SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Nucleic Acid Based Assay, Microsatellite Instability Assay

Device Trade Name: Idylla™ CDx MSI Test

Device Procode: SFL

Applicant's Name and Address: Biocartis N.V.
Generaal De Wittelaan 11B
2800 Mechelen, Belgium

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P250005

Date of FDA Notice of Approval: August 15, 2025

II. INDICATIONS FOR USE

For *in vitro* diagnostic use. For Prescription Use Only.
For use on the Biocartis Idylla System only.

The Idylla CDx MSI Test is an *in vitro* diagnostic test intended for the qualitative detection of a panel of seven monomorphic biomarkers (ACVR2A, BTBD7, DIDO1, MRE11, RYR3, SEC31A and SULF2) for identification of microsatellite instability (MSI) in colorectal cancer (CRC) tissue. The Idylla CDx MSI Test uses formalin-fixed, paraffin embedded (FFPE) tissue sections from patients with CRC, from which nucleic acids are extracted, then analyzed using PCR amplification and subsequent melt-curve analysis. The Idylla CDx MSI Test reports MSI status as either Microsatellite Stable (MSS) or Microsatellite Instability-High (MSI-H) or invalid.

The test is intended as a companion diagnostic to identify CRC patients with MSI-H status, who may benefit from treatment with OPDIVO (nivolumab) as a monotherapy and/or treatment with OPDIVO (nivolumab) in combination with YERVOY (ipilimumab).

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the Idylla CDx MSI Test labeling.

V. DEVICE DESCRIPTION

The Idylla CDx MSI test is an automated test on the Idylla System with fully integrated specimen preparation of FFPE tissue sections followed by Real Time Polymerase Chain Reaction (RT-PCR) amplification and high-resolution melting fluorescence detection of the targeted sequences with a turnaround time in under 150 minutes.

The Idylla System consists of the Idylla Instrumentation (i.e. the Idylla Console connected to up to eight Idylla instruments) and the Idylla Tests, which come in the form of Idylla Cartridges designed for specific applications. The Reagent Specific Software (RSSW), packaged in the Test Type Package (TTP), is available for download to the Idylla Console to be executed on the Idylla Instrument.

The Idylla CDx MSI Test qualitatively detects a panel of seven monomorphic biomarkers listed in Table 1 below. Specifically, the 1bp deletion of any one of the biomarkers is considered the mutant allele, while the normal length (no deletion) is considered the wild type (WT) allele. These mutants are frequently present in samples with MSI-H status.

Table 1. Seven biomarkers in the Idylla CDx MSI Test

Biomarkers
ACVR2A
BTBD7
DIDO1
MRE11
RYR3
SEC31A
SULF2

The Idylla CDx MSI Test cartridges are ready-for-use and contain the necessary reagents to perform sample preparation, PCR amplification and high-resolution detection, starting from insertion of FFPE tissue sections. The MSI TTP directs the processing of the sample within the Cartridge, including result determination.

Sample Processing: The Cartridge processing steps that instruct how the instrument should process the input sample to obtain the raw fluorescence data include:

- **FFPE liquefaction and cell lysis**

After insertion of the FFPE tissue section into the Cartridge, a combination of chemical reagents, enzymes, heat, and High-intensity Focused Ultrasound (HiFU) induces deparaffinization, disruption of the tissue and lysis of the cells. The nucleic acids are liberated for subsequent PCR amplification.

- **PCR amplification using biomarker-specific primers**

  All necessary PCR reagents are present in a stable formulation and are used to amplify seven biomarkers indicative for MSI Status.

Detection, analysis, and reporting: The post-processing steps during which the fluorescence intensity measurements are retrieved and summarized into curve characteristics that are converted into a diagnostic call by the classification algorithm according to the rule set defined in the design specifications of the Test include:

- **Detection and Analysis**

  Detection of these specific targets is performed using fluorescently labeled molecular beacons after PCR amplification. These beacons differentially melt from the wild type or mutated amplicons with increasing temperature. The fluorescence differences at melting temperatures are further analyzed by the MSI TTP and translated into genetic calls on biomarker level and MSI status on sample level.

- **Reporting**

  At the end of the run, the MSI status and the number of mutated biomarkers in the analyzed sample is displayed on the Console screen.

The principles of the test specific software include:

After PCR amplification, molecular beacons dissociate from the wild type or mutated amplicons beacon specifically with increasing temperature. The MSI Reagent Specific Software automatically checks the validity of the measured fluorescence profiles: presence of specific PCR amplicons results in biomarker-specific fluorescence profiles, which eliminates the need for a sample processing control in the Cartridge. Next, a powerful pattern-recognition approach trained on several thousands of profiles determines the genotype for each of the seven MSI biomarkers.

The Idylla CDx MSI Test automatically interprets the test results and makes them available for viewing on the Console. A sample is called Microsatellite Instability-High or MSI-H if the sample has ≥2 mutant biomarkers. A sample is called Microsatellite Stable or MSS if the sample has <2 mutant biomarkers (Table 2).

Table 2. Result Interpretation

Number of Positive Biomarkers	MSI Status
<2	MSS
≥2	MSI-H

An 'Invalid' call for a biomarker is observed when no PCR product was formed in a particular sample and therefore no characteristic fluorescent profile could be analyzed. This may occur when using poor quality samples. A Cartridge is considered valid if ≥ 5 out of 7 MSI biomarkers show a valid marker result for the sample. An invalid Cartridge will report the MSI status being 'Invalid'. An 'Invalid' result is reported if >2 out of 7 MSI biomarkers show an invalid biomarker result for a particular sample (Table 3). In the case of an invalid result, it is recommended that testing is repeated on a new sample from the same patient with a new Idylla CDx MSI Test cartridge. If the repeat test is invalid, it is considered the final result for the patient material tested.

Table 3. Invalid Reporting

Number of Invalid Biomarkers	The Quality Status Shown on The Result Report is...
0 Invalid biomarkers	7 MSI biomarkers have been properly amplified and therefore the Test result is VALID
1 Invalid biomarkers	6 of the 7 MSI biomarkers have been properly amplified and therefore the Test result is VALID
2 Invalid biomarkers	5 of the 7 MSI biomarkers have been properly amplified and therefore the Test result is VALID
3 or more Invalid biomarkers	Less than 5 MSI biomarkers have been properly amplified and therefore the Test result is INVALID

Materials Required But Not Provided

The following materials are not provided, but are required to perform an Idylla CDx MSI Test:

Table 4. Materials required but not provided

Item	Specification
Idylla Instrument	Cat. No. P0010
Idylla Console	Cat. No. P1010
Positive and Negative Reference Controls	Cat. No. SID-000114, manufactured and sold by SensID GmbH. For more information on external control materials, please see below.
Nuclease-Free Water	N/A
Whatman Filter Papers	Grade 1: 500 circles 10 mm
Tweezers	Preferably non-ribbed tweezers
Razor Blades	Single edge blade

Quality Control

Blank or No Template Controls are not included in the design of the Idylla CDx MSI Test.

Idylla MSI FFPE Reference Set, manufactured by SensID GmbH, is sold separately from the device kit, and intended as qualitative control material for MSI markers in FFPE format based on human cell lines. This Reference Set should be used as the external controls to demonstrate that the reagents and the assay procedure perform properly. The quality control results should pass before testing patient samples. If the controls do not perform as expected, the test must be repeated and/or contact Biocartis technical support before testing patient samples. Controls should be tested:

- Before each new lot of Biocartis ldylla CDx MSI Test (IVD) Cartridges are placed into use.
- After each major service of the ldylla Instrument and/or critical Instrument part replacement.
- As required by your laboratory's standard quality control procedures.
- As required by federal, state and local guidelines.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA-cleared or -approved alternatives for the testing of FFPE tissue sections for the purposes of determining MSI status in the selection of CRC patients who are eligible for treatment with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab).

VII. MARKETING HISTORY

The Idylla CDx MSI Test has not been marketed in the United States or any foreign country.

The Idylla MSI Test has been cleared under K211181, and marketed in the United States and EU countries. The Idylla CDx MSI Test and the cleared Idylla MSI Test (K211181) have identical biomarkers, reagents, instruments and testing procedures. The configurations for these two tests are equivalent since they meet the same essential design specifications.

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 test results, and subsequently, inappropriate patient management decisions. Patients with false positive results may be inappropriately treated without clinical benefit and may experience adverse reactions associated with inappropriate therapy. Patients with false negative results may not be considered for treatment. There is also a risk of delayed results, which may lead to delay of treatment.

For the specific adverse events that occurred in the OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) clinical studies, please see the FDA approved package inserts, which are available at Drugs@FDA.

IX. SUMMARY OF NON-CLINICAL STUDIES

The Idylla CDx MSI Test and the Idylla MSI Test, cleared under K211181/S001, have identical biomarkers, reagents, instruments and testing procedures. The configurations for these two tests are equivalent since they meet the same essential design specifications. Therefore, the analytical data generated to support the Idylla MSI Test clearance is leveraged to support the Idylla CDx MSI Test after demonstrating that the samples used in the previous 510(k) are representative of the intended use population in the clinical trial to support the therapeutic product approval. These data are presented below.

A. Laboratory Studies

Accuracy: Method Comparison Study

A method comparison study was conducted to validate the accuracy of the Idylla MSI Test against a 510(k) cleared comparator device, the OncoMate MSI Dx Analysis System for the detection of MSI status in clinical samples.

The study was conducted by testing FFPE tissue specimens from 123 sequentially enrolled colorectal cancer patient samples. The sequential sample set was supplemented with 20 confirmed Lynch cases (enrichment cohort) obtained from the Colorectal Cancer Family Registry (CCFR) for a total of 143 samples. All stages of CRC (stages I – IV), and tumors from different sites of the colorectal area were represented.

Of the total 143 samples included in the study (123 sequential and 20 confirmed Lynch), valid test results were obtained for all 143 with Idylla MSI Test, and for 140 with OncoMate MSI Dx.

The agreement parameters PPA, NPA and OPA were calculated between Idylla MSI Test and OncoMate MSI for MSI status calls.

The concordance and agreement analysis between Idylla MSI Test and the OncoMate MSI Dx for the 143 samples is presented in Table 5 and Table 6 respectively. A total of 32 samples were detected as MSI-H by the OncoMate MSI Dx out of which 31 were also detected as MSI-H by the Idylla MSI Test. Of the total 108 samples detected as MSS by OncoMate MSI Dx, 107 were also MSS by Idylla MSI Test. The PPA was 96.88% and the NPA was 99.07% between the two methods.

Table 5. Concordance for MSI status between Idylla MSI Test and the OncoMate MSI Dx Analysis System for all samples

Idylla MSI Test	ONCOMATE MSI DX				
	MSI-H	MSS	Invalid	No Call	Total
MSI-H	31	1**	0	3	35
MSS	1*	107	0	0	108
Invalid	0	0	0	0	0
Total	32	108	0	3	143

*: One (1) sample that tested MSS by Idylla MSI Test and MSI-H by the OncoMate MSI Dx Analysis System is a confirmed Lynch case by NGS.
**: One (1) sample that tested MSI-H by Idylla MSI Test and MSS by the OncoMate MSI Dx Analysis System is a confirmed Lynch case by NGS.

Table 6. Percent agreement for Idylla MSI Test and the OncoMate MSI Dx Analysis System for all samples

Measure	Rate	Point Estimate (%)	95% CI
PPA	31/32	96.88	83.78 – 99.92
NPA	107/108	99.07	94.95 – 99.98
OPA	138/140	98.57	94.93 – 99.83

Analytical Sensitivity

Limit of Blank (LoB)

The LoB study was conducted to evaluate the non-specific amplification and cross-reactivity between the mutant and wild type targets. Specifically, the LoB study

evaluated whether the Idylla MSI Test can correctly generate an 'Invalid' MSI status call when there is no sample in the Cartridge. This study also evaluated whether there is cross-reactivity between the MSI mutant primers and beacons with MSI wild type DNA.

For the no template control (NTC) study, 60 cartridges from three lots were tested to examine the contamination in the investigational use only (IUO) MSI Test cartridges. The results obtained from NTC study were calculated for the proportion of invalid calls (# of invalid/60). For the Wild Type (WT) study, 20 FFPE MSS clinical samples were tested in duplicate using three different lots of IUO MSI Test cartridges to evaluate the analytical specificity of the test.

