VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody

INTRODUCTION

This document contains two instructions for use. Both panels consist of the same formulations and packaging, utilize the same ancillary reagents, and are run on the BenchMark ULTRA instrument. Both panels use the same scoring algorithm for interpretation.

VENTANA MMR RxDx Panel

Refer to Table 1 for indication of use.

Includes the following antibodies:

- VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody
- VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody
- VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody
- VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody

VENTANA MMR IHC Panel

Colorectal Carcinoma for Lynch Syndrome

Includes the following antibodies:

- VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody
- VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody
- VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody
- VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody
- VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody

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VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody

For use with the VENTANA MMR RxDx Panel

REF 790-5091

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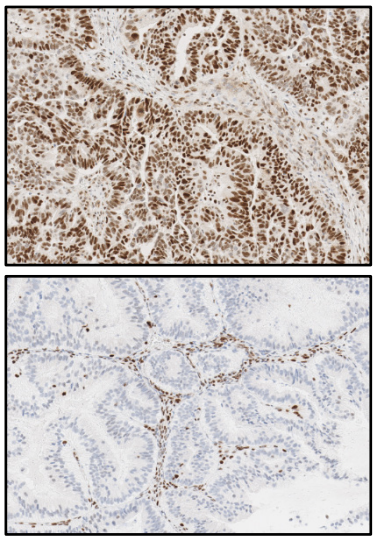


Figure 1. VENTANA anti-MLH1 (M1) antibody staining with Intact (top) or Loss (bottom) of expression in endometrial carcinoma tissue.

INTENDED USE

VENTANA MMR RxDx Panel

VENTANA MMR RxDx Panel is a qualitative immunohistochemistry test intended for use in the assessment of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2 and MSH6) in formalin-fixed, paraffin-embedded (FFPE) tissue specimens by light microscopy. The OptiView DAB IHC Detection Kit is used for MLH1, MSH2 and MSH6, and the OptiView DAB IHC Detection Kit with the OptiView Amplification Kit is used for PMS2 on a VENTANA BenchMark ULTRA instrument.

VENTANA MMR RxDx Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody, and VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody.

VENTANA MMR RxDx Panel is indicated as an aid in identifying patients eligible for treatment with the therapies listed in Table 1 for the indication and MMR status in accordance with the approved therapeutic product labeling.

Table 1. VENTANA MMR RxDx Panel companion diagnostic indications.

Indication for use	Therapy	MMR Status
Solid Tumors	JEMPERLI (dostarlimab-gxly)	deficient MMR (dMMR)
Solid Tumors	KEYTRUDA (pembrolizumab)	deficient MMR (dMMR)
Endometrial Carcinoma (EC)	KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib)	proficient MMR (pMMR)

Results of the VENTANA MMR RxDx Panel should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This product is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody (VENTANA anti-MLH1 (M1) antibody) is a mouse monoclonal antibody produced against a full-length recombinant MLH1 protein with a glutathione-S-transferase (GST) tag. VENTANA anti-MLH1 (M1) antibody recognizes MLH1, which is one of several clinically important DNA MMR proteins.[1,2] VENTANA anti-MLH1 (M1) antibody is part of VENTANA MMR RxDx Panel, an immunohistochemical (IHC) assay system for identifying tumors with loss of expression of any of 4 MMR proteins that ordinarily are ubiquitously expressed in proliferating normal and malignant cells: MLH1, PMS2, MSH2, and MSH6.[3] These tumors are considered MMR-deficient (dMMR).

MMR is a conserved molecular mechanism that functions to correct the improper base substitutions that spontaneously occur during DNA replication.[4] Polymerase chain reaction (PCR)-based methods have shown that MMR failure frequently leads to microsatellite instability (MSI), a condition in which short, tandem nucleotide repeats are inserted into the DNA.[5-7] When the number of repeats is equal to or greater than 30% of the examined microsatellite loci, MSI can be further characterized as MSI-High (MSI-H). Defects in the MMR machinery have been attributed to mutations in the MMR proteins, most commonly MLH1, PMS2, MSH2, and MSH6.

The MLH1 and PMS2 proteins normally function together in a heterodimeric complex, as do the MSH2 and MSH6 proteins. When MMR is functioning normally, the MSH6/MSH2 heterodimer binds to mismatched DNA. This binding induces a conformational change that allows the MLH1/PMS2 heterodimer to bind the DNA-bound MSH6/MSH2 complex, resulting in excision repair of the affected DNA.[7,8] Mutations or deficiencies in these proteins result in frequent MSI and somatic mutation due to replication error. MMR IHC testing can be useful in identifying tumors with alterations in MMR.[9]

CLINICAL SIGNIFICANCE

Solid Tumors, Including Endometrial Carcinoma

Cancer is the second leading cause of death in the United States (US) and worldwide.[10,16] In the US, approximately 1.8 million new cancer cases were expected to be diagnosed in 2020.[10] Worldwide, 17 million new cases were expected to be diagnosed in 2018.[11] Of these cases, the vast majority will consist of solid tumors, approximately 14% of which have been shown to have defective MMR protein expression.[12] The prevalence of MSI-H/dMMR in solid tumors can vary by tumor indication and stage of disease.[12] The tumor types having the highest prevalence of MMR defects include colorectal and endometrial carcinomas. In particular, a significant proportion of cancers originating from colon and rectum (CRC), which remain the third most prevalent cancers (excluding skin cancers) in both sexes develop through defective function of the MMR mechanism. As a consequence of the MMR deficiency, CRC tumors exhibit MSI resulting from the inability of MMR proteins to repair DNA replication errors.[13,14]

Endometrial carcinoma is the most common gynecological malignant disease and the fourth most common cancer in North American women.[15,16] It is one of the leading causes of cancer-related death in the world.[17] EC is frequently noted to have many genetic alterations including MSI.[15] MSI-H and dMMR has been reported in 20-40% of endometrial cancers.[18,19,20] While the treatment of EC varies depending on the grade, histology and stage of disease, evaluation of the MMR status of EC tumors is helpful for prognosis and guiding treatment.[21]

PD-1/PD-L1 Checkpoint Inhibition and DNA Mismatch Repair (MMR)

Emerging immunotherapies, particularly those that modify cellular pathways involving the programmed death 1 (PD-1) or programmed death ligand 1 (PD-L1) proteins, are reshaping clinicians' therapeutic strategies. PD-1 is an inhibitory receptor expressed on T-cells after T-cell activation, which is sustained in states of chronic stimulation such as in chronic infection or cancer.[22] PD-L1 expression has been observed in immune cells and malignant cells, and aberrant expression of PD-L1 on tumor cells has been reported to impede anti-tumor immunity, resulting in immune evasion.[22,23] Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity. Multiple studies have demonstrated that MMR deficiency correlates with higher expression of PD-1 or PD-L1, possibly due to increased neoantigen expression associated with the tumor mutation burden that results from replication errors.[15,24] Thus, MMR proteins may be useful as predictive biomarkers for PD-1–targeted therapy; specifically, a loss of expression of one or more MMR proteins might predict an increased likelihood of response to such therapy.[25,26,27] PD-1 inhibitors can be beneficial in cancers with a high frequency of MMR deficiency and /or MSI-H.[25,27] Hence, patients with solid tumors including endometrial carcinoma who are considering PD-1 targeted therapy will benefit from a companion diagnostic assay to determine if they may be eligible for treatment with PD-1 or PD-L1 checkpoint inhibition therapy.

In syngeneic mouse tumor models, lenvatinib decreased tumor-associated macrophages, increased activated cytotoxic T cells, and demonstrated greater antitumor activity in combination with an anti-PD-1 monoclonal antibody compared to either treatment alone.[28,29]

A loss of expression of any of the essential MMR proteins, including MLH1, PMS2, MSH2, or MSH6, causes MMR deficiency. As part of the VENTANA MMR RxDx Panel,

VENTANA anti-MLH1 (M1) antibody aids in determining the MMR IHC status of tumors by classifying them as intact or loss for MMR protein expression. The presence of staining for all four MMR protein markers in the tumor using VENTANA MMR RxDx Panel indicates that the case is MMR-Proficient (pMMR). The absence of staining for any of the MMR protein markers using VENTANA MMR RxDx Panel indicates that the case is MMR-Deficient (dMMR).

PRINCIPLE OF THE PROCEDURE

The VENTANA anti-MLH1 (M1) antibody is a mouse monoclonal antibody produced against a full-length recombinant MLH1 protein with a GST tag. The VENTANA anti-MLH1 (M1) antibody binds to the MLH1 protein in FFPE tissue sections. The antibody can be localized using a haptenated secondary antibody followed by a multimer anti-hapten-HRP conjugate (OptiView DAB IHC Detection Kit, Cat. No. 760-700 / 06396500001). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the BenchMark ULTRA instrument washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies ULTRA LCS (ULTRA LCS (Predilute), Cat. No. 650-210 / 05424534001), which minimizes evaporation of the aqueous reagents from the specimen slide.

In addition to staining with VENTANA anti-MLH1 (M1) antibody, a second slide should be stained with the mouse monoclonal negative reagent, Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001). The negative reagent control is used to assess background staining.

MATERIAL PROVIDED

VENTANA anti-MLH1 (M1) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-MLH1 (M1) antibody contains approximately 5 µg of a mouse monoclonal antibody.

The antibody is diluted in a TBS buffer containing 0.3% carrier protein.

Specific antibody concentration is approximately 1 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-MLH1 (M1) antibody is a mouse monoclonal antibody produced as a cell culture supernatant.

Refer to the appropriate interpretation guide for detailed instructions for interpretation of MMR Panel staining in specific indications:

- VENTANA MMR RxDx Panel Interpretation Guide for EC indication (P/N 1019382US)
- VENTANA MMR RxDx Panel Interpretation Guide for Solid Tumors indication (P/N 1020156US)

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed, but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody (Cat. No. 790-5094 / 07862261001)
2. VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody (Cat. No. 790-5093 / 07862253001)
3. VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody (Cat. No. 790-5092 / 07862245001)
4. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
5. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
6. Microscope slides, positively charged
7. Bar code labels (appropriate for negative reagent control and primary antibody being tested)
8. Xylene (Histological grade)
9. Ethanol or reagent alcohol (Histological grade)
   - 100% solution: Undiluted ethanol or reagent alcohol
   - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
   - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
10. Deionized or distilled water
11. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
12. For VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 or Cat. No. 860-099 / 06718663001)
13. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
14. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
15. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
16. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
17. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
18. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
19. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
20. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
21. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
22. Light microscope
23. Absorbent wipes

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. Tissue should be fixed immediately following excision for use with the VENTANA MMR antibodies. A delay to fixation of more than 6 hours has been shown to have an adverse effect on stain intensity of the tissue. Tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours is recommended. Fixation times of less than 6 hours and more than 72 hours may result in a loss of staining for MLH1. The amount of NBF used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).[30-32]

Fixatives such as zinc formalin, Z-5, 95% alcohol, alcohol-formalin-acetic acid (AFA) and PREFER fixative have demonstrated weak or variable staining; they are not recommended for use with this assay. Users who deviate from the specified specimen preparation must accept responsibility for interpretation of patient results.

Sections should be cut at 4 µm thick and mounted on positively charged glass slides. No other thicknesses have been validated. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time and may be compromised 45 days after cutting from the FFPE tissue block. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

It is recommended that positive and negative controls be run simultaneously with test specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only).
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.

6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[33,34]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument Operator's Manual, and instructions for use of all necessary components located at dialog.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

STAINING PROCEDURE

VENTANA anti-MLH1 (M1) antibody has been developed for use on a BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit and ancillary reagents. Refer to Table 2 for the recommended staining protocol for VENTANA MMR RxDx Panel.

This antibody has been optimized for specific incubation times, but the user must validate results obtained with this reagent. The effect of varying time and temperature of the antigen retrieval (cell conditioning) and antibody incubation from the recommended staining protocol in Table 2 may result in sub optimal staining and false MMR results. It is strongly recommended not to deviate from the recommended staining protocol in Table 2. Appropriate controls should be employed and documented. Users who deviate from the listed protocol must accept responsibility for interpretation of patient results.

The parameters for the automated procedures can be displayed, printed, and edited according to the procedure in the instrument Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding IHC staining procedures.

Table 2. Recommended staining procedure and protocol for VENTANA anti-MLH1 (M1) antibody and Negative Control (Monoclonal) with OptiView DAB IHC Detection Kit on a BenchMark ULTRA instrument for VENTANA MMR RxDx Panel.

Staining Procedure: U MMR Panel	
Protocol Step	**Parameter Input**
Antibody (Primary)	anti-MLH1 Mouse Mono Ab Selected Or Negative Control Selected
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 64 minutes
Pre-Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	24 minutes
OptiView HQ Linker	8 minutes
OptiView HRP Multimer	8 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Note: Any deviation from recommended test procedures may invalidate results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patients' results.

Due to variations in tissue fixation and processing methods, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation time and cell conditioning time based on individual specimens and pathologist preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[31]

If the internal positive controls fail to demonstrate appropriate staining, results with the test specimen should be considered invalid.

QUALITY CONTROL PROCEDURES

Internal Positive Controls

Normal tissue elements (e.g., lymphocytes, fibroblasts, or normal epithelium) in the immediate vicinity of the tumor will serve as internal positive controls. Unequivocal nuclear staining in these cells validates the staining run. If the internal positive controls fail to demonstrate appropriate staining, results with the test specimen should be considered invalid.

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. Optimal laboratory practice is to include a positive control section on the same slide as the patient tissue. This practice helps to identify a failure to apply primary antibody or other critical reagent to the patient test slide. A tissue with weak positive staining is more suitable for optimal quality control. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy, or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Such tissues may monitor all steps of the procedure from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

Pre-qualified solid tumor tissue including EC tissue with an MMR status of intact or tonsil may be used as a positive system-level control. Normal tonsil will stain intact for MLH1 using the VENTANA anti-MLH1 (M1) antibody. The positive tissue control should exhibit unequivocal nuclear staining in viable tumor and/or normal tissue elements. For all tissues, internal positive control cells (i.e., lymphocytes, fibroblasts or normal epithelium in the vicinity of the tumor) should stain positive in the nucleus.

Negative Reagent Control for Test Tissue

Ventana Medical Systems, Inc. strongly recommends a negative reagent control be used to stain an adjacent section of the patient specimen tissue on a separate slide from the VENTANA anti-MLH1 (M1) antibody-stained slide. A negative reagent control mouse monoclonal antibody (Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001) is recommended for use in place of the primary antibody to evaluate nonspecific staining. The staining parameters for the negative reagent control antibody should be the same as those for the primary antibody.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing on a series of tissues with known IHC performance characteristics representing tissues Intact for MLH1 Clinical Status. (Refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist,[35] or the CLSI Approved Guideline.[36])

STAINING INTERPRETATION / EXPECTED RESULTS

VENTANA anti-MLH1 (M1) antibody has a nuclear staining pattern in actively proliferating cells. Tumor tissue stained with the VENTANA anti-MLH1 (M1) antibody is assigned a clinical status by a trained pathologist based on their evaluation of the presence or absence of specific nuclear staining in the tumor. A clinical status of Intact is assigned to cases with unequivocal nuclear staining in viable tumor cells, in the presence of acceptable internal positive controls (nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor). A clinical status of Loss is assigned to cases with unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls as shown in Table 3.

If unequivocal nuclear staining is absent in internal positive controls and/or background staining interferes with interpretation, then the assay should be considered unacceptable and repeated. Punctate nuclear staining of tumor cells should be considered negative (Loss). In cases with focal tumor cell staining, some specimens may exhibit focal staining

in the tumor cells and staining intensity may vary from weak to strong. Based on the VENTANA MMR RxDx Panel scoring algorithm, focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls should be given a Clinical Status of Loss. On the other hand, focal strong unequivocal nuclear staining in the viable tumor cells in the presence of internal positive controls should be given a Clinical Status of Intact.

Table 3. Staining interpretation for the VENTANA anti-MLH1 (M1) antibody.

