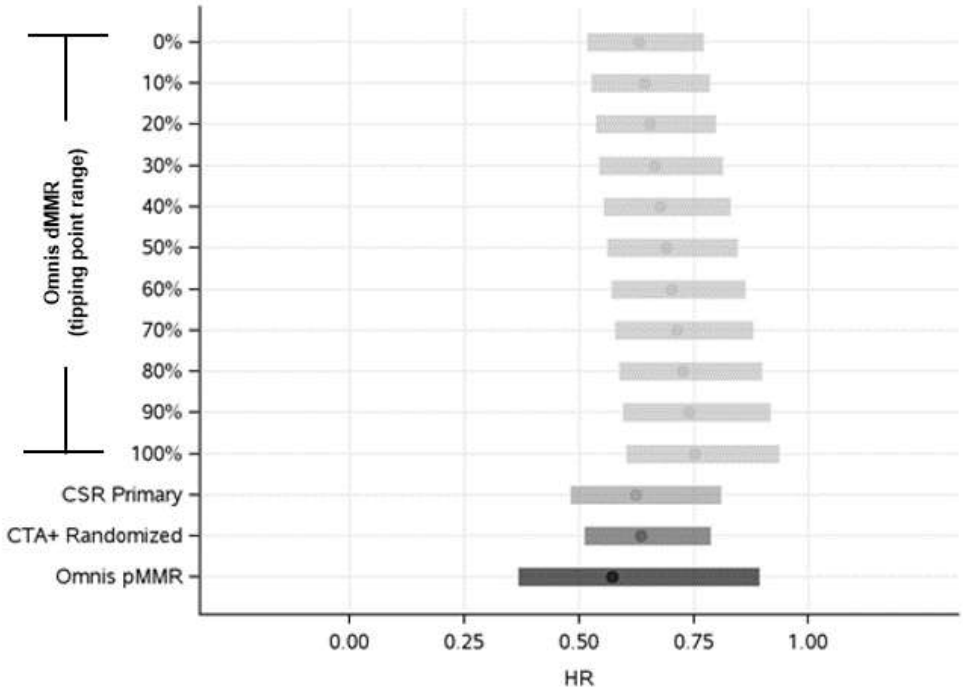

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) and prior lines of therapy (0, 1, ≥2) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first 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 OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW).**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, 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 HR of PFS of the subjects not enrolled (CTA-negative/CDx-positive, HR=1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.63). The tipping point PFS HR CI values ranged from a minimum of 0.52 (0% tipping point range CI lower-bound) to a maximum of 0.94 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.52-0.77, and the 100% tipping point 95% CI range at 0.60-0.94.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 582). **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). 3L+, all lines treatment; BICR, blinded independent central review; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; MSI-H, microsatellite instability high; Nivo, nivolumab; pMMR, mismatch repair proficient.

**Figure 6. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CHECKMATE-8HW).**

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in Objective Response Rate (ORR) over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA, with ORR 63.6% (95% CI: 58.4, 68.7) vs. 49.3% (95% CI: 44.0, 54.6), respectively. ORR benefit observed in 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) vs. 58.3% (95% CI: 52.2, 64.2), respectively (Table 15). No clinically meaningful ORR benefit was observed in all lines subjects with the CTA-positive/CDx-negative status (p=0.0568).

**Table 15: ORR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[c]**

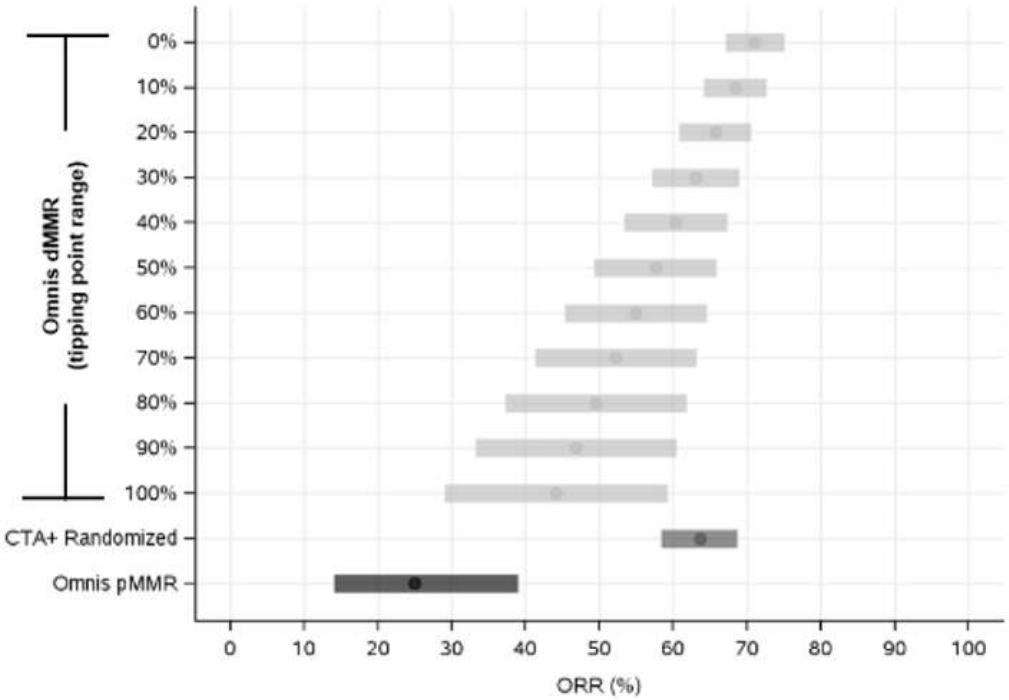
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed dMMR/MSI-H status using clinical trial assay modalities; CDx-positive: deficient mismatch repair (dMMR); CDx-negative: proficient mismatch repair (pMMR); ORR, overall response rate. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.

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. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

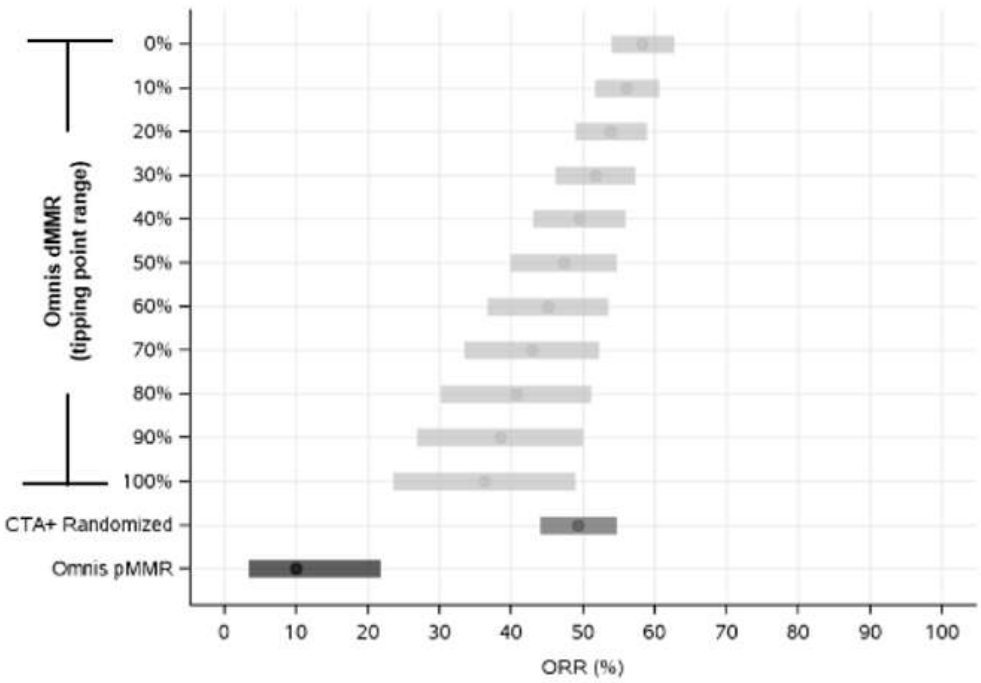
Zero to 100% of the tipping point range was assumed for the missing ORR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of these patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for the ORR of the subjects not enrolled (ORR = 0 for CTA-negative/CDx-positive). The tipping point range of 0% assumes a best-case scenario where the ORR for these subjects is equal to the observed ORR for enrolled subjects (ORR = 0.711 for nivolumab plus ipilimumab in CTA-positive/CDx-positive subjects; ORR = 0.583 for nivolumab monotherapy in CTA-positive/CDx-positive subjects). For nivolumab plus ipilimumab, the tipping point ORR point estimate values ranged from 0.438 to 0.7107 and CI values ranged from a minimum of 0.2881 (100% tipping point range CI lower-bound) to a maximum of 0.7501 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2881-0.5878, and the 0% tipping point 95% CI range at 0.6714-0.7501. For nivolumab monotherapy, the tipping point ORR point estimates ranged from 0.3593 to 0.5830 and CI values ranged from a minimum of 0.233 (100% tipping point range CI lower-bound) to a maximum of 0.6265 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2330-0.4856, and the 0% tipping point 95% CI range at 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).




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 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 = 0.583).**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

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

### 15.3.5 Clinical Performance Summary

In summary, successful bridging between CTA-positive and dMMR status by MMR IHC Panel pharmDx (Dako Omnis) was achieved based on similar clinical utility between patients with dMMR/MSI-H status by CTA and those with dMMR status by MMR IHC Panel pharmDx (Dako Omnis).

A clinically meaningful improvement in PFS per BICR was demonstrated with:

- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to chemotherapy in 1L treatment of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).
- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to OPDIVO (nivolumab) monotherapy in all lines of therapy of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).

Re-estimation of efficacy results using imputations and sensitivity analyses by considering the missing assessments of MMR IHC Panel pharmDx (Dako Omnis) supported these findings.

These results support the clinical performance of MMR IHC Panel pharmDx (Dako Omnis) by demonstrating the assay's clinical utility in identifying patients with dMMR CRC who may benefit from treatment with OPDIVO (nivolumab) alone or in combination with YERVOY (ipilimumab) in accordance with their approved US package inserts.

## 16. Troubleshooting

Refer to the Troubleshooting section in Agilent's Education Guide for remedial action or contact Agilent Pathology Support to report unusual staining.[8]

Dako Omnis is an automated system designed to alert the user if anything in the run has been outside of specifications. Please refer to the Dako Omnis User Guide for details on what conditions are flagged and how. Table 16 is a troubleshooting guide for results and conditions that are not easily identified through the Dako Omnis warning and alert system.

The user should always ensure adherence to the maintenance schedule for the Dako Omnis instrument. Always ensure to use the appropriate controls as described in 'Quality Control', Section 11.

**Table 16. Troubleshooting.**

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| 1. No or weak staining of slides. | 1a. Excessive heating of mounted tissue sections prior to loading on Dako Omnis may lead to loss of visible MSH2 immunoreactivity and/or tissue morphology. | 1a. Dry the tissue sections at 58 ± 2 °C for a maximum of 1 hour, using a calibrated oven with uniform heat distribution.[10] |
| | 1b. Wrong storage conditions used for reagents. | 1b. Check that reagents have been stored correctly according to listed storage conditions. |
| | 1c. Inappropriate fixation method used. | 1c. Ensure that patient tissue is not fixed for too short or too long a time period, that ischemia time has been minimized, and that the correct fixative (10% NBF) was used. |
| | 1d. Reagent is used past its expiration date. | 1d. Check Dako Link Omnis Workstation software to determine if slides were flagged suspicious. Ensure reagent is not used past its expiration date. |
| | 1e. Reagent is used past its onboard stability. | 1e. Check Dako Link Omnis Workstation software to determine if slides were flagged as suspicious. Ensure reagent is not used past its onboard stability. |
| | 1f. Incorrect placement of dynamic gap lids in staining modules. | 1f. Check placement of dynamic gap lids. |
| | 1g. Damaged dynamic gap lids. | 1g. Check integrity of dynamic gap lids. |
| | 1h. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution (50x) concentrate. | 1h. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 1i. Incorrect Target Retrieval Solution is used. | 1i. Ensure that correct Target Retrieval Solution specified in 'Materials Required, but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |
| | 1j. 1x Target Retrieval Solution does not meet pH specifications. | 1j. Check pH of 1x Target Retrieval Solution. If pH is outside acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. Refer to Problem #6 for additional troubleshooting. |
| 2. Excessive nonspecific staining of slides. | 2a. Starch additives used in mounting sections to slides. | 2a. Avoid using starch additives for adhering sections to glass slides. Many additives are immunoreactive. |
| | 2b. Sections dried after staining procedure/prior to coverslipping. | 2b. Verify that the Unloading Station is filled with sufficient water. |
| | | 2b. Avoid stained slides drying out after staining completion (e.g., between removal from Dako Omnis and coverslipping). |
| | 2d. Inappropriate fixation method used. | 2d. Ensure that approved fixative was used. Alternative fixative may cause excessive nonspecific staining. |
| | 2e. Paraffin incompletely removed. | 2e. Check appearance of solvent coupling. Refer to Dako Omnis User Guide for details. |

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| | 2f. Nonspecific binding of reagents to tissue. | 2f. Ensure that correct fixation method of the specimen is used and avoid large areas of necrosis. |
| | 2g. Re-use of mixing strip. | 2g. Ensure that new mixing strips are used. |
| 3. Excessively strong specific staining. | 3. Inappropriate fixation method used. | 3. Ensure that only approved fixatives and fixation methods are used. |
| 4. Tissue detaches from slides. | 4. Use of incorrect slides. | 4. Use FLEX IHC Microscope Slides (Code K8020), or SuperFrost Plus slides. |
| 5. Slide is flagged as suspicious. | 5a. Reagent is used beyond its expiration date. | 5. Slides flagged as suspicious should be evaluated by qualified personnel, contact an Agilent Technologies representative if further action is needed. |
| | 5b. Reagent is stored onboard Dako Omnis beyond its validated onboard stability. | |
| | 5c. Maintenance overdue or other factors. | |
| 6. 1x Target Retrieval Solution does not meet pH specifications | 6a. pH meter is not calibrated correctly. | 6a. Ensure pH meter is calibrated per manufacturer's recommendations. After recalibration, retest pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If pH is outside of the acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. |
| | 6b. Target Retrieval Solution pH is measured at incorrect temperature. | 6b. Ensure that 1x Target Retrieval Solution pH is measured at ambient temperature. |
| | 6c. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution concentrate. | 6c. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 6d. Incorrect Target Retrieval Solution is used. | 6d. Ensure that the correct Target Retrieval Solution specified in 'Materials Required but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |

**NOTE:** If the problem cannot be attributed to any of the causes in Table 16, or if the suggested corrective action fails to resolve the problem, please contact Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found in Agilent's Education Guide (available from www.agilent.com), Atlas of Immunohistology, and Immunoperoxidase Techniques. A Practical Approach to Tumor Diagnosis.[8,12,13]

## 17. References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**Explanation of symbols**

| | | | | | |
|---|---|---|---|---|---|
| REF | Catalogue number | | Temperature limitation | IVD | In vitro diagnostic medical device |
| | Manufacturer | LOT | Batch code | Σ | Contains sufficient for <n> tests |
| | Use by | | Consult instructions for use | BIO | Contains biological material of animal origin |
| | Caution | EC REP | Authorized representative in the European Community | | |

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TX02438/6.00

Revision 2025.08

# MSH6 IHC pharmDx (Dako Omnis)
# Rx Only
# Code GE086

Primary antibody for use with MMR IHC Panel pharmDx (Dako Omnis)
60 tests for use with Dako Omnis

**Table of Contents**

## 1. Intended 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).

## 2. Summary and Explanation

The MMR pathway is used by normal proliferating cells to repair mutations that may occur during DNA replication. Loss of function of any of the following four MMR proteins, MLH1, PMS2, MSH2, MSH6, results in MMR deficiency (dMMR) and can lead to an increased mutation rate, promotion of tumorigenesis, and generation of neoantigens. dMMR tumors may be more responsive to immunotherapies than tumors with functioning MMR pathways due to the increased presence of neoantigens and immune cell recruitment.[1,2] MSH6 IHC pharmDx (Dako Omnis) is part of MMR IHC Panel pharmDx (Dako Omnis), which is an IHC panel that is used to detect loss of function of any of the four MMR proteins.

Bristol-Myers Squibb sponsored trial, CHECKMATE-8HW (CA2098HW), investigated the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in identifying MMR deficient metastatic CRC patients who may respond to treatment with OPDIVO alone or in combination with YERVOY.[3,4]

*OPDIVO and YERVOY are trademarks owned by Bristol-Myers Squibb Company.*

## 3. Principle of Procedure

MSH6 IHC pharmDx (Dako Omnis) is an optimized antibody reagent with the protocol required to complete an IHC staining procedure of FFPE specimens using the Dako Omnis instrument. Following incubation with the primary monoclonal antibody to MSH6, the specimen is sequentially incubated with peroxidase block, two 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 chromogen results in precipitation of a visible reaction product at the site of antigen. The specimen may then be counterstained and coverslipped. Results are interpreted using a bright field microscope. MMR Negative Control Reagent, Rabbit (GE102) slide should be run alongside the MSH6 IHC pharmDx (Dako Omnis) slide. Please consult Dako Omnis User Guide for detailed instructions on loading and unloading of slides, reagents, bulk fluids and waste.

## 4. Materials Provided

The product includes 12 mL of primary antibody to MSH6 protein (approximately 0.18µg/mL) sufficient for 60 tests. The product has been optimized for use with the Dako Omnis instrument. Please refer to the Dako Omnis User Guide for further information.

| *Quantity* | *Description* |
|---|---|
| 1 x 12 mL | **MSH6 IHC pharmDx (Dako Omnis)** |

**MSH6 IHC pharmDx**

**(Dako Omnis)**

Monoclonal rabbit anti-human MSH6, clone EP49, in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.

## 5. Materials Required, but Not Supplied

Dako Omnis (Code GI100)
MLH1 IHC pharmDx (Dako Omnis (Code GE079)
MSH2 IHC pharmDx (Dako Omnis) (Code GE085)
PMS2 IHC pharmDx (Dako Omnis) (Code GE087)
MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101)
MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102)
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 (see 'Quality Control', Section 11)
pH meter

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

*__NOTE__: *Use Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) for MSH6 IHC pharmDx (Dako Omnis) (Code GE086) testing. Do not use EnVision FLEX Target Retrieval Solution, High pH (50x) from EnVision FLEX, High pH (Dako Omnis), Code GV800 or GV823.*

**__NOTE__: *Not all sources of distilled or de-ionized water may be of sufficient quality for IHC reagent preparation. Agilent recommends reagent-grade distilled or de-ionized water (corresponding to Clinical Laboratory Reagent Water [CLRW] standard as specified by CLSI), or water similar in quality to be used for reagent preparation.*[5]

## 6. Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, $NaN_3$ may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.[6]
4. MSH6 IHC pharmDx (Dako Omnis) contains material of animal origin. As with any product derived from biological sources, proper handling procedures should be used in accordance with local requirements.
5. Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection, and disposed of with proper precautions.[7]
6. Incubation times, temperatures, or methods other than those specified may give erroneous results.
7. Reagents have been optimally diluted. Further dilution may result in loss of visible MSH6 immunoreactivity.
8. Paraffin residue may lead to false negative results.
9. Use of reagent volumes other than recommended may result in loss of visible MSH6 immunoreactivity.
10. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
11. Unused solution should be disposed of in accordance with all local, regional, national and international regulations.
12. Safety Data Sheets are available on www.agilent.com or on request.
13. Lack of adherence to the maintenance schedule for the Dako Omnis instrument may give erroneous results. Refer to Dako Omnis User Guides for additional information and for additional instrument-related precautions.
14. Contact Agilent Pathology Support via www.agilent.com to report any unusual staining.

## 7. Storage

Store MSH6 IHC pharmDx (Dako Omnis) in the original product packaging at 2–8 °C when not in use on Dako Omnis. During storage, the flip top vial cap should be closed.

**Do not use the reagent after the expiration date printed on the reagent vial label.** If the reagents are stored under any conditions other than those specified in the instructions for use, they must be validated by the user. The expiry date on the label is valid for unopened vials as well as opened (in-use) vials when handled according to instructions.

Onboard reagent stability for MSH6 IHC pharmDx (Dako Omnis) has been validated to 375 hours. After staining completion, the reagents should be removed from Dako Omnis and stored in the original product packaging at 2–8 °C with flip top vial caps closed securely on the vials. For onboard stability of all ancillary components including diluted working solutions of Wash Buffer and Target Retrieval Solution, pH 9, refer to respective instructions for use. Onboard time of reagents is tracked by the Dako Omnis software; refer to Dako Omnis User Guide for details.