The LoB study results demonstrate that the Idylla MSI Test can correctly generate an 'Invalid' MSI status call when there is no sample in the Cartridge and the MSI status 'MSS' when a wild type sample is tested with the Idylla MSI Test.

Limit of Detection (LoD)

The LoD is defined as the lowest average mutant/total allele ratio (of weighted combined MSI biomarkers based on prevalence) that generates a positive MSI status in 95% of cases (at a 95% confidence level). The initial LoD estimation was determined using contrived samples from cell lines designed to be representative of an average heterozygous clinical sample.

For clinical samples, the LoD represents the minimum proportion of cells required to obtain a correct MSI call using clinical samples. Estimation was performed using four clinical MSI-H samples diluted to various neoplastic cell contents with MSS samples. Final LoD confirmation studies were performed using seven clinical samples (MSI-H and MSS) at approximately 33% neoplastic cell content.

LoD estimation with reference samples: The LoD estimation study was performed using reference cell lines (0% and 100% allelic frequency (AF) samples), by a titration experiment using varying levels of biomarker allelic frequency (5% - 50% mimicking heterozygous clinical samples with 10 – 100% neoplastic cell content) at two different sample input levels (high and low input) representative for clinical samples. This study result showed the estimated LoD was 30% allelic frequency, as demonstrated in Table 7 and Table 8 below.

Table 7. Estimation of LoD results (contrived samples)

Allelic Frequency Biomarkers	Number of Replicates Tested	LoD Estimation Results	
		Low Sample Input	High Sample Input
		Number of Correct Calls (%)	Number of Correct Calls (%)
50%	24	24/24 (100%)	24/24 (100%)
30%	24	24/24 (100%)	24/24 (100%)

Allelic Frequency Biomarkers	Number of Replicates Tested	LoD Estimation Results	
		Low Sample Input	High Sample Input
		Number of Correct Calls (%)	Number of Correct Calls (%)
20%	24	24/24 (100%)	24/24 (100%)
15%	24	24/24 (100%)	24/24 (100%)
10%	24	22/24 (91.7%)	24/24 (100%)
5%	24	4/24 (16.7%)	0/24 (0%)

Table 8. LoD estimation hit rates* and 95% confidence interval per biomarker and per sample in high and low input (contrived samples)

Sample Dilution Series (AF%)		Individual Biomarker Results (Point Estimate or Hit Rate), 95% CI						
		BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
High Input Background	**5% MSI-H**	4% (1/24)	0% (0/24)	0% (0/24)	0% (0/24)	0% (0/24)	0% (0/24)	0% (0/24)
		0.74-20.24%	0.00-13.80%	0.00-13.80%	0.00-13.80%	0.00-13.80%	0.00-13.80%	0.00-13.80%
	10% MSI-H	100% (24/24)	88% (21/24)	100% (24/24)	100% (24/24)	75% (18/24)	12% (3/24)	83% (20/24)
		86.20-100%	69.00-95.66%	86.20-100%	86.20-100%	55.10-88.00%	4.34-31.00%	64.15-93.32%
	15% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	92% (22/24)	42% (10/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	74.15-97.68%	24.47-61.17%	86.20-100%
	20% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	92% (22/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	74.15-97.68%	86.20-100%
	30% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%
	50% MSI-H	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)	100% (24/24)

Sample Dilution Series (AF%)		Individual Biomarker Results (Point Estimate or Hit Rate), 95% CI						
		BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
		86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%	86.20-100%
Low Input Background	5% MSI-H	17% (4/24)	8% (2/24)	4% (1/24)	17% (4/24)	4% (1/24)	0% (0/24)	8% (2/24)
		6.68-35.85%	2.32-25.85%	0.74-20.24%	6.68-35.85%	0.74-20.24%	0.00-13.80%	2.32-25.85%
	10% MSI-H	83% (20/24)	50% (12/24)	88% (21/24)	75% (18/24)	88% (21/24)	17% (4/24)	75% (18/24)
		64.15-93.32%	31.43-68.57%	69.00-95.66%	55.10-88.00%	69.00-95.66%	6.68-35.85%	55.10-88.00%
	15% MSI-H	88% (21/24)	88% (21/24)	100% (24/24)	96% (23/24)	92% (22/24)	33% (8/24)	83% (20/24)
		69.00-95.66%	69.00-95.66%	86.20-100%	79.76-99.26%	74.15-97.68%	17.97-53.29%	64.15-93.32%

Clinical LoD estimation: The clinical LoD was estimated using four FFPE samples through a titration study with varying levels of MSI-H neoplastic cell content (5-50%) as shown in Table 9. The call rates for Sample 4 were variable because this sample had a ddPCR measurement that was well below the validated linear range of the ddPCR method before dilutions were made. This sample was also originally characterized as challenging, with only two positive markers. Therefore, the call rate for this sample could also be indicative of the performance with challenging samples.

Table 9. Estimation of clinical LoD results

% Neoplastic cell content	Correct MSI-H Call (Hit Rate)				Results (correct calls)
	Sample 1	Sample 2	Sample 3	Sample 4	
50%	6/6	6/6	6/6	6/6	24/24
40%	6/6	6/6	6/6	6/6	24/24
30%	6/6	6/6	6/6	4/6	22/24
20%	6/6	6/6	6/6	5/6	23/24
10%	6/6	6/6	6/6	5/6	23/24
5%	0/6	3/6	6/6	1/6	10/24

Clinical LoD confirmation: The clinical LoD was confirmed using seven FFPE clinical samples (MSI-H and MSS including challenging samples) at approximately 33% neoplastic cell content and the minimum required tissue level (25 mm² per 10 µm section). The samples were tested across two Cartridge lots in ten replicates per lot (n=20) over five days on multiple instruments (total n=140 from all seven samples). As presented in Table 10 below, all seven clinical samples (MSI-H and MSS) including those with neoplastic cell content of ≤33% and the lowest sample input size of 25 mm² per 10 µm section, generated reports with 100% correct calls. This study confirms the clinical analytical sensitivity (LoD) of the Idylla MSI Test at approximately ≤33% neoplastic cell content in clinical samples at the lowest sample input size (25 mm^2 per 10 µm section). A secondary analysis of the data was performed at the biomarker level as shown in Table 11 for the MSI-H samples and in Table 12 for the MSS samples.

Table 10. Clinical LoD confirmation study results (clinical samples tested at ≤33 % neoplastic cell content and the lowest sample input)

Sample ID	Cartridge Lot	Total Runs	MSI Status (Idylla Results)	Concordant (correct) Call %
Sample 1 (MSI-H) **Sample 2 (MSI-H)** **Sample 3 (MSI-H)** **Sample 4 (MSI-H)**	Lot 1	40	MSI-H	100% (80/80)
	Lot 2	40		
Sample 5 (MSS) **Sample 6 (MSS)** **Sample 7 (MSS)**	Lot 1	30	MSS	100% (60/60)
	Lot 2	30		

Table 11. LoD confirmation study: Point estimate and 95% CI presented per biomarker and per sample for MSI-H samples (clinical specimens)

Sample	Individual Biomarker Results (Point Estimate or Hit Rate*) 95% CI at 33% Neoplastic Cell Content						
	BTBD7	**RYR3**	**SEC31A**	**ACVR2A**	**DIDO1**	**MRE11**	**SULF2**
Sample 1	100% (20/20 mut)	95.00% (19/20 wt)	100% (20/20 mut)	100% (20/20 mut)	70% (14/20 mut)	95.00% (19/20 wt)	100% (20/20 mut)
	83.89-100%	76.39- 99.11%	83.89-100%	83.89-100%	48.1-85.45%	76.39- 99.11%	83.89-100%
Sample 2	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	95.00% (19/20 mut)	65% (13/20 mut)
	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	76.39- 99.11%	43.29- 81.88%
Sample 3	100% (20/20 mut)	100% (20/20 mut)	85% (17/20 wt)	100% (20/20 wt)	100% (20/20 mut)	100% (20/20 wt)	100% (20/20 wt)

Sample	Individual Biomarker Results (Point Estimate or Hit Rate*) 95% CI at 33% Neoplastic Cell Content						
	BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
	83.89-100%	83.89-100%	63.96-94.76%	83.89-100%	83.89-100%	83.89-100%	83.89-100%
Sample 4	100% (20/20 mut)	100% (20/20 wt)	35% (7/20 wt)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)	100% (20/20 mut)
	83.89-100%	83.89-100%	18.12-57.00%	83.89-100%	83.89-100%	83.89-100%	83.89-100%

*Hit rates of biomarkers n/N: mutant detected/total observations.

Table 12. LoD confirmation study: mutant hit rates and 95% CI presented per biomarker and per sample for MSS samples

Sample	Individual Biomarker Results (Point Estimate or Hit Rate*) 95% CI at 33% Neoplastic Cell Content						
	BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 5	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)
	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%
Sample 6	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)
	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%
Sample 7	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)	100% (20/20 wt)
	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%	83.89-100%

*Hit rates of biomarkers n/N: mutant detected/total observations.
Note: 'mut' refers to a mutant biomarker and 'wt' refers to a wild type biomarker

For MSS samples, individual biomarkers score consistently as wild type (Table 12). For clinical MSI-H samples, a concordance of ≥ 95%-point estimate can be observed for 4 biomarkers (BTBD7, RYR3, ACVR2A and MRE11) across all 4 MSI-H samples. The remaining biomarkers showed concordance below 95% for some samples. For SEC31A, two samples had hit rates below 95%, Sample 3 at 85% and Sample 4 at 35%. DIDO1 demonstrated a 70% hit rate in sample 1, and SULF2 demonstrated a hit rate of 65% with sample 2 (Table 11).

Precision/Reproducibility

The inter-lab reproducibility study was conducted at three laboratory sites. Seven individual clinical specimens (MSI-H and MSS FFPE CRC tissue sections) that satisfied

the sample requirement conditions were tested at all three sites with three lots of Idylla MSI Test Cartridges, on the Idylla Platform. This study was designed to evaluate the impact of lot-to-lot, instrument/operator, inter-day and inter-lab variability on the Idylla MSI Test. At each site, each sample was tested by one operator on four Instruments with one replicate per Instrument per day, for three non-consecutive days using three lots of Idylla MSI Test Cartridges, resulting in a total of twelve replicates per sample per site. Overall, this study produced 36 results (tests) in total per sample from all three sites. All 252 valid runs from the three sites were used for data analysis. As presented in Table 13 below, all three sites generated identical and correct MSI status calls across the sites for all seven clinical samples tested. Reproducibility data showed no relevant effects of the different variation sources analyzed (inter-lab, inter-Instrument, inter-lot Cartridge and inter-day) in this study. A secondary analysis of the data from the multi-site reproducibility study using FFPE clinical samples was performed for every biomarker and for each of the specimens. Table 14 provides a global overview of the analysis for every sample, including the agreement for both MSI status and individual biomarkers with 95% CI.