Clinical Status	Description
Intact MLH1 Expression	Unequivocal nuclear staining in viable tumor cells in the presence of acceptable internal positive controls (eg nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor)
Loss of MLH1 Expression	Unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of acceptable internal positive controls. Punctate nuclear staining will be considered negative.

VENTANA anti-MLH1 (M1) antibody-stained cases are categorized as Intact or Loss according to the presence or absence of specific staining in the tumor.

The interpretation for overall panel-level MMR Status is provided below in Table 4.

Table 4. Staining interpretation for VENTANA MMR RxDx Panel.

Proficient	Deficient
All 4 markers (MLH1, PMS2, MSH2, and MSH6) in the panel exhibit intact protein expression	At least 1 marker (MLH1, PMS2, MSH2, and MSH6) in the panel exhibits loss of protein expression

SPECIFIC LIMITATIONS

Ventana Medical Systems, Inc. provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Deviation from the recommended conditions for antigen retrieval (Cell Conditioning) provided in the staining protocol (Table 2) may invalidate the results. Appropriate controls should be employed and documented. Users who deviate from the listed protocol must accept responsibility for interpretation of patient results.

VENTANA anti-MLH1 (M1) antibody has been solely cleared for use on the BenchMark ULTRA instrument with OptiView DAB IHC Detection Kit and is not cleared for use with any other detection methods or automated staining instruments.

Some cases may be particularly challenging due to the following issues:

- Nonspecific background: Some specimens may exhibit nonspecific background staining for reasons that are not well understood. For this reason, evaluation of a VENTANA anti-MLH1 (M1) antibody slide must include a comparison of the slide to the negative reagent control slide to determine the level of nonspecific background staining. Cytoplasmic staining, if present, should be disregarded in VENTANA anti-MLH1 (M1) antibody IHC interpretation.
- Focal Staining: Some specimens may exhibit focal staining in tumor cells; the staining intensity may vary from weak to strong. As specified by the VENTANA anti-MLH1 (M1) antibody IHC scoring algorithm, focal weak equivocal nuclear staining in viable tumor cells in the presence of acceptable internal positive control staining should be categorized as Loss status.
- Punctate Staining: Some specimens may exhibit discrete punctate staining within a few nuclei of the tumor; the staining intensity may vary from weak to strong. This staining pattern should be ignored. If only this type of staining pattern is present, the clinical status is Loss.
- Speckling: In contrast to punctate staining, speckling has a finer, more granular appearance and can be focal or occur across many tumor cells. This staining pattern, if seen in the tumor cell nuclei, should be ignored and the slide given a clinical status of Loss.
- Tissue or Staining Artifact: Histologic artifacts originating from the sample processing and microtomy processes can also complicate the determination of VENTANA anti-MLH1 (M1) antibody IHC clinical status. These artifacts may include, but are not limited to, fixation gradients and edge effects, DAB trapping, nuclear bubbling, lack of staining in some regions of the tissue, tearing or folding of the tissue, and loss of the tissue section. In some instances, repeat staining of new sections or acquisition of a new specimen may be required.

The clinical performance of the MMR RxDx panel with KEYTRUDA (pembrolizumab) in gastric cancer has not been fully established. Data collection to further establish the clinical performance in gastric cancer patients with the MMR RxDx is ongoing.

PERFORMANCE CHARACTERISTICS

Analytical Performance

Staining tests for staining sensitivity, specificity, repeatability, and intermediate precision, as well as tests for reader precision, inter-laboratory reproducibility, and clinical outcome were conducted and the results are listed in the following section.

Sensitivity and Specificity

Analytical sensitivity was evaluated by characterizing dMMR prevalence in 3056 tissue samples from the intended use solid tumor population represented by seven organ systems (gastrointestinal, reproductive, urinary, hepatopancreatobiliary, endocrine, soft tissue, and thoracic). The prevalence for MMR deficiency within the overall pan tumor tissue samples was 5.8% (where dMMR prevalence was calculated using total samples assessed). The prevalence of loss status on an individual MMR marker basis was 4.2% in MLH1, 4.6% in PMS2, 0.9% in MSH2, and 1.2% in MSH6. On a MMR panel level, 8.3% of all enrolled pan tumor cases were deemed non-evaluable during the final reads. The final failure rate for each individual MMR marker was 3.5% for MLH1, 7.9% for PMS2, 4.6% for MSH2, and 2.4% for MSH6.[12]

Analytical specificity was determined by staining multiple cases of normal and neoplastic human tissues with VENTANA anti-MLH1 (M1) antibody. The results are listed in Table 5 and Table 6. Positive staining is nuclear unless otherwise specified. No unexpected staining was observed with VENTANA anti-MLH1 (M1) antibody on the normal and neoplastic tissues. As expected, since MMR is present in all actively proliferating cells, all normal and neoplastic tissues demonstrated positive staining.

Table 5. Specificity of VENTANA anti-MLH1 antibody staining on formalin-fixed, paraffin-embedded normal tissues.

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Adrenal gland	3/3	Lung	4/4
Bladder	3/3	Lymph node	3/3
Bone marrow	3/3	Mesothelium	4/4
Ovary	5/5	Pancreas	3/3
Breast	3/3	Parathyroid gland	3/3
Cerebellum	3/3	Peripheral nerve	5/5
Cerebrum	3/3	Prostate	3/3
Cervix	3/3	Skeletal Muscle	3/3
Colon	3/3	Skin	3/3
Endometrium	3/3	Spleen	3/3
Esophagus	3/3	Stomach	3/3
Heart	3/3	Testis	3/3
Hypophysis (Pituitary)	3/3	Thymus	3/3
Intestine	3/3	Thyroid	4/4
Kidney	3/3	Tongue/Salivary gland	3/3
Liver	3/3	Tonsil	3/3

Note: Mismatch repair proteins such as MLH1 are present in all actively proliferating cells. For all tissues, positive/negative staining was determined for tissue-specific elements in the presence of positive staining in normal control cells (lymphocytes, fibroblasts, and epithelial cells).

Table 6. Specificity of VENTANA anti-MLH1 (M1) antibody staining on a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# Positive / Total Cases
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebrum)	1/1
Serous adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Ovary)	1/1
Pancreatic neuroendocrine neoplasm (Pancreas)	1/1
Seminoma (Testis)	2/2
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	1/1
Microinvasive ductal carcinoma (Breast)	1/1
Invasive ductal carcinoma (Breast)	1/1
Small cell carcinoma (Lung)	1/1
Squamous cell carcinoma (Lung)	1/1
Neuroendocrine carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	1/1
Signet ring carcinoma (Stomach)	1/1
Adenocarcinoma (Small intestine)	1/1
Stromal sarcoma (Small intestine)	1/1
Gastrointestinal stromal tumor (Peritoneum)	1/1
Adenocarcinoma (Colon)	1/1
Adenocarcinoma (Rectum)	1/1
Gastrointestinal stromal tumor (GIST) (Rectum)	1/1
Hepatoblastoma (Liver)	1/1
Clear cell carcinoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Leiomyoma (Uterus)	1/1
Adenocarcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	1/1
Squamous cell carcinoma (Skin)	1/1
Neurofibroma (Lumbar)	1/1
Neuroblastoma (Retroperitoneum)	1/1
Mesothelioma (Peritoneum)	1/1
B-cell lymphoma; NOS (Lymph node)	3/3
Hodgkin's lymphoma (Lymph node)	1/1
Leiomyosarcoma (Bladder)	1/1
Osteosarcoma	1/1
Spindle cell rhabdomyosarcoma (Peritoneum)	1/1
Leiomyosarcoma (Smooth muscle)	1/1

Note: Mismatch repair proteins such as MLH1 are present in all actively proliferating cells. For all tissues, positive/negative staining was determined for tumor cells in the presence of positive staining in normal control cells (lymphocytes, fibroblasts, and epithelial cells).

ANALYTICAL PERFORMANCE FOR VENTANA MMR RXDX PANEL IN ENDOMETRIAL CARCINOMA

Repeatability and Intermediate Precision- Marker Level Study

In this study, EC samples were supplemented with samples from a variety of other solid tumor tissues. The sample distribution was as follows: 6 EC (3 intact and 3 loss), 27 (15 intact and 12 loss) FFPE samples from variety of solid tumor tissues from each of the following organ systems were included in this study: urinary (6 samples), reproductive (9 samples), gastrointestinal (6 samples), hepato-pancreatobiliary (3 samples) soft tissue (1 sample), and thoracic (2 samples). The study designs verified staining precision on tumor tissues stained with VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody.

- Three lots of MLH1 (M1) (between-antibody lots)
- Three lots of OptiView DAB IHC Detection Kits (between-detection kits)
- Three BenchMark ULTRA instruments (between instruments)
- Across three days (between-day)
- Across all intermediate precision conditions (within-run)

Each sample was assigned one mode based on the samples aggregated per test condition for between-antibody lots, between-detection kit lots, between-instruments and between-day. For within-run condition, each sample was compared within its duplicate samples per test run. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti-MLH1 (M1) antibody (Table 3). Results are summarized in Table 7 for EC tissues and Table 8 for variety of tumor tissues including EC.

Table 7. Repeatability and intermediate precision of VENTANA anti-MLH1 (M1) antibody on EC tissues as measured by MLH1 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	LPA	18/18	100.0	(82.4, 100.0)
	IPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)
Between-Detection Kits	LPA	18/18	100.0	(82.4, 100.0)
	IPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)
Between-Instruments (BenchMark ULTRA)	LPA	18/18	100.0	(82.4, 100.0)
	IPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)
Between-Day	LPA	18/18	100.0	(82.4, 100.0)
	IPA	18/18	100.0	(82.4, 100.0)
	OPA	36/36	100.0	(90.4, 100.0)

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Within-Run	LPA	27/27	100.0	(87.5, 100.0)
	IPA	27/27	100.0	(87.5, 100.0)
	OPA	54/54	100.0	(93.4, 100.0)

Note: LPA =Loss Percent Agreement (agreement rate for MLH1 Loss status); IPA = Intact Percent Agreement (agreement rate for MLH1 Intact status); OPA = Overall Percent Agreement (overall agreement rate for MLH1 clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% LPA, IPA and OPA were calculated using Wilson score method.

Table 8. Repeatability and intermediate precision of VENTANA anti-MLH1 (M1) antibody on a variety of solid tumor tissues including EC as measured by MLH1 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	LPA	66/66	100.0	(94.5, 100.0)
	IPA	96/96	100.0	(96.2, 100.0)
	OPA	162/162	100.0	(97.7, 100.0)
Between-Detection Kits	LPA	65/65	100.0	(94.4, 100.0)
	IPA	95/95	100.0	(96.1, 100.0)
	OPA	160/160	100.0	(97.7, 100.0)
Between-Instruments (BenchMark ULTRA)	LPA	64/64	100.0	(94.3, 100.0)
	IPA	96/96	100.0	(96.2, 100.0)
	OPA	160/160	100.0	(97.7, 100.0)
Between-Day	LPA	65/65	100.0	(94.4, 100.0)
	IPA	96/96	100.0	(96.2, 100.0)
	OPA	161/161	100.0	(97.7, 100.0)
Within-Run	LPA	97/97	100.0	(96.2, 100.0)
	IPA	143/143	100.0	(97.4, 100.0)
	OPA	240/240	100.0	(98.4, 100.0)

Note: LPA =Loss Percent Agreement (agreement rate for MLH1 Loss status); IPA = Intact Percent Agreement (agreement rate for MLH1 Intact status); OPA = Overall Percent Agreement (overall agreement rate for MLH1 clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% LPA, IPA and OPA were calculated using Wilson score method.

Between Day Intermediate Precision- Marker Level Study

In this study, EC samples were supplemented with samples from a variety of other solid tumor tissues. The sample distribution was as follows: 8 EC (4 intact and 4 loss), 24 (12 intact and 12 loss) FFPE samples from a variety of solid tumor tissues from each of the following organ systems were included in this study: urinary (2 samples), reproductive (8 samples), and gastrointestinal (14 samples). The study design verified staining precision on tumor tissues stained with VENTANA anti- MLH1 (M1) Mouse Monoclonal Primary Antibody across five non-consecutive days.

For each sample, the mode of the staining result was determined as the most frequently observed staining result among the 10 test samples stained on the five non-consecutive days using a single lot of antibody and single lot of detection on one instrument. The result from each test sample was then compared to the respective mode and deemed concordant or discordant. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti- MLH1 (M1) antibody (Table 3). Results are summarized in Table 9 for EC tissues and Table 10 for variety of solid tumor tissues including EC.

Table 9. Between day intermediate precision of VENTANA anti-MLH1 (M1) antibody on EC tissues as measured by MLH1 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Day	LPA	40/40	100.0	(91.2, 100.0)
	IPA	40/40	100.0	(91.2, 100.0)
	OPA	80/80	100.0	(95.4, 100.0)

Note: LPA =Loss Percent Agreement (agreement rate for MLH1 Loss status); IPA = Intact Percent Agreement (agreement rate for MLH1 Intact status); OPA = Overall Percent Agreement (overall agreement rate for MLH1 clinical status).

Note: Two-sided 95% confidence intervals (CIs) was calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% LPA, IPA and OPA were calculated using Wilson score method.

Table 10. Between day intermediate precision of VENTANA anti-MLH1 (M1) antibody on a variety of solid tumor tissues including EC as measured by MLH1 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Day	LPA	120/120	100.0	(96.9, 100.0)
	IPA	120/120	100.0	(96.9, 100.0)
	OPA	240/240	100.0	(98.4, 100.0)

Note: LPA =Loss Percent Agreement (agreement rate for MLH1 Loss status); IPA = Intact Percent Agreement (agreement rate for MLH1 Intact status); OPA = Overall Percent Agreement (overall agreement rate for MLH1 clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% LPA, IPA and OPA were calculated using Wilson score method.

Reader Precision- Panel Level Study

Between-Reader and Within-Reader precision was assessed by evaluating concordance of MMR RxDx Panel status between three readers and within individual readers. In this study, EC samples were supplemented with samples from a variety of other tumor tissues. The sample distribution was as follows: 34 EC (17 proficient and 17 deficient), 134 (72 proficient and 62 deficient) FFPE samples from a variety of solid tumor types from each of the following organ systems were included in the study: urinary (14 samples), reproductive (46 samples), gastrointestinal (33 samples), endocrine (7 samples), hepato-pancreatobiliary (11 samples), soft tissue/skin (9 samples), thoracic (9 samples) and other (head and neck- 5 samples). Specimens were blinded and randomized prior to evaluation for MLH1 status (intact or loss) and panel-level status (proficient of deficient) using the VENTANA MMR RxDx Panel scoring algorithm (Table 4). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers and within-reader are summarized in Table 11 for EC tissues and Table 12 for variety of tumor tissues including EC.

Table 11. Within-Reader and Between-Reader Precision of the VENTANA MMR RxDx Panel on EC tissues as measured by MMR Clinical Status (Proficient/ Deficient).

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	ADPA	100/101	99.0	(97.0 ,100.0)
	APPA	102/103	99.0	(97.1 ,100.0)
	OPA	101/102	99.0	(97.1 ,100.0)
Between-Reader	ADPA	98/100	98.0	(93.8 ,100.0)
	APPA	102/104	98.1	(94.4 ,100.0)
	OPA	100/102	98.0	(94.1 ,100.0)

Note: ADPA = Average dMMR Percent Agreement (pairwise agreement rate for dMMR status); APPA = Average pMMR Percent Agreement (pairwise agreement rate for pMMR status); OPA = Overall Percent Agreement (overall agreement rate for MMR clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples.

Table 12. Within-Reader and Between-Reader Precision of the VENTANA MMR RxDx Panel on a variety of solid tumor tissues including EC as measured by MMR Clinical Status (Proficient/ Deficient).

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	ADPA	(362/365)	99.2	(98.2 ,100.0)
	APPA	(432/435)	99.3	(98.5 ,100.0)
	OPA	(397/400)	99.3	(98.4 ,100.0)
Between-Reader	ADPA	(356/362)	98.3	(96.6 ,100.0)
	APPA	(428/434)	98.6	(97.2 ,100.0)
	OPA	(392/398)	98.5	(96.9 ,100.0)

Note: ADPA = Average dMMR Percent Agreement (pairwise agreement rate for dMMR status); APPA = Average pMMR Percent Agreement (pairwise agreement rate for pMMR status); OPA = Overall Percent Agreement (overall agreement rate for MMR clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples.

