

## SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

### I. GENERAL INFORMATION

|                                              |                                                                 |
|----------------------------------------------|-----------------------------------------------------------------|
| Device Generic Name:                         | Nucleic Acid Based Assay,<br>Microsatellite Instability         |
| Device Trade Name:                           | OncoMate® MSI Dx Analysis<br>System                             |
| Device Procode:                              | SFL                                                             |
| Applicant's Name and Address:                | Promega Corporation, 2800 Woods<br>Hollow Rd, Madison, WI 53711 |
| Date(s) of Panel Recommendation:             | None                                                            |
| Premarket Approval Application (PMA) Number: | P240026                                                         |
| Date of FDA Notice of Approval:              | November 5, 2025                                                |

### II. INDICATIONS FOR USE

The OncoMate® MSI Dx Analysis System is a qualitative multiplex polymerase chain reaction (PCR) test intended to detect the deletion of mononucleotides in five microsatellite loci (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) for the identification of microsatellite instability (MSI) using DNA obtained from formalin-fixed, paraffin-embedded (FFPE) endometrial carcinoma tissue specimens, and DNA isolated from matched normal FFPE specimen or whole blood. The OncoMate® MSI Dx Analysis System is for use with the Applied Biosystems® 3500 Dx Genetic Analyzer and OncoMate® MSI Dx Analysis Software.

The OncoMate® MSI Dx Analysis System is indicated for use as a companion diagnostic test to identify patients with microsatellite stable (MSS; defined as not MSI-high [not MSI-H]) endometrial carcinoma who may benefit from treatment with Keytruda® (pembrolizumab) in combination with Lenvima® (lenvatinib) in accordance with the approved therapeutic product labeling.

### III. CONTRAINDICATIONS

There are no known contraindications.

#### IV. WARNINGS AND PRECAUTIONS

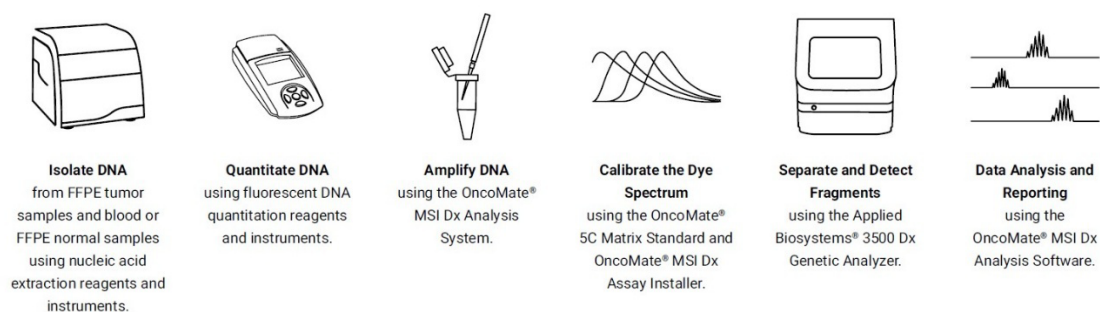
The warnings and precautions can be found in the OncoMate MSI Dx Analysis System labeling.

#### V. DEVICE DESCRIPTION

OncoMate MSI Dx Analysis System is a distributed *in vitro* diagnostic (IVD) test. The assay system contains reagents, software and procedures to determine MSI status from DNA extracted from formalin-fixed, paraffin-embedded (FFPE) endometrial solid tumors and is for use with the Applied Biosystems 3500 Dx Genetic Analyzer.

The OncoMate MSI Dx Analysis System employs multiplexed polymerase chain reaction (PCR) to amplify microsatellite markers: five mononucleotide repeat biomarkers to assess MSI status (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) and two pentanucleotide repeat markers utilized for quality control (QC) purposes (Penta C and Penta D). Microsatellites are short, DNA-repeat regions [e.g., (A)<sub>n</sub>, (CA)<sub>n</sub>, (AAT)<sub>n</sub>, (AGAT)<sub>n</sub>, (AAAAG)<sub>n</sub>,] that are distributed throughout the human genome and are prone to insertion and deletion copying errors during DNA replication. The resulting amplification products are resolved by fragment size using capillary electrophoresis (CE) with fluorescent detection. Fragment data are analyzed by the user-facing software to compare a tumor sample profile to its matched normal sample (run in separate capillaries) to determine microsatellite instability (Figure 1).

**Figure 1. OncoMate MSI Dx Analysis System assay workflow.**



#### Test Output

The test provides an MSI-H (negative), MSS (positive) sample result, or invalid. Please note that MSS and not MSI-H are interchangeable in the context of this device. If the sample data are not suitable for result interpretation, an Invalid result will be generated.

## A. Test Kit Contents

The OncoMate MSI Dx Analysis System contains sufficient reagents to perform 100 reactions (50 paired reactions). The following materials are included within the amplification kit:

**Table 1: OncoMate MSI Dx Analysis System Contents**

| Component                  |
|----------------------------|
| OncoMate MSI 5X Primer Mix |
| OncoMate MSI 5X Master Mix |
| 2800M Control DNA, 10ng/μL |
| Water, Amplification Grade |
| Size Standard 500          |

## B. Materials Required but Not Provided with the OncoMate MSI Dx Analysis System

### Reagents

- OncoMate 5C Matrix Standard
- IVD-labeled DNA extraction systems for FFPE and blood (as applicable)
- Hi-Di™ Formamide 3500 Dx Series
- POP-7 Performance Optimized Polymer 3500 Dx Series
- Conditioning Reagent 3500 Dx Series

### Instruments and Accessories

- Fluorometer compatible with fluorescent-dye-based dsDNA quantification reagents
- Applied Biosystems 3500Dx Genetic Analyzer
- Thermal cycler compatible with 96-well plates or reaction tubes
- 3500 Dx Capillary Array 50 cm
- 3500 Dx Series Septa 96-Well Plate
- Anode Buffer Container 3500 Dx Series
- Cathode Buffer Container 3500 Dx Series
- 3500 Dx Series Septa Cathode Buffer Container

### Software

- OncoMate MSI Dx Assay Installer
- OncoMate MSI Dx Analysis Software

## C. Principles of Operation

### 1. Specimen Requirements, Collection, and Preparation for Analysis

The OncoMate MSI Dx Analysis System is intended for use with separate tumor and normal samples collected from the same cancer patient. FFPE tumor tissue

is appropriate, and blood or FFPE normal tissue may be used as the required matched normal sample.

The user prepares FFPE tissue samples using 10% neutral-buffered formalin following standard pathology practices. A pathology review of prepared tissue sections confirms that the material is appropriate for downstream use. Tissue suitable for use in the assay should contain  $\geq 30\%$  tumor cells and sufficient nucleated cells to yield adequate DNA for quantification and amplification with the OncoMate MSI Dx Analysis System. Tumor specimens may be macrodissected to reach the 30% tumor-cell requirement.

The OncoMate MSI Dx Analysis System has been validated for use with whole blood collected in BD Vacutainer® K2EDTA tubes.

## **2. Nucleic Acid Extraction**

Deparaffinization, protease digestion, and RNase treatment of the FFPE tissue section is performed. Samples are separated into aqueous and mineral oil/paraffin phases, and the aqueous phase is further processed to capture the DNA. The OncoMate MSI Dx Analysis System should be used with appropriate extraction methods to process the sample through nucleic acid binding, washing and elution into water or an elution buffer. When blood is used as the matched normal sample, the extraction process is similar except that deparaffination involving mineral oil is not required. Once eluted, the extracted dsDNA is quantified using fluorescent DNA-binding dyes. A standard curve is prepared using control DNA and analyzed in parallel with the dye-stained sample DNA, and fluorescence is measured using a compatible fluorometer. The results of this analysis determine DNA concentration and inform the sample volume requirement for subsequent PCR amplification. DNA extracts must yield a minimum concentration of 0.17ng/ $\mu$ l to provide 1ng of DNA for PCR when amplification reactions (10 $\mu$ l) are formulated to accept the maximum DNA eluate volume (6 $\mu$ l).

## **3. DNA Target Amplification and Labeling**

During a multiplex PCR, several distinct DNA targets are amplified in parallel within the same reaction. When DNA primers are conjugated with a fluorescent dye molecule, the PCR products generated are dye-labeled, permitting their downstream detection via fluorescence. Fluorophore-labeled primers are used to co-amplify seven microsatellite markers: five mononucleotide repeat markers (BAT-25, BAT 26, NR-21, NR-24 and MONO-27) and two pentanucleotide repeat markers, Penta C and Penta D. Following multiplex PCR, the dye-labeled OncoMate MSI Dx Analysis System amplification products proceed to analysis.

## **4. Fragment Analysis via Capillary Electrophoresis**

Following PCR, dye-labeled OncoMate MSI Dx Analysis System amplification products from patient tumor and normal samples are separated and analyzed using capillary electrophoresis. The double-stranded amplified DNA is heat-

denatured in formamide alongside fluorescently labeled DNA fragments of known size, the Size Standard 500. The resulting single-stranded DNA is electrokinetically injected into a capillary of the Applied Biosystems 3500 Dx Genetic Analyzer, where the DNA fragments are separated based on size and detected through the incorporated fluorescent label. The raw fragment data are contained within a data file (.fsa file) generated by the capillary electrophoresis instrument.

## 5. Data Analysis and Reporting

The OncoMate MSI Dx Analysis Software is used to determine tumor MSI status by analyzing microsatellite fragment data (.fsa files) generated by the Applied Biosystems 3500 Dx Genetic Analyzer. Following fragment sizing, the software analyzes the required positive and negative amplification controls to verify expected PCR and capillary electrophoresis performance and applies additional QC checks to evaluate patient data quality. To confirm correct sample pairing, the software analyzes pentanucleotide-repeat markers for similarity. The software then evaluates mononucleotide-repeat markers for evidence of nucleotide deletions indicative of microsatellite instability. Stability results from the five mononucleotide-repeat markers are collectively interpreted to assign an overall tumor MSI status.

The OncoMate® MSI Dx Analysis Software requires data from a paired normal sample (FFPE tissue or blood) to determine tumor MSI status. Comparison to a normal sample mitigates incorrect interpretation of instability in individuals exhibiting a) heterozygosity and polymorphisms at mononucleotide-repeat markers that could be mistaken as instability and b) subtle differences indicative of instability that are difficult to identify without the matched normal sample.

A mononucleotide-repeat marker is considered “Unstable” when a  $\geq 2$ -base-pair (bp) deletion (implemented in the software as  $\geq 1.75$ bp to account for the sizing precision of capillary electrophoresis) is detected in the tumor sample relative to the normal sample. When no such deletion is detected, the marker is interpreted as “Stable”. A marker result of “Invalid” is assigned when critical quality control failures prevent an MSI result from being assigned. Please refer to the Section 6.3 of the Technical Manual (TM-543) for a summary of data quality control tests implemented in the OncoMate MSI Dx Analysis Software. A marker result of “No Call” is assigned when peak intensities are too low or too divergent from the matched normal sample to make a confident stability assessment.

Stability results from the five mononucleotide-repeat markers are collectively interpreted to assign an overall tumor MSI status (Table 2):

- A tumor sample is interpreted as “MSI-H” when two or more markers are unstable.
- A tumor sample is interpreted as “MSS” when four or more markers are interpreted as stable. A sample interpreted as “MSS” by the software is a not MSI-H result.

- A sample is assigned an MSI result of “Invalid” when the sample identity check fails, any Invalid marker results are observed, or No Call results preclude sample interpretation.

**Table 2: Results Interpretation**

| Number of Biomarkers    | Sample MSI Status |
|-------------------------|-------------------|
| ≥ 2 unstable biomarkers | MSI-H             |
| ≥ 4 stable biomarkers   | MSS (not MSI-H)   |

## D. Test Controls

### *Spectral Calibration*

Prior to analysis, the Applied Biosystems 3500 Dx Genetic Analyzer is calibrated with matrix standards so that the fluorescent signals resulting from the set of specific dyes used in the assay can be distinguished. During capillary electrophoresis, dye-labeled OncoMate MSI Dx Analysis System amplification products are separated and detected using the Applied Biosystems 3500 Dx Genetic Analyzer. The OncoMate 5C Matrix Standard consists of DNA fragments labeled with five different fluorescent dyes (fluorescein, JOE, TMR-ET, CXR-ET and WEN) in one tube. The spectral calibration is performed using the ‘OncoMate\_MSI’ dye set, which is installed on the Applied Biosystems 3500 Dx Genetic Analyzer using the Promega OncoMate MSI Dx Assay Installer v2.0. Once generated, the spectral calibration file is applied automatically during sample detection to account for the spectral overlap among the dyes and to separate the raw fluorescent signals into individual dye signals.

