SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Nucleic Acid Based Assay, Microsatellite Instability
Device Trade Name:	OncoMate® MSI Dx Analysis System
Device Procode:	SFL
Applicant's Name and Address:	Promega Corporation. 2800 Woods Hollow Rd, Madison, WI 53711
Date(s) of Panel Recommendation:	None

Premarket Approval Application (PMA) Number: P250046

Date of FDA Notice of Approval: September 15, 2026

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) solid tumor 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 who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1 OncoMate® MSI Dx Analysis System Companion Diagnostic Indications

Indication	Biomarker	Therapy
Endometrial Carcinoma	MSS; defined as not MSI-high [not MSI-H]	KEYTRUDA® (pembrolizumab) in combination with LENVIMA® (lenvatinib)
Solid Tumors	MSI-H	KEYTRUDA® (pembrolizumab)

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

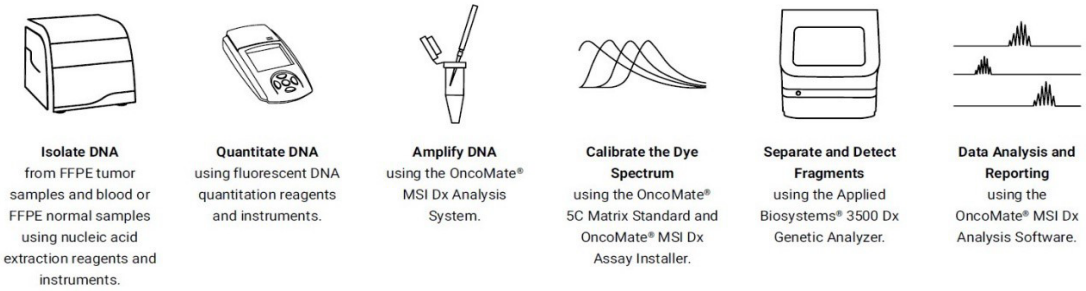
The warnings and precautions can be found in the OncoMate MSI Dx Analysis System labeling.

V. DEVICE DESCRIPTION

The 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) 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 for quality control (QC) purposes (Penta C and Penta D). Microsatellites are short, DNA-repeat regions [e.g., (A)n, (CA)n, (AAT)n, (AGAT)n, (AAAAG)n] 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 (analyzed 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, MSS or Invalid sample result. For the pan solid tumor indication, MSI-H is the positive result and MSS is the negative result. If sample data are not suitable for result interpretation, an Invalid result is 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 specimens obtained from the same patient. FFPE tumor tissue is used for tumor analysis, and either whole blood or FFPE normal tissue can be used as the required matched normal specimen.

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.
Analytical validation supports performance with FFPE tumor specimens that contain ≥20% tumor cells for colorectal, gastric and small bowel tumors and ≥30% tumor cells for all other solid tumor types, including endometrial carcinoma. Specimens below the applicable tumor-content threshold may be macrodissected to enrich tumor content prior to DNA extraction. Whole blood matched normal samples were validated using 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 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, 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/µl to provide 1ng of DNA for PCR when amplification reactions (10µl) are formulated to accept the maximum DNA eluate volume (6µl).

3. DNA Target Amplification and Labeling

During 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 resulting PCR products are dye-labeled, permitting downstream detection via fluorescence. Dye-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 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 ≥2-base-pair (bp) deletion (implemented in the software as ≥1.75bp to account for 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 Section 6.3 of the OncoMate MSI Dx Analysis System Technical Manual (TM543) 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 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 amplification 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 alternative tests to assess MSI status or mismatch repair (MMR) protein expressions in tumors based on the biomarker of interest and indication for use. The approved companion diagnostic (CDx) tests are listed in Table 3. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his or her physician to select the method that best meets expectations and lifestyle.

Table 3. FDA-Approved CDx Alternatives to Assess MSI Status or MMR Proteins with a Solid Tumor Indication

Biomarker	Device	Company	Technology	Therapy/Use
MSI-H/dMMR	*VENTANA RxDx MMR Panel*	*Ventana Medical Systems, Inc.*	IHC[1]	KEYTRUDA (pembrolizumab)
	MI Cancer Seek	*Caris Life Sciences*	NGS[2]	KEYTRUDA (pembrolizumab)
	FoundationOne CDx	*Foundation Medicine, Inc.*	NGS	KEYTRUDA (pembrolizumab)

Abbreviations:
[1]IHC = Immunohistochemistry
[2]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 was cleared as a Class II device under 510(k) K200129 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. 510(k) 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 OncoMate MSI Dx Interpretive Software.

The OncoMate MSI Dx Analysis System was subsequently approved as a Class III device under PMA P240026 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. PMA P240026 was approved on November 5, 2025, by the United States FDA. The device is comprised of the OncoMate MSI Dx Analysis System, OncoMate 5C Matrix Standard, OncoMate MSI Dx Assay Installer v2.0, Applied Biosystems 3500 Dx Genetic Analyzer and OncoMate MSI Dx Analysis Software.

The OncoMate MSI Dx Analysis System is also marketed 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 configuration includes the OncoMate MSI Dx Analysis System and OncoMate 5C Matrix Standard. The device formulations and methods for the European device are identical to those for the U.S. device. However, the European device includes instructions for capillary electrophoresis instrument run method setup in place of the OncoMate MSI Dx Assay Installer and uses manual data interpretation following fragment analysis in place of software-based data interpretation.

The device has not been withdrawn from any market for any reasons 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 solid tumors. 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), which are available at Drugs@FDA.

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

Promega executed analytical validation studies to support the companion diagnostic claims indicated in the intended use statement using MSI-H and MSS specimens from 29 solid tumor tissue types, 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 and approved under PMA P240026, were leveraged for a subset of studies, including DNA interference, cross-contamination and stability

studies, to support the current PMA. The OncoMate MSI Dx Analysis System MSS test result is equivalent to a not MSI-H result.

1. Analytical Accuracy

Data from 611 solid tumor samples were used to evaluate accuracy of the OncoMate MSI Dx Analysis System by comparison to the validated immunohistochemistry (IHC) panel which detects mismatch repair (MMR) status. The results of this comparison are shown in Table 4. Of the 496 samples that yielded a result of pMMR with the IHC panel, 482 yielded an "MSS" result with the OncoMate MSI Dx Analysis System and 14 samples yielded an MSI result of "MSI-H". Of the 115 samples that yielded a result of dMMR with the IHC panel, 13 yielded an "MSS" result with the OncoMate MSI Dx Analysis System and 102 samples yielded an MSI result of "MSI-H".

Table 4. Results Generated Using the OncoMate MSI Analysis System and an MMR IHC Panel

OncoMate MSI Dx Analysis System Result	**MMR IHC Panel Result**		**Total**
	pMMR	dMMR	
MSS	482	13	495
MSI-H	14	102	116
Total	496	115	611

The discordant samples were retested using a third validated molecular assay, and agreement between the OncoMate MSI Dx and that assay was observed in 19 of 20 evaluable cases.

With the MMR IHC panel as the reference method, agreement between MSI-H and dMMR results (NPA) was 88.7%, with a 95% confidence interval of 81.6–93.3%; agreement between MSS and pMMR results (PPA) was 97.2%, with a 95% confidence interval of 95.3–98.3%; and overall agreement (OPA) was 95.6%, with a 95% confidence interval of 93.6–96.9%. These results are presented in Table 5.

Table 5. Agreement Estimates for MSI Results Generated Using the OncoMate MSI Dx Analysis System as Compared to MMR Results

	Estimate	**95% Confidence Interval**	
		Lower Limit	**Upper Limit**
MSI-H/dMMR Agreement	88.7% (102/115)	81.6%	93.3%
MSS/pMMR Agreement	97.2% (482/496)	95.3%	98.3%
Overall Agreement	95.6% (584/611)	93.6%	96.9%

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 ≥2bp (implemented in the software as ≥1.75bp to account for 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).

Nine MSS tumor sample pairs, divided equally among endometrial, gastric and breast tumors, 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 2 nonconsecutive days. Across the 9 samples, 36 replicates were analyzed in the presence of resolution markers and 36 replicates in the absence of resolution markers (72 MSI results total).

When 2 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 6) Based on the study results, 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 6. Differences in Observed Sizes of Resolution Markers

Resolution Marker Pair	Known Size Difference (bp)	Observed Average Size Difference (bp)
Res_Large 1 and Res_Large 3	2.00	2.11
Res_Large 2 and Res_Large 4	2.00	2.01
Res_Large 3 and Res_Large 5	2.00	1.91
Res_Large 4 and Res_Large 6	2.00	2.02
Res_Large 5 and Res_Large 7	2.00	2.12
Res_Small 1 and Res_Small 3	2.00	2.37
Res_Small 2 and Res_Small 4	2.00	2.21
Res_Small 3 and Res_Small 5	2.00	2.06
Res_Small 4 and Res_Small 6	2.00	2.21

Resolution Marker Pair	Known Size Difference (bp)	Observed Average Size Difference (bp)
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 3 days, 2 amplification kit lots and 5 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 (one sample each at 30% and 80% TC and 2 samples at 60% TC). Therefore, a total of 120 replicates were assessed across all samples.

All tests resulted in MSS results (100%; 120/120) with a 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 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 four-part study estimated 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 across a two-part study (part 1, determination, and part 2, confirmation). In the determination study, two MSI-H FFPE tumor samples—one endometrial and one colorectal—were analyzed at 5 different levels of tumor content (10%, 20%, 30%, 40% and the original tumor content [≥50%]) at a constant DNA input amount (1.0ng). DNA from each MSI-H sample tumor was blended with DNA from the matched normal sample to achieve the prescribed tumor contents. This LoD determination study used 2 reagent lots, with 5 runs per lot on nonconsecutive days and at least 1 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 that achieved a percent positivity ≥95% (hit rate ≥0.95) across all factors and FFPE tumor specimens was 20%. Frequencies of MSI-H results for each tumor content percentage are summarized in Table 7. Locus-level calls for each locus are shown in Table 8.