Inter-laboratory Reproducibility Study- Panel Level Study for EC Indication

An Inter-Laboratory Reproducibility Study of the VENTANA MMR RxDx Panel was completed to demonstrate reproducibility of each VENTANA MMR RxDx Panel assay to determine the mismatch repair (MMR) status of tumor specimens. The study included 30 archival, de-identified, formalin-fixed, paraffin-embedded (FFPE) specimens of EC run across 3 BenchMark ULTRA instruments on each of 3 non-consecutive days over 20 days at three external laboratories.

Each set of 5 stained slides per sample per staining day was randomized and evaluated by a total of 6 readers (2 readers/site) for a marker level status (Intact or Loss) and panel level status (Deficient or Proficient). The study included a total of 540 observations for thirty samples stained over 3 days across 3 sites with 2 readers per site. The MMR status results for all readers, sites, and days for the samples were combined and analyzed versus the reader modes for the same samples to determine the overall reproducibility of MMR status. The summary of the agreement rates across all evaluable observations, using the sample-level reader modes for MMR panel level status as the reference can be found in Table 13.

Table 13. Inter-Laboratory Reproducibility for overall agreement rates for VENTANA MMR RxDx Panel in EC

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	DMPA	263/268	98.1	(95.5, 100.0)
	PMPA	269/269	100.0	(98.6, 100.0)
	OPA	532/537	99.1	(97.8, 100.0)
Within-Site	DMPA	263/268	98.1	(95.5, 100.0)
	PMPA	269/269	100.0	(98.6, 100.0)
	OPA	532/537	99.1	(97.8, 100.0)
Within-Reader	DMPA	263/265	99.2	(98.1, 100.0)
	PMPA	272/272	100.0	(98.6, 100.0)
	OPA	535/537	99.6	(99.1, 100.0)

Note: DMPA = dMMR Percent Agreement (agreement rate for dMMR status); PMPA = pMMR Percent Agreement (agreement rate for pMMR status); OPA = Overall Percent Agreement (overall agreement rate for MMR clinical status).

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates.

In addition, pairwise comparisons of MMR status were made between-site, between-reader and between-day for panel level MMR status. A summary of the results can be found in Table 14. The data indicate assay reproducibility across 3 days, 3 sites, and 6 readers.

Table 14. Inter-Laboratory Reproducibility Pairwise Agreement Rates for the VENTANA MMR RxDx Panel in EC

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-Site	ADPA	3072/3132	98.1	(95.3, 100.0)
	APPA	3216/3276	98.2	(95.7, 100.0)
	OPA	3144/3204	98.1	(95.5, 100.0)
Between-Reader	ADPA	258/263	98.1	(95.3, 100.0)
	APPA	268/273	98.2	(95.7, 100.0)
	OPA	263/268	98.1	(95.5, 100.0)
Between-Day	ADPA	518/522	99.2	(98.1, 100.0)
	APPA	542/546	99.3	(98.2, 100.0)
	OPA	530/534	99.3	(98.1, 100.0)

Note: ADPA: Average dMMR Percent Agreement (pairwise agreement rate for dMMR status); APPA: Average pMMR Percent Agreement (pairwise agreement rate for pMMR status); OPA = Overall Percent Agreement (overall agreement rate for MMR clinical status).

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates.

CLINICAL PERFORMANCE IN ENDOMETRIAL CARCINOMA

Clinical Performance of dostarlimab (JEMPERLI) in GARNET Study

The efficacy of JEMPERLI was evaluated in the GARNET study (NCT02715284), a multicenter, multicohort, open-label study conducted in patients with recurrent or advanced solid tumors. The efficacy population consisted of patients with mismatch repair deficient (dMMR) recurrent or advanced solid tumors including EC tested with local or central IHC who had progressed on or after treatment with a platinum-containing regimen.

Patients with prior treatment with PD-1/PD-L1–blocking antibodies or other immune checkpoint inhibitor therapy and patients with autoimmune disease that required systemic therapy with immunosuppressant agents within 2 years were excluded from the study. Patients received JEMPERLI 500 mg intravenously every 3 weeks for 4 doses followed by 1000 mg intravenously every 6 weeks. Treatment continued until disease progression or unacceptable toxicity. The major efficacy outcome measures were Overall Response Rate (ORR) and Duration of Response (DOR) as assessed by blinded independent central review (BICR) according to the Response Evaluation Criteria in Solid Tumors (RECIST) v 1.1.

The first planned interim analysis for GARNET study included dMMR EC patients enrolled in GARNET before clinical cutoff date (CCOD) of 08-Jul-2019. The efficacy population for the first interim analysis consisted of a cohort of 71 dMMR EC patients. The second planned interim analysis included dMMR solid tumor patients including EC enrolled in GARNET before CCOD of 01-Mar-2020. The efficacy population for the second interim analysis consisted of a cohort of 209 dMMR solid tumor patients including EC.

Performance of the VENTANA MMR RxDx assay was measured by evaluating its ability to identify patients with EC for first interim analysis and solid tumors (including EC) for second interim analysis, who were likely to respond to treatment with JEMPERLI (ie, the efficacy results observed in GARNET) and additionally by evaluating how often the clinical trial assay (CTA) was able to yield a valid result (i.e., staining acceptability rates). Specifically, the diagnostic performance evaluation sought to determine the efficacy of JEMPERLI among patients with recurrent or advanced MMR deficient EC or recurrent or advanced MMR deficient solid tumors (including EC) who could have been enrolled in GARNET had VENTANA MMR RxDx Panel (CDx assay) been used for enrollment screening rather than the CTA. A bridging approach was required for these analyses because patients were not screened for GARNET study enrollment using the CDx assay. For the diagnostic performance evaluation for VENTANA MMR RxDx Panel, only a subset of cases from Biologics License Application (BLA) safety population which met the diagnostic study criteria were included for each of the interim analysis.

GARNET Study Clinical Results- EC (dMMR)

The results described in this section are for the first planned interim analysis of GARNET study for dMMR EC patients enrolled before cutoff date of 08-Jul-2019.

A summary of efficacy results for subjects with dMMR EC in the primary BLA efficacy analysis set is presented in Table 15.

Table 15. Efficacy Results in GARNET dMMR Endometrial Cancer Population.

Endpoint	JEMPERLI N = 71
Confirmed Overall Response Rate	
ORR	42.3%
(95% CI)	(30.6, 54.6)
Complete response rate	12.7%
Partial response rate	29.6%
Duration of Response	
Median in months	Not reached
(range)[a]	(2.6, 22.4+)
Patients with duration ≥ 6 months	93.3%

Note: CI = Confidence interval, + = ongoing at last assessment.

[a] Median follow-up for duration of response was 14.1 months, measured from time of first response.

VENTANA MMR RxDx Panel Clinical Performance for GARNET dMMR Endometrial Cancer Population

The population for the diagnostic performance evaluation of VENTANA MMR RxDx Panel efficacy (defined as PMA Cohorts) in EC included patients enrolled in GARNET study who received study treatment prior to CCOD for the first planned interim analysis and whose eligibility for that study was confirmed using a CTA, defined as any locally or centrally performed MMR IHC other than VENTANA MMR RxDx Panel.

As part of the bridging analysis, the agreement of MMR status between CTA and CDx results were calculated using the CTA results as the reference. For the purpose of the analyses, a proficient MMR status was considered negative, and a deficient MMR status was considered positive. Among all the clinical samples from the original GARNET study BLA safety population with both evaluable CDx result and CTA results, the inter-assay concordance results are shown in Table 16.

Table 16. MMR Status Concordance between the GARNET Study (NCT02715284) Clinical Trial Assay and VENTANA MMR RxDx Panel.

Analysis Population [a]	Agreement		
	Measure [b]	n/N	% (95% CI [c])
IU Concordance	PPA	51/55	92.7 (82.7, 97.1)
	NPA	68/68	100.0 (94.7, 100.0)
	OPA	119/123	96.7 (91.9, 98.7)
ITD Concordance	PPA	70/76	92.1 (83.8, 96.3)
	NPA	90/91	98.9 (94.0, 99.8)
	OPA	160/167	95.8 (91.6, 98.0)

[a] Analyses were performed in the Concordance population (all patients in the Safety Population with an evaluable VENTANA MMR RxDx Panel (CDx) staining result). The Intent-to-Diagnose (ITD) and Concordance populations were equivalent in this study. The Intended Use (IU) Concordance population includes only the subset of patients who are also in the IU population (ie, for whom VENTANA MMR RxDx Panel testing attempt was performed according to the requirements of the diagnostic protocol).

[b] For the purpose of the analyses, a proficient MMR status was considered negative, and a deficient MMR status was considered positive. PPA = positive percent agreement; NPA = negative percent agreement; OPA = overall percent agreement.

[c] Two-sided 95% confidence intervals (CIs) were calculated using the Wilson score method.

Additional analyses were conducted to estimate the drug efficacy for CDx assay, including primary analysis using different multiple imputation (MI) approaches and sensitivity analysis using lower bound of 95% CI of PPA and NPA for inter-assay concordance and adjusted prevalence for CTA+. Based on efficacy results from this analyses utilizing the imputed CDx status, the ORR and DOR for EC patients with dMMR status, as determined by the VENTANA MMR RxDx Panel assay, was similar to that observed in the CTA dMMR population.

Staining acceptability rates for VENTANA MMR RxDx Panel and each of its component biomarker assays were evaluated at the subject level in the Intent-to-Diagnose (ITD) and Intended Use (IU) population for EC patients with dMMR status. The staining acceptability was 100% for each biomarker and panel level for both ITD and IU populations.

Clinical Performance of pembrolizumab (KEYTRUDA) in combination with lenvatinib (LENVIMA) in KEYNOTE Study

The efficacy of KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib) was investigated in KEYNOTE-775/Study-309 (KEYNOTE-775), a multicenter, open-label, randomized, active-controlled trial that enrolled 827 patients with advanced endometrial carcinoma who had been previously treated with at least one prior platinum-based chemotherapy regimen in any setting, including in the neoadjuvant and adjuvant settings. Patients were stratified by their MMR status (dMMR versus pMMR (not-dMMR)) and pMMR participants were further stratified by ECOG performance status, geographic region, and history of pelvic radiation. MMR status was determined by a CTA. Patients were randomized (1:1) to one of the following treatment arms:

- KEYTRUDA 200 mg intravenously every 3 weeks in combination with LENVIMA 20 mg orally once daily.
- Investigator's choice, consisting of either doxorubicin 60 mg/ m^2 every 3 weeks or paclitaxel 80 mg/ m^2 given weekly, 3 weeks on/ 1 week off.

The major efficacy outcome measures were OS and PFS as assessed by BICR according to RECISTv1.1, modified to follow a maximum of 10 target lesions and a maximum of 5 target lesions per organ. Additional efficacy outcome measures included ORR and DoR, as assessed by BICR.

Among the 697 pMMR patients, 346 patients were randomized to KEYTRUDA in combination with LENVIMA, and 351 patients were randomized to investigator's choice of doxorubicin or paclitaxel.

A clinical bridging study was conducted to establish the clinical validity of the VENTANA MMR RxDx Panel as a pMMR endometrial carcinoma CDx for KEYTRUDA in combination with LENVIMA. Samples from 429 patients from KEYNOTE-775 were retrospectively tested with the VENTANA MMR RxDx Panel and 409 had evaluable MMR status. Three hundred thirty-five were determined to be pMMR, of which, 168 had been randomized to KEYTRUDA in combination with LENVIMA, and 167 had been randomized to investigator's choice of doxorubicin or paclitaxel.

Concordance Analysis

As part of the bridging analysis, the agreement of MMR status between CTA and VENTANA MMR RxDx Panel results was calculated using the CTA results as the reference. Refer to Table 17 for concordance analysis when CTA and VENTANA MMR RxDx Panel MMR results were evaluable. VENTANA MMR RxDx Panel MMR status was not obtained for 20 participants. For the purpose of the analyses, a pMMR status was considered positive, and a dMMR status was considered negative.

Table 17. Concordance Analysis of MMR status between CTA and VENTANA MMR RxDx Panel: KEYNOTE-775 samples

	CTA pMMR	CTA dMMR	All
CDx pMMR	330	5	335
CDx dMMR	9	65	74
All	339	70	409
PPA [a] = 330/339, 97.3% (95% CI: 95.0, 98.6)			
NPA [a] = 65/70, 92.9% (95% CI: 84.3, 96.9)			
OPA = 395/409, 96.6% (95% CI: 94.3, 98.0)			

[a] Note: For the purpose of the analyses, a proficient MMR status was considered positive, and a deficient MMR status was considered negative. PPA = positive percent agreement; NPA = negative percent agreement; OPA = overall percent agreement

Clinical Efficacy Results

Efficacy results for the pMMR patients based on the CTA status and VENTANA MMR RxDx Panel are summarized in Table 18. As shown in Table 18, efficacy in pMMR patients identified by the VENTANA MMR RxDx Panel as part of the bridging study was comparable to that in the overall clinical trial.

Table 18. Efficacy results for the pMMR patients based on the CTA status and VENTANA MMR RxDx Panel

Endpoint	Pembrolizumab and Lenvatinib n=346	Inv. choice n=351	Pembrolizumab and Lenvatinib n=168	Inv. choice n=167
	pMMR status by CTA		pMMR status by VENTANA MMR RxDx Panel	
OS Median in months (95% CI)	17.4 (14.2, 19.9)	12.0 (10.8, 13.3)	17.2 (13.9, 18.5)	10.5 (8.5, 12.2)
OS HR [a] (95% CI)	0.68 (0.56, 0.84)		0.58 (0.43, 0.78)	
p-Value [b]	0.0001		NA	
PFS Median in months (95% CI)	6.6 (5.6, 7.4)	3.8 (3.6, 5.0)	6.8 (5.6, 7.8)	3.9 (3.5, 5.6)
PFS HR [a] (95% CI)	0.60 (0.50, 0.72)		0.56 (0.43, 0.72)	
p-Value [b]	< 0.0001		NA	
ORR [c] (95% CI)	30% (26, 36)	15% (12,19)	27% (20.3, 34.2)	14% (8.9, 19.9)
DOR Median in months (range)	9.2 (1.6+, 23.7+)	5.7 (0.0+, 24.2+)	9.1 (1.9+, 16.7+)	11.7 (1.8+, 15.5)

[a] Based on the stratified Cox regression model

[b] Based on stratified log-rank test

[c] Response: Best objective response as confirmed complete response or partial response.

Sensitivity analysis was performed based on multiple imputation to assess the impact of the missing VETNANA MMR RxDx Panel pMMR results. The sensitivity analysis results support the robustness of the primary analysis results.

Staining acceptability rates for VENTANA MMR RxDx Panel and each of its component biomarker assays were evaluated at the subject level in the Intended Use (IU) population for EC patients with pMMR status. The final staining acceptability rate was greater than 99% for each biomarker and panel level for IU population.

ANALYTICAL PERFORMANCE FOR VENTANA MMR RXDX PANEL IN SOLID TUMORS

Repeatability and Intermediate Precision- Marker Level Study

In this study, a variety of solid tumor tissues were analyzed. The sample distribution was as follows: 27 (15 intact and 12 loss) FFPE samples from variety of solid tumor tissues from each of the following organ systems were included in this study: urinary (6 samples), reproductive (9 samples), gastrointestinal (6 samples), hepato-pancreatobiliary (3 samples) soft tissue (1 sample), and thoracic (2 samples). The study designs verified staining precision on tumor tissues stained with VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody.

- Three lots of MLH1 (M1) (between-antibody lots)
- Three lots of OptiView DAB IHC Detection Kits (between-detection kits)
- Three BenchMark ULTRA instruments (between instruments)
- Across three days (between-day)
- Across all intermediate precision conditions (within-run)

Each sample was assigned one mode based on the samples aggregated per test condition for between-antibody lots, between-detection kit lots, between-instruments and between-day. For within-run condition, each sample was compared within its duplicate samples per test run. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti-MLH1 (M1) antibody (Table 3). Results are summarized in Table 19 for variety of tumor tissues including EC.