**NOTE**: There are no obvious visual signs to indicate incorrect product storage or handling of this product during the product's shelf life. Positive and negative controls should be run simultaneously with patient specimens, preferably on the same slide, to monitor product performance during the product's shelf life. If a problem is suspected with the antibody during the shelf life that cannot be explained by incorrect product storage or handling, or other variations in laboratory procedures, contact Agilent Pathology Support. Refer to 'Quality Control', Section 11 and 'Troubleshooting', Section 16 for more information.

## 8. Specimen Preparation

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

### 8.1. Paraffin-embedded tissue

FFPE tissues are suitable for use with the primary antibody to the MSH6 protein. 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 [such as 10% unbuffered formalin, Bouin's fixative, and acetic formalin alcohol (AFA)] have not been validated and may give erroneous results. 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.

### 8.2. 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.

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.

**NOTE**: The tissue specimens must be positioned on the glass within the defined slide staining area. Please consult the Dako Omnis User Guide for dimensions of slide staining area.

## 9. Reagent Preparation

The user should adhere to appropriate personal protective equipment requirements and become familiar with all components prior to use (see 'Precautions', Section 6).

Target Retrieval Solution, pH 9 (50x) (Code GC309) and Wash Buffer (20x) (Code GC807) must be prepared according to their respective instructions for use. Refer to the GC309 and GC807 instructions for use 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.

## 10. Staining Procedure

**Procedural Notes**

The user should read these instructions carefully and become familiar with all the components and the instrumentation prior to use (see 'Precautions', Section 6).

The automated staining procedure for MSH6 IHC pharmDx (Dako Omnis) on Dako Omnis includes deparaffinization of tissue sections, target retrieval, and staining. The slides are unloaded in the Unloading Station. All protocol steps are preprogrammed into the Dako Link Omnis Workstation software. The "MMR MSH6 IHC pDx GE086**"** protocol is used for MSH6 IHC pharmDx (Dako Omnis). If the appropriate MSH6 IHC pharmDx (Dako Omnis) protocol is not on your server, please contact your local Technical Service Representative or Agilent Pathology Support to obtain the protocols. Refer to the Dako Omnis User Guide for further information on how to operate and maintain the instruments.

**NOTE:** The MSH6 reagent and instructions supplied with this product have been designed for optimal performance. Further dilution of the antibody or alteration of staining protocol may give erroneous or discordant results. Differences in tissue processing and technical procedures in the user's laboratory may invalidate the assay results.

**NOTE**: Laboratories located at high elevations should determine the best method of maintaining the required temperature (97 °C) during heat-induced epitope retrieval. Any adjustments required to address elevation concerns must be validated by the user. Refer to the Dako Omnis User Guide for additional information.

**Prestaining procedure**

1. Choose the MMR MSH6 IHC pDx GE086 protocol to be applied for each slide from the Dako Link Omnis Workstation software.
2. Ensure the Dako Link Omnis Workstation software is configured to print slide labels with the protocol name displayed.
3. Print slide labels and attach them to the glass slides.
4. Place the slides in the Slide Rack. A Slide Rack can hold from one to five slides.
5. Ensure that the bulk bottles with fluids are onboard and registered by the Dako Omnis instrument. Bulk bottle fluids:
    a. Clearify™ clearing agent (Code GC810)
    b. Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) **diluted to 1x working concentration with distilled or de-ionized water.**
    c. Wash buffer (Code GC807) **diluted to 1x working concentration with distilled or de-ionized water.**
6. Ensure that all flip top vial caps are open and locked in place before loading all required reagents in the Reagent Storage Module:
    a. MSH6 IHC pharmDx (Dako Omnis) (Code GE086)
    b. EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis) (Code GV800)
    c. EnVision FLEX Visualization Reagent (Dako Omnis) (Code GV800)
    d. EnVision FLEX Substrate Buffer (Dako Omnis) (Code GV800)
    e. EnVision FLEX DAB+ Chromogen (Dako Omnis) (Code GV800)
    f. EnVision FLEX+ Mouse LINKER (Dako Omnis) (Code GV821)
    g. EnVision FLEX+ Rabbit LINKER (Dako Omnis) (Code GV809)
    h. Optional: Hematoxylin (Dako Omnis) (Code GC808) or equivalent
    i. Sulfuric Acid (Code GC203)
7. Load the Slide Rack onto Dako Omnis.
8. Follow the instructions on the Touch Screen and tap "Done" to initiate the staining procedure.
9. Ensure the slide Unloading Station is filled with distilled or de-ionized water to prevent slides from drying.

**NOTE:** When using the overnight staining feature (delayed start) slides must be removed from the Unloading Station the morning the staining has been completed.

**NOTE:** The MSH6 IHC pharmDx (Dako Omnis) protocol on the Dako Omnis instrument can be monitored on the Dako Link Omnis Workstation.

**Dako Omnis Staining Protocol**

When processing slides for staining with the MMR IHC Panel pharmDx (Dako Omnis) assay, the Dako Omnis automated platform executes the protocols for MMR MSH6 IHC pDx GE086 as stated in Table 1. Refer to MMR Negative Control Reagent, Rabbit (GE102) IFU for details on the GE102 staining protocol. The MMR MSH6 IHC pDx GE086 protocol has been designed for optimal performance. Any changes to the staining protocol may alter the performance of the device and must be validated by the user. Unless otherwise noted, each step of the staining procedure is executed at the instrument's fixed temperature of 32ºC. The instrument's fixed temperature is not an editable parameter.

**Table 1. MMR MSH6 IHC pDx GE086 Staining Protocol**

| Protocol Step | Reagent | Setting |
|---|---|---|
| **Dewax** | Clearify Clearing Agent | 25 °C, 10 s incubation top, 1 min incubation bottom, 1 cycle |
| | DI Water | 5 s incubation, 1 cycle |
| **Target retrieval** | TRS, pH 9* | 97 °C*, 30 min incubation* |
| | Cooling fluid DI Water | N/A |
| **Staining** | Wash Buffer | 2:40 min incubation, 2 cycles |
| | Primary antibody (MSH6*) | 20 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Peroxidase-Blocking Reagent | 3 min incubation |
| | Wash buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Rabbit LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Mouse LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX/HRP | 20 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Substrate Working Solution | 5 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| **Counterstaining** | Hematoxylin* | 3 min incubation* |
| | DI Water* | 2 min incubation, 10 cycles |
| | Wash Buffer* | 2 min incubation, 10 cycles |

*Parameter is editable by the end user when creating a copy. Any changes to the staining protocol may alter the performance of the device and must be validated by the user.

**Counterstain**

Slides may be counterstained with Hematoxylin (Dako Omnis) (Code GC808). The "MMR MSH6 IHC pDx GE086" protocol on Dako Omnis includes a counterstaining step that is set as editable for the user. If a counterstain other than the recommended Hematoxylin (Dako Omnis) (Code GC808) is used, the alternative counterstain must be validated by the user. See the Dako Omnis User Guide for further information on editing protocols.

Preprogrammed:

Slides are counterstained for 3 minutes with Hematoxylin (Dako Omnis) (Code GC808). The Hematoxylin incubation time is preprogrammed in the protocol. Slides are ready for mounting when removed from the Unloading Station.

User Defined:

If the selected protocol does not include an automated counterstaining process, it is the responsibility of the user to counterstain the specimen(s) per internally validated procedure prior to mounting.

**Mounting**

After staining onboard Dako Omnis, the sections must be dehydrated, cleared, and mounted using nonaqueous, permanent mounting methods.

**NOTE:** Some fading of stained slides may occur, depending on several factors including, but not limited to, counterstaining, mounting materials and methods, and slide storage conditions. To minimize fading, store stained slides in the dark at room temperature (20–25 °C).

## 11. Quality Control

MSH6 IHC pharmDx (Dako Omnis) has been quality controlled for IHC using the required reagents and staining procedures outlined in 'Reagent Preparation', Section 9 and 'Staining Procedure', Section 10. Deviations from the recommended procedures may lead to significant variability in results. Consult the quality control guidelines of the College of American Pathologists (CAP) Accreditation Program for Immunohistochemistry. See also Agilent's Education Guide: Immunohistochemical Staining Methods and CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[8,9]

### 11.1. 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 must be used, where possible, as internal positive controls instead of a separate positive control tissue. In very rare cases, nonmalignant elements may have loss of biomarker expression, in which case nonmalignant elements of the negative control tissue may be used to qualify the staining procedure.

Refer to Table 4 for further information on positive and negative control tissues.

### 11.2. Negative control reagent

MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102) should be used in place of the 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 Rb GE102" for slides stained with the negative control reagent (NCR). Refer to the MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102) instructions for use for details.

### 11.3. Assay verification

Prior to initial use of a staining system in a diagnostic procedure, the user should verify the assay's performance by testing it on a series of lab-supplied tissues with known IHC performance characteristics representing known positive and negative tissues. Refer to the quality control procedures outlined in 'Quality Control', Section 11, as well as to the quality control requirements of the CAP Certification Program for Immunohistochemistry and/or CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[9] These quality control procedures should be repeated for each new primary antibody lot. Troubleshooting options for potential problems, their causes and suggested corrective actions are outlined in Table 16.

## 12. Staining Interpretation

A Hematoxylin and Eosin (H&E) stained section is used to determine if a specimen is acceptable for IHC. MMR IHC Panel pharmDx (Dako Omnis) and H&E staining should be performed on serial sections from the same FFPE block of the specimen to confirm:

1. The histologic diagnosis of CRC.
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.

Each specimen should be evaluated using 4x–20x magnification. The specific staining pattern 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.

The entire tissue section should be considered, avoiding edge effects, noninvasive components, necrotic areas, and areas with obvious fixation artifacts. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.

Nonspecific cytoplasmic staining may be present in some tissues. 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, epithelia cells
2. Edge effects

3. Necrotic areas
4. Areas with noninvasive components (normal epithelium, adenoma)
5. Areas with obvious fixation artifacts should not be scored or scored with caution

Protein status of Intact or Loss is determined for MSH6 using the guidelines described in Table 2.

**Table 2. Determination of MSH6 Intact or Loss status.**

| | |
|---|---|
| Intact | Nuclear staining in viable malignant cells must be unequivocal, with at least the same overall staining intensity as in adjacent internal positive controls.<br>If focal staining is present, the tissue is considered intact if:<br>1) continuous in multiple glands/nests<br>and<br>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.<br>If focal staining is present, the tissue is considered loss if:<br>1) continuous in only a single gland/nest,<br>2) discontinuous in multiple glands/nests,<br>or<br>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.

After a protein status of Intact or Loss is assigned to each biomarker (MLH1, PMS2, MSH2, and MSH6) for a given specimen, a diagnostic status of MMR proficient or MMR deficient is given using the definitions in Table 3.

**Table 3. Definitions of MMR proficient and MMR deficient.**

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

Some cases may be more challenging to interpret due to particular staining patterns, morphology, nonspecific staining, and/or tissue or staining artifacts. For additional guidance on MMR staining interpretation and examples of challenging cases, refer to the MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual for details.

### 13. Tissue Evaluation

The following table provides the order of slide evaluation for interpretation of MSH6 IHC pharmDx (Dako Omnis). Per the intended use and MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual, evaluation must be performed in conjunction with other required biomarkers.

**Table 4. Recommended order of tissue evaluation.**

| Tissue | Rationale | Requirements |
|---|---|---|
| 1. Patient tissue stained with H&E | An H&E stain of the patient tissue is evaluated first to assess tissue histology and preservation quality.<br>Note: The H&E may be reviewed again in the context of the patient tissue slides stained with the NCR and primary antibody (Steps 5 and 6) | The H&E and MMR IHC Panel pharmDx (Dako Omnis) stains should be performed on serial sections from the same FFPE block of the specimen.<br>Tissue specimens should be well preserved, confirm the diagnosis of CRC, and include at least 50 viable malignant tumor cells. |

| Tissue | Rationale | Requirements |
|---|---|---|
| 2. Positive control stained with primary antibody to the MSH6 protein (GE086) | The positive control tissue stained with primary antibody to the MSH6 protein should be examined next.<br><br>**Patient CRC tissues** contain positive nonmalignant elements that serve as an internal positive control. This internal positive control eliminates the need for a separate control tissue.<br><br>In rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In cases that demonstrate complete loss of biomarker expression in nonmalignant elements, internal positive controls within the negative control tissue should be reviewed to qualify the staining procedure.<br><br>Positive tissue controls should be used for monitoring the performance of processed tissues and test reagents. | Nuclear staining of benign or normal epithelium, lymphocytes, and stromal cells present in the patient tissue must demonstrate moderate to strong staining intensity.<br><br>If patient tissue demonstrates focal loss of biomarker expression in nonmalignant elements, the remaining areas with nuclear staining in the internal positive control elements may still be used to qualify the staining procedure.<br><br>If patient tissue demonstrates complete loss of biomarker expression in both malignant and nonmalignant elements, use internal positive control within the negative control tissue.<br><br>If the internal positive controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid. |
| 3. Negative control tissue stained with primary antibody to the MSH6 protein (GE086)<br><br>(Lab-supplied) | The negative control tissue stained with primary antibody to the MSH6 protein should be examined next to verify labeling specificity of the target antigen by the primary antibody. | Negative control tissue should be prescreened CRC tissues with loss of MSH6 expression.<br><br>At least one negative control tissue section should be included in each staining procedure, either as an on-slide control or as a separate negative control slide. On-slide tissue controls are recommended and eliminate the need for a separate control slide.<br><br>Slides stained with primary antibody to the MSH6 protein should exhibit no nuclear staining or focal weak nuclear staining in malignant tumor cells in the presence of moderate to strong staining in internal positive controls in the tumor area. Internal positive control elements include nuclear staining in normal epithelium, lymphocytes, or stroma.<br><br>If the negative tissue controls fail to demonstrate appropriate staining, results with the test specimens should be considered invalid. |
| 4. Optional:<br><br>Negative control tissue stained with MMR Negative Control Reagent, Rabbit (GE102)<br><br>(Lab-supplied) | NCR may be used to stain the negative control tissue specimen if needed for troubleshooting purposes. | NCR slides must exhibit no or weak staining in malignant tumor cells. |

| Tissue | Rationale | Requirements |
|---|---|---|
| 5. Patient tissue stained with MMR Negative Control Reagent, Rabbit (GE102) | Examine patient specimens stained with NCR. NCR is used in place of the primary antibody and aids in interpretation of specific staining at the antigen site. | NCR slides must exhibit no or weak staining in malignant tumor cells. If weak staining is present in tumor nuclei, it should be used as a baseline to evaluate the MSH6 slide. Staining at the same intensity or lower that may occur in the MSH6 antibody slide should be disregarded upon interpretation.<br><br>NCR slides with moderate or strong staining in malignant tumor cells are invalid and the corresponding MSH6 slide is considered nonevaluable. The patient tissue must be retested. |
| 6. Patient tissue stained with primary antibody to the MSH6 protein (GE086) | Examine the patient specimen stained with the primary antibody to the MSH6 protein last to assess MSH6 protein status. | All malignant tumor cells should be evaluated for MSH6 protein expression and included in the MSH6 protein scoring assessment. Positive staining intensity should be assessed within the context of any nonspecific staining observed on the patients NCR slide. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.<br><br>In very rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In such cases, the patient tissue may still be evaluated if the staining procedure is qualified using the internal positive controls within the negative control tissue.<br><br>As with any IHC test, absence of staining means that the antigen was not detected, not necessarily that the antigen was absent in the cells/tissue assayed.<br><br>For staining interpretation guidelines, refer to 'Staining Interpretation', Section 12. |

## 14. Limitations

### 14.1. General limitations

1. For prescription use only (Rx only).
2. IHC is a multistep process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
3. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any staining or its absence must be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls. It is the responsibility of a qualified pathologist, who is familiar with the antibodies, reagents and methods used, to interpret the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
6. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[10]
7. Reagents may demonstrate unexpected reactions in previously untested tissue types. The possibility of unexpected reactions even in tested tissue types cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Agilent Pathology Support with documented unexpected reactions.
8. False-positive results may be seen due to nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes) and endogenous peroxidase activity (cytochrome C).[10]
9. The reagents and instructions supplied for this assay have been designed for optimal performance. Further dilution of the reagents or alteration of incubation times or temperatures may give erroneous or discordant results.
10. Slides flagged in the slide log on the Dako Omnis Workstation should be investigated by qualified personnel. Refer to the Dako Omnis User Guide for further details.
11. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date. Improper storage and use of reagents may lead to erroneous results.
12. Canceled slides indicate a significant issue occurred during staining and should not be used. The specimen will require restaining. Refer to the Dako Omnis User Guide for further details.
13. This device is not intended to be used to identify patients with Lynch syndrome or to differentiate between sporadic CRC and Lynch syndrome.

### 14.2. Product-specific limitations

1. False-negative results could be caused by degradation of the antigen in the tissues over time. Once mounted on slides, specimens should be stored in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C. Tissue sections should be stained within 2 months of sectioning.

2. Use of MSH6 IHC pharmDx (Dako Omnis) on specimens fixed in fixatives other than NBF has not been validated.
3. Use of MSH6 IHC pharmDx (Dako Omnis) on decalcified tissues has not been validated.
4. Reduced staining was observed with 10% unbuffered formalin, Bouin's fixative, and AFA, so they are not acceptable for use with this assay.
5. This product has undergone a transport simulation study to account for anticipated temperature variations during ambient condition shipping. However, it is possible that this product, when shipped under ambient conditions, may be exposed to shipping conditions outside of tested ranges (-20ºC to 37ºC). Therefore, it is essential to use controls, as specified in this IFU, to confirm expected performance of this product.

## 15. Performance Evaluation

### 15.1. Analytical performance evaluation: normal and neoplastic tissues

Table 5 summarizes monoclonal rabbit anti-MSH6, clone EP49, immunoreactivity on the recommended panel of normal tissues. Table 6 summarizes monoclonal rabbit anti-MSH6, clone EP49, immunoreactivity on a panel of neoplastic tissues. All tissues were FFPE and stained with MSH6 IHC pharmDx (Dako Omnis) according to the instructions in this package insert. Nuclear staining was observed in a majority of tissue types tested. In some cases, cytoplasmic and/or extracellular staining was observed.

**Table 5. Summary of MSH6 IHC pharmDx (Dako Omnis) normal tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|---|---|
| Adrenal (3) | 2/3 | Lung (3) | 3/3 | Salivary gland (3) | 3/3 |
| Bladder (3) | 3/3 | Mesothelial cells (3) | 3/3 | Skin (3) | 3/3 |
| Bone marrow (3) | 2/3* | Muscle, cardiac (3) | 2/3 | Small intestine (3) | 3/3 |
| Breast (3) | 2/3 | Muscle, skeletal (3) | 3/3 | Spleen (3) | 2/3 |
| Cerebellum (3) | 1/3 | Nerve, peripheral (3) | 1/3 | Stomach (3) | 2/3 |
| Cerebrum (3) | 1/3 | Ovary (3) | 3/3 | Testis (3) | 3/3 |
| Cervix (3) | 3/3 | Pancreas (3) | 3/3 | Thymus (3) | 3/3** |
| Colon (3) | 3/3 | Parathyroid (3) | 3/3 | Thyroid (3) | 2/3 |
| Esophagus (3) | 3/3 | Pituitary (3) | 1/3 | Tonsil (3) | 3/3 |
| Kidney (3) | 3/3 | Prostate (3) | 3/3 | Uterus (3) | 3/3 |
| Liver (3) | 3/3* | | | | |

*cytoplasmic staining pattern for at least one case
**cytoplasmic and extracellular staining pattern for at least one case

**Table 6. Summary of MSH6 IHC pharmDx (Dako Omnis) neoplastic tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|
| Bladder carcinoma (2) | 2/2 | Pancreatic adenocarcinoma (1) | 1/1 |
| Breast carcinoma (5) | 4/5 | Pancreatic glucagonoma (1) | 1/1 |
| Cholangiocarcinoma (1) | 1/1 | Pleomorphic rhabdomyosarcoma (1) | 1/1 |
| Colon adenocarcinoma (1) | 1/1 | PNET scrotum (1) | 1/1 |
| Endometrial sarcoma (1) | 1/1 | Prostate adenocarcinoma (1) | 1/1 |
| Ewing's sarcoma (1) | 1/1 | Prostate benign prostatic hyperplasia (1) | 1/1 |
| Gastric adenocarcinoma (2) | 2/2 | Squamous carcinoma of ear (1) | 1/1 |
| Lung carcinoma (3) | 3/3 | Testicular embryonal carcinoma (1) | 1/1 |
| Lymphoma of cecum (1) | 1/1 | Testicular yolk sac tumor (1) | 1/1 |
| Melanoma (3) | 3/3 | Thymic carcinoid tumor (1) | 1/1 |
| Merkel cell tumor (1) | 1/1 | Thymoma (1) | 1/1 |
| Ovarian carcinoma (2) | 2/2 | Thyroid carcinoma (2) | 2/2 |
| Ovarian dysgerminoma (1) | 1/1 | Uterine adenomatoid tumor (1) | 1/1 |
| Ovarian granulosa cell tumor (1) | 1/1 | | |

### 15.2. Analytical performance evaluation: CRC

#### 15.2.1. Analytical sensitivity

Analytical sensitivity of MSH6 IHC pharmDx (Dako Omnis) was evaluated across 171 unique specimens of FFPE CRC tissues. The prevalence of loss of MSH6 expression observed was 1.2% (2/171). Assessment of MMR IHC Panel pharmDx (Dako Omnis) staining in these 171 unique specimens demonstrated a dMMR prevalence of 8.8% (15/171).