Table 7. MSI Result Frequencies for Tumor Content Limit of Detection Determination

Sample Number	Sample or Tissue Type	MSI-H Frequencies 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
1	Colorectal	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
2	Endometrial	75.0% (15/20)	90.0% (18/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)

Table 8. MSI Result Frequencies for Tumor Content Limit of Detection Determination per Locus

Sample Number	Sample or Tissue Type	Tumor Content	Known MSI Result	MSI-H Frequencies of Unstable Calls									
				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
1	Colorectal	10%	MSI-H	95.0% (19/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	90.0% (18/20)	95.0% (19/20)	100% (20/20)	100% (20/20)
		20%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		40%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		Original (50%)		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
2	Endometrial	10%	MSI-H	5.0% (1/20)	10.0% (2/20)	5.0% (1/20)	0% (0/20)	65.0% (13/20)	75.0% (15/20)	95.0% (19/20)	90.0% (18/20)	35.0% (7/20)	30.0% (6/20)
		20%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		40%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		Original (60%)		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)

To confirm the LOD for tumor content, 10 MSI-H tumor samples from 8 tissues (prostate, melanoma, endometrial, liver, gastric, ovarian, colorectal and small bowel) were tested on 5 nonconsecutive days, with 4 replicates per sample within each run. All samples were

evaluated at 30% tumor content using a 1.0ng DNA input. The 5 gastrointestinal tumor samples, which were derived from gastric, colorectal and small bowel tissues, also were evaluated at 20% tumor content and 1.0ng DNA. DNA from each MSI-H sample was blended with DNA from the matched normal sample to achieve the prescribed tumor content.

At 30% tumor content and 1.0ng DNA input, all 10 tumor samples yielded a percent positivity of 100% (hit rate of 1) for MSI-H results across 200 total replicates. At 20% tumor content and 1.0ng DNA input, all 5 gastrointestinal tumor samples yielded a percent positivity of 100% (hit rate of 1) for MSI-H results across 100 total replicates. These data are summarized in Table 9. Locus-level results are summarized in Table 10.

Table 9. MSI Result Frequencies for Tumor-Content Limit of Detection Confirmation

	Sample Number	Tissue Type	Tumor Content	Observed MSI Result		Hit Rate
				MSI-H	MSS	
Nongastrointestinal	3	Prostate	30%	100% (20/20)	0% (0/20)	1
	4	Melanoma (Skin)	30%	100% (20/20)	0% (0/20)	1
	5	Biliary-cholangio (Liver)	30%	100% (20/20)	0% (0/20)	1
	6	Ovarian	30%	100% (20/20)	0% (0/20)	1
	7	Endometrial	30%	100% (20/20)	0% (0/20)	1
Gastrointestinal	8	Small Bowel	20%	100% (20/20)	0% (0/20)	1
			30%	100% (20/20)	0% (0/20)	1
	9	Small Bowel	20%	100% (20/20)	0% (0/20)	1
			30%	100% (20/20)	0% (0/20)	1
	10	Gastric	20%	100% (20/20)	0% (0/20)	1
			30%	100% (20/20)	0% (0/20)	1
	11	Gastric	20%	100% (20/20)	0% (0/20)	1

	Sample Number	Tissue Type	Tumor Content	Observed MSI Result		Hit Rate
				MSI-H	MSS	
			30%	100% (20/20)	0% (0/20)	1
	12	Colorectal	20%	100% (20/20)	0% (0/20)	1
			30%	100% (20/20)	0% (0/20)	1
Total 30% Tumor Content Nongastrointestinal Tumors				100% (100/100)	0% (0/100)	1
Total 20% Tumor Content Gastrointestinal Tumors				100% (100/100)	0% (0/100)	1
Total 30% Tumor Content, Gastrointestinal Tumors				100% (100/100)	0% (0/100)	1
Total				100% (300/300)	0% (0/300)	1

Table 10. MSI Result Frequencies for Tumor-Content Limit of Detection Confirmation per Locus

Sample Number	Tissue Type	Tumor Content	Known MSI Result	MSI-H Frequencies of Unstable Calls				
				NR-21	BAT-25	BAT-26	NR-24	MONO-27
3	**Prostate**	30%	MSI-H	100% (20/20)	100% (20/20)	0% (0/20)[1]	100% (20/20)	100% (20/20)
4	**Skin**	30%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	95.0% (19/20)
5	**Biliary-cholangio**	30%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	90.0% (18/20)	0% (0/20)[2]
6	**Ovarian**	30%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
7	**Endometrial**	30%	MSI-H	95.0% (19/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
8	**Small Bowel**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
9	**Small Bowel**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)

Sample Number	Tissue Type	Tumor Content	Known MSI Result	MSI-H Frequencies of Unstable Calls				
				NR-21	BAT-25	BAT-26	NR-24	MONO-27
3	**Prostate**	30%	MSI-H	100% (20/20)	100% (20/20)	0% (0/20)[1]	100% (20/20)	100% (20/20)
10	**Gastric**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
11	**Gastric**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	5.0% (1/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	15.0% (3/20)	100% (20/20)
12	**Colorectal**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)

[1]The prostate tumor sample had a locus-level stability call of "Unstable" at all loci except BAT-26, which had a stability call of "Stable".

[2]The biliary-cholangio tumor sample had a locus-level stability call of "Unstable" at all loci except MONO-27, which had a stability call of "Stable".

2. Limit of Detection – DNA Input

DNA input LoD was established across a two-part study (part 1, determination and part 2, confirmation). In the determination study, a dilution series of DNA purified from 2 colorectal and 2 endometrial FFPE tumors (1 MSI-H and 1 MSS each) and matched normal tissue was performed. Each sample was analyzed at 6 different DNA input amounts (0.05ng, 0.2ng, 0.5ng, 0.75ng, 1.0ng and 1.25ng per reaction). DNA from each MSI-H sample was blended with DNA from the matched normal sample to represent the minimum tumor content requirement. All samples were assayed at 30% tumor content, while the MSI-H colorectal sample was also assayed at 20% tumor content. MSS tumor samples were analyzed at the original tumor content. Each tumor sample was analyzed across 5 nonconsecutive days using 2 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). Across samples and factors, the DNA input amount corresponding to a 95% probability of detection (C95), estimated by probit regression, was 0.76ng. Results from this determination study are summarized in Table 11 and Table 12 below.

Table 11. MSI Result Frequencies for DNA-Input Limit of Detection Determination

Sample Number	Sample or Tissue Type	Known MSI Result	Tumor Content	MSI Result Frequencies by DNA Input Amount (ng)											
				0.05ng		0.2ng		0.5ng		0.75ng		1.0ng		1.25ng	
				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	Colorectal	MSI-H	20%	0% (0/20)	0% (0/20)	80.0% (16/20)	90.0% (18/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
			30%	0% (0/20)	0% (0/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
2	Endometrial	MSI-H	30%	5.0% (1/20)	15.0% (3/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
13	Colorectal	MSS	90%	ND[1]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	Endometrial	MSS	95%	ND[1]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[1]ND = Not determined.

Table 12: MSI Result Frequencies for DNA-Input Limit of Detection Determination per Locus

Sample Number	Sample or Tissue Type	Known MSI Result	Tumor Content	DNA Input Amount	MSI Results Frequencies of Unstable Calls									
					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
1	Colorectal	MSI-H	20%	0.05ng	10.0% (2/20)	10.0% (2/20)	5.0% (1/20)	0% (0/20)	0% (0/20)	5.0% (1/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				0.2ng	75.0% (15/20)	100% (20/20)	75.0% (15/20)	90.0% (18/20)	65.0% (13/20)	55.0% (11/20)	25.0% (5/20)	45.0% (9/20)	45.0% (9/20)	50.0% (10/20)
				0.5ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	95.0% (19/20)	100% (20/20)	100% (20/20)
				0.75ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				1.0ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				1.25ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
			30%	0.05ng	30.0% (6/20)	25.0% (5/20)	5.0% (1/20)	0% (0/20)	10.0% (2/20)	10.0% (2/20)	0% (0/20)	5.0% (1/20)	0% (0/20)	0% (0/20)
				0.2ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	90.0% (18/20)	85.0% (17/20)	95.0% (19/20)	90.0% (18/20)
				0.5ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				0.75ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				1.0ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				1.25ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)

Sample Number	Sample or Tissue Type	Known MSI Result	Tumor Content	DNA Input Amount	MSI Results Frequencies of Unstable Calls									
					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
2	**Endometrial**	**MSI-H**	30%	0.05ng	85.0% (17/20)	90.0% (18/20)	60.0% (12/20)	55.0% (11/20)	50.0% (10/20)	55.0% (11/20)	20.0% (4/20)	15.0% (3/20)	35.0% (7/20)	50.0% (10/20)
				0.2ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				0.5ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				0.75ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				1.0ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
				1.25ng	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
13	**Colorectal**	**MSS**	90%	0.05ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				0.2ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				0.5ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				0.75ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				1.0ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				1.25ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
14	**Endometrial**	**MSS**	95%	0.05ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				0.2ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				0.5ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				0.75ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				1.0ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
				1.25ng	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)

To confirm the LoD for sample DNA input amount, 16 FFPE tumor samples (the same 10 MSI-H tumor samples used to verify tumor-content LoD plus an additional 6 MSS tumor samples) were tested on 5 nonconsecutive days, with 4 replicates per sample within each run using a single OncoMate MSI Dx Analysis System lot. Twenty replicates for each of ten MSI-H tumor samples were evaluated at 30% tumor content using a 0.76ng DNA input, and 20 replicates for each of 6 MSS tumor samples were evaluated at their original tumor content using a 0.76ng DNA input. MSI-H tissues evaluated comprised prostate,

melanoma, endometrial, liver, gastric, ovarian, colorectal and small bowel tumors, and MSS tissues comprised endometrial, melanoma and renal tumors. The 5 MSI-H gastrointestinal tumor samples were also evaluated with 20% tumor content and a 0.76ng DNA input.

At 30% tumor content and 0.76ng DNA input, all 10 tumor samples yielded a percent positivity of 100% (hit rate of 1) for MSI-H results across 200 total replicates. At 20% tumor content and 0.76ng DNA input, all 5 gastrointestinal tumor samples yielded a percent positivity of 100% (hit rate of 1) for MSI-H results across 100 total replicates. These data are summarized in Table 13 and Table 14. At their original tumor contents, the 6 MSS tumor samples yielded 100% correct MSS results for the 120 total replicates examined using 0.76ng DNA input.