Table 13. Reproducibility study results

Clinical Sample	Number of Replicates Tested	Reproducibility Results	
		Number of correct MSI Status Calls	Concordance Rate (%)
Sample 1 (MSI-H)	36	36/36	100%
Sample 2 (MSI-H)	36	36/36	100%
Sample 3 (MSI-H)	36	36/36	100%
Sample 4 (MSI-H)	36	36/36	100%
Sample 5 (MSS)	36	36/36	100%
Sample 6 (MSS)	36	36/36	100%
Sample 7 (MSS)	36	36/36	100%
WITHIN SITE			
Site 1	84	84/84	100%
Site 2	84	84/84	100%
Site 3	84	84/84	100%
All Sites	252	252 / 252	100%

Table 14. Summary of PPA (MUT) and NPA (WT) and 95% Cl for MSI status and individual biomarkers in FFPE clinical samples

Sample	Reference MSI Status	Tissue size (mm^2)	%Neoplastic Cells in Tissue	Agreement To Reference Status % PPA (MUT) And NPA (WT) (Hit Rate), 95% CI	Individual Biomarker Results (Point Estimate (Hit Rate), 95% CI)						
					BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 1	MSI-H	75	30 - 40	100% (36/36)	100% (36/36 MUT)	100% ** (36/36 MUT)	100% (36/36 WT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)
				90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%
Sample 2*	MSI-H	30	50 - 60	100% (36/36)	77.78% (28/36 WT)	97.22% ** (35/36 MUT)	100% (36/36 WT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	86.11% (31/36 WT)
				90.36-100%	61.92-88.28%	85.83-99.51%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	71.34-93.92%
Sample 3	MSI-H	160	30-40	100% (36/36)	80.56 % (29/36 MUT)	97.22% (35/36 MUT)	97.22% (35/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	94.44% (34/36 MUT)	97.22% (35/36 MUT)
				90.36-100%	64.97-90.25%	85.83-99.51%	85.83-99.51%	90.36-100%	90.36-100%	81.86-98.46%	85.83-99.51%
Sample 4	MSI-H	125	40	100% (36/36)	100% (36/36 MUT)	66.67% (24/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	100% (36/36 MUT)	86.11% (31/36 MUT)	97.22% (35/36 WT)
				90.36-100%	90.36-100%	50.33-79.79%	90.36-100%	90.36-100%	90.36-100%	71.34-93.92%	85.83-99.51%
Sample 5	MSS	37	35	100% (36/36)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)
				90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%

Sample	Reference MSI Status	Tissue size (mm^2)	%Neoplastic Cells in Tissue	Agreement To Reference Status % PPA (MUT) And NPA (WT) (Hit Rate), 95% CI	Individual Biomarker Results (Point Estimate (Hit Rate), 95% CI)						
					BTBD7	RYR3	SEC31A	ACVR2A	DIDO1	MRE11	SULF2
Sample 6	MSS	35	50	100% (36/36)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)
				90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%	90.36-100%
Sample 7*	MSS	110	40	100% (36/36)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)	88.89%** (32/36 WT)	100% (36/36 WT)	100% (36/36 WT)	100% (36/36 WT)
				90.36-100%	90.36-100%	90.36-100%	90.36-100%	74.69 – 95.59	90.36-100%	90.36-100%	90.36-100%

* Borderline samples per Idylla Test v2.0 initial screening results

** While reproducibility for this marker across 36 replicates is > 85%, the biomarker call observed is discordant to the biomarker status of the clinical sample that was obtained during sample characterization.

All three sites generated correct MSI status calls across the sites for all seven clinical samples tested. The reproducibility across 36 replicates for each biomarker per sample was between 77.8 and 100%, for some occasions a biomarker call was observed that was discordant to the initial biomarker status of the clinical sample (** indicated in Table 14).

Analytical Specificity/Interfering Substances

Interference testing was performed using seven FFPE colorectal cancer clinical samples (MSI-H and MSS) that met the sample requirement criteria, including commonly encountered interfering substances such as hemoglobin, triglycerides and paraffin. For hemoglobin and triglycerides, interference was tested at the highest possible concentration levels (2 mg/ml and 37 mmol/l, respectively) per CLSI guidelines Interference Testing in Clinical Chemistry, EP07-A2. To assess the paraffin interference, one additional 10 µm paraffin blank section was added to the Cartridge along with the sample. Each sample was tested with an interfering substance in five replicates using a single lot of Idylla MSI Cartridges. Additionally, the samples were tested without an interfering substance as a control. The results obtained for each interfering substance were checked for a correct MSI status call, and compared with the control results (runs without interfering substance) for the proportion (%) of concordance with control results.

As presented in Table 15 below, all seven samples tested with interference substances i.e., hemoglobin, triglycerides and excess paraffin generated correct MSI status calls, and showed 100% concordance with control results (with no interference substance). Therefore hemoglobin, triglycerides and paraffin have no impact on the Idylla MSI Test.

Table 15. Interference results for Hemoglobin, Triglycerides and paraffin

Interference Substance	Total Runs* with Each Interference Substance	Interference Test Results	
		Correct MSI Status Calls (%)	Concordance with Control Results (%)
Control (No Interferent)	35	35 / 35 (100%)	-
Hemoglobin (2 mg / ml)	35	35 / 35 (100%)	100%
Triglycerides (37 mm)	35	35 / 35 (100%)	100%
Paraffin (1 x 10 µm section)	35	35 / 35 (100%)	100%

* Total runs from all seven clinical samples tested (at five runs per sample)

Mucin (i.e., % mucinous cells in FFPE sample) impact on the Idylla MSI Test was evaluated using nineteen individual FFPE clinical samples with various levels of mucinous cell content (0% - 60%). These samples included both MSI-H and MSS samples that met sample requirement criteria for the test. Each mucinous sample was tested in three replicates using one lot of Idylla MSI Test Cartridges. The results obtained were summarized for the mucinous cell content for each sample tested and their valid MSI status call. The highest mucinous cell content (%)at which the sample still generated a valid and correct MSI status call is considered the tested interference limit for mucinous content impact on the Idylla MSI Test results. All 19 mucinous samples results showed correct MSI calls for approximately 96.5% runs (55 of 57 runs) performed, except one sample which generated 'Invalid' results for two out of three runs performed. This study indicated that samples with mucinous cell content up to 60% have no impact on the performance of the Idylla MSI Test, and can still generate correct MSI status calls.

The impact of necrosis content >50% on the Idylla MSI Test was evaluated using four FFPE clinical samples with various levels of necrosis content (66-73%). The samples included both MSI-H and MSS samples. Each sample was tested in triplicate with and without necrosis content. All four samples generated a correct MSI status call for testing with and without necrosis. Therefore, necrosis content up to 73% has no impact on the Idylla MSI Test results.

Stability

Specimen Stability

To establish the specimen stability, four (4) different FFPE blocks (2 MSS and 2 MSI-H) ranging from good quality to bad quality were selected. Per block, two (2) slices and two (2) slides were tested in duplicates for 6 time points. The study results show that the FFPE tissue sections can be stored at 15-30°C for up to 366 days.

Long Term Stability

A real time stability study was performed. The study used clinical specimens consisting of three (3) MSI-H samples (two (2) replicates per sample tested at each timepoint per storage temperature) and three (3) MSS samples (two (2) replicates per sample tested at each timepoint per storage temperature). Cartridges were stored for 19 months at 5 ± 3°C and 30 ± 2°C storage conditions. The study results support 18 months stability when stored at 5 ± 3°C and 12 months stability when stored at 30 ± 2°C storage conditions for the Idylla MSI Test. However, of the total N = 367 cartridges tested over all three cartridge batches and multiple time points, there were a total of 14 invalid cartridge calls. Therefore, an ongoing confirmatory stability study was initiated in January 2025, and the first timepoint (3 months) was evaluated on April 10, 2025. Additionally, the stability study of the external controls has been incorporated into the ongoing long term stability study.

Shipping Stability

Cartridges underwent temperature cycling between -20°C for 72 hours and +25°C for 24 hours across a total of two cycles. The third winter cycle of 48 hours at -20°C and 24 hours at +25°C hours followed immediately. After completion of winter cycling, the cartridges were exposed to the temperature of 40°C for 72 hours.

Immediately after temperature cycles, 9 MSI cartridges were tested with 6 FFPE MSI slices and 3 FFPE MSS slices to generate the time point 0 data. The reference baseline for data analysis was time point 0 data (QC release). The cartridges exposed to the thermal cycling were stored at 30 ± 2 °C and further tested according to the real time stability study protocol.

The results show that exposing the Idylla MSI Test to high temperatures (up to 40°C) and low temperatures (to -20°C) for a limited time of up to 72 hours does not impact product performance.

In Use Stability

For this study, 84 MSI cartridges were removed from the pouch and kept in the incubator at 30 ± 2 °C and 75 % Relative humidity (RH) for 6 days. First 12 cartridges were removed from the incubator after two hours (= day 0) and tested with 6 MSI-H

samples and 6 MSS samples. The remaining cartridges were run on day 1 to 6 (six cartridges per day). In-use stability study (without pouch, without specimen) was verified at the beginning (within 3 months after T0) and at the end of shelf life.

For in-use study 1, MSI cartridges were removed from their pouches and placed in an incubator (at 30 ± 2 °C and 75 ± 5 % RH) for up to 6 days. The first cartridges were tested with MSS and MSI FFPE slices after 2 hours (= day 0) in the incubator, and the remaining cartridges were tested at the 5 subsequent time points, i.e., day 2 to day 6. Six cartridges were tested per time point: 3 cartridges with FFPE MSS slices and 3 cartridges with FFPE MSI slices.

For In-use study 2, MSI cartridges were removed from their pouches, loaded with a MSS FFPE slice or MSI slice. Six cartridges loaded with the two input sample types were run on Idylla immediately after the sample was loaded (hour 0). The other loaded cartridges were placed in an incubator (at 30 ± 2 °C and 75 ± 5 % RH) for 3 and 9 hours before running them on Idylla. Six cartridges were tested per time point: 3 cartridges with FFPE MSS slices and 3 cartridges with FFPE MSI slices.

The in-use stability data obtained at the beginning and end of shelf life support that MSI cartridges can be used up to:

- 5 days after removal from the pouch and stored on the bench (at laboratory ambient conditions) without the sample
- 8 hours after removal from the pouch, loaded with a sample and stored at laboratory ambient conditions.

B. Animal Studies

Not Applicable

C. Additional Studies

Not Applicable

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of the Idylla CDx MSI test as a companion diagnostic (CDx) device to aid in the identification of patients with CRC who are likely to benefit from treatment with OPDIVO (nivolumab) alone or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) was established in the CHECKMATE-8HW clinical study and the associated clinical bridging study.

A. Study Design

CHECKMATE-8HW is a Phase 3, randomized, 3-arm open-label study of nivolumab monotherapy (Arm A), nivolumab plus ipilimumab combination therapy (Arm B) or investigator's choice chemotherapy (Arm C) for the treatment of subjects with deficient

mismatch repair/microsatellite instability-high (dMMR/MSI-H) metastatic colorectal cancer (mCRC). In this study, subjects were enrolled and randomized based on local dMMR/MSI-H test results, referred to as Clinical Trial Assay positive (CTA+) in this document. The clinical performance of the Idylla CDx MSI Test was demonstrated through the assessment of concordance between CTA and the Idylla CDx MSI Test.

A total of 839 patients with unresectable or metastatic microsatellite instability high or mismatch repair-deficient colorectal cancer by local testing were initially enrolled in the CA209-8HW trial. Two participant samples were later determined to be local negatives. Therefore, the bridging study was conducted with 837 subjects randomly assigned to receive nivolumab plus ipilimumab (352 patients), nivolumab (353 patients), or chemotherapy (132 patients) across all lines of therapy (Table 16).

First Line Setting

Among 303 patients in the first line setting who were randomly assigned to nivolumab in combination with ipilimumab (200) and to chemotherapy (101), 147 and 71 patients had Idylla CDx MSI test confirmed MSI-H status in nivolumab in combination with ipilimumab arm and chemotherapy arm, respectively.

All Lines Setting

Among 707 patients across all treatment lines who were randomly assigned to nivolumab in combination with ipilimumab (352) and to nivolumab (353) single agent, 257 and 247 patients had Idylla CDx MSI test confirmed MSI-H status in the nivolumab in combination with ipilimumab arm and in the nivolumab arm, respectively.

Table 16: Number of Participants for Different Treatments

Treatment Arm	**All Randomized Patients**	**All Randomized Patients Biocartis Confirmed dMMR/MSI-H**	**All Randomized Patients**	**All Randomized Patients Biocartis Confirmed dMMR/MSI-H**
	All Lines	**All Lines**	**First Line**	**First Line**
Arm A: Nivolumab	N=353	N=247	N=201	N=170
Arm B: Nivolumab plus Ipilimumab	N=352*	N=257	N=200	N=147
Arm C: Chemotherapy	N=132	N=113	N=101	N=71

* Two participant (2) samples were later determined to be local negatives

1. Clinical Inclusion and Exclusion Criteria

The sample inclusion and exclusion criteria for the retrospective testing of the clinical bridging study were:

Inclusion Criteria

- Specimens meet the screening enrollment requirements for the study CHECKMATE-8HW
- Specimens meet the screening tissue requirements for the Idylla CDx MSI Test
  - The minimal tissue volume available before input in the cartridge for one test run should be 0.25 μm^3, which corresponds to one slice, 62.5 mm2 for 4 µm sections, 50 mm^2 for 5 µm or 25 mm2 for 10 µm sections. Minimal Tissue Volume available should be sufficient for one test run on the Idylla system and to accommodate one repeat in the event of an invalid result.
  - Samples for evaluations should contain a minimum neoplastic cell content of 33%.