Table 19. Repeatability and intermediate precision of VENTANA anti-MLH1 (M1) antibody on a variety of solid tumor tissues including EC as measured by MLH1 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Antibody Lots	LPA	66/66	100.0	(94.5, 100.0)
	IPA	96/96	100.0	(96.2, 100.0)
	OPA	162/162	100.0	(97.7, 100.0)
Between-Detection Kits	LPA	65/65	100.0	(94.4, 100.0)
	IPA	95/95	100.0	(96.1, 100.0)
	OPA	160/160	100.0	(97.7, 100.0)
Between-Instruments (BenchMark ULTRA)	LPA	64/64	100.0	(94.3, 100.0)
	IPA	96/96	100.0	(96.2, 100.0)
	OPA	160/160	100.0	(97.7, 100.0)
Between-Day	LPA	65/65	100.0	(94.4, 100.0)
	IPA	96/96	100.0	(96.2, 100.0)
	OPA	161/161	100.0	(97.7, 100.0)
Within-Run	LPA	97/97	100.0	(96.2, 100.0)
	IPA	143/143	100.0	(97.4, 100.0)
	OPA	240/240	100.0	(98.4, 100.0)

Note: LPA =Loss Percent Agreement (agreement rate for MLH1 Loss status); IPA = Intact Percent Agreement (agreement rate for MLH1 Intact status); OPA = Overall Percent Agreement (overall agreement rate for MLH1 clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% LPA, IPA and OPA were calculated using Wilson score method.

Between Day Intermediate Precision- Marker Level Study

In this study, a variety of solid tumor tissues were analyzed. The sample distribution was as follows: 24 (12 intact and 12 loss) FFPE samples from a variety of solid tumor tissues from each of the following organ systems were included in this study: urinary (2 samples), reproductive (8 samples), and gastrointestinal (14 samples). The study design verified staining precision on tumor tissues stained with VENTANA anti- MLH1 (M1) Mouse Monoclonal Primary Antibody across five non-consecutive days.

For each sample, the mode of the staining result was determined as the most frequently observed staining result among the 10 test samples stained on the five non-consecutive days using a single lot of antibody and single lot of detection on one instrument. The result from each test sample was then compared to the respective mode and deemed concordant or discordant. All slides were blinded and randomized, and then evaluated using the staining interpretation for VENTANA anti- MLH1 (M1) antibody (Table 3). Results are summarized in Table 20 for variety of tumor tissues including EC.

Table 20. Between day intermediate precision of VENTANA anti-MLH1 (M1) antibody on a variety of solid tumor tissues including EC as measured by MLH1 Clinical Status (Intact/ Loss).

Repeatability/ Precision	Agreement			
	Type	n/N	%	95% CI
Between-Day	LPA	120/120	100.0	(96.9, 100.0)
	IPA	120/120	100.0	(96.9, 100.0)
	OPA	240/240	100.0	(98.4, 100.0)

Note: : LPA =Loss Percent Agreement (agreement rate for MLH1 Loss status); IPA = Intact Percent Agreement (agreement rate for MLH1 Intact status); OPA = Overall Percent Agreement (overall agreement rate for MLH1 clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples. CIs for 100% LPA, IPA and OPA were calculated using Wilson score method.

Reader Precision- Panel Level Study

Between-Reader and Within-Reader precision was assessed by evaluating concordance of MMR RxDx Panel status between three readers and within individual readers. In this study, samples from a variety of tumor tissues were analyzed. The sample distribution was as follows: 162 (100 proficient and 62 deficient) FFPE samples from a variety of solid tumor types from each of the following organ systems were included in the study: urinary (15 samples), reproductive (48 samples), gastrointestinal (56 samples), endocrine (7 samples), hepato-pancreatobiliary (13 samples), soft tissue/skin (9 samples), thoracic (9 samples) and other (head and neck- 5 samples). Specimens were blinded and randomized prior to evaluation for MLH1 status (intact or loss) and panel-level status (proficient of deficient) using the VENTANA MMR RxDx Panel scoring algorithm (Table 4). Readers scored all specimens twice, with a minimum of two weeks between reads. The agreement rates between the readers and within-reader are summarized in Table 21 for variety of tumor tissues including EC.

Table 21. Within-Reader and Between-Reader Precision of the VENTANA MMR RxDx Panel on a variety of solid tumor tissues including EC as measured by MMR Clinical Status (Proficient/ Deficient).

Precision	Agreement			
	Type	n/N	%	95% CI
Within-Reader	ADPA	364/366	99.5	(98.6 ,100.0)
	APPA	598/600	99.7	(99.2 ,100.0)
	OPA	481/483	99.6	(99.0 ,100.0)
Between-Reader	ADPA	364/366	99.5	(98.3 ,100.0)
	APPA	596/598	99.7	(99.0 ,100.0)
	OPA	480/482	99.6	(98.8 ,100.0)

Note: ADPA = Average dMMR Percent Agreement (pairwise agreement rate for dMMR status); APPA = Average pMMR Percent Agreement (pairwise agreement rate for pMMR status); OPA = Overall Percent Agreement (overall agreement rate for MMR clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples.

Inter-laboratory Reproducibility Study- Panel Level Study for Solid Tumors

The reproducibility of VENTANA MMR RxDx Panel was examined in 3 independent Inter-Laboratory Reproducibility (ILR) studies (diverse solid tumors, EC only and CRC only) conducted using the same study design. In each study, a set of de-identified FFPE tumor specimens was stained on a BenchMark ULTRA instrument at each of 3 external laboratories on each of 3 non-consecutive days (spanning at least 20 days in total). Each staining day at each site produced a 5-slide panel [4 biomarker antibody-stained slides

and 1 slide stained with Negative Control (Monoclonal) using the PMS2 staining protocol] that was independently evaluated for the status of each marker (Intact or Loss) and for MMR status (Deficient or Proficient) by 2 pathologists at the site.

The solid tumor ILR study used 60 diverse solid tumor cases (30 dMMR cases and 30 pMMR cases), of which 6 were considered challenging; EC only and CRC only ILR studies used 30 EC cases and 30 CRC cases respectively. The EC and CRC studies each used 15 dMMR cases and 15 pMMR cases, and each included 4 cases that were considered challenging.

The marker-level and MMR results for all cases in the 3 studies were combined (120 cases in total) and analyzed for the same cases to assess the performance of VENTANA MMR RxDx Panel as a diagnostic device for determining MMR status in diverse solid tumor specimens. The combined analysis of MMR status across all readers, sites, and days included a total of 2155 observations. The summary of MMR status agreement rates across all evaluable observations vs the reader modes, using the reader modes as the reference, is shown in Table 22. The agreement rates for the pooled within-site and within-reader analyses vs the reader modes, using the within-site and within-reader modes as the references, are also shown in Table 22.

Table 22. Inter-Laboratory Reproducibility for overall agreement rates for VENTANA MMR RxDx Panel in variety of solid tumor tissues including EC.

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Overall	DMPA	1066/1076	99.1	(98.3, 99.6)
	PMPA	1075/1079	99.6	(99.2, 100.0)
	OPA	2141/2155	99.4	(98.9, 99.7)
Within-Site	DMPA	1066/1076	99.1	(98.3, 99.6)
	PMPA	1075/1079	99.6	(99.2, 100.0)
	OPA	2141/2155	99.4	(98.9, 99.7)
Within-Reader	DMPA	1066/1073	99.3	(98.9, 99.7)
	PMPA	1078/1082	99.6	(99.2, 100.0)
	OPA	2144/2155	99.5	(99.2, 99.8)

Note: DMPA = dMMR Percent Agreement (agreement rate for dMMR status); PMPA = pMMR Percent Agreement (agreement rate for pMMR status); OPA = Overall Percent Agreement (overall agreement rate for MMR clinical status).

Note: Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates.

In addition, pairwise comparisons of MMR status were made between-site, between-reader and between-day for panel level MMR status. A summary of the results can be found in Table 23. The data indicate assay reproducibility across 3 days, 3 sites, and 6 readers.

Table 23. Inter-Laboratory Reproducibility Pairwise Agreement Rates for the VENTANA MMR RxDx Panel in a variety of solid tumor tissues including EC.

Inter-Laboratory Reproducibility	Agreement			
	Type	n/N	%	95% CI
Between-Site	ADPA	12628/12794	98.7	(97.8, 99.4)
	APPA	12840/13006	98.7	(97.8, 99.4)
	OPA	12734/12900	98.7	(97.8, 99.4)
Between-Reader	ADPA	1056/1068	98.9	(98.1, 99.5)
	APPA	1072/1084	98.9	(98.1, 99.5)
	OPA	1064/1076	98.9	(98.1, 99.5)
Between-Day	ADPA	2110/2132	99.0	(98.3, 99.5)
	APPA	2146/2168	99.0	(98.3, 99.5)
	OPA	2128/2150	99.0	(98.3, 99.5)

Note: ADPA: Average dMMR Percent Agreement (pairwise agreement rate for dMMR status); APPA: Average pMMR Percent Agreement (pairwise agreement rate for pMMR status); OPA = Overall Percent Agreement (overall agreement rate for MMR clinical status).

Note: Two-sided 95% confidence intervals (CIs) were calculated using the percentile bootstrap method from 2000 bootstrap samples.

Note: The same sites and readers were used for EC and CRC ILR studies. For the solid tumor ILR study, two sites were different from the EC and CRC ILR.

CLINICAL PERFORMANCE IN SOLID TUMORS

Clinical Performance of dostarlimab (JEMPERLI) in GARNET Study

The efficacy of JEMPERLI was evaluated in the GARNET study (NCT02715284), a multicenter, multicohort, open-label study conducted in patients with recurrent or advanced solid tumors. The efficacy population consisted of patients with mismatch repair deficient (dMMR) recurrent or advanced solid tumors including EC tested with local or central IHC who had progressed on or after treatment with a platinum-containing regimen. Patients with prior treatment with PD-1/PD-L1–blocking antibodies or other immune checkpoint inhibitor therapy and patients with autoimmune disease that required systemic therapy with immunosuppressant agents within 2 years were excluded from the study. Patients received JEMPERLI 500 mg intravenously every 3 weeks for 4 doses followed by 1000 mg intravenously every 6 weeks. Treatment continued until disease progression or unacceptable toxicity. The major efficacy outcome measures were Overall Response Rate (ORR) and Duration of Response (DOR) as assessed by blinded independent central review (BICR) according to the Response Evaluation Criteria in Solid Tumors (RECIST) v 1.1.

The first planned interim analysis for GARNET study included dMMR EC patients enrolled in GARNET before clinical cutoff date (CCOD) of 08-Jul-2019. The efficacy population for the first interim analysis consisted of a cohort of 71 dMMR EC patients. The second planned interim analysis included dMMR solid tumor patients including EC enrolled in GARNET before CCOD of 01-Mar-2020. The efficacy population for the second interim analysis consisted of a cohort of 209 dMMR solid tumor patients including EC.

Performance of the VENTANA MMR RxDx assay was measured by evaluating its ability to identify patients with EC for first interim analysis and solid tumors (including EC) for second interim analysis, who were likely to respond to treatment with JEMPERLI (ie, the efficacy results observed in GARNET) and additionally by evaluating how often the investigational assay was able to yield a valid result (i.e., staining acceptability rates). Specifically, the diagnostic performance evaluation sought to determine the efficacy of JEMPERLI among patients with recurrent or advanced MMR deficient EC or recurrent or advanced MMR deficient solid tumors (including EC) who could have been enrolled in GARNET had VENTANA MMR RxDx Panel (CDx assay) been used for enrollment screening rather than the CTA. A bridging approach was required for these analyses because patients were not screened for GARNET study enrollment using the CDx assay. For the diagnostic performance evaluation for VENTANA MMR RxDx Panel, only a subset of cases from Biologics License Application (BLA) safety population which met the diagnostic study criteria were included for each of the interim analysis.

Solid Tumors including EC

The results described in this section are for the second planned interim analysis of GARNET study for dMMR solid tumors patients including EC enrolled before CCOD of 01-Mar-2020.

GARNET Study Clinical Results- Solid Tumors including EC (dMMR)

A summary of efficacy results for subjects with dMMR solid tumors including EC in the primary BLA efficacy analysis set is presented in Table 24.

Table 24. Efficacy Results in GARNET dMMR Recurrent or Advanced Solid Tumor (including EC) Population

Endpoint	JEMPERLI N = 209
Confirmed Overall Response Rate	
ORR	41.6%
(95% CI)	(34.9, 48.6)
Complete response rate	9.1%
Partial response rate	32.5%
Duration of Response	
Median in months	34.7
(range)[a]	2.6, 35.8+
Patients with duration ≥ 6 months	95.4%

Note: CI = Confidence interval, + = ongoing at last assessment.

[a] Median follow-up for duration of response was 17.5 months, measured from time of first response.

VENTANA MMR RxDx Panel Clinical Performance in the GARNET dMMR Solid Tumor (including EC) Population

The population for the diagnostic performance evaluation of VENTANA MMR RxDx Panel efficacy (defined as PMA Cohorts) in solid tumors included patients enrolled in the GARNET study who received study treatment prior to CCOD for the second planned interim analysis and whose eligibility for that study was confirmed using a CTA, defined as any locally or centrally performed MMR IHC test.

As part of the bridging analysis, the agreement of MMR status between CTA and CDx results was calculated using the CTA results as the reference. For the purpose of the analyses, a proficient MMR status was considered negative, and a deficient MMR status was considered positive. Among all the clinical samples from the original GARNET study BLA safety population with both an evaluable CDx result and an evaluable CTA result, the inter-assay concordance results are shown in Table 25.

Table 25. MMR Status Concordance between the GARNET Study (NCT02715284) Clinical Trial Assay and VENTANA MMR RxDx Panel in the Solid Tumor (including EC) Population (IU Concordance Population)[a].

Groups	Agreement		
	Measure [b]	% (n/N)	95% CI [c]
EC	PPA	93.2 (68/73)	(84.9, 97.0)
	NPA	98.7 (74/75)	(92.8, 99.8)
	OPA	95.9 (142/148)	(91.4, 98.1)
Non-EC	PPA	83.1 (69/83)	(73.7, 89.7)
	NPA [d]	N/E	N/E
	OPA [d]	N/E	N/E
dMMR solid tumors	PPA	87.8 (137/156)	(81.8, 92.1)
	NPA	N/E	N/E
	OPA [d]	N/E	N/E

[a] All patients in the Safety Population who had evaluable Clinical Trial Assay (CTA) MMR and VENTANA MMR RxDx Panel (CDx) MMMR results, excluding patients whose final CDx result was associated with an diagnostic protocol deviation (i.e., was not Intended Use (IU).

[b] For the purposes of the concordance analysis, an MMR Status of Deficient was considered Positive and an MMR Status of Proficient was considered Negative.

[c] Two-sided 95% CI were calculated using the Wilson score method.

[d] Because very few CTA– patients were enrolled in dMMR solid tumor (except EC) cohort, NPA and OPA was not evaluable.

Note: N/E = Not Evaluated; N/A = Not Applicable.

Additional analyses were conducted to estimate the drug efficacy for CDx assay, including primary analysis using multiple imputation (MI) approach for missing data imputation under different assumptions for non-EC screen failed patients. Based on efficacy results from this analyses after missing CDx status imputation for enrolled patients without an evaluable CDx status and missing efficacy simulation for non-EC screen failed, the ORR and DOR for solid tumor (including EC) patients with dMMR status, as determined by the VENTANA MMR RxDx Panel assay, was similar to that observed in the CTA dMMR population.

Staining acceptability rates for VENTANA MMR RxDx Panel and each of its component biomarker assays were evaluated at the subject level in the Intent-to-Diagnose (ITD) and Intended Use (IU) population for solid tumor (including EC) patients with dMMR status. The final staining acceptability rate was greater than 98% for each biomarker and panel level for ITD and IU population.

