SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	FoundationOne®CDx (F1CDx)
Device Procode:	PQP
Applicant's Name and Address:	Foundation Medicine, Inc. 150 Second Street, Cambridge, MA 02141

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P170019/S029

Date of FDA Notice of Approval: February 18, 2022

The original PMA (P170019) was approved on November 30, 2017 and is indicated for the detection of genetic alterations in patients who may benefit from one of fifteen FDA-approved therapies for non-small cell lung cancer (NSCLC), melanoma, breast cancer, colorectal cancer (CRC), and ovarian cancer. The SSED to support the indication is available on the CDRH website and is incorporated by reference here. Subsequently, thirteen PMA supplements were approved for expanding the indications for use of F1CDx since its original approval. A PMA supplement (P170019/S005) for adding genomic loss of heterozygosity (LOH) was approved on April 10, 2019. A PMA supplement (P170019/S004) for adding an indication for LYNPARZA® (olaparib) in ovarian cancer patients with *BRCA1/2* alterations was approved on July 1, 2019. A PMA supplement (P170019/S008) for adding an indication for TAGRISSO® (osimertinib) in NSCLC patients with *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations was approved on July 1, 2019. A PMA supplement (P170019/S006) for adding an indication for PIQRAY® (alpelisib) in breast cancer patients with *PIK3CA* alterations was approved on December 3, 2019. A PMA supplement (P170019/S010) for adding a second site in Morrisville, NC, where the F1CDx assay will be performed, was approved on December 16, 2019. A PMA supplement (P170019/S013) for adding an indication for PEMZYRE® (pemigatinib) in cholangiocarcinoma patients with *FGFR2* fusions was approved on April 17, 2020. A PMA supplement (P170019/S011) for adding an indication for TABRECTA® (capmatinib) in NSCLC patients with *MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping was approved on May 6, 2020. A PMA supplement (P170019/S015) for adding an indication for LYNPARZA® (olaparib) in metastatic castration resistant prostate cancer (mCRPC) patients with mutations in homologous recombination repair (HRR) genes was approved on May 19, 2020. A PMA

supplement (P170019/S016) for adding an indication for KEYTRUDA® (pembrolizumab) in patients with solid tumors high tumor mutational burden (TMB) at the cut-off of 10 mutations per megabase (mut/Mb) was approved on June 16, 2020. A PMA supplement (P170019/S017) for adding an indication for VITRAKVI® (larotrectinib) in patients with *NTRK1/2/3* fusions was approved on October 23, 2020. PMA supplement (P170019/S021) for adding an indication for TRUSELTIQ™ (infigratinib) in cholangiocarcinoma patients with FGFR2 fusions and select rearrangements was approved on May 28, 2021. A PMA supplement (P170019/S023) for adding an indication for ALUNBRIG® (brigatinib) in NSCLC patients with *ALK* rearrangements was approved on June 30, 2021. A PMA supplement (P170019/S026) was approved July 30, 2021 for a tumor profiling claim to replace the Principal Component Analysis (PCA) MSI caller used within F1CDx to the Fraction-Based (FB) MSI caller. A PMA supplement (P170019/S025) was approved on November 10, 2021, for the class labeling for BRAF Inhibitors approved by FDA. A PMA supplement (P170019/S030) was approved on January 19, 2022, for adding an indication TECENTRIQ® (atezolizumab) in combination with COTELLIC® (cobimetinib) and ZELBORAF® (vemurafenib) in melanoma patients with BRAF V600 mutations.

The current supplement was submitted to expand the indication for F1CDx to include a companion diagnostic (CDx) indication for the detection of microsatellite instability – High (MSI-H) status in patients with solid tumors who may benefit from treatment with KEYTRUDA® (pembrolizumab).

II. INDICATIONS FOR USE

FoundationOne®CDx (F1CDx) is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology for detection of substitutions, insertion and deletion alterations (indels) and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Table 1. Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (afatinib), Iressa® (gefitinib), Tagrisso® (osimertinib), or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib),

		Alunbrig® (brigatinib) Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Tabrecta® (capmatinib)
Melanoma	*BRAF* V600E	BRAF Inhibitors approved by FDA*
	BRAF V600E and V600K	Mekinist® (trametinib) or BRAF/MEK Inhibitor Combinations approved by FDA*
	BRAF V600 mutation-positive	Tecentriq® (atezolizumab) in combination with Cotellic® (cobimetinib) and Zelboraf® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	Piqray® (alpelisib)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild type (absence of mutations in exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Lynparza® (olaparib) or Rubraca® (rucaparib)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre® (pemigatinib) or Truseltiq™ (infigratinib)
Prostate cancer	Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations	Lynparza® (olaparib)
Solid tumors	TMB ≥ 10 mutations per megabase	Keytruda® (pembrolizumab)
	NTRK1/2/3 fusions	Vitrakvi® (larotrectinib)
	MSI-High	Keytruda® (pembrolizumab)

*For the most current information about the therapeutic products in this group, go to: https://www.fda.gov/medical-devices/in-vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-in-vitro-and-imaging-tools

The test is also used for detection of genomic loss of heterozygosity (LOH) from formalin-fixed, paraffin-embedded (FFPE) ovarian tumor tissue. Positive homologous recombination deficiency (HRD) status (F1CDx HRD defined as tBRCA-positive and/or LOH high) in ovarian cancer patients is associated with improved progression-free survival (PFS) from Rubraca (rucaparib) maintenance therapy in accordance with the Rubraca product label.

The F1CDx assay will be performed at Foundation Medicine, Inc. sites located in Cambridge, MA and Morrisville, NC.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the FoundationOne®CDx labeling.

V. DEVICE DESCRIPTION

FoundationOne®CDx (F1CDx) is performed at Foundation Medicine, Inc. sites located in Cambridge, MA and Morrisville, NC. The assay includes reagents, software, instruments and procedures for testing DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples.

The assay employs a single DNA extraction method from routine FFPE biopsy or surgical resection specimens, 50-1000 ng of which undergoes whole-genome shotgun library construction and hybridization-based capture of all coding exons from 309 cancer-related genes, 1 promoter region, 1 non-coding RNA (ncRNA), and select intronic regions from 34 commonly rearranged genes, 21 of which also include the coding exons (refer to Table 2 and Table 3, below, for the complete list of genes included in F1CDx). In total, the assay therefore detects alterations in 324 genes. Using the Illumina® HiSeq 4000 platform, hybrid-capture selected libraries are sequenced to high uniform depth (