SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Immunohistochemistry test, DNA mismatch repair (MMR) Protein assay
Device Trade Name:	VENTANA MMR RxDx Panel
Device Procode	QNH
Applicant's Name and Address:	Ventana Medical Systems, Inc. 1910 East Innovation Park Drive Tucson, AZ 85755
Date of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P200019
Date of FDA Notice of Approval:	April 22, 2021

II. INDICATIONS FOR USE

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) endometrial carcinoma tissue 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 JEMPERLI (dostarlimab-gxly) as listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1. VENTANA MMR RxDx Panel companion diagnostic indications.

Indication for use	Therapy	MMR Status
Endometrial Carcinoma (EC)	JEMPERLI (dostarlimab-gxly)	deficient MMR (dMMR)

Test results of this 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.

III. CONTRAINDICATIONS

There are no known contraindications associated with these tests.

IV. WARNINGS AND PRECAUTIONS

Warnings and precautions can be found in the anti-MLH1 (M1) Mouse Monoclonal Primary Antibody, 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 product labeling.

V. DEVICE DESCRIPTION

A. Device Kit Components

The VENTANA MMR RxDx Panel is comprised of four primary antibodies used to detect the MMR proteins MLH1, PMS2, MSH2 and MSH6 in endometrial tissue specimens. The primary antibodies are used in combination with individually optimized detection reagents and in conjunction with ancillary reagents in order to complete specimen testing. The VENTANA MMR RxDx Panel is optimized to run on the VENTANA BenchMark Ultra platform with OptiView DAB detection kit or in the case of PSM2 antibody the OptiView DAB detection Kit with the OptiView Amplification Kit. The presence or absence of target proteins is determined by visual examination of the specimen slide under light microscope by a qualified pathologist.

The VENTANA MMR RxDx Panel antibodies are packaged as individual products in single ready to use reagent dispensers. The VENTANA MMR RxDx Panel uses four separate endometrial tissue slides that are stained on the BenchMark ULTRA instrument. The primary antibody reagents are listed below.

1. VENTANA anti-MLH1 (M1) Mouse Monoclonal Primary Antibody

The anti-MLH1 (M1) antibody is a mouse monoclonal antibody produced against a full-length recombinant MLH1 protein with a glutathione-S-transferase tag. The antibody binds to the MLH1 protein in FFPE tissue sections, where it can be localized using the OptiView DAB IHC Detection Kit. Antibody concentration is ~1 µg/mL.

2. VENTANA anti-PMS2 (A16-4) Mouse Monoclonal Primary Antibody

The VENTANA anti-PMS2 (A16-4) antibody is a mouse monoclonal antibody raised against a recombinant PMS2 protein. The antibody binds to PMS2 protein

in FFPE tissue sections, where it can be localized using the OptiView DAB IHC Detection Kit and OptiView Amplification Kit. Antibody concentration is ~1 µg/mL.

3. VENTANA anti-MSH2 (G219-1129) Mouse Monoclonal Primary Antibody

The anti-MSH2 (G219-1129) antibody is a mouse monoclonal antibody produced against a recombinant human MSH2 protein. The antibody binds to the MSH2 protein in FFPE tissue sections, where it can be localized using the OptiView DAB IHC Detection Kit. Antibody concentration is ~ 20 µg/mL.

4. VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody

The VENTANA anti-MSH6 (SP93) antibody is a rabbit monoclonal antibody raised against a recombinant MSH6 protein. It binds to MSH6 protein in FFPE tissue sections, where it can be localized using the OptiView DAB IHC Detection Kit. Antibody concentration is ~1 µg/mL.

Detection and ancillary reagents required but not provided with VENTANA MMR RxDx Panel are listed below:

- OptiView DAB IHC detection Kit containing the following components
  - OptiView peroxidase Inhibitor
  - OptiView HQ universal Linker
  - OptiView HRP Multimer
  - OptiView H_2O_2
  - OptiView DAB
  - OptiView Copper
- OptiView Amplification kit
  - OptiView Amplification (0.003% HQ conjugated tyramide complex)
  - OptiView H_2O_2
  - OptiView Multimer
- Hematoxylin II
- Bluing Reagent
- Reaction Buffer (10x)
- EZ Prep Reagent (10x)
- ULTRA Cell Conditioning (CC1) (Pre-dilute)
- ULTRA Liquid Cover Slip (LCS) (Pre-dilute)
- Negative reagent control mouse monoclonal antibody
- Negative reagent control rabbit monoclonal antibody

B. Device Instrument and Software

The VENTANA MMR RxDx Panel test is fully automated. The VENTANA MMR RxDx panel antibodies are for use on the BenchMark ULTRA instrument using Ventana System Software (VSS) software version 12.5.4 or earlier.

C. Specimen Preparation

Routinely processed, formalin-fixed, paraffin-embedded (FFPE) tissues are suitable for use with the MMR primary antibodies when used with VENTANA OptiView DAB detection kit (with OptiView Amplification Kit for PMS2) and BenchMark ULTRA instruments. Tissue should be fixed in 10% neutral buffered formalin (NBF) for at least 6 hours and for a maximum of 72 hours at room temperature (15-25 °C). Fixation times of less than 6 hours may result in a loss of staining for the MMR antibodies. The amount of NBF used should be 15 to 20 times the volume of tissue. Fixatives such as alcohol-formalin-acetic acid (AFA), PREFER fixative, or other alcohol-containing fixatives are not recommended for use with this assay.

Sections should be cut 4μm thick and mounted on positively charged glass slides. Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time.

D. Test Controls

Run controls should be included in each staining run to establish the validity of the test results. The following controls should be run with the assay:
Pre-qualified endometrial cancer (EC) tissue with an MMR status of intact may be used as a positive system-level control for MMR antibodies to detect the intact protein. Alternatively, pre-qualified normal endometrial tissue fixed and processed in the same manner as the patient tissue can also be used as a positive system-level control. Normal endometrium will stain positive for all antibodies in the MMR IHC Panel. Since the MSH6, PMS2, MSH2 and MLH1 proteins are expressed in all tissues, a normal negative tissue control does not exist for these biomarkers. For a negative system level control, EC tissue with loss of an MMR protein can be used as an appropriate tissue control for mismatch repair protein deficiency status. However, lymphocytes, fibroblast and epithelial cells should exhibit staining and serve as positive internal control cells in EC samples with MMR protein deficiency (dMMR).

1. Internal Positive Controls

Normal tissue elements (e.g. lymphocytes, fibroblasts, or normal epithelium) in the immediate vicinity of the tumor may 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.

2. Negative Reagent Control

Negative reagent control should be used to stain an adjacent section of the patient specimen tissue on a separate slide from the VENTANA MMR antibody stained slides. A negative reagent control mouse monoclonal antibody (for MLH1, PMS2, and MSH2) and a negative reagent control rabbit monoclonal antibody (for MSH6) is recommended for use in place of the primary antibodies to evaluate nonspecific staining.

Additional information about positive and negative controls are available in the product labeling.

E. Principles of Procedure

The VENTANA MMR RxDx Panel is an immunohistochemistry test system used to stain FFPE EC specimens to detect expression of the MMR proteins (MLH1, PMS2, MSH2 and MSH6). The 4 antibodies of the VENTANA MMR RxDx Panel have individualized staining protocols that are created using available staining parameters provided in staining procedures in the VSS software that drives the BenchMark ULTRA automated staining platform. The panel test is run individually on 4 separate tissue sections and the test process involves sequential application of specific primary antibodies against the panel protein, followed by detection reagents and chromogen deposition for visualization of the target protein expression.

The VENTANA MMR RxDx Panel is automated for use on the BenchMark ULTRA automated slide stainer from deparaffinization through counterstaining. Patient FFPE tissue specimens are cut 4µm thick and mounted on positively charged glass slides. These slides are loaded into the Benchmark ULTRA instrument. This system first removes the paraffin wax from the tissue, and then subjects the tissue to heated antigen retrieval (cell conditioning). Antigen retrieval is the process by which the ability of antibodies to bind to the epitopes is restored to formalin-fixed tissues.

Endogenous peroxidases that could potentially react with the horseradish peroxidase conjugates (HRP) are blocked with OptiView Inhibitor (3% H2O2). After the endogenous peroxidase block, the Antibody is dispensed during the antibody incubation step and allowed to bind to its antigen. The slides are then incubated with the reagents in the OptiView DAB IHC Detection Kit and OptiView Amplification Kit to achieve preferred staining of target cells. The OptiView DAB IHC Detection Kit is an indirect, biotin-free system for detecting mouse IgG, mouse IgM, and rabbit primary antibodies which produces a visible dark brown precipitate (3,3'-Diaminobenzidine) via an HRP enzymatic reaction at the antigen site. The PMS2 test uses the OptiView amplification in addition of to the OptiView DAB detection system for signal amplification. The OptiView Amplification Kit includes an HQ hapten conjugate (OptiView Amplifier), corresponding substrate (OptiView Amplification H2O2), and mouse anti-HQ monoclonal antibody containing HRP (OptiView Amplification Multimer). Tissues are then counterstained blue using Hematoxylin II and Bluing Reagent to create brown/blue contrast to aid the pathologist when reviewing the slides using bright field microscopy.