### *Positive and Negative Controls*

Positive and negative amplification controls are used within the system. The positive control is 2800M Control DNA (10ng/μl), which is DNA extracted from a microsatellite-stable human cell line. The negative control is Water, Amplification Grade, which is used as a reagent blank.

Positive and no-template ("negative") control amplification reactions using 2800M Control DNA and Water, Amplification Grade, respectively, must be analyzed concurrently with patient samples to verify assay performance. At least one 2800M Control DNA amplification reaction and one negative control amplification reaction must be completed on each plate (i.e., batch) of patient samples to be uploaded and analyzed using the OncoMate MSI Dx Analysis Software. The negative control reaction is analyzed to ensure that no unexpected amplification occurred in no-template reactions, which would indicate the presence of DNA contamination and lead to an Invalid assay result. The positive control reaction is analyzed to demonstrate that the amplification chemistry performed as expected.

### *Capillary Electrophoresis Standards*

All analyzed samples and controls must contain Size Standard 500 (added prior to CE analysis). The Size Standard 500 contains a series of 21 DNA fragments of known

lengths (60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500bp), also referred to as a DNA ladder. Each fragment is labeled with WEN dye and is detected separately in the presence of OncoMate MSI Dx Analysis System-amplified products using the Applied Biosystems 3500 Dx Genetic Analyzer. For each sample or control, amplified DNA fragments are sized with reference to the size standard fragments using the Local Southern method. The size standard controls for capillary-to-capillary variations in sizing precision during capillary electrophoresis and allows direct comparison of samples across the capillary electrophoresis run. Only the 60-base to 300-base fragments are analyzed for fragment sizing in the OncoMate MSI Dx Analysis Software.

## VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are other alternatives for the assessment of MSI status or mismatch repair (MMR) protein expressions in endometrial cancers. The approved CDx tests are listed in Table 3. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

**Table 3: FDA-Approved CDx Alternatives for Assessment of MSI Status or MMR Proteins.**

| Biomarker                                                                                                  | Device                        | Company                             | Technology       | Therapy/Use                                                       | Indication         |
|------------------------------------------------------------------------------------------------------------|-------------------------------|-------------------------------------|------------------|-------------------------------------------------------------------|--------------------|
| <b>Not MSI-H/pMMR</b>                                                                                      | <i>VENTANA RxDx MMR Panel</i> | <i>Ventana Medical Systems, Inc</i> | IHC <sup>1</sup> | Keytruda (pembrolizumab) in combination with Lenvima (lenvatinib) | Endometrial Cancer |
|                                                                                                            | <i>MI Cancer Seek</i>         | <i>Caris Life Sciences</i>          | NGS <sup>2</sup> | Keytruda (pembrolizumab) in combination with Lenvima (lenvatinib) | Endometrial Cancer |
| Abbreviations:<br><sup>1</sup> IHC = Immunohistochemistry<br><sup>2</sup> NGS = Next Generation Sequencing |                               |                                     |                  |                                                                   |                    |

For additional details see the FDA List of Cleared or Approved Companion Diagnostic Devices at: <https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools>

## VII. MARKETING HISTORY

The OncoMate MSI Dx Analysis System is currently a cleared Class II device to aid in the identification of probable Lynch syndrome in colorectal cancer patients to help identify patients who would benefit from additional genetic testing to diagnose Lynch



syndrome (K200129). K200129 was approved on July 26, 2021, by the United States FDA. The device is comprised of the Maxwell® CSC Instrument, Maxwell CSC DNA FFPE Kit, OncoMate MSI Dx Analysis System, OncoMate 5C Matrix Standard, OncoMate MSI Dx Assay Installer, Applied Biosystems 3500 Dx Genetic Analyzer and the OncoMate MSI Dx Interpretive Software.

The OncoMate MSI Dx Analysis System is on market for use as an in vitro diagnostic in select European countries, which are France, Germany, Austria, Poland, United Kingdom, Ireland, Belgium, Netherlands, Luxembourg, Spain, Italy, Switzerland, Denmark, Sweden, Norway, Czech Republic, Hungary, Romania and Slovakia. The device was introduced on June 9, 2020, in France, Germany, Austria, Poland, United Kingdom, Ireland, Belgium, Netherlands, Luxembourg, Spain, Italy, Switzerland, Denmark, Sweden and Norway and on September 7, 2021, in Czech Republic, Hungary, Romania and Slovakia. The device is comprised of the OncoMate MSI Dx Analysis System and OncoMate 5C Matrix Standard. The device formulations and methods for the European device are identical to the US device. However, the European device has instructions for capillary electrophoresis instrument run method setup in place of the OncoMate MSI Dx Assay Installer and uses manual data interpretation after fragment analysis in place of the software data interpretation.

The device has not been withdrawn from any market for any reason related to safety or effectiveness.

## **VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH**

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect OncoMate MSI Dx Analysis System results and subsequently improper patient management and treatment decisions for patients with endometrial cancer. Patients with false positive results may undergo treatment with the therapy listed in the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

No device-related adverse events were reported in connection with the clinical studies used to support this PMA as the studies were performed retrospectively using banked samples.

For the specific adverse events related to the approved therapeutics, please see the FDA approved drug product labels for KEYTRUDA (pembrolizumab) and LENVIMA (lenvatinib), which are available at [Drugs@FDA](https://www.fda.gov/drugs@fda).



## **IX. SUMMARY OF NON-CLINICAL STUDIES**

### **A. Laboratory Studies**

Promega executed analytical validation studies to support the CDx claims indicated in the intended use statement using MSI-H and MSS endometrial cancer tumor tissues, including challenging samples with an observed allele size difference between tumor and normal sample close to the cutoff (i.e., 2bp). Additionally, analytical data generated to support the OncoMate MSI Dx Analysis System, cleared under K200129, was leveraged for a subset of studies, including DNA interference, cross-contamination, and stability studies, to support the OncoMate MSI Dx Analysis System CDx test. For the analytical studies summarized below, an MSS result is considered the positive result, and an MSI-H result is considered the negative result to support the intended use/indications for use statement. The OncoMate MSI Dx Analysis System MSS test result is equivalent to a not MSI-H result.

#### **1. Analytical Accuracy**

Accuracy of the OncoMate MSI Dx Analysis System was evaluated by comparing agreement of the OncoMate MSI Dx Analysis System test results with those generated by an immunohistochemistry (IHC) assay, which assesses mismatch repair (MMR) status, using data generated during two separate bridging studies associated with Merck's Clinical Trial KEYNOTE-775 (KN775): one evaluating the OncoMate MSI Dx Analysis System (i.e., the OncoMate KN775 bridging study) and another evaluating the immunohistochemistry (IHC) assay which assesses mismatch repair (MMR) status. When these sample sets were compared, 255 tumor samples had a valid MSI result ["MSS" (not MSI-H) or "MSI-H"] and a valid MMR result from the IHC assay. Therefore, MMR protein staining and MSI testing results from these 255 endometrial tumor samples were compared to estimate accuracy of the OncoMate® MSI Dx Analysis System.

Treating the IHC comparator assay results as the reference method, the positive percent agreement (PPA) was defined as the percent of samples with a positive OncoMate MSI Dx Analysis System result [MSS (or not MSI-H)] that were in agreement with the positive IHC result (pMMR). The resulting PPA was 99.0% (205/207), with a 95% confidence interval of 96.5–99.7%. The negative percent agreement (NPA) between the OncoMate MSI Dx Analysis System and the IHC comparator assay was defined as the percent of samples with a negative OncoMate MSI Dx Analysis System result (MSI-H) that were in agreement with the negative IHC result (dMMR). The resulting NPA was 91.7% (44/48), with a 95% confidence interval of 80.4–96.7%. The overall percent agreement was 97.6% (249/255), with a 95% confidence interval of 95.0–98.9%. See Table 4, below.

**Table 4: Concordance for MSI Status between the OncoMate MSI Analysis System and the MMR IHC Assay**

| OncoMate MSI Dx Analysis System Result | MMR IHC Assay Result                 |      | Total |
|----------------------------------------|--------------------------------------|------|-------|
|                                        | pMMR                                 | dMMR |       |
| MSS (not MSI-H)                        | 205                                  | 4    | 209   |
| MSI-H                                  | 2                                    | 44   | 46    |
| Total                                  | 207                                  | 48   | 255   |
| <b>PPA</b>                             | 99.0% (205/207) [95% CI: 96.5, 99.7] |      |       |
| <b>NPA</b>                             | 91.7% (44/48) [95% CI: 80.4, 96.7]   |      |       |
| <b>OPA</b>                             | 97.6 (249/255) [95% CI: 95.0, 98.9]  |      |       |

The two discordant samples called mismatch repair proficient (pMMR) by the IHC orthogonal method and MSI-H by the OncoMate MSI Dx Analysis System had deletion sizes near the cutoff (i.e., 2bp) across nearly all loci, and were therefore challenging cases. The four discordant samples called deficient MMR (dMMR) by the IHC orthogonal method and MSS (not MSI-H) by the OncoMate MSI Dx Analysis System were attributed to the potential dilution of MSI-H tumor cells by MSS tumor and/or immune cells, potential delayed onset of MSI in tumors that have recently acquired mismatch repair deficiency, and possible subjectivity in pathology for MMR status. Of these 4 samples which were dMMR by the IHC assay/MSS by the OncoMate MSI Dx Analysis System, 3 were close to the tumor content percent limit of detection (1 – 2X limit of detection [LoD]). The remaining 1 discordant sample, although not considered challenging, resulted in a false-positive call. Please refer to Section XIV.C for discussion of the clinical benefit-risk assessment for this device regarding false positive or false negative calls.

## 2. Analytical Sensitivity

### a. Normal Range and Cut-off

The Normal Range study was conducted to verify the system’s capability to resolve amplicons that differ by  $\geq 2$ bp (implemented in the software as  $\geq 1.75$ bp to account for the sizing precision of capillary electrophoresis). This study evaluated sizing linearity and precision using two sets of synthetic DNA fragments with known sizes that differ by 1bp (“resolution markers”). Two sets of seven resolution markers were analyzed during this study. These fragments consist of dye-labeled amplicons of known size that are separated by 1bp within each set, with the two sets designed to bracket the upper (Large) and lower (Small) ends of the amplicon size range of the MSI markers (86–188bp).

Three MSS endometrial cancer tumor sample pairs were amplified in duplicate using the OncoMate MSI Dx Analysis System. Amplified products were then analyzed by capillary electrophoresis in the presence and absence of resolution

markers on each of two nonconsecutive days. Across the three samples, 12 replicates were analyzed in the presence of resolution markers and 12 replicates in the absence of resolution markers (24 MSI results total).

When two sets of resolution markers were analyzed by CE and actual sizes were characterized, the precision of DNA fragment size determination was sufficient to detect single-base-pair size differences across the amplicon size range (Table 5). Based on the results of this study, the OncoMate MSI Dx Analysis System can resolve DNA fragments that differ by a 2-base deletion, the assay threshold for identifying marker instability.

**Table 5: Absolute Differences in Observed Sizes of Resolution Markers.**

| <b>Resolution Marker Pair</b> | <b>Known Size Difference (bp)</b> | <b>Observed Average Size Difference (bp)</b> |
|-------------------------------|-----------------------------------|----------------------------------------------|
| Res_Large 1 and Res_Large 3   | 2.00                              | 2.12                                         |
| Res_Large 2 and Res_Large 4   | 2.00                              | 2.00                                         |
| Res_Large 3 and Res_Large 5   | 2.00                              | 1.90                                         |
| Res_Large 4 and Res_Large 6   | 2.00                              | 2.02                                         |
| Res_Large 5 and Res_Large 7   | 2.00                              | 2.13                                         |
| Res_Small 1 and Res_Small 3   | 2.00                              | 2.35                                         |
| Res_Small 2 and Res_Small 4   | 2.00                              | 2.22                                         |
| Res_Small 3 and Res_Small 5   | 2.00                              | 2.08                                         |
| Res_Small 4 and Res_Small 6   | 2.00                              | 2.19                                         |
| Res_Small 5 and Res_Small 7   | 2.00                              | 2.34                                         |

**b. Limit of Blank**

This study was designed to estimate the Limit of Blank (LoB) or false positive call rate of the OncoMate MSI Dx Analysis System using cancer patient samples that do not exhibit microsatellite instability (i.e., have MSS status). MSI results were generated across three days, two amplification kit lots and five replicates per sample per run for a total of 30 replicates of each MSS sample. Four MSS endometrial cancer samples with 30–80% tumor content (TC) were tested (30%, 60%, 60%, and 80% TC). Therefore, a total of 120 replicates were assessed across all samples.