Table 13. MSI Result Frequencies for DNA-Input Limit of Detection Confirmation

	Tissue Type	Sample Number	Known MSI Result	Tumor Content	Observed MSI Result		Hit Rate
					MSI-H	MSS	
Nongastrointestinal	**Prostate**	3	MSI-H	30%	100% (20/20)	0% (0/20)	1
	Skin	4	MSI-H	30%	100% (20/20)	0% (0/20)	1
	Biliary-cholangio (liver)	5	MSI-H	30%	100% (20/20)	0% (0/20)	1
	Ovarian	6	MSI-H	30%	100% (20/20)	0% (0/20)	1
	Endometrial	7	MSI-H	30%	100% (20/20)	0% (0/20)	1
		15	MSS	70%	0% (0/20)	100% (20/20)	1[1]
		16	MSS	90%	0% (0/20)	100% (20/20)	1[1]
	Melanoma	17	MSS	80%	0% (0/20)	100% (20/20)	1[1]
		18	MSS	90%	0% (0/20)	100% (20/20)	1[1]
	Kidney	19	MSS	98%	0% (0/20)	100% (20/20)	1[1]
		20	MSS	98%	0% (0/20)	100% (20/20)	1[1]

	Tissue Type	Sample Number	Known MSI Result	Tumor Content	Observed MSI Result		Hit Rate
					MSI-H	MSS	
Gastrointestinal	**Small Bowel**	8	MSI-H	20%	100% (20/20)	0% (0/20)	1
				30%	100% (20/20)	0% (0/20)	1
	Small Bowel	9	MSI-H	20%	100% (20/20)	0% (0/20)	1
				30%	100% (20/20)	0% (0/20)	1
	Gastric	10	MSI-H	20%	100% (20/20)	0% (0/20)	1
				30%	100% (20/20)	0% (0/20)	1
	Gastric	11	MSI-H	20%	100% (20/20)	0% (0/20)	1
				30%	100% (20/20)	0% (0/20)	1
	Colorectal	12	MSI-H	20%	100% (20/20)	0% (0/20)	1
				30%	100% (20/20)	0% (0/20)	1
[1]Hit rates for MSS tumor samples are not informative due to the absence of microsatellite instability.							

Table 14. MSI Result Frequencies for DNA-Input Limit of Detection Confirmation per Locus

Sample Number	Tissue Type	Tumor Content	Known MSI Result	MSI Results Frequencies of Unstable Calls				
				NR-21	BAT-25	BAT-26	NR-24	MONO-27
3	**Prostate**	30%	MSI-H	100% (20/20)	100% (20/20)	0% (0/20)	100% (20/20)	100% (20/20)
4	**Skin**	30%	MSI-H	100% (20/20)	100% (20/20)	95.0% (19/20)	95.0% (19/20)	85.0% (17/20)
5	**Biliary-cholangio**	30%	MSI-H	100% (20/20)	95.0% (19/20)	100% (20/20)	80.0% (16/20)	0% (0/20)
6	**Ovarian**	30%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
7	**Endometrial**	30%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)

Sample Number	Tissue Type	Tumor Content	Known MSI Result	MSI Results Frequencies of Unstable Calls				
				NR-21	BAT-25	BAT-26	NR-24	MONO-27
8	**Small Bowel**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
9	**Small Bowel**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
10	**Gastric**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
11	**Gastric**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	0% (0/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	55.0% (11/20)	100% (20/20)
12	**Colorectal**	20%	MSI-H	100% (20/20)	100% (20/20)	100% (20/20)	90.0% (18/20)	100% (20/20)
		30%		100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)	100% (20/20)
15	**Endometrial**	70%	MSS	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
16	**Endometrial**	90%	MSS	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
17	**Melanoma**	80%	MSS	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
18	**Melanoma**	90%	MSS	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
19	**Kidney**	98%	MSS	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)
20	**Kidney**	98%	MSS	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)	0% (0/20)

Based on the analytical validation studies, the established limit of detection (LoD) for the OncoMate MSI Dx Analysis System is 30% tumor content with 0.76ng DNA input for non-gastrointestinal tumor samples and 20% tumor content with 0.76ng DNA input for gastrointestinal tumor samples.

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 samples. Nine tumors were tested in this study with matched normal DNA purified from FFPE tissues: 3 endometrial cancer samples (2 MSI-H and 1 MSS), 3 FFPE gastric cancer samples (2 MSI-H and 1 MSS), 1 lung cancer sample (MSI-H), 1 colorectal cancer sample (MSI-H) and 1 breast cancer sample (MSS). The percent tumor content (%TC) for each sample is provided in Table 15. These samples were intentionally selected to span the expected range of deletion sizes to ensure analytical comprehensiveness across the solid tumor intended use. Deletions at the assay's 2bp cutoff were represented at each microsatellite locus.

Table 15. Tumor Content of Samples Used to Evaluate Precision

Tissue Type	Sample Number	Known MSI Status	Tumor Content of Tumor Sample
Endometrial	21	MSI-H	95%
	22	MSI-H	70%
	23	MSS	80%
Colorectal	24	MSI-H	30%
Breast	25	MSS	50%
Lung	26	MSI-H	80%
Gastric	27	MSS	50%
	28	MSI-H	70%
	29	MSI-H	30%
Endometrial	30	MSI-H	30%
	31	MSI-H	30%
	32	MSI-H	30%
	33	MSS	30%
	34	MSS	30%
	35	MSS	30%

To assess within-lot reproducibility, samples were analyzed on 5 nonconsecutive days using the same OncoMate MSI Dx Analysis System lot at 3 different study sites. A different operator team at each study site analyzed 5 replicates for each sample per day using a single CE instrument.

To assess between-lot reproducibility, samples were analyzed on 5 nonconsecutive days, across 5 replicates per day, using 3 OncoMate MSI Dx Analysis System lots and a single CE instrument at a single study site.

PPA and NPA values for MSS and MSI-H results, respectively, across site, day, lot or run were 100%, demonstrating high reproducibility for each factor tested. Across all study factors, PPA was 100% (750/750, 95% CI of 99.5–100%) and NPA was 100% (375/375, 95% CI of 99.0–100%). These data are shown in Table 16.

Individual loci for these FFPE tumor samples exhibited ≥88.8% agreement for all markers except BAT 25 for one MSI H sample (sample 22). This locus was identified as Stable during sample screening prior to study execution but yielded Unstable results in 58.4% (73/125) of replicates during study execution. Locus-level agreement for MSI H and MSS tumor samples are reported in Table 17.

Table 16. Agreement Estimates for Observed MSI Result Compared to Majority MSI Result by Study Site, Lot and Day

		PPA			NPA		
			95% CI			95% CI	
		Estimate	Lower Limit	Upper Limit	Estimate	Lower Limit	Upper Limit
Site	1 (Lot 1)	100% (150/150)	97.6%	100%	100% (75/75)	95.2%	100%
	2 (Lot 1)	100% (150/150)	97.6%	100%	100% (75/75)	95.2%	100%
	3 (Lot 1)	100% (150/150)	97.6%	100%	100% (75/75)	95.2%	100%
	Overall, Sites 1–3	**100% (450/450)**	**99.2%**	**100%**	**100% (225/225)**	**98.4%**	**100%**
Lot	1 (Site 1)	100% (150/150)	97.6%	100%	100% (75/75)	95.2%	100%
	2 (Site 1)	100% (150/150)	97.6%	100%	100% (75/75)	95.2%	100%
	3 (Site 1)	100% (150/150)	97.6%	100%	100% (75/75)	95.2%	100%
	Overall, Lots 1–3	**100% (450/450)**	**99.2%**	**100%**	**100% (225/225)**	**98.4%**	**100%**
Day (Lot	1	100% (90/90)	96.0%	100%	100%	92.1%	100%

		PPA			NPA		
			95% CI			95% CI	
		Estimate	Lower Limit	Upper Limit	Estimate	Lower Limit	Upper Limit
1, Sites 1–3)					(45/45)		
	2	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	3	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	4	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	5	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	Overall, Sites 1–3	**100% (450/450)**	**99.2%**	**100%**	**100% (225/225)**	**98.4%**	**100%**
Day (Site 1, Lots 1–3)	1	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	2	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	3	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	4	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	5	100% (90/90)	96.0%	100%	100% (45/45)	92.1%	100%
	Overall, Lots 1–3	**100% (450/450)**	**99.2%**	**100%**	**100% (225/225)**	**98.4%**	**100%**
Overall, Across All Factors		**100% (750/750)**	**99.5%**	**100%**	**100% (375/375)**	**99.0%**	**100%**

Table 17. Percent Agreement of Sample-Level MSI Results and Locus-Level Stability Results as Compared to the Majority Result

Sample Number	Tissue Type	Majority MSI Result	MSI Result		NR-21		BAT-25		BAT-26		NR-24		MONO-27	
			Agreement, % (n/n)	95% CI Lower Limit / 95% CI Upper Limit	Agreement, % (n/n)	95% CI Lower Limit / 95% CI Upper Limit	Agreement, % (n/n)	95% CI Lower Limit / 95% CI Upper Limit	Agreement, % (n/n)	95% CI Lower Limit / 95% CI Upper Limit	Agreement, % (n/n)	95% CI Lower Limit / 95% CI Upper Limit	Agreement, % (n/n)	95% CI Lower Limit / 95% CI Upper Limit
21	Endometrial	MSI-H	100% (125/125)	97.1% / 100%	99.2% (124/125)	95.6% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
22	Endometrial	MSI-H	100% (125/125)	97.1% / 100%	98.4% (123/125)	94.3% / 99.8%	58.4% (73/125)	49.3% / 67.2%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
23	Endometrial	MSS	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
24	Colorectal	MSI-H	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
25	Breast	MSS	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
26	Lung	MSI-H	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	99.2% (124/125)	95.6% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
27	Gastric	MSS	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
28	Gastric	MSI-H	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%
29	Gastric	MSI-H	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	100% (125/125)	97.1% / 100%	88.8% (111/125)	81.9% / 93.7%
Overall for MSI-H Tumor Samples			100% (750/750)	99.5% / 100%	99.6% (747/750)	98.8% / 99.9%	92.9% (697/750)	90.9% / 94.7%	100% (750/750)	99.5% / 100%	100% (750/750)	99.5% / 100%	98.1% (736/750)	96.9% / 99.0%
Overall for MSS Tumor Samples			100% (375/375)	99.0% / 100%	100% (375/375)	99.0% / 100%	100% (375/375)	99.0% / 100%	100% (375/375)	99.0% / 100%	100% (375/375)	99.0% / 100%	100% (375/375)	99.0% / 100%

To assess precision and reproducibility near the assay's limit of detection for tumor content, 6 endometrial tumor samples (3 MSI-H and 3 MSS) with 30% tumor content were evaluated in a supplemental reproducibility study. In this supplemental study, each sample was analyzed on 5 nonconsecutive days at 2 sites using 2 OncoMate MSI

Dx Analysis System lots. At each site, a single operator analyzed 5 replicates for each sample per day using a single CE instrument.