Clinical Performance of Pembrolizumab (KEYTRUDA) in KEYNOTE-158 and KEYOTE-164

A clinical bridging study using 444 patient samples from KEYNOTE-158 Cohort K (n=321) and KEYNOTE-164 (n=123) was conducted to establish the clinical validity of the VENTANA MMR RxDx Panel as a CDx for pembrolizumab in dMMR solid tumors. KEYNOTE-158 is an ongoing multicenter, global, open label trial of KEYTRUDA in patients with multiple types of advanced (unresectable and/or metastatic) cancers who have failed prior therapy. All patients enrolled in this study had a histologically or cytologically documented, advanced solid tumor that was incurable and for which prior standard first-line treatment had failed. Patients had progressed on or were intolerant to therapies that are known to provide clinical benefit. All patients received pembrolizumab 200 mg every three weeks (Q3W). KEYNOTE-158 Cohort K enrolled patients with unresectable or metastatic MSI-H/dMMR solid tumors (except CRC). KEYNOTE-164 is a multicenter, multicohort, single arm, open-label trial designed to evaluate the efficacy of pembrolizumab in previously treated patients with unresectable or metastatic MSI-H/dMMR CRC tumors. Local IHC or PCR assays were primarily used to enroll KEYNOTE-158 Cohort K and KEYNOTE-164 participants. All participants received pembrolizumab 200 mg Q3W.

Primary objectives of KEYNOTE-158 Cohort K and KEYNOTE-164 were to evaluate ORR per RECIST 1.1 (assessed by central imaging) to pembrolizumab. Secondary objectives included assessment of DOR, PFS and OS in the pembrolizumab treated participants. The data cut-off date for the clinical efficacy analyses for KEYNOTE-158 was October 05, 2020 and September 09, 2019 for KEYNOTE 164.

Samples from KEYNOTE-177 (trial in first line MSI-H/dMMR CRC patients), KEYNOTE-158 Cohorts A-J patients (patients in rare tumor non-CRC cohorts who had failed prior therapy) and tumor bank samples were additionally tested with VENTANA MMR RxDx Panel for concordance assessment with CTA. Sample accounting is included in Table 26.

Table 26. CDx Sample Accounting Across Cohorts.

Study	CDx Tested	CDx Evaluable
All	971	937
KN158 Cohort K	169	160
KN158 Cohort A to J	310	300
KN164	28	28
KN177	37	36
Tumor Bank	427	413

All KEYNOTE Study Concordance Analysis

Table 27 shows concordance between the CTA and CDx evaluable samples across solid tumors. There were 3 CTA non-evaluable samples (tumor bank) out of 937 CDx evaluable samples and these samples were excluded from the concordance analyses, leaving 934 for the analyses. For the purpose of the analyses, a proficient MMR status was considered negative, and a deficient MMR status was considered positive.

Table 27. Concordance between CTA and CDx.

Tumor Type	N	CTA Pos-	CTA Pos-	CTA Neg-ative	CTA Neg-ative	PPA %	NPA %	OPA %
All	934	193	75	10	656	72.0 (66.4, 77.0)	98.5 (97.3, 99.2)	90.9 (88.9, 92.6)
CRC	185	78	10	3	94	88.6 (80.3, 93.7)	96.9 (91.3, 98.9)	93.0 (88.3, 95.8)
GC	91	28	1	2	60	96.6 (82.8, 99.4)	96.8 (89.0, 99.1)	96.7 (90.8, 98.9)
EC	74	40	9	0	25	81.6 (68.6, 90.0)	100.0 (86.7, 100.0)	87.8 (78.5, 93.5)
SI CA	16	11	0	0	5	100.0 (74.1, 100.0)	100.0 (56.6, 100.0)	100.0 (80.6, 100.0)
OC	13	7	6	0	0	53.8 (29.1, 76.8)	NA	53.8 (29.1, 76.8)
Other	555	29	49	5	472	37.2 (27.3, 48.3)	99.0 (97.6, 99.6)	90.3 (87.5, 92.5)

Note: CTA-Positive includes samples from KEYNOTE-158 Cohort K, KEYNOTE-164, KEYNOTE-177 and tumor bank. CTA-Negative includes samples from KEYNOTE-158 Cohorts A-J and tumor bank.

Note: Tumor type abbreviations defined as follows: CRC=Colorectal carcinoma, GC=Gastric carcinoma, EC=Endometrial carcinoma; SI CA= Small intestine carcinoma, OC=Ovarian carcinoma.

Demographic Characteristics

For the clinical device bridging study, baseline characteristics were compared between the CTA positive, CDx evaluable and CDx non-evaluable populations. As noted above, the CTA-positive population consists of 444 patients, 321 from KEYNOTE-158 Cohort K and 123 from KEYNOTE-164. Among these 444 participants, the baseline characteristics were: median age of 59 years, 36% ≥ 65 years of age; 46% male; 78% White, 13% Asian, and 4% Black; and 44% had an ECOG PS of 0 and 56% had an ECOG PS of 1. Ninety-three percent of participants had metastatic disease. Sixty-two percent of participants received 2 or more prior lines of therapy.

All KEYNOTE Study Clinical Efficacy Results

The clinical validity of CDx for the detection of MSI-H status in patients with solid tumors was based on estimation of clinical efficacy in the CDx-positive, CTA-positive population. The major efficacy outcome measure was ORR per RECIST 1.1 (assessed by central imaging). ORR for the CTA positive, CDx positive/CTA positive, CDx negative/CTA positive, and CDx missing/CTA positive are presented in Table 28.

Table 28. Efficacy Results in KEYNOTE-164 and KEYNOTE-158 Cohort K Combined.

Clinical outcome	CTA positive (N=444)	CDx positive and CTA positive (N=101)	CDx negative and CTA positive (N=61)	CDx result missing and CTA positive (N=282)
ORR% (95% CI)	31.8% (27.4, 36.3)	34.7% (25.5, 44.8)	9.8% (3.7, 20.2)	35.5% (29.9, 41.4)
Complete response	38 (8.6%)	7 (6.9%)	2 (3.3%)	29 (10.3%)
Partial response	103 (23.2%)	28 (27.7%)	4 (6.6%)	71 (25.2%)
Duration of Response	N=141	N=35	N=6	N=100
Median in months (range)	NR (2.1+ - 51.1+)	NR (3.7+ - 49.0+)	13.4 (6.5 - 32.7+)	47.5 (2.1+ - 51.1+)
% with duration ≥ 6 months	129 (95.6)	30 (93.8)	6 (100.0)	93 (95.9)
% with duration ≥ 12 months	104 (90.1)	21 (90.6)	3 (83.3)	80 (90.5)

Note: Database Cutoff Date: KEYNOTE 164: September 09, 2019, KEYNOTE 158: October 05, 2020

The ORR in the CTA-positive population was 31.8% (141/444), (95% CI: 27.4, 36.3). There were 41 CTA-positive participants who also had CDx results with partial or complete responses. Among them 85.4% (35/41) were positive by CDx (95% CI: 70.8, 94.4). There were 121 CTA-positive participants who also had CDx results with no responses. Among the 121 CTA positive patients who did not respond to KEYTRUDA, only 54.5% (66/121) were positive by CDx (95% CI: 45.2, 63.6). Taken together, CDx has a higher percent of positive results among participants with responses than among participants without responses [difference between 85.4% (35/41) and 54.5% (66/121) was 30.8% with 95% CI: (14.8, 43.2).

The ORR in CDx-positive/CTA-positive participants was 34.7% (35/101), (95% CI: 25.5, 44.8). The ORR in CDx-negative/CTA-positive participants was 9.8% (6/61), (95% CI: 3.7, 20.2). The ORR in CDx-positive/CTA-positive participants was higher than the ORR in CDx negative/ CTA-positive participants [difference between 34.7% (35/101) and 9.8% (6/61) was 24.8% with 95% CI: 11.9, 36.3].

The similarity of the ORR for the CTA-positive population (n=444) overall (31.8%, 95% CI: 27.4, 36.3) and for those missing a valid CDx result (n=282; 35.5%, 95% CI: 29.9, 41.4) suggests no overt imbalance in efficacy effect of pembrolizumab between patients on whom the CDx was or was not obtained.

Sensitivity Analysis

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with missing/invalid CDx results and the missing CDx-positive, CTA-negative population that was not enrolled and evaluated by KEYNOTE-158 Cohort K and KEYNOTE-164 clinical studies.

To evaluate the impact of missing/invalid CDx results, the distribution of patients for baseline covariates, disease characteristics, tumor organ system, and tumor types was compared among the CTA-positive population, the CDx-evaluable/CTA-positive subpopulation, and CDx-missing CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable CDx MSI tumor status (n=282). The imputation model included the clinical outcome and covariates that are considered predictive of missingness of the CDx tumor status and showing some predictive value of the CDx tumor status.

The clinical efficacy (ORR) for the CDx-positive subjects in the device intended use population was estimated under different assumed scenarios based on observed and imputed CDx results.

For the CDx-positive, CTA-negative population that was not enrolled and evaluated by KEYNOTE-158 Cohort K and KEYNOTE-164 clinical studies, bridging equations that involved an ORR attenuation factor that ranges from 0 (assume full attenuation of the efficacy in CTA-negative/CDx-positive) to 1 (assume no attenuation of the efficacy in CTA-negative/CDx-positive compared to the observed ORR in CDx-positive patients in the efficacy population) were used for the clinical efficacy analysis in this missing population.

Sensitivity analysis considering the NPA and assuming different CTA positivity rates in the CDx intended use population, which ranged 2-5%, were investigated to assess influence on the efficacy estimated for the intended use, i.e., CDx-positive subjects. These sensitivity analyses demonstrated the robustness of the clinical efficacy estimate from the primary analysis.

Subgroup Analyses

Response to KEYTRUDA for the CTA positive, CDx-positive/CTA-positive, CDx negative/CTA-positive and CDx-missing/CTA-positive patients was analyzed by primary tumor type. Within the CDx positive patients for the efficacy set, 10 tumor types were represented. Response rates by tumor types are included in Table 29.

Table 29. ORR Estimates per tumor type in Subpopulations by CDx Status.

Tumor Type	CTA-Positive	CTA-Positive and CDx dMMR	CTA-Positive and CDx pMMR	CTA-Positive and CDx Missing
CRC	42/123 34.1% (25.8, 43.2)	10/22 45.5% (24.4, 67.8)	1/5 20.0% (0.5, 71.6)	31/96 32.3% (23.1, 42.6)
Non-CRC	99/321 30.8% (25.8, 36.2)	25/79 31.6% (21.6 43.1)	5/56 8.9% (3.0, 19.6)	69/186 37.1% (30.1, 44.5)
EC	33/68 48.5% (36.2, 61.0)	11/26 42.3% (23.4, 63.1)	1/7 14.3% (0.4, 57.9)	21/35 60.0% (42.1, 76.1)
GC	13/42 31.0% (17.6, 47.1)	3/18 16.7% (3.6, 41.4)	NA	10/24 41.7% (22.1, 63.4)
SI CA	12/25 48.0% (27.8, 68.7)	3/8 37.5% (8.5, 75.5)	NA	9/17 52.9% (27.8, 77.0)
OC	8/24 33.3% (15.6, 55.3)	3/6 50.0% (11.8, 88.2)	1/6 16.7% (0.4, 64.1)	4/12 33.3% (9.9, 65.1)
Cholangioca	9/22 40.9% (20.7, 63.6)	1/4 25.0% (0.6, 80.6)	0/1 0.0% (0.0, 97.5)	8/17 47.1% (23.0, 72.2)
Pancreatic CA	4/22 18.2% (5.2, 40.3)	NA	1/4 25.0% (0.6, 80.6)	3/18 16.7% (3.6, 41.4)
Brain	1/17 5.9% (0.1, 28.7)	NA	1/3 33.3% (0.8, 90.6)	0/14 0.0% (0.0, 23.2)
Sarcoma	3/14 21.4% (4.7, 50.8)	NA	0/8 0.0% (0.0, 36.9)	3/6 50.0% (11.8, 88.2)
NE CA	2/12 16.7% (2.1, 48.4)	0/2 0.0% (0.0, 84.2)	0/4 0.0% (0.0, 60.2)	2/6 33.3% (4.3, 77.7)
Breast CA	1/11 9.1% (0.2, 41.3)	1/3 33.3% (0.8, 90.6)	0/4 0.0% (0.0, 60.2)	0/4 0.0% (0.0, 60.2)
Others	13/64 20.3% (11.3, 32.2)	3/12 25.0% (5.5, 57.2)	1/19 5.3% (0.1, 26.0)	9/33 27.3% (13.3, 45.5)

Note: Database Cutoff Date: KEYNOTE 164: September 09, 2019, KEYNOTE 158: October 05, 2020

Note: Tumor type abbreviations defined as follows: CRC=Colorectal carcinoma, GC= Gastric carcinoma, EC=Endometrial carcinoma; SI CA= Small intestine carcinoma, OC=Ovarian carcinoma, CHOLANGIOCA=Cholangiocarcinoma, Pancreatic CA=Pancreatic carcinoma, NE CA= Neuroendocrine carcinoma, BREAST CA= Breast carcinoma.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

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VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody

For use with the VENTANA MMR IHC Panel

REF 790-5091

07862237001

IVD Σ 50

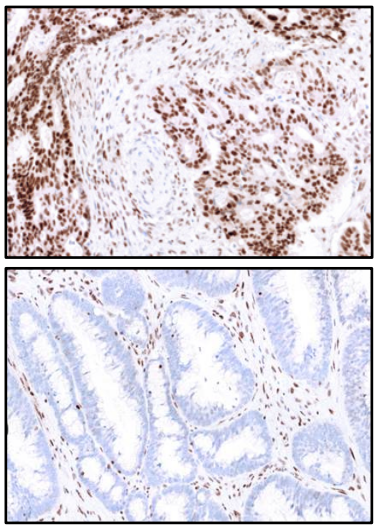


Figure 2. VENTANA anti-MLH1 (M1) antibody staining with Intact (top) or Loss (bottom) of expression in colon cancer tissue.

INTENDED USE

VENTANA MMR IHC Panel

The VENTANA MMR IHC Panel is a qualitative immunohistochemistry (IHC) test intended for use in the light microscopic assessment of mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, and MSH6) and BRAF V600E proteins in formalin-fixed, paraffin-embedded colorectal cancer (CRC) tissue sections. The OptiView DAB IHC Detection Kit is used with MLH1, MSH2, MSH6 and BRAF V600E, and the OptiView DAB IHC Detection Kit with OptiView Amplification Kit is used for PMS2 detection. The VENTANA MMR IHC Panel is for use on the VENTANA BenchMark ULTRA instrument. The VENTANA MMR IHC Panel includes VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody, VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody, and VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody.

The VENTANA MMR IHC Panel is indicated in patients diagnosed with colorectal cancer (CRC) to detect mismatch repair (MMR) proteins deficiency as an aid in the identification of probable Lynch syndrome and to detect BRAF V600E protein as an aid to differentiate between sporadic CRC and probable Lynch syndrome.

Results from the VENTANA MMR IHC Panel should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

Intended for in vitro diagnostic (IVD) use. Prescription Use Only.

SUMMARY AND EXPLANATION

VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody (VENTANA anti-MLH1 (M1) antibody) is a mouse monoclonal antibody produced against a full-length recombinant MLH1 protein with a glutathione-S-transferase (GST) tag. VENTANA anti-MLH1 (M1) antibody recognizes MLH1, which is one of several clinically important DNA mismatch repair (MMR) proteins.[37,38] VENTANA anti-MLH1 (M1) antibody is part of VENTANA MMR RxDx Panel, an immunohistochemical (IHC) assay system for identifying tumors with MMR deficiency. MMR proteins (MLH1, PMS2, MSH2, and MSH6) are ubiquitously expressed in proliferating normal and malignant cells.[39]

MMR is a conserved molecular mechanism that functions to correct the improper base substitutions that spontaneously occur during DNA replication.[40] Polymerase chain reaction (PCR)-based methods have shown that MMR failure frequently leads to microsatellite instability (MSI), a condition in which short, tandem nucleotide repeats are inserted into the DNA.[41-43] When the number of repeats is equal to or greater than 30% of the examined microsatellite loci, MSI can be further characterized as MSI-High (MSI-H). Defects in the MMR machinery have been attributed to mutations in the MMR proteins, most commonly MLH1, PMS2, MSH2, and MSH6.