Table 1. VENTANA MMR RxDx Panel Staining Protocol

Protocol Parameter	MLH1	PMS2	MSH2	MSH6
Deparaffinization	Selected	Selected	Selected	Selected
Cell Conditioning	Cell Conditioning 1, 64 minutes, 100°C	Cell Conditioning 1, 92 minutes, 100°C	Cell Conditioning 1, 40 minutes, 100°C	Cell Conditioning 1, 48 minutes, 100°C
Pre-primary antibody peroxidase	Selected	Selected	Selected	Selected
Antibody Incubation or Negative Reagent Control	24 minutes, 36°C	32 minutes, 36°C	12 minutes, 36°C	16 minutes, 36°C

Protocol Parameter	MLH1	PMS2	MSH2	MSH6
OptiView HQ Linker	8 minutes (default)	8 minutes (default)	8 minutes (default)	8 minutes (default)
OptiView HRP Multimer	8 minutes (default)	8 minutes (default)	8 minutes (default)	8 minutes (default)
OptiView Amplification	Not Selected	Selected	Not Selected	Not Selected
Amplifier and Amplification H_2O_2	Not Selected	4 minutes	Not Selected	Not Selected
Amplification Multimer	Not Selected	4 minutes	Not Selected	Not Selected
Hematoxylin II	4 minutes	4 minutes	4 minutes	4 minutes
Bluing Reagent	4 minutes	4 minutes	4 minutes	4 minutes

F. Staining Interpretation

Stained slides are interpreted by a qualified pathologist and the MMR status (intact or loss) for each of the MMR proteins (MLH1, PMS2, MSH2 and MSH6) is assigned based on the presence or absence of specific nuclear staining in the tumor. An MMR 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). An MMR 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.

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.

MMR Intact (or proficient) Status: Detection of all four proteins (MLH1, PMS2, MSH2 and MSH6) in the tumor.

MMR Loss (or deficient) Status: Loss of expression of at least one protein (MLH1, PMS2, MSH2 and MSH6) in the tumor.

Interpretation of Challenging Cases: The VENTANA MMR RxDx Panel scoring algorithm for each of the four MMR antibodies is binary: either the protein is expressed (a clinical status of Intact) or it is not expressed (a clinical status of Loss). Once each of the four antibodies is interpreted, the case may be assigned an overall status of proficient (all four antibodies are intact) or deficient (at least one antibody is a loss). As a result, there are no true "borderline" cases. While the vast majority of cases stained with VENTANA MMR RxDx Panel are clearly proficient or deficient in their staining results, a few cases have been observed that present a challenge in interpretation. These cases may be challenging due to the following issues: Non-specific background staining, focal staining, punctate staining, speckling, and tissue or staining artifacts resulting from sample processing and microtomy processes.

Interpretation for MMR proteins status is detailed in Table 2 below.

Table 2. Protein Expression in Endometrial Carcinoma (EC) Tissue Stained with the VENTANA MMR RxDx Panel

Clinical Status	Description
Intact MMR Protein Expression	Unequivocal nuclear staining in viable tumor cells, in the presence of acceptable internal positive controls (e.g. nuclear staining in lymphocytes, fibroblasts, or normal epithelium in the vicinity of the tumor)
Loss of MMR Protein 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.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Other devices utilizing immunohistochemistry, next generation sequencing, or fluorescent *in situ* hybridization for detection of MMR deficiency are commercially available.

VII. MARKETING HISTORY

VENTANA MMR RxDx Panel is currently marketed globally in several countries, including in the United States as a Class II device for the identification of Lynch syndrome (DEN170030). The device in the US and ex-US products contain the same reagents. The device has not been withdrawn to date from the market in any country for reasons relating to safety and effectiveness of the device.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

As with any IVD test, the potential risks are associated with an incorrect test result or incorrect interpretation of results, rather than with the device itself. Failure of the device to perform as expected or failure to correctly interpret results may lead to improper patient management decisions.

IX. SUMMARY OF NONCLINICAL STUDIES

Nonclinical studies were performed using the VENTANA MMR RxDx Panel to support the analytical performance of the device for the EC indication. These studies were performed using EC specimens (as well as a variety of other tumor tissue for certain studies). Since the prevalence of EC with dMMR status is low, some studies were supplemented with proficient and deficient cases as needed, from a variety of additional tumor types. Studies were conducted to characterize the assay, demonstrate the impact of pre-analytical variables on assay performance, verify precision and robustness of the assay, and establish assay stability.

A. Laboratory Studies

1. Analytical Sensitivity

Prevalence of the absence of staining (loss) in individual MMR antibodies were assessed on 227 EC specimens. Prevalence of MMR loss cases (MMR Panel status), as well as VENTANA MMR RxDx Panel failure rates (Not Evaluable in the below Table) within EC tissue samples were also assessed. The results of this assessment for the overall MMR Panel status is shown in the last row of Table 3 below.

Table 3. Prevalence of individual MMR antibodies

Antibody	Assessed	Evaluable	Not Evaluable	Loss Cases	Prevalence % (95% CI)	Failure Rate % (95% CI)
MLH1	227	227	0	35	15.4 (11.3, 20.7)	0.0 (0.0, 1.7)
PMS2	227	211	16	37	17.5 (13.0, 23.2)	7.0 (4.4, 11.1)
MSH2	227	220	7	6	2.7 (1.3, 5.8)	3.1 (1.5, 6.2)
MSH6	227	226	1	17	7.5 (4.7, 11.7)	0.4 (0.1, 2.5)
Panel	227	208	19	50	24.0 (18.7, 30.3)	8.4 (5.4, 12.7)

2. Analytical Specificity

a. Western Blot

Western blots analyses were conducted to demonstrate that the antibodies specifically detect the proteins of predicted molecular weight for each of the 4 VENTANA MMR RxDx Panel antibodies using cell lines with known MMR loss or intact status. Cell lines used in the study were matched pair of human cell line HD PAR595 that expressed wild type MMR proteins or were engineered with frame shift knockouts for PMS2 and MLH1 and complete knockout for MSH6 and MSH2. Western Blots confirmed presence of reactive bands at expected molecular weighs for each of the 5 panel markers.

IHC tests using the same cell lines formalin-fixed, paraffin-embedded conducted to assess nonspecific binding in the context of use. The results of the IHC with engineered cell lines was consistent with expected reactivity.

The combined results from western blots and cell line IHC demonstrated specific antibody reactivity for each of the 4 markers included in the VENTANA MMR RxDx Panel.

b. Peptide Inhibition

The objective of this study was to confirm that the VENTANA MMR RxDx Panel antibodies bind their epitopes within the corresponding protein biomarker. The presence of excess peptide containing the specific epitope will inhibit the antibody from binding to the specific epitope within the corresponding protein biomarker. This information contributes to the characterization of the primary antibody, confirming the antibody binding site and demonstrating the staining specificity of the antibody for the biomarker

in an immunohistochemical assay. The tissue was stained in duplicate with one lot of primary antibody co-incubated with and without varying concentrations of its corresponding peptide using OptiView detection kit chemistry (and OptiView Amplification where applicable) on the BenchMark ULTRA automated slide staining platform. Samples were scored according to stain intensity.

MLH1 peptide inhibition data: In this peptide inhibition study, complete inhibition of VENTANA anti-MLH1 (M1) antibody binding was observed in the presence of 5×10-5 to 5×10-8 M concentrations of the MLH1 peptide, and partial restoration of staining was observed at 5×10-9 M peptide. No inhibition was observed in the presence of the non-specific, unrelated PMS2 cross-react peptide at 5×10-7 M. All controls and background levels were acceptable.

PMS2 peptide inhibition data: Complete inhibition of VENTANA anti-PMS2 (A16-4) antibody binding was observed in the presence of 5×10-5 to 5×10-6 M concentrations of the PMS2-specific 19-mer peptide, partial restoration of staining was observed at 5×10-7 M peptide, and full restoration of staining at 5×10-8 M peptide. No inhibition was observed in the presence of unrelated peptide at 5×10-7 M. All controls and background levels were acceptable.

MSH2 peptide inhibition data: Similarly, complete inhibition of VENTANA anti-MSH2 (G219-1129) antibody binding was observed in the presence of 5×10-5 to 5×10-8 M concentrations of the MSH2 peptide, and partial restoration of staining was observed at 5×10-9 M peptide. No inhibition was observed in the presence of the non-specific, unrelated PMS2 cross-react peptide at 5×10-7 M. All controls and background levels were acceptable.

MSH6 peptide inhibition data: In this assay the 19mer MSH6 peptide successfully inhibited the VENTANA anti-MSH6 (SP93) antibody by showing complete loss of specific staining at high peptide concentrations (5×10-6, 5×10-7, and 5×10-8 M). Partial recovery of staining was observed when the peptide concentration decreased to 5×10-9 M. These results demonstrate that the epitope for the antibody is contained within the 19mer MSH6 peptide. All controls and background levels were acceptable.

c. Immunoreactivity in Human Tissues

The purpose of this study was to assess the analytical specificity of VENTANA MMR RxDx Panel on non-neoplastic and neoplastic tissue samples performed in accordance with the FDA's "Guidance for Submission of Immunohistochemistry Applications to the FDA" recommendation, including non-specific staining, background, cross-reactivity and biomarker prevalence. No unexpected staining was observed with VENTANA MMR RxDx Panel on the normal and neoplastic tissues. As expected, since mismatch repair is present in all actively proliferating cells, most normal and neoplastic tissues demonstrated positive staining (and acceptable levels of nonspecific staining that did not interfere with sample interpretation). The results are presented in Tables 4 and 5 below.