At the sample-level and locus-level, overall agreement for MSI-H and MSS endometrial cancer tumor samples were reported, with an overall locus-level 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 tumor samples and 98.0% (95% CI: 94.3, 99.6) for MSS endometrial tumor samples. For the MONO-27 locus, overall agreement was 86.0% (95% CI: 79.4, 91.1) for MSI-H endometrial tumor samples and 98.7% (95% CI: 95.3, 99.8) for MSS endometrial 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 samples close to the cutoff (i.e., 2bp), which resulted in 58.0% (29/50) agreement with the majority Unstable result. Sample-level and locus-level results are summarized in Table 18 and Table 19.

Table 18. Agreement Estimates for Observed MSI Result as Compared to Majority MSI Result for Samples Near the Assay's Limit of Detection for Tumor Content

		MSI-H Agreement				MSS Agreement			
				95% CI				95% CI	
		N	Observed Agreement	Lower Limit	Upper Limit	N	Observed Agreement	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%
	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%

		MSI-H Agreement				MSS Agreement			
				95% CI				95% CI	
		N	Observed Agreement	Lower Limit	Upper Limit	N	Observed Agreement	Lower Limit	Upper Limit
	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%
Overall, Across All Factors		150	100%	97.6%	100%	150	100%	97.6%	100%

Table 19. Percent Agreement of Sample-Level MSI Results and Locus-Level Stability Results Compared to the Majority Result for Samples Near the Assay's Limit of Detection for Tumor Content

		MSI Result		NR-21		BAT-25		BAT-26		NR-24		MONO-27	
			95% CI Lower Limit		95% CI Lower Limit		95% CI Lower Limit		95% CI Lower Limit		95% CI Lower Limit		95% CI Lower Limit
Sample ID	Majority MSI Result	Agreement, % (n/n)	95% CI Upper Limit	Agreement, % (n/n)	95% CI Upper Limit	Agreement, % (n/n)	95% CI Upper Limit	Agreement, % (n/n)	95% CI Upper Limit	Agreement, % (n/n)	95% CI Upper Limit	Agreement, % (n/n)	95% CI Upper Limit
30	MSI-H	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%	100% (50/50)	92.9%
			100%		100%		100%		100%		100%		100%
31	MSI-H	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%	100% (50/50)	92.9%
			100%		100%		100%		100%		100%		100%
32	MSI-H	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%	58.0% (29/50)	43.2%
			100%		100%		100%		100%		100%		71.8%
33	MSS	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%	100% (50/50)	92.9%
			100%		100%		100%		100%		100%		100%
34	MSS	100% (50/50)	92.9%	100% (50/50)	92.9%	100% (50/50)	92.9%	100% (50/50)	92.9%	94.0%[1] (47/50)	83.5%	96.0%[2] (48/50)	86.3%
			100%		100%		100%		100%		98.7%		99.5%
35	MSS	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%	100% (50/50)	92.9%
			100%		100%		100%		100%		100%		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%

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

[1] -- Three "No Call" results were observed for NR-24 because of low peak intensity in the tumor sample.

2 -- Two "No Call" results were observed at MONO-27 because of low peak intensity in the tumor sample.

4. Analytical Specificity

a. Interfering Substances

The DNA Interference study evaluated the potential effect of endogenous and exogenous interfering substances associated with FFPE tissue samples, blood, DNA purification reagents and blood collection devices on performance of the OncoMate MSI Dx Analysis System. Two supplemental studies also were performed to clarify the influence of melanin on assay performance. The studies specifically characterized the 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.

A total of 18 FFPE cancer clinical samples (five MSI-H and two MSS endometrial, five MSI-H and two MSS colorectal, two MSS gastric and two MSS kidney), along with matched normal FFPE tissue and matched normal blood samples, were tested using three different DNA extraction systems to purify 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 sample replicates (48/48 for each potential interferent) when using a matched normal sample from blood or FFPE tissue and with all three DNA extraction systems. For mucin, concordant results were obtained for 95.8% of all sample replicates (46/48). Finally, 100% of sample replicates (48/48) with high necrosis yielded concordant MSI results to sample replicates with low to no necrosis with each of the three DNA extraction systems used. See Table 20 and Table 21.

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. The reanalysis reported 100% agreement between test and control conditions.

Table 20. 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/24	n/24	n/48	n/48	Percent
Conjugated Bilirubin (475µM)	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%
EDTA (5.4mg/ml)	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%
Hemoglobin (10mg/ml)	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%
Triglycerides (16.94mM)	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%
Mucin (1mg/ml)	Tissue	22/24	24/24	2/48	46/48	95.8%
Necrosis (40–90%)	Tissue	24/24	24/24	0/48	48/48	100%
No Interferent, Water	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%

Table 21. 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/24	n/24	n/48	n/48	Percent
Melanin (0.005%)	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%
Unconjugated Bilirubin (684µM)	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%
No Interferent, DMSO	Blood	24/24	24/24	0/48	48/48	100%
	Tissue	24/24	24/24	0/48	48/48	100%

b. Cross-Contamination

This study assessed the potential for 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 were not associated with evidence of cross-contamination. When these nine replicates were excluded as per the study protocol, 100% (461/461) of analyses returned the expected result. No false MSI H or false MSS results were observed.

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 with 8 colorectal cancer samples (4 MSS and four MSI-H) and 4 endometrial carcinoma samples (2 MSS and 2 MSI-H). The stability study design and reagent lot kits used were leveraged from the stability study that supports the original 510(k) (K200129). Time points tested included baseline (T0), 6, 13, 19, 25, 37, 43 and 49 months stored at their specified storage temperatures. Stability was assessed by calculating concordance between T0 and all subsequent timepoints.

Sample-level agreement was 100% for all time points 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 evaluated 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 that supported the original 510(k) (K200129). Supplemental to the data leveraged from K200129, open kit stability was evaluated using 3 amplification reagent lots with 8 colorectal cancer samples (4 MSS and four MSI-H) and 4 endometrial carcinoma samples (two MSS and two MSI-H). The timepoints tested included baseline (T0), 1, 2, 3 and 4 months at +2°C to +10°C. Stability was assessed by calculating concordance between T0 and all subsequent timepoints.

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

c. Ship Stress Stability

This study evaluated 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 that supports the original 510(k) (K200129). Ship stress stability was assessed using 4 amplification reagent lots with eight colorectal cancer samples (four MSS and four MSI-H) and four endometrial carcinoma samples (two MSS and two MSI H). Reagent lots underwent 5 freeze/thaw cycles prior to exposure to one of the following ship stress conditions:

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

Stability was assessed by calculating concordance between nonshipped and shipped reagent lots. The sample-level agreement was 100% for all data points.

d. Slide Stability

This study evaluated stability of the BAT-25, BAT-26, NR-21, NR-24 and MONO 27 markers in FFPE endometrial and colorectal 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 2, 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 100% of locus-level stability calls were concordant with T0 across all time points. Based on the study results, the recommended cut slide stability is 24 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. OncoMate MSI Dx Analysis System results from whole blood at baseline (T0) were compared to results from whole blood exposed to specified temperatures at subsequent time points. Since no matched tumor sample was assessed in this study, the baseline condition was considered the “normal” reference sample in the software analysis and the

experimental storage condition was considered the “tumor” sample. Sample-level and locus-level stability calls were determined for each test condition using this approach.

For all temperature profiles and all time points, 100% of sample results were “MSS”. Based on the study results, 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 6 colorectal cancer sample pairs (3 MSS and 3 MSI-H) consisting of tumor and matched normal FFPE tissue and 6 endometrial cancer sample pairs (3MSS and 3 MSI-H) consisting of tumor FFPE tissue and matched normal blood, and storing the amplified products at 2–10°C or –30°C to –10°C. MSI results generated at a given time point were compared to results observed at Time 0 to assess stability. The time points tested were 1, 3 and 8 days (to assess the 2–10°C storage condition) and 3 days, 2 weeks and 5 weeks (to assess 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 study results, 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 stability of DNA purified from 18 cancer specimens (12 MSS and 6 MSI-H) using multiple DNA extraction methods (see Section 9) and stored at –30°C to –10°C. The specimens tested comprised 3 MSI-H and 1 MSS endometrial, 3 MSI-H and 1 MSS colorectal, 1 MSS pancreatic, 1 MSS gastric, 1 MSS melanoma, 1 MSS kidney, 1 MSS liver, 1 MSS thyroid, 1 MSS lung, 1 MSS prostate, 1 MSS bladder and 1 MSS small bowel. MSI results generated at a baseline time point (T0) were compared to MSI results at subsequent time points (5, 27, 53 and 79 weeks) to assess agreement.

100% of MSI results at each time point were concordant with Time 0 results. Based on the study results, DNA eluates from various DNA extraction methods using FFPE and blood samples are stable when stored for up to 18 months at
–30°C to –10°C.

6. General Lab Equipment and Reagent Evaluation - Thermal Cycler Compatibility

To demonstrate thermal cycler compatibility, data generated using 3 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. Please refer to Section 3.5 of the OncoMate MSI Dx Analysis System Technical Manual for a list of thermal cycler specifications for OncoMate MSI Dx Analysis System amplification reactions.

7. Robustness - Guard Banding

The robustness of the OncoMate MSI Dx Analysis System formulations and methods was evaluated by varying amplification conditions from those in the instructions for use using one MSS and two MSI-H endometrial and one MSS and two MSI-H gastric cancer specimens. The sample-level MSI results observed at each test condition were compared to the applicable MSI results at the nominal condition, and agreement estimates and two-sided 95% CI across all tumor samples were calculated. For each of the eight test conditions, sample-level agreement for MSS specimens was 100% (54/54), with a lower 95% CI limit of 93.4%; agreement for MSI-H specimens was 100% (108/108), with a lower 95% CI limit of 96.6%; and overall agreement was 100% (162/162), with a lower 95% CI limit of 97.7%.

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 the Maxwell CSC DNA FFPE Kit and Maxwell CSC Blood DNA Kit using matched tumor and normal FFPE samples and matched normal blood samples. Two DNA purification runs were performed per lot and per sample. Twenty MSI-H and 20 MSS samples were evaluated, derived from colorectal, endometrial, adrenal, pancreatic, gastric, melanoma, breast, lung, prostate, bladder, renal, head and neck, and cervical tumors. MSI-H samples ranged from 30% to 90% tumor content, and MSS samples ranged from 30% to 95% tumor content. The study generated 240 MSI results using tumor FFPE samples with matched normal blood samples and 240 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. Agreement estimates with two-sided 95% confidence intervals (95% CI) were calculated.