#### 15.2.2. Precision

The precision of MSH6 IHC pharmDx (Dako Omnis) was evaluated. Diagnostic status was recorded as 'Intact' or 'Loss'. The inter-observer analysis was conducted to evaluate the scoring precision of MSH6 IHC pharmDx (Dako Omnis) across multiple observers at a single site. The study was not designed with this supplemental analysis in mind which led to an imbalance in cases with MSH6 'Intact' and 'Loss' status; therefore, any results reliant on sample size in the 'Loss' population supplemental analysis will be underpowered. Percent agreement of loss (LPA), percent agreement of intact (IPA) and overall percent agreement (OA) 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 100.0%.

**Table 7. Precision of MSH6 IHC pharmDx (Dako Omnis) tested at one site.**

| 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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (92.6, 100.0)<br>(92.6, 100.0)<br>(96.2, 100.0) |
| 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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (92.0, 100.0)<br>(92.6, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (94.9, 100.0)<br>(94.9, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>98.6<br>99.2 | (92.9, 100.0)<br>(95.7, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (94.9, 100.0)<br>(94.9, 100.0)<br>(97.4, 100.0) |
| Inter-Observer | 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<br>IPA<br>OA | 82.4<br>99.4<br>97.7 | (70.6, 94.4)<br>(98.1, 100.0)<br>(95.9, 99.4) |

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

Additionally, the precision of MMR IHC Panel pharmDx (Dako Omnis) scoring across multiple observers at a single site was evaluated. Diagnostic status was recorded as 'pMMR' or 'dMMR'. dMPA, pMPA and OA were computed with corresponding two-sided 95% percentile bootstrap CIs.

**Table 8. Inter-Observer precision of MMR IHC Panel pharmDx (Dako Omnis) tested at one site.**

| Precision Study | Study Design | % Agreement (95% CI) | | |
|---|---|---|---|---|
| Inter-Observer | One set of 58 CRC 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<br>pMPA<br>OA | 95.7<br>98.8<br>97.1 | (91.3, 98.9)<br>(96.2, 100.0)<br>(94.7, 99.4) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

### 15.2.3. External reproducibility

The reproducibility of MSH6 IHC pharmDx (Dako Omnis) was evaluated at three external testing sites. Diagnostic status was recorded as 'Intact' or 'Loss'. LPA, IPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 9. Reproducibility of MSH6 IHC pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>IPA<br>OA | 97.2<br>99.1<br>98.6 | (91.3, 100.0)<br>(97.7, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>99.1<br>99.3 | (94.7, 100.0)<br>(97.7, 100.0)<br>(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<br>IPA<br>OA | 98.1<br>100.0<br>99.6 | (95.4, 100.0)<br>(99.1, 100.0)<br>(99.1, 100.0) |

| Reproducibility Study | Study Design | % Agreement (95% CI) | | |
|---|---|---|---|---|
| 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. | NPA<br>PPA<br>OA | 98.1<br>100.0<br>99.6 | (95.4, 100.0)<br>(99.1, 100.0)<br>(99.1, 100.0) |

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

In the same study, the reproducibility of MMR IHC pharmDx (Dako Omnis) was analyzed. MMR diagnostic status was recorded as 'Proficient' (pMMR) or 'Deficient' (dMMR). dMPA, pMPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 10. Reproducibility of MMR IHC Panel pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>pMPA<br>OA | 98.6<br>99.3<br>99.0 | (95.7, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 100.0<br>99.3<br>99.7 | (97.3, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |
| 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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

### 15.3. Clinical performance evaluation: colorectal cancer (OPDIVO [nivolumab] alone and OPDIVO [nivolumab] in combination with YERVOY [ipilimumab])

CHECKMATE-8HW was a phase 3, randomized, open-label, multi-center, three-arm clinical trial of nivolumab monotherapy, nivolumab plus ipilimumab combination therapy, or standard chemotherapy in recurrent or metastatic dMMR/microsatellite instability high (MSI-H) CRC across lines of therapy. CHECKMATE-8HW had dual primary objectives: comparing clinical efficacy as evaluated through progression-free survival (PFS) per blinded independent central review (BICR) of nivolumab plus ipilimumab vs chemotherapy in first-line treatment (1L) and comparing clinical efficacy as evaluated through PFS per BICR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of therapy.

#### 15.3.1. Clinical Study Overview

CHECKMATE-8HW was a randomized, 3-arm, open-label trial in immunotherapy-naive patients across all lines of therapy with unresectable or metastatic CRC with known tumor MSI-H or dMMR (MSI-H/dMMR) status as determined in accordance with local standard of practice.

Eligible patients were ≥ 18 years of age, with recurrent or metastatic dMMR or MSI-H CRC not amenable to surgery. Enrollment was based on confirmation of dMMR/MSI-H status by local standard of practice, referred to here as the Clinical Trial Assay (CTA). Modalities for the CTA included: IHC, polymerase chain reaction (PCR), or next generation sequencing (NGS). Patients were considered CTA-positive and eligible for enrollment if they were identified as dMMR and/or MSI-H by at least one of the CTA modalities. Patients were randomized to OPDIVO (nivolumab) monotherapy, OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy, or investigator's choice chemotherapy in a 2:2:1 ratio. Patients who progressed after 2 prior lines of therapy were randomized in a 1:1 ratio to OPDIVO (nivolumab) monotherapy or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. Randomization was stratified by tumor location (right vs left) and by prior lines of therapy (0, 1, 2L+).

The clinical efficacy of the OPDIVO (nivolumab) and OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination was evaluated in the randomized patient population with centrally confirmed MSI-H/dMMR status. Central assessment of MSI-H status using PCR (Idylla MSI) test and dMMR status using MMR IHC Panel pharmDx (Dako Omnis) was conducted retrospectively on patient tumor specimens used for local MSI-H/dMMR status determination. Patients with confirmed MSI-H/dMMR status by either central test comprised the primary drug efficacy population.

The evaluation of the drug efficacy relied on the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) versus chemotherapy in the first-line (1L) setting and the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines setting. The major efficacy outcome measure was BICR-assessed PFS per RECIST 1.1.

#### 15.3.2. IVD Bridging Study

Specimens from CHECKMATE-8HW were analyzed in an IVD bridging study to establish the clinical performance of the companion diagnostic MMR IHC Panel pharmDx (Dako Omnis) for detection of dMMR in patients with unresectable or metastatic CRC (mCRC) who would benefit from OPDIVO (nivolumab) alone or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. The

IVD bridging study was designed to bridge the clinical efficacy from the CTA-positive population (dMMR and/or MSI-H by at least one of the local modalities, including IHC, PCR, and/or NGS) to the intended use population of dMMR by MMR IHC Panel pharmDx (Dako Omnis), which will be demonstrated by the clinical utility analysis through concordance (positive percent agreement [PPA] and negative percent agreement [NPA]) of MMR IHC Panel pharmDx (Dako Omnis) against CTA.

The endpoints to demonstrate the clinical utility of the companion diagnostic are:

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects with mCRC.

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab)

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines randomized subjects with mCRC.

Analyses were performed to demonstrate comparable efficacy based on dMMR by MMR IHC Panel pharmDx (Dako Omnis). These analyses support the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population.

**15.3.3 Concordance Analysis**

For the concordance analysis, PPA between CTA-positive+ and dMMR by MMR IHC Panel pharmDx (Dako Omnis) was estimated using CHECKMATE-8HW clinical samples. 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, NPA was assessed using commercially procured samples that were predetermined as pMMR and/or MSS using test methods representative of the CTA.

The PPA and the two-sided 95% confidence interval (CI) were calculated between CTA-positive and dMMR by MMR IHC Panel pharmDx (Dako Omnis) using clinical specimens and CTA-positive status as the reference. The NPA and the two-sided 95% CI were calculated between procured samples with pMMR/MSS status (CTA-negative) and pMMR by MMR IHC Panel pharmDx (Dako Omnis) with CTA-negative as the reference. There are no formal acceptance criteria for PPA and NPA. The success criteria for the IVD bridging study depends on the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) dMMR for its intended use.

The clinical efficacy of OPDIVO (nivolumab) plus YERVOY (ipilimumab) from CHECKMATE-8HW was based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the randomized patients as well as the PFS comparisons of OPDIVO (nivolumab) plus ipilimumab vs OPDIVO (nivolumab) alone in all lines of the randomized patients. Each PFS comparison was estimated by hazard ratio (HR) and the 95% CI in a stratified Cox proportional hazards model using the randomized arm as a single covariate; line of therapy (for all lines comparison) and tumor sidedness as the stratification factors. PFS curves were estimated and presented using Kaplan-Meier product-limit methodology from the patients with the concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1). Median PFS with two-sided 95% CI using the Brookmeyer and Crowley method (with log-log transformation) was computed. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 2.

Additionally, to assess the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population to identify dMMR patients with mCRC treated with OPDIVO (nivolumab) plus YERVOY (ipilimumab), a tipping point analysis was conducted to consider the missing patients who were not enrolled due to their local pMMR/MSS status, which were potentially misclassified by the CTA and may be dMMR by MMR IHC Panel pharmDx (Dako Omnis). Tipping point analysis was conducted by assuming that the PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of these patients ranged from the best scenario (i.e., HR equal to that estimable from the enrolled patients with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis)), to the worst scenario (i.e. HR equal to 1). A full range of the tipping point analysis results were assessed for the clinical utility of dMMR status by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population. The tipping point analysis is shown graphically in Figure 3.

The clinical utility for MMR IHC Panel pharmDx (Dako Omnis) to identify dMMR patients in the intended use population for treatment with OPDIVO (nivolumab) plus YERVOY (ipilimumab) was also based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines of the randomized patients and of the centrally confirmed dMMR patients by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4) per the same methods outlined above. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 5. Tipping point analysis was also conducted for this clinical endpoint per the same methods outlined above. The tipping point analysis is shown graphically in Figure 6.

A total of 839 subjects were randomized in CHECKMATE-8HW, and of those, 837 were CTA-positive. Of the 837 CTA-positive randomized subjects, 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). To avoid introducing bias to the concordance and the clinical performance evaluation, imputations and sensitivity analyses were conducted after these analyses were done with the available data and are reflected in the clinical performance results presented below.

**15.3.2.1. Concordance results**

Concordance between the CTA and MMR IHC Panel pharmDx (Dako Omnis) was assessed via PPA using CHECKMATE-8HW clinical samples and NPA using commercially-procured samples (Tables 11 and 12). The conservative estimate for MMR IHC Panel pharmDx (Dako Omnis) positivity rate is 79.1% (662/837), assuming all 60 excluded samples would have resulted in a negative status by MMR IHC Panel pharmDx (Dako Omnis). Point estimates for positive percent agreement (PPA) and negative percent agreement (NPA) were 85.2% and 97.5%, respectively. The level of agreement achieved between the CTA and MMR IHC Panel pharmDx (Dako Omnis) is shown in Table 12.

**Table 11. Specimen distribution of comparison between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| | | CTA | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| MMR IHC Panel PharmDx (Dako Omnis) | Positive | 662 | 5 | 667 |
| | Negative | 115 | 199 | 314 |
| Total | | 777 | 204 | 981 |

**Table 12. Analytical concordance between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| Performance Criteria | Point Estimate of Percent Agreement (95% CI, Wilson Score) |
|---|---|
| PPA | 85.2 (82.5, 87.5) |
| NPA | 97.5 (94.4, 98.9) |

CI, confidence interval by Wilson score method; NPA, negative percent agreement; PPA, positive percent agreement.

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 utility analysis 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.

### 15.3.3. Clinical Efficacy Results

#### 15.3.3.1. OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects

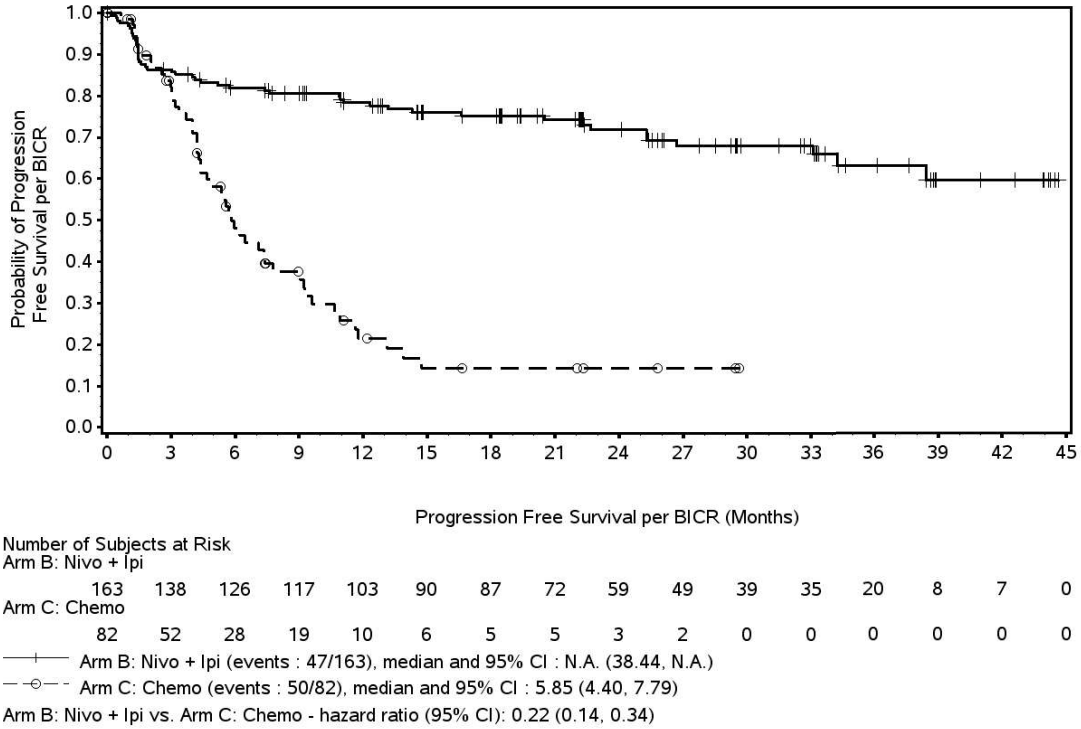
A total of 301 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 200) or chemotherapy (n = 101). The study included 88 sites in 22 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, 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. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the chemotherapy group was 65 years. Most subjects were white (n=259, 86%), 32 (10.6%) were Asian, 4 (1.3%) were black. The ethnicity of subjects was 32 (10.6%) Hispanic,150 (49.8%) non-Hispanic, and 119 (39.5%) not reported. The number of male and female subjects was 139 (46.2%) and 162 (53.8%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR (median PFS not reached) over chemotherapy (PFS 6.21 months) in 1L randomized subjects with dMMR/MSI-H status determined by the CTA (HR 0.32) (Table 12). The PFS benefit observed in 1L randomized subjects with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CDx-positive) is consistent with that in 1L CTA-positive randomized subjects (HR 0.22) (Table 12, Figure 1). A similar improvement in PFS is not observed in the CTA-positive/CDx-negative population, which showed PFS worsening when comparing OPDIVO (nivolumab) plus YERVOY (ipilimumab) (median PFS 1.81 months) with chemotherapy (median PFS 11.53 months).

**Table 13: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

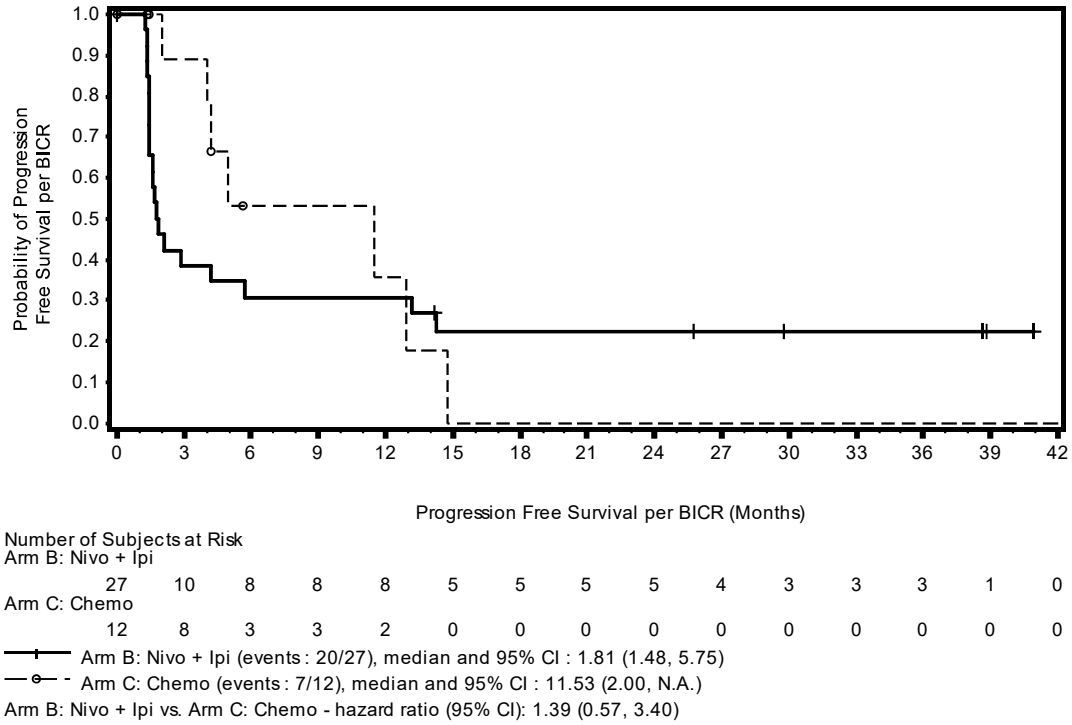
| | CTA-positive/CDx-positive 1L randomized subjects | | CTA-positive/CDx-negative 1L randomized subjects | | 1L Randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N=163 | Chemotherapy N=82 | Nivolumab plus ipilimumab N=27 | Chemotherapy N=12 | Nivolumab plus ipilimumab N=200 | Chemotherapy 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), mo[a] | NR (38.44, NR) | 5.85 (4.40, 7.79) | 1.81 (1.48, 5.75) | 11.53 (2.00, NR) | NR (34.30, NR) | 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. PFS 95% CI upper-bound values of NR 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) 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NR, not reached; PFS, progression-free survival. Subject number totals for 1L randomized CTA-positive (n=301) and 1L randomized subjects with CDx results (n=284) are not equal due to insufficient tissue availability or invalid test status of some subjects 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 1. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**



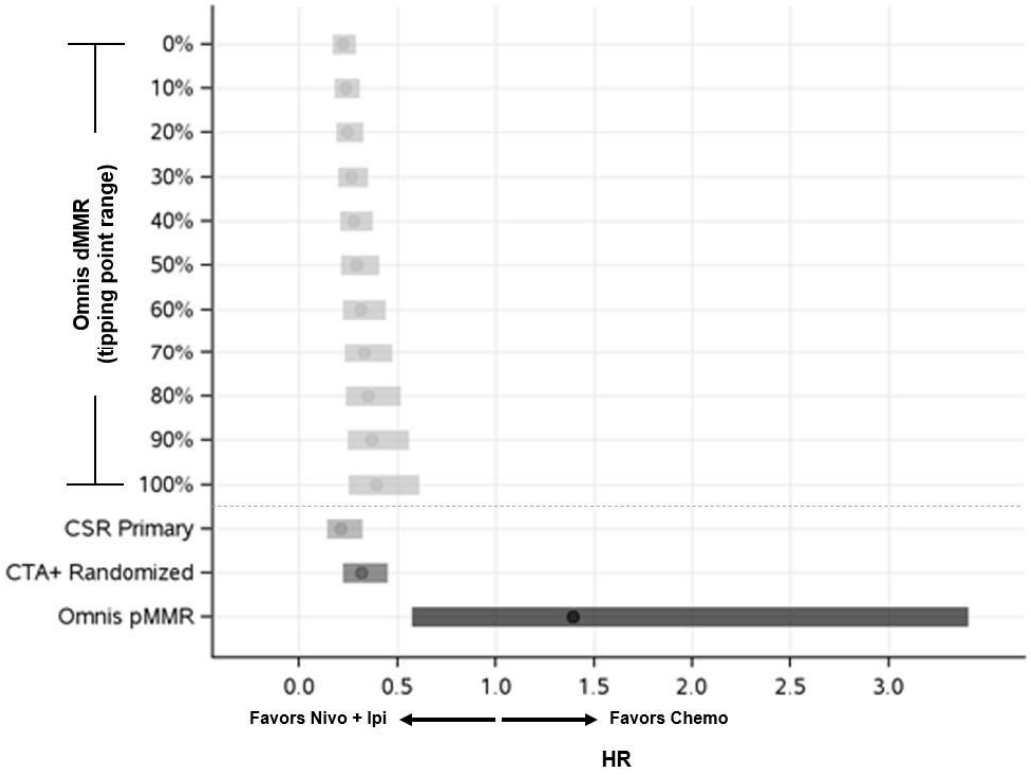

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date.
1L, first-line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

**Figure 2. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2023Oct12 when only the 1L subjects in OPDIVO (nivolumab) plus YERVOY (ipilimumab) and chemotherapy arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for HR of the PFS in these subjects (CTA-negative/CDx-positive, HR = 1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.22). The tipping point PFS HR CI values ranged from a minimum of 0.16 (0% tipping point range CI lower-bound) to a maximum of 0.61 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.16-0.30, and the 100% tipping point 95% CI range at 0.25-0.61.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in the 1L CTA-positive randomized patients and in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 255). **CTA+ Randomized**: Subjects randomized in CHECKMATE-8HW with locally confirmed MSI-H/dMMR status (N = 301). **Omnis pMMR**: Subjects randomized in CHECKMATE-8HW with centrally tested pMMR status and locally confirmed MSI-H/dMMR status (N = 39). chemo, chemotherapy; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab;.