All tests resulted in MSS results (100%; 120/120) with sample level false positive rate of 0%. At the locus level, the 120 test results represent 600 mononucleotide locus allele calls. For the mononucleotide loci, 99.8% (599/600) of the marker stability calls were “Stable”. There was a single instance of one locus, BAT-26, being called unstable. A single unstable locus results in an MSS final interpretive result, and the one unstable locus did not affect the final test result. The LoB study results demonstrate that the OncoMate MSI Dx Analysis System can correctly generate MSS results with no significant background that contributes to false MSI-H results.

**c. Limit of Detection**

This study was designed to estimate the Limit of Detection (LoD) of the OncoMate MSI Dx Analysis System in terms of lowest tumor content and DNA input amount required to correctly assign an MSI-H result with 95% percent positivity.

**1. Limit of Detection - Tumor Content**

Tumor content LoD was established using one MSI-H sample, as depicted in Tables 6 and 7, from an endometrial tumor that was analyzed at five different levels of tumor content (10%, 20%, 30%, 40% and the original tumor content) and a constant DNA input amount (1.0ng). This LoD establishment study used two reagent lots, with five runs per lot on nonconsecutive days and at least one CE instrument, for a total of 40 replicates per sample at each level of tumor content (4 replicates/run•lot × 5 runs × 2 lots). The lowest tumor content achieving a percent positivity ≥95% (hit rate ≥0.95) across all factors and samples was 20%. The absolute and relative frequency for each tumor content percentage is summarized in Tables 6 and 7.

**Table 6: Percent Tumor Content Limit of Detection for MSI**

| Absolute and Relative MSI-H Frequency by % Tumor Content |                  |                 |                 |                 |                 |                 |                 |                        |                 |
|----------------------------------------------------------|------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|------------------------|-----------------|
| 10%                                                      |                  | 20%             |                 | 30%             |                 | 40%             |                 | Original Tumor Content |                 |
| Lot 1                                                    | Lot 2            | Lot 1           | Lot 2           | Lot 1           | Lot 2           | Lot 1           | Lot 2           | Lot 1                  | Lot 2           |
| 75.0%<br>(15/20)                                         | 90.0%<br>(18/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)        | 100%<br>(20/20) |

**Table 7: Percent Tumor Content Limit of Detection per Locus**

| Lot # | Tumor Content | Absolute and Relative Unstable Call Frequencies at Locus-Level, % (n/n) |                |                  |                  |                 |
|-------|---------------|-------------------------------------------------------------------------|----------------|------------------|------------------|-----------------|
|       |               | NR-21                                                                   | BAT-25         | BAT-26           | NR-24            | MONO-27         |
| 1     | 10%           | 5.0%<br>(1/20)                                                          | 5.0%<br>(1/20) | 65.0%<br>(13/20) | 95.0%<br>(19/20) | 35.0%<br>(7/20) |

| Lot # | Tumor Content | Absolute and Relative Unstable Call Frequencies at Locus-Level, % (n/n) |                 |                  |                  |                 |
|-------|---------------|-------------------------------------------------------------------------|-----------------|------------------|------------------|-----------------|
|       |               | NR-21                                                                   | BAT-25          | BAT-26           | NR-24            | MONO-27         |
|       | 20%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |
|       | 30%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |
|       | 40%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |
|       | 60%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |
| 2     | 10%           | 10.0%<br>(2/20)                                                         | 0%<br>(0/20)    | 75.0%<br>(15/20) | 90.0%<br>(18/20) | 30.0%<br>(6/20) |
|       | 20%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |
|       | 30%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |
|       | 40%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |
|       | 60%           | 100%<br>(20/20)                                                         | 100%<br>(20/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) |

## 2. Limit of Detection – DNA Input

DNA input LoD was established using a dilution series of DNA purified from two endometrial FFPE tumor (one MSI-H and one MSS) and matched normal tissue as depicted in Tables 8 and 9. Each sample was analyzed at six different DNA input amounts (0.05ng, 0.2ng, 0.5ng, 0.75ng, 1.0ng and 1.25ng per reaction). DNA from MSI-H tumor samples was blended with DNA from the matched normal sample, as described above, to represent the minimum tumor content requirement of 30%. MSS tumor samples were analyzed at the original tumor content. Each tumor sample was analyzed using two OncoMate MSI Dx Analysis System lots, for a total of 40 replicates per sample at each DNA input amount (4 replicates/run•lot × 5 runs × 2 lots). Results from this study are summarized in Tables 8 and 9, below.

**Table 8: DNA Input Amount (ng) Limit of Detection for MSI**

| Sample ID | Known MSI Result | Absolute and Relative MSI Status Frequency by DNA Input Amount (ng) |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |                 |
|-----------|------------------|---------------------------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|           |                  | 0.05 ng                                                             |                 | 0.2 ng          |                 | 0.5 ng          |                 | 0.75 ng         |                 | 1 ng            |                 | 1.25 ng         |                 |
|           |                  | Lot 1                                                               | Lot 2           | Lot 1           | Lot 2           | Lot 1           | Lot 2           | Lot 1           | Lot 2           | Lot 1           | Lot 2           | Lot 1           | Lot 2           |
| 1         | MSS              | 0%<br>(0/20)                                                        | 0%<br>(0/20)    | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) |
| 2         | MSI-H            | 5.0%<br>(1/20)                                                      | 15.0%<br>(3/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20) |

**Table 9: DNA Input Amount (ng) Limit of Detection per Locus**

| Known MSI Result | DNA Input (ng) | Absolute and Relative Stability Call Frequencies at Locus-Level, % (n/n) |                  |                  |                  |                  |                  |                  |                 |                 |                  |
|------------------|----------------|--------------------------------------------------------------------------|------------------|------------------|------------------|------------------|------------------|------------------|-----------------|-----------------|------------------|
|                  |                | NR-21                                                                    |                  | BAT-25           |                  | BAT-26           |                  | NR-24            |                 | MONO-27         |                  |
|                  |                | Lot 1                                                                    | Lot 2            | Lot 1            | Lot 2            | Lot 1            | Lot 2            | Lot 1            | Lot 2           | Lot 1           | Lot 2            |
| MSS              | 0.05           | 0%<br>(0/20)                                                             | 10.0%<br>(2/20)  | 0%<br>(0/20)     | 0%<br>(0/20)     | 0%<br>(0/20)     | 0%<br>(0/20)     | 0%<br>(0/20)     | 0%<br>(0/20)    | 0%<br>(0/20)    | 0%<br>(0/20)     |
|                  | 0.2            | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 85.0%<br>(17/20) | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 95.0%<br>(19/20) | 100%<br>(20/20) | 100%<br>(20/20) | 95.0%<br>(19/20) |
|                  | 0.5            | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
|                  | 0.75           | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
|                  | 1              | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
|                  | 1.25           | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
| MSI-H            | 0.05           | 85.0%<br>(17/20)                                                         | 90.0%<br>(18/20) | 60.0%<br>(12/20) | 55.0%<br>(11/20) | 50.0%<br>(10/20) | 55.0%<br>(11/20) | 20.0%<br>(4/20)  | 15.0%<br>(3/20) | 35.0%<br>(7/20) | 50.0%<br>(10/20) |
|                  | 0.2            | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
|                  | 0.5            | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
|                  | 0.75           | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
|                  | 1              | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |
|                  | 1.25           | 100%<br>(20/20)                                                          | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20)  | 100%<br>(20/20) | 100%<br>(20/20) | 100%<br>(20/20)  |

Based on the results of the study, the LoD for endometrial cancer specimens is 20% tumor content at 1 ng of DNA input and 0.2 ng of DNA input at 30% tumor content.

### 3. **Precision**

This study was performed to evaluate precision and reproducibility of the OncoMate MSI Dx Analysis System when testing MSS and MSI-H FFPE tumors. A total of 3 FFPE endometrial cancer samples (2 MSI-H and 1 MSS) and DNA purified from matched normal FFPE tissues were used in this study. The percent tumor content (%TC) of the three endometrial samples were 95%, 80% and 70% (See Table 11, below). To assess within-lot reproducibility and precision, samples were analyzed on five nonconsecutive days using the same OncoMate MSI Dx Analysis System lot at three different study sites. A different operator team at each study site analyzed five replicates for each sample per day using a single CE instrument. To assess between-lot reproducibility and precision, the samples were analyzed on five nonconsecutive days, across five replicates per day, using three OncoMate MSI Dx Analysis System lots, and a single CE instrument, at a single study site.

Agreement rates were calculated by comparing observed MSI results for each sample replicate to the majority MSI result observed across all replicates. Data across individual factors (site, lot and day) and all factors were summarized to evaluate the reproducibility of the OncoMate MSI Dx Analysis System. Across all sites and lots, all replicates reported a final result of “MSS” or “MSI-H”, and none had a terminal result of “Invalid”. Positive percent agreement values for MSS results across site, lot or day were 100%, and NPA values for MSI-H results were 100%. Across all study factors, PPA was 100% (95% CI of 97.1–100%) and NPA was 100% (95% CI of 98.4–100%). These data are shown in Table 10.

**Table 10: PPA and NPA Estimates for Observed MSI Result as Compared to Majority MSI Result by Study Site, Lot and Day.**

|      |           | PPA                |             |             | NPA                |             |             |
|------|-----------|--------------------|-------------|-------------|--------------------|-------------|-------------|
|      |           | Observed Agreement | 95% CI      |             | Observed Agreement | 95% CI      |             |
|      |           |                    | Lower Limit | Upper Limit |                    | Lower Limit | Upper Limit |
| Site | 1 (Lot 1) | 100%<br>(25/25)    | 86.3%       | 100%        | 100%<br>(50/50)    | 92.9%       | 100%        |
|      | 2 (Lot 1) | 100%<br>(25/25)    | 86.3%       | 100%        | 100%<br>(50/50)    | 92.9%       | 100%        |
|      | 3 (Lot 1) | 100%<br>(25/25)    | 86.3%       | 100%        | 100%<br>(50/50)    | 92.9%       | 100%        |



|                               |                           | PPA                |             |             | NPA                |             |             |
|-------------------------------|---------------------------|--------------------|-------------|-------------|--------------------|-------------|-------------|
|                               |                           | Observed Agreement | 95% CI      |             | Observed Agreement | 95% CI      |             |
|                               |                           |                    | Lower Limit | Upper Limit |                    | Lower Limit | Upper Limit |
|                               | <b>Overall, Sites 1–3</b> | 100%<br>(75/75)    | 95.2%       | 100%        | 100%<br>(150/150)  | 97.6%       | 100%        |
| <b>Lot</b>                    | 1 (Site 1)                | 100%<br>(25/25)    | 86.3%       | 100%        | 100%<br>(50/50)    | 92.9%       | 100%        |
|                               | 2 (Site 1)                | 100%<br>(25/25)    | 86.3%       | 100%        | 100%<br>(50/50)    | 92.9%       | 100%        |
|                               | 3 (Site 1)                | 100%<br>(25/25)    | 86.3%       | 100%        | 100%<br>(50/50)    | 92.9%       | 100%        |
|                               | <b>Overall, Lots 1–3</b>  | 100%<br>(75/75)    | 95.2%       | 100%        | 100%<br>(150/150)  | 97.6%       | 100%        |
| <b>Day (Lot 1, Sites 1–3)</b> | 1                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | 2                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | 3                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | 4                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | 5                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | <b>Overall, Sites 1–3</b> | 100%<br>(75/75)    | 95.2%       | 100%        | 100%<br>(150/150)  | 97.6%       | 100%        |
| <b>Day (Site 1, Lots 1–3)</b> | 1                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | 2                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | 3                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                               | 4                         | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |

|                                    |                          | PPA                |             |             | NPA                |             |             |
|------------------------------------|--------------------------|--------------------|-------------|-------------|--------------------|-------------|-------------|
|                                    |                          | Observed Agreement | 95% CI      |             | Observed Agreement | 95% CI      |             |
|                                    |                          |                    | Lower Limit | Upper Limit |                    | Lower Limit | Upper Limit |
|                                    | 5                        | 100%<br>(15/15)    | 78.2%       | 100%        | 100%<br>(30/30)    | 88.4%       | 100%        |
|                                    | <b>Overall, Lots 1–3</b> | 100%<br>(75/75)    | 95.2%       | 100%        | 100%<br>(150/150)  | 97.6%       | 100%        |
| <b>Overall, Across All Factors</b> |                          | 100%<br>(125/125)  | 97.1%       | 100%        | 100%<br>(250/250)  | 98.4%       | 100%        |

At the sample-level and locus-level, overall agreement for MSI-H and MSS endometrial cancer tumor samples were reported, with a PPA of  $\geq 98.8\%$  (lowest 95% CI bound: 96.5%) across all samples at all biomarkers apart from BAT-25. For the BAT-25 locus, overall PPA was 79.2% (95% CI: 73.6, 84.1) for MSI-H endometrial cancer tumor samples and 100% (95% CI: 97.1, 100) for MSS endometrial cancer tumor samples. The variability at the BAT-25 locus was due to a single MSI-H sample having an observed allele size difference between tumor and normal sample close to the cutoff (i.e., 2bp), which resulted in 58.4% (73/125) agreement with the majority result. These results are summarized in Table 11, below.