Agreement values for MSS and MSI-H specimens were both 100% (120/120 and 120/120, respectively), with equivalent lower 95% CI limits of 97.0%. Overall agreement was 100%, with a lower 95% CI limit of 98.5%. DNA yields for the three intended-use sample types (tumor FFPE tissue, normal FFPE tissue and normal whole blood) were also evaluated. All extractions resulted in a DNA concentration ≥0.17ng/µl, the minimum yield required for a 1ng DNA input when using the maximum DNA eluate volume of 6µl for PCR.

Based on the study results, 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

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

Each DNA extraction method for both blood and FFPE samples was performed by two operator teams using two replicate extractions per sample, repeated on three nonconsecutive days. Extractions were performed on FFPE tumor and matched FFPE samples from seven MSI-H and 12 MSS samples from 12 tissues (colorectal, endometrial, pancreatic, gastric, melanoma, renal, liver, thyroid, lung, prostate, bladder and small bowel). Matched normal blood samples were available for 10 of the 12 MSS tumor samples.

Across all DNA extraction methods and sample sources, DNA yield for 99.7% (1974/1980) 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 a concentration ≥0.17ng/µl). MSI results generated using FFPE tissue or blood as the matched normal sample were compared across all DNA purification methods to calculate average agreement values. Across all DNA purification methods evaluated, average agreement values for both MSS and MSI-H samples were 100% when MSI results were generated using FFPE or blood tissue as the matched normal sample.

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

B. Animal Studies

Not applicable.

C. Additional Studies

Not applicable.

X. SUMMARY OF PRIMARY CLINICAL STUDY(IES)

The clinical performance of the OncoMate MSI Dx Analysis System (hereafter referred to as the "CDx") for detection of MSI-H status in patients with solid tumors who may be eligible for treatment with KEYTRUDA (pembrolizumab) was established through a clinical bridging study. The bridging study used archived FFPE tumor tissue specimens from patients enrolled in Merck clinical studies KEYNOTE-158 and KEYNOTE-164, supplemented by a set of commercially procured tumor bank specimens that served as an additional source of biomarker-negative samples to estimate NPA. The clinical study is summarized below.

A. Study Design

Therapeutic Clinical Study KEYNOTE-158

KEYNOTE-158 is a multicenter, global, open-label trial of pembrolizumab in participants with multiple types of advanced (unresectable and/or metastatic) cancers who had failed prior therapy. All enrolled participants had a histologically or cytologically documented advanced solid tumor that was incurable and for which prior standard first-line therapy had failed; all had progressed on or were intolerant to therapies known to provide clinical benefit. All participants received pembrolizumab 200mg every 3 weeks. Enrollment in Cohorts A–K was completed prior to CDx testing and clinical validation analyses (last participant enrolled October 2020); the study remains active, with enrolled participants continuing pembrolizumab treatment and/or protocol-specified follow-up.
Participants with the following solid tumor types were enrolled:

- Cohorts A–J: anal squamous cell carcinoma, biliary adenocarcinoma, neuroendocrine tumors, endometrial carcinoma, cervical squamous cell carcinoma, vulvar squamous cell carcinoma, small cell lung carcinoma, mesothelioma, thyroid carcinoma, and salivary gland carcinoma. These cohorts enrolled all-comer participants irrespective of biomarker status.
- Cohort K: participants with any advanced solid tumor (except CRC) determined to be MSI-H or dMMR by local PCR or IHC testing.

Eligibility criteria included an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, life expectancy greater than 3 months and prior treatment with

standard therapies. Participants were ineligible if they had known CNS metastases or autoimmune disease, were receiving immunosuppressive therapy or had received an anticancer monoclonal antibody (mAb) within 4 weeks prior to trial Day 1. For Cohorts A–J, participants were required to have adequate tumor tissue for PD-L1, gene expression profiling (GEP) and MSI-H testing.

A primary objective of KEYNOTE-158 was to evaluate the ORR per RECIST 1.1 as assessed by blinded independent central review (BICR) to pembrolizumab in Cohort A–K participants with MSI-H tumors. ORR was defined as the proportion of participants in the analysis population with a confirmed complete response (CR) or partial response (PR) per BICR-assessed RECIST 1.1. Secondary objectives included duration of response (DOR), progression-free survival (PFS) and overall survival (OS). DOR was defined as the time from first documented CR or PR to disease progression or death from any cause, whichever occurred first. PFS was defined as the time from first dose of study treatment to documented progressive disease or death from any cause. OS was defined as the time from first dose of study treatment to death from any cause.

Local testing was used to determine the MSI/MMR tumor status of Cohort K participants for purposes of enrollment.

Enrollment in KEYNOTE-158 Cohorts A–K is complete; the last participant was enrolled in October 2020. In total, 1,066 participants were enrolled and received at least one dose of pembrolizumab in the combined Cohorts A–J, and 355 participants were enrolled and received at least one dose of pembrolizumab in Cohort K. All 355 Cohort K participants are part of the efficacy population for this submission, reflecting a data cutoff of 12 January 2022.

Therapeutic Clinical Study KEYNOTE-164

KEYNOTE-164 was a single-arm, multisite, multicohort, Phase 2 study evaluating efficacy of pembrolizumab in participants with locally advanced unresectable or metastatic (Stage IV) MSI-H or dMMR colorectal cancer (CRC). Participants meeting eligibility criteria were enrolled into one of 2 cohorts:

- Cohort A: participants with locally advanced unresectable or metastatic MSI H/dMMR CRC previously treated with at least two lines of standard therapy that included fluoropyrimidine, oxaliplatin and irinotecan.
- Cohort B: participants with locally advanced unresectable or metastatic MSI H/dMMR CRC previously treated with at least one line of systemic standard-of-care (SOC) therapy (fluoropyrimidine plus oxaliplatin, or fluoropyrimidine plus irinotecan, with or without anti-VEGF or anti-EGFR mAb).

All participants received pembrolizumab 200mg every 3 weeks. Eligibility required an ECOG PS of 0 or 1, life expectancy greater than 3 months and histologically proven locally

advanced unresectable or metastatic (Stage IV) CRC. Tumor response was assessed every 9 weeks. Participants continued pembrolizumab until progressive disease, unacceptable adverse events, receipt of 35 trial treatments (approximately 2 years) or other discontinuation criteria per the trial protocol. All participants were followed for OS. Adverse events were monitored throughout the trial and graded per National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0.

The primary objective of KEYNOTE-164 was to evaluate the ORR per central-imaging-assessed RECIST 1.1. Secondary objectives included DOR, disease control rate (DCR), PFS per central-imaging-assessed RECIST 1.1 and OS.

Local testing was used to determine the MSI/MMR tumor status of participants for purposes of enrollment. The study has been completed (last participant enrolled September 2016). A total of 124 participants received pembrolizumab, including 61 in Cohort A and 63 in Cohort B. One of these 124 participants was determined not to be MSI H/dMMR by local testing and is therefore excluded from the efficacy population for this submission; 123 KEYNOTE-164 participants comprise the CRC component of the efficacy population. The KEYNOTE-164 data cutoff for analyses in this submission is 19 February 2021.

Clinical Bridging Study Design

The bridging study was performed through retrospective testing of available FFPE tumor tissue from participants in studies KEYNOTE-158 and KEYNOTE-164, and through retrospective testing of an additional set of commercially procured FFPE tumor tissue specimens (tumor bank). As described above, KEYNOTE-158 Cohorts A–J enrolled participants with specific tumor types irrespective of biomarker status, while Cohort K enrolled participants with nonCRC solid tumors that were MSI-H/dMMR by local testing. KEYNOTE-164 enrolled participants with CRC tumors that were MSI-H/dMMR by local testing.

Eligible participants from KEYNOTE-158 Cohort K and KEYNOTE-164, who were enrolled on the basis of MSI-H/dMMR results from local CTA testing, collectively define the CTA-positive efficacy population for the bridging study. At the time KEYNOTE-158 Cohort K was initiated, tumor sample submission was not required, resulting in incomplete and nonsequential sample availability for retrospective CDx testing; tumor sample submission was subsequently added as an eligibility criterion under Protocol Amendment 7. KEYNOTE-164 was initiated in August 2015 and completed enrollment in September 2016; its protocol likewise did not require collection of tumor samples from enrolled patients, resulting in incomplete sample availability for retrospective CDx testing.

Clinical outcome data from KEYNOTE-158 Cohorts A–J were not used for the primary efficacy analyses; however, MSI/MMR tumor status from a subset of these participants was used in the concordance analyses.

Because KEYNOTE-158 Cohort K and KEYNOTE-164 enrolled only participants who were MSI-H/dMMR by local CTA testing, the enrolled population is distinct from the CDx intent-to-test population. Tumors that are pMMR or not MSI-H by local testing could potentially be identified as MSI-H by the CDx; however, such cases were not enrolled into KEYNOTE-158 Cohort K or KEYNOTE-164, and pembrolizumab response data are therefore not directly available for these cases. Statistical bridging methodology was used to estimate efficacy for CDx-positive patients in the intent-to-test population, incorporating estimates of PPA, NPA and clinical efficacy data from CTA MSI H/dMMR patients.

Retrospective testing of samples from enrolled CTA-positive participants with the CDx supported estimation of positive percent agreement (PPA) between the local MSI/MMR CTAs and CDx. Because tumor samples from participants who were CTA-negative at the time of study screening were not retained, NPA could not be assessed directly from KEYNOTE-158 Cohort K or KEYNOTE-164. To support NPA estimation, a simulated local-CTA-negative population was assembled from KEYNOTE-158 Cohort A–J and tumor bank samples. Details of the simulated CTA-negative population are provided below.

1. Clinical Bridging Study Inclusion and Exclusion Criteria

The inclusion and exclusion criteria for selection into the clinical bridging study were:

Sample Inclusion Criteria

- Samples must meet CDx processing requirements.
- Tumor samples must be FFPE blocks or slides.
- A matched normal sample from the same patient must be available, as follows:
- DNA from matched normal blood was used with KEYNOTE-158 and KEYNOTE-164 tumor specimens
- Adjacent normal FFPE tissue was used with tumor bank specimens
- Samples must be obtained following HIPAA guidelines with patients' consent in compliance with 21 CFR Part 50 "Protection of Human Subjects" and 21 CFR Part 56 "Institutional Review Boards".

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

2. Follow-up Schedule

The OncoMate clinical bridging study involved only retrospective testing of FFPE tumor tissue samples. No additional patient follow-up was conducted as part of the bridging study.

3. Clinical Endpoints

The objectives of the OncoMate clinical bridging study were:

- To estimate agreement between the CTA and CDx for MSI test outcomes.
- To estimate ORR for pembrolizumab treatment among clinical study participants whose archived solid tumor specimens were determined to be MSI-H by retrospective testing with the CDx. The primary analysis was based on participants with observed CDx results. A pre-specified sensitivity analysis included participants with and without observed CDx results, with missing or non-evaluable CDx results imputed using a Bayesian multiple-imputation methodology.