Table 4. Specificity of VENTANA MMR RxDx Panel in FFPE normal tissues

Tissue	MLH1 # Positive / Total Cases	PMS2 # Positive / Total Cases	MSH2 # Positive / Total Cases	MSH6 # Positive / Total Cases
Adrenal gland	3/3	3/3	3/3	3/3
Bladder	3/3	3/3	3/3	3/3
Bone marrow	3/3	3/3	3/3	3/3
Ovary	5/5	4/4	5/5	5/5
Breast	3/3	3/3	3/3	3/3
Cerebellum	3/3	3/3	3/3	3/3
Cerebrum	3/3	3/3	3/3	3/3
Cervix	3/3	3/3	3/3	3/3
Colon	3/3	3/3	3/3	3/3
Endometrium	3/3	3/3	3/3	2/3
Esophagus	3/3	3/3	3/3	3/3
Heart	3/3	2/3	1/3	3/3
Hypophysis (Pituitary)	3/3	3/3	3/3	3/3
Intestine	3/3	3/3	3/3	3/3
Kidney	3/3	3/3	3/3	3/3
Liver	3/3	3/3	3/3	3/3
Lung	4/4	3/3	3/3	4/4
Lymph node	3/3	3/3	3/3	3/3
Mesothelium	4/4	2/3	3/3	3/3
Pancreas	3/3	3/3	3/3	3/3
Parathyroid gland	5/5	3/3	3/3	3/3
Peripheral nerve	5/5	4/4	5/5	5/5
Prostrate	3/3	3/3	3/3	3/3
Skeletal muscle	3/3	2/3	3/3	3/3
Skin	3/3	3/3	3/3	3/3
Spleen	3/3	3/3	3/3	3/3
Stomach	3/3	3/3	3/3	3/3
Testis	3/3	3/3	3/3	3/3
Thymus	3/3	3/3	3/3	3/3
Thyroid	4/4	4/4	3/3	4/4
Tongue/Salivary gland	3/3	3/3	2/3	3/3
Tonsil	3/3	3/3	3/3	3/3

Note: Mismatch repair proteins such as MLH1, PMS2, MSH6, and MSH2 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 5. Specificity of VENTANA MMR RxDx Panel in FFPE neoplastic tissues

Tissue	MLH1 # Positive / Total Cases	PMS2 # Positive / Total Cases	MSH2 # Positive / Total Cases	MSH6 # Positive / Total Cases
Glioblastoma (Cerebrum)	1/1	1/1	1/1	1/1
Meningioma (Cerebrum)	1/1	0/0	1/1	1/1
Ependymoma (Cerebrum)	1/1	1/1	1/1	1/1
Oligodendroglioma (Cerebrum)	1/1	1/1	1/1	1/1
Serous adenocarcinoma (Ovary)	1/1	1/1	1/1	1/1
Adenocarcinoma (Ovary)	1/1	1/1	1/1	1/1
Pancreatic neuroendocrine neoplasm (Pancreas)	1/1	1/1	1/1	1/1
Adenocarcinoma (Pancreas)	0/0	0/0	1/1	0/0
Seminoma (Testis)	1/1	2/2	2/2	2/2
Medullary carcinoma (Thyroid)	1/1	1/1	1/1	1/1
Papillary carcinoma (Thyroid)	1/1	1/1	1/1	1/1
Ductal carcinoma in situ (Breast)	1/1	1/1	1/1	1/1
Microinvasive ductal carcinoma (Breast)	1/1	1/1	1/1	1/1
Invasive ductal carcinoma (Breast)	1/1	1/1	1/1	1/1
B-cell lymphoma, NOS (spleen)	0/0	0/0	1/1	1/1
Small cell carcinoma (Lung)	1/1	1/1	1/1	1/1
Squamous cell carcinoma (Lung)	1/1	1/1	1/1	1/1
Neuroendocrine carcinoma (Esophagus)	1/1	1/1	1/1	1/1
Adenocarcinoma (Lung)	0/0	0/0	1/1	1/1
Adenocarcinoma (Esophagus)	1/1	0/0	1/1	1/1
Signet ring carcinoma (Stomach)	1/1	1/1	1/1	1/1
Adenocarcinoma (Small intestine)	1/1	1/1	1/1	1/1
Stromal sarcoma (Small intestine)	1/1	1/1	1/1	1/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	1/1	0/0	1/1	0/0
Adenocarcinoma (Colon)	1/1	1/1	1/1	1/1
Adenocarcinoma (Rectum)	1/1	1/1	1/1	1/1
Gastrointestinal stromal tumor (GIST) (Rectum)	1/1	1/1	1/1	1/1
Hepatoblastoma (Liver)	1/1	1/1	1/1	1/1
Hepatocellular carcinoma (Liver)	0/0	0/0	0/0	1/1
Clear cell carcinoma (Kidney)	1/1	1/1	1/1	1/1
Adenocarcinoma (Prostate)	2/2	1/1	2/2	2/2
Leiomyoma (Uterus)	1/1	0/0	1/1	0/0
Adenocarcinoma (Uterus)	1/1	0/0	1/1	1/1

Tissue	MLH1 # Positive / Total Cases	PMS2 # Positive / Total Cases	MSH2 # Positive / Total Cases	MSH6 # Positive / Total Cases
Squamous cell carcinoma (Cervix)	2/2	1/1	2/2	2/2
Clear cell carcinoma (Endometrium)	0/0	0/0	0/0	1/1
Embryonal rhabdomyosarcoma (Striated muscle)	1/1	1/1	1/1	1/1
Squamous cell carcinoma (Skin)	1/1	1/1	1/1	1/1
Neurofibroma (Lumbar)	1/1	0/0	0/0	0/0
Neuroblastoma (Retroperitoneum)	1/1	1/1	1/1	1/1
Mesothelioma (Peritoneum)	1/1	1/1	1/1	1/1
B-cell lymphoma; NOS (Lymph node)	3/3	2/2	2/2	2/2
Hodgkin's lymphoma (Lymph node)	1/1	1/1	1/1	1/1
Leiomyosarcoma (Bladder)	1/1	1/1	1/1	1/1
Osteosarcoma	1/1	1/1	1/1	1/1
Spindle cell rhabdomyosarcoma (Peritoneum)	1/1	0/0	0/0	1/1
Leiomyosarcoma (Smooth muscle)	1/1	1/1	1/1	1/1

Note: Mismatch repair proteins 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).

3. Robustness

a. Tissue Thickness

Staining performance of the VENTANA MMR RxDx Panel was assessed on FFPE solid tumor tissue sections including EC tissues at the standard tissue thickness of 4 µm. These results show that a tissue thickness of 4 µm is suitable for consistent VENTANA MMR RxDx Panel antibody staining performance, however, no other tissue thicknesses have been validated.

b. Protocol Limitations and Failure Modes

The purpose of this study was to identify protocol conditions that might lead to a potential false positive, false negative, or unacceptable result and prevent these conditions from affecting the end user. Tumor Stain Intensity, Internal Control Stain Intensity, Background Staining, and Antibody Status were used to evaluate the impact of varied protocol conditions on solid tumor tissue. The conditions tested were; primary antibody incubation time and concentration, cell conditioning duration, counterstain times, critical dispense failure modes, and HRP Multimer and HQ Linker incubation times. Since the prevalence of EC with dMMR status is low, the protocol limitations and failure modes studies were supplemented with a variety of additional tumor types.

The sample distribution was as follows:

Four EC samples (2 intact and 2 loss) for each biomarker of the MMR panel.

MLHI: 9 (5 intact and 4 loss); ovary (2 cases), stomach (1 case), bladder (2 cases).

PMS2:10 (5 intact and 5 loss); gastric (2 case), ovary (2 case) and bladder (2 case), and. intact cases included a challenging bladder case.

MSH2: 10 (5 intact and 5 loss); ovary (2 cases), stomach (2 cases), bladder (1 case), ureter (1 case).

MSH6: 9 (5 intact and 4 loss); ovary (1 case), ureter (1 case), gastric (2 cases), renal pelvis (1 case).

Results from failure modes tested for each antibody of the VENTANA MMR RxDx Panel are summarized in Tables 6-9. Standard staining protocol conditions for the VENTANA MMR RxDx Panel antibodies are provided in Table 1 (refer to sub-section E (Principles of Procedure) under Section V (Device Description) above.

Table 6. MLH1 Protocol Limitations and Failure Modes

Protocol Limitations and Failure Modes for the VENTANA anti-MLH1 (M1) Primary Antibody	
Protocol Step	**Results**
Cell Conditioning (CC1)	Standard Antigen Retrieval: 64 min. Antigen retrieval duration (40 min CC1 and 80 min CC1) resulted in no change of MLH1 status. No protocol limitation required.
Primary Antibody	Standard anti-MLH1 Antibody Incubation: 24 min. Primary antibody Incubation (4 min and 32 min) resulted in no change of MLH1 status. No protocol limitation required. Ab Titer: 1/4x of standard resulted in greater than 1.0 pt. difference in the stain intensity as compared to the reference. This has been identified as a potential failure mode.
OptiView HQ Linker	No reagent on slide (no dispense) resulted in unevaluable MLH1 status. This has been identified as a potential failure mode.
OptiView Multimer	No reagent on slide (no dispense) resulted in unevaluable MLH1 status. This has been identified as a potential failure mode.
Counterstain: Hematoxylin II	Standard Counterstain: 4 min Counterstain duration (32 min) resulted in no change of MLH1 status. No protocol limitation required.
Post Counterstain: Bluing Reagent	Standard Post Counterstain: 4 min Post Counterstain duration (32 min) resulted in no change of MLH1 status. No protocol limitation required.

Table 7. PMS2 Protocol Limitations and Failure Modes

Protocol Limitations and Failure Modes for the VENTANA anti-PMS2 (A16-4) Primary Antibody	
Protocol Step	**Results**
Cell Conditioning (CC1)	Standard Antigen Retrieval: 92 min. Antigen retrieval duration (56 min CC1 and 104 min CC1) resulted in no change of PMS2 status. No protocol limitation required.
Primary Antibody	Standard PMS2 Antibody Incubation: 32 min. 1) Ab incubation duration (8 min) resulted in unevaluable PMS2 status. This has been identified as a potential protocol limitation. 2) Ab Titer: 0.25x and 0.5x the optimal titer resulted in greater than 1.0 pt. difference in the stain intensity as compared to the reference but there was no change in status. 1.5x and 2x the optimal titer resulted in unacceptable high background. This has been identified as a potential failure mode.
OptiView HQ Linker	No reagent on slide (no dispense) resulted in unevaluable PMS2 status. This has been identified as a potential failure mode.
OptiView HRP Multimer	No reagent on slide (no dispense) resulted in unevaluable PMS2 status. This has been identified as a potential failure mode.
OptiView Amplification	No reagent on slide (no dispense) resulted in either unevaluable PMS2 status or greater than 1.0 pt. difference in the stain intensity as compared to the reference without change in status. This has been identified as a potential failure mode.
Counterstain: Hematoxylin II	Standard Counterstain: 4 min. Counterstain duration (32 min) resulted in no change of PMS2 status. No protocol limitation required.
Post Counterstain: Bluing Reagent	Standard Post Counterstain: 4 min. Post Counterstain duration (32 min) resulted in no change of PMS2 status. No protocol limitation required.