Exclusion Criteria

- Specimens that fail to meet the inclusion criteria are excluded from the study.
- Specimens from subjects that withdrew their Informed Consent.

2. Follow-up Schedule

For the CHECKMATE-8HW clinical study, PFS and OS are critical endpoints. Participants continue to be followed for collection of outcomes and/or survival follow-up data until death or the conclusion of the study. All participants must be followed for at least 100 days after last dose of study treatment with the exception of participants in the chemotherapy arm that experience progressive disease on treatment and who enter the Crossover Cohort. These participants are required to complete at least Follow Up Visit 1 following end of treatment in the chemotherapy arm.

3. Clinical Bridging Study Endpoints

The endpoints for the clinical efficacy of Idylla CDx MSI-H for the intended use are:

- Progression-free survival (PFS) comparison, in HR of nivolumab + ipilimumab vs. chemotherapy in first line, for mCRC patients determined as Idylla CDx MSI-H in the Intended Use.
- Objective Response Rate (ORR) and PFS per blinded independent central review (BICR) for nivolumab + ipilimumab vs nivolumab in all lines of treatment, for mCRC patients determined as Idylla CDx MSI-H in the Intended Use.

The acceptance criteria: the clinical efficacy results across the range of the tipping point analysis are clinically meaningful, i.e., the clinical efficacy results are consistent with the efficacy of the CTA+ randomized participants.

B. Accountability of PMA Cohort

The total number of CTA+ participants randomized to the CHECKMATE-8HW trial was 837 (2 randomized samples were later determined to be local negatives). Of these, 725 subjects had valid Idylla CDx MSI Test results, and 112 were missing due to cancellation for not meeting the test requirements or other quality requirements as listed in Table 17 below.

Table 17. Accountability of PMA cohort

Description	Number
Randomized CTA+ Subjects	837
Subjects not received by central testing lab for evaluation by the Idylla CDx MSI test	6
Subjects cancelled prior to testing by the Idylla CDx MSI test*	96
Subjects with tests results removed from dataset**	10
Subjects included in Idylla CDx MSI test PMA dataset	725

*: includes subjects that did not meet testing protocol inclusion criteria.
**: includes 7 subjects with invalid Idylla™ CDx MSI Test results, 3 subjects were removed since they were tested under enrollment or test protocol deviations.

C. Study Population Demographics and Baseline Parameters

The demographics, baseline disease and specimen characteristics of patients evaluated or not evaluated by the Idylla CDx MSI Test are presented in Tables 18 to 20. The distributions of demographics and other baseline disease and specimen characteristics of the patients were generally balanced between the evaluated or not evaluated groups by the Idylla CDx MSI Test and similar between the CTA+ randomized patients and those centrally confirmed as MSI-H by the Idylla CDx MSI Test.

Table 18. Summary of age, sex, race, and ethnicity by Idylla CDx MSI valid and missing status

	Idylla CDx MSI Valid N = 725	Idylla CDx MSI Missing N = 112	Total, CTA+ N = 837
Age (years)			
Mean	60.9	57.9	60.5

	Idylla CDx MSI Valid N = 725	Idylla CDx MSI Missing N = 112	Total, CTA+ N = 837
Median	63.0	60.0	63.0
Min, Max	20, 87	21, 86	20, 87
Q1, Q3	52.0, 71.0	49.0, 69.0	51.0, 71.0
SD	13.5	14.6	13.7
Age Categorization, n (%)			
0 - 49	161 (22.2)	28 (25.0)	189 (22.6)
50 - 64	221 (30.5)	42 (37.5)	263 (31.4)
65 - 79	301 (41.5)	37 (33.0)	338 (40.4)
>= 80	42 (5.8)	5 (4.5)	47 (5.6)
Sex, n (%)			
Male	352 (48.6)	63 (56.3)	415 (49.6)
Female	373 (51.4)	49 (43.8)	422 (50.4)
Race, n (%)			
White	630 (86.9)	97 (86.6)	727 (86.9)
Black or African American	10 (1.4)	3 (2.7)	13 (1.6)
Asian	72 (9.9)	6 (5.4)	78 (9.3)
Other	13 (1.8)	6 (5.4)	19 (2.3)
Ethnicity, n (%)			
Hispanic or Latino	67 (9.2)	10 (8.9)	77 (9.2)
Not Hispanic or Latino	362 (49.9)	58 (51.8)	420 (50.2)
Not Reported	296 (40.8)	44 (39.3)	340 (40.6)

Table 19. Summary of baseline disease characteristics by Idylla CDx MSI by valid and missing status

	MSI Valid N=725	MSI Missing N=112	Total N=837
ECOG Performance Status, n (%)			
0	387 (53.4)	49 (43.8)	436 (52.1)
>= 1	338 (46.6)	63 (56.3)	401 (47.9)
Weight (kg)			

	MSI Valid N=725	MSI Missing N=112	Total N=837
Mean	70.12	72.22	70.40
Median	68.00	70.35	68.00
Min, max	34.0, 152.0	34.9, 178.0	34.0, 178.0
Q1, Q3	58.00, 80.00	60.00, 80.90	58.00, 80.00
SD	17.26	18.92	17.50
Tobacco Use, n (%)			
Yes	87 (12.0)	12 (10.7)	99 (11.8)
No	537 (74.1)	82 (73.2)	619 (74.0)
Not reported	101 (13.9)	18 (16.1)	119 (14.2)
Alcohol Use, n (%)			
Yes	31 (4.3)	4 (3.6)	35 (4.2)
No	593 (81.8)	90 (80.4)	683 (81.6)
Not reported	101 (13.9)	18 (16.1)	119 (14.2)
Disease Stage at Initial Diagnosis, n (%)			
Stage 0	0	0	0
Stage I	6 (0.8)	1 (0.9)	7 (0.8)
Stage II	131 (18.1)	15 (13.4)	146 (17.4)
Stage III	293 (40.4)	23 (20.5)	316 (37.8)
Stage IV	293 (40.4)	72 (64.3)	365 (43.6)
Not reported	2 (0.3)	1 (0.9)	3 (0.4)
Histological Grade, n (%)			
Gx	101 (13.9)	23 (20.5)	124 (14.8)
G1	74 (10.2)	17 (15.2)	91 (10.9)
G2	283 (39.0)	44 (39.3)	327 (39.1)
G3	249 (34.3)	22 (19.6)	271 (32.4)
G4	17 (2.3)	3 (2.7)	20 (2.4)
Not reported	1 (0.1)	3 (2.7)	4 (0.5)
Cell Type, n (%)			
Adenocarcinoma	689 (95.0)	107 (95.5)	796 (95.1)
Other type	35 (4.8)	5 (4.5)	40 (4.8)
Not reported	1 (0.1)	0	1 (0.1)
Tumor Location, n (%)			
Rectum/Rectosigmoid junction	69 (9.5)	22 (19.6)	91 (10.9)
Cecum	92 (12.7)	12 (10.7)	104 (12.4)

	MSI Valid N=725	MSI Missing N=112	Total N=837
Colon ascending/hepatic flexure	311 (42.9)	46 (41.1)	357 (42.7)
Colon descending/splenic flexure	70 (9.7)	8 (7.1)	78 (9.3)
Colon sigmoid	79 (10.9)	15 (13.4)	94 (11.2)
Colon transverse	98 (13.5)	9(8.0)	107 (12.8)
Unknown	6 (0.8)	0	6 (0.7)
Tumor Sidedness (CRF), n (%)			
Left	218 (30.1)	45 (40.2)	263 (31.4)
Right	507 (69.9)	67 (59.8)	574 (68.6)
Number of Prior Lines of Therapy (CRF), n (%)			
0	404 (55.7)	69 (61.6)	473 (56.5)
1	179 (24.7)	23 (20.5)	202 (24.1)
>= 2	140 (19.3)	20 (17.9)	160 (19.1)
Not reported	2 (0.3)	0	2 (0.2)
Time From Initial Disease Diagnosis to Randomization, n (%)			
< 1 year	354 (48.8)	67 (59.8)	421 (50.3)
>= 1 and < 3 years	256 (35.3)	28 (25.0)	284 (33.9)
>= 3 years	114 (15.7)	17 (15.2)	131 (15.7)
Not reported	1 (0.1)	0	1 (0.1)
Liver Metastasis per BICR, n (%)			
Yes	292 (40.3)	55 (49.1)	347 (41.5)
No	430 (59.3)	57 (50.9)	487 (58.2)
Not reported	3 (0.4)	0	3 (0.4)
Lung Metastasis per BICR, n (%)			
Yes	188 (25.9)	29 (25.9)	217 (25.9)
No	534 (73.7)	83 (74.1)	617 (73.7)
Not reported	3 (0.4)	0	3 (0.4)
Peritoneal Metastasis per BICR, n (%)			
Yes	282 (38.9)	47 (42.0)	329 (39.3)
No	440 (60.7)	65 (58.0)	505 (60.3)
Not reported	3 (0.4)	0	3 (0.4)
PD-L1 Status, n (%)			

	MSI Valid N=725	MSI Missing N=112	Total N=837
>= 1%	140 (19.3)	16 (14.3)	156 (18.6)
< 1%	562 (77.5)	60 (53.6)	622 (74.3)
Non evaluable/indeterminate	4 (0.6)	2 (1.8)	6 (0.7)
Not available	19 (2.6)	34 (30.4)	53 (6.3)
IHC Test Results per Central Assessment, n (%)			
dMMR	595 (82.1)	67 (59.8)	662 (79.1)
pMMR	102 (14.1)	13 (11.6)	115 (13.7)
Not available	28 (3.9)	32 (28.6)	60 (7.2)
IHC Test Results per Local Assessment, n (%)			
dMMR	596 (82.2)	95 (84.8)	691 (82.6)
pMMR	11 (1.5)	0	11 (1.3)
Not available	118 (16.3)	17 (15.2)	135 (16.1)
PCR Test Results per Central Assessment, n (%)			
MSI-H	600 (82.8)	0	600 (71.7)
MSI-L/MSS	125 (17.2)	0	125 (14.9)
Not available	0	112 (100.0)	112 (13.4)
PCR Test Results per Local Assessment, n (%)			
MSI-H	264 (36.4)	42 (37.5)	306 (36.6)
MSI-L/MSS	4 (0.6)	3 (2.7)	7 (0.8)
Not available	457 (63.0)	67 (59.8)	524 (62.6)
NGS Test Results per Local Assessment, n (%)			
MSI-H	81 (11.2)	12 (10.7)	93 (11.1)
MSI-L/MSS	6 (0.8)	3 (2.7)	9 (1.1)
Not available	638 (88.0)	97 (86.6)	735 (87.8)
BRAF Mutation Status, n (%)			
Wild type/No mutation	312 (43.0)	51 (45.5)	363 (43.4)
Mutant	209 (28.8)	22 (19.6)	231 (27.6)
Not available	204 (28.1)	39 (34.8)	243 (29.0)

	MSI Valid N=725	MSI Missing N=112	Total N=837
KRAS Mutation Status, n (%)			
Wild type/no mutation	369 (50.9)	47 (42.0)	416 (49.7)
Mutant	169 (23.3)	28 (25.0)	197 (23.5)
Not available	187 (25.8)	37 (33.0)	224 (26.8)
NRAS Mutation Status, n (%)			
Wild type/no mutation	458 (63.2)	64 (57.1)	522 (62.4)
Mutant	21 (2.9)	3 (2.7)	24 (2.9)
Not available	246 (33.9)	45 (40.2)	291 (34.8)
Lynch Syndrome, n (%)			
Yes	103 (14.2)	17 (15.2)	120 (14.3)
No	424 (58.5)	64 (57.1)	488 (58.3)
Unknown	185 (25.5)	30 (26.8)	215 (25.7)
Not reported	13 (1.8)	1 (0.9)	14 (1.7)
Time From Completion of Most Recent Prior Adjuvant/Neoadjuvant Therapy to Treatment, n (%)			
< 6 months	61 (8.4)	6 (5.4)	67 (8.0)
6 -< 12 months	58 (8.0)	4 (3.6)	62 (7.4)
>= 12 months	125 (17.2)	13 (11.6)	138 (16.5)
Not reported	481 (66.3)	89 (79.5)	570 (68.1)
Prior Surgery Related to Current Cancer, n (%)			
Yes	674 (93.0)	63 (56.3)	737 (88.1)
No	51 (7.0)	49 (43.8)	100 (11.9)
Prior Radiotherapy, n (%)			
Yes	67 (9.2)	13 (11.6)	80 (9.6)
No	658 (90.8)	99 (88.4)	757 (90.4)