B. Accountability of PMA Cohort

Overall, 682 tumor samples were tested with the CDx and 647 (94.9%) returned a valid ("MSS" or "MSI-H") result. Sample accounting by study cohort is shown in Table 23. Of the 682 samples tested, 35 samples (5.1%) returned "Invalid" MSI results after troubleshooting. The invalid rate includes 7 samples with evidence of DNA contamination (1.0%), 24 samples with QC failures associated with low peak intensity (3.5%), 1 sample with high peak intensity (0.1%), 1 sample with sample-identity check failure (0.1%) and 2 samples with unexpected peaks detection.

Table 23. Sample Accountability

Study	N	CDx Tested	CDx Evaluable	CDx-Positive	CDx-Negative
KEYNOTE-158 Cohort K	355	149	145	87	58
KEYNOTE-158 Cohorts A–J	1,066	220	211	0	211
KEYNOTE-164	123	35	34	30	4
Tumor Bank	423	278	257	20	237
Total	1,967	682	647	137	510

The 478 participants treated with pembrolizumab in KEYNOTE-158 Cohort K (n = 355) and KEYNOTE-164 (n = 123) constitute the CTA-positive efficacy population for the bridging study. Of these 478 participants, 184 had tumor samples tested with the CDx and 179 returned evaluable results (MSI-H or MSS). The efficacy analysis was performed for these 179 participants, while the sensitivity analyses were performed using Bayesian multiple imputation for the 299 participants without evaluable results.

The 299 participants without evaluable results are accounted for as follows: tumor samples from 294 participants (206 from KEYNOTE-158 Cohort K and 88 from KEYNOTE-164) were

not available for retrospective testing with the CDx because tumor blocks were not available; tumor samples for five participants (four from KEYNOTE-158 Cohort K and one from KEYNOTE-164) were tested but returned non-evaluable CDx results.

In addition, 1,066 samples from KEYNOTE-158 Cohorts A–J participants and 423 tumor bank samples were considered for CTA testing to support assembly of the simulated CTA-negative population. For KEYNOTE-158 Cohorts A–J participants, 846 tumor samples were unavailable for testing, primarily because tumor blocks were not available. For the tumor bank cohort, 145 samples were unavailable for testing, primarily because of H&E QC failures or low DNA concentrations. Therefore, 220 KEYNOTE-158 Cohort A–J samples and 278 tumor bank samples were tested with the CDx, with 211 and 257 evaluable, respectively.

In total, evaluable CDx results from 647 samples (179 from KEYNOTE-158 Cohort K and KEYNOTE-164 combined plus 211 from KEYNOTE-158 Cohorts A–J, plus 257 from the tumor bank) were available to support concordance analyses.

C. Study Population Demographics and Baseline Parameters

For the clinical device bridging study, baseline characteristics for the CTA-positive efficacy population (355 from KEYNOTE KEYNOTE-158 Cohort K and 123 from KEYNOTE-164 combined; n = 478) and for the same population stratified by CDx test outcome (CDx-positive, CDx-negative and CDx non-evaluable or missing) are presented in Table 24. The distribution of tumor types is reported in Table 25. Among these 478 participants, the baseline characteristics were: median age of 60.0 years (range 20 to 89), 35.8% ≥65 years of age; 44.4% male; 77.6% White, 13.4% Asian and 3.3% Black; and 44.8% had an ECOG PS of 0 and 55.2% had an ECOG PS of 1. 92.5% of participants had metastatic disease. 60.9% of participants received two or more prior lines of therapy.

Table 24. Patient Characteristics in CTA-Positive Population and by CDx Result in CTA-Positive Population (KEYNOTE-158 Cohort K and KEYNOTE-164)

	CTA-Positive (N = 478)	CDx-Positive (N = 117)	CDx-Negative (N = 62)	CDx Non-evaluable or Missing (N = 299)
	n (%)	n (%)	n (%)	n (%)
Sex				
Male	212 (44.4)	45 (38.5)	30 (48.4)	137 (45.8)

	CTA-Positive (N = 478)	CDx-Positive (N = 117)	CDx-Negative (N = 62)	CDx Non-evaluable or Missing (N = 299)
	n (%)	n (%)	n (%)	n (%)
Female	266 (55.6)	72 (61.5)	32 (51.6)	162 (54.2)
Age (years)				
<65	307 (64.2)	59 (50.4)	48 (77.4)	200 (66.9)
≥65	171 (35.8)	58 (49.6)	14 (22.6)	99 (33.1)
Mean	58.4	62.1	55.0	57.6
SD	13.7	13.2	14.6	13.4
Median	60.0	64.0	57.5	59.0
Range	20–89	20–89	20–87	23–86
Race				
American Indian/Alaska Native	18 (3.8)	5 (4.3)	7 (11.3)	6 (2.0)
Asian	64 (13.4)	10 (8.5)	2 (3.2)	52 (17.4)
Black or African American	16 (3.3)	1 (0.9)	0 (0.0)	15 (5.0)
Multiple	7 (1.5)	3 (2.6)	2 (3.2)	2 (0.7)
White	371 (77.6)	97 (82.9)	51 (82.3)	223 (74.6)
Missing	2 (0.4)	1 (0.9)	0 (0.0)	1 (0.3)
Ethnicity				
Hispanic or Latino	62 (13.0)	11 (9.4)	10 (16.1)	41 (13.7)
Not Hispanic or Latino	378 (79.1)	90 (76.9)	47 (75.8)	241 (80.6)
Not Reported	37 (7.7)	15 (12.8)	5 (8.1)	17 (5.7)
Unknown	1 (0.2)	1 (0.9)	0 (0.0)	0 (0.0)

	CTA-Positive (N = 478)	CDx-Positive (N = 117)	CDx-Negative (N = 62)	CDx Non-evaluable or Missing (N = 299)
	n (%)	n (%)	n (%)	n (%)
ECOG				
0	214 (44.8)	47 (40.2)	32 (51.6)	135 (45.2)
1	264 (55.2)	70 (59.8)	30 (48.4)	164 (54.8)
Metastatic Staging				
MX	21 (4.4)	3 (2.6)	6 (9.7)	12 (4.0)
M0	15 (3.1)	1 (0.9)	1 (1.6)	13 (4.3)
M1	442 (92.5)	113 (96.6)	55 (88.7)	274 (91.6)
Cancer Staging				
III	3 (0.6)	0 (0.0)	1 (1.6)	2 (0.7)
IIIA	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.3)
IIIB	4 (0.8)	1 (0.9)	0 (0.0)	3 (1.0)
IIIC	2 (0.4)	0 (0.0)	0 (0.0)	2 (0.7)
IV	424 (88.7)	100 (85.5)	50 (80.6)	274 (91.6)
IVA	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.3)
IVB	34 (7.1)	15 (12.8)	6 (9.7)	13 (4.3)
IVC	9 (1.9)	1 (0.9)	5 (8.1)	3 (1.0)
Brain Metastasis				
Yes	6 (1.3)	0 (0.0)	4 (6.5)	2 (0.7)
No	472 (98.7)	117 (100.0)	58 (93.5)	297 (99.3)
Prior Line of Therapy				

	CTA-Positive (N = 478)	CDx-Positive (N = 117)	CDx-Negative (N = 62)	CDx Non-evaluable or Missing (N = 299)
	n (%)	n (%)	n (%)	n (%)
0	8 (1.7)	1 (0.9)	2 (3.2)	5 (1.7)
Adjuvant/Neoadjuvant/Definitive	2 (0.4)	0 (0.0)	1 (1.6)	1 (0.3)
1	177 (37.0)	52 (44.4)	15 (24.2)	110 (36.8)
2	137 (28.7)	35 (29.9)	12 (19.4)	90 (30.1)
3	79 (16.5)	19 (16.2)	14 (22.6)	46 (15.4)
4	32 (6.7)	6 (5.1)	7 (11.3)	19 (6.4)
5 or more	43 (9.0)	4 (3.4)	11 (17.7)	28 (9.4)

Database cutoff dates: KEYNOTE-164: 19 February 2021. KEYNOTE-158: 12 January 2022.

Table 25. Tumor Type Distribution in CTA-Positive Population and by CDx Result (KEYNOTE-158 Cohort K and KEYNOTE-164)

Tumor Type	CTA-Positive (N = 478)	CDx-Positive (N = 117)	CDx-Negative (N = 62)	CDx Non-evaluable (N = 299)
Colorectal	123	30	4	89
Endometrial	83	33	3	47
Gastric	51	23	3	25
Cholangiocarcinoma	22	4	2	16
Ovarian	25	3	5	17
Pancreas	22	1	3	18
Small intestine	27	7	1	19

Tumor Type	CTA-Positive (N = 478)	CDx-Positive (N = 117)	CDx-Negative (N = 62)	CDx Non-evaluable (N = 299)
Breast	13	2	4	7
Glioma	21	3	6	12
NET	11	1	5	5
Sarcoma	14	N/A	8	6
Other*	66	10	18	38
Total	478	117	62	299

*Other includes tumors recorded as "Other" and tumor types with fewer than 10 samples in the CTA-positive efficacy population.

Tumor bank samples and KEYNOTE-158 Cohort A–J samples were used together to assemble the simulated local-CTA-negative population for NPA estimation. Of the 423 tumor bank samples available for the bridging study, 278 met inclusion criteria and were tested with the CDx and 257 returned an evaluable result.

D. Safety and Effectiveness Results

1. Safety Results

No adverse events were reported in connection with the OncoMate bridging study used to support this PMA supplement, as the bridging study was performed retrospectively on archived samples.

The evaluation of safety was based on the analysis of adverse events (AEs, clinical laboratory evaluations, physical examinations, and vital signs). Refer to the drug label available at Drugs@FDA for complete safety information on KEYTRUDA.

2. Effectiveness Results

Concordance Results

KEYNOTE-158 Cohort K and KEYNOTE-164 enrolled only participants with MSI H/dMMR solid tumors. Tumors that were pMMR or not MSI-H (i.e., MSS) by local CTA testing were not enrolled, and corresponding samples were not retained. Therefore, KEYNOTE-158 Cohorts A–J and tumor bank samples were used to assemble a simulated local-CTA-negative population. For purposes of generating

simulated local MSI/MMR results, PCR testing for MSI status and IHC testing for MMR status were performed at an external laboratory.