The MLH1 and PMS2 proteins normally function together in a heterodimeric complex, as do the MSH2 and MSH6 proteins. When MMR is functioning normally, the MSH6/MSH2 heterodimer binds to mismatched DNA. This binding induces a conformational change that allows the MLH1/PMS2 heterodimer to bind the DNA-bound MSH6/MSH2 complex, resulting in excision repair of the affected DNA.[43,44] Mutations or deficiencies in these proteins result in frequent MSI and somatic mutation due to replication error. MMR IHC testing can be useful in identifying tumors with alterations in MMR.[45]

CLINICAL SIGNIFICANCE

Colorectal Carcinoma and Lynch Syndrome

Colorectal cancer is the third most common cancer and the fourth most prevalent cause of death in the world.[46] The majority of CRCs show chromosomal instability, however approximately 15% of cancers develop through an alternative pathway characterized by defective function of the DNA mismatch repair (MMR) system. As a consequence of the MMR deficiency, tumors exhibit microsatellite instability (MSI) resulting from the inability of MMR proteins to repair DNA replication errors. CRCs with MMR defects are denoted as deficient MMR (dMMR) tumors. In contrast, CRCs with no MMR defects are denoted as proficient MMR (pMMR) tumors. The dMMR colorectal cancers are often poorly differentiated and frequently show proximal colon predominance, mucinous, medullary, or signet ring histologic features and increased numbers of tumor-infiltrating lymphocytes.[47,48] In general, MMR deficiency may be caused either by germline mutations in one of the MMR genes with subsequent loss of the corresponding normal allele through genetic or epigenetic mechanisms, somatic mutations in the alleles, or by epigenetic inactivation of the MLH1 gene through methylation.[49]

Lynch syndrome was described in the 1960s and identified a link between the loss of MMR function and cancer.[50] The MLH1, PMS2, MSH2, and MSH6 proteins are clinically important MMR proteins encoded by genes that may be mutated in families with Lynch syndrome. [37,38] Loss of MMR proteins (MLH1, PMS2, MSH2, or MSH6) may lead to MSI and a higher lifetime risk of not only CRC, but also cancers of the stomach, brain, pancreas, skin, endometrium and ovaries. Patients with Lynch syndrome have a 50-80% lifetime risk for CRC.[43,51,52] Lynch syndrome is unique from other hereditary cancer syndromes as direct testing on tumor tissue aids in the identification of patients at risk for Lynch syndrome and helps inform subsequent germline genetic testing. Families with Lynch syndrome benefit from advanced cancer screening protocols. Various guidelines, including National Comprehensive Cancer Network (NCCN) guidelines, recommend that all CRCs should be screened for potential Lynch syndrome to identify patients and families that will benefit from further genetic testing and counseling.[50,53-56] Carriers of these mutations have a high lifetime risk of developing colorectal and other cancers due to accumulation of DNA replication errors in proliferating cells. Lynch syndrome represents 1-6% of all CRCs. These tumors result from the inheritance of a germline autosomal dominant mutation in one of the four MMR genes, with MLH1 loss occurring in the majority of these Lynch syndrome associated CRCs.[43,57,58] More than 300 different mutations in the MMR family of proteins have been identified in patients with Lynch syndrome. The Lynch syndrome-associated tumor phenotype is generally characterized by immunohistochemical loss of expression in MMR proteins, particularly MLH1, PMS2, MSH2, and MSH6.[58-61] MMR IHC testing has been shown to be useful in the identification of the specific MMR gene in which either a germline or a somatic alteration is most likely to be found.[61] Using the VENTANA MMR IHC Panel will aid in determining the MMR status of CRCs by classifying them as intact or loss for MMR protein expression. Detection of all four MMR proteins in the tumor indicates normal or intact MMR. Loss of MLH1 or MSH2 expression is almost invariably accompanied with the loss of its heterodimer partner, PMS2 or MSH6, respectively. However, loss of PMS2 or MSH6 does not lead to loss of MLH1 or MSH2. Loss of PMS2, MSH2 and/or MSH6 is consistent with probable Lynch syndrome, and patients should be referred for additional testing and counseling consistent with clinical practice.

Loss of MLH1 protein may indicate a sporadic occurrence or potential Lynch syndrome. In 15% or more of sporadic CRC, loss of MLH1 protein is due to hypermethylation of the MLH1 promoter.[43,62,63] Importantly, the BRAF V600E mutation is observed in about two thirds of tumors with loss of MLH1 expression from MLH1 promoter hypermethylation. In contrast, the BRAF V600E mutation is very rarely observed in Lynch syndrome tumors.[62] Therefore, if the result of the VENTANA anti-MLH1 (M1) Mouse Monoclonal Antibody (VENTANA anti-MLH1 (M1) antibody) indicates loss of MLH1 protein, VENTANA anti-BRAF V600E (VE1) antibody may stratify the tumor as sporadic or probable Lynch syndrome.[43,64] In CRC, loss of MLH1 protein is frequently the result of hypermethylation of the MLH1 promoter and indicates a sporadic occurrence.[65] The presence of the BRAF V600E protein is tightly linked with hypermethylation of the MLH1 promoter. As a result,

loss of MLH1 protein with a BRAF V600E status of positive strongly indicates that the tumor is the result of a sporadic occurrence, virtually eliminating Lynch syndrome as the underlying cause of malignancy.[65,66] When loss of MLH1 protein is accompanied with a BRAF V600E status of negative, the MLH1 loss is consistent with a high probability of Lynch syndrome.[67]

PRINCIPLE OF THE PROCEDURE

The VENTANA anti-MLH1 (M1) antibody is a mouse monoclonal antibody produced against a full-length recombinant MLH1 protein with a GST tag. The VENTANA anti-MLH1 (M1) antibody binds to the MLH1 protein in FFPE tissue sections. The antibody can be localized using a haptenated secondary antibody followed by a multimer anti-hapten-HRP conjugate (OptiView DAB IHC Detection Kit, Cat. No. 760-700 / 06396500001). The specific antibody-enzyme complex is then visualized with a precipitating enzyme reaction product. Each step is incubated for a precise time and temperature. At the end of each incubation step, the BenchMark ULTRA instrument washes the sections to stop the reaction and to remove unbound material that would hinder the desired reaction in subsequent steps. It also applies ULTRA LCS (ULTRA LCS (Predilute), Cat. No. 650-210 / 05424534001), which minimizes evaporation of the aqueous reagents from the specimen slide.

In addition to staining with VENTANA anti-MLH1 (M1) antibody, a second slide should be stained with the mouse monoclonal negative reagent, Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001). The negative reagent control is used to assess background staining.

MATERIAL PROVIDED

VENTANA anti-MLH1 (M1) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA anti-MLH1 (M1) antibody contains approximately 5 µg of a mouse monoclonal antibody.

The antibody is diluted in a TBS buffer containing 0.3% carrier protein.

Specific antibody concentration is approximately 1 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA anti-MLH1 (M1) antibody is a mouse monoclonal antibody produced as a cell culture supernatant.

Refer to the appropriate interpretation guide for detailed instructions for interpretation of MMR Panel staining in specific indications: VENTANA MMR IHC Panel Interpretation Guide for Staining of Colorectal Tissues (P/N 1016702US)

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed, but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody (Cat. No. 790-5094 / 07862261001)
2. VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody (Cat. No. 790-5093 / 07862253001)
3. VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody (Cat. No. 790-5092 / 07862245001)
4. VENTANA anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody (Cat. No. 790-5095 / 07862270001), if staining with VENTANA MMR IHC Panel
5. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
6. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
7. Microscope slides, positively charged
8. Bar code labels (appropriate for negative reagent control and primary antibody being tested)
9. Xylene (Histological grade)
10. Ethanol or reagent alcohol (Histological grade)
    - 100% solution: Undiluted ethanol or reagent alcohol
    - 95% solution: Mix 95 parts of ethanol or reagent alcohol with 5 parts of deionized water
    - 80% solution: Mix 80 parts of ethanol or reagent alcohol with 20 parts of deionized water
11. Deionized or distilled water
12. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
13. For VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, OptiView Amplification Kit (Cat. No. 760-099 / 06396518001 or Cat. No. 860-099 / 06718663001)
14. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
15. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
16. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
17. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
18. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
19. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
20. Permanent mounting medium (Permount Fisher Cat. No. SP15-500 or equivalent)
21. Cover glass (sufficient to cover tissue, such as VWR Cat. No. 48393-060)
22. Automated coverslipper (such as the Tissue-Tek SCA Automated Coverslipper)
23. Light microscope
24. Absorbent wipes

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. Tissue should be fixed immediately following excision for use with the VENTANA MMR antibodies. A delay to fixation of more than 6 hours has been shown to have an adverse effect on stain intensity of the tissue. Tissue fixation in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours is recommended. Fixation times of less than 6 hours and more than 72 hours may result in a loss of staining for MLH1. The amount of NBF used should be 15 to 20 times the volume of tissue. No fixative will penetrate more than 2 to 3 mm of solid tissue or 5 mm of porous tissue in a 24-hour period. Fixation can be performed at room temperature (15-25°C).[68-70]

Fixatives such as zinc formalin, Z-5, 95% alcohol, alcohol-formalin-acetic acid (AFA) and PREFER fixative have demonstrated weak or variable staining; they are not recommended for use with this assay. Users who deviate from the specified specimen preparation must accept responsibility for interpretation of patient results.

Sections should be cut at 4 µm thick and mounted on positively-charged glass slides. No other thicknesses have been validated. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time and may be compromised 45 days after cutting from the FFPE tissue block. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. **CAUTION**: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only).
4. Do not use beyond the specified number of tests.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[71,72]

7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument Operator's Manual, and instructions for use of all necessary components located at dialog.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

STAINING PROCEDURE

VENTANA anti-MLH1 (M1) antibody has been developed for use on a BenchMark ULTRA instrument in combination with OptiView DAB IHC Detection Kit and ancillary reagents. Refer to Table 30 for the recommended staining protocol for VENTANA MMR IHC Panel.

This antibody has been optimized for specific incubation times, but the user must validate results obtained with this reagent. The effect of varying time and temperature of the antigen retrieval (cell conditioning) and antibody incubation from the recommended staining protocol in Table 30 may result in sub-optimal staining and false deficient and false proficient results. It is strongly recommended not to deviate from the recommended staining protocol in Table 30. Any deviation from recommended test procedures may invalidate expected results. Appropriate controls must be employed and documented. Users who deviate from recommended test procedures must accept responsibility for interpretation of patients' results.

The parameters for the automated procedures can be displayed, printed, and edited according to the procedure in the instrument Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding IHC staining procedures.

Table 30. Recommended staining procedure and protocol for VENTANA anti-MLH1 (M1) antibody and Negative Control (monoclonal) with OptiView DAB IHC Detection Kit on a BenchMark ULTRA instrument for VENTANA MMR IHC Panel.

Staining Procedure: U OptiView DAB IHC	
Protocol Step	**Parameter Input**
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 64 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	24 minutes, 36°C
OptiView HQ Linker	8 minutes (default)
OptiView HRP Multimer	8 minutes (default)
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Note: Users have the option to use 'U MMR Panel' staining procedure in Table 2 to stain CRC tissues.

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation and cell conditioning based on individual specimens and pathologist preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."[69]

If the internal positive controls fail to demonstrate appropriate staining, results with the test specimen should be considered invalid.

QUALITY CONTROL PROCEDURES

Internal Positive Controls

Normal tissue elements (e.g., lymphocytes, fibroblasts, or normal epithelium) in the immediate vicinity of the tumor will serve as internal positive controls. Unequivocal nuclear staining in these cells validates the staining run. If the internal positive controls fail to demonstrate appropriate staining, results with the test specimen should be considered invalid.

Negative Reagent Control for Test Tissue

Ventana Medical Systems, Inc. strongly recommends a negative reagent control be used to stain an adjacent section of the patient specimen tissue on a separate slide from the VENTANA anti-MLH1 (M1) antibody stained slide. A negative reagent control mouse monoclonal antibody (Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001) is recommended for use in place of the primary antibody to evaluate nonspecific staining. The staining parameters for the negative reagent control antibody should be the same as that for the primary antibody.

Positive Tissue Control

A positive tissue control must be run with every staining procedure performed. Optimal laboratory practice is to include a positive control section on the same slide as the patient tissue. This practice helps to identify a failure to apply primary antibody or other critical reagent to the patient test slide. A tissue with weak positive staining is more suitable for optimal quality control. The positive staining tissue components are used to confirm that the antibody was applied and the instrument functioned properly. This tissue may contain both positive and negative staining cells or tissue components and serve as both the positive and negative control tissue. Control tissues should be fresh autopsy, biopsy, or surgical specimens prepared or fixed as soon as possible in a manner identical to the test sections. Such tissues may monitor all steps of the procedure from tissue preparation through staining. Use of a tissue section fixed or processed differently from the test specimen will provide control for all reagents and method steps except fixation and tissue processing.

Known positive tissue controls should be utilized only for monitoring the correct performance of processed tissues and test reagents, not as an aid in determining a specific diagnosis of patient samples. If the positive tissue controls fail to demonstrate positive staining, results with the test specimens should be considered invalid.

CRC tissue with an MLH1 Clinical Status of Intact or normal colon tissue pre-qualified with VENTANA anti-MLH1 (M1) antibody may be used as a positive tissue control. Normal colon will stain Intact for MLH1 using the VENTANA anti-MLH1 (M1) antibody. The positive tissue control should exhibit unequivocal nuclear staining in viable tumor and/or normal colon tissue elements. For all tissues, internal positive control cells (i.e., lymphocytes, fibroblasts or normal epithelium in the vicinity of the tumor) should stain positive in the nucleus.

Negative Tissue Control

Since the MLH1, PMS2, MSH2, and MSH6 proteins are expressed in all tissues, a normal negative tissue control does not exist for these biomarkers. However, CRC tissue with an MLH1 Clinical Status of Loss pre-qualified with VENTANA anti-MLH1 (M1) antibody may be used as a negative tissue control. The negative tissue control should be used only to monitor the correct performance of processed tissues, test reagents and instruments and not as an aid in formulating a specific diagnosis of patient samples.

Assay Verification

Prior to initial use of an antibody or staining system in a diagnostic procedure, the specificity of the antibody should be verified by testing on a series of tissues with known IHC performance characteristics representing tissues Intact for MLH1 Clinical Status. (Refer to the Quality Control Procedures previously outlined in this section of the product insert and to the Quality Control recommendations of the College of American Pathologists Laboratory Accreditation Program, Anatomic Pathology Checklist,[73] or the CLSI Approved Guideline.[74])

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for VENTANA anti-MLH1 (M1) antibody is nuclear in actively proliferating cells. Tumor tissue stained with the VENTANA anti-MLH1 (M1) antibody is assigned a clinical status by a trained pathologist based on their evaluation of the presence or absence of specific nuclear staining in the tumor. A clinical status of Intact is assigned to cases with unequivocal nuclear staining in viable tumor cells, in the presence of acceptable internal positive controls (nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor). A clinical status of Loss is assigned to

cases with unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls as shown in Table 31.

If unequivocal nuclear stain is absent in internal positive controls and/or background staining interferes with interpretation, the assay should be considered unacceptable and repeated. Punctate nuclear staining of tumor cells should be considered negative (Loss). In cases with focal tumor cell staining, some specimens may exhibit focal staining in the tumor cells and staining intensity may vary from weak to strong. Based on the MMR RxDx scoring algorithm, focal weak equivocal nuclear staining in the viable tumor cells in the presence of internal positive controls should be given a Clinical Status of Loss. On the other hand, focal strong unequivocal nuclear staining in the viable tumor cells in the presence of internal positive controls should be given a Clinical Status of Intact.

Table 31. Staining interpretation for VENTANA anti-MLH1 (M1) antibody.