Table 8. MSH2 Protocol Limitations and Failure Modes

Protocol Limitations and Failure Modes for the VENTANA anti-MSH2 (G219-1129) Primary Antibody	
Protocol Step	**Results**
Cell Conditioning (CC1)	Standard Antigen Retrieval: 40 min. Antigen retrieval duration (24 min CC1 and 72 min CC1) resulted in unevaluable MSH2 status. These conditions have been identified as potential protocol limitations.
Primary Antibody	Standard Primary Antibody Incubation: 12 min. Ab incubation duration (4 min) resulted in unevaluable MSH2 status. This condition has been identified as a potential protocol limitation. Ab Titer: 1/4x, 1.5x, 2x of standard resulted in unevaluable MSH2 status. This condition has been identified as a potential failure mode.

Protocol Limitations and Failure Modes for the VENTANA anti-MSH2 (G219-1129) Primary Antibody	
OptiView HQ Linker	No reagent on slide (no dispense) resulted in unevaluable MSH2 status. This condition has been identified as a potential failure mode.
OptiView Multimer	No reagent on slide (no dispense) resulted in unevaluable MSH2 status. This condition has been identified as a potential failure mode.
Counterstain: Hematoxylin II	Standard Counterstain: 4 min. Counterstain duration (32 min) resulted in no change of MSH2 status. No protocol limitation required.
Post Counterstain: Bluing Reagent	Standard Post Counterstain: 4 min. Post Counterstain duration (32 min) resulted in no change of MSH2 status. No protocol limitation required.

Table 9. MSH6 Protocol Limitations and Failure Modes

Protocol Limitations and Failure Modes for the VENTANA anti-MSH6 (SP93) Rabbit Monoclonal Primary Antibody	
Protocol Step	**Results**
Cell Conditioning (CC1)	Standard Antigen Retrieval: 48 min. Antigen retrieval duration (32 min and 96 min) resulted in no change of MSH6 status. No protocol limitation required.
Primary Antibody	Standard Primary Antibody Incubation: 16 min. Antibody Incubation duration (32 min) resulted in no change of status. No protocol limitation required. Antibody Incubation duration (4 min) resulted in a greater than 1.0 pt. stain intensity for one case (VT0000237595) when compared to the reference slide but there was no change in MSH6 status. This has been identified as a potential protocol limitation. Ab Titer of 2x that of the optimal titer resulted in unacceptable background and non-evaluable internal controls for one case when compared to the reference slide and there was also a change in MSH6 status. This has been identified as a potential failure mode.
OptiView HQ Linker	No reagent on slide (no dispense) resulted in change of MSH6 status. This has been identified as a potential failure mode.
OptiView Multimer	No reagent on slide (no dispense) resulted in change of MSH6 status. This has been identified as a potential failure mode.
Counterstain: Hematoxylin II	Standard Counterstain: 4 min. Counterstain duration of 32 min resulted in no change of MSH6 status. No protocol limitation required.
Post Counterstain: Bluing Reagent	Standard Post Counterstain: 4 min. Post Counterstain duration of 32 min resulted in no change of MSH6 status. No protocol limitation required.

Results from Failure Modes tested for each Antibody of the VENTANA MMR RxDx Panel (primary antibody titer and failed reagent dispenses for critical components) did result in failures and the internal controls were able to accurately identify the failures. A higher than optimal concentration of antibody could lead to a false status change (loss to intact). Varied protocol conditions (primary antibody incubation time, cell conditioning duration, counterstain time and HRP Multimer and HQ Linker incubation times) do not adversely affect the ability to properly interpret and determine loss or intact status.

4. Precision

The purpose of this study was to evaluate precision of the VENTANA MMR RxDx Panel. For each biomarker of the MMR panel, the intermediate precision studies used 6 cases of EC with a balanced loss/intact status distribution. Since the prevalence of EC with dMMR status is low, the intermediate precision studies were supplemented with a variety of additional tumor types.

The sample distribution was as follows:

Six EC samples (3 intact and 3 loss) for each biomarker of the MMR panel.

MLH1: 27 (15 intact and 12 loss): urinary (6 cases), reproductive (9 cases), gastrointestinal (6 cases), hepato-pancreatobiliary (3 cases) soft tissue (1 case), and thoracic (2 cases).

PMS2: 26 (14 intact and 12 loss): urinary (3 cases), reproductive (9 cases), gastrointestinal (7 cases), endocrine (2), hepato-pancreatobiliary (1 case), soft tissue (2 cases), and thoracic (2 cases).

MSH2: 27 (15 loss and 12 intact loss): urinary (6 cases), reproductive (12 cases), gastrointestinal (4 cases), hepato-pancreatobiliary (1 case), soft tissue (2 cases), and thoracic (2 cases).

MSH6: 28 (15 intact and 13 loss): urinary (4 cases), reproductive (11 cases), gastrointestinal (2 cases), endocrine (3 cases), hepato-pancreatobiliary (2 cases) soft tissue (4 cases), and thoracic (2 cases).

Total number of observations for each test condition is depicted in Tables 10-14 below.

The following parameters were tested:

- Three lots of antibody (between-antibody lots)
- Three lots of OptiView DAB IHC Detection Kits (between-detection kits) and OptiView Amplification (where applicable)
- Three ULTRA instruments (between-instruments)
- Across 3 days (between-day)
- Across all intermediate precision conditions (within-run).

Each case was assigned one mode based on the samples aggregated per test condition for:

- Between-antibody lots
- Between-detection kit lots
- Between-instruments
- Between-day

Each case was compared within its duplicate samples per test run for:

- Within-run

Analyses included evaluation of overall percent agreement (OPA), positive percent agreement (PPA) for Loss cases, and negative percent agreement (NPA) for Intact cases between antibody lot, between detection kit lot, between day, between instrument and within run. Table a is analysis for EC tissue. However, since the prevalence of each of the biomarkers of the dMMR panel is low for EC, the study was supplemented with samples from other tissue types. Therefore, Table b for all test conditions below is analyses of other tissues including EC.

Tables 10 (a and b). Precision, Between-antibody lots

Table 10a. Between-Antibody Lots - EC Tissue								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)
Intact	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Loss	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Table 10b. Between-Antibody Lots - Variety of Tumor Tissues Including EC								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	100.0 (162/162)	(97.7, 100.0)	100.0 (156/156)	(97.6, 100.0)	100.0 (162/162)	(97.7, 100.0)	100 (168/168)	(97.8, 100.0)
Intact	100.0 (96/96)	(96.2, 100.0)	100.0 (84/84)	(95.6, 100)	100.0 (90/90)	(95.9, 100.0)	100 (90/90)	(95.9, 100.0)
Loss	100.0 (66/66)	(94.5, 100.0)	100.0 (72/72)	(94.9, 100)	100.0 (72/72)	(94.9, 100.0)	100 (78/78)	(95.3, 100.0)

Tables 11 (a and b). Precision, Between-detection kit lots

Table 11a. Between-Detection Kit Lots - EC Tissue								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)
Intact	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Loss	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Table 11b. Between-Detection Kit Lots - Variety of Tumor Tissues Including EC								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	98.8 (160/162)	(96.9, 100.0)	94.2 (147/156)	(89.7, 98.1)	100.0 (162/162)	(97.7, 100.0)	100 (168/168)	(97.8, 100.0)
Intact	99.0 (95/96)	(96.7, 100.0)	97.6 (82/84)	(92.9, 100)	100.0 (90/90)	(95.9, 100.0)	100 (90/90)	(95.9, 100.0)
Loss	98.5 (65/66)	(95.2, 100.0)	94.2 (65/72)	(83.3, 97.2)	100.0 (72/72)	(94.9, 100.0)	100 (78/78)	(95.3, 100.0)

Tables 12 (a and b). Precision, Between-instruments

Table 12a. Between-Instruments - EC Tissue								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)
Intact	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Loss	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Table 12b. Between-Instruments - Variety of Tumor Tissues Including EC								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	98.8 (160/162)	(96.9, 100.0)	100.0 (156/156)	(97.6, 100.0)	100.0 (162/162)	(97.7, 100.0)	100 (168/168)	(97.8, 100.0)
Intact	100.0 (96/96)	(96.2, 100.0)	100.0 (84/84)	(95.6, 100)	100.0 (90/90)	(95.9, 100.0)	100 (90/90)	(95.9, 100.0)
Loss	97.0 (64/66)	(92.4, 100.0)	100.0 (72/72)	(94.9, 100)	100.0 (72/72)	(94.9, 100.0)	100 (78/78)	(95.3, 100.0)

Tables 13 (a and b). Precision, Between-days

Table 13a. Between-Days - EC Tissue								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)	100.0 (36/36)	(90.4, 100.0)
Intact	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Loss	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)	100.0 (18/18)	(82.4, 100.0)
Table 13b. Between-Days - Variety of Tumor Tissues Including EC								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	99.4 (161/162)	(98.1, 100.0)	100.0 (156/156)	(97.6, 100.0)	100.0 (162/162)	(97.7, 100.0)	100 (168/168)	(97.8, 100.0)
Intact	100.0 (96/96)	(96.2, 100.0)	100.0 (84/84)	(95.6, 100)	100.0 (90/90)	(95.9, 100.0)	100 (90/90)	(95.9, 100.0)
Loss	98.5 (65/66)	(95.2, 100.0)	100.0 (72/72)	(94.9, 100)	100.0 (72/72)	(94.9, 100.0)	100 (78/78)	(95.3, 100.0)