The CTA status for each sample was derived from available PCR and/or IHC results. A sample was considered CTA-positive if the CTA PCR result was MSI-H or the CTA IHC result was dMMR. A sample was considered CTA-negative if the CTA PCR result was not MSI-H (i.e., MSS) and the CTA IHC result was pMMR. CTA status was considered non-evaluable if none of the CTA PCR or IHC results were evaluable.

Of the 682 samples tested with the CDx, 647 were CDx-evaluable. The concordance analysis population comprised 646 samples with both CDx-evaluable and CTA-evaluable results—199 CTA-positive (KEYNOTE-158 Cohort K, KEYNOTE-164 and tumor bank)—and 447 CTA-negative (KEYNOTE-158 Cohorts A–J and tumor bank). Note, one sample (of 647) lacked an evaluable CTA result and was excluded from the concordance analysis.

Agreement between the CDx and CTAs, with CTAs as the reference, is summarized in Table 26. Point estimates of PPA and NPA were 68.8% and 100.0%, respectively.

Table 26. Concordance Between CTA and CDx

	CTA-Positive	CTA-Negative	Total
CDx-Positive	137	0	137
CDx-Negative	62	447	509
Total	199	447	646
PPA (95% CI)	68.8 (62.1, 74.9)		
NPA (95% CI)	100.0 (99.1, 100.0)		
OPA (95% CI)	90.4 (87.9, 92.4)		

CTA-Positive includes samples from KEYNOTE-158 Cohort K, KEYNOTE-164 and tumor bank. CTA-Negative includes samples from KEYNOTE-158 Cohorts A–J and tumor bank.
Database Cutoff: KEYNOTE-164 19 February 2021; KEYNOTE-158 12 January 2022.

In general, there was an unexpectedly high proportion of CTA-positive samples from KEYNOTE-158 and KEYNOTE-164 that were CDx-negative, leading to further investigation to understand the likely correct MSI status of these samples. The reason for the observed discordances may be due to heterogeneity in MSI/MMR local testing caused by differences in reagents and/or instrumentation and varying levels of assay characterization; KEYNOTE-158 clinical trial testing alone spanned 18 different countries. To further investigate the low PPA, evaluable CTA+/CDx- samples across different tumor types were retested using two independent validated assays, one IHC-based and one NGS-based, and agreement was observed in 45 of 47 evaluable

cases, supporting the likely correct MSI status as determined by the CDx. Of note, none of the CTA-positive/CDx-negative patients responded to pembrolizumab, and CDx-positive participants across the efficacy population had a substantially higher objective response rate (45.3%) than CTA-positive/CDx-negative participants (3.2%), demonstrating that the CDx enriches for MSI-H patients who respond to pembrolizumab. See efficacy results below.

Agreement measures, PPA, NPA and OPA, between the CDx and CTAs for samples with valid CDx results and CTA results were also calculated per tumor type using the CTA results as the reference and are shown in Table 27. Point estimates of PPA and OPA per tumor type ranged from 0% to 92.1% and from 20.0% to 100%, respectively. Point estimates of NPA were all 100%. Agreement measures for non PCR-based and PCR-based CTAs are shown in Table 28 and Table 29.

Table 27. Concordance Between CTA and CDx per Tumor Type

Tumor Type	N	CTA+/ CDx+	CTA+/ CDx–	CTA–/ CDx+	CTA–/ CDx–	PPA % (95% CI)	NPA % (95% CI)	OPA % (95% CI)
Colorectal	104	43	4	0	57	91.5 (80.1, 96.6)	100.0 (93.7, 100.0)	96.2 (90.5, 98.5)
Esophageal	88	0	0	0	88	N/A	100.0 (95.8, 100.0)	100.0 (95.8, 100.0)
Gastric	65	24	3	0	38	88.9 (71.9, 96.1)	100.0 (90.8, 100.0)	95.4 (87.3, 98.4)
Endometrial	54	35	3	0	16	92.1 (79.2, 97.3)	100.0 (80.6, 100.0)	94.4 (84.9, 98.1)
Cholangiocarcinoma	40	5	2	0	33	71.4 (35.9, 91.8)	100.0 (89.6, 100.0)	95.0 (83.5, 98.6)
Anal	31	1	1	0	29	50.0 (9.5, 90.5)	100.0 (88.3, 100.0)	96.8 (83.8, 99.4)
NET	31	1	5	0	25	16.7 (3.0, 56.4)	100.0 (86.7, 100.0)	83.9 (67.4, 92.9)
Vulvar	29	0	0	0	29	N/A	100.0 (88.3, 100.0)	100.0 (88.3, 100.0)

Tumor Type	N	CTA+/ CDx+	CTA+/ CDx−	CTA−/ CDx+	CTA−/ CDx−	PPA % (95% CI)	NPA % (95% CI)	OPA % (95% CI)
Mesothelioma	27	0	3	0	24	0.0 (0.0, 56.1)	100.0 (86.2, 100.0)	88.9 (71.9, 96.1)
Thyroid	26	1	4	0	21	20.0 (3.6, 62.4)	100.0 (84.5, 100.0)	84.6 (66.5, 93.8)
Cervical	25	1	3	0	21	25.0 (4.6, 69.9)	100.0 (84.5, 100.0)	88.0 (70.0, 95.8)
Small Cell Lung	19	0	1	0	18	0.0 (0.0, 79.3)	100.0 (82.4, 100.0)	94.7 (75.4, 99.1)
Salivary	16	0	0	0	16	N/A	100.0 (80.6, 100.0)	100.0 (80.6, 100.0)
Pancreatic	12	1	3	0	8	25.0 (4.6, 69.9)	100.0 (67.6, 100.0)	75.0 (46.8, 91.1)
Small Intestine	12	8	1	0	3	88.9 (56.5, 98.0)	100.0 (43.9, 100.0)	91.7 (64.6, 98.5)
Bladder	11	1	1	0	9	50.0 (9.5, 90.5)	100.0 (70.1, 100.0)	90.9 (62.3, 98.4)
HNSCC	10	0	0	0	10	N/A	100.0 (72.2, 100.0)	100.0 (72.2, 100.0)
Sarcoma	10	1	8	0	1	11.1 (2.0, 43.5)	100.0 (20.7, 100.0)	20.0 (5.7, 51.0)
Other*	36	15	20	0	1	42.9 (28.0, 59.1)	100.0 (20.7, 100.0)	44.4 (29.5, 60.4)
Pooled	646	137	62	0	447	68.8 (62.1, 74.9)	100.0 (99.1, 100.0)	90.4 (87.9, 92.4)

*Other includes tumors recorded as “Other” and tumor types with fewer than 10 samples in the CTA-positive efficacy population.

Table 28. Agreement Between CTA (IHC) and CDx, with CTA (IHC) as Reference

	CTA (IHC)-Positive	CTA (IHC)-Negative	Total
CDx-Positive	120	3	123
CDx-Negative	47	428	475
Total	167	431	598
PPA (95% CI)	71.9 (64.6, 78.1)		
NPA (95% CI)	99.3 (98.0, 99.8)		
OPA (95% CI)	91.6 (89.1, 93.6)		

The CTA (IHC) concordance population consists of samples/subjects with an evaluable CDx result and either an evaluable CTA IHC result (n = 591) or an evaluable CTA "Other" result (n = 7). CTA methods categorized as "Other" include "IHC and MLH1 mutation 1411del4 confirmed by specific mutation analysis", "PCR and IHC", "IHC and PCR" and "other both IHC and PCR".
Database cutoff: KEYNOTE-164 19 February 2021; KEYNOTE-158 12 January 2022.

Table 29. Agreement Between CTA (PCR) and CDx, with CTA (PCR) as Reference

	CTA (PCR)-Positive	CTA (PCR)-Negative	Total
CDx-Positive	44	0	44
CDx-Negative	15	438	453
Total	59	438	497
PPA (95% CI)	74.6 (62.2, 83.9)		
NPA (95% CI)	100.0 (99.1, 100.0)		
OPA (95% CI)	97.0 (95.1, 98.2)		

The CTA (PCR) concordance population consists of samples/subjects with an evaluable CDx result and an evaluable CTA PCR result. For samples with both a CTA PCR and IHC result, only the CTA PCR result is considered in this table.
Database cutoff: KEYNOTE-164 19 February 2021; KEYNOTE-158 12 January 2022.

Clinical Efficacy Results

Clinical validity of the CDx for detection of MSI-H status in patients with solid tumors was established by estimating clinical efficacy in the CDx-positive / CTA-positive efficacy population, and compared to CTA-positive and CTA-positive / CDx negative population. The major efficacy outcome measure was ORR per RECIST 1.1 as assessed by blinded independent central review (BICR) and secondary efficacy outcome measure was DOR. ORR and DoR values for the CTA positive, CDx positive/CTA positive, CDx negative/CTA positive and CDx missing/CTA positive KEYNOTE study participants are presented in Table 30.

Table 7. Efficacy Results in KEYNOTE-164 and KEYNOTE-158 Cohort K Combined

Clinical Outcome	All CTA-Positive (Total) (N=478)	CDx-Positive (MSI-H) and CTA-Positive (N=117)	CDx-Negative (MSS) and CTA-Positive (N=62)	CDx-Missing and CTA-Positive (N=299)
ORR (95% CI[a])	33.5% (29.3, 37.9)	45.3% (36.1, 54.8)	3.2% (0.4, 11.2)	35.1% (29.7, 40.8)
Complete Response	48 (10.0%)	14 (12.0%)	0 (0.0%)	34 (11.4%)
Partial Response	112 (23.4%)	39 (33.3%)	2 (3.2%)	71 (23.7%)
Duration of Response[b]	**N=160**	**N=53**	**N=2**	**N=105**
Median[c] in months (range)	63.2 (1.9+ – 63.2)	NR (3.6 – 61.0+)	NR (18.9 – 28.1+)	63.2 (1.9+ – 63.2)
n (%[c]) with duration ≥6 months	149 (94.9%)	49 (92.5%)	2 (100%)	98 (96.1%)
n (%[c]) with duration ≥12 months	123 (90.3%)	39 (88.4%)	2 (100%)	82 (91.1%)

[a] Based on binomial exact confidence interval method.

[b] Includes participants with confirmed complete response or partial response.

[c] From product-limit (Kaplan-Meier) method for censored data.

"+" indicates there is no progressive disease by the time of last disease assessment.

NR = Not Reached.

Database Cutoff Date: KEYNOTE-164: 2021-02-19, KEYNOTE-158: 2022-01-12

The efficacy subpopulations were analyzed by tumor type and response rates by tumor type are provided in Table 31.