**Figure 3. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus ipilimumab) vs chemotherapy in 1L subjects (CHECKMATE-8HW)**

#### 15.3.3.2. OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects

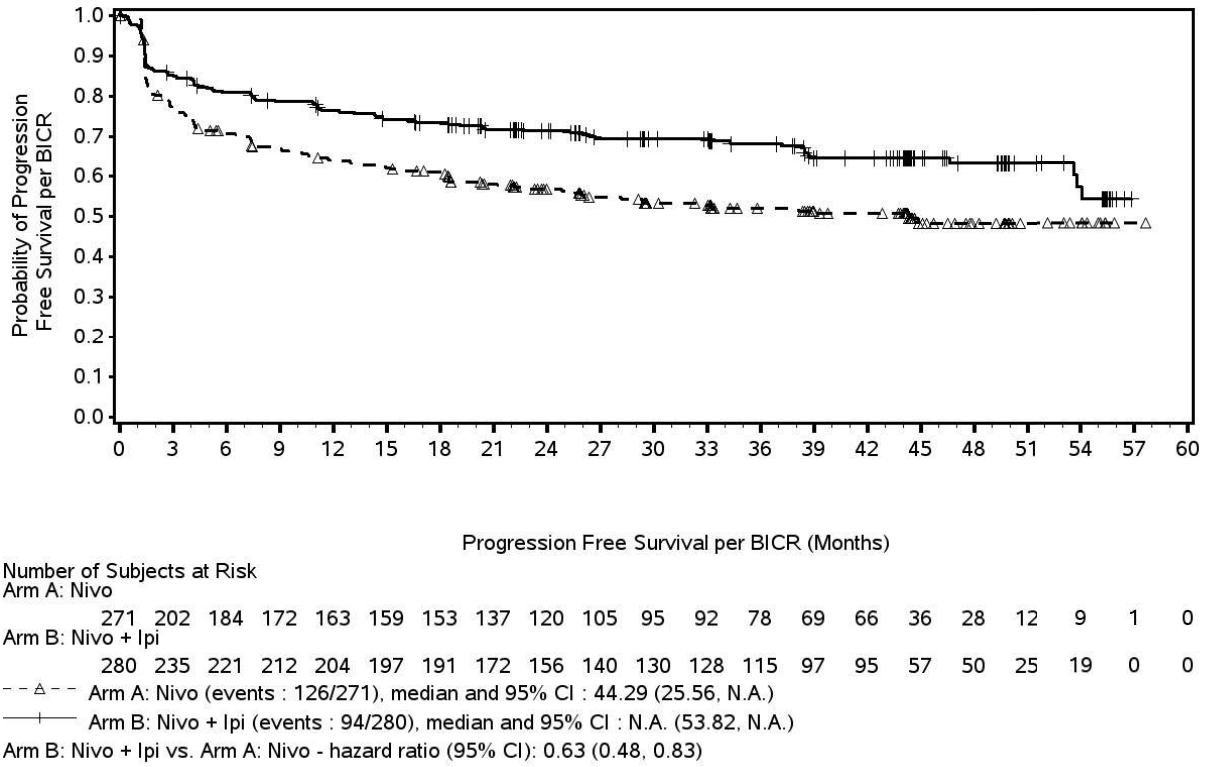
A total of 705 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 352) or OPDIVO (nivolumab) (n= 353). The study included 88 sites in 23 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, Denmark, France, Germany, Greece, Ireland, Italy, Japan, Netherlands, Norway, Romania, Spain, Turkey, UK, and US). Most subjects were from US/Canada/European Union (n = 495, 70.2%), 59 (8.4%) were from Asia, and 151 (21.4%) were from the rest of the world. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the OPDIVO (nivolumab) group was 63 years. Most subjects were white (n=614, 87.1%), 63 (8.9%) were Asian, 11 (1.6%) were black. The ethnicity of subjects was 66 (9.4%) Hispanic, 353 (50.0%) non-Hispanic, and 286 (40.6%) not reported. The number of male and female subjects was 351 (49.8%) and 354 (50.2%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA (HR 0.63). PFS benefit observed in all lines of therapy with concordant CTA-positive/CDx-positive population is consistent with that in all lines CTA-positive randomized subjects (HR 0.63) (Table 14, Figure 4). No clinically meaningful PFS benefit was observed in all lines subjects with the CTA-positive/CDx-negative status, shown in median PFS < 2.5 months in both OPDIVO (nivolumab) plus YERVOY (ipilimumab) and OPDIVO (nivolumab) monotherapy.

**Table 14: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[d]**

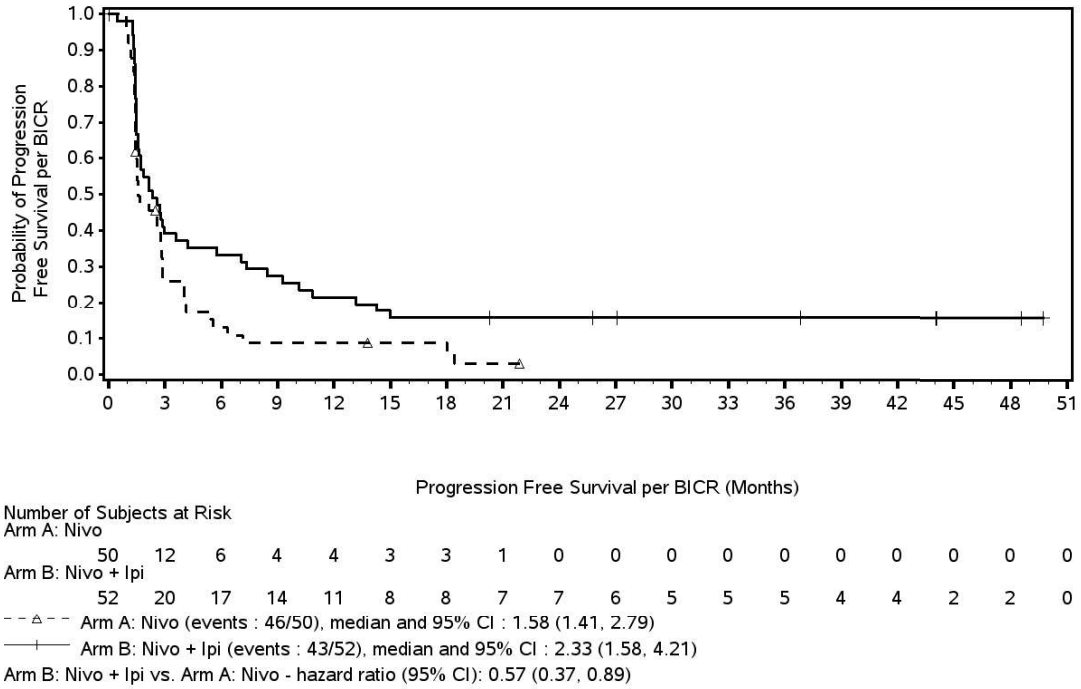
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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), mo[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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NA, not available; NR, not reached; PFS, progression-free survival. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.




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 4. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW)**



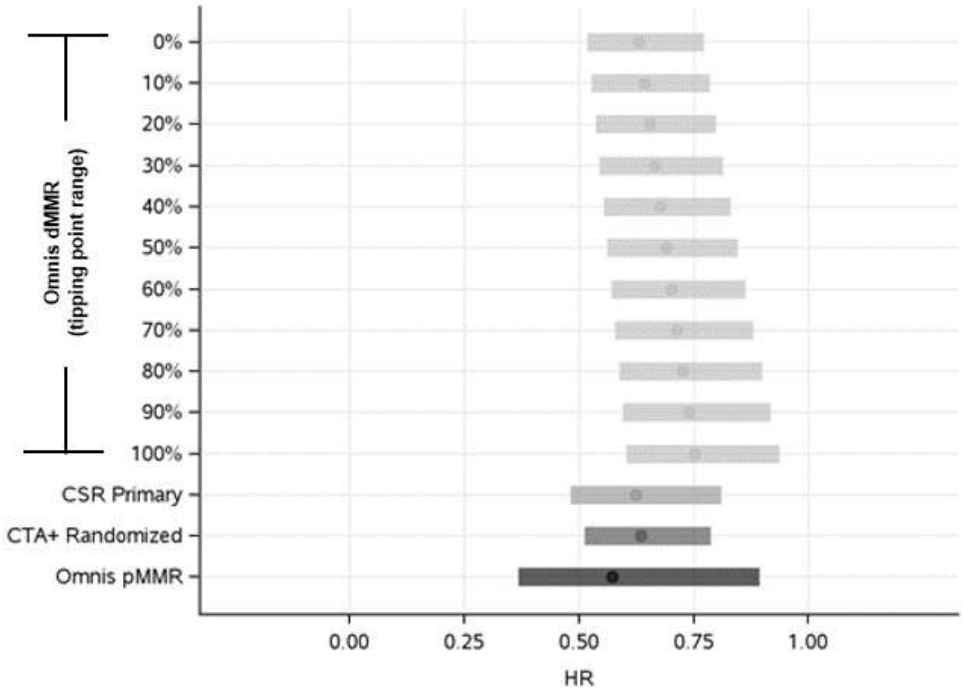

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) and prior lines of therapy (0, 1, ≥2) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first 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 OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW).**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, 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 HR of PFS of the subjects not enrolled (CTA-negative/CDx-positive, HR=1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.63). The tipping point PFS HR CI values ranged from a minimum of 0.52 (0% tipping point range CI lower-bound) to a maximum of 0.94 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.52-0.77, and the 100% tipping point 95% CI range at 0.60-0.94.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 582). **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). 3L+, all lines treatment; BICR, blinded independent central review; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; MSI-H, microsatellite instability high; Nivo, nivolumab; pMMR, mismatch repair proficient.

**Figure 6. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CHECKMATE-8HW).**

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in Objective Response Rate (ORR) over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA, with ORR 63.6% (95% CI: 58.4, 68.7) vs. 49.3% (95% CI: 44.0, 54.6), respectively. ORR benefit observed in 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) vs. 58.3% (95% CI: 52.2, 64.2), respectively (Table 15). No clinically meaningful ORR benefit was observed in all lines subjects with the CTA-positive/CDx-negative status (p=0.0568).

**Table 15: ORR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[c]**

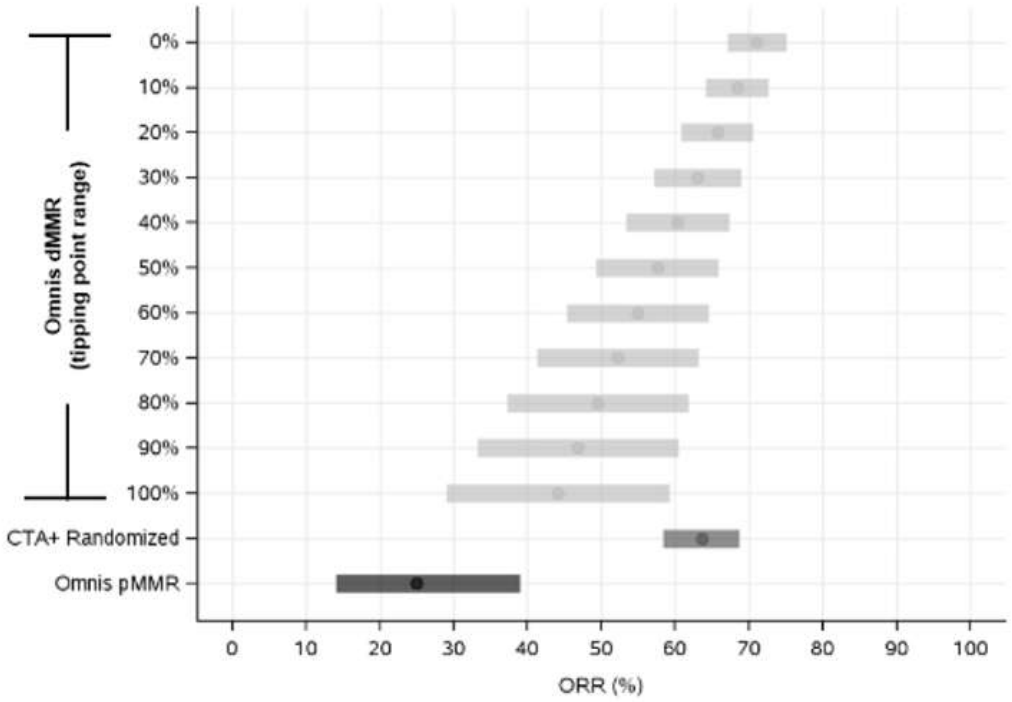
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed dMMR/MSI-H status using clinical trial assay modalities; CDx-positive: deficient mismatch repair (dMMR); CDx-negative: proficient mismatch repair (pMMR); ORR, overall response rate. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.

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. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

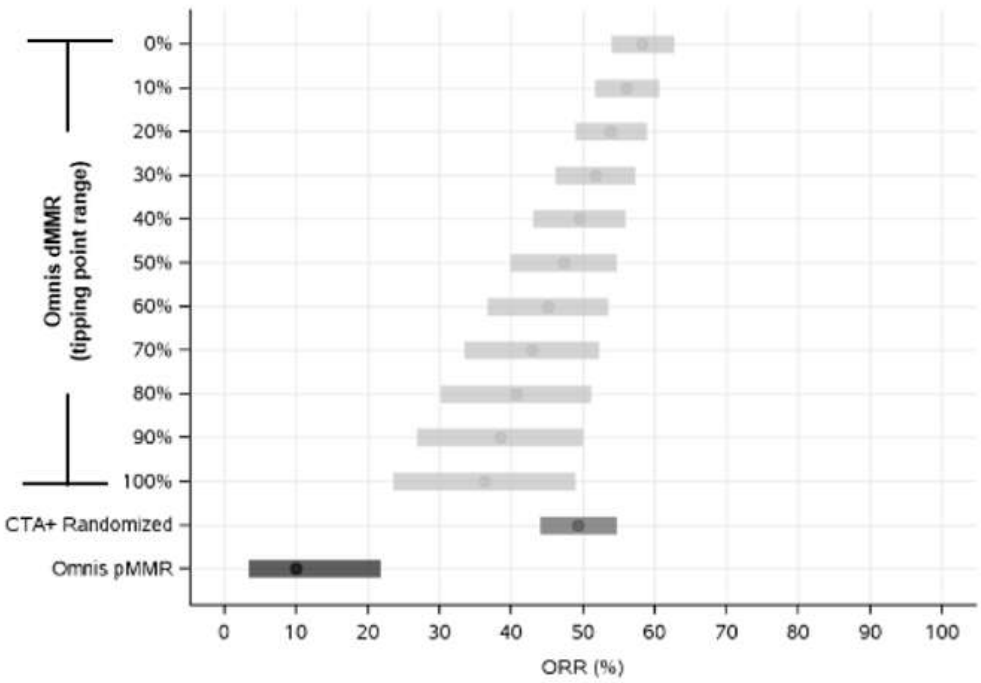
Zero to 100% of the tipping point range was assumed for the missing ORR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of these patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for the ORR of the subjects not enrolled (ORR = 0 for CTA-negative/CDx-positive). The tipping point range of 0% assumes a best-case scenario where the ORR for these subjects is equal to the observed ORR for enrolled subjects (ORR = 0.711 for nivolumab plus ipilimumab in CTA-positive/CDx-positive subjects; ORR = 0.583 for nivolumab monotherapy in CTA-positive/CDx-positive subjects). For nivolumab plus ipilimumab, the tipping point ORR point estimate values ranged from 0.438 to 0.7107 and CI values ranged from a minimum of 0.2881 (100% tipping point range CI lower-bound) to a maximum of 0.7501 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2881-0.5878, and the 0% tipping point 95% CI range at 0.6714-0.7501. For nivolumab monotherapy, the tipping point ORR point estimates ranged from 0.3593 to 0.5830 and CI values ranged from a minimum of 0.233 (100% tipping point range CI lower-bound) to a maximum of 0.6265 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2330-0.4856, and the 0% tipping point 95% CI range at 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).




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 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 = 0.583).**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

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

### 15.3.4. Clinical Performance Summary

In summary, successful bridging between CTA-positive and dMMR status by MMR IHC Panel pharmDx (Dako Omnis) was achieved based on similar clinical utility between patients with dMMR/MSI-H status by CTA and those with dMMR status by MMR IHC Panel pharmDx (Dako Omnis).

A clinically meaningful improvement in PFS per BICR was demonstrated with:

- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to chemotherapy in 1L treatment of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).
- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to OPDIVO (nivolumab) monotherapy in all lines of therapy of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).

Re-estimation of efficacy results using imputations and sensitivity analyses by considering the missing assessments of MMR IHC Panel pharmDx (Dako Omnis) supported these findings.

These results support the clinical performance of MMR IHC Panel pharmDx (Dako Omnis) by demonstrating the assay's clinical utility in identifying patients with dMMR CRC who may benefit from treatment with OPDIVO (nivolumab) alone or in combination with YERVOY (ipilimumab) in accordance with their approved US package inserts.

## 16. Troubleshooting

Refer to the Troubleshooting section in Agilent's Education Guide for remedial action or contact Agilent Pathology Support to report unusual staining.[8]

Dako Omnis is an automated system designed to alert the user if anything in the run has been outside of specifications. Please refer to the Dako Omnis User Guide for details on what conditions are flagged and how. Table 16 is a troubleshooting guide for results and conditions that are not easily identified through the Dako Omnis warning and alert system.

The user should always ensure adherence to the maintenance schedule for the Dako Omnis instrument. Always ensure to use the appropriate controls as described in 'Quality Control', Section 11.