Table 20. Summary of specimen characteristics by Idylla CDx MSI by valid and missing status

	MSI Valid N =725	MSI Missing N = 112	Total N = 837
Anatomic Location by Site of Collection of Specimen, n (%)			
Primary	628 (86.6)	78 (69.6)	706 (84.3)
Colon	440 (60.7)	53 (47.3)	493 (58.9)

	MSI Valid N =725	MSI Missing N = 112	Total N = 837
Metastatic	89 (12.3)	28 (25.0)	117 (14.0)
Cervix	1 (0.1)	0	1 (0.1)
Liver	28 (3.9)	12 (10.7)	40 (4.8)
Lung	6 (0.8)	2 (1.8)	8 (1.0)
Lymph node	16 (2.2)	5 (4.5)	21 (2.5)
Ovary	6 (0.8)	0	6 (0.7)
Peritoneum	11 (1.5)	2 (1.8)	13 (1.6)
Skin	1 (0.1)	0	1 (0.1)
Soft tissue	0	1 (0.9)	1 (0.1)
Stomach	1 (0.1)	0	1 (0.1)
Unknown/not reported	19 (2.6)	6 (5.4)	25 (3.0)
Recurrent	8 (1.1)	0	8 (1.0)
Colon	5 (0.7)	0	5 (0.6)
Unknown/not reported	3 (0.4)	0	3 (0.4)
Specimen Type, n (%)			
Slides	351 (48.4)	40 (35.7)	391 (46.7)
Tissue block	373 (51.4)	66 (58.9)	439 (52.4)
Tissue in formalin	1 (0.1)	0	1 (0.1)
Unknown/ not reported	0	6 (5.4)	6 (0.7)
Biopsy Type, n (%)			
Core needle biopsy	37 (5.1)	35 (31.3)	72 (8.6)
Excisional biopsy	49 (6.8)	22 (19.6)	71 (8.5)
Incisional biopsy	22 (3.0)	20 (17.9)	42 (5.0)
Surgical resection	602 (83.0)	26 (23.2)	628 (75.0)
Other	15 (2.1)	3 (2.7)	18 (2.2)
Unknown/ not reported	0	6 (5.4)	6 (0.7)
Fixative Time, n (%)			
< 12 H	56 (7.7)	9 (8.0)	65 (7.8)

	MSI Valid N =725	MSI Missing N = 112	Total N = 837
12 -< 24 H	123 (17.0)	24 (21.4)	147 (17.6)
24 -< 48 H	80 (11.0)	9 (8.0)	89 (10.6)
48-72 H	6 (0.8)	1 (0.9)	7 (0.8)
> 72 H	9 (1.2)	1 (0.9)	10 (1.2)
Thickness of Section, n (%)			
4 microns	331 (45.7)	39 (34.8)	370 (44.2)
Other	22 (3.0)	2 (1.8)	24 (2.9)
Time of Excision to Fixative Immersion, n (%)			
<= 30 min	151 (20.8)	26 (23.2)	177 (21.1)
> 30 min	89 (12.3)	15 (13.4)	104 (12.4)

The Procured Negative Samples

A total of 156 samples with test results from IHC (proficient mismatch repair or pMMR) or PCR (MSS), single negative, were procured from commercial vendors. The median age of patients from which samples were collected was 68 years with a range of 38 – 88 years of age. The sex distribution is comparable with 48% female and 52% male. The race distribution was predominantly white (94.231%). Samples were represented from Stage I – IV of the disease, with Stage IV alone contributing 36.5%. The distributions of demographics and other baseline disease and specimen characteristics of the patients were generally representative and balanced.

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with nivolumab in combination with ipilimumab, or as a single agent, in MSI-H mCRC was addressed in BLA (125377/S135 and 125554/S132) based on the results from the CHECKMATE-8HW trial. Please refer to Drugs@FDA for complete safety information. The Idylla CDx MSI Test was used as one of the CDx tests in CHECKMATE-8HW trial which is the basis for this PMA submission.

The safety of the device is based on nonclinical laboratory testing results as well as data collected in a clinical bridging study conducted to support PMA approval as described above. The validation data supports the Idylla CDx MSI Test as a reliable method for reporting MSI status as MSS or MSI-H.

2. Effectiveness Results

Concordance Results between CTA and Idylla CDx MSI Test

The concordance analysis between CTA+ and Idylla CDx MSI-H was based on valid Idylla CDx MSI Test results from the randomized subjects to all arms of the CHECKMATE-8HW trial. Of the total 837 CTA+ samples, there were 600 MSI-H, 125 MSS and 112 non-evaluable or invalid results by Idylla CDx MSI Test. Since patients with local pMMR/MSS results were not eligible for enrollment to CHECKMATE-8HW, samples from pMMR/MSS (negative) locally enrolled population were unavailable for the bridging study. Therefore, for the NPA estimation, procured specimens were tested in a central laboratory with the Idylla CDx MSI Test and compared to results used to characterize the specimens as either MSS or pMMR, to represent the screen failed CTA- population. A total of 156 CTA- procured samples, either pMMR by IHC or MSS by PCR at a ratio of 3:1 (3/4 tested by IHC and 1/4 tested by PCR to represent the ratio of local test modalities of CHECKMATE -8HW), were tested by the Idylla CDx MSI Test, with 152 MSS, 3 MSI-H and 1 invalid results. Table 21 presents the concordance, and Table 22 presents the PPA and NPA estimates with 95% CI.

Table 21. Concordance between CTA+ and Idylla CDx MSI-H and CTA- and Idylla CDx MSS

Idylla CDx MSI Test	CTA	
	CTA+ (CHECKMATE-8HW Trial)	CTA-* (Procured Samples)
MSI-H	600	3
MSS	125	152
Not Evaluable (cancelled) or invalid	112	1
Total	837	156

*CTA-: pMMR or MSS by IHC or PCR, respectively, from procured samples.

Table 22. PPA between CTA+ and Idylla CDx MSI-H and NPA between CTA- and Idylla CDx MSS

Measure	Rate	Point Estimate* (%)	95% CI (Wilson Score)
PPA	600/725	82.8	79.8 - 85.3
NPA	152/155	98.06	94.46 - 99.34

*CTA-: proficient mismatch repair (pMMR) or MSS by IHC or PCR, respectively, from procured samples.

The better drug efficacy in $CDx^{+}CTA^{+}$ population than in the $CDx^{-}CTA^{+}$ population, as presented in Table 23 below, can partially address any potential concerns due to the lower PPA observed in the bridging concordance study. Additionally, re-estimation of efficacy results using imputations and sensitivity analyses by considering the missing assessments of Idylla CDx MSI Test, as presented in Figure 3 below, also supported these findings.

Clinical Efficacy of the Idylla CDx MSI Test for First Line OPDIVO (Nivolumab) in Combination with Ipilimumab

The HR PFS per BICR analysis for the clinical efficacy was performed with a total of 301 CTA+ participants in CHECKMATE-8HW randomized to Arm B, nivolumab + ipilimumab (n = 200), and Arm C, chemotherapy (n = 101) in 1L mCRC with valid Idylla CDx MSI-H Test results (n = 218, 147 in Arm B and 71 in Arm C) and was bridged to the Idylla CDx MSI Test.

For the patients with the centrally confirmed Idylla CDx MSI-H status, the median ages for Arm B and Arm C were 63 years and 65 years, respectively; the sex distribution was 42.9% Male and 57.1% Female in Arm B, and 43.7% Male and 56.3% Female in Arm C. For comparison, in the CTA+ participants, the median ages for Arm B and Arm C were 62 years and 65 years, respectively; the sex distribution was 47.0% Male and 53.0% Female in Arm B, and 44.6% Male and 55.4% Female in Arm C. The distributions of demographics and other baseline disease and specimen characteristics of the patients were generally balanced between the two arms and similar between the CTA+ randomized patients and those centrally confirmed as MSI-H by the Idylla CDx MSI Test.

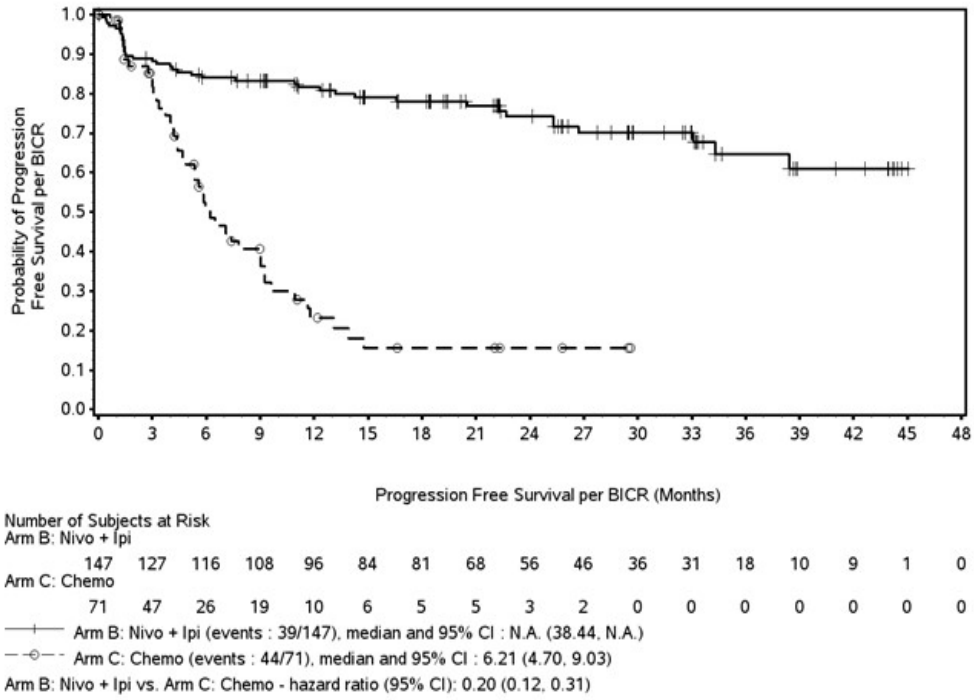
Of the total 301 subjects with the CTA^{+} status randomized to receive nivolumab + ipilimumab or chemotherapy, nivolumab + ipilimumab demonstrated a clinically meaningful improvement in PFS per BICR over chemotherapy (HR = 0.32) in 1L. Additionally, of these randomized subjects with centrally confirmed MSI-H status by Idylla CDx MSI test, the PFS per BICR benefit by nivolumab + ipilimumab over chemotherapy (HR = 0.20) was observed. Median PFS per BICR was not reached for nivolumab + ipilimumab (95% CI: 38.44 months – not reached), compared to 6.21 months for chemo ((95% CI: 4.70 – 9.03 months) Table 23 and Figure 1). In the CTA^{+} randomized subjects with centrally confirmed MSS status by Idylla CDx, nivolumab + ipilimumab vs chemotherapy showed HR of 1.51, with median PFS per BICR of 1.81 months (95% CI, 1.45 - 5.75 months) for nivolumab + ipilimumab compared to 7.36 months (95% CI, 4.01 - 12.88 months) for chemotherapy (Figure 2).