Table 14 (a and b). Precision, Within Run

Table 14a. Within-Run - EC Tissue								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	100.0 (54/54)	(93.4, 100.0)	100.0 (54/54)	(93.4, 100.0)	100.0 (54/54)	(93.4, 100.0)	100.0 (54/54)	(93.4, 100.0)
Intact	100.0 (27/27)	(87.5, 100.0)	100.0 (27/27)	(87.5, 100.0)	100.0 (27/27)	(87.5, 100.0)	100.0 (27/27)	(87.5, 100.0)
Loss	100.0 (27/27)	(87.5, 100.0)	100.0 (27/27)	(87.5, 100.0)	100.0 (27/27)	(87.5, 100.0)	100.0 (27/27)	(87.5, 100.0)
Table 14b. Within-Run - Variety of Tumor Tissues Including EC								
Mode	**MLH1**		**PMS2**		**MSH2**		**MSH6**	
	%(n/N)	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**	**%(n/N)**	**95% CI**
Overall	98.8 (240/243)	(97.3, 100.0)	100.0 (228/228)	(98.3, 100.0)	100.0 (243/243)	(98.4, 100.0)	100.0 (252/252)	(98.5, 100.0)
Intact	99.3 (143/144)	(97.8, 100.0)	100.0 (125/125)	(97.0, 100.0)	100.0 (135/135)	(97.2, 100.0)	100.0 (135/135)	(97.2, 100.0)
Loss	98.0 (97/99)	(94.9, 100.0)	100.0 (103/103)	(96.4, 100.0)	100.0 (108/108)	(96.6, 100.0)	100.0 (117/117)	(96.8, 100.0)

Between Day Precision for VENTANA MMR RxDx Panel

The precision of the VENTANA MMR RxDx Panel on EC samples when stained across multiple days (5 non-consecutive) was also evaluated. For biomarkers of the MMR panel, the between-day precision study used 6 cases of EC for PMS2 and MSH2, and 8 cases of EC for MLH1 and MSH6 with a balanced loss/intact status distribution for each biomarker. OPA, PPA, and NPA were assessed, and the agreement results are presented in Table 15 below.

Table 15. Precision, Between Day for VENTANA MMR RxDx Panel

Overall Agreement Between Days							
Antibody	Slide-Level Marker Status	Marker Mode Status			Measure	%(n/N)	95% CI
		Loss	Intact	Total			
MLH1	Loss	40	0	40	PPA	100.0 (40/40)	(91.2, 100.0)
	Intact	0	40	40	NPA	100.0 (40/40)	(91.2, 100.0)
	Total	40	40	80	OPA	100.0 (80/80)	(95.4, 100.0)
PMS2	Loss	30	0	30	PPA	100.0 (30/30)	(88.6, 100.0)
	Intact	0	30	30	NPA	100.0 (30/30)	(88.6, 100.0)
	Total	30	30	60	OPA	100.0 (60/60)	(94.0, 100.0)
MSH2	Loss	30	0	30	PPA	100.0 (30/30)	(88.6, 100.0)
	Intact	0	30	30	NPA	100.0 (30/30)	(88.6, 100.0)
	Total	30	30	60	OPA	100.0 (60/60)	(94.0, 100.0)
MSH6	Loss	40	0	40	PPA	100.0 (40/40)	(91.2, 100.0)
	Intact	0	40	40	NPA	100.0 (40/40)	(91.2, 100.0)
	Total	40	40	80	OPA	100.0 (80/80)	(95.4, 100.0)

Staining with the VENTANA MMR RxDx Panel antibodies on EC tissue met study requirements, which stated that the primary antibody shall produce concordant staining results in at least 90% of samples.

5. Reader Precision

Between-Reader and Within-Reader precision was assessed by evaluating concordance of VENTANA MMR RxDx Panel status between 3 readers and within individual readers using 134 (72 proficient and 62 deficient) cases from a variety of tumor types including 34 (17 proficient and 17 deficient) EC cases.

The sample distribution was as follows:

Thirty-four EC samples (17 proficient and 17 deficient) for each biomarker of the MMR panel.

134 (72 proficient and 62 deficient): urinary (14 cases), reproductive (46 cases), gastrointestinal (33 cases), endocrine (7 cases), hepato-pancreatobiliary (11 cases), soft tissue/skin (9 cases), thoracic (9 cases) and other (head and neck- 5 cases)

Specimens were blinded and randomized prior to evaluation of each of the 4 individual antibody status (intact or loss) and panel-level status (proficient of deficient) for each case using the VENTANA MMR RxDx Panel scoring algorithm (Staining Interpretation Section F of the Device Description Section V, and Table 2 above). 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 16 for EC tissues and Table 17 for variety of tumor tissues including EC.

Table 16. Within-Reader and Between-Reader Precision of the VENTANA MMR RxDx Panel on EC tissues as measured by MMR (Proficient/ Deficient)

Precision	Clinical Status	Agreement			
		Type	n/N	%	95% CI
Within-Reader	Deficient	APA	100/101	99.0	(97.0 ,100.0)
	Proficient	ANA	102/103	99.0	(97.1 ,100.0)
	Total	OPA	101/102	99.0	(97.1 ,100.0)
Between-Reader	Deficient	APA	98/100	98.0	(93.8 ,100.0)
	Proficient	ANA	102/104	98.1	(94.4 ,100.0)
	Total	OPA	100/102	98.0	(94.1 ,100.0)

Note: Average Positive Agreement (ANA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

2-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples.

Table 17. Within-Reader and Between-Reader Precision of the VENTANA MMR RxDx Panel on a variety of tumor tissues including EC as measured by MMR Status (Proficient/ Deficient)

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

Note: Average Positive Agreement (ANA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

2-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples.

Table 18. Within-Reader and Between-Reader Precision of the VENTANA MMR RxDx Panel on EC tissue as measured for each marker (Intact/ Loss)

Marker	Parameter	Clinical Status	Agreement			
			Type	n/N	%	95% CI
MLH1	Within-Reader	Loss	APA	78/78	100.0	(95.3, 100.0)
		Intact	ANA	126/126	100.0	(97.0, 100.0)
		Total	OPA	102/102	100.0	(96.4, 100.0)
	Between-Reader	Loss	APA	78/78	100.0	(95.3, 100.0)
		Intact	ANA	126/126	100.0	(97.0, 100.0)
		Total	OPA	102/102	100.0	(96.4, 100.0)
PMS2	Within-Reader	Loss	APA	78/78	100.0	(95.3, 100.0)
		Intact	ANA	126/126	100.0	(97.0, 100.0)
		Total	OPA	102/102	100.0	(96.4, 100.0)
	Between-Reader	Loss	APA	78/78	100.0	(95.3, 100.0)
		Intact	ANA	126/126	100.0	(97.0, 100.0)
		Total	OPA	102/102	100.0	(96.4, 100.0)
MSH2	Within-Reader	Loss	APA	16/17	94.1	(80.0 ,100.0)
		Intact	ANA	186/187	99.5	(98.2 ,100.0)
		Total	OPA	101/102)	99.0	(97.1 ,100.0)
	Between-Reader	Loss	APA	14/16	87.5	(50.0 ,100.0)
		Intact	ANA	186/188	98.9	(96.6 ,100.0)
		Total	OPA	100/102	98.0	(94.1 ,100.0)
MSH6	Within-Reader	Loss	APA	22/23	95.7	(84.6 ,100.0)
		Intact	ANA	180/181	99.4	(98.2 ,100.0)
		Total	OPA	101/102	99.0	(97.1 ,100.0)
	Between-Reader	Loss	APA	20/22	90.9	(63.6 ,100.0)
		Intact	ANA	180/182	98.9	(96.4 ,100.0)
		Total	OPA	100/102	98.0	(94.1 ,100.0)

Note: Average Positive Agreement (ANA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

2-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples.

Table 19. Within-Reader and Between-Reader Precision of the VENTANA MMR RxDx Panel on a variety of tumor tissues including EC as measured for each marker (Intact/ Loss)

Marker	Parameter	Clinical Status	Agreement			
			Type	n/N	%	95% CI
MLH1	Within-Reader	Loss	APA	290/291	99.7	(98.9 ,100.0)
		Intact	ANA	510/511	99.8	(99.4 ,100.0)
		Total	OPA	400/401	99.8	(99.3 ,100.0)
	Between-Reader	Loss	APA	286/290	98.6	(96.4 ,100.0)
		Intact	ANA	506/510	99.2	(98.0 ,100.0)
		Total	OPA	396/400	99.0	(97.5 ,100.0)
PMS2	Within-Reader	Loss	APA	290/293	99.0	(97.7 ,100.0)
		Intact	ANA	504/507	99.4	(98.7 ,100.0)
		Total	OPA	397/400	99.3	(98.3 ,100.0)
	Between-Reader	Loss	APA	284/290	97.9	(95.3 ,100.0)
		Intact	ANA	500/506	98.8	(97.4 ,100.0)
		Total	OPA	392/398	98.5	(96.5 ,100.0)
MSH2	Within-Reader	Loss	APA	44/47	93.6	(86.7 ,100.0)
		Intact	ANA	752/755	99.6	(99.1 ,100.0)
		Total	OPA	398/401	99.3	(98.4 ,100.0)
	Between-Reader	Loss	APA	40/46	87.0	(69.2 ,100.0)
		Intact	ANA	748/754	99.2	(98.1 ,100.0)
		Total	OPA	394/400	98.5	(96.5 ,100.0)
MSH6	Within-Reader	Loss	APA	70/72	97.2	(93.3 ,100.0)
		Intact	ANA	728/730	99.7	(99.3 ,100.0)
		Total	OPA	399/401	99.5	(98.8 ,100.0)
	Between-Reader	Loss	APA	62/70	88.6	(76.9 ,97.7)
		Intact	ANA	722/730	98.9	(97.7 ,99.7)
		Total	OPA	392/400	98.0	(96.0 ,99.5)

Note: Average Positive Agreement (ANA), Average Negative Agreement (ANA), Overall Percent Agreement (OPA).