**Table 11: Percent Agreement of Sample-Level MSI Results and Locus-Level Stability Results compared to the Majority Result**

| Sample ID                 | Majority MSI Result | Percent Tumor Content | Agreement with Majority MSI Result, % (n/n) | NR-21                                   |                                         | BAT-25             |                                         | BAT-26                                  |                    | NR-24                                   |                                         | MONO-27            |                                         |                                         |                    |
|---------------------------|---------------------|-----------------------|---------------------------------------------|-----------------------------------------|-----------------------------------------|--------------------|-----------------------------------------|-----------------------------------------|--------------------|-----------------------------------------|-----------------------------------------|--------------------|-----------------------------------------|-----------------------------------------|--------------------|
|                           |                     |                       | 95% CI                                      | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit                      | 95% CI Upper Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit                      | 95% CI Upper Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit                      | 95% CI Upper Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit                      | 95% CI Upper Limit |
|                           |                     |                       |                                             | 95% CI                                  | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | 95% CI Upper Limit                      | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | 95% CI Upper Limit                      | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | 95% CI Upper Limit                      | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit |
| 1                         | MSI-H               | 95%                   | 100% (125/125)                              | 99.2% (124/125)                         | 95.6%                                   | 100% (125/125)     | 97.1%                                   | 100% (125/125)                          | 97.1%              | 100% (125/125)                          | 97.1%                                   | 100% (125/125)     | 97.1%                                   |                                         |                    |
|                           |                     |                       | 97.1, 100                                   |                                         | 100%                                    |                    | 100%                                    |                                         | 100%               |                                         | 100%                                    |                    | 100%                                    |                                         |                    |
| 2                         | MSI-H               | 70%                   | 100% (125/125)                              | 98.4% (123/125)                         | 94.3%                                   | 58.4% (73/125)     | 49.3%                                   | 100% (125/125)                          | 97.1%              | 100% (125/125)                          | 97.1%                                   | 100% (125/125)     | 97.1%                                   |                                         |                    |
|                           |                     |                       | 97.1, 100                                   |                                         | 99.8%                                   |                    | 67.2%                                   |                                         | 100%               |                                         | 100%                                    |                    | 100%                                    |                                         |                    |
| 3                         | MSS                 | 80%                   | 100% (125/125)                              | 100% (125/125)                          | 97.1%                                   | 100% (125/125)     | 97.1%                                   | 100% (125/125)                          | 97.1%              | 100% (125/125)                          | 97.1%                                   | 100% (125/125)     | 97.1%                                   |                                         |                    |
|                           |                     |                       | 97.1, 100                                   |                                         | 100%                                    |                    | 100%                                    |                                         | 100%               |                                         | 100%                                    |                    | 100%                                    |                                         |                    |
| Overall for MSI-H Samples |                     |                       |                                             | 98.8% (247/250)                         | 96.5%                                   | 79.2% (198/250)    | 73.6%                                   | 100% (250/250)                          | 98.5%              | 100% (250/250)                          | 98.5%                                   | 100% (250/250)     | 98.5%                                   |                                         |                    |
|                           |                     |                       |                                             |                                         | 99.8%                                   |                    | 84.1%                                   |                                         | 100%               |                                         | 100%                                    |                    | 100%                                    |                                         |                    |
| Overall for MSS Samples   |                     |                       |                                             | 100%                                    | 97.1%                                   | 100%               | 97.1%                                   | 100%                                    | 97.1%              | 100%                                    | 97.1%                                   | 100%               | 97.1%                                   |                                         |                    |

| Sample ID | Majority MSI Result | Percent Tumor Content | Agreement with Majority MSI Result, % (n/n) | NR-21                                   |                    | BAT-25                                  |                    | BAT-26                                  |                    | NR-24                                   |                    | MONO-27                                 |                    |
|-----------|---------------------|-----------------------|---------------------------------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|
|           |                     |                       |                                             | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit |
|           |                     |                       | 95% CI                                      |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |
|           |                     |                       |                                             | (125/125)                               | 100%               | (125/125)                               | 100%               | (125/125)                               | 100%               | (125/125)                               | 100%               | (125/125)                               | 100%               |

To assess precision and reproducibility near the assay's limit of detection for tumor content, six endometrial tumor samples (three MSI-H and three MSS) with 30% tumor content were evaluated in a supplemental reproducibility study. In this supplemental study, each sample was analyzed on five nonconsecutive days at two sites using two OncoMate MSI Dx Analysis System lots. At each site, a single operator analyzed five replicates for each sample per day using a single CE instrument.

Across the study sites and the reagent lots, PPA for MSS results was 100% and NPA for MSI-H results was 100%. Across all study factors, PPA was 100% (95% CI of 97.6–100%) and NPA was 100% (97.6–100%). See Table 12.

**Table 12: PPA and NPA Estimates for Observed MSI Result as Compared to Majority MSI Result by Study Site, Lot and Day for Challenging Samples.**

|              |                        | PPA |                    |             |             | NPA |                    |             |             |
|--------------|------------------------|-----|--------------------|-------------|-------------|-----|--------------------|-------------|-------------|
|              |                        | N   | Observed Agreement | 95% CI      |             | N   | Observed Agreement | 95% CI      |             |
|              |                        |     |                    | Lower Limit | Upper Limit |     |                    | Lower Limit | Upper Limit |
| Site         | 1                      | 75  | 100%               | 95.2%       | 100%        | 75  | 100%               | 95.2%       | 100%        |
|              | 2                      | 75  | 100%               | 95.2%       | 100%        | 75  | 100%               | 95.2%       | 100%        |
| Lot          | 1                      | 75  | 100%               | 95.2%       | 100%        | 75  | 100%               | 95.2%       | 100%        |
|              | 2                      | 75  | 100%               | 95.2%       | 100%        | 75  | 100%               | 95.2%       | 100%        |
| Day (Site 1) | 1 (Lot 1)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|              | 2 (Lot 2)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|              | 3 (Lot 1)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|              | 4 (Lot 2)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|              | 5 (Lot 1)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|              | Overall, Site 1, Lot 1 | 45  | 100%               | 92.1%       | 100%        | 45  | 100%               | 92.1%       | 100%        |
|              | Overall, Site 1, Lot 2 | 30  | 100%               | 88.4%       | 100%        | 30  | 100%               | 88.4%       | 100%        |
| Day (Site 2) | 1 (Lot 2)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|              | 2 (Lot 1)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|              | 3 (Lot 2)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |

|                    |                        | PPA |                    |             |             | NPA |                    |             |             |
|--------------------|------------------------|-----|--------------------|-------------|-------------|-----|--------------------|-------------|-------------|
|                    |                        | N   | Observed Agreement | 95% CI      |             | N   | Observed Agreement | 95% CI      |             |
|                    |                        |     |                    | Lower Limit | Upper Limit |     |                    | Lower Limit | Upper Limit |
|                    | 4 (Lot 1)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|                    | 5 (Lot 2)              | 15  | 100%               | 78.2%       | 100%        | 15  | 100%               | 78.2%       | 100%        |
|                    | Overall, Site 2, Lot 1 | 30  | 100%               | 88.4%       | 100%        | 30  | 100%               | 88.4%       | 100%        |
|                    | Overall, Site 2, Lot 2 | 45  | 100%               | 92.1%       | 100%        | 45  | 100%               | 92.1%       | 100%        |
| Across All Factors |                        | 150 | 100%               | 97.6%       | 100%        | 150 | 100%               | 97.6%       | 100%        |

At the sample-level and locus-level, overall agreement for MSI-H and MSS endometrial cancer tumor samples were reported, with an overall agreement of 100% (95% CI: 97.6, 100.0) for both sample types at all biomarkers apart from NR-24 and MONO-27. For the NR-24 locus, overall agreement was 100% (95% CI: 97.6, 100.0) for MSI-H endometrial cancer tumor samples and 98% (95% CI: 94.3, 99.6) for MSS endometrial cancer tumor samples. For the MONO-27 locus, the overall agreement was 86.0% (95% CI: 79.4, 91.1) for MSI-H endometrial cancer tumor samples and 98.7% (95% CI: 95.3, 99.8) for MSS endometrial cancer tumor samples. The variability at the MONO-27 locus was due to a single MSI-H sample having an observed allele size difference between tumor and normal sample close to the cutoff (i.e., 2bp), which resulted in 58.0% (29/50) agreement with the majority result. These results are summarized in Table 13, below.

**Table 13: Percent Agreement of Sample-Level MSI Results and Locus-Level Stability Results compared to the Majority Result for Challenging Samples**

| Sample ID | Majority MSI Result | Agreement with Majority MSI Result, % (n/n) | NR-21                                   |                    | BAT-25                                  |                    | BAT-26                                  |                    | NR-24                                   |                    | MONO-27                                 |                    |
|-----------|---------------------|---------------------------------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|
|           |                     | 95% CI                                      | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit |
|           |                     |                                             |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |
| 1         | MSI-H               | 100% (50/50)                                | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              |
|           |                     | 92.9, 100                                   |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |
| 2         | MSI-H               | 100% (50/50)                                | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              |
|           |                     | 92.9, 100                                   |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |
| 3         | MSI-H               | 100% (50/50)                                | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 58.0% (29/50)                           | 43.2%              |
|           |                     | 92.9, 100                                   |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 71.8%              |
| 4         | MSS                 | 100% (50/50)                                | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              |
|           |                     | 92.9, 100                                   |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |
| 5         | MSS                 | 100% (50/50)                                | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 100% (50/50)                            | 92.9%              | 94.0% (47/50)                           | 83.5%              | 96.0% (48/50)                           | 86.3%              |
|           |                     | 92.9, 100                                   |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 98.7%              |                                         | 99.5%              |
| 6         | MSS                 | 100% (50/50)                                | 100%                                    | 92.9%              | 100%                                    | 92.9%              | 100%                                    | 92.9%              | 100% (50/50)                            | 92.9%              | 100%                                    | 92.9%              |

| Sample ID                 | Majority MSI Result | Agreement with Majority MSI Result, % (n/n) | NR-21                                   |                    | BAT-25                                  |                    | BAT-26                                  |                    | NR-24                                   |                    | MONO-27                                 |                    |
|---------------------------|---------------------|---------------------------------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|-----------------------------------------|--------------------|
|                           |                     |                                             | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit | Agreement with Majority Result, % (n/n) | 95% CI Lower Limit |
|                           |                     |                                             |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |                                         | 95% CI Upper Limit |
|                           |                     | 92.9, 100                                   | (50/50)                                 | 100%               | (50/50)                                 | 100%               | (50/50)                                 | 100%               |                                         | 100%               | (50/50)                                 | 100%               |
| Overall for MSI-H Samples |                     |                                             | 100% (150/150)                          | 97.6%              | 100% (150/150)                          | 97.6%              | 100% (150/150)                          | 97.6%              | 100% (150/150)                          | 97.6%              | 86.0% (129/150)                         | 79.4%              |
|                           |                     |                                             |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 91.1%              |
| Overall for MSS Samples   |                     |                                             | 100% (150/150)                          | 97.6%              | 100% (150/150)                          | 97.6%              | 100% (150/150)                          | 97.6%              | 98.0% (147/150)                         | 94.3%              | 98.7% (148/150)                         | 95.3%              |
|                           |                     |                                             |                                         | 100%               |                                         | 100%               |                                         | 100%               |                                         | 99.6%              |                                         | 99.8%              |

#### 4. Analytical Specificity

##### a. Interfering Substances

The DNA Interference studies evaluated the potential effect of endogenous and exogenous interfering substances associated with FFPE tissue samples, blood, DNA purification reagents and blood collection devices on the performance of the OncoMate MSI Dx Analysis System. The study specifically characterized effects of hemoglobin (10mg/ml), triglycerides (16.94mM), mucin (1mg/ml), melanin (0.005%), conjugated bilirubin (475µM), unconjugated bilirubin (684µM), EDTA (5.4mg/ml) and necrosis (40–90%) on MSI results. Additionally, data generated during the Interference study for K200129, which characterized the effects of proteinase K, ethanol and guanidine hydrochloride, were reanalyzed using the OncoMate MSI Dx Analysis Software. A total of 6 FFPE endometrial cancer clinical samples (4 MSI-H and 2 MSS), along with matched normal FFPE tissue and matched normal blood samples, were tested using three different DNA extraction systems to extract DNA from each sample source. Mucin and necrosis were tested with matched tumor and normal FFPE tissue only.