Table 23. PFS per BICR of First Line OPDIVO (Nivolumab) in Combination with Ipilimumab Estimated from CHECKMATE-8HW

Source	Arm B: Nivolumab + Ipilimumab			Arm C: Chemotherapy			Hazard Ratio***	
	N	Median PFS (months)	95% CI	N	Median PFS** (months)	95% CI	Estimate	95% CI
All CTA+ Randomized	200	N.A*	(34.30, N.A.)	101	6.21	(4.70, 9.00)	0.32	(0.22 - 0.45)
Idylla CDx MSI-H and CTA+	147	N.A*	(38.44, N.A.)	71	6.21	(4.70, 9.03)	0.20****	(0.12 - 0.31)
Idylla CDx MSS and CTA+	30	1.81	(1.45, 5.75)	15	7.36	(4.01, 12.88)	1.51	(0.68 - 3.33)

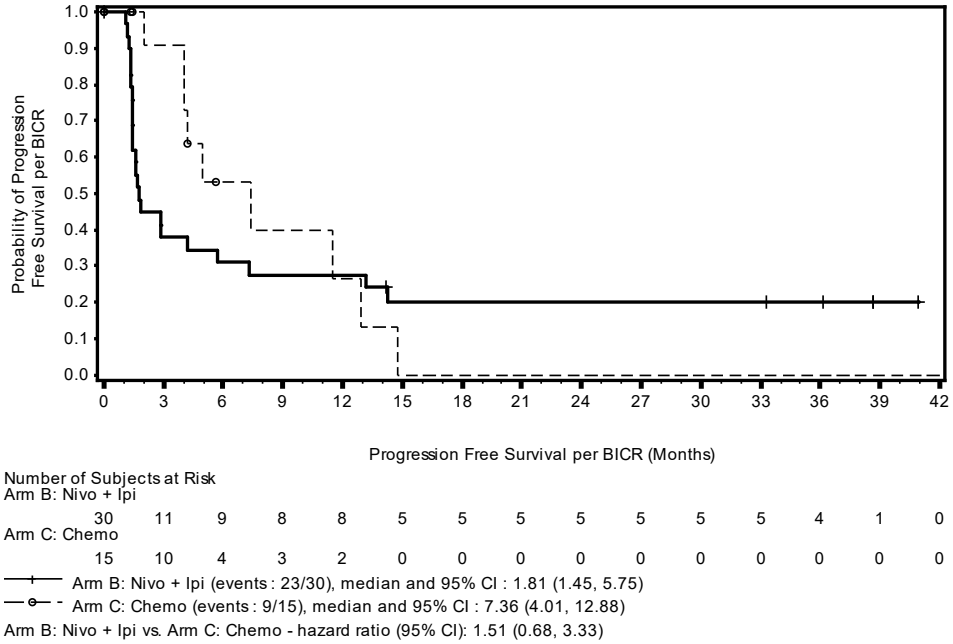
*: N.A.: Not available since the median PFS is not reached
**: Median PFS is based on Kaplan-Meier Estimates
***: Hazard Ratio (HR) is Arm B over Arm C from a Cox Model stratified by tumor sidedness (left vs. right) as entered into the Interactive Response Technology (IRT) system.
**** The HR is based on the Idylla CDx MSI-H and CTA+ subjects. The difference between the HRs (0.20 in the table above vs. 0.21 in the drug labeling) for the 1L PFS comparison of nivolumab plus ipilimumab versus chemotherapy is due to the slightly different population since the intended use population in the drug labeling is based on the two centrally confirmed CDx tests on the CTA+ randomized subjects (Idylla CDx MSI Test or MMR IHC Panel pharmDx).

Figure 1. Kaplan-Meier Curve of PFS per BICR for Nivolumab plus Ipilimumab vs Chemotherapy in 1L CTA+ Randomized Subjects with Centrally Confirmed MSI-H by Idylla CDx



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

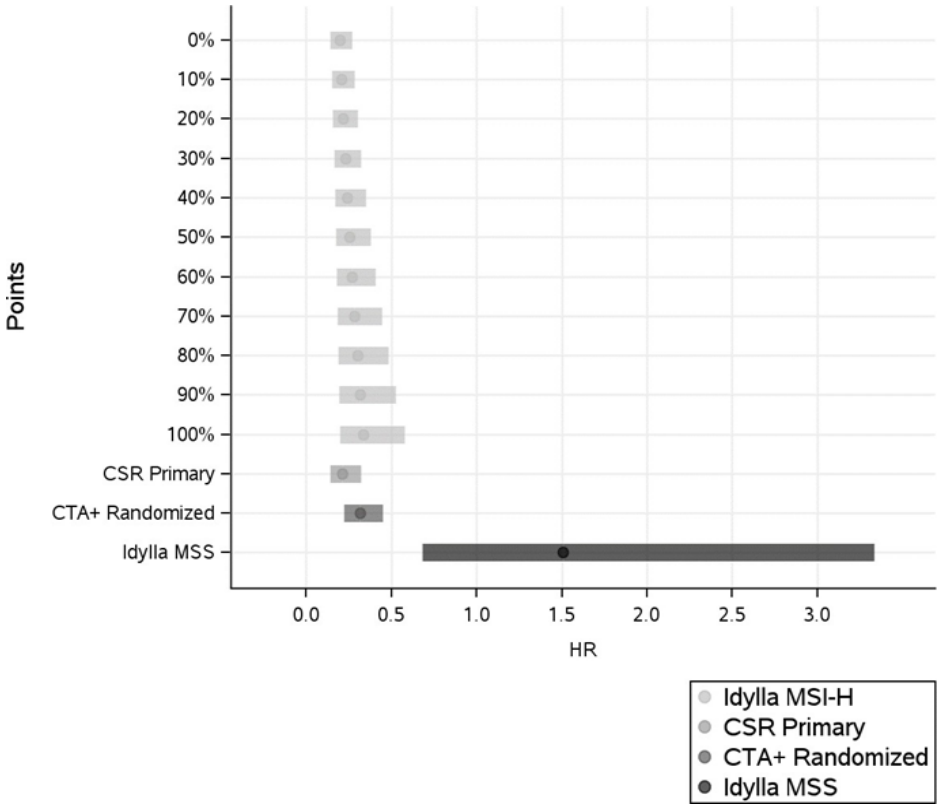
Figure 2. Kaplan-Meier Curve of PFS per BICR for Nivolumab plus Ipilimumab vs Chemotherapy in 1L CTA+ Randomized Subjects with Centrally Confirmed MSS by Idylla CDx



Statistical model for hazard ratio: Stratified Cox proportional hazard model by tumor sidedness (left vs. right) as entered into the interactive response system. Symbols represent censored observations. Excludes data collected on or after first crossover dose date. KM plot will be generated only if there are at least 10 subjects in each treatment arm in population or subgroup.

The clinical efficacy was further estimated using a tipping point analysis with a simulated range for the missing HR of PFS potentially with Idylla CDx MSI-H status and unenrolled to CHECKMATE-8HW due to their locally tested negative status (CTA-). It was assumed the best scenario of the missing HR to be equal to the HR in the concordant population with CTA+ and Idylla CDx MSI-H status, i.e., HR = 0.2, and the worst scenario to be HR=1. The overall PFS HR estimated for nivolumab + ipilimumab vs. chemotherapy in 1L from the tipping point analysis ranges from 0.20 (95% CI, 0.14 - 0.28) to 0.34 (95% CI, 0.20 - 0.58). The results showed that, across the tipping point range, the clinical efficacy for the intended use of Idylla CDx MSI-H is similar to the efficacy observed from the CTA^+ randomized population, and different with the patients with the enrolled CTA^+ but Idylla CDx MSS status (depicted in Figure 3). In addition, the clinical efficacy results for the MSI-H status by Idylla CDx intended use are clinically meaningful, meeting the preset acceptance criteria.

Figure 3. Forest Plot of PFS per BICR of Nivolumab plus Ipilimumab vs. Chemotherapy in 1L for Idylla CDx MSI-H of the Intended Use - Estimated from Tipping Point Analysis Compared to Efficacies in CTA+ Randomized, Clinical Study Report (CSR) Primary and Idylla CDx MSS Participants in 1L



Idylla CDx MSI-H population is the intended use population. CSR primary population is randomized subjects with centrally confirmed dMMR/MSI-H status. CTA+ randomized subjects are subjects who were randomized in CHECKMATE-8HW with locally tested dMMR/MSI-H status. Idylla MSS population is subjects with centrally confirmed Idylla MSS status and locally confirmed dMMR/MSI-H status. Tipping point analysis results range from the best (0%) to the worst scenario (100%), where the best scenario represents HR equal to the estimated value from the data of the concordant population with CTA+ & Idylla CDx MSI-H and the worst scenario represents HR equal to 1.

Clinical Efficacy of the Idylla CDx MSI Test for All Lines OPDIVO (Ipilimumab) and OPDIVO (Ipilimumab) in Combination with Ipilimumab

The HR PFS per BICR analysis was performed with a total of 705 CTA^+ participants randomized to Arm B, nivolumab plus ipilimumab (n = 352) and Arm A, nivolumab, (n = 353) in all lines of mCRC with valid Idylla CDx MSI-H Test results (n = 504) and was bridged to the Idylla CDx MSI Test. For the patients with the centrally confirmed Idylla CDx MSI-H status, the median ages for Arm B and Arm A were 63 years and 64 years, respectively; the sex distribution was 43.2% Male and 56.8% Female in Arm B, and 53.4% Male and 46.6% Female in Arm A. For comparison, in the CTA+ participants, the median ages for Arm B

and Arm A were 62 years and 63 years, respectively; the sex distribution was 45.7% Male and 54.3% Female in Arm B, and 53.8% Male and 46.2% Female in Arm A. The distributions of demographics and other baseline disease and specimen characteristics of the patients were generally balanced between the Arm A and Arm B and similar between the CTA+ randomized patients and those centrally confirmed as MSI-H by the Idylla CDx MSI Test (Table 18-20).

Of the total subjects with the CTA+ status from all lines randomized to receive nivolumab + ipilimumab or nivolumab monotherapy, nivolumab + ipilimumab demonstrated a clinically meaningful improvement in PFS per BICR over nivolumab (HR = 0.63). Additionally, of these randomized subjects with centrally confirmed MSI-H status by Idylla CDx, the PFS benefit by nivolumab + ipilimumab vs. nivolumab was observed (HR = 0.60). Median PFS per BICR was not reached for nivolumab + ipilimumab (95% CI: 53.82 – N.A. months), compared to 44.85 months for nivolumab ((95% CI: 22.97 – N.A. months) Table 24, Figure 4). Of the CTA^{+} randomized subjects with centrally confirmed MSS status by Idylla CDx, The PFS HR for nivolumab + ipilimumab vs. nivolumab was 0.76. The median PFS per BICR was 2.66 months (95% CI, 1.58 - 3.58 months) for nivolumab + ipilimumab compared to 1.94 months (95% CI, 1.45 - 2.83 months) for nivolumab (Table 24 and Figure 5).