2-sided 95% confidence interval (CI) was calculated using the percentile bootstrap method from 2000 bootstrap samples.

Reader concordance of VENTANA MMR RxDx Panel staining on a variety of tumor cases met all the requirements and acceptance criteria for Within-Reader and Between-Reader precision studies.

6. External Reproducibility

The reproducibility of VENTANA MMR RxDx Panel, as used on the BenchMark ULTRA instrument with OptiView DAB detection and OptiView amplification, in determining MMR status in EC was evaluated.

Thirty archival, de-identified, FFPE EC specimens were used in the study. Fifteen of the cases were deficient for overall MMR status (dMMR) and 15 cases were proficient for overall MMR status (pMMR). Four cases considered to be challenging to interpret (refer to sub-section F (Staining Interpretation) under section V (Device Description) were included.

Multiple slides containing serial sections of the specimens were distributed to each of 3 external clinical laboratories (study sites) for staining with VENTANA MMR RxDx Panel on BenchMark ULTRA automated staining instruments. On each of 3 non-consecutive staining days at each site, site study staff stained 5 sections of each of the 30 cases, using the 4 antibody components of MMR RxDx Panel and a negative control antibody. The first and last staining days at a site were at least 20 days apart. All sites used the same investigational product lots.

Each set of 5 stained slides per case per staining day was combined with a case-matched hematoxylin and eosin (H&E)-stained slide and provided as a 6-slide panel to 2 trained, blinded pathologists (readers) at the site. Each pathologist independently interpreted each case panel for the status (Intact or Loss) of each of the 4 VENTANA MMR RxDx Panel biomarkers (MLH1, PMS2, MSH2 and MSH6) and for the overall MMR status of the case (dMMR or pMMR). A case was considered pMMR if all 4 biomarkers were Intact and was considered dMMR if any one of the four biomarkers had a Loss status.

PPA and NPA rates for MMR status (dMMR or pMMR) across all evaluable observations, using the case-level reader modes for MMR status as the reference were assessed. Study results are presented for panel-level status in Table 20, and for marker-level status in Tables 21 below.

Table 20. External Reproducibility Study for VENTANA MMR RxDx Panel

	Overall		**1. Between-Site**		**Between-Reader**	
Analysis	**% (n/N)**	**95% CI***	**% (n/N)**	**95% CI***	**% (n/N)**	**95% CI***
PPA	98.1 (263/268)	(95.5, 100.0)	98.1 (263/268)	(95.5, 100.0)	99.2 (263/265)	(98.1, 100.0)
NPA	100.0 (269/269)	(98.6, 100.0)	100.0 (269/269)	(98.6, 100.0)	100.0 (272/272)	(98.6, 100.0)
OPA	99.1 (532/537)	(97.8, 100.0)	99.1 (532/537)	(97.8, 100.0)	99.6 (535/537)	(99.1, 100.0)

* Two-sided 95% CIs for point estimates (PEs) of 100% were calculated using the Wilson score method; for other PEs, they were calculated using the percentile bootstrap method with 2000 replicates.

Table 21. External Reproducibility Study for VENTANA MMR RxDx Panel on EC tissue as measured for each marker

		Agreement with Mode [b]					
		PPA		NPA		OPA	
	Marker	% (n/N)[a]	95% CI [c]	% (n/N)	95% CI	% (n/N)	95% CI
Agreement of Reader Biomarker Status with the Case-Level Reader Modal Status	MLH1	98.8 (160/162)	(97.2, 100.0)	100.0 (377/377)	(99.0, 100.0)	99.6 (537/539)	(99.1, 100.0)
	PMS2	98.1 (159/162)	(96.3, 100.0)	100.0 (377/377)	(99.0, 100.0)	99.4 (536/539)	(98.9, 100.0)
	MSH2	96.2 (102/106)	(90.0, 100.0)	99.5 (429/431)	(98.9, 100.0)	98.9 (531/537)	(97.6, 99.8)
	MSH6	88.9 (96/108)	(78.9, 98.1)	99.3 (428/431)	(98.6, 100.0)	97.2 (524/539)	(95.2, 99.3)
Within Site Agreement of Biomarker Status with the Case-Level Mode	MLH1	98.8 (160/162)	(97.2, 100.0)	100.0 (377/377)	(99.0, 100.0)	99.6 (537/539)	(99.1, 100.0)
	PMS2	98.1 (159/162)	(96.3, 100.0)	100.0 (377/377)	(99.0, 100.0)	99.4 (536/539)	(98.9, 100.0)
	MSH2	96.2 (102/106)	(90.0, 100.0)	99.5 (429/431)	(98.9, 100.0)	98.9 (531/537)	(97.6, 99.8)
	MSH6	94.1 (96/102)	(88.2, 98.6)	99.3 (434/437)	(98.6, 100.0)	98.3 (530/539)	(97.0, 99.3)
Within-Reader Agreement of Biomarker Status with the Case-Level Mode	MLH1	98.8 (160/162)	(97.2, 100.0)	100.0 (377/377)	(99.0, 100.0)	99.6 (537/539)	(99.1, 100.0)
	PMS2	98.1 (159/162)	(96.3, 100.0)	100.0 (377/377)	(99.0, 100.0)	99.4 (536/539)	(98.9, 100.0)
	MSH2	99.0 (102/103)	(96.9, 100.0)	99.5 (432/434)	(98.9, 100.0)	99.4 (534/537)	(98.9, 100.0)
	MSH6	97.0 (96/99)	(94.4, 100.0)	99.3 (437/440)	(98.7, 100.0)	98.9 (533/539)	(98.1, 99.6)

NPA = negative percent agreement; PPA = positive percent agreement OPA = overall percent agreement
[a] Counts indicate numbers of reader observations, not numbers of unique cases.
[b] For the purpose of agreement rate calculations, a biomarker status of Loss was considered Positive and a biomarker status of Intact was considered Negative.
[c] Two-sided 95% CIs were calculated using the percentile bootstrap method with 2000 replicates, except that those for point estimates of 100% were calculated using the Wilson score method.

Results of the External Reproducibility Study for VENTANA MMR RxDx Panel showed that one of the biomarkers, MSH6, presented relatively low PPA with point estimate 88.9% and lower bound for its 95% CI as 78.9%. It was observed that the discordant observations for MSH6 (relative to a Loss mode) were largely concentrated at a single Site C (11 of 15 discordant MSH6 observations) and mostly associated with a single Reader C1 (7 of the 11 discordant Site C observations). Further, the decreased PPA for MSH6 relative to that for the other MMR biomarkers had little effect on MMR status reproducibility, as all study cases with a Loss (positive) MSH6 status also had a Loss MSH2 status, and the PPA for MSH2 status was high (PPA = 96.2%; 95% CI: 90.0, 100.0)." Based on these observations, the relatively low PPA of MSH6 may not be assay related or a major issue for the final assessment of the VENTANA MMR RxDx Panel assay results.

7. Stability Studies

a. Cut Slide Stability

The purpose of the cut slide stability study was to determine the stability of VENTANA MMR RxDx Panel antibody epitopes in FFPE EC tissues sections. Two EC tissues with

intact MMR status were sectioned and stored at two different conditions for the duration of the study (5±3ºC and 30±5ºC). Slides were stained with each of the VENTANA MMR RxDx Panel antibodies at different time points and compared to the Time 0 reference as follows: Day 0 (reference), Day 15, Day 30 and Day 45.

Based on the study results, the cut slide stability is 45 days at both the 5±3ºC and 30±5ºC storage conditions.

b. Real Time Stability

The purpose of this study was to evaluate the stability (shelf-life, in-use and shipping) of VENTANA MMR antibodies (MLH1, PMS2, MSH2 and MSH6). Stability testing evaluated staining performance on FFPE tumor tissue. Three production lots of each MMR panel antibody were subjected to the ship stress conditions and tested at specified intervals until 26 months or failure, whichever occurred first. The conditions tested were as follows:

- Ship Stress
  - Intended Storage (2°C -8°C)
  - Hot Ship Stress (30°C±5ºC 192 hours)
  - Hot Ship Stress (15°C±5ºC 192 hours)
  - Cold Ship Stress Freeze/Thaw (-20°C±5ºC 192 hours)
- On-Board Stability
- Open Vial Stability

FFPE tumor tissues were tested in triplicate at multiple time points. Based on the data that is provided the assigned reagent stability is as follows:

1. MLH1- 24 months
2. PMS2 - 12 months
3. MSH2 - 24 months
4. MSH6 - 24 months

B. Animal Studies

None

C. Additional Studies

1. Fixative Type and Time

This study evaluated the effect of fixative type, fixation time, and delay to fixation on each of the VENTANA MMR RxDx Panel antibodies using a BenchMark ULTRA instrument with OptiView DAB IHC detection (with OptiView Amplification where applicable). Tonsil tissues were fixed for 1 hour (hr), 6 hr, 12 hr, 24 hr, 48 hr, or 72 hr in each of six different fixatives: 10% neutral buffered formalin [NBF], zinc formalin, 95% alcohol, alcohol-formalin-acetic acid [AFA], Z-5, or PREFER. Additionally, tonsil tissue was initially harvested and then stored for 0 min, 30 min, 1 hr, 2 hr, 6 hr, or 24 hr prior to fixation in 10% NBF.

Based on the results of this study, fixation of tissue in 10% NBF, zinc formalin, or Z-5 for 6 to 72 hours produced acceptable staining results with the VENTANA MMR antibody assays. Tissue fixation in 95% alcohol, AFA, or PREFER resulted in weak and variable staining. These fixatives (95% alcohol, AFA, PREFER) are not recommended for use with the VENTANA MMR RxDx Panel antibody assays.

2. Tissue Heterogeneity

This study investigated the prevalence of case heterogeneity in EC tissue blocks when stained with VENTANA MMR RxDx Panel Assay on the BenchMark ULTRA instrument.