For hemoglobin, triglycerides, conjugated bilirubin, unconjugated bilirubin, melanin and EDTA, concordant MSI results were obtained for 100% of the sample replicates (18/18 for each potential interferent) when using a matched normal sample from either blood or FFPE tissue and with all three DNA extraction systems. For mucin, concordant results were obtained for 88.9% of all sample replicates (16/18). Finally, 100% of sample replicates (18/18) with high necrosis yielded concordant MSI results to sample replicates with low to no necrosis with each of the three DNA extraction systems used (Tables 8 and 9). For ethanol, proteinase K and guanidine hydrochloride, a reanalysis of the Interference study for FDA submission K200129 using the OncoMate MSI Dx

Analysis Software reported 100% agreement between test and control conditions. These results are summarized below in Tables 14 and 15.

**Table 14: Number and Percent Concordant MSI Results by Interferent in Water Diluent.**

| Interferent (Final Concentration) | Matched Normal Sample Type | MSI Result |       |         | Total |         |
|-----------------------------------|----------------------------|------------|-------|---------|-------|---------|
|                                   |                            | MSS        | MSI-H | Invalid |       |         |
|                                   |                            | n/6        | n/12  | n/18    | n/18  | Percent |
| Conjugated Bilirubin (475µM)      | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
| EDTA (5.4mg/ml)                   | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
| Hemoglobin (10mg/ml)              | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
| Triglycerides (16.94mM)           | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
| Mucin <sup>1</sup> (1mg/ml)       | Tissue                     | 4/6        | 12/12 | 2/18    | 16/18 | 88.9%   |
| Necrosis <sup>1</sup> (50–90%)    | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
| No Interferent, Water             | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |

<sup>1</sup> Necrosis and mucin were not evaluated for blood samples

**Table 15: Number and Percent Concordant MSI Results by Interferent in DMSO Diluent.**

| Interferent (Final Concentration) | Matched Normal Sample Type | MSI Result |       |         | Total |         |
|-----------------------------------|----------------------------|------------|-------|---------|-------|---------|
|                                   |                            | MSS        | MSI-H | Invalid |       |         |
|                                   |                            | n/6        | n/12  | n/18    | n/18  | Percent |
| Melanin (0.005%)                  | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
| Unconjugated Bilirubin (684µM)    | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
| No Interferent, DMSO              | Blood                      | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |
|                                   | Tissue                     | 6/6        | 12/12 | 0/18    | 18/18 | 100%    |

**b. Cross-Contamination**

This study was designed to assess the potential of erroneous results due to cross-contamination (within run) throughout the complete OncoMate MSI Dx Analysis System workflow. Data generated during the K200129 Cross-Contamination study were reanalyzed using the OncoMate MSI Dx Analysis Software.

During reanalysis, no cross-contamination was detected. No batch QC failures were observed, and the software assigned no inaccurate MSI results. Expected results were observed for all 160 MSI-H replicates. Of 160 MSS replicates, 151 were assigned an MSI result of “MSS”. The remaining nine replicates were assigned a result of “Invalid” due to the enhanced quality control implemented in the OncoMate MSI Dx Analysis Software and for reasons not attributable to cross-contamination. When these nine replicates were excluded as per the study protocol, 100% (461/461) of analyses returned the expected result.

**5. Stability**

**a. Closed Kit Reagent Stability**

The closed-kit stability of OncoMate MSI Dx Analysis System reagents was evaluated using seven amplification reagent lots and four endometrial cancer samples (2 MSS and 2 MSI-H). The stability study design and reagent lot kits used were leveraged from the stability study supporting the original 510(k) (K200129). The timepoints tested included baseline (T0), 13, 25, 37, 43, and 49 months stored at their specified storage temperatures. Assessment of stability was completed by calculating concordance between T0 and all subsequent timepoints.

The sample-level agreement was 100% for all timepoints tested. The results demonstrated that the reagents were stable for 36 months at –30°C to –10°C and for 36 months (33 months at –30°C to –10°C followed by 3 months at +2°C to +10°C).

**b. Open Kit Reagent Stability**

This study was performed to evaluate in-use (open kit) stability for the OncoMate MSI Dx Analysis System. The stability study design and reagent lot kits used were leveraged from the stability study supporting the original 510(k) (K200129). Supplemental to the data leveraged from K200129, open kit stability was evaluated using one amplification reagent lot and four endometrial cancer samples (2 MSS and 2 MSI-H). The timepoints tested included baseline (T0), 1, 2, 3, and 4 months at +2°C to +10°C. Assessment of stability was completed by calculating concordance between T0 and all subsequent timepoints.

The sample-level agreement was 100% for all timepoints tested. The results demonstrated that the reagents were stable for 3 months at +2°C to +10°C.



**c. Ship Stress Stability**

This study was performed to evaluate reagent stability for the OncoMate MSI Dx Analysis System when exposed to challenging shipping conditions. The stability study design and reagent lot kits used were leveraged from the stability study supporting the original 510(k) (K200129). Ship stress stability was assessed using four amplification reagent lots and four endometrial cancer samples (2 MSS and 2 MSI-H). Reagent lots underwent five freeze/thaw cycles prior to exposure to one of the following ship stress conditions:

- i. Summer Ship Stress: Reagent kit lots packed in shipping containers and stored at ambient temperature for 5 days total
- ii. Winter Ship Stress: Reagent kit lots packed in dry ice shipping containers for 5 days total

Assessment of stability was completed by calculating concordance between non-shipped and shipped reagent lots. The sample-level agreement was 100% for all datapoints. The results demonstrated that the reagents can withstand winter and summer shipping conditions.

**d. Slide Stability**

This study evaluated the stability of the BAT-25, BAT-26, NR-21, NR-24 and MONO-27 loci in FFPE endometrial cancer tissue sectioned and mounted on glass slides and stored at 20–25°C by comparing sample-level MSI results generated at baseline (T0) and later time points. Stability of FFPE tissue sections was evaluated after 6, 12, 18, and 24 months of storage at 20–25°C and compared to baseline MSI status.

100% of sample-level MSI results and  $\geq 95\%$  locus-level stability calls were concordant results across all timepoints with T0. Based on the results of this study, the recommended cut slide stability is 18 months at the 20–25°C storage condition.

**e. Whole Blood Stability**

Stability of whole blood collected in BD Vacutainer K2EDTA tubes was assessed using whole blood from 16 healthy donors (i.e., MSS) exposed to winter and summer shipping conditions and routine laboratory storage and handling. A comparison of OncoMate MSI Dx Analysis System results from whole blood at baseline (T0) to results from whole blood exposed to specified temperatures at subsequent timepoints was performed. Since no matched tumor sample was assessed in this study, the baseline condition was considered as the “normal” sample and the experimental conditions were considered as the “tumor” sample, and this is how the final sample- and locus-level stability calls were made.

For all temperature profiles and all time points, 100% of sample results were “MSS”. Based on the results of this study, whole blood collected into BD

Vacutainer K2EDTA tubes is stable for use with the OncoMate MSI Dx Analysis System following winter and summer shipping, refrigeration (2–10°C) or room-temperature storage (18–23°C) for 2 weeks, and frozen storage (–30°C to –10°C) for 1 month.

**f. Amplified Product Stability**

Stability of amplified (“intermediate”) PCR products generated using the OncoMate MSI Dx Analysis System was assessed by amplifying DNA purified from six endometrial cancer sample pairs (3 MSS and 3 MSI-H), consisting of tumor FFPE tissue and matched normal blood, and storing the amplified products at either 2–10°C or –30°C to –10°C. The MSI results generated at a given time point were compared to results observed at Time 0 to assess stability. The timepoints tested which were compared to baseline were 8 days (for assessment of the 2–10°C storage condition) and 5 weeks (for assessment of the –30°C to –10°C storage condition).

Across all storage conditions and time points, 100% of sample MSI results were concordant with the results at Time 0. Based on the results of this study, amplified products generated using the OncoMate MSI Dx Analysis System are stable when stored at 2–10°C for up to 7 days or at –30°C to –10°C for up to 30 days.

**g. Extracted DNA Stability**

This study evaluated the stability of DNA purified from 3 MSI-H and 1 MSS endometrial cancer specimens and stored at –30°C to –10°C. A comparison of MSI results generated at a baseline time point (T0) to subsequent time points (5, 27, 53, 79, 106, and 161 weeks) was performed and agreement was assessed.

100% of the MSI results at each time point were concordant with Time 0 results. Based on the results of this study, DNA eluates from various DNA extraction methods using FFPE and blood samples are stable when stored up to 12 months at –30°C to –10°C.

**6. General Lab Equipment and Reagent Evaluation**

**a. Thermal Cycler Compatibility**

To demonstrate thermal cycler compatibility, data generated using three different thermal cycler models during the K200129 Analytical Specificity study were reanalyzed using the OncoMate MSI Dx Analysis Software to determine amplicon sizes. The results of this analysis demonstrated that the OncoMate MSI Dx Analysis System is compatible with multiple thermal cycler models. Refer to Section 3.5 of the OncoMate MSI Dx Analysis System Instructions for Use of Product Technical Manual for a list of thermal cycler specifications for OncoMate MSI Dx Analysis System amplification reactions.

## **7. Robustness**

### **a. Guard Banding**

The robustness of the OncoMate MSI Dx Analysis System formulations and methods were evaluated by varying amplification conditions from those in the instructions for use using endometrial cancer specimens. The sample-level MSI results observed at each test condition were compared to the applicable MSI results at the nominal condition, and PPA, NPA, OPA and two-sided 95% CI across all tumor samples were calculated. For each of the eight test conditions, sample-level MSI result PPA was 100% (27/27), with a lower 95% CI limit of 87.2%; NPA was 100% (54/54), with a lower 95% CI limit of 93.4%; and OPA was 100% (81/81), with a lower 95% CI limit of 95.6%.

Based on the results of this study, the OncoMate MSI Dx Analysis System is robust to the varied test conditions evaluated.

## **8. Matrix Equivalence of Extracted Tissue and Blood**

The Matrix Equivalence study assessed the equivalence of matched normal FFPE tissue and matched normal blood samples when determining MSI status using the OncoMate MSI Dx Analysis System.

DNA extractions were performed with three lots each of both the Maxwell CSC DNA FFPE and Maxwell CSC Blood DNA Kits using matched tumor and normal FFPE samples and matched normal blood samples. Two DNA purification runs were performed per lot and per sample. Seven MSI-H and two MSS endometrial cancer tumor samples were used, including samples at/near the LoD. The study generated 54 MSI results using tumor FFPE samples with matched normal blood samples and 54 MSI results using tumor FFPE samples with matched normal FFPE samples.

For each donor, the MSI result obtained using the FFPE sample as the matched normal sample type was compared to the MSI result obtained using blood as the matched normal sample type. PPA, NPA, and OPA estimates with two-sided 95% confidence intervals (95% CI) were calculated.

PPA and NPA values were both 100% (12/12 and 42/42, respectively), with lower 95% CI limits of 73.5% and 91.6% respectively; OPA was 100% with a lower 95% CI limit of 93.4%. DNA yields for the three intended-use sample types (tumor FFPE tissue, normal FFPE tissue and normal whole blood) were also evaluated. All extractions produced a DNA concentration  $\geq 0.17$  ng/ $\mu$ l, the minimum yield required for a 1 ng DNA input when using the maximum DNA eluate volume of 6  $\mu$ l for PCR.

Based on the results of this study, concordant MSI results are obtained using the OncoMate MSI Dx Analysis System when either blood or FFPE samples are used as the matched normal sample. Additionally, sufficient DNA is consistently purified from

FFPE tissue and blood samples using the Maxwell CSC DNA FFPE Kit and Maxwell CSC Blood DNA Kit for use with the OncoMate MSI Dx Analysis System.

## **9. DNA Extraction Method Equivalence**

The suitability of various DNA purification methods for use with the OncoMate MSI Dx Analysis System was assessed by comparing results generated from DNA extracts derived from FFPE endometrial tissue, normal FFPE tissue, and normal blood using six DNA extraction methods, three each for FFPE tissue and blood sample sources.

Each of the DNA extraction methods for both blood and FFPE samples were performed by two operators using two replicate extractions per sample, repeated on three nonconsecutive days. Extractions were performed on matched normal blood and endometrial cancer tumor FFPE samples (4 MSI-H and 1 MSS).

Across all DNA extraction methods and sample sources, DNA yield for 100% (540/540) of extractions was greater than or equal to the minimum DNA input amount required by the OncoMate MSI Dx Analysis System (1ng total DNA at an eluate concentration  $\geq 0.17\text{ng}/\mu\text{l}$ ). MSI results generated using FFPE tissue or blood as the matched normal sample were compared across all DNA purification methods to calculate average positive agreement (APA) and average negative agreement (ANA) values. Across all DNA purification methods evaluated, both the APA and ANA were 100% when MSI results were generated using FFPE or blood tissue as the matched normal sample.

Based on the results of this study, the OncoMate MSI Dx Analysis System is suitable for use with a range of appropriate extraction methods for blood and FFPE samples.