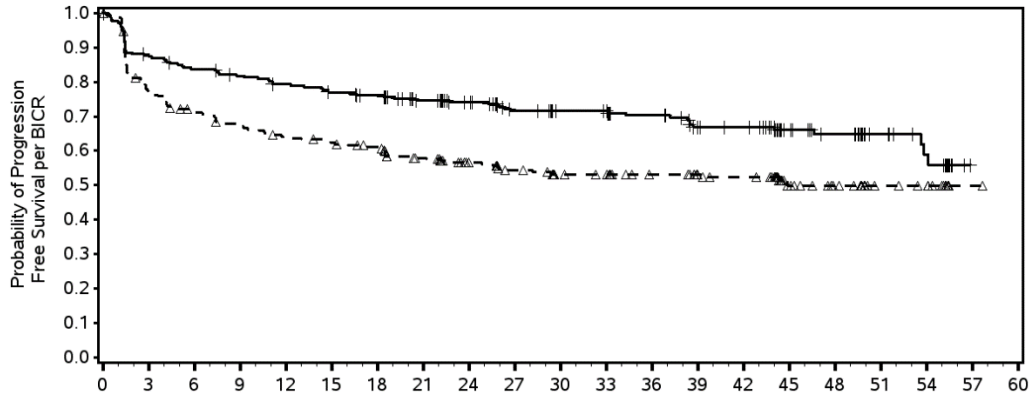
Table 24. PFS per BICR of All Lines OPDIVO (Nivolumab) and OPDIVO (Nivolumab) in Combination with Ipilimumab Estimated from CHECKMATE-8HW

	Arm B: Nivolumab + Ipilimumab			Arm A: Nivolumab			Hazard Ratio***	
Source	**N**	**Median PFS** (months)**	**95% CI**	**N**	**Median PFS** (months)**	**95% CI**	**Estimate**	**95% CI**
All CTA+ Randomized	352	54.08	(46.62, N.A.*)	353	18.43	(9.20, 28.16)	0.63	(0.51 - 0.79)
Idylla CDx MSI-H and CTA+	257	N.A.*	(53.82, N.A.*)	247	44.85	(22.97, N.A.)	0.60****	(0.45 - 0.80)
Idylla CDx MSS and CTA+	55	2.66	(1.58, 3.58)	53	1.94	(1.45, 2.83)	0.76	(0.50 - 1.16)

*: N.A.: Not available since the median PFS is not reached
**: Median PFS is based on Kaplan-Meier Estimates
***: Hazard Ratio (HR) is Arm B over Arm A from a Cox Model stratified by tumor sidedness (left vs. right) and prior lines of therapy (0, 1, >=2) as entered into the Interactive Response Technology (IRT) system.
**** The HR is based on the Idylla CDx MSI-H and CTA+ subjects. The difference between the HRs (0.60 in the table above vs. 0.62 in the drug labeling) for all lines PFS comparison of nivolumab plus ipilimumab versus nivolumab is due to the slightly different population since the intended use population in the drug

labeling is based on the two centrally confirmed CDx tests on the CTA+ randomized subjects (Idylla CDx MSI Test or MMR IHC Panel pharmDx).

Figure 4. Kaplan-Meier Curve of PFS per BICR for Nivolumab plus Ipilimumab vs. Nivolumab in all lines CTA+ Randomized Subjects with Centrally Confirmed MSI-H by Idylla CDx



Number of Subjects at Risk

Arm A: Nivo	247	185	170	160	150	146	140	125	108	94	84	82	73	67	64	36	29	13	11	1	0
Arm B: Nivo + Ipi	257	221	210	202	195	188	181	163	148	131	121	120	108	91	89	55	48	26	20	0	0

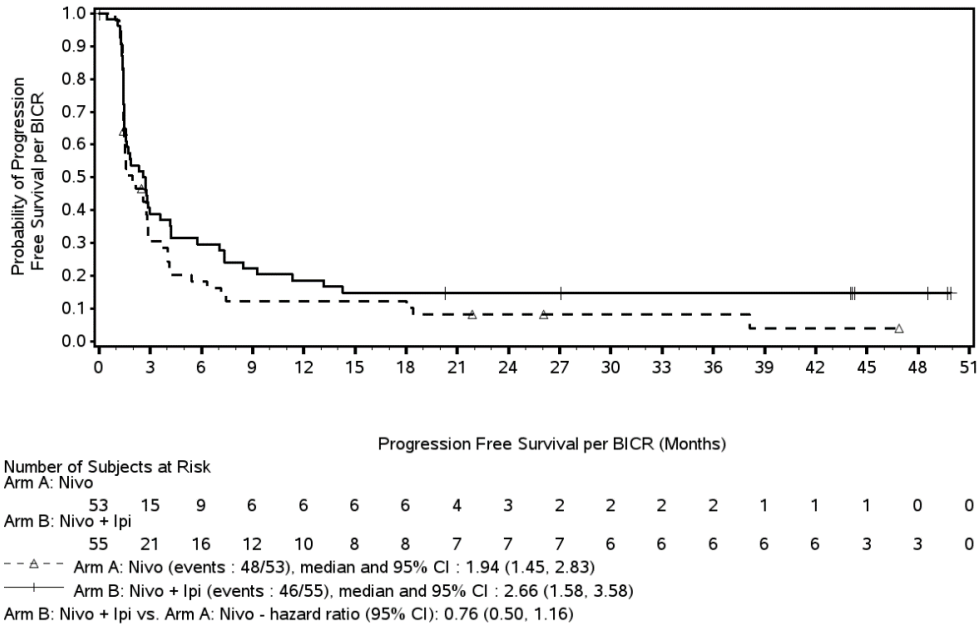
- - △ - - Arm A: Nivo (events : 113/247), median and 95% CI : 44.85 (22.97, N.A.)

——+—— Arm B: Nivo + Ipi (events : 81/257), median and 95% CI : N.A. (53.82, N.A.)

Arm B: Nivo + Ipi vs. Arm A: Nivo - hazard ratio (95% CI): 0.60 (0.45, 0.80)

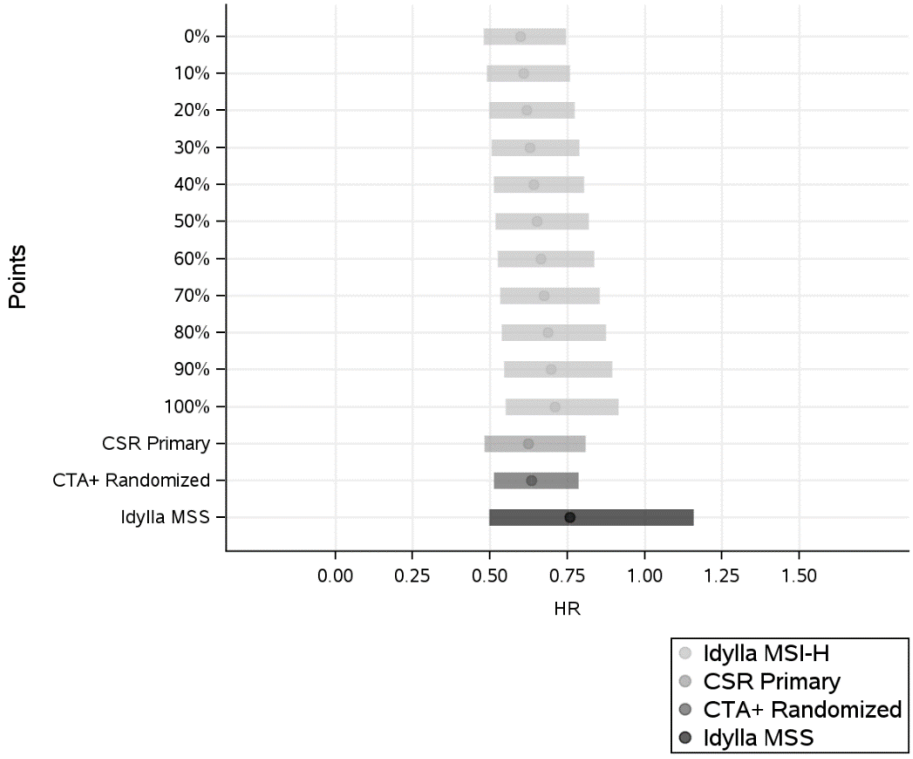
Statistical model for hazard ratio: Stratified Cox proportional hazard model by tumor sidedness (left vs. right) and prior lines of therapy (0, 1, >= 2) as entered into the IRT. Symbols represent censored observations. KM plot was generated only if there were at least 10 subjects in each treatment arm in population or subgroup.

Figure 5. Kaplan-Meier Curve of PFS per BICR for nivolumab plus ipilimumab vs nivolumab in all lines CTA+ Randomized Subjects with Centrally Confirmed MSS by Idylla CDx



The clinical efficacy was further estimated using a tipping point analysis with a simulated range for the missing HR of PFS potentially with Idylla CDx MSI-H status and unenrolled to CHECKMATE-8HW due to their locally tested negative status (CTA^-). It was assumed the best scenario of the missing HR to be equal to the HR in the concordant population with CTA^+ and Idylla CDx MSI-H status, i.e., HR = 0.6, and the worst scenario to be HR=1. The overall PFS HR estimated from the tipping point analysis ranges from 0.60 (95% CI, 0.48 - 0.75) to 0.71 (95% CI, 0.55 - 0.92). The results showed that, across the tipping point range, the clinical efficacy for the intended use of Idylla CDx MSI-H is similar to the efficacy observed from the CTA^+ randomized patients in all lines of therapy (depicted in Figure 6), showing clinically meaningful improvement.

Figure 6. Forest Plot of PFS per BICR of nivolumab plus ipilimumab vs. nivolumab in all lines for Idylla CDx MSI-H of Intended Use - Estimated from Tipping Point Analysis Compared to Efficacies in CTA+ Randomized, CSR Primary and Idylla CDx MSS Participants in all lines.



Idylla CDx MSI-H population is the intended use population. CSR primary population is randomized subjects with centrally confirmed dMMR/MSI-H status. CTA+ randomized subjects are subjects who were randomized in CHECKMATE-8HW with locally tested dMMR/MSI-H status. Idylla CDx MSS population is subjects with centrally tested MSS status and locally confirmed dMMR/MSI-H status. Tipping point analysis results range from the best (0%) to the worst scenario (100%), where the best scenario represents HR equal to the estimated value from the data of the concordant population and the worst scenario represents HR equal to 1.

The ORR in all lines for nivolumab and nivolumab + ipilimumab is provided in Table 25. The ORR for nivolumab + ipilimumab and nivolumab alone in all lines with centrally confirmed MSI-H status by Idylla CDx MSI Test were 73.5% (95% CI: 67.7, 78.8) and 58.7% (95% CI: 52.3, 64.9), respectively. The results are consistent with the ORR observed in CTA+ patients randomized to nivolumab + ipilimumab (63.6%, 95% CI: 58.4, 68.7) and nivolumab monotherapy (49.3%, 95% CI: 44.0, 54.6) arms, respectively. In contrast, the ORR in patients with enrolled CTA+ but Idylla CDx MSS status were 23.6% (95% CI: 13.2, 37.0) in nivolumab + ipilimumab and 11.3% (95% CI: 4.3, 23.0) nivolumab alone (Table 25).

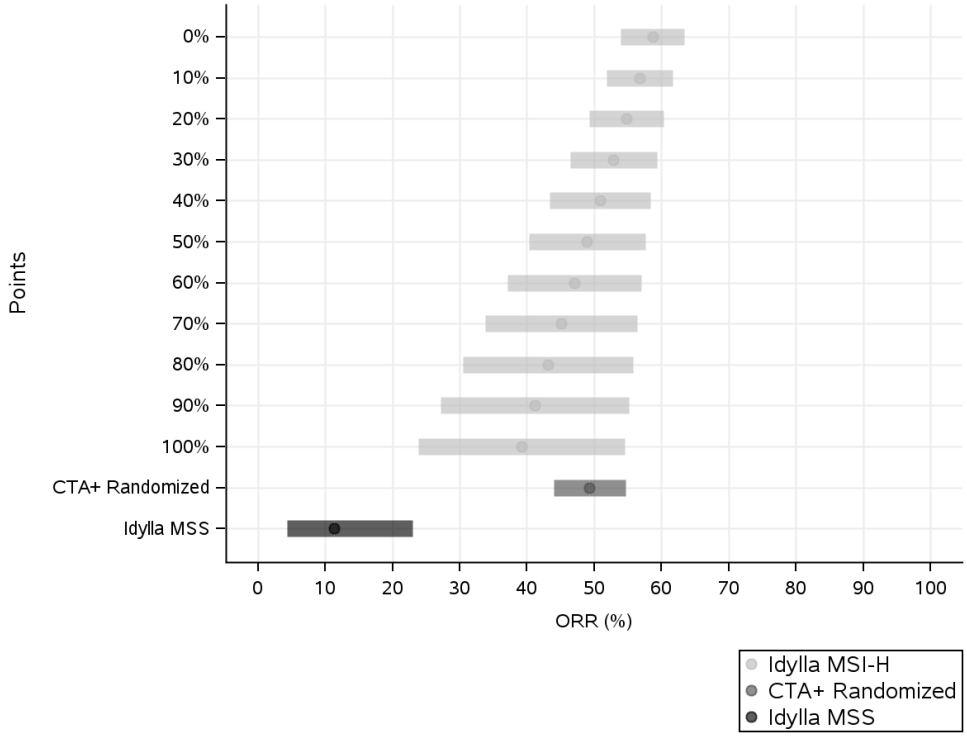
Table 25. ORR per BICR of All Lines OPDIVO (Nivolumab) and OPDIVO (Nivolumab) in Combination with YERVOY (Ipilimumab) Estimated from CHECKMATE-8HW

	Idylla CDx MSI-H and CTA+		Idylla CDx MSS and CTA+		All CTA+ Randomized	
	Arm B: Nivolumab + Ipilimumab N = 257	Arm A: Nivolumab N = 247	Arm B: Nivolumab + Ipilimumab N = 55	Arm A: Nivolumab N = 53	Arm B: Nivolumab + Ipilimumab N = 352	Arm A: Nivolumab N = 353
ORR (95% CI)*	73.5% (67.7, 78.8)	58.7% (52.3, 64.9)	23.6% (13.2, 37.0)	11.3% (4.3, 23.0)	63.6% (58.4, 68.7)	49.3% (44.0, 54.6)
CRR	32.7%	29.6%	14.5%	1.9%	27.8%	23.2%
PRR	40.9%	29.1%	9.1%	9.4%	35.8%	26.1%
p-value**	0.0004	N.A	0.2293	N.A	0.0001	N.A

CRR = Complete Response Rate, PRR = Partial Response Rate, N.A = not applicable.
*ORR (CRR+PRR), confidence interval based on the Clopper and Pearson method.
**Based on Cochran-Mantel-Haenszel (CMH) test stratified by the same factors as used in the Cox proportional hazards model and not evaluated for statistical significance.