For the sample set of 44 tissues (22 pairs) evaluated, 10 of 44 EC tissues demonstrated dMMR status. All 22 pairs exhibited equivalent marker and panel level MMR status. Therefore, case heterogeneity was not observed in any of patient pairs for overall panel-level nor marker-level MMR status. Although the trend in this study indicates case heterogeneity is unlikely to be observed in EC, the small sample size of the available dMMR tissues provide a limited assessment of case heterogeneity for MMR panel status.

3. Primary versus Metastatic

This study assessed the concordance of MMR panel status between FFPE matched EC primary and metastatic tumors when stained with the MMR RxDx Panel assay on the BenchMark ULTRA instrument.

For the sample set of 20 tissue cases (10 pairs) evaluated, 4 cases exhibited an MMR status of deficient (dMMR), and 16 cases exhibited an MMR status of proficient (pMMR). All 10 pairs exhibited equivalent marker and panel level MMR status. Therefore, concordance between each matched primary vs. metastatic pair was observed with no change in individual status. Due to the difficulty of procuring matching patient samples from commercial vendors and the overall low prevalence of MMR biomarkers, the sample distribution for MMR status in this study was unequal, with only 4 of 20 cases representing a dMMR status. Although the trend in this study indicates discordance is unlikely to be observed between EC primary and their matched metastatic tumor, the small sample size of the available dMMR tissues provide a limited assessment of primary vs. metastatic concordance for MMR panel status. An OPA of 100% and the corresponding 95% Wilson Score confidence intervals of 72.2-100.0% were observed.

X. SUMMARY OF CLINICAL STUDIES

The clinical performance of VENTANA MMR RxDx Panel as a CDx device for the EC indication for dostarlimab was based on the clinical trial 4010-01-001 **(GARNET)** – Subpart 2B, Cohort A1.

A. Study Design

GARNET is a multicenter, open-label, study with Subpart 2B, designed to evaluate the antitumor activity of dostarlimab in patients with recurrent or advanced mismatch repair deficient (dMMR)/microsatellite instability-high (MSI-H) cancers, including dMMR/MSI-H EC. The Subpart 2B expansion cohorts A1 and A2 are specific for dMMR (loss or biomarker-positive) and pMMR (intact or biomarker-negative) EC, respectively.

1. Clinical Inclusion and Exclusion Criteria (Cohorts A1 and A2)

Key Trial Inclusion Criteria

1. Histologically or cytologically proven recurrent or advanced EC. All EC histologies are allowed except endometrial sarcoma (including carcinosarcoma).
2. Patients who have progressed on or after platinum doublet therapy
3. Patients have received no more than 2 lines of anti-cancer therapy for recurrent or advanced (≥Stage IIIB) disease. Prior treatment with hormone therapies is acceptable and does not count towards the number of anti-cancer therapies noted in the criterion above for this cohort.
4. Patients must submit 2 scans demonstrating increase in tumor measurement that meet criteria for PD on or after the latest systemic anticancer therapy based on RECIST 1.1 to Central Radiology prior to the first dose of dostarlimab.
5. Presence of at least 1 measurable lesion on baseline scan will be confirmed by central radiology review.
6. Status of tumor MMR status as determined by immunohistochemistry (IHC) testing.

Key Trial Exclusion Criteria

1. Patient has received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent.
2. Patient has known uncontrolled central nervous system metastases and/or carcinomatous meningitis.
3. Patient has a known additional malignancy that progressed or required active treatment within last 2 years.

2. Follow-up Schedule

Up to 2 years, or until the subject meets protocol specific discontinuation criteria

3. Clinical Endpoints

Primary endpoints:

Objective response rate (ORR) and duration of response (DOR) by independent blinded central review using Response Evaluation Criteria in Solid Tumors (RECIST) v1.1

Secondary endpoints:

Progression-free survival (PFS) based on independent blinded central review using RECIST v1.1

Immune-related overall response rate (irORR) based on Investigators' assessment using irRECIST

B. Accountability of PMA Cohort

Study participants with recurrent or advanced endometrial carcinoma in the GARNET study comprise the primary analysis population for this application. Of the 249 patients enrolled in the study, 175 subjects were in the BLA efficacy population and 74 were in the BLA non-efficacy population. 167 (118 in the BLA efficacy population and 49 in the BLA non-efficacy population) were able to be tested at the central laboratory to determine their MMR status, including 71 CDx+ and 96 CDx-, although 44 of the samples were associated with one or more deviations from the protocol-defined sample eligibility requirements for testing. Of 118 patients in BLA efficacy population retested by CDx, 53 was detected as CDx+ and 65 as CDx- (Complete cases population). After excluding the cases with protocol deviation, 42 cases were identified as CDx+ and 57 as CDx- (Complete cases IU population).

Table 22. Accountability of PMA Cohort

	Number of Study Subjects
Enrolled in GARNET Trial	249
MMR Proficient (pMMR) by clinical trial assay (CTA)	145 (BLA Cohort A2)
MMR Deficient (dMMR) by CTA	104 (BLA Cohort A1)
Tumor sample collected at baseline	185
Tissue available but excluded from VENTANA MMR RxDx Panel (CDx) testing [i.e., from Intent-to-Diagnose (ITD) population]	18*
Tested by CDx (ITD population)	167
Total ITD with evaluable CDx MMR assessment	167
pMMR	96 (CDx Cohort A2)
dMMR	71 (CDx Cohort A1)
In ITD but associated with diagnostic protocol deviation [i.e., excluded from Intended Use (IU) population]	44**
Total ITD with evaluable CDx MMR assessment	123
pMMR	72 (CDx Cohort A2–IU)
dMMR	51 (CDx Cohort A1–IU)
Specimens with insufficient tissue	4 HE not acceptable, 14 test results not complete
Sample Procedure	
Biopsy	4
Resection	25
Unknown	94

*Sample characteristics unsuitable for CDx testing (4 cases) or CDx testing not complete as of diagnostic data snapshot date (14 cases).

**Sample tested but excluded from IU population due to diagnostic protocol deviation(s) concerning sample eligibility for CDx testing. For 43 cases in the ITD population, the deviation was staining of a sample for which the fixative was unknown. Additional deviations concerning 5 cases with unknown fixatives also occurred; An additional case was stained with the CDx test outside of the cut slide stability date.

C. Study Population Demographics and Baseline Parameters

The table below summarizes patient demographic information and MMR status for patients tested with VENTANA MMR RxDx Panel (the ITD population).

Table 23. Study Population Demographics and Baseline Parameters

Characteristic	VENTANA MMR RxDx Panel Status		Overall (N=167)
	dMMR (CDx Cohort A1) (N=71)	pMMR (Cohort A2) (N=96)	
Age (yr)			
Mean (SD)	64.0 (8.31)	64.3 (9.54)	64.1 (9.01)
Median	65.0	66.0	66.0
Minimum, maximum	39, 80	30, 80	30, 80
Age Group (yr), n (%)			
<65 years	35 (49.3)	39 (40.6)	74 (44.3)
≥65 years to <75 years	28 (39.4)	48 (50.0)	76 (45.5)
≥75 years	8 (11.3)	9 (9.4)	17 (10.2)
Ethnicity, n (%)			
Hispanic or Latino	3 (4.2)	3 (3.1)	6 (3.6)
Not Hispanic or Latino	56 (78.9)	87 (90.6)	143 (85.6)
Not reported	11 (15.5)	5 (5.2)	16 (9.6)
Unknown	1 (1.4)	1 (1.0)	2 (1.2)
Race, n (%)			
American Indian or Alaska Native	3 (4.2)	1 (1.0)	4 (2.4)
Asian	2 (2.8)	4 (4.2)	6 (3.6)
Black	0	8 (8.3)	8 (4.8)
White	56 (78.9)	74 (77.1)	130 (77.8)
Other	0	3 (3.1)	3 (1.8)
Not reported	10 (14.1)	5 (5.2)	15 (9.0)
Unknown	0	1 (1.0)	1 (0.6)
Country, n (%)			
Canada	14 (19.7)	15 (15.6)	29 (17.4)
Denmark	1 (1.4)	4 (4.2)	5 (3.0)
France	9 (12.7)	2 (2.1)	11 (6.6)
Italy	2 (2.8)	0	2 (1.2)
Poland	0	1 (1.0)	1 (0.6)
Spain	21 (29.6)	19 (19.8)	40 (24.0)

Characteristic	VENTANA MMR RxDx Panel Status		Overall (N=167)
	dMMR (CDx Cohort A1) (N=71)	pMMR (Cohort A2) (N=96)	
United Kingdom of Great Britain	5 (7.0)	7 (7.3)	12 (7.2)
United States	19 (26.8)	48 (50.0)	67 (40.1)
Clinical Trial Assay (CTA) MMR Status [a], n (%)			
MMR-deficient (dMMR)	70 (98.6)	6 (6.3)	76 (45.5)
MMR-proficient (pMMR)	1 (1.4)	90 (93.8)	91 (54.5)
Stage at Initial Diagnosis, n (%)			
Stage I	29 (40.8)	24 (25.0)	53 (31.7)
Stage II	4 (5.6)	6 (6.3)	10 (6.0)
Stage III	25 (35.2)	33 (34.4)	58 (34.7)
Stage IV	12 (16.9)	32 (33.3)	44 (26.3)
Unknown	1 (1.4)	1 (1.0)	2 (1.2)
Grade Endometrial Cancer at First Diagnosis, n (%)			
Grade 1	21 (29.6)	7 (7.3)	28 (16.8)
Grade 2	25 (35.2)	14 (14.6)	39 (23.4)
Grade 3	21 (29.6)	56 (58.3)	77 (46.1)
Not Assessable	4 (5.6)	19 (19.8)	23 (13.8)
Baseline ECOG Performance, n (%)			
0	27 (38.0)	55 (57.3)	82 (49.1)
1	44 (62.0)	41 (42.7)	85 (50.9)
Histology, n (%)			
Adenocarcinoma	1 (1.4)	0	1 (0.6)
Adenosquamous	1 (1.4)	0	1 (0.6)
Carcinoma epidermoide	0	1 (1.0)	1 (0.6)
Carcinosarcoma	0	1 (1.0)	1 (0.6)
Clear cell carcinoma	0	8 (8.3)	8 (4.8)
Endometrial carcinoma Type II	14 (19.7)	15 (15.6)	29 (17.4)
Endometrial neuroendocrine carcinoma	0	1 (1.0)	1 (0.6)
Endometrioid carcinoma (type unknown)	1 (1.4)	0	1 (0.6)
Endometrioid carcinoma Type I	46 (64.8)	24 (25.0)	70 (41.9)
High-grade uterine carcinoma	0	1 (1.0)	1 (0.6)
Mixed carcinoma	2 (2.8)	5 (5.2)	7 (4.2)
Moderately differentiated adenocarcinoma	0	1 (1.0)	1 (0.6)