## **B. Animal Studies**

Not applicable.

## **C. Additional Studies**

Not applicable.

## **X. SUMMARY OF PRIMARY CLINICAL STUDY**

The clinical performance of the OncoMate MSI Dx Analysis System as a companion diagnostic (CDx) device to aid in the identification of endometrial carcinoma patients with MSS (i.e., not MSI-H) tumor status for treatment with pembrolizumab in combination with lenvatinib was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the Study 309/KEYNOTE-775 study (KEYNOTE-775).

## **A. Therapeutic Study Design**

The KEYNOTE-775/Study-309 (NCT03517449) was a multicenter, open-label, randomized, phase three trial that compared efficacy and safety of pembrolizumab in combination with lenvatinib versus treatment of physician's choice (TPC) in participants (also referred to as patients or subjects) with advanced endometrial cancer. In this study, patients were enrolled based on centrally-assessed mismatch repair protein (MMR) proficiency status, as measured by the immunohistochemistry (IHC)-based clinical trial assay (CTA). Patients were further stratified into treatment arms based on MMR status, either proficient MMR status (pMMR) or deficient MMR status (dMMR). The clinical performance of the OncoMate MSI Dx Analysis System was demonstrated through the assessment of concordance between CTA and the OncoMate MSI Dx Analysis System.

## **B. OncoMate MSI Dx Analysis System Clinical Bridging Study**

The objectives of the clinical bridging study were to determine the concordance of results for the MSI status between the OncoMate MSI Dx Analysis System (CDx) and the CTA and to demonstrate that the therapeutic drug efficacy was maintained when the OncoMate MSI Dx Analysis System was used to identify patients with MSS (not MSI-H) endometrial solid tumors from the KEYNOTE-775 study who may benefit from treatment with pembrolizumab in combination with lenvatinib.

Clinical validity of the OncoMate MSI Dx Analysis System was evaluated using remnant clinical study samples from the KEYNOTE-775 study. Agreement to the IHC-based CTA enrolling method and treatment efficacy within the CDx-identified cohorts were evaluated. When evaluating device clinical performance with KEYNOTE-775 specimens (summarized below), a MSS (not MSI-H) OncoMate MSI Dx Analysis System result is considered the positive result, and an MSI-H result is considered the negative result.

### **1. Clinical Bridging Study Inclusion and Exclusion Criteria**

All tumor specimens submitted for the KEYNOTE-775 study that also satisfied the inclusion/exclusion criteria outlined below were tested with the OncoMate MSI Dx Analysis System.

#### *Inclusion Criteria*

Specimens had to meet all the following criteria to have been included in the clinical bridging study:

- i. Human FFPE tissue (whole FFPE tissue blocks or 5µm tissue sections mounted on glass slides) from endometrial tumor cancer cases with matched normal blood from the same patient.
- ii. Tumor samples have  $\geq 30\%$  tumor content.
  - a. If the sample contains  $< 30\%$  tumor content, the sample may be macrodissected according to the laboratory's standard operating procedures, if an area containing a minimum of 30% tumor content

- can be identified and the amount of tissue is within the requirements described in the DNA extraction method instructions for use.
- iii. Purified DNA concentration  $\geq 0.17\text{ng}/\mu\text{l}$  for normal and tumor DNA eluates.

#### *Exclusion Criteria*

Samples that did not meet all relevant inclusion criteria were excluded from testing.

### **C. Accountability of PMA Cohort**

The OncoMate MSI Dx Analysis System (hereafter referred to as the “CDx”) clinical bridging study included a total of 489 (59.1%) of the 827 patient samples from the population enrolled into the KEYNOTE-775 study. A total of 338 (40.9%) samples were missing and excluded from the study due to insufficient tissue for retesting (287/827; 34.7%), failure to meet clinical bridging study inclusion criteria (17/827; 2.1%), or failure of the CDx to provide a result (34/827; 4.1%). Patient accountability is summarized in the Table 16, below.

**Table 16: Sample Accountability for the KEYNOTE-775 Clinical Bridging Study**

| Population Description                        |                            | CTA MMR Status |               | Total<br>N=827 |
|-----------------------------------------------|----------------------------|----------------|---------------|----------------|
|                                               |                            | pMMR<br>N=697  | dMMR<br>N=130 |                |
| Drug Study (KEYNOTE-775) Population Status, n |                            |                |               |                |
| Patients enrolled into<br>KEYNOTE-775         | Lenvatinib + Pembrolizumab | 346            | 65            | 411            |
|                                               | TPC                        | 351            | 65            | 416            |
| CDx Bridging Study Population Status, n       |                            |                |               |                |
| CDx Evaluable                                 |                            | 407            | 82            | 489            |
| CDx Unevaluable                               |                            | 290            | 48            | 338            |
| Final Bridging Study Population               |                            | 407            | 82            | 489            |

### **D. Study Population Demographics and Baseline Parameters**

The key patient demographics and baseline clinical characteristics of the OncoMate MSI Dx Analysis System evaluable and unevaluable populations are shown below (Table 17). Please refer to Section X.E.2, below, for information on the sensitivity analysis that was performed to evaluate the impact of missing OncoMate MSI Dx Analysis System results on the effectiveness of the device.

**Table 17: Clinical Bridging Study Population Demographics and Clinical Characteristics by CDx-evaluable/unevaluable Status**

| Characteristic                        | CDx Status           |                        | Total<br>(n=827) | P-Value <sup>d</sup> |
|---------------------------------------|----------------------|------------------------|------------------|----------------------|
|                                       | Evaluable<br>(n=489) | Unevaluable<br>(n=338) |                  |                      |
| Age (years), n (%)                    |                      |                        |                  |                      |
| <65 years                             | 257 (52.6)           | 153 (45.3)             | 410 (49.6)       | 0.0405               |
| ≥65 years                             | 232 (47.4)           | 185 (54.7)             | 417 (50.4)       |                      |
| Mean                                  | 62.9                 | 64.4                   | 63.5             |                      |
| SD                                    | 9.1                  | 9.1                    | 9.1              |                      |
| Median                                | 64.0                 | 65.0                   | 65.0             |                      |
| Range                                 | 30 to 86             | 30 to 84               | 30 to 86         |                      |
| Race, n (%)                           |                      |                        |                  |                      |
| Asian                                 | 136 (27.8)           | 41 (12.1)              | 177 (21.4)       | 2.6e-07              |
| Black or African American             | 19 (3.9)             | 12 (3.6)               | 31 (3.7)         |                      |
| White                                 | 271 (55.4)           | 236 (69.8)             | 507 (61.3)       |                      |
| Other                                 | 13 (2.7)             | 19 (5.6)               | 32 (3.9)         |                      |
| Not Reported                          | 50 (10.2)            | 30 (8.9)               | 80 (9.7)         |                      |
| Ethnicity, n (%)                      |                      |                        |                  |                      |
| Hispanic or Latino                    | 54.0 (11.0)          | 79 (23.4)              | 133 (16.1)       | 1.37e-05             |
| Not Hispanic or Latino                | 375 (76.7)           | 220 (65.1)             | 595 (71.9)       |                      |
| Not Reported                          | 60 (12.2)            | 39 (11.6)              | 99 (12.0)        |                      |
| Region <sup>a</sup> , n (%)           |                      |                        |                  |                      |
| Region 1                              | 293 (59.9)           | 181 (53.6)             | 474 (57.3)       | 0.074                |
| Region 2                              | 196 (40.1)           | 157 (46.4)             | 353 (42.7)       |                      |
| ECOG Performance Status, n (%)        |                      |                        |                  |                      |
| 0                                     | 292 (59.7)           | 195 (57.7)             | 487 (58.9)       | 0.566                |
| ≥1                                    | 197 (40.3)           | 143 (42.3)             | 340 (41.1)       |                      |
| History of Pelvic Radiation, n (%)    |                      |                        |                  |                      |
| Yes                                   | 197 (40.3)           | 163 (48.2)             | 360 (43.5)       | 0.027                |
| No                                    | 292 (59.7)           | 175 (51.8)             | 467 (56.5)       |                      |
| Histology of Initial Diagnosis, n (%) |                      |                        |                  |                      |
| Endometrioid carcinoma                | 286 (58.5)           | 211 (62.4)             | 497 (60.1)       | 0.279                |
| Serous carcinoma                      | 134 (27.4)           | 84 (24.9)              | 218 (26.3)       |                      |
| Clear cell carcinoma                  | 26 (5.3)             | 21 (6.2)               | 47 (5.7)         |                      |



| Characteristic                                                                            | CDx Status           |                        | Total<br>(n=827) | P-Value <sup>d</sup> |
|-------------------------------------------------------------------------------------------|----------------------|------------------------|------------------|----------------------|
|                                                                                           | Evaluable<br>(n=489) | Unevaluable<br>(n=338) |                  |                      |
| Mixed cell carcinoma                                                                      | 23 (4.7)             | 15 (4.4)               | 38 (4.6)         |                      |
| Other                                                                                     | 20 (4.1)             | 7 (2.1)                | 27 (3.3)         |                      |
| FIGO Stage at Initial Diagnosis, n (%)                                                    |                      |                        |                  |                      |
| Stage 0                                                                                   | 0                    | 0                      | 0                | 0.189                |
| Stage I                                                                                   | 147 (30.1)           | 103 (30.4)             | 250 (30.2)       |                      |
| Stage II                                                                                  | 26 (5.3)             | 32 (9.5)               | 58 (7.0)         |                      |
| Stage III                                                                                 | 147 (30.1)           | 99 (29.3)              | 246 (29.7)       |                      |
| Stage IV                                                                                  | 169 (34.5)           | 104 (30.8)             | 273 (33.0)       |                      |
| Brain - Primary Lesion or Metastasis at Study Enrollment, n (%) <sup>b</sup>              |                      |                        |                  |                      |
| Yes                                                                                       | 4 (0.8)              | 0 (0.0)                | 4 (0.5)          | N/A*                 |
| No                                                                                        | 485 (99.2)           | 338 (100)              | 823 (99.5)       |                      |
| Bone - Primary Lesion or Metastasis at Study Enrollment, n (%) <sup>b</sup>               |                      |                        |                  |                      |
| Yes                                                                                       | 45 (9.2)             | 31 (9.2)               | 76 (9.2)         | 1                    |
| No                                                                                        | 444 (90.8)           | 307 (90.8)             | 751 (90.8)       |                      |
| Liver - Primary Lesion or Metastasis at Study Enrollment, n (%) <sup>b</sup>              |                      |                        |                  |                      |
| Yes                                                                                       | 120 (24.5)           | 85 (25.1)              | 205 (24.8)       | 0.87                 |
| No                                                                                        | 369 (75.5)           | 253 (74.9)             | 622 (75.2)       |                      |
| Lung - Primary Lesion or Metastasis at Study Enrollment, n (%) <sup>b</sup>               |                      |                        |                  |                      |
| Yes                                                                                       | 180 (36.8)           | 143 (42.3)             | 323 (39.1)       | 0.128                |
| No                                                                                        | 309 (63.2)           | 195 (57.7)             | 504 (60.9)       |                      |
| Intra-abdominal - Primary Lesion or Metastasis at Study Enrollment, n (%) <sup>b, c</sup> |                      |                        |                  |                      |
| Yes                                                                                       | 230 (47.0)           | 151 (44.7)             | 381 (46.1)       | 0.523                |
| No                                                                                        | 259 (53.0)           | 187 (55.3)             | 446 (53.9)       |                      |
| Lymph Node - Primary Lesion or Metastasis at Study Enrollment, n (%) <sup>b</sup>         |                      |                        |                  |                      |
| Yes                                                                                       | 287 (58.7)           | 205 (60.7)             | 492 (59.5)       | 0.614                |
| No                                                                                        | 202 (41.3)           | 133 (39.3)             | 335 (40.5)       |                      |
| Brain - Primary Lesion or Metastasis at Initial Diagnosis, n (%) <sup>b</sup>             |                      |                        |                  |                      |
| Yes                                                                                       | 0 (0)                | 1 (0.3)                | 1 (0.1)          | N/A*                 |
| No                                                                                        | 489 (100.0)          | 337 (99.7)             | 826 (99.9)       |                      |
| Bone - Primary Lesion or Metastasis at Initial Diagnosis, n (%) <sup>b</sup>              |                      |                        |                  |                      |
| Yes                                                                                       | 12 (2.5)             | 9 (2.7)                | 21 (2.5)         | 0.827                |
| No                                                                                        | 477 (97.5)           | 329 (97.3)             | 806 (97.5)       |                      |

| Characteristic                                                                             | CDx Status           |                        | Total<br>(n=827) | P-Value <sup>d</sup> |
|--------------------------------------------------------------------------------------------|----------------------|------------------------|------------------|----------------------|
|                                                                                            | Evaluable<br>(n=489) | Unevaluable<br>(n=338) |                  |                      |
| Liver - Primary Lesion or Metastasis at Initial Diagnosis, n (%) <sup>b</sup>              |                      |                        |                  |                      |
| Yes                                                                                        | 24 (4.9)             | 12 (3.6)               | 36 (4.4)         | 0.39                 |
| No                                                                                         | 465 (95.1)           | 326 (96.4)             | 791 (95.6)       |                      |
| Lung - Primary Lesion or Metastasis at Initial Diagnosis, n (%) <sup>b</sup>               |                      |                        |                  |                      |
| Yes                                                                                        | 28 (5.7)             | 20 (5.9)               | 48 (5.8)         | 1                    |
| No                                                                                         | 461 (94.3)           | 318 (94.1)             | 779 (94.2)       |                      |
| Intra-abdominal - Primary Lesion or Metastasis at Initial Diagnosis, n (%) <sup>b, c</sup> |                      |                        |                  |                      |
| Yes                                                                                        | 125 (25.6)           | 75 (22.2)              | 200 (24.2)       | 0.283                |
| No                                                                                         | 364 (74.4)           | 263 (77.8)             | 627 (75.8)       |                      |
| Lymph Node - Primary Lesion or Metastasis at Initial Diagnosis, n (%) <sup>b</sup>         |                      |                        |                  |                      |
| Yes                                                                                        | 165 (33.7)           | 100 (29.6)             | 265 (32.0)       | 0.225                |
| No                                                                                         | 324 (66.3)           | 238 (70.4)             | 562 (68.0)       |                      |

SD: Standard Deviation

<sup>a</sup> Region 1: Europe, USA, Canada, Australia, New Zealand, Israel; Region 2: Rest of World.