Clinical Status	Description
Intact MLH1 Expression	Unequivocal nuclear staining in viable tumor cells in the presence of acceptable internal positive controls (eg nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor)
Loss of MLH1 Expression	Unequivocal loss of nuclear staining or focal weak equivocal nuclear staining in the viable tumor cells in the presence of acceptable internal positive controls. Punctate nuclear staining will be considered negative.

VENTANA anti-MLH1 (M1) antibody stained cases are categorized as Intact or Loss according to the presence or absence of specific staining in the tumor.

The interpretation for overall panel-level MMR Status is provided below in Table 32.

Table 32. Staining interpretation for VENTANA MMR RxDx Panel.

Proficient/ Negative	Deficient/ Positive
All 4 markers (MLH1, PMS2, MSH2, and MSH6) in the panel exhibit intact protein expression	At least 1 marker (MLH1, PMS2, MSH2, and MSH6) in the panel exhibits loss of protein expression

SPECIFIC LIMITATIONS

Ventana Medical Systems, Inc. provides antibodies and reagents at optimal dilution for use when the provided instructions are followed. Deviation from the recommended conditions for antigen retrieval (Cell Conditioning) provided in the staining protocol (Table 30) may invalidate the results. Appropriate controls should be employed and documented. Users who deviate from the listed protocol must accept responsibility for interpretation of patient results.

VENTANA anti-MLH1 (M1) antibody has been solely cleared for use on the BenchMark ULTRA instrument with OptiView DAB IHC Detection Kit and is not cleared for use with any other detection methods or automated staining instruments.

Some cases may be particularly challenging due to the following issues:

- Nonspecific background: Some specimens may exhibit nonspecific background staining for reasons that are not well understood. For this reason, evaluation of a VENTANA anti-MLH1 (M1) antibody slide must include a comparison of the slide to the negative reagent control slide to determine the level of nonspecific background staining. Cytoplasmic staining, if present, should be disregarded in VENTANA anti-MLH1 (M1) antibody IHC interpretation.
- Focal Staining: Some specimens may exhibit focal staining in tumor cells; the staining intensity may vary from weak to strong. As specified by the VENTANA anti-MLH1 (M1) antibody IHC scoring algorithm, focal weak equivocal nuclear staining in viable tumor cells in the presence of acceptable internal positive control staining should be categorized as Loss status.
- Punctate Staining: Some specimens may exhibit discrete punctate staining within a few nuclei of the tumor; the staining intensity may vary from weak to strong. This staining pattern should be ignored. If only this type of staining pattern is present, the clinical status is Loss.
- Tissue or Staining Artifact: Histologic artifacts originating from the sample processing and microtomy processes can also complicate the determination of VENTANA anti-MLH1 (M1) antibody IHC clinical status. These artifacts may include, but are not limited to, fixation gradients and edge effects, DAB trapping, nuclear bubbling, lack of staining in some regions of the tissue, tearing or folding of the tissue, and loss of the tissue section. In some instances, repeat staining of new sections or acquisition of a new specimen may be required.

PERFORMANCE CHARACTERISTICS

Analytical Performance

Staining tests for staining sensitivity, specificity, repeatability, and intermediate precision, as well as tests for reader precision, inter-laboratory reproducibility, and clinical outcome were conducted and the results are listed in the following section.

Sensitivity and Specificity

Analytical sensitivity and specificity were determined by staining multiple cases of normal and neoplastic human tissues with VENTANA anti-MLH1 (M1) antibody. The results are listed in Table 33 and Table 34. Positive staining is nuclear unless otherwise specified. No unexpected staining was observed with VENTANA anti-MLH1 (M1) antibody on the normal and neoplastic tissues. As expected, since mismatch repair is present in all actively proliferating cells, all normal and neoplastic tissues demonstrated positive staining.

Table 33. Sensitivity/Specificity of VENTANA anti-MLH1 antibody staining on formalin-fixed, paraffin-embedded normal tissues.

Tissue	# Positive / Total Cases	Tissue	# Positive / Total Cases
Adrenal gland	3/3	Lung	4/4
Bladder	3/3	Lymph node	3/3
Bone marrow	3/3	Mesothelium	4/4
Ovary	5/5	Pancreas	3/3
Breast	3/3	Parathyroid gland	3/3
Cerebellum	3/3	Peripheral nerve	5/5
Cerebrum	3/3	Prostate	3/3
Cervix	3/3	Skeletal Muscle	3/3
Colon	3/3	Skin	3/3
Endometrium	3/3	Spleen	3/3
Esophagus	3/3	Stomach	3/3
Heart	3/3	Testis	3/3
Hypophysis (Pituitary)	3/3	Thymus	3/3
Intestine	3/3	Thyroid	4/4
Kidney	3/3	Tongue/Salivary gland	3/3
Liver	3/3	Tonsil	3/3

Note: Mismatch repair proteins such as MLH1 are present in all actively proliferating cells. For all tissues, positive/negative staining was determined for tissue specific elements in the presence of positive staining in normal control cells (lymphocytes, fibroblasts, and epithelial cells).

Table 34. Sensitivity/Specificity of VENTANA anti-MLH1 (M1) antibody staining on a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# Positive / Total Cases
Glioblastoma (Cerebrum)	1/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	1/1
Oligodendroglioma (Cerebrum)	1/1
Serous adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Ovary)	1/1
Pancreatic neuroendocrine neoplasm (Pancreas)	1/1
Seminoma (Testis)	2/2
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	1/1
Microinvasive ductal carcinoma (Breast)	1/1
Invasive ductal carcinoma (Breast)	1/1
Small cell carcinoma (Lung)	1/1
Squamous cell carcinoma (Lung)	1/1
Neuroendocrine carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	1/1
Signet ring carcinoma (Stomach)	1/1
Adenocarcinoma (Small intestine)	1/1
Stromal sarcoma (Small intestine)	1/1
Gastrointestinal stromal tumor (Peritoneum)	1/1
Adenocarcinoma (Colon)	1/1
Adenocarcinoma (Rectum)	1/1
Gastrointestinal stromal tumor (GIST) (Rectum)	1/1
Hepatoblastoma (Liver)	1/1
Clear cell carcinoma (Kidney)	1/1
Adenocarcinoma (Prostate)	2/2
Leiomyoma (Uterus)	1/1
Adenocarcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	1/1
Squamous cell carcinoma (Skin)	1/1
Neurofibroma (Lumbar)	1/1
Neuroblastoma (Retroperitoneum)	1/1
Mesothelioma (Peritoneum)	1/1
B-cell lymphoma; NOS (Lymph node)	3/3
Hodgkin's lymphoma (Lymph node)	1/1
Leiomyosarcoma (Bladder)	1/1
Osteosarcoma	1/1
Spindle cell rhabdomyosarcoma (Peritoneum)	1/1
Leiomyosarcoma (Smooth muscle)	1/1

Note: Mismatch repair proteins such as MLH1 are present in all actively proliferating cells. For all tissues, positive/negative staining was determined for tumor cells in the presence of positive staining in normal control cells (lymphocytes, fibroblasts, and epithelial cells).

ANALYTICAL PERFORMANCE FOR VENTANA MMR IHC PANEL

Within-Day Repeatability and Day-to-Day Precision

The repeatability and precision of VENTANA anti-MLH1 (M1) antibody was evaluated on the BenchMark ULTRA instrument in combination with the OptiView DAB IHC Detection Kit.

Within-Day Repeatability was evaluated using 10 CRC specimens (5 Intact and 5 Loss for MLH1 expression). Five replicate slides from each of the CRC specimens were stained with VENTANA anti-MLH1 (M1) antibody on a single BenchMark ULTRA instrument within a single day. Each VENTANA anti-MLH1 (M1) antibody-stained slide was paired with a negative reagent control stained slide from the same case. All slides were randomized, and then evaluated as Intact or Loss by a single pathologist blinded to the case diagnosis.

Day-to-Day Precision was also evaluated using 10 CRC specimens (5 Intact and 5 Loss for MLH1 expression). Replicate slides from each of the CRC specimens were stained with VENTANA anti-MLH1 (M1) antibody on a BenchMark ULTRA instrument on each of 5 non-consecutive days. Each VENTANA anti-MLH1 (M1) antibody-stained slide was paired with a negative reagent control stained slide from the same case. All slides were randomized, and then evaluated as Intact or Loss by a single pathologist blinded to the case diagnosis.

None of the slides stained with the negative reagent control showed specific staining, and background staining was ≤ 0.5. Using pooled data of all possible pairings, both Within-Day Repeatability and Day-to-Day Precision studies demonstrated 100% positive percent agreement (PPA), 100% negative percent agreement (NPA) and 100% overall percent agreement (OPA). A summary of the results is shown in Table 35.

Table 35. Within-Day Repeatability and Day-to-Day Precision of the VENTANA anti-MLH1 (M1) antibody as Measured by Clinical Status (Intact or Loss).

Repeatability/ Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Day Repeatability	Intact	PPA	25/25	100.0	(86.7, 100.0)
	Loss	NPA	25/25	100.0	(86.7, 100.0)
	Total	OPA	50/50	100.0	(92.9, 100.0)
Day-to-Day Precision	Intact	PPA	50/50	100.0	(92.9, 100.0)
	Loss	NPA	50/50	100.0	(92.9, 100.0)
	Total	OPA	100/100	100.0	(96.3, 100.0)

Note: 95% CIs were calculated using the Wilson Score method.

BenchMark ULTRA Instrument-to-Instrument Precision

BenchMark ULTRA Instrument-to-Instrument Precision of the VENTANA anti-MLH1 (M1) antibody was determined by staining replicate slides of 10 CRC specimens (5 Intact and 5 Loss for MLH1 expression) across 3 BenchMark ULTRA instruments with VENTANA anti-MLH1 (M1) antibody using the OptiView DAB IHC Detection kit. Replicate slides from each of the CRC specimens were stained with VENTANA anti-MLH1 (M1) antibody on 3 BenchMark ULTRA instruments.

Each VENTANA anti-MLH1 (M1) antibody-stained slide was paired with a negative reagent control stained slide from the same case. All slides were randomized, and then

evaluated for Clinical Status (Intact or Loss) by a single pathologist blinded to the case diagnosis. None of the slides stained with the negative reagent control showed specific staining, and background staining was ≤ 0.5.

For BenchMark ULTRA Instrument-to-Instrument Precision, pairwise comparisons of the Clinical Status of slides for each specimen were made between instruments and demonstrated 100% PPA, NPA, and OPA. A summary of the results can be found in Table 36.

Table 36. BenchMark ULTRA Instrument-to-Instrument Precision of the VENTANA anti-MLH1 (M1) antibody as Measured by Clinical Status (Intact or Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Instrument-to-Instrument	Intact	PPA	30/30	100.0	(88.6, 100.0)
	Loss	NPA	30/30	100.0	(88.6, 100.0)
	Total	OPA	60/60	100.0	(94.0, 100.0)

Note: 95% CIs were calculated using the Wilson Score method.

Reader Precision Studies

Within- and Between-Reader precision was evaluated on 20 CRC (11 Intact and 9 Loss cases) stained with VENTANA anti-MLH1 (M1) antibody and the OptiView DAB IHC Detection Kit. Each VENTANA anti-MLH1 (M1) antibody-stained slide was paired with a negative reagent control stained slide from the same case.

All slides were randomized and evaluated by 3 pathologists for Intact or Loss MLH1 Clinical Status. Pathologists were blinded to the case diagnosis. Following a two week washout period, the VENTANA anti-MLH1 (M1) antibody-stained slides were re-randomized for a second evaluation of the MLH1 Clinical Status by each of the 3 pathologists. None of the slides stained with the negative reagent control showed specific staining and background staining was ≤ 0.5.

Within-Reader precision compared initial and final slide evaluations from a single pathologist providing 20 CRC slide comparisons per pathologist. Comparisons from the 3 pathologists were pooled and demonstrated 98.5% average positive agreement (APA), 98.1% average negative agreement (ANA), and 98.3% OPA for Within-Reader precision. A summary of the results can be found in Table 37.

Between-Reader precision compared all slide evaluations (20 CRC x 2 evaluations/case x 3 pathologists = 120 slide evaluations) to a modal case status for each CRC case. The results demonstrate 100% PPA, 98.1% NPA and 99.2% OPA for Between-Reader precision. A summary of the results can be found in Table 37.

Table 37. Within-Reader and Between-Reader Precision of the VENTANA anti-MLH1 (M1) antibody on CRC Cases as Measured by MLH1 Clinical Status (Intact/Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Reader	Intact	APA	66/67	98.5	(88.0, 100.0)
	Loss	ANA	52/53	98.1	(83.3, 100.0)
	Total	OPA	59/60	98.3	(85.0, 100.0)
Between Reader	Intact	PPA	66/66	100.0	(94.5, 100.0)
	Loss	NPA	53/54	98.1	(90.2, 99.7)
	Total	OPA	119/120	99.2	(95.4, 99.9)

Note: For Within-Reader, the APA and ANA 95% CIs were calculated using the Clopper-Pearson based method; the OPA 95% CI was calculated using the percentile bootstrap method. For Between-Reader, 95% CIs were calculated using the Wilson Score method.

Lot-to-Lot Precision

Lot-to-lot Precision of VENTANA anti-MLH1 (M1) antibody was determined by testing 3 production lots of the VENTANA anti-MLH1 (M1) antibody each on triplicate slides of 10 CRC (5 Intact and 5 Loss for MLH1 expression) on a BenchMark ULTRA instrument using the OptiView DAB IHC Detection Kit.

Each VENTANA anti-MLH1 (M1) antibody-stained slide was paired with a negative reagent control stained slide from the same case. Slide sets were randomized, and evaluated by a single pathologist blinded to the case diagnosis and VENTANA anti-MLH1 (M1) antibody lot number. None of the slides stained with the negative reagent control showed specific staining, and background staining was ≤ 0.5.

For VENTANA anti-MLH1 (M1) antibody Lot-to-Lot Precision, all slide evaluations were compared to a modal case status for each CRC case. The OPA between the VENTANA anti-MLH1 (M1) antibody lots was 100%; demonstrating that VENTANA anti-MLH1 (M1) antibody staining is reproducible across antibody lots.

A summary of the results for Lot-to-Lot Precision of the VENTANA anti-MLH1 (M1) antibody is shown in Table 38.

Table 38. Lot-to-Lot Precision of the VENTANA anti-MLH1 (M1) antibody as measured by Clinical Status (Intact or Loss).

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Lot-to-Lot	Intact	PPA	45/45	100.0	(92.1, 100.0)
	Loss	NPA	45/45	100.0	(92.1, 100.0)
	Total	OPA	90/90	100.0	(95.9, 100.0)

Note: 95% CIs were calculated using the Wilson Score method.

Inter-Laboratory Reproducibility Study

An Inter-Laboratory Reproducibility Study of the VENTANA MMR IHC Panel was completed to demonstrate reproducibility of each VENTANA MMR IHC Panel assay to determine Clinical Status. The study included 6 CRC tissue specimens (3 Intact and 3 Loss) for each MMR protein and 16 CRC tissue specimens (8 Positive and 8 Negative) for BRAF V600E run across 3 BenchMark ULTRA instruments on each of 5 non-consecutive days over 21 days at three external laboratories. The specimens were randomized and evaluated by a total of 6 readers (2 readers/site) who were blinded to the MMR Clinical Status of the study set. Each of the 40 cases in the study had 30 observations across all days, sites, and readers. The modal case reference status was derived for each case based on the most often observed status of the 30 observations. The study included a total of 1200 observations for all five proteins. For all evaluable cases, the acceptability rate for morphology and background in this study was 100%. A summary of the pooled (all five proteins) agreement statistics between the modal case reference status and individual observations can be found in Table 39.

Table 39. Agreement between the VENTANA MMR IHC Panel and Modal Case Reference Status.

Inter-Laboratory Reproducibility	Clinical Status	Agreement			
		Type	n/N	%	95% CI
All Proteins	Intact/Positive	PPA	598/600	99.8	(98.7, 100.0)
	Loss/Negative	NPA	593/600	98.9	(97.4, 99.5)
	Total	OPA	1191/1200	99.4	(98.6, 99.7)

Note: Clinical Status is defined as Intact or Loss for protein expression for MMR or Positive or Negative for BRAF V600E protein. 95% CIs were calculated using a generalized linear mixed model (GLMM) approach.