Characteristic	VENTANA MMR RxDx Panel Status		Overall (N=167)
	dMMR (CDx Cohort A1) (N=71)	pMMR (Cohort A2) (N=96)	
Papillary serous carcinoma	0	1 (1.0)	1 (0.6)
Serous carcinoma	2 (2.8)	31 (32.3)	33 (19.8)
Squamous carcinoma	0	3 (3.1)	3 (1.8)
Undifferentiated carcinoma	3 (4.2)	4 (4.2)	7 (4.2)
Unknown	1 (1.4)	0	1 (0.6)
Number of Prior Therapies, n (%)			
One prior therapy	43 (60.6)	54 (56.3)	97 (58.1)
≥Two prior therapies	28 (39.4)	42 (43.8)	70 (41.9)
Prior Radiation, n (%)			
No prior radiation	14 (19.7)	34 (35.4)	48 (28.7)
Prior radiation	57 (80.3)	62 (64.6)	119 (71.3)
Prior Bevacizumab, n (%)			
No prior bevacizumab	70 (98.6)	90 (93.8)	160 (95.8)
Prior bevacizumab	1 (1.4)	6 (6.3)	7 (4.2)

[a] MMR result from local or central testing using an IHC assay other than VENTANA MMR RxDx Panel.

D. Safety and Effectiveness Results

1. Safety Results

Adverse events (AEs) in the GARNET trial were specific to the toxicity of the investigational agent. There were no device-specific adverse events in the diagnostic study. In this trial, observed AEs included events that were in line with those expected in subjects with recurrent or advanced endometrial cancer, as well as those consistent with reported safety profiles of monoclonal antibodies blocking the PD 1 interactions. Based on the treatment-emergent adverse events (TEAEs) in this trial, the product labeling for dostarlimab reflects that there were 5 (4.8%) TEAEs leading to permanent discontinuation of study drug. Please refer to Drugs@FDA for complete safety information on dostarlimab.

2. Effectiveness Results

The analysis of effectiveness was based on the BLA efficacy population (71 in cohort A1 (dMMR)). Table 24 shows the efficacy results.

Table 24: Efficacy Results in GARNET dMMR Endometrial Cancer Population

Endpoints	JEMPERLI N = 71 (dMMR)
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 DOR was 14.1 months, measured from time of first response.

Clinical Bridging Study

Bridging study was performed to evaluate agreements of MMR status (PPA for dMMR status, and NPA for pMMR status) between VENTANA MMR RxDx Panel and the CTA (testing using an IHC assay other than VENTANA MMR RxDx Panel) and to assess the clinical efficacy (ORR, DOR) of the patients enrolled in the GARNET Study.

The agreement of MMR status between CTA and CDx results was calculated in the IU and ITD subsets of the Concordance population using the CTA results as reference. For the purpose of the analyses, a pMMR status was considered negative, and dMMR status was considered positive. The estimated PPA and NPA rates were 92.1% (95% CI: 83.8, 96.3) and 98.9% (95% CI: 94.0, 99.8), respectively for the 167 retest samples (the ITD population). Among the subset of cases for which VENTANA MMR RxDx Panel staining was performed according to the requirements of the Dx protocol (the IU population), the PPA and NPA rates were slightly higher, at 92.7% (95% CI: 82.7, 97.1) and 100.0% (95% CI: 94.7, 100.0), respectively.

Table 25: MMR Status Concordance between the GARNET Clinical Trial Assay and VENTANA MMR RxDx Panel

Analysis Population [a]	CDx MMR Status	CTA MMR Status			Agreement		
		dMMR	pMMR	Total	Measure	n/N	% (95% CI [b])
IU Concordance	dMMR	51	0	51	PPA	51/55	92.7 (82.7, 97.1)
	pMMR	4	68	72	NPA	68/68	100.0 (94.7, 100.0)
	Total	55	68	123	OPA	119/123	96.7 (91.9, 98.7)
ITD Concordance	dMMR	70	1	71	PPA	70/76	92.1 (83.8, 96.3)
	pMMR	6	90	96	NPA	90/91	98.9 (94.0, 99.8)
	Total	76	91	167	OPA	160/167	95.8 (91.6, 98.0)

[a] Analyses were performed in the Concordance population [all EC patients in the BLA Safety population with an evaluable VENTANA MMR RxDx Panel (CDx) testing result]. The ITD Concordance population comprised all patients whose samples were tested with the CDx assay (n = 167). The 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 Dx protocol).

[b] Two-sided 95% 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 these 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 population (cohort A1).

3. Subgroup Analyses

There was no subgroup analysis performed.

4. Pediatric Extrapolation

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

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included 1 principal investigator. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

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

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Hematology and Pathology Devices Panel, an FDA advisory committee, for review and recommendation because the information in the PMA did not raise any new safety and effectiveness questions compared with information previously reviewed by this panel.

XIII. CONCLUSIONS DRAWN FROM THE STUDIES

A. Effectiveness Conclusions

Effectiveness of the of VENTANA MMR RxDx Panel in determining MMR status (proficient or deficient) is based on the clinical performance and benefit to patients with recurrent or advanced EC and other solid tumors as assessed in the GARNET study which is an ongoing evaluation on the safety and the efficacy of dostarlimab in these patients who had not received prior therapy with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent. The clinical benefit of the VENTANA MMR RxDx Panel was demonstrated for patients enrolled in GRANET clinical study, in which the MMR status was determined using CTA. Overall, the observed clinical benefit in the subset of patients tested with the VENTANA MMR RxDx Panel Assay was comparable to that observed in the BLA efficacy population. Additional sensitivity analyses combined with multiple imputation approaches for missing CDx values consistently support the clinical benefit of the VENTANA MMR RxDx Panel.

The performance of the VENTANA MMR RxDx Assay was also supported by the analytical validation studies.

B. Safety Conclusions

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

The VENTANA MMR RxDx Panel is an *in vitro* diagnostic device, which involves tumor specimens collected from patients with EC. The risks of the device are based on data collected in the clinical study conducted to support PMA approval as described above. Risks of the VENTANA MMR RxDx Panel are associated with failure of the device to perform as expected or failure to correctly interpret test results. As VENTANA MMR RxDx Panel is intended for use to identify patients for dostarlimab therapy, if incorrect, or false, results are reported, then EC patients may not receive the proper treatment. Patients with false positive results may undergo treatment with dostarlimab without much clinical benefit and may experience adverse reactions associated with dostarlimab therapy. Patients with false negative results may not be considered for

treatment with dostarlimab, and therefore, may receive other treatment options. There is also a risk of delayed results, which may lead to a delay in treatment with dostarlimab.

C. Benefit-Risk Determination

The probable benefits of this device are based on the data collected in the GARNET clinical study. When the VENTANA MMR RxDx Panel is used in the designated intended use population, recurrent/ advanced endometrial cancer, according to the approved instructions for use, and whose tumors demonstrate deficient MMR with this device, the benefits of the use of dostarlimab are expected to be an overall response rate of approximately 43% and, for those who respond to this therapy, a duration of response in the range of approximately 1.9-19.6 months (median not reached). This level of benefit is considered clinically significant.

With the use of the VENTANA MMR RxDx Panel in the designated intended use population, recurrent/ advanced endometrial cancer, according to the approved instructions for use, and whose tumors demonstrate deficient MMR with this device, the risks of a false positive device result and subsequent use of dostarlimab include a failure of the patient's tumor to respond to this therapy, and the experiencing of toxicity/adverse events.

In this trial, observed AEs included events that were in line with those expected in subjects with recurrent or advanced endometrial cancer, as well as those consistent with reported safety profiles of monoclonal antibodies blocking the PD 1 interactions. Based on the treatment-emergent adverse events (TEAE) in this trial, the product labeling for dostarlimab reflects that there were 5 (4.8%) TEAEs leading to permanent discontinuation of study drug on Study 4010-01-001. CDER indicates that dostarlimab demonstrated an acceptable safety profile with manageable toxicities based on data from 104 patients with dMMR endometrial cancer and from a larger safety database of 444 patients treated with dostarlimab monotherapy on Study 4010-01-001.

In addition to a false positive device result, a failure to respond could also be due to the particular biology of the patient's tumor, or other idiosyncratic biological issues specific to the individual patient, even if the device result is a true positive, as not all true positives will respond to this therapy. With respect to a false negative device result, such a patient could be deprived of a potentially beneficial endometrial cancer treatment and associated overall response rate and durability. The risks are mitigated by the expected poor prognosis of recurrent endometrial cancer.

Patient Perspectives

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

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Study 4010-01-001 (GARNET) is a multi-center, open-label ongoing clinical study of dostarlimab efficacy

and safety in adult patients who have recurrent or advanced EC and other solid tumors with limited available treatment options. Data from this study support the use of the MMR RxDx Panel as an aid in selecting patients with MMR deficient endometrial cancer who are likely to benefit from dostarlimab therapy. The response rate in EC patients with deficient MMR expression is better than what would be expected of available therapy and represents an improvement that is reasonably likely to predict clinical benefit.

XIV. CDRH DECISION

CDRH issued an approval order on April 22, 2021.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

None