<sup>b</sup> Lesion location as determined by investigator review

<sup>c</sup> Includes reported locations of colon, abdominal cavity, omentum, small intestine, peritoneal cavity, and peritoneum. Does not include lymph nodes or other organs.

<sup>d</sup> T-test for continuous covariate and fisher exact test for categorical and ordinal covariate between CDx-evaluable and CDx-unevaluable populations

\* Covariate excluded from significance assessment and from imputation model due to very small sample size

## **E. Safety and Effectiveness Results**

### **1. Safety Results**

The safety with respect to treatment with pembrolizumab in combination with lenvatinib, or pembrolizumab as a single agent, in MSS (not MSI-H) endometrial cancer was addressed in the pembrolizumab (BLA #125514/S-105) and lenvatinib (NDA #206947/S-20) submissions and is summarized in their respective drug labels. Please refer to [Drugs@FDA](#) for complete safety information on pembrolizumab in combination with lenvatinib.

No adverse events were reported in the conduct of the diagnostic study to support this PMA as testing by the OncoMate MSI Dx Analysis System was done retrospectively on banked study specimens and test results were not reported to patients.

## 2. Effectiveness Results

### Concordance Results between CTA and OncoMate MSI Dx Analysis System

The concordance analysis between patients assigned pMMR or dMMR status by the CTA and a MSS (not MSI-H) or MSI-H status by the OncoMate MSI Dx Analysis System is shown in Table 18 below. Of the 489 participants that had evaluable CTA results, the OncoMate MSI Dx Analysis System determined 409 to be MSS (not MSI-H) and 80 to be MSI-H. The estimates for PPA (MSS vs pMMR), NPA (MSI-H vs dMMR) and OPA were 98.8% (95% CI: 97.2, 99.5) , 91.5% (95% CI: 83.4, 95.8) and 97.5% (95% CI: 95.8, 98.6), respectively.

**Table 18: Concordance Between OncoMate MSI Dx Analysis System and CTA Testing**

| OncoMate MSI Dx Analysis System | CTA MMR IHC Panel         |      | Total |
|---------------------------------|---------------------------|------|-------|
|                                 | pMMR                      | dMMR |       |
| MSS (not MSI-H)                 | 402                       | 7    | 409   |
| MSI-H                           | 5                         | 75   | 80    |
| Total                           | 407                       | 82   | 489   |
| PPA                             | 98.8 (95% CI: 97.2, 99.5) |      |       |
| NPA                             | 91.5 (95% CI: 83.4, 95.8) |      |       |
| OPA                             | 97.5 (95% CI: 95.8, 98.6) |      |       |

The discordance between the CTA and the OncoMate MSI Dx Analysis System (CDx) were evaluated. Of the 5 samples which were pMMR by the CTA and MSI-H by the CDx, all 5 were found to have instability at all five biomarker loci. Two were in the pembrolizumab in combination with lenvatinib treatment arm of the KEYNOTE-775 study and neither responded to the treatment. Of the 7 samples which were dMMR by the CTA and MSS (not MSI-H) by the CDx, 4 were in the pembrolizumab in combination with lenvatinib treatment arm of the KEYNOTE-775 study and none responded to the treatment. Of these 4 samples which were dMMR by the IHC assay/MSS by the OncoMate MSI Dx Analysis System, 3 were close to the tumor content percent limit of detection (1 – 2X LoD). The remaining 1 discordant sample, although not considered challenging, resulted in a false-positive call. Please refer to Section XIV.C for discussion of the clinical benefit-risk assessment for this device regarding false positive or false negative calls.

### Clinical Efficacy Results

In the clinical bridging study, the efficacy of pembrolizumab in combination with lenvatinib was evaluated in the KEYNOTE-775 clinical study subjects whose

tumors were MSS [not MSI-H (n=409)] as defined by OncoMate MSI Dx Analysis System (Table 17). Of the subjects who were MSS (not MSI-H) by the CDx, the overall survival (OS) per BICR benefit by pembrolizumab in combination with lenvatinib over TPC (HR = 0.60) was observed. Median OS per BICR was 18.0 months (95% CI: 14.0, 20.5), compared to 11.3 months for TPC (95% CI: 10.0, 12.8). These results are comparable to the primary efficacy population which had pMMR status by the CTA. Specifically, the OS per BICR benefit by pembrolizumab in combination with lenvatinib over TPC (HR = 0.68) and a median OS per BICR was 17.4 months (95% CI: 14.2, 19.9), compared to 12.0 months for TPC (95% CI: 10.8, 13.3) were observed.

Of the subjects who were MSS (not MSI-H) by the CDx, the progression-free survival (PFS) per BICR benefit by pembrolizumab in combination with lenvatinib over TPC (HR = 0.59) was observed. Median PFS per BICR was 7.2 months (95% CI: 5.6, 7.6), compared to 3.7 months for TPC (95% CI: 3.6, 5.4). These results are comparable to the primary efficacy patient population which were pMMR status by the CTA. Specifically, in this population, the PFS per BICR benefit by pembrolizumab in combination with lenvatinib over TPC (HR = 0.60) and a median PFS per BICR was 6.6 months (95% CI: 5.6, 7.4), compared to 3.8 months for TPC (95% CI: 3.6, 5.0) were observed.

The overall response rate (ORR) results for the MSS (not MSI-H) by CDx population (ORR: 32.6%) is comparable to that for the patients which had pMMR status by the CTA (ORR: 30.3%). The median duration of response (DoR) for the MSS (not MSI-H) by CDx population was 9.2 months, which is comparable to the DoR for the pMMR status by CTA population (9.2 months).

These results are summarized below in Table 19 and in Figures 2 and 3.

**Table 19: Clinical Benefit of Pembrolizumab in Combination with Lenvatinib Estimated from KEYNOTE-775**

| Endpoint                                 | pMMR status by CTA<br>(n=697)     |                         | MSS (not MSI-H)<br>by CDx* (n=409) |                         | CDx unevaluable<br>(n=338)        |                         |
|------------------------------------------|-----------------------------------|-------------------------|------------------------------------|-------------------------|-----------------------------------|-------------------------|
|                                          | Pembro +<br>Lenvatinib<br>(N=346) | TPC<br>group<br>(N=351) | Pembro +<br>Lenvatinib<br>(N=218)  | TPC<br>group<br>(N=191) | Pembro +<br>Lenvatinib<br>(N=155) | TPC<br>group<br>(N=183) |
| <b>OS median in months<br/>(95% CI)</b>  | 17.4<br>(14.2, 19.9)              | 12.0<br>(10.8, 13.3)    | 18.0<br>(14.0, 20.5)               | 11.3<br>(10.0, 12.8)    | 17.1<br>(12.2, 20.0)              | 13.0<br>(10.7, 15.2)    |
| <b>OS HR<sup>1</sup> (95% CI)</b>        | 0.68 (0.56, 0.84)                 |                         | 0.60 (0.46, 0.78)                  |                         | 0.80 (0.59, 1.08)                 |                         |
| <b>PFS median in months<br/>(95% CI)</b> | 6.6<br>(5.6, 7.4)                 | 3.8<br>(3.6, 5.0)       | 7.2<br>(5.6, 7.6)                  | 3.7<br>(3.6, 5.4)       | 6.1<br>(5.4, 7.5)                 | 4.1<br>(3.5, 5.6)       |
| <b>PFS HR<sup>1</sup> (95% CI)</b>       | 0.60 (0.50, 0.72)                 |                         | 0.59 (0.47, 0.74)                  |                         | 0.63 (0.48, 0.83)                 |                         |
| <b>ORR<sup>2</sup> (%)</b>               | 30.3<br>(25.5, 35.5)              | 15.1<br>(11.5, 19.3)    | 32.6<br>(26.4, 39.2)               | 15.7<br>(10.9, 21.7)    | 26.5<br>(19.7, 34.1)              | 15.8<br>(10.9, 22.0)    |

|                                                          |                     |                     |                     |                     |                      |                     |
|----------------------------------------------------------|---------------------|---------------------|---------------------|---------------------|----------------------|---------------------|
| <b>ORR<sup>2</sup> Difference (%)</b><br><b>(95% CI)</b> | 15.2 (9.1, 21.4)    |                     | 16.9 (8.6, 24.9)    |                     | 10.6 (1.9, 19.5)     |                     |
| <b>DOR median in months</b><br><b>(range)</b>            | 9.2<br>(1.6, 23.7+) | 5.7<br>(0.0, 24.2+) | 9.2<br>(1.6, 23.7+) | 5.8<br>(1.8, 24.2+) | 10.9<br>(1.6, 18.8+) | 5.2<br>(0.0, 15.6+) |

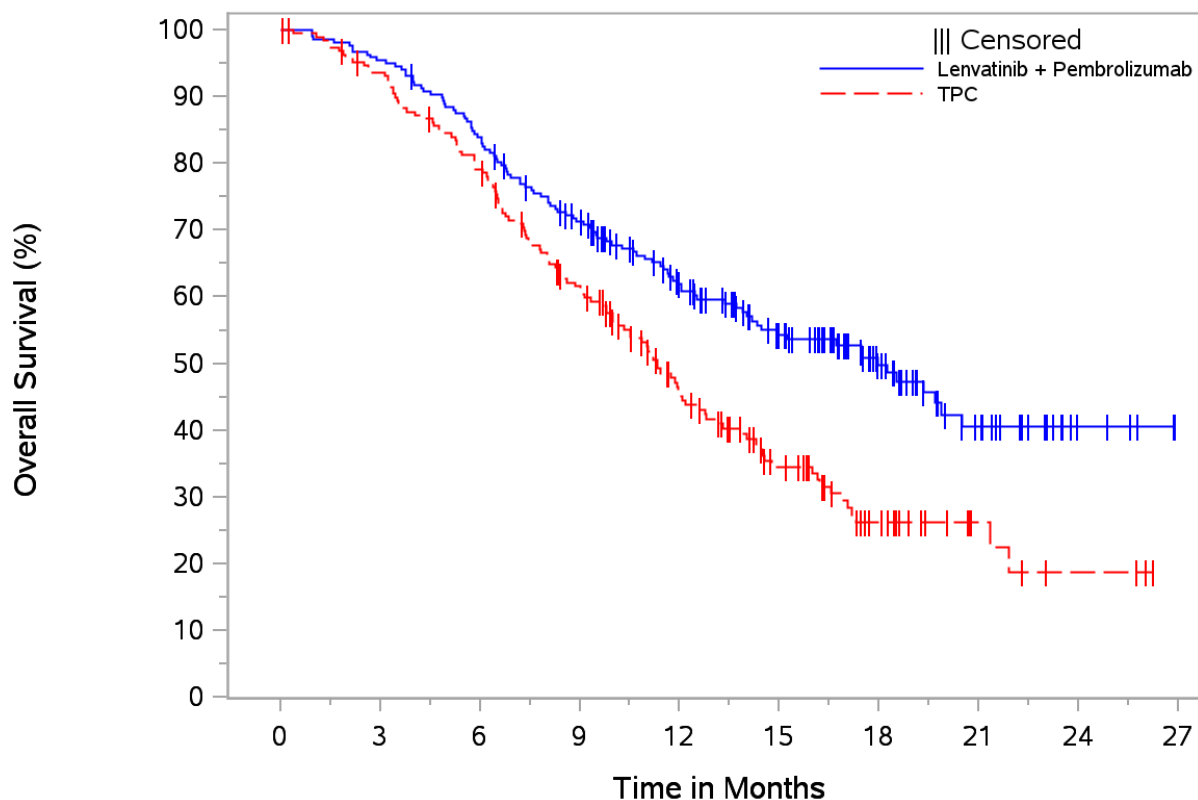
OS: Overall Survival. PFS: Progression-Free Survival. ORR: Overall Response Rate. DOR: Duration of Response

\*MSS (not MSI-H) population consists of n=402 participants with pMMR status by CTA and MSS (not MSI-H) by the CDx (drug efficacy population) and n=7 participants with dMMR status by the CTA and MSS (not MSI-H) by the CDx. The n=7 participants with dMMR status by CTA were not included in the n=697 drug efficacy population from KEYNOTE-775. The KEYNOTE-775 study enrolled 827 subjects total, with 697 classified as pMMR and 130 as dMMR by the CTA. When evaluating participants using the CDx, the total population was 747 subjects (combining CDx-evaluable and CDx-unevaluable participants). The discrepancy between the pMMR population by CTA (697 subjects) and the total CDx population (747 subjects) occurs because the CDx analysis included dMMR participants when assessing clinical benefit.