In addition, pairwise comparisons were made Between-Site, Between-Day and Between-Reader for the VENTANA anti-MLH1 (M1) antibody. For MLH1, this study set included a total of 180 observations. A summary of the results can be found in Table 40. The data indicate assay reproducibility across 5 days, 3 sites, and 6 readers.

Table 40. Inter-Laboratory Reproducibility Pairwise Agreement Rates for the VENTANA anti-MLH1 (M1) antibody as Measured by Clinical Status (Intact or Loss).

Inter-Laboratory Reproducibility		Agreement			
		Type	n/N	%	95% CI
Between-Site (3 sites)		APA	360/360	100.0	(98.9, 100.0)
		ANA	360/360	100.0	(98.9, 100.0)
		OPA	360/360	100.0	(98.9, 100.0)
Between-Day (5 non-consecutive days)	Site A	APA	120/120	100.0	(96.9, 100.0)
		ANA	120/120	100.0	(96.9, 100.0)
		OPA	120/120	100.0	(96.9, 100.0)
	Site B	APA	120/120	100.0	(96.9, 100.0)
		ANA	120/120	100.0	(96.9, 100.0)
		OPA	120/120	100.0	(96.9, 100.0)
	Site C	APA	120/120	100.0	(96.9, 100.0)
		ANA	120/120	100.0	(96.9, 100.0)
		OPA	120/120	100.0	(96.9, 100.0)
Between-Reader (2 pathologists per site)		APA	90/90	100.0	(95.9, 100.0)
		ANA	90/90	100.0	(95.9, 100.0)
		OPA	90/90	100.0	(95.9, 100.0)

Note: 95% CIs were calculated using the Wilson Score method.

Accuracy Study: Concordance of VENTANA MMR IHC Panel Results to DNA Sequencing Results

A study was conducted to compare the performance of the VENTANA MMR IHC Panel to a comprehensive DNA sequencing colon panel for the identification of CRCs that result from potential Lynch syndrome. The DNA sequencing colon panel included genomic analysis of variants present in MMR genes (MLH1, PMS2, MSH2, MSH6, EPCAM), BRAF, and other genes important in carcinogenesis (e.g., PIK3CA, KRAS, NRAS, ERBB2, etc.). Sequencing included all exons, intronic and flanking sequences as well as large deletions, duplications, and mosaicism.

For the study, 150 sequential CRC cases were stained by H&E and evaluated for indications of proper fixation and morphology including the presence of cellular elements (tumor and internal control cells). Each case was evaluated to determine if the specimen contained a minimum of 50% tumor content to provide sufficient representation of tumor cells in the sample as recommended for DNA sequencing. Of the 150 sequential CRC cases, 7 cases were excluded from the study set due to insufficient viable tumor (inadequate cellularity or lack of tumor content), 3 cases due to misclassification as CRC, and 1 due to clerical error. The remaining sequential study set cases were sectioned and a second H&E evaluation of bracketing slides was completed to ensure tissue integrity and tumor were represented through all sections, until sufficient cases (minimum of 100 cases) were enrolled into the study. Two cases were removed from the study due to lack of sufficient viable tumor content throughout the block and 1 was not evaluated due to clerical error. Following review, the sequential study set included 111 cases meeting the selection criteria and were enrolled into the study. The remaining 25 sequential CRC cases were not evaluated and not enrolled into the study. In addition, an enrichment study set of 15 CRC cases showing a Clinical status of Loss by IHC were included to ensure that Loss of each protein was represented in the study. Tissue sections of all cases in the study were stained by IHC with the VENTANA MMR IHC Panel and appropriate negative reagent controls. Additional tissue sections from each case were subjected to the DNA sequencing colon panel. Of the 126 enrolled cases, 7 cases were excluded from analyses due to failure of sequencing.

In the final study set of 119 cases (including one case that failed to produce IHC results due to tissue loss), the analysis compared the results of the VENTANA MMR IHC Panel to those for DNA sequencing at the case level, where DNA sequencing acted as the reference status for IHC comparison as shown in Table 41. For IHC, the MMR status (Intact / Loss) was stratified by BRAF V600E status, and for DNA sequencing, results were characterized by the presence or absence of potential pathogenic mutations. For this study, a pathogenic mutation within the tumor is defined as a germline or somatic mutation predicted to result in the loss of MMR protein expression. Point estimates for this comparison were 77.8% PPA, 97.0% NPA and 94.1% OPA.

IHC MMR status and DNA sequencing status were compared separately for individual MMR proteins within the study in Table 42. For MLH1 and PMS2 loss cases, results were stratified by BRAF V600E. The OPA of each MMR protein, when compared to the results of the DNA sequencing colon panel, was 95.8% for VENTANA anti-MLH1 (M1) antibody, 94.1% for VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody, 98.3% for VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody and 96.6% for VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary antibody. Out of three cases containing a potential pathogenic mutation in the MLH1 gene, all were MLH1 loss cases by IHC. One of these cases was also BRAF V600E positive suggesting sporadic CRC. It is likely given the variable allelic frequency that the pathogenic mutation identified in the tumor does not represent a germline mutation, but an acquired mutation in this tumor. Four of the eight cases that contained a potential pathogenic mutation affecting MSH6 expression demonstrated MSH6 Intact status by IHC. Of these, two contained POLE mutations which variably affect the expression of MMR proteins [75-77] and do not represent Lynch syndrome mutations. One case demonstrated MSH6 IHC staining in a small portion of the tumor and was designated Intact, but DNA sequencing showed several mutations in the MSH6 gene which likely result from somatic mutations. An analysis of the VENTANA MMR IHC Panel and DNA sequencing results was also performed for the sequential (Table 43) and enrichment (Table 44) study sets at the case level. In addition, an evaluation of the IHC results for each MMR protein and DNA sequencing within the sequential and enrichment study sets are presented in Table 45 and Table 46, respectively.

The VENTANA anti-BRAF V600E (VE1) antibody is included in the VENTANA MMR IHC panel for the stratification of CRC cases showing a loss of MLH1 protein expression to sporadic or likely Lynch syndrome cancers. Of the 24 BRAF V600E IHC positive cases in this study, 20 cases had loss of MLH1 protein by IHC. The remaining four cases were pMMR (intact for all MMR proteins). All 24 BRAF V600E positive specimens were identified as sporadic CRC. Thus the data support the use of VENTANA anti-BRAF V600E (VE1) antibody to differentiate between sporadic and probable Lynch syndrome CRC in the absence of MLH1 expression. In addition, BRAF V600E Clinical status in CRC obtained by IHC using the VENTANA anti-BRAF V600E (VE1) antibody was also compared to BRAF mutational status results determined by DNA sequencing. For 23 BRAF V600E IHC positive cases (one case failed to yield DNA sequencing results), the PPA, NPA, and OPA of the VENTANA anti-BRAF V600E (VE1) antibody based IHC testing using DNA sequencing results as the reference all were 100%. These results verified that the VENTANA anti-BRAF V600E (VE1) antibody correctly identifies CRC having the BRAF V600E mutation.

Table 41. Evaluation of the VENTANA MMR IHC Panel and DNA Sequencing Results at the case level – ALL CASES.

A) Comparison of VENTANA MMR IHC Panel and DNA Sequencing Results.

VENTANA MMR IHC Panel Results		DNA Sequencing Results			
		Pathogenic Mutation	No Pathogenic Mutation	Invalid	Total
MMR Loss	BRAF V600E +	1	19	0	20
	BRAF V600E -	14	3	1	18
MMR Intact	BRAF V600E +	0	3	1	4
	BRAF V600E -	2	76	5	83
Invalid		1	0	0	1
Total		18	101	7	126

Note: Invalids are defined as failure to produce results by IHC and/or DNA sequencing.

B) Agreement between VENTANA MMR IHC Panel and DNA Sequencing Results.

Agreement			
Type	**n/N**	**%**	**95% CI**
PPA	14/18	77.8	(54.8, 91.0)
NPA	98/101	97.0	(91.6, 99.0)
OPA	112/119	94.1	(88.4, 97.1)

Note: Only Invalids resulting from a failure by IHC are included in the analysis. 95% CIs were calculated using the Wilson Score method.

The association between the test results and the final diagnosis with respect to potential Lynch syndrome is an estimate because the study was enriched with potential Lynch syndrome positive cases.

Table 42. Evaluation of the VENTANA MMR IHC Panel and DNA Sequencing Results at the Individual MMR Protein Level – ALL CASES

A) Comparison of Individual MMR Protein Status and DNA Sequencing Results.

IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	**No Pathogenic Mutation**	**Total**
MLH1 Loss	BRAF V600E +	1*	19	20
	BRAF V600E -	2	4	6
MLH1 Intact		0	92	92
Total		3	115	118
PMS2 Loss	BRAF V600E +	0	20	20
	BRAF V600E -	3	7	10
PMS2 Intact		0	88	88
Total		3	115	118
MSH2 Loss		3	2	5
MSH2 Intact		0	113	113
Total		3	115	118
MSH6 Loss		4	0	4
MSH6 Intact		4	110	114
Total		8	110	118

*Variant allele frequency indicates this is a rare MLH1 (p.K196Nfs*6) somatic mutation event and not a germline mutation.

B) Agreement between Individual MMR Protein and DNA Sequencing Results.

Agreement				
Protein	**Type**	**n/N**	**%**	**95% CI**
MLH1	PPA	2/3	66.7	(20.8, 93.9)
	NPA	111/115	96.5	(91.4, 98.6)
	OPA	113/118	95.8	(90.5, 98.2)
PMS2	PPA	3/3	100.0	(43.9, 100.0)
	NPA	108/115	93.9	(88.0, 97.0)
	OPA	111/118	94.1	(88.3, 97.1)
MSH2	PPA	3/3	100.0	(43.9, 100.0)
	NPA	113/115	98.3	(93.9, 99.5)
	OPA	116/118	98.3	(94.0, 99.5)
MSH6	PPA	4/8	50.0	(21.5, 78.5)
	NPA	110/110	100.0	(96.6, 100.0)
	OPA	114/118	96.6	(91.6, 98.7)

Note: 95% CIs were calculated using the Wilson Score method.

Table 43. Evaluation of the VENTANA MMR IHC Panel and DNA Sequencing Results at the Case Level – SEQUENTIAL STUDY SET.

A) Comparison of VENTANA MMR IHC Panel and DNA Sequencing Results.

VENTANA MMR IHC Panel Results		DNA Sequencing Results			
		Pathogenic Mutation	**No Pathogenic Mutation**	**Invalid**	**Total**
MMR Loss	BRAF V600E +	1	18	0	19
	BRAF V600E -	4	2	0	6
MMR Intact	BRAF V600E +	0	3	1	4
	BRAF V600E -	1	76	5	82
Invalid		0	0	0	0
Total		6	99	6	111

Note: Invalids are defined as failure to produce results by IHC and/or DNA sequencing.

B) Agreement of VENTANA MMR IHC Panel and DNA Sequencing Results.

Agreement			
Type	**n/N**	**%**	**95% CI**
PPA	4/6	66.7	(30.0, 90.3)
NPA	97/99	98.0	(92.9, 99.4)
OPA	101/105	96.2	(90.6, 98.5)

Note: Only Invalids resulting from a failure by IHC are included in the analysis. 95% CIs were calculated using the Wilson Score method.

Table 44. Evaluation of the VENTANA MMR IHC Panel and DNA Sequencing Results at the Case Level – ENRICHMENT STUDY SET.

A) Comparison of VENTANA MMR IHC Panel and DNA Sequencing Results.

VENTANA MMR IHC Panel Results		DNA Sequencing Results			
		Pathogenic Mutation	**No Pathogenic Mutation**	**Invalid**	**Total**
MMR Loss	BRAF V600E +	0	1	0	1
	BRAF V600E -	10	1	1	12
MMR Intact	BRAF V600E +	0	0	0	0
	BRAF V600E -	1	0	0	1
Invalid		1	0	0	1

VENTANA MMR IHC Panel Results	DNA Sequencing Results			
	Pathogenic Mutation	No Pathogenic Mutation	Invalid	Total
Total	12	2	1	15

Note: Invalids are defined as failure to produce results by IHC and/or DNA sequencing.

B) Agreement of VENTANA MMR IHC Panel and DNA Sequencing Results.

Agreement			
Type	n/N	%	95% CI
PPA	10/12	83.3	(55.2, 95.3)
NPA	1/2	50.0	(9.5, 90.5)
OPA	11/14	78.6	(52.4, 92.4)

Note: Only Invalids resulting from a failure by IHC are included in the analysis. 95% CIs were calculated using the Wilson Score method.

Table 45. Evaluation of MMR protein and DNA Sequencing Results at the Individual MMR Protein Level – SEQUENTIAL STUDY SET.

A) Comparison of Individual MMR Protein Status and DNA Sequencing Results.

IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MLH1 Loss	BRAF V600E +	1	18	19
	BRAF V600E -	1	4	5
MLH1 Intact		0	81	81
Total		2	103	105
PMS2 Loss	BRAF V600E +	0	19	19
	BRAF V600E -	0	5	5
PMS Intact		0	81	81
Total		0	105	105
MSH2 Loss		1	0	1
MSH2 Intact		0	104	104
Total		1	104	105
MSH6 Loss		0	0	0
MSH6 Intact		3	102	105
Total		3	102	105

B) Agreement of Individual MMR Protein Status and DNA Sequencing Results.

Agreement				
Protein	Type	n/N	%	95% CI
MLH1	PPA	1/2	50.0	(9.5, 90.5)
	NPA	99/103	96.1	(90.4, 98.5)
	OPA	100/105	95.2	(89.3, 97.9)
PMS2	PPA	n.e.	n.e.	n.e.
	NPA	100/105	95.2	(89.3, 97.9)
	OPA	100/105	95.2	(89.3, 97.9)
MSH2	PPA	1/1	100.0	(20.7, 100.0)
	NPA	104/104	100.0	(96.4, 100.0)
	OPA	105/105	100.0	(96.5, 100.0)
MSH6	PPA	0/3	0.0	(0.0, 56.1)
	NPA	102/102	100.0	(96.4, 100.0)
	OPA	102/105	97.1	(91.9, 99.0)

Note: 95% CIs were calculated using the Wilson Score method. n.e.= not estimable.

Table 46. Evaluation of MMR protein and DNA Sequencing Results at the Individual MMR Protein Level – ENRICHMENT STUDY SET.

A) Comparison of Individual MMR Protein Status and DNA Sequencing Results.

IHC Results		DNA Sequencing Results		
		Pathogenic Mutation	No Pathogenic Mutation	Total
MLH1 Loss	BRAF V600E +	0	1	1
	BRAF V600E -	1	0	1
MLH1 Intact		0	11	11
Total		1	12	13
PMS2 Loss	BRAF V600E +	0	1	1
	BRAF V600E -	3	2	5
PMS2 Intact		0	7	7
Total		3	10	13
MSH2 Loss		2	2	4
MSH2 Intact		0	9	9
Total		2	11	13
MSH6 Loss		4	0	4
MSH6 Intact		1	8	9
Total		5	8	13

B) Agreement of Individual MMR Protein Status and DNA Sequencing Results.

Agreement				
Protein	Type	n/N	%	95% CI
MLH1	PPA	1/1	100.0	(20.7, 100.0)
	NPA	12/12	100.0	(75.8, 100.0)
	OPA	13/13	100.0	(77.2, 100.0)
PMS2	PPA	3/3	100.0	(43.9, 100.0)
	NPA	8/10	80.0	(49.0, 94.3)
	OPA	11/13	84.6	(57.8, 95.7)
MSH2	PPA	2/2	100.0	(34.2, 100.0)
	NPA	9/11	81.8	(52.3, 94.9)
	OPA	11/13	84.6	(57.8, 95.7)
MSH6	PPA	4/5	80.0	(37.6, 96.4)

Agreement				
Protein	Type	n/N	%	95% CI
	NPA	8/8	100.0	(67.6, 100.0)
	OPA	12/13	92.3	(66.7, 98.6)

Note: 95% CIs were calculated using the Wilson Score method.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

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