**Table 16. Troubleshooting.**

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| 7. No or weak staining of slides. | 1a. Excessive heating of mounted tissue sections prior to loading on Dako Omnis may lead to loss of visible MSH6 immunoreactivity and/or tissue morphology. | 1a. Dry the tissue sections at 58 ± 2 °C for a maximum of 1 hour, using a calibrated oven with uniform heat distribution.[10] |
| | 1b. Wrong storage conditions used for reagents. | 1b. Check that reagents have been stored correctly according to listed storage conditions. |
| | 1c. Inappropriate fixation method used. | 1c. Ensure that patient tissue is not fixed for too short or too long a time period, that ischemia time has been minimized, and that the correct fixative (10% NBF) was used. |
| | 1d. Reagent is used past its expiration date. | 1d. Check Dako Link Omnis Workstation software to determine if slides were flagged suspicious. Ensure reagent is not used past its expiration date. |
| | 1e. Reagent is used past its onboard stability. | 1e. Check Dako Link Omnis Workstation software to determine if slides were flagged as suspicious. Ensure reagent is not used past its onboard stability. |
| | 1f. Incorrect placement of dynamic gap lids in staining modules. | 1f. Check placement of dynamic gap lids. |
| | 1g. Damaged dynamic gap lids. | 1g. Check integrity of dynamic gap lids. |
| | 1h. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution (50x) concentrate. | 1h. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 1i. Incorrect Target Retrieval Solution is used. | 1i. Ensure that correct Target Retrieval Solution specified in 'Materials Required, but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |
| | 1j. 1x Target Retrieval Solution does not meet pH specifications. | 1j. Check pH of 1x Target Retrieval Solution. If pH is outside acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. Refer to Problem #6 for additional troubleshooting. |
| 8. Excessive nonspecific staining of slides. | 2a. Starch additives used in mounting sections to slides. | 2a. Avoid using starch additives for adhering sections to glass slides. Many additives are immunoreactive. |
| | 2b. Sections dried after staining procedure/prior to coverslipping. | 2b. Verify that the Unloading Station is filled with sufficient water. |
| | | 2b. Avoid stained slides drying out after staining completion (e.g., between removal from Dako Omnis and coverslipping). |
| | 2d. Inappropriate fixation method used. | 2d. Ensure that approved fixative was used. Alternative fixative may cause excessive nonspecific staining. |
| | 2e. Paraffin incompletely removed. | 2e. Check appearance of solvent coupling. Refer to Dako Omnis User Guide for details. |

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| | 2f. Nonspecific binding of reagents to tissue. | 2f. Ensure that correct fixation method of the specimen is used and avoid large areas of necrosis. |
| | 2g. Re-use of mixing strip. | 2g. Ensure that new mixing strips are used. |
| 9. Excessively strong specific staining. | 3. Inappropriate fixation method used. | 3. Ensure that only approved fixatives and fixation methods are used. |
| 10. Tissue detaches from slides. | 4. Use of incorrect slides. | 4. Use FLEX IHC Microscope Slides (Code K8020), or SuperFrost Plus slides. |
| 11. Slide is flagged as suspicious. | 5a. Reagent is used beyond its expiration date. | 5. Slides flagged as suspicious should be evaluated by qualified personnel, contact an Agilent Technologies representative if further action is needed. |
| | 5b. Reagent is stored onboard Dako Omnis beyond its validated onboard stability. | |
| | 5c. Maintenance overdue or other factors. | |
| 12. 1x Target Retrieval Solution does not meet pH specifications | 6a. pH meter is not calibrated correctly. | 6a. Ensure pH meter is calibrated per manufacturer's recommendations. After recalibration, retest pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If pH is outside of the acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. |
| | 6b. Target Retrieval Solution pH is measured at incorrect temperature. | 6b. Ensure that 1x Target Retrieval Solution pH is measured at ambient temperature. |
| | 6c. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution concentrate. | 6c. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 6d. Incorrect Target Retrieval Solution is used. | 6d. Ensure that the correct Target Retrieval Solution specified in 'Materials Required but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |

**NOTE:** If the problem cannot be attributed to any of the causes in Table 16, or if the suggested corrective action fails to resolve the problem, please contact Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found in Agilent's Education Guide (available from www.agilent.com), Atlas of Immunohistology, and Immunoperoxidase Techniques. A Practical Approach to Tumor Diagnosis.[8,12,13]

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**Explanation of symbols**

| | | | | | |
|---|---|---|---|---|---|
| REF | Catalogue number | | Temperature limitation | IVD | In vitro diagnostic medical device |
| | Manufacturer | LOT | Batch code | Σ | Contains sufficient for <n> tests |
| | Use by | | Consult instructions for use | BIO | Contains biological material of animal origin |
| | Caution | EC REP | Authorized representative in the European Community | | |

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Revision 2025.08

# PMS2 IHC pharmDx (Dako Omnis)
# Rx Only
# Code GE087

Primary antibody for use with MMR IHC Panel pharmDx (Dako Omnis)
60 tests for use with Dako Omnis

**Table of Contents**

## 1. Intended 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).

## 2. Summary and Explanation

The MMR pathway is used by normal proliferating cells to repair mutations that may occur during DNA replication. Loss of function of any of the following four MMR proteins, MLH1, PMS2, MSH2, MSH6, results in MMR deficiency (dMMR) and can lead to an increased mutation rate, promotion of tumorigenesis, and generation of neoantigens. dMMR tumors may be more responsive to immunotherapies than tumors with functioning MMR pathways due to the increased presence of neoantigens and immune cell recruitment.[1,2] PMS2 IHC pharmDx (Dako Omnis) is part of MMR IHC Panel pharmDx (Dako Omnis), which is an IHC panel that is used to detect loss of function of any of the four MMR proteins.

Bristol-Myers Squibb sponsored trial, CHECKMATE-8HW (CA2098HW), investigated the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in identifying MMR deficient metastatic CRC patients who may respond to treatment with OPDIVO alone or in combination with YERVOY.[3,4]

*OPDIVO and YERVOY are trademarks owned by Bristol-Myers Squibb Company.*

## 3. Principle of Procedure

PMS2 IHC pharmDx (Dako Omnis) is an optimized antibody reagent with the protocol required to complete an IHC staining procedure of FFPE specimens using the Dako Omnis instrument. Following incubation with the primary monoclonal antibody to PMS2, the specimen is sequentially incubated with peroxidase block, two 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 chromogen results in precipitation of a visible reaction product at the site of antigen. The specimen may then be counterstained and coverslipped. Results are interpreted using a bright field microscope. MMR Negative Control Reagent, Rabbit (GE102) slide should be run alongside the PMS2 IHC pharmDx (Dako Omnis) slide. Please consult Dako Omnis User Guide for detailed instructions on loading and unloading of slides, reagents, bulk fluids and waste.

## 4. Materials Provided

The product includes 12 mL of primary antibody to PMS2 protein (approximately 3.05µg/mL) sufficient for 60 tests. The product has been optimized for use with the Dako Omnis instrument. Please refer to the Dako Omnis User Guide for further information.

| *Quantity* | *Description* |
|---|---|
| 1 x 12 mL | **PMS2 IHC pharmDx (Dako Omnis)** |

**PMS2 IHC pharmDx**

**(Dako Omnis)**

Monoclonal rabbit anti-human PMS2, clone EP51, in a buffered solution, containing stabilizing protein, and 0.015 mol/L sodium azide.

## 5. Materials Required, but Not Supplied

Dako Omnis (Code GI100)
MLH1 IHC pharmDx (Dako Omnis) (Code GE079)
MSH2 IHC pharmDx (Dako Omnis) (Code GE085)
MSH6 IHC pharmDx (Dako Omnis) (Code GE086)
MMR Negative Control Reagent, Mouse (Dako Omnis) (Code GE101)
MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102)
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 (see 'Quality Control', Section 11)
pH meter

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

*__NOTE__: *Use Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) for PMS2 IHC pharmDx (Dako Omnis) (Code GE087) testing. Do not use EnVision FLEX Target Retrieval Solution, High pH (50x) from EnVision FLEX, High pH (Dako Omnis), Code GV800 or GV823.*

**__NOTE__: *Not all sources of distilled or de-ionized water may be of sufficient quality for IHC reagent preparation. Agilent recommends reagent-grade distilled or de-ionized water (corresponding to Clinical Laboratory Reagent Water [CLRW] standard as specified by CLSI), or water similar in quality to be used for reagent preparation.*[5]

## 6. Precautions

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide (NaN3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, NaN3 may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.[6]
4. PMS2 IHC pharmDx (Dako Omnis) contains material of animal origin. As with any product derived from biological sources, proper handling procedures should be used in accordance with local requirements.
5. Specimens, before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection, and disposed of with proper precautions.[7]
6. Incubation times, temperatures, or methods other than those specified may give erroneous results.
7. Reagents have been optimally diluted. Further dilution may result in loss of visible PMS2 immunoreactivity.
8. Paraffin residue may lead to false negative results.
9. Use of reagent volumes other than recommended may result in loss of visible PMS2 immunoreactivity.
10. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
11. Unused solution should be disposed of in accordance with all local, regional, national and international regulations.
12. Safety Data Sheets are available on www.agilent.com or on request.
13. Lack of adherence to the maintenance schedule for the Dako Omnis instrument may give erroneous results. Refer to Dako Omnis User Guides for additional information and for additional instrument-related precautions.
14. Contact Agilent Pathology Support via www.agilent.com to report any unusual staining.

## 7. Storage

Store PMS2 IHC pharmDx (Dako Omnis) in the original product packaging at 2–8 °C when not in use on Dako Omnis. During storage, the flip top vial cap should be closed.

**Do not use the reagent after the expiration date printed on the reagent vial label.** If the reagents are stored under any conditions other than those specified in the instructions for use, they must be validated by the user. The expiry date on the label is valid for unopened vials as well as opened (in-use) vials when handled according to instructions.

Onboard reagent stability for PMS2 IHC pharmDx (Dako Omnis) has been validated to 375 hours. After staining completion, the reagents should be removed from Dako Omnis and stored in the original product packaging at 2–8 °C with flip top vial caps closed securely on the vials. For onboard stability of all ancillary components including diluted working solutions of Wash Buffer and Target Retrieval Solution, pH 9, refer to respective instructions for use. Onboard time of reagents is tracked by the Dako Omnis software; refer to Dako Omnis User Guide for details.

**NOTE**: . There are no obvious visual signs to indicate incorrect product storage or handling of this product during the product's shelf life. Positive and negative controls should be run simultaneously with patient specimens, preferably on the same slide, to monitor product performance during the product's shelf life. If a problem is suspected with the antibody during the shelf life that cannot be explained by incorrect product storage or handling, or other variations in laboratory procedures, contact Agilent Pathology Support. Refer to 'Quality Control', Section 11 and 'Troubleshooting', Section 16 for more information.

## 8. Specimen Preparation

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

### 8.1. Paraffin-embedded tissue

FFPE tissues are suitable for use with the primary antibody to the PMS2 protein. 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 [such as % unbuffered formalin, Bouin's fixative, and acetic formalin alcohol (AFA)] have not been validated and may give erroneous results. 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.

### 8.2. 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.

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.

**NOTE**: The tissue specimens must be positioned on the glass within the defined slide staining area. Please consult the Dako Omnis User Guide for dimensions of slide staining area.

## 9. Reagent Preparation

The user should adhere to appropriate personal protective equipment requirements and become familiar with all components prior to use (see 'Precautions', Section 6).

Target Retrieval Solution, pH 9 (50x) (Code GC309) and Wash Buffer (20x) (Code GC807) must be prepared according to their respective instructions for use. Refer to the GC309 and GC807 instructions for use 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.

## 10. Staining Procedure

**Procedural Notes**

The user should read these instructions carefully and become familiar with all the components and the instrumentation prior to use (see 'Precautions', Section 6).

The automated staining procedure for PMS2 IHC pharmDx (Dako Omnis) on Dako Omnis includes deparaffinization of tissue sections, target retrieval, and staining. The slides are unloaded in the Unloading Station. All protocol steps are preprogrammed into the Dako Link Omnis Workstation software. The "MMR PMS2 IHC pDx GE087**"** protocol is used for PMS2 IHC pharmDx (Dako Omnis). If the appropriate PMS2 IHC pharmDx (Dako Omnis) protocol is not on your server, please contact your local Technical Service Representative or Agilent Pathology Support to obtain the protocols. Refer to the Dako Omnis User Guide for further information on how to operate and maintain the instruments.

**NOTE:** The PMS2 reagent and instructions supplied with this product have been designed for optimal performance. Further dilution of the antibody or alteration of staining protocol may give erroneous or discordant results. Differences in tissue processing and technical procedures in the user's laboratory may invalidate the assay results.

**NOTE**: Laboratories located at high elevations should determine the best method of maintaining the required temperature (97 °C) during heat-induced epitope retrieval. Any adjustments required to address elevation concerns must be validated by the user. Refer to the Dako Omnis User Guide for additional information.

**Prestaining procedure**

1. Choose the MMR PMS2 IHC pDx GE087 protocol to be applied for each slide from the Dako Link Omnis Workstation software.
2. Ensure the Dako Link Omnis Workstation software is configured to print slide labels with the protocol name displayed.
3. Print slide labels and attach them to the glass slides.
4. Place the slides in the Slide Rack. A Slide Rack can hold from one to five slides.
5. Ensure that the bulk bottles with fluids are onboard and registered by the Dako Omnis instrument. Bulk bottle fluids:
    a. Clearify™ clearing agent (Code GC810)
    b. Target Retrieval Solution, pH 9 (50x) (Dako Omnis) (Code GC309) **diluted to 1x working concentration with distilled or de-ionized water.**
    c. Wash buffer (Code GC807) **diluted to 1x working concentration with distilled or de-ionized water.**
6. Ensure that all flip top vial caps are open and locked in place before loading all required reagents in the Reagent Storage Module:
    a. PMS2 IHC pharmDx (Dako Omnis) (Code GE087)
    b. EnVision FLEX Peroxidase-Blocking Reagent (Dako Omnis) (Code GV800)
    c. EnVision FLEX Visualization Reagent (Dako Omnis) (Code GV800)
    d. EnVision FLEX Substrate Buffer (Dako Omnis) (Code GV800)
    e. EnVision FLEX DAB+ Chromogen (Dako Omnis) (Code GV800)
    f. EnVision FLEX+ Mouse LINKER (Dako Omnis) (Code GV821)
    g. EnVision FLEX+ Rabbit LINKER (Dako Omnis) (Code GV809)
    h. Optional: Hematoxylin (Dako Omnis) (Code GC808) or equivalent
    i. Sulfuric Acid (Code GC203)
7. Load the Slide Rack onto Dako Omnis.

8. Follow the instructions on the Touch Screen and tap “Done” to initiate the staining procedure.
9. Ensure the slide Unloading Station is filled with distilled or de-ionized water to prevent slides from drying.

**NOTE:** When using the overnight staining feature (delayed start) slides must be removed from the Unloading Station the morning the staining has been completed.

**NOTE:** The PMS2 IHC pharmDx (Dako Omnis) protocol on the Dako Omnis instrument can be monitored on the Dako Link Omnis Workstation.

**Dako Omnis Staining Protocol**

When processing slides for staining with the MMR IHC Panel pharmDx (Dako Omnis) assay, the Dako Omnis automated platform executes the protocols for MMR PMS2 IHC pDx GE087 as stated in Table 1. Refer to MMR Negative Control Reagent, Rabbit (GE102) IFU for details on the GE102 staining protocol. The MMR PMS2 IHC pDx GE087 protocol has been designed for optimal performance. Any changes to the staining protocol may alter the performance of the device and must be validated by the user. Unless otherwise noted, each step of the staining procedure is executed at the instrument’s fixed temperature of 32ºC. The instrument’s fixed temperature is not an editable parameter.

**Table 1. MMR PMS2 IHC pDx GE087 Staining Protocol**

| Protocol Step | Reagent | Setting |
|---|---|---|
| **Dewax** | Clearify Clearing Agent | 25 °C, 10 s incubation top, 1 min incubation bottom, 1 cycle |
| | DI Water | 5 s incubation, 1 cycle |
| **Target retrieval** | TRS, pH 9* | 97 °C*, 30 min incubation* |
| | Cooling fluid DI Water | N/A |
| **Staining** | Wash Buffer | 2:40 min incubation, 2 cycles |
| | Primary antibody (PMS2*) | 20 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Peroxidase-Blocking Reagent | 3 min incubation |
| | Wash buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Rabbit LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX+ Mouse LINKER | 10 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX/HRP | 20 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | EnV FLEX Substrate Working Solution | 5 min incubation |
| | Wash Buffer | 2 min incubation, 10 cycles |
| | DI Water | 31 s incubation, 1 cycle |
| | Wash Buffer | 2 min incubation, 10 cycles |
| **Counterstaining** | Hematoxylin* | 3 min incubation* |
| | DI Water* | 2 min incubation, 10 cycles |
| | Wash Buffer* | 2 min incubation, 10 cycles |

*Parameter is editable by the end user when creating a copy. Any changes to the staining protocol may alter the performance of the device and must be validated by the user.

**Counterstain**

Slides may be counterstained with Hematoxylin (Dako Omnis) (Code GC808). The “MMR PMS2 IHC pDx GE087” protocol on Dako Omnis includes a counterstaining step that is set as editable for the user. If a counterstain other than the recommended Hematoxylin (Dako Omnis) (Code GC808) is used, the alternative counterstain must be validated by the user. See the Dako Omnis User Guide for further information on editing protocols.

Preprogrammed:

Slides are counterstained for 3 minutes with Hematoxylin (Dako Omnis) (Code GC808). The Hematoxylin incubation time is preprogrammed in the protocol. Slides are ready for mounting when removed from the Unloading Station.

User Defined:

If the selected protocol does not include an automated counterstaining process, it is the responsibility of the user to counterstain the specimen(s) per internally validated procedure prior to mounting.

**Mounting**

After staining onboard Dako Omnis, the sections must be dehydrated, cleared, and mounted using nonaqueous, permanent mounting methods.

**NOTE:** Some fading of stained slides may occur, depending on several factors including, but not limited to, counterstaining, mounting materials and methods, and slide storage conditions. To minimize fading, store stained slides in the dark at room temperature (20–25 °C).

## 11. Quality Control

PMS2 IHC pharmDx (Dako Omnis) has been quality controlled for IHC using the required reagents and staining procedures outlined in 'Reagent Preparation', Section 9 and 'Staining Procedure', Section 10. Deviations from the recommended procedures may lead to significant variability in results. Consult the quality control guidelines of the College of American Pathologists (CAP) Accreditation Program for Immunohistochemistry. See also Agilent's Education Guide: Immunohistochemical Staining Methods and CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[8,9]

### 11.1. 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 must be used, where possible, as internal positive controls instead of a separate positive control tissue. In very rare cases, nonmalignant elements may have loss of biomarker expression, in which case nonmalignant elements of the negative control tissue may be used to qualify the staining procedure.

Refer to Table 4 for further information on positive and negative control tissues.

### 11.2. Negative control reagent

MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102) should be used in place of the 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 Rb GE102" for slides stained with the negative control reagent (NCR). Refer to the MMR Negative Control Reagent, Rabbit (Dako Omnis) (Code GE102) instructions for use for details.

### 11.3. Assay verification

Prior to initial use of a staining system in a diagnostic procedure, the user should verify the assay's performance by testing it on a series of lab-supplied tissues with known IHC performance characteristics representing known positive and negative tissues. Refer to the quality control procedures outlined in 'Quality Control', Section 11, as well as to the quality control requirements of the CAP Certification Program for Immunohistochemistry and/or CLSI Quality Assurance for Design Control and Implementation of Immunohistochemistry Assays, CLSI Document for additional information.[9] These quality control procedures should be repeated for each new primary antibody lot. Troubleshooting options for potential problems, their causes and suggested corrective actions are outlined in Table 16.

## 12. Staining Interpretation

A Hematoxylin and Eosin (H&E) stained section is used to determine if a specimen is acceptable for IHC. MMR IHC Panel pharmDx (Dako Omnis) and H&E staining should be performed on serial sections from the same FFPE block of the specimen to confirm:

1. The histologic diagnosis of CRC.
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.

Each specimen should be evaluated using 4x–20x magnification. The specific staining pattern 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.

The entire tissue section should be considered, avoiding edge effects, noninvasive components, necrotic areas, and areas with obvious fixation artifacts. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.

Nonspecific cytoplasmic staining may be present in some tissues. 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, epithelia cells
2. Edge effects
3. Necrotic areas
4. Areas with noninvasive components (normal epithelium, adenoma)
5. Areas with obvious fixation artifacts should not be scored or scored with caution

Protein status of Intact or Loss is determined for PMS2 using the guidelines described in Table 2.