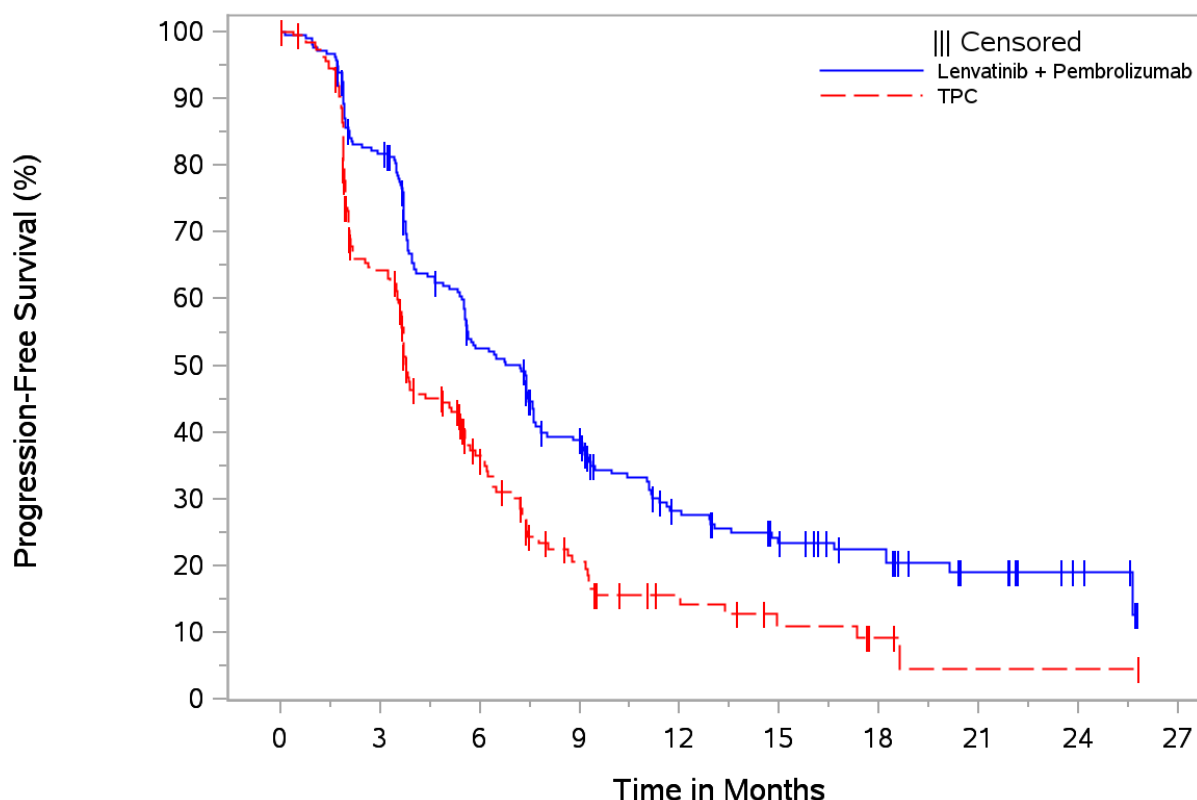
<sup>1</sup>Based on the stratified Cox regression model for the CTA-identified pMMR participants and on non-stratified Cox regression model for the other two populations

<sup>2</sup>Response: Best objective response as confirmed complete response or partial response

**Figure 2: Kaplan-Meier Estimates of Overall Survival Observed in MSS (not MSI-H) by CDx Participants**



**Figure 3: Kaplan-Meier Estimates of Progression-Free Survival Observed in MSS (not MSI-H) by CDx Participants**



### Sensitivity Analysis

Sensitivity analyses with regard to the missing CDx test results were conducted to evaluate the robustness of the efficacy estimates considering OncoMate MSI Dx Analysis System unevaluable patients enrolled in the KEYNOTE-775 trial. To conduct a missing data sensitivity analysis, data sets were created through multiple imputation, each containing the observed CDx results and imputed CDx results when missing. Efficacy endpoint estimates and hypothesis tests were conducted on each complete data set and summarized over the multiple imputations. The final imputation model included the covariates which were found to be predictive of CDx MSI status after adjusting for the CTA MMR status.

The imputed PFS HR and OS HR was estimated to be 0.60 (95% CI: 0.59, 0.61) and 0.68 (95% CI: 0.56, 0.84), respectively. This is comparable to the PFS HR and OS HR for the CTA-positive population (i.e., pMMR) [0.60 (0.50, 0.72) and 0.68 (0.56, 0.84), respectively]. The imputed ORR was estimated to be 30.1% (95% CI: 29.7, 30.5), which is comparable to the ORR for the CTA-positive population [30.3% (25.5, 35.5)]. The imputed median DOR was estimated to be

9.2 months, which is comparable to the median DOR for the CTA-positive population (9.2 months)

### 3. Pediatric Extrapolation

In this premarket application, existing clinical data was leveraged to support approval of a pediatric patient population.

Patients aged 18 years and older were eligible for enrollment into the KEYNOTE-775 trial if they fulfilled other inclusion criteria. The youngest patient enrolled in the study was 30 years old. Data from adult patients aged 30 years and older in the KEYNOTE-775 trial are considered generalizable to the pediatric population aged 18-21 years and can be relied on to establish safety and effectiveness within the 18-21 years age group, as discussed below.

From a technical standpoint, polymerase chain reaction (PCR)-based microsatellite instability (MSI) assays function by comparing tumor DNA to matched normal DNA to detect instability in microsatellite regions—short, repetitive DNA sequences. These assays are validated to identify instability regardless of patient age, as the PCR amplification process is not influenced by age-related factors.

There is no evidence of age-related attenuation or resistance to MSI signal detection. Microsatellite repeat patterns are quasi-monomorphic in germline DNA, so their lengths in pediatric tissues are equivalent to those observed in adult tissues. The underlying mechanism of MSI (replication slippage with defective mismatch repair) is conserved across age and tumor types. In the presence of MSI, length alterations in established pentaplex markers (BAT-25, BAT-26, NR-21, NR-24 and MONO-27) will be equally detectable and interpretable in pediatric and adult endometrial tumors using standard MSI PCR protocols.

From a scientific and technical standpoint, pediatric tumor samples are expected to yield results consistent with adult samples when subjected to MSI PCR analysis and, therefore, the data supporting the safety and effectiveness of the Promega OncoMate MSI Dx Analysis System presented in this premarket submission can be extrapolated to pediatric patients up 18 to 21 years of age.

## **XI. FINANCIAL DISCLOSURE**

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



## **XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION**

Not applicable.

## **XIII. 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 Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

## **XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES**

### **A. Effectiveness Conclusions**

Analytical performance studies were conducted with the OncoMate MSI Dx Analysis System using FFPE tissue from endometrial cancer tumor specimens. The performance for detecting the MSI status of the five biomarkers, when the test is used in accordance with the directions provided, has been evaluated based on clinical and non-clinical studies conducted for the device as described above. The clinical benefit of the OncoMate MSI Dx Analysis System in the detection of Not-MSI-H (MSS) status in endometrial carcinoma patients for treatment with pembrolizumab and lenvatinib combination therapy was demonstrated through a clinical bridging study of participants from the KEYNOTE-775 clinical trial. The concordance observed between the OncoMate MSI Dx Analysis System and the clinical trial assay (CTA) used for enrollment into the KEYNOTE-775 clinical trial supports the effectiveness of the OncoMate MSI Dx Analysis System to identify patients with not MSI-H endometrial solid tumors who may benefit from treatment with pembrolizumab in combination with lenvatinib in accordance with the approved therapeutic product labeling.

### **B. Safety Conclusions**

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

The OncoMate MSI Dx Analysis System is an IVD test, performed using DNA extracted from FFPE tumor tissue. Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results and/or inappropriate patient management decision in cancer treatment. Patients with false positive results may undergo treatment with pembrolizumab in combination with lenvatinib and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy, from which they might have received meaningful clinical benefit. There is

also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

### **C. Benefit-Risk Determination**

#### **Probable Benefit:**

The probable benefits of the OncoMate MSI Dx Analysis System for identification of not-MSI-H endometrial carcinoma patients for treatment with KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib) are based on data collected in the KEYNOTE-775 clinical trial and the clinical bridging study. The clinical benefit of the OncoMate MSI Dx Analysis System for the selection of endometrial carcinoma patients with not-MSI-H tumor status was demonstrated in a retrospective bridging study using samples from patients with advanced endometrial cancer who had been previously treated with at least one prior platinum-based chemotherapy regimen enrolled in KEYNOTE-775.

Clinical efficacy of MSS (not MSI-H) patients identified by the OncoMate MSI Dx Analysis System demonstrated significant clinical benefit, for treatment with KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib), compared to treatment of physician's choice, with a progression-free survival hazard ratio of 0.59 (95% CI: 0.47, 0.74) and median PFS of 7.2 months (95% CI: 5.6, 7.6) versus 3.7 months (95% CI: 3.6, 5.4) for the control arm. Overall survival showed a hazard ratio of 0.60 (95% CI: 0.46, 0.78) with median OS of 18.0 months (95% CI: 14.0, 20.5), for treatment with KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib), versus 11.3 months (95% CI: 10.0, 12.8) for the control arm. Objective response rate was 32.6% (95% CI: 26.4, 39.2) versus 15.7% (95% CI: 10.9, 21.7) for the control arm. These results maintained the efficacy observed in the NDA intent-to-treat (ITT) population. Sensitivity analyses accounting for missing OncoMate MSI Dx Analysis System results demonstrated robust efficacy estimates with imputed PFS HR of 0.60 (95% CI: 0.59, 0.61), OS HR of 0.68 (95% CI: 0.56, 0.84), and ORR of 30.1% (95% CI: 29.7, 30.5), confirming the reliability of the primary analysis results.

This supports that the OncoMate MSI Dx Analysis System provides meaningful clinical benefit, in selecting not-MSI-H patients with advanced endometrial carcinoma for treatment with KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib), providing clear evidence for the benefit of this device in identification of the correct patient population, for treatment with KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib), in this population with significant unmet medical need.

#### **Probable Risks:**

There is potential risk associated with the use of this device, mainly due to 1) false positive, false negative, or failure to provide a result, and 2) incorrect interpretation of test results by the user. The risks of the OncoMate MSI Dx Analysis System are associated with the potential mismanagement of patients resulting from false results

of the test. Patients who are determined to be false positive by the test may be exposed to KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib) treatment that is not beneficial, which may lead to adverse events or delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing potentially beneficial KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib) therapy. The risk of false results is partially mitigated by clinical and analytical studies presented above. The clinical and analytical performance of the device demonstrate that the assay is expected to perform with reasonable accuracy, mitigating the potential for false results.

**Patient Perspective:**

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

In conclusion, given the available information above, the data support that the probable benefits outweigh the probable risks for selecting patients with MSS (not MSI-H) endometrial solid tumors for treatment with KEYTRUDA (pembrolizumab) in combination with LENVIMA (lenvatinib).

**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. Data from the analytical and clinical validation studies support the performance of the OncoMate MSI Dx Analysis System as an aid in identifying patients with MSS (not MSI-H) endometrial solid tumors who may benefit from treatment with pembrolizumab in combination with lenvatinib in accordance with the approved therapeutic product labeling.

**XV. CDRH DECISION**

CDRH issued an approval order on November 5, 2025.

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

**XVI. 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.