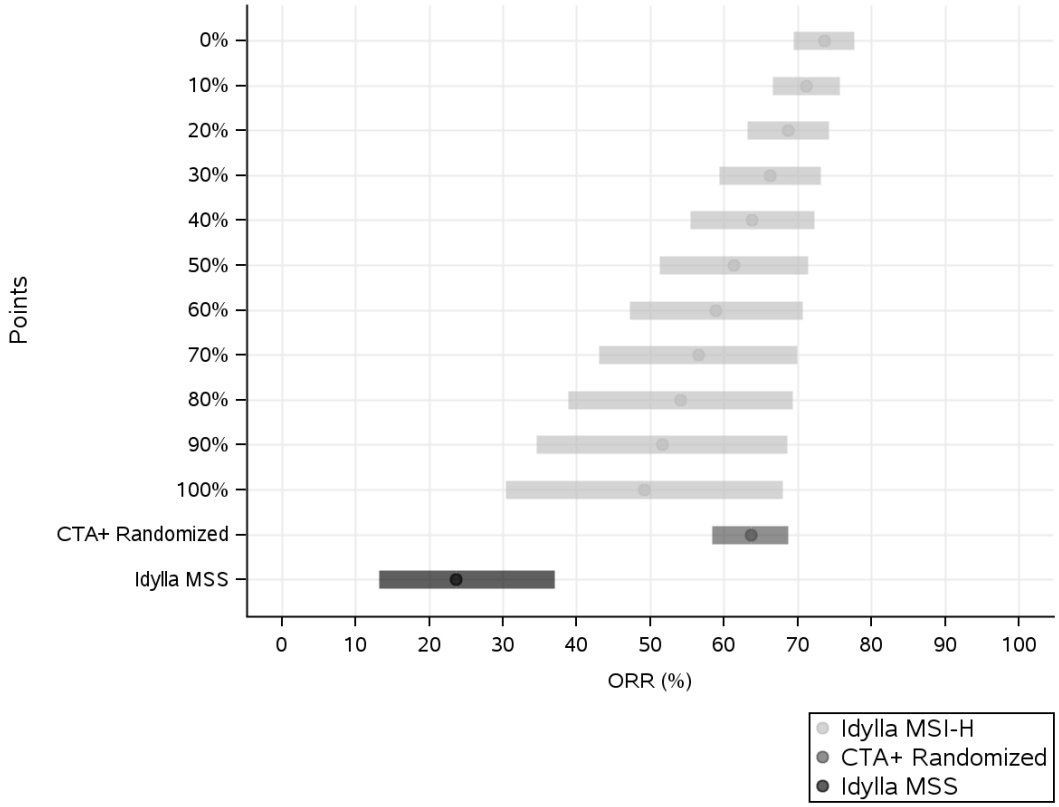
As depicted in Figure 7 and Figure 8, the ORRs for all lines of nivolumab monotherapy, and for nivolumab plus ipilimumab for the intended use of Idylla MSI-H estimated from tipping point analysis are comparable with the ORR in all lines, CTA+ patients randomized to nivolumab monotherapy and nivolumab plus ipilimumab arms, respectively, and different with the enrolled CTA+ but Idylla CDx MSS status.

Figure 7. Forest Plot of ORR per BICR for Idylla CDx MSI-H of the Intended Use - All Lines Subjects by Nivolumab Monotherapy



Idylla CDx MSI-H population is the intended use population. CTA+ randomized Subjects are subjects who were randomized in CHECKMATE-8HW with locally confirmed dMMR/MSI-H status. Idylla CDx MSS population is subjects with centrally tested MSS status and locally confirmed dMMR/MSI-H status. Tipping point analysis results range from the best (0%) to the worst scenario (100%), where the best scenario assumes the inestimable ORR in locally misclassified as pMMR/MSS status & Idylla CDx MSI-H population equal to the estimated value from the data of the concordant population and the worst scenario assume the inestimable ORR equal to 0%.

Figure 8. Forest Plot of ORR per BICR for Idylla CDx MSI-H of the Intended Use - All Lines Subjects by Nivolumab and Ipilimumab Combination Therapy



Idylla CDx MSI-H population is the intended use population. CTA+ randomized Subjects are subjects who were randomized in CHECKMATE-8HW with locally confirmed dMMR/MSI-H status. Idylla CDx MSS population is subjects with centrally tested MSS status and locally confirmed dMMR/MSI-H status. Tipping point analysis results range from the best (0%) to the worst scenario (100%), where the best scenario assumes the inestimable ORR in locally misclassified as pMMR/MSS status & Idylla CDx MSI-H population equal to the estimated value from the data of the concordant population and the worst scenario assume the inestimable ORR equal to 0%.

Imputation and Sensitivity Analysis

Out of the total 837 of the CTA^+ randomized patients to the study, 112 subjects (13.38%) didn't have a valid result by Idylla CDx MSI Test, of which 105 subjects with missing Idylla results were due to insufficient remaining samples or tumor tissue content for the Idylla test. To evaluate the robustness of the PPA and the clinical efficacy results estimated from the valid results, also applicable for all randomized patients, the multiple imputation was conducted based on the data structure of the demographics and baseline disease and specimen characteristics. With 500 imputed results for a single patient's missing status, the PPA was re-estimated that resulted in a range of 83.15% (95% CI: 80.47% - 85.54%) to 83.63% (95% CI: 80.97% - 85.98%). During NPA estimation with procured samples, there was only one (1) missing data as a result of invalid result from the

Idylla CDx MSI Test. To estimate the NPA in the worst-case and best-case scenarios, imputation was performed assuming the invalid sample as false positive, 'Idylla CDx MSI-H', and as 'Idylla CDx MSS'. The worst-case NPA estimate was 97.44% (95% CI: 93.59% - 99%), and the best-case was 98.08% (95% CI: 94.5% - 99.34%).

Conclusion of Clinical Studies

The clinical validation of the Idylla CDx test for patients with CRC with MSI-H status was evaluated from the CHECKMATE-8HW trial in terms of PFS per BICR of nivolumab plus ipilimumab vs chemotherapy in first line, and ORR and PFS per BICR of nivolumab plus ipilimumab vs nivolumab in all lines of therapy. Bridging study between CTA+ and Idylla CDx MSI-H demonstrated similar clinical efficacy (PFS) between patients with dMMR/MSI-H status by CTA and those with MSI-H status by Idylla CDx MSI Test in the intended use population. Re-estimation of efficacy results using imputations and sensitivity analyses by considering the missing assessments of Idylla CDx MSI Test also supported these findings.

4. **Pediatric Extrapolation**

In this premarket submission, existing clinical data from CHECKMATE-8HW can be leveraged to support the reasonable assurance of safety and effectiveness of the proposed device in the pediatric subpopulation aged 12-21 years old to identify MSI-H CRC patients eligible for potential treatment with OPDIVO (nivolumab) alone and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab). Two pediatric samples (from individuals ages 18 and 21) were enrolled in clinical validation study; both had concordant CTA and CDx results (i.e., MSI-H). Also, data from adult patients in this trial is considered generalizable to the pediatric population aged 12-21 years. Additionally, since there is no change in the specimen type (FFPE) to be used or assay workflow prescribed in the Instructions for Use based on the age of the patient, the validation data can be extrapolated to the pediatric population and can be relied upon to establish safety and effectiveness within the 12-21 years age group.

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 for the device included three investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XII. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

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

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIV. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of Idylla CDx MSI Test to identify patients with MSI-H CRC who may benefit from treatment with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) is supported by the results from the clinical study. Analytical and clinical performance studies were conducted with the Idylla CDx MSI Test using nucleic acids extracted from FFPE CRC tumor tissue. This study was performed using specimens from CRC patients with MSI-H status enrolled in the CHECKMATE-8HW clinical trial supplemented with negative samples (either pMMR by IHC or MSS by PCR) that were commercially procured. The bridging between CTA+ and Idylla MSI-H was conducted based on similar clinical efficacy (PFS and ORR outcomes) between patients with MSI-H status by CTA and those with MSI-H status by the Idylla CDx MSI Test. The results from analytical validation and clinical studies support the reasonable assurance of safety and effectiveness of the Idylla CDx MSI Test when used in accordance with the indications for use in identifying patients with MSI-H CRC who may benefit from treatment with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) in accordance with their approved therapeutic product labeling.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies as well as data collected in a clinical study conducted to support PMA approval as described above. The bridging study was an observational retrospective study. No Unanticipated Adverse Device Effects (UADE) as defined in 21 CFR 812.150 and ISO 20916 occurred during the study.

Idylla CDx MSI Test is an IVD test, performed using nucleic acids 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 decisions in CRC cancer treatment. Patients with false positive results

may undergo treatment without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment, from which they might have received meaningful clinical benefit. There is also a risk of delayed results, which may lead to delay of treatment.

C. Benefit-Risk Determination

The probable benefits of the device are also based on data collected in a clinical study conducted to support PMA approval as described above, as well as the analytical validation supporting the device. For the intended use of identifying CRC patients with MSI-H to be treated with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab), in the metastatic CRC population, the probable benefit of the Idylla CDx MSI Test was demonstrated through a clinical efficacy analysis in the CHECKMATE-8HW trial. In the MSI-H CRC, in the advanced or metastatic setting, the population defined by the Idylla CDx MSI Test, nivolumab + ipilimumab combination therapy demonstrated clinically meaningful benefit over chemotherapy in 1L. In all lines studied, for MSI-H CRC, as defined by the Idylla CDx MSI Test, nivolumab + ipilimumab or nivolumab alone (in the refractory population) also had meaningful clinical efficacy.

The probable risks of the device are also based on data collected in a clinical study conducted to support PMA approval as described above. There is risk associated with the use of this device, mainly due to false positives, false negatives, and delay in providing results. Risks associated with the Idylla CDx MSI Test include the possibility of inaccurate results that may lead to mismanagement of patients. Patients with false positive results may undergo treatment with OPDIVO (nivolumab) as monotherapy or in combination with YERVOY (ipilimumab), without clinical benefit and may experience adverse reactions associated with the therapy(ies). Patients with false negative results may not be considered for treatment with OPDIVO (nivolumab) as monotherapy or in combination with YERVOY (ipilimumab). There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy. These risks are mitigated by the analytical and clinical performance of the device.

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

In conclusion, given the available information above, the data support that for identification of CRC patients with MSI-H status, for whom treatment with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) as a combination therapy the probable benefits outweigh the probable risks.

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 studies and clinical bridging study using patients enrolled in the CHECKMATE-8HW study support the performance of the Idylla CDx MSI Test in identification of CRC patients with MSI-H status, for whom treatment with OPDIVO (nivolumab) as a monotherapy and/or OPDIVO (nivolumab) in combination with YERVOY (ipilimumab) as a combination therapy is indicated in approved therapeutic product labeling.

XV. CDRH DECISION

CDRH issued an approval order on August 15, 2025.

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

XVI. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.