**Table 2. Determination of PMS2 Intact or Loss status.**

| | |
|---|---|
| Intact | Nuclear staining in viable malignant cells must be unequivocal, with at least the same overall staining intensity as in adjacent internal positive controls.<br>If focal staining is present, the tissue is considered intact if:<br>1) continuous in multiple glands/nests<br>and<br>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.<br>If focal staining is present, the tissue is considered loss if:<br>1) continuous in only a single gland/nest,<br>2) discontinuous in multiple glands/nests,<br>or<br>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.

After a protein status of Intact or Loss is assigned to each biomarker (MLH1, PMS2, MSH2, and MSH6) for a given specimen, a diagnostic status of MMR proficient or MMR deficient is given using the definitions in Table 3.

**Table 3. Definitions of MMR proficient and MMR deficient.**

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

Some cases may be more challenging to interpret due to particular staining patterns, morphology, nonspecific staining, and/or tissue or staining artifacts. For additional guidance on MMR staining interpretation and examples of challenging cases, refer to the MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual for details.

### 13. Tissue Evaluation

The following table provides the order of slide evaluation for interpretation of PMS2 IHC pharmDx (Dako Omnis). Per the intended use and MMR IHC Panel pharmDx (Dako Omnis) Interpretation Manual, evaluation must be performed in conjunction with other required biomarkers.

**Table 4. Recommended order of tissue evaluation.**

| Tissue | Rationale | Requirements |
|---|---|---|
| 1. Patient tissue stained with H&E | An H&E stain of the patient tissue is evaluated first to assess tissue histology and preservation quality.<br>Note: The H&E may be reviewed again in the context of the patient tissue slides stained with the NCR and primary antibody (Steps 5 and 6) | The H&E and MMR IHC Panel pharmDx (Dako Omnis) stains should be performed on serial sections from the same FFPE block of the specimen.<br>Tissue specimens should be well preserved, confirm the diagnosis of CRC, and include at least 50 viable malignant tumor cells. |
| 2. Positive control stained with primary antibody to the PMS2 protein (GE087) | The positive control tissue stained with primary antibody to the PMS2 protein should be examined next.<br>**Patient CRC tissues** contain positive nonmalignant elements that serve as an internal positive control. This internal positive control eliminates the need for a separate control tissue.<br>In rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In cases that demonstrate complete loss of biomarker expression in nonmalignant elements, internal positive controls within the negative control tissue should be reviewed to qualify the staining procedure.<br>Positive tissue controls should be used for monitoring the performance of processed tissues and test reagents. | Nuclear staining of benign or normal epithelium, lymphocytes, and stromal cells present in the patient tissue must demonstrate moderate to strong staining intensity.<br>If patient tissue demonstrates focal loss of biomarker expression in nonmalignant elements, the remaining areas with nuclear staining in the internal positive control elements may still be used to qualify the staining procedure.<br>If patient tissue demonstrates complete loss of biomarker expression in both malignant and nonmalignant elements, use internal positive control within the negative control tissue.<br>If the internal positive controls fail to demonstrate appropriate positive staining, results with the test specimens should be considered invalid. |
| 3. Negative control tissue stained with primary antibody to the PMS2 protein (GE087)<br>(Lab-supplied) | The negative control tissue stained with primary antibody to the PMS2 protein should be examined next to verify labeling specificity of the target antigen by the primary antibody. | Negative control tissue should be prescreened CRC tissues with loss of PMS2 expression.<br>At least one negative control tissue section should be included in each staining procedure, either as an on-slide control or as a separate negative control slide. On-slide tissue controls are recommended and eliminate the need for a separate control slide.<br>Slides stained with primary antibody to the PMS2 protein should exhibit no nuclear staining or focal weak nuclear staining in malignant tumor cells in the presence of moderate to strong staining in internal positive controls in the tumor area. Internal positive control elements include nuclear staining in normal epithelium, lymphocytes, or stroma.<br>If the negative tissue controls fail to demonstrate appropriate staining, results with the test specimens should be considered invalid. |

| Tissue | Rationale | Requirements |
|---|---|---|
| 4. Optional:<br>Negative control tissue stained with MMR Negative Control Reagent, Rabbit (GE102)<br>(Lab-supplied) | NCR may be used to stain the negative control tissue specimen if needed for troubleshooting purposes. | NCR slides must exhibit no or weak staining in malignant tumor cells. |
| 5. Patient tissue stained with MMR Negative Control Reagent, Rabbit (GE102) | Examine patient specimens stained with NCR. NCR is used in place of the primary antibody and aids in interpretation of specific staining at the antigen site. | NCR slides must exhibit no or weak staining in malignant tumor cells. If weak staining is present in tumor nuclei, it should be used as a baseline to evaluate the PMS2 slide. Staining at the same intensity or lower that may occur in the PMS2 slide should be disregarded upon interpretation.<br>NCR slides with moderate or strong staining in malignant tumor cells are invalid and the corresponding PMS2 slide is considered nonevaluable. The patient tissue must be retested. |
| 6. Patient tissue stained with primary antibody to the PMS2 protein (GE087) | Examine the patient specimen stained with the primary antibody to the PMS2 protein last to assess PMS2 protein status. | All malignant tumor cells should be evaluated for the PMS2 protein expression and included in the PMS2 protein scoring assessment. Positive staining intensity should be assessed within the context of any nonspecific staining observed on the patients NCR slide. Areas of tumor with no nuclear staining in internal positive controls (lymphocytes, stromal cells, or normal epithelium) should be ignored.<br>In very rare cases, patient tissue can have loss of biomarker expression in both malignant and nonmalignant elements and therefore will exhibit no signal when properly stained. In such cases, the patient tissue may still be evaluated if the staining procedure is qualified using the internal positive controls within the negative control tissue.<br>As with any IHC test, absence of staining means that the antigen was not detected, not necessarily that the antigen was absent in the cells/tissue assayed.<br>For staining interpretation guidelines, refer to 'Staining Interpretation', Section 12. |

## 14. Limitations

### 14.1. General limitations

1. For prescription use only (Rx only).
2. IHC is a multistep process that requires specialized training in the selection of the appropriate reagents; tissue selection, fixation, and processing; preparation of the IHC slide; and interpretation of the staining results.
3. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.
4. Excessive or incomplete counterstaining may compromise proper interpretation of results.
5. The clinical interpretation of any staining or its absence must be evaluated within the context of clinical presentation, morphology and other histopathological criteria. The clinical interpretation of any staining, or its absence, must be complemented by morphological studies and proper controls. It is the responsibility of a qualified pathologist, who is familiar with the antibodies, reagents and methods used, to interpret the stained preparation. Staining must be performed in a certified, licensed laboratory under the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.
6. Tissues from persons infected with hepatitis B virus and containing hepatitis B surface antigen (HBsAg) may exhibit nonspecific staining with horseradish peroxidase.[10]
7. Reagents may demonstrate unexpected reactions in previously untested tissue types. The possibility of unexpected reactions even in tested tissue types cannot be completely eliminated due to biological variability of antigen expression in neoplasms, or other pathological tissues. Contact Agilent Pathology Support with documented unexpected reactions.

8. False-positive results may be seen due to nonimmunological binding of proteins or substrate reaction products. They may also be caused by pseudoperoxidase activity (erythrocytes) and endogenous peroxidase activity (cytochrome C).[10]
9. The reagents and instructions supplied for this assay have been designed for optimal performance. Further dilution of the reagents or alteration of incubation times or temperatures may give erroneous or discordant results.
10. Slides flagged in the slide log on the Dako Omnis Workstation should be investigated by qualified personnel. Refer to the Dako Omnis User Guide for further details.
11. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date. Improper storage and use of reagents may lead to erroneous results.
12. Canceled slides indicate a significant issue occurred during staining and should not be used. The specimen will require restaining. Refer to the Dako Omnis User Guide for further details.
13. This device is not intended to be used to identify patients with Lynch syndrome or to differentiate between sporadic CRC and Lynch syndrome.

### 14.2. Product-specific limitations

1. False-negative results could be caused by degradation of the antigen in the tissues over time. Once mounted on slides, specimens should be stored in the dark at 2–8 °C (preferred) or at room temperature up to 25 °C. Tissue sections should be stained within 2 months of sectioning.
2. Use of PMS2 IHC pharmDx (Dako Omnis) on specimens fixed in fixatives other than NBF has not been validated.
3. Use of PMS2 IHC pharmDx (Dako Omnis) on decalcified tissues has not been validated.
4. Reduced staining was observed with 10% unbuffered formalin, Bouin's fixative, and AFA, so they are not acceptable for use with this assay
5. This product has undergone a transport simulation study to account for anticipated temperature variations during ambient condition shipping. However, it is possible that this product, when shipped under ambient conditions, may be exposed to shipping conditions outside of tested ranges (-20ºC to 37ºC). Therefore, it is essential to use controls, as specified in this IFU, to confirm expected performance of this product.

## 15. Performance Evaluation

### 15.1. Analytical performance evaluation: normal and neoplastic tissues

Table 5 summarizes monoclonal rabbit anti-PMS2, clone EP51, immunoreactivity on the recommended panel of normal tissues. Table 6 summarizes monoclonal rabbit anti-PMS2, clone EP51, immunoreactivity on a panel of neoplastic tissues. All tissues were FFPE and stained with PMS2 IHC pharmDx (Dako Omnis) according to the instructions in this package insert. Nuclear staining was observed in a majority of tissue types tested. In some cases, cytoplasmic and/or extracellular staining was observed.

**Table 5. Summary of PMS2 IHC pharmDx (Dako Omnis) normal tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|---|---|
| Adrenal (3) | 2/3 | Lung (3) | 3/3 | Salivary gland (3) | 3/3 |
| Bladder (3) | 3/3 | Mesothelial cells (3) | 3/3 | Skin (3) | 3/3 |
| Bone marrow (3) | 2/3* | Muscle, cardiac (3) | 3/3* | Small intestine (3) | 3/3 |
| Breast (3) | 2/3 | Muscle, skeletal (3) | 3/3 | Spleen (3) | 3/3 |
| Cerebellum (3) | 3/3 | Nerve, peripheral (3) | 3/3 | Stomach (3) | 3/3 |
| Cerebrum (3) | 3/3 | Ovary (3) | 3/3 | Testis (3) | 3/3 |
| Cervix (3) | 3/3 | Pancreas (3) | 3/3 | Thymus (3) | 3/3 |
| Colon (3) | 3/3 | Parathyroid (3) | 3/3 | Thyroid (3) | 3/3 |
| Esophagus (3) | 3/3 | Pituitary (3) | 3/3 | Tonsil (3) | 3/3 |
| Kidney (3) | 3/3 | Prostate (3) | 3/3 | Uterus (3) | 3/3 |
| Liver (3) | 3/3* | | | | |

*cytoplasmic staining pattern for at least one case
**cytoplasmic and extracellular staining pattern for at least one case

**Table 6 Summary of PMS2 IHC pharmDx (Dako Omnis) neoplastic tissue reactivity.**

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|
| Bladder carcinoma (2) | 2/2 | Ovarian dysgerminoma (1) | 1/1 |
| Breast carcinoma (4) | 3/4 | Ovarian granulosa cell tumor (1) | 1/1 |
| Colon Mucinous Adenocarcinoma (1) | 0/1 | Pleomorphic rhabdomyosarcoma (1) | 1/1 |
| Cholangiocarcinoma (1) | 1/1 | Prostate adenocarcinoma (1) | 1/1 |
| Endometrial sarcoma (1) | 1/1 | Prostate benign prostatic hyperplasia (1) | 1/1 |
| Ewing's sarcoma (1) | 1/1 | Renal cell carcinoma (1) | 1/1 |
| Gastric adenocarcinoma (2) | 2/2 | Squamous carcinoma of ear (1) | 1/1 |
| Kidney transitional cell carcinoma (1) | 1/1 | Testicular embryonal carcinoma (1) | 1/1 |
| Lung carcinoma (2) | 2/2 | Testicular yolk sac tumor (1) | 1/1 |
| Lymphoma of cecum (1) | 1/1 | Thymoma (1) | 1/1 |

| Tissue Type (# Tested) | Positive Tissue Elements | Tissue Type (# Tested) | Positive Tissue Elements |
|---|---|---|---|
| Melanoma (3) | 3/3 | Thyroid carcinoma (1) | 1/1 |
| Merkel cell tumor (1) | 1/1 | Uterine adenomatoid tumor (1) | 1/1 |
| Ovarian carcinoma (2) | 2/2 | | |

### 15.2. Analytical performance evaluation: CRC

#### 15.2.1. Analytical sensitivity

Analytical sensitivity of PMS2 IHC pharmDx (Dako Omnis) was evaluated across 171 unique specimens of FFPE CRC tissues. The prevalence of loss of PMS2 expression observed was 8.8% (15/171). Assessment of MMR IHC Panel pharmDx (Dako Omnis) staining in these 171 unique specimens demonstrated a dMMR prevalence of 8.8% (15/171).

#### 15.2.2. Precision

The precision of PMS2 IHC pharmDx (Dako Omnis) was evaluated. Diagnostic status was recorded as 'Intact' or 'Loss'. The inter-observer analysis was conducted to evaluate the scoring precision of PMS2 IHC pharmDx (Dako Omnis) across multiple observers at a single site. Percent agreement of loss (LPA), percent agreement of intact (IPA) and overall percent agreement (OA) 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 100.0%.

**Table 7. Precision of PMS2 IHC pharmDx (Dako Omnis) tested at one site.**

| 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)<br>IPA 100.0 (92.0, 100.0)<br>OA 100.0 (96.2, 100.0) |
| Inter-rack | Each of 24 CRC specimens (13 loss, 11 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 (93.1, 100.0)<br>IPA 100.0 (92.0, 100.0)<br>OA 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)<br>IPA 98.6 (95.8, 100.0)<br>OA 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)<br>IPA 100.0 (94.0, 100.0)<br>OA 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)<br>IPA 100.0 (94.0, 100.0)<br>OA 100.0 (97.4, 100.0) |
| Inter-Observer | 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)<br>IPA 97.7 (94.2, 100.0)<br>OA 96.5 (93.6, 98.8) |

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

Additionally, the precision of MMR IHC Panel pharmDx (Dako Omnis) scoring across multiple observers at a single site was evaluated. Diagnostic status was recorded as 'pMMR' or 'dMMR'. dMPA, pMPA and OA were computed with corresponding two-sided 95% percentile bootstrap CIs.

**Table 8. Inter-Observer precision of MMR IHC Panel pharmDx (Dako Omnis) tested at one site.**

| Precision Study | Study Design | % Agreement (95% CI) |
|---|---|---|
| Inter-Observer | One set of 58 CRC 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)<br>pMPA 98.8 (96.2, 100.0)<br>OA 97.1 (94.7, 99.4) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

#### 15.2.3. External reproducibility

The reproducibility of PMS2 IHC pharmDx (Dako Omnis) was evaluated at three external testing sites. Diagnostic status was recorded as 'Intact' or 'Loss'. LPA, IPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 9. Reproducibility of PMS2 IHC pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>IPA<br>OA | 98.6<br>99.5<br>99.3 | (95.8, 100.0)<br>(98.6, 100.0)<br>(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<br>IPA<br>OA | 98.6<br>99.5<br>99.3 | (95.8, 100.0)<br>(98.6, 100.0)<br>(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. | NPA<br>PPA<br>OA | 100.0<br>100.0<br>100.0 | (97.7, 100.0)<br>(99.0, 100.0)<br>(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<br>IPA<br>OA | 100.0<br>100.0<br>100.0 | (97.7, 100.0)<br>(99.0, 100.0)<br>(99.3, 100.0) |

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

In the same study, the reproducibility of MMR IHC pharmDx (Dako Omnis) was analyzed. MMR diagnostic status was recorded as 'Proficient' (pMMR) or 'Deficient' (dMMR). dMPA, pMPA, and OA were computed with corresponding two-sided 95% percentile bootstrap CIs. The Wilson score limits were used to calculate CIs for agreement parameters with point estimates equal to 100.0%.

**Table 10. Reproducibility of MMR IHC Panel pharmDx (Dako Omnis) tested at three external sites.**

| 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<br>pMPA<br>OA | 98.6<br>99.3<br>99.0 | (95.7, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 100.0<br>99.3<br>99.7 | (97.3, 100.0)<br>(97.9, 100.0)<br>(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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |
| 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<br>pMPA<br>OA | 99.6<br>100.0<br>99.8 | (98.9, 100.0)<br>(98.6, 100.0)<br>(99.4, 100.0) |

dMPA = Percent Agreement of dMMR; pMPA = Percent Agreement of pMMR; OA = Overall Percent Agreement

### 15.3. Clinical performance evaluation: colorectal cancer (OPDIVO [nivolumab] alone and OPDIVO [nivolumab] in combination with YERVOY [ipilimumab])

CHECKMATE-8HW was a phase 3, randomized, open-label, multi-center, three-arm clinical trial of nivolumab monotherapy, nivolumab plus ipilimumab combination therapy, or standard chemotherapy in recurrent or metastatic dMMR/microsatellite instability high (MSI-H) CRC across lines of therapy. CHECKMATE-8HW had dual primary objectives: comparing clinical efficacy as evaluated through progression-free survival (PFS) per blinded independent central review (BICR) of nivolumab plus ipilimumab vs chemotherapy in first-line treatment (1L) and comparing clinical efficacy as evaluated through PFS per BICR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of therapy.

#### 15.3.1. Clinical Study Overview

CHECKMATE-8HW was a randomized, 3-arm, open-label trial in immunotherapy-naive patients across all lines of therapy with unresectable or metastatic CRC with known tumor MSI-H or dMMR (MSI-H/dMMR) status as determined in accordance with local standard of practice.

Eligible patients were ≥ 18 years of age, with recurrent or metastatic dMMR or MSI-H CRC not amenable to surgery. Enrollment was based on confirmation of dMMR/MSI-H status by local standard of practice, referred to here as the Clinical Trial Assay (CTA). Modalities for the CTA included: IHC, polymerase chain reaction (PCR), or next generation sequencing (NGS). Patients were considered CTA-positive and eligible for enrollment if they were identified as dMMR and/or MSI-H by at least one of the CTA modalities. Patients were randomized to OPDIVO (nivolumab) monotherapy, OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy, or investigator's choice chemotherapy in a 2:2:1 ratio. Patients who progressed after 2 prior lines of therapy were randomized in a 1:1 ratio to OPDIVO (nivolumab) monotherapy or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. Randomization was stratified by tumor location (right vs left) and by prior lines of therapy (0, 1, 2L+).

The clinical efficacy of the OPDIVO (nivolumab) and OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination was evaluated in the randomized patient population with centrally confirmed MSI-H/dMMR status. Central assessment of MSI-H status using PCR (Idylla MSI) test and dMMR status using MMR IHC Panel pharmDx (Dako Omnis) was conducted retrospectively on patient tumor specimens used for local MSI-H/dMMR status determination. Patients with confirmed MSI-H/dMMR status by either central test comprised the primary drug efficacy population.

The evaluation of the drug efficacy relied on the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) versus chemotherapy in the first-line (1L) setting and the comparison of patients with centrally confirmed MSI-H/dMMR mCRC randomized to OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines setting. The major efficacy outcome measure was BICR-assessed PFS per RECIST 1.1.

#### 15.3.2. IVD Bridging Study

Specimens from CHECKMATE-8HW were analyzed in an IVD bridging study to establish the clinical performance of the companion diagnostic MMR IHC Panel pharmDx (Dako Omnis) for detection of dMMR in patients with unresectable or metastatic CRC (mCRC) who would benefit from OPDIVO (nivolumab) alone or OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy. The IVD bridging study was designed to bridge the clinical efficacy from the CTA-positive population (dMMR and/or MSI-H by at least one of the local modalities, including IHC, PCR, and/or NGS) to the intended use population of dMMR by MMR IHC Panel pharmDx (Dako Omnis), which will be demonstrated by the clinical utility analysis through concordance (positive percent agreement [PPA] and negative percent agreement [NPA]) of MMR IHC Panel pharmDx (Dako Omnis) against CTA.

The endpoints to demonstrate the clinical utility of the companion diagnostic are:

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects with mCRC.

- PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab)

  The clinical performance study bridged the efficacy from CTA-positive to dMMR by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population with the interim analysis results in PFS per BICR in OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines randomized subjects with mCRC.

Analyses were performed to demonstrate comparable efficacy based on dMMR by MMR IHC Panel pharmDx (Dako Omnis). These analyses support the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population.

#### 15.3.3. Concordance Analysis

For the concordance analysis, PPA between CTA-positive+ and dMMR by MMR IHC Panel pharmDx (Dako Omnis) was estimated using CHECKMATE-8HW clinical samples. 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, NPA was assessed using commercially procured samples that were predetermined as pMMR and/or MSS using test methods representative of the CTA.

The PPA and the two-sided 95% confidence interval (CI) were calculated between CTA-positive and dMMR by MMR IHC Panel pharmDx (Dako Omnis) using clinical specimens and CTA-positive status as the reference. The NPA and the two-sided 95% CI were calculated between procured samples with pMMR/MSS status (CTA-negative) and pMMR by MMR IHC Panel pharmDx (Dako Omnis) with CTA-negative as the reference. There are no formal acceptance criteria for PPA and NPA. The success criteria for the IVD bridging study depends on the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) dMMR for its intended use.

The clinical efficacy of OPDIVO (nivolumab) plus YERVOY (ipilimumab) from CHECKMATE-8HW was based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the randomized patients as well as the PFS comparisons of OPDIVO (nivolumab) plus ipilimumab vs OPDIVO (nivolumab) alone in all lines of the randomized patients. Each PFS comparison was estimated by hazard ratio (HR) and the 95% CI in a stratified Cox proportional hazards model using the randomized arm as a single covariate; line of therapy (for all lines comparison) and tumor sidedness as the stratification factors. PFS curves were estimated and presented using Kaplan-Meier product-limit methodology from the patients with the concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1). Median PFS with two-sided 95% CI using the Brookmeyer and Crowley method (with log-log transformation) was computed. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 2.

Additionally, to assess the clinical utility of MMR IHC Panel pharmDx (Dako Omnis) in the intended use population to identify dMMR patients with mCRC treated with OPDIVO (nivolumab) plus YERVOY (ipilimumab), a tipping point analysis was conducted to consider the missing patients who were not enrolled due to their local pMMR/MSS status, which were potentially misclassified by the CTA and may be dMMR by MMR IHC Panel pharmDx (Dako Omnis). Tipping point analysis was conducted by assuming that the PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of these patients ranged from the best scenario (i.e., HR equal to that estimable from the enrolled patients with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis)), to the worst scenario (i.e. HR equal to 1). A full range of the tipping point analysis

results were assessed for the clinical utility of dMMR status by MMR IHC Panel pharmDx (Dako Omnis) in the intended use population. The tipping point analysis is shown graphically in Figure 3.

The clinical utility for MMR IHC Panel pharmDx (Dako Omnis) to identify dMMR patients in the intended use population for treatment with OPDIVO (nivolumab) plus YERVOY (ipilimumab) was also based on the PFS comparisons of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in all lines of the randomized patients and of the centrally confirmed dMMR patients by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4) per the same methods outlined above. PFS curves are presented from the patients with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) in Figure 5. Tipping point analysis was also conducted for this clinical endpoint per the same methods outlined above. The tipping point analysis is shown graphically in Figure 6.

A total of 839 subjects were randomized in CHECKMATE-8HW, and of those, 837 were CTA-positive. Of the 837 CTA-positive randomized subjects, 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). To avoid introducing bias to the concordance and the clinical performance evaluation, imputations and sensitivity analyses were conducted after these analyses were done with the available data and are reflected in the clinical performance results presented below.

#### 15.3.3.1. Concordance results

Concordance between the CTA and MMR IHC Panel pharmDx (Dako Omnis) was assessed via PPA using CHECKMATE-8HW clinical samples and NPA using commercially-procured samples (Tables 11 and 12). The conservative estimate for MMR IHC Panel pharmDx (Dako Omnis) positivity rate is 79.1% (662/837), assuming all 60 excluded samples would have resulted in a negative status by MMR IHC Panel pharmDx (Dako Omnis). Point estimates for positive percent agreement (PPA) and negative percent agreement (NPA) were 85.2% and 97.5%, respectively. The level of agreement achieved between the CTA and MMR IHC Panel pharmDx (Dako Omnis) is shown in Table 12.

**Table 11. Specimen distribution of comparison between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| | | CTA | | Total |
|---|---|---|---|---|
| | | Positive | Negative | |
| MMR IHC Panel PharmDx (Dako Omnis) | Positive | 662 | 5 | 667 |
| | Negative | 115 | 199 | 314 |
| Total | | 777 | 204 | 981 |

**Table 12. Analytical concordance between CTA and MMR IHC Panel PharmDx (Dako Omnis)**

| Performance Criteria | Point Estimate of Percent Agreement (95% CI, Wilson Score) |
|---|---|
| PPA | 85.2 (82.5, 87.5) |
| NPA | 97.5 (94.4, 98.9) |

CI, confidence interval by Wilson score method; NPA, negative percent agreement; PPA, positive percent agreement.

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 utility analysis 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.

### 15.3.4. Clinical Efficacy Results

#### 15.3.4.1. OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects

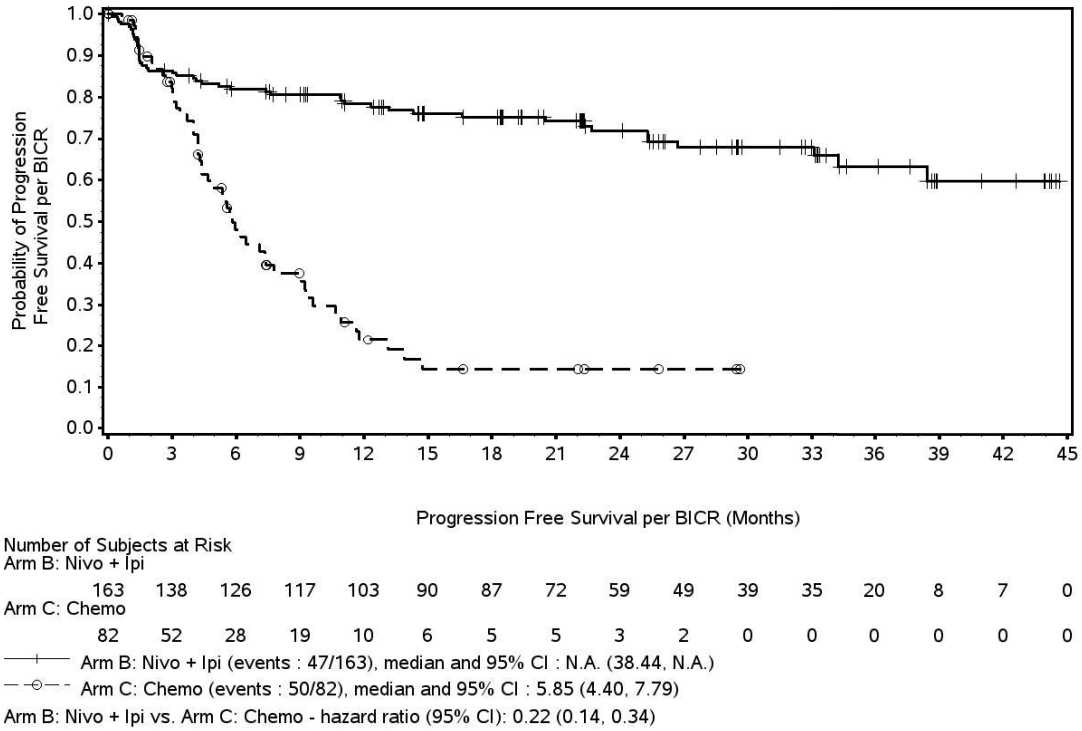
A total of 301 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 200) or chemotherapy (n = 101). The study included 88 sites in 22 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, 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. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the chemotherapy group was 65 years. Most subjects were white (n=259, 86%), 32 (10.6%) were Asian, 4 (1.3%) were black. The ethnicity of subjects was 32 (10.6%) Hispanic,150 (49.8%) non-Hispanic, and 119 (39.5%) not reported. The number of male and female subjects was 139 (46.2%) and 162 (53.8%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR (median PFS not reached) over chemotherapy (PFS 6.21 months) in 1L randomized subjects with dMMR/MSI-H status determined by the CTA (HR 0.32) (Table 12). The PFS benefit observed in 1L randomized subjects with concordant CTA-positive and centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CDx-positive) is consistent with that in 1L CTA-positive randomized subjects (HR 0.22) (Table 12, Figure 1). A similar improvement in PFS is not observed in the CTA-positive/CDx-negative population, which showed PFS worsening when comparing OPDIVO (nivolumab) plus YERVOY (ipilimumab) (median PFS 1.81 months) with chemotherapy (median PFS 11.53 months).

**Table 13: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

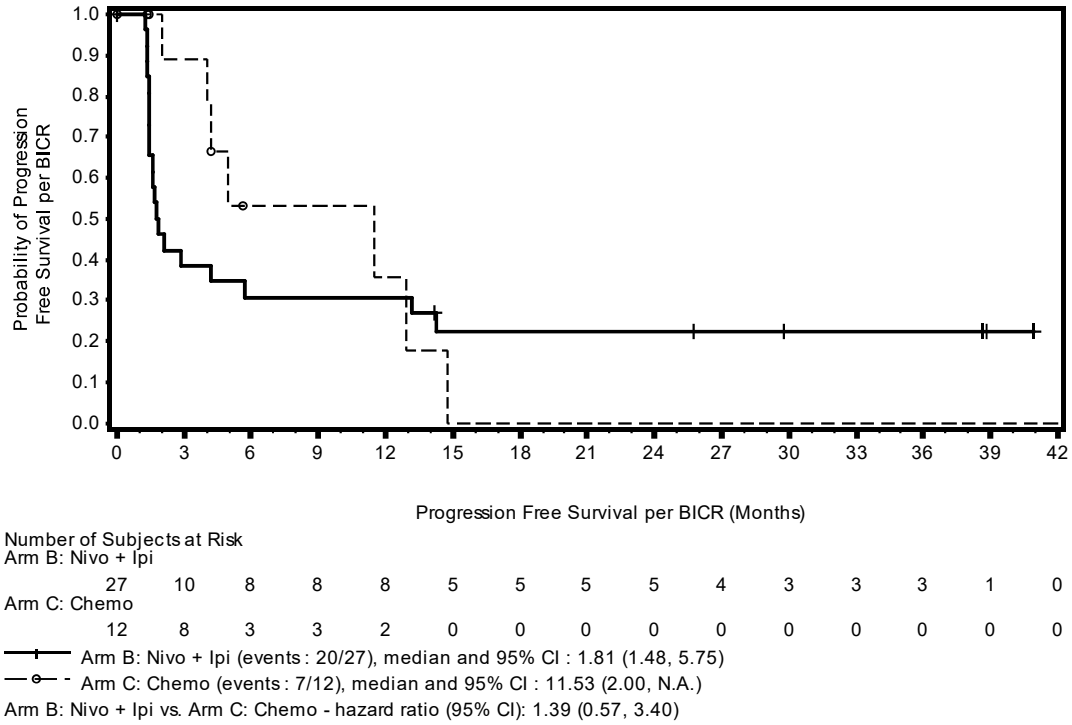
| | CTA-positive/CDx-positive 1L randomized subjects | | CTA-positive/CDx-negative 1L randomized subjects | | 1L Randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N=163 | Chemotherapy N=82 | Nivolumab plus ipilimumab N=27 | Chemotherapy N=12 | Nivolumab plus ipilimumab N=200 | Chemotherapy 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), mo[a] | NR (38.44, NR) | 5.85 (4.40, 7.79) | 1.81 (1.48, 5.75) | 11.53 (2.00, NR) | NR (34.30, NR) | 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. PFS 95% CI upper-bound values of NR 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) 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NR, not reached; PFS, progression-free survival. Subject number totals for 1L randomized CTA-positive (n=301) and 1L randomized subjects with CDx results (n=284) are not equal due to insufficient tissue availability or invalid test status of some subjects 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 1. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**



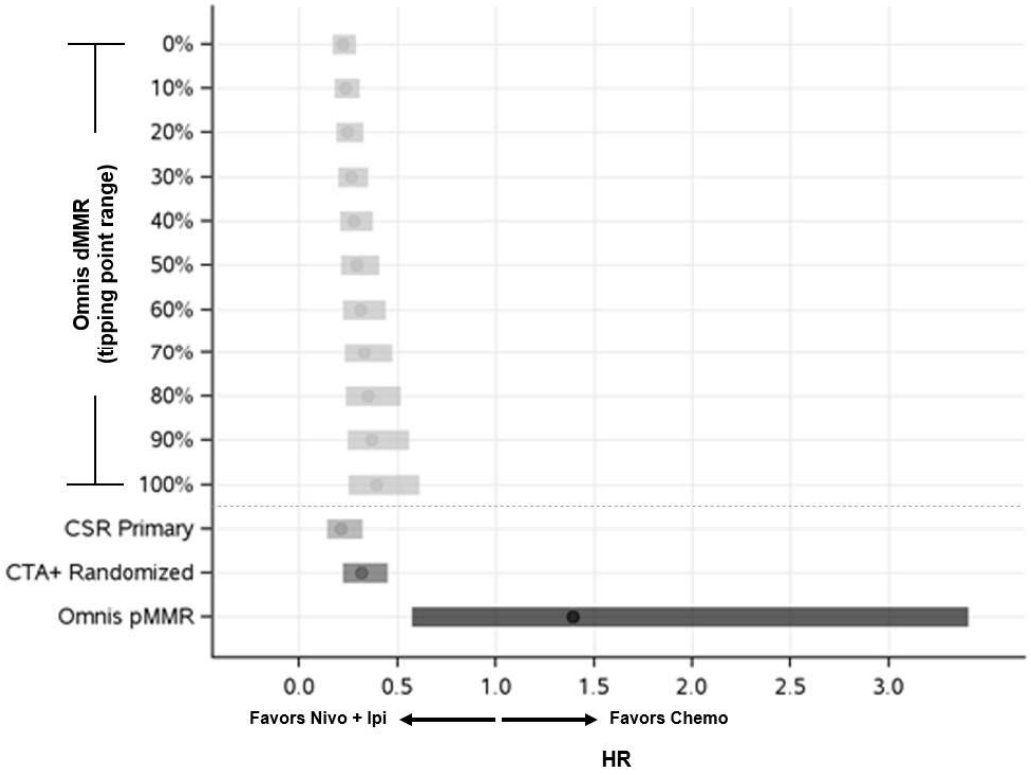

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first crossover dose date.
1L, first-line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

**Figure 2. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2023Oct12 when only the 1L subjects in OPDIVO (nivolumab) plus YERVOY (ipilimumab) and chemotherapy arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, of OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in 1L of the patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for HR of the PFS in these subjects (CTA-negative/CDx-positive, HR = 1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.22). The tipping point PFS HR CI values ranged from a minimum of 0.16 (0% tipping point range CI lower-bound) to a maximum of 0.61 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.16-0.30, and the 100% tipping point 95% CI range at 0.25-0.61.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs chemotherapy in the 1L CTA-positive randomized patients and in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 1).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 255). **CTA+ Randomized**: Subjects randomized in CHECKMATE-8HW with locally confirmed MSI-H/dMMR status (N = 301). **Omnis pMMR**: Subjects randomized in CHECKMATE-8HW with centrally tested pMMR status and locally confirmed MSI-H/dMMR status (N = 39). chemo, chemotherapy; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; Nivo, nivolumab;.

**Figure 3. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus ipilimumab) vs chemotherapy in 1L subjects (CHECKMATE-8HW)**

#### 15.3.4.2. OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects

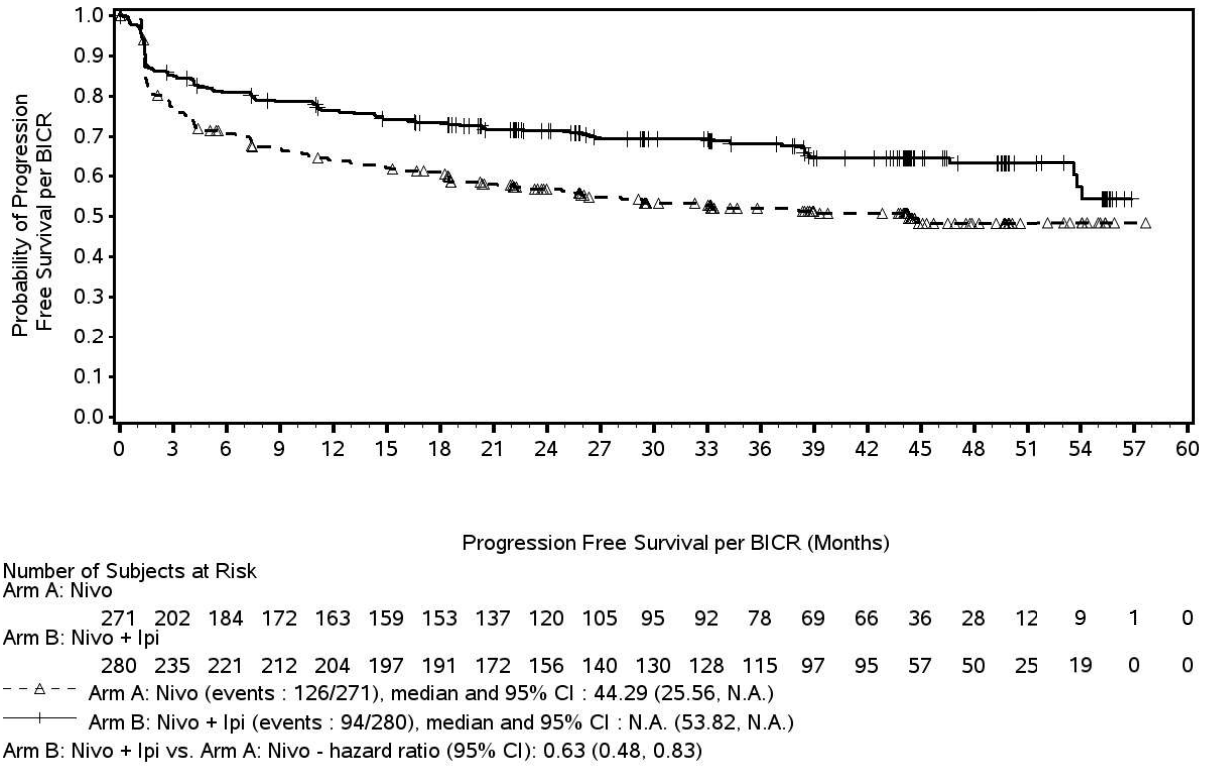
A total of 705 subjects with dMMR/MSI-H status determined by the CTA were randomized to receive OPDIVO (nivolumab) plus YERVOY (ipilimumab) (n = 352) or OPDIVO (nivolumab) (n= 353). The study included 88 sites in 23 countries (Argentina, Australia, Austria, Belgium, Brazil, Canada, Chile, China, Czechia, Denmark, France, Germany, Greece, Ireland, Italy, Japan, Netherlands, Norway, Romania, Spain, Turkey, UK, and US). Most subjects were from US/Canada/European Union (n = 495, 70.2%), 59 (8.4%) were from Asia, and 151 (21.4%) were from the rest of the world. The median age in the OPDIVO (nivolumab) plus YERVOY (ipilimumab) group was 62 years, and the median age in the OPDIVO (nivolumab) group was 63 years. Most subjects were white (n=614, 87.1%), 63 (8.9%) were Asian, 11 (1.6%) were black. The ethnicity of subjects was 66 (9.4%) Hispanic, 353 (50.0%) non-Hispanic, and 286 (40.6%) not reported. The number of male and female subjects was 351 (49.8%) and 354 (50.2%), respectively.

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in PFS per BICR over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA (HR 0.63). PFS benefit observed in all lines of therapy with concordant CTA-positive/CDx-positive population is consistent with that in all lines CTA-positive randomized subjects (HR 0.63) (Table 14, Figure 4). No clinically meaningful PFS benefit was observed in all lines subjects with the CTA-positive/CDx-negative status, shown in median PFS < 2.5 months in both OPDIVO (nivolumab) plus YERVOY (ipilimumab) and OPDIVO (nivolumab) monotherapy.