Table 31. Efficacy Results in KEYNOTE-164 and KEYNOTE-158 Cohort K Combined / ORR Estimates per Tumor Type

Efficacy Subpopulation (All CTA-Positive)	Overall (All CTA-Positive) (N = 478)	CDx-Positive (OncoMate MSI-H) (N = 117)	CDx-Negative (OncoMate MSS) (N = 62)	CDx Missing (N = 299)
Tumor Type	**Responder (n) / Subpopulation (N), ORR% (95% CI)**			
All	160/478, 33.5% (29.3, 39.7)	53/117, 45.3% (36.1, 54.8)	2/62, 3.2% (0.4, 11.2)	105/299, 35.1% (29.7, 40.8)
Breast	1/13, 7.7% (0.2, 36.0)	1/2, 50.0% (1.3, 98.7)	0/4, 0.0% (0, 60.2)	0/7, 0.0% (0, 41.0)
Cholangiocarcinoma	9/22, 40.9% (20.7, 63.6)	3/4, 75.0% (19.4, 99.4)	0/2, 0.0% (0, 84.2)	6/16, 37.5% (15.2, 64.6)

Efficacy Subpopulation (All CTA-Positive)	Overall (All CTA-Positive) (N = 478)	CDx-Positive (OncoMate MSI-H) (N = 117)	CDx-Negative (OncoMate MSS) (N = 62)	CDx Missing (N = 299)
Tumor Type	**Responder (n) / Subpopulation (N), ORR% (95% CI)**			
CRC	42/123, 34.1% (25.8, 43.2)	14/30, 46.7% (28.3, 65.7)	0/4, 0.0% (0, 60.2)	28/89, 31.5% (22.0, 42.2)
Endometrial	42/83, 50.6% (39.4, 61.8)	16/33, 48.5% (30.8, 66.5)	0/3, 0.0% (0, 70.8)	26/47, 55.3% (40.1, 69.8)
Gastric	20/51, 39.2% (25.8, 53.9)	9/23, 39.1% (19.7, 61.5)	0/3, 0.0% (0, 70.8)	11/25, 44.0% (24.4, 65.1)
Glioma	1/21, 4.8% (0.1, 23.8)	0/3, 0.0% (0, 70.8)	1/6, 16.7% (0.4, 64.1)	0/12, 0.0% (0.0, 26.5)
NET	1/11, 9.1% (0.2, 41.3)	0/1, 0.0% (0, 97.5)	0/5, 0.0% (0, 52.2)	1/5, 20% (0.5, 71.6)
Ovarian	8/25, 32.0% (14.9, 53.5)	2/3, 66.7% (9.4, 99.2)	0/5, 0.0% (0, 52.2)	6/17, 35.3% (14.2, 61.7)
Pancreas	4/22, 18.2% (5.2, 40.3)	1/1, 100% (2.5, 100)	0/3, 0.0% (0, 70.8)	3/18, 16.7% (3.6, 41.4)
Sarcoma	3/14, 21.4% (4.7, 50.8)	N/A	0/8, 0.0% (0, 36.9)	3/6, 50.0% (11.8, 88.2)
Small intestine	16/27, 59.3% (38.8, 77.6)	5/7, 71.4% (29.0, 96.3)	0/1, 0.0% (0, 97.5)	11/19, 57.9% (33.5, 79.7)
Other*	13/66, 19.7% (10.9, 31.3)	2/10, 20.0% (2.5, 55.6)	1/18, 5.6% (0.1, 27.3)	10/38, 26.3% (13.4, 43.1)

* Other category includes those tumors that were recorded as "other" and all remaining tumor types with less than 10 samples in the CTA-positive (efficacy) population.
Database Cutoff: KEYNOTE-164 2021-02-19; KEYNOTE-158 2022-01-12.

The ORR in the CTA-positive efficacy population was 33.5% (160/478; 95% CI: 29.3, 37.9). The ORR in the CDx-positive/CTA-positive subpopulation was 45.3% (53/117; 95% CI: 36.1, 54.8), while the ORR was 3.2% (2/62; 95% CI: 0.4, 11.2) in the CDx-negative/CTA-positive subpopulation.

The ORR in participants with missing or non-evaluable CDx results (n = 299; 35.1%; 95% CI: 29.7, 40.8) was similar to the ORR in the overall efficacy population irrespective of CDx status (n = 478; 33.5%; 95% CI: 29.3, 37.9), suggesting no overt imbalance in pembrolizumab efficacy between participants for whom a CDx result was or was not obtained.

Sensitivity Analysis

Sensitivity analyses were performed to evaluate robustness of the ORR estimate in light of (i) missing or non-evaluable CDx results among CTA-positive efficacy

participants (62.5%, 299/478) and (ii) the absence of direct observation of pembrolizumab response in any members of the CTA-negative/CDx-positive population who were not enrolled in KEYNOTE-158 Cohort K or KEYNOTE-164.

To evaluate (i), a multiple-imputation methodology was used to impute CDx tumor status for participants with missing or non-evaluable results. The imputation-based ORR over the imputed data sets was 47.3% (95% CI: 41.0, 53.6)
To address (ii), the pre-specified bridging equation incorporated a range of MSI H/dMMR prevalence estimates (2–5%) in the intent-to-test population and a range of ORR attenuation factors (0 to 1, where 0 represents full attenuation of pembrolizumab efficacy in CTA-negative/CDx-positive participants and 1 represents no attenuation). Because the NPA point estimate was 100.0%, the bridging equation simplifies such that the bridged ORR is equal to the observed ORR in the CDx-positive/CTA-positive ("double-positive") population, independent of the prevalence and attenuation factor inputs. The primary bridged ORR estimate was therefore 45.3% (95% CI: 36.3, 54.3).

3. Pediatric Extrapolation

In this premarket application, existing clinical data was submitted to support the reasonable assurance of safety and effectiveness of the proposed device in pediatric patients. The therapeutic indication for KEYTRUDA in patients with MSI-H/dMMR solid tumors is for both the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) with solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options.

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.

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 three investigators. None of the clinical investigators had disclosable financial interests or 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

The analytical and clinical performance for detecting the MSI status of CDx 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 identifying MSI-H solid tumor patients for treatment with pembrolizumab monotherapy was demonstrated through clinical evidence from KEYNOTE-158 and KEYNOTE-164 bridging studies. In the CDx-positive efficacy subpopulation, ORR was 45.3% (95% CI: 36.1, 54.8), consistent with the ORR that formed the basis for pembrolizumab authorization in this indication (ORR = 33.8%, 95% CI: 29.7, 38.1), and meaningfully higher than in CTA-positive/CDx-negative participants (ORR = 3.2%, 95% CI: 0.4, 11.2). These data support the effectiveness of the OncoMate MSI Dx Analysis System to identify patients with MSI-H solid tumors who may benefit from treatment with pembrolizumab 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 MSI-H results may undergo treatment with pembrolizumab and may experience adverse reactions associated with the therapy. Patients with false MSS 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 to identify patients with MSI-H solid tumors for treatment with KEYTRUDA (pembrolizumab) are based on data collected in the KEYNOTE-158 and KEYNOTE-164 studies. The clinical benefit of the OncoMate MSI Dx Analysis System to select cancer patients with MSI-H tumor status was demonstrated using samples from patients who had a histologically or cytologically documented, advanced MSI H solid tumor that was incurable and for which prior standard first-line treatment had failed (KEYNOTE-158, Cohort K) and patients with locally advanced unresectable or metastatic MSI H/dMMR CRC previously treated with at least one line of systemic standard-of-care therapy (KEYNOTE-164).

Clinical efficacy of MSI-H patients identified by the OncoMate MSI Dx Analysis System demonstrated significant and clinically meaningful benefit in the CDx-positive efficacy population for treatment with KEYTRUDA (pembrolizumab); the ORR in this population was 45.3% (95% CI: 36.1, 54.8), which is meaningful and maintains the efficacy observed in the BLA (BLA 125514/S 132), where the ORR for pembrolizumab treated solid tumor patients was 33.5% (95% CI: 29.3, 37.9). ORR was higher in participants with CTA+/CDx-positive tumors compared to participants with CTA+/CDx-negative tumors (ORR = 3.2%, 95% CI: 0.4, 11.2). Sensitivity analyses accounting for missing OncoMate MSI Dx Analysis System results demonstrated robust efficacy in the efficacy subpopulation of patients with missing or non-evaluable CDx status; ORR was 35.1% (95% CI: 29.7, 40.8), which is similar to that for the overall efficacy population irrespective of CDx status (ORR = 33.5%, 95% CI: 29.3, 37.9).

Median overall survival (OS) in the CDx-positive efficacy population was 44.6 (95% CI: 19.9, NR), and OS rates at 12 months and 36 months were 66.5% and 53.0%, respectively. Pembrolizumab treatment resulted in clinically meaningful durable responses in the CDx-positive efficacy subpopulation that had a confirmed complete or partial response. Response continued through ≥12 months and ≥36 months for the majority of responders (88.4% and 67.3%, respectively).

Probable Risks:

There is probable risk associated with the use of this device, mainly due to 1) false MSI-H or MSS results 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 potential mismanagement of patients due to false test results. Patients who are determined to be false MSI-H may be exposed to KEYTRUDA (pembrolizumab) treatment that may not be beneficial, and which may lead to adverse events or delayed access to more effective therapies. A false MSS result may prevent a patient from accessing potentially beneficial KEYTRUDA (pembrolizumab) 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.

The risks of false results are mitigated through multiple layers of analytical safeguards, including matched normal comparison, pentanucleotide identity verification, multiple locus consensus interpretation (≥2 unstable markers for MSI-H), positive and negative amplification controls, capillary electrophoresis spectral calibration and software-based quality control algorithms designed to detect signal intensity imbalance and sizing irregularities. In addition, the risks of false results are mitigated by the rigorous clinical bridging studies performed, which demonstrated acceptable clinical efficacy in the Oncomate MSI Dx Analysis System MSI-H solid tumor population.

In conclusion, given the totality of the available information, the data supports the use of the Oncomate MSI Dx Analysis System to identify MSI-H solid tumor patients, for treatment with pembrolizumab (Keytruda), the probable benefits exceed the probable risks.

1. Patient Perspective

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

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. 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 MSI-H solid tumors who may benefit from treatment with pembrolizumab in accordance with the approved therapeutic product labeling.

XV. CDRH DECISION

CDRH issued an approval order on September 15, 2026

The applicant's manufacturing facilities have been inspected and found to be in compliance with the device Quality Management System Regulation (QMSR, 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.