**Table 14: PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[d]**

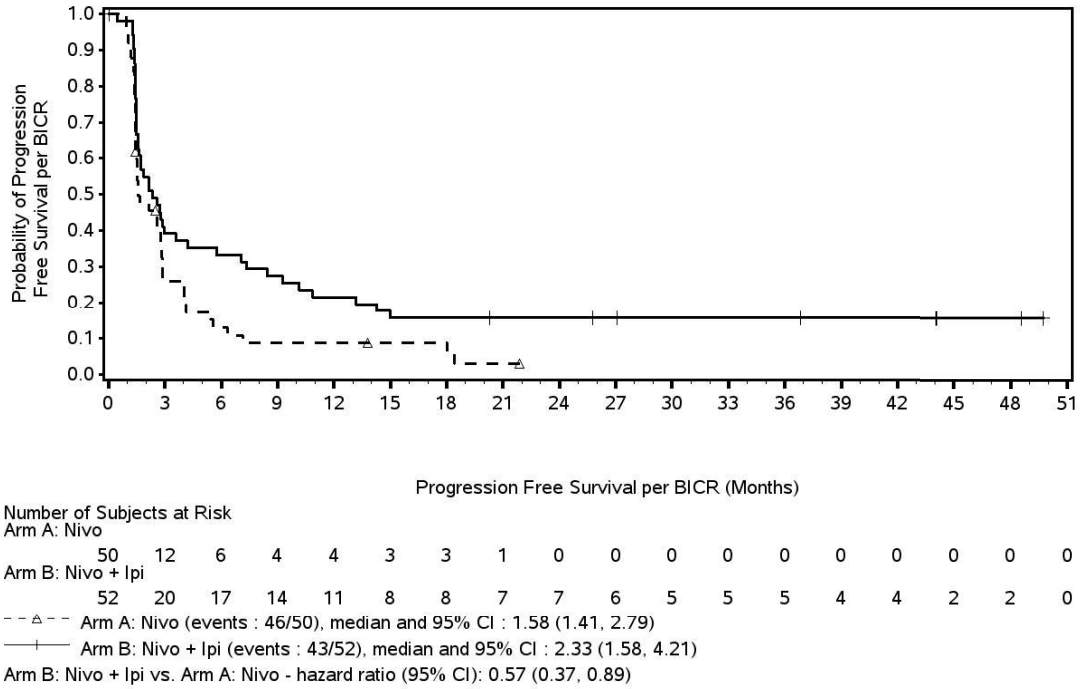
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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), mo[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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed 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; NA, not available; NR, not reached; PFS, progression-free survival. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.




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 4. Kaplan-Meier curve of PFS per BICR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed dMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW)**



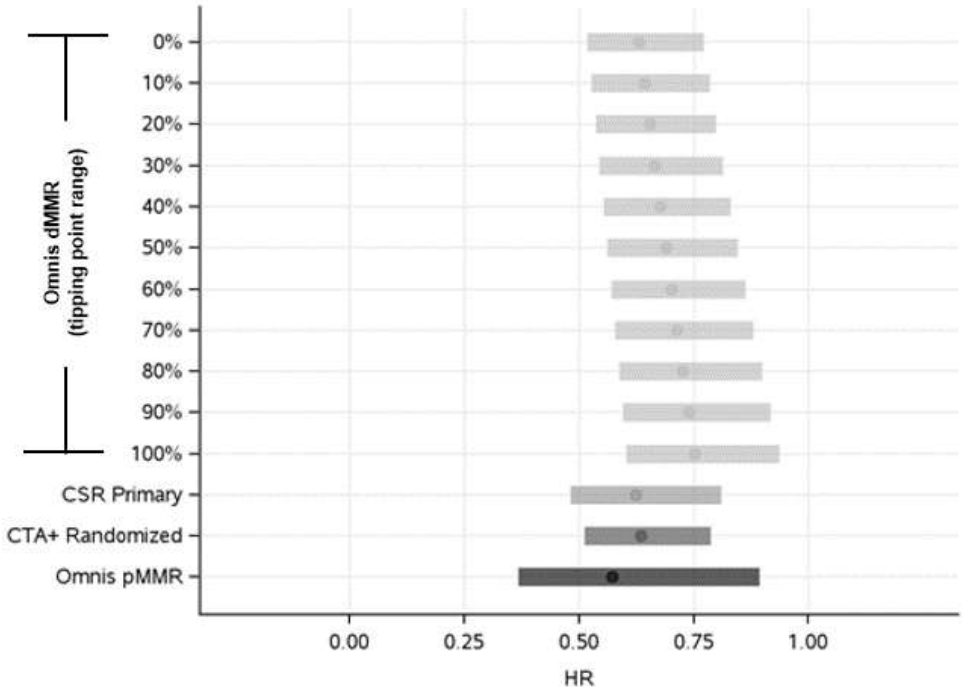

Statistical model for HR: stratified Cox proportional hazard model and stratified log-rank test by tumor sidedness (left vs right) and prior lines of therapy (0, 1, ≥2) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after the first 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 OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all line CTA-positive randomized subjects with centrally confirmed pMMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE 8HW).**

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 HR 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 HR equal to 1. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

Zero to 100% of the tipping point range was assumed for the missing PFS comparison, in HR, 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 HR of PFS of the subjects not enrolled (CTA-negative/CDx-positive, HR=1). The tipping point range of 0% assumes a best-case scenario where the HR of PFS for these subjects is equal to the observed HR for enrolled subjects (CTA-positive/CDx-positive, HR = 0.63). The tipping point PFS HR CI values ranged from a minimum of 0.52 (0% tipping point range CI lower-bound) to a maximum of 0.94 (100% tipping point CI upper-bound), with the 0% tipping point 95% CI range at 0.52-0.77, and the 100% tipping point 95% CI range at 0.60-0.94.

These results are also comparable with the PFS benefits by OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) alone in the patients with the concordant CTA-positive and centrally confirmed dMMR determined by MMR IHC Panel pharmDx (Dako Omnis) (Figure 4).




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 centrally confirmed dMMR by MMR IHC Panel pharmDx (Dako Omnis) or MSI-H by Idylla MSI (N = 582). **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). 3L+, all lines treatment; BICR, blinded independent central review; CTA, clinical trial assay; dMMR, mismatch repair deficient; HR, hazard ratio; Ipi, ipilimumab; MSI-H, microsatellite instability high; Nivo, nivolumab; pMMR, mismatch repair proficient.

**Figure 6. Forest Plot of PFS per BICR for Omnis dMMR of the intended use - OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CHECKMATE-8HW).**

OPDIVO (nivolumab) plus YERVOY (ipilimumab) showed a clinically meaningful improvement in Objective Response Rate (ORR) over OPDIVO (nivolumab) monotherapy in all lines of therapy with dMMR/MSI-H status determined by the CTA, with ORR 63.6% (95% CI: 58.4, 68.7) vs. 49.3% (95% CI: 44.0, 54.6), respectively. ORR benefit observed in 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) vs. 58.3% (95% CI: 52.2, 64.2), respectively (Table 15). No clinically meaningful ORR benefit was observed in all lines subjects with the CTA-positive/CDx-negative status (p=0.0568).

**Table 15: ORR for OPDIVO (nivolumab) plus YERVOY (ipilimumab) vs OPDIVO (nivolumab) in all lines randomized subjects (CTA-positive) with and without centrally confirmed MMR status by MMR IHC Panel pharmDx (Dako Omnis) (CHECKMATE-8HW)[c]**

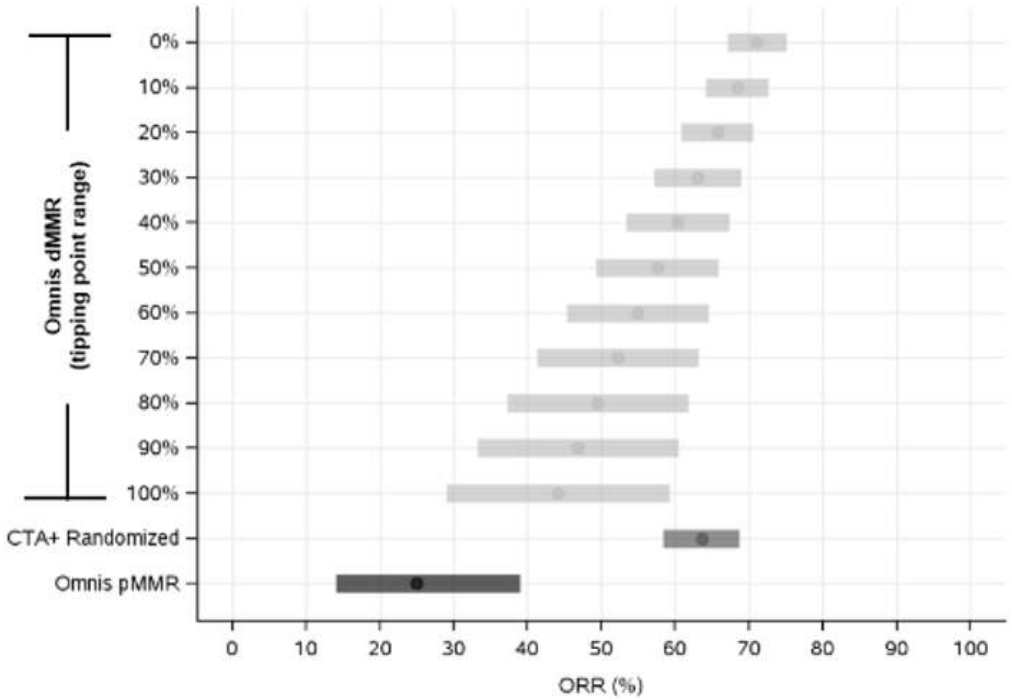
| | CTA-positive/CDx-positive all lines randomized subjects | | CTA-positive/CDx-negative all lines randomized subjects | | All lines randomized subjects (CTA-positive) | |
|---|---|---|---|---|---|---|
| | Nivolumab plus ipilimumab N = 280 | Nivolumab N = 271 | Nivolumab plus ipilimumab N = 52 | Nivolumab 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.
Clinical efficacy data cutoff: 2023Oct12. 1L, first-line treatment; BICR, blinded independent central review; CI, confidence interval; CTA-positive, subjects with locally confirmed dMMR/MSI-H status using clinical trial assay modalities; CDx-positive: deficient mismatch repair (dMMR); CDx-negative: proficient mismatch repair (pMMR); ORR, overall response rate. 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). Please see drug labels for the patient populations included in the approved therapeutic indications.

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. The results are based on the data from a data cutoff on 2024Sept25 when subjects in OPDIVO (nivolumab) plus ipilimumab and OPDIVO (nivolumab) arms were unblinded.

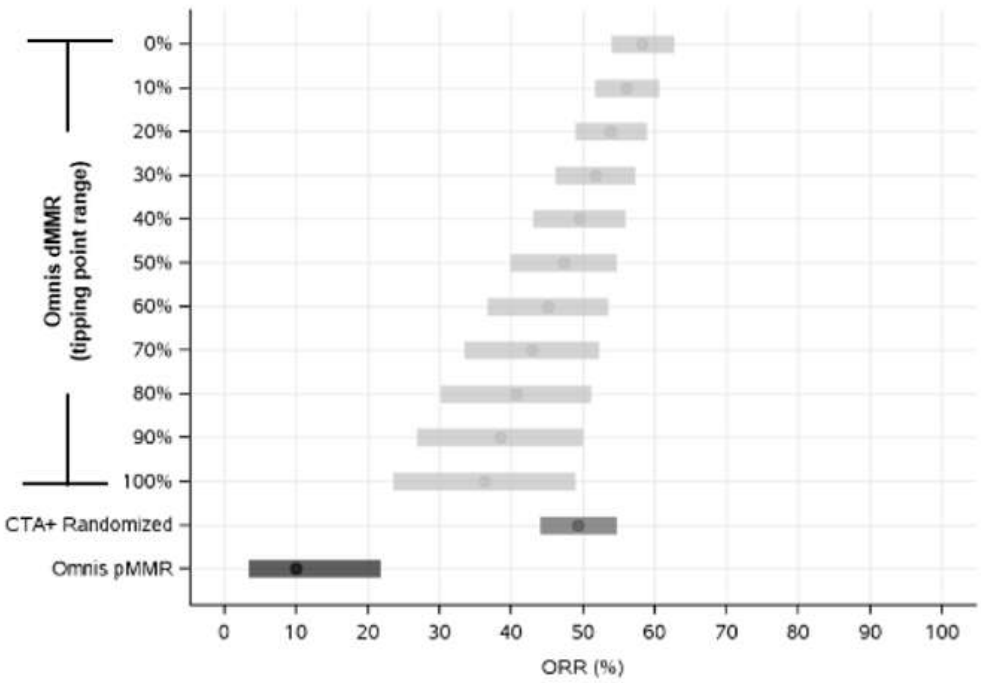
Zero to 100% of the tipping point range was assumed for the missing ORR of nivolumab plus ipilimumab vs nivolumab monotherapy in all lines of these patients who were not enrolled. The tipping point range of 100% assumes a worst-case scenario for the ORR of the subjects not enrolled (ORR = 0 for CTA-negative/CDx-positive). The tipping point range of 0% assumes a best-case scenario where the ORR for these subjects is equal to the observed ORR for enrolled subjects (ORR = 0.711 for nivolumab plus ipilimumab in CTA-positive/CDx-positive subjects; ORR = 0.583 for nivolumab monotherapy in CTA-positive/CDx-positive subjects). For nivolumab plus ipilimumab, the tipping point ORR point estimate values ranged from 0.438 to 0.7107 and CI values ranged from a minimum of 0.2881 (100% tipping point range CI lower-bound) to a maximum of 0.7501 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2881-0.5878, and the 0% tipping point 95% CI range at 0.6714-0.7501. For nivolumab monotherapy, the tipping point ORR point estimates ranged from 0.3593 to 0.5830 and CI values ranged from a minimum of 0.233 (100% tipping point range CI lower-bound) to a maximum of 0.6265 (0% tipping point CI upper-bound), with the 100% tipping point 95% CI range at 0.2330-0.4856, and the 0% tipping point 95% CI range at 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).




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 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 = 0.583).**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

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

### 15.3.5. Clinical Performance Summary

In summary, successful bridging between CTA-positive and dMMR status by MMR IHC Panel pharmDx (Dako Omnis) was achieved based on similar clinical utility between patients with dMMR/MSI-H status by CTA and those with dMMR status by MMR IHC Panel pharmDx (Dako Omnis).

A clinically meaningful improvement in PFS per BICR was demonstrated with:

- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to chemotherapy in 1L treatment of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).
- OPDIVO (nivolumab) plus YERVOY (ipilimumab) combination therapy compared to OPDIVO (nivolumab) monotherapy in all lines of therapy of mCRC subjects with dMMR determined by MMR IHC Panel pharmDx (Dako Omnis).

Re-estimation of efficacy results using imputations and sensitivity analyses by considering the missing assessments of MMR IHC Panel pharmDx (Dako Omnis) supported these findings.

These results support the clinical performance of MMR IHC Panel pharmDx (Dako Omnis) by demonstrating the assay's clinical utility in identifying patients with dMMR CRC who may benefit from treatment with OPDIVO (nivolumab) alone or in combination with YERVOY (ipilimumab) in accordance with their approved US package inserts.

## 16. Troubleshooting

Refer to the Troubleshooting section in Agilent's Education Guide for remedial action or contact Agilent Pathology Support to report unusual staining.[8]

Dako Omnis is an automated system designed to alert the user if anything in the run has been outside of specifications. Please refer to the Dako Omnis User Guide for details on what conditions are flagged and how. Table 16 is a troubleshooting guide for results and conditions that are not easily identified through the Dako Omnis warning and alert system.

The user should always ensure adherence to the maintenance schedule for the Dako Omnis instrument. Always ensure to use the appropriate controls as described in 'Quality Control', Section 11.

**Table 16. Troubleshooting.**

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| 1. No or weak staining of slides. | 1a. Excessive heating of mounted tissue sections prior to loading on Dako Omnis may lead to loss of visible PMS2 immunoreactivity and/or tissue morphology. | 1a. Dry the tissue sections at 58 ± 2 °C for a maximum of 1 hour, using a calibrated oven with uniform heat distribution.[10] |
| | 1b. Wrong storage conditions used for reagents. | 1b. Check that reagents have been stored correctly according to listed storage conditions. |
| | 1c. Inappropriate fixation method used. | 1c. Ensure that patient tissue is not fixed for too short or too long a time period, that ischemia time has been minimized, and that the correct fixative (10% NBF) was used. |
| | 1d. Reagent is used past its expiration date. | 1d. Check Dako Link Omnis Workstation software to determine if slides were flagged suspicious. Ensure reagent is not used past its expiration date. |
| | 1e. Reagent is used past its onboard stability. | 1e. Check Dako Link Omnis Workstation software to determine if slides were flagged as suspicious. Ensure reagent is not used past its onboard stability. |
| | 1f. Incorrect placement of dynamic gap lids in staining modules. | 1f. Check placement of dynamic gap lids. |
| | 1g. Damaged dynamic gap lids. | 1g. Check integrity of dynamic gap lids. |
| | 1h. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution (50x) concentrate. | 1h. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 1i. Incorrect Target Retrieval Solution is used. | 1i. Ensure that correct Target Retrieval Solution specified in 'Materials Required, but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |
| | 1j. 1x Target Retrieval Solution does not meet pH specifications. | 1j. Check pH of 1x Target Retrieval Solution. If pH is outside acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. Refer to Problem #6 for additional troubleshooting. |
| 2. Excessive nonspecific staining of slides. | 2a. Starch additives used in mounting sections to slides. | 2a. Avoid using starch additives for adhering sections to glass slides. Many additives are immunoreactive. |
| | 2b. Sections dried after staining procedure/prior to coverslipping. | 2b. Verify that the Unloading Station is filled with sufficient water. |
| | | 2b. Avoid stained slides drying out after staining completion (e.g., between removal from Dako Omnis and coverslipping). |
| | 2d. Inappropriate fixation method used. | 2d. Ensure that approved fixative was used. Alternative fixative may cause excessive nonspecific staining. |

| Problem | Probable Cause | Suggested Action |
|---|---|---|
| | 2e. Paraffin incompletely removed. | 2e. Check appearance of solvent coupling. Refer to Dako Omnis User Guide for details. |
| | 2f. Nonspecific binding of reagents to tissue. | 2f. Ensure that correct fixation method of the specimen is used and avoid large areas of necrosis. |
| | 2g. Re-use of mixing strip. | 2g. Ensure that new mixing strips are used. |
| 3. Excessively strong specific staining. | 3. Inappropriate fixation method used. | 3. Ensure that only approved fixatives and fixation methods are used. |
| 4. Tissue detaches from slides. | 4. Use of incorrect slides. | 4. Use FLEX IHC Microscope Slides (Code K8020), or SuperFrost Plus slides. |
| 5. Slide is flagged as suspicious. | 5a. Reagent is used beyond its expiration date. | 5. Slides flagged as suspicious should be evaluated by qualified personnel, contact an Agilent Technologies representative if further action is needed. |
| | 5b. Reagent is stored onboard Dako Omnis beyond its validated onboard stability. | |
| | 5c. Maintenance overdue or other factors. | |
| 6. 1x Target Retrieval Solution does not meet pH specifications | 6a. pH meter is not calibrated correctly. | 6a. Ensure pH meter is calibrated per manufacturer's recommendations. After recalibration, retest pH of 1x Target Retrieval Solution. Do not modify the pH of 1x Target Retrieval Solution. If pH is outside of the acceptable range (pH 9.0 ± 0.2), discard 1x Target Retrieval Solution. Prepare new 1x Target Retrieval Solution. Check pH of new 1x Target Retrieval Solution. |
| | 6b. Target Retrieval Solution pH is measured at incorrect temperature. | 6b. Ensure that 1x Target Retrieval Solution pH is measured at ambient temperature. |
| | 6c. Distilled or de-ionized water is not used to dilute the Target Retrieval Solution concentrate. | 6c. Ensure that distilled or de-ionized water is used to prepare 1x Target Retrieval Solution. |
| | 6d. Incorrect Target Retrieval Solution is used. | 6d. Ensure that the correct Target Retrieval Solution specified in 'Materials Required but Not Supplied', Section 5 and/or 'Reagent Preparation', Section 9 is used. |

**NOTE:** If the problem cannot be attributed to any of the causes in Table 16, or if the suggested corrective action fails to resolve the problem, please contact Agilent Pathology Support for further assistance. Additional information on staining techniques and specimen preparation can be found in Agilent's Education Guide (available from www.agilent.com), Atlas of Immunohistology, and Immunoperoxidase Techniques. A Practical Approach to Tumor Diagnosis.[8,12,13]

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**Explanation of symbols**

| | | | | | |
|---|---|---|---|---|---|
| REF | Catalogue number | | Temperature limitation | IVD | In vitro diagnostic medical device |
| | Manufacturer | LOT | Batch code | Σ | Contains sufficient for <n> tests |
| | Use by | | Consult instructions for use | BIO | Contains biological material of animal origin |
| | Caution | EC REP | Authorized representative in the European Community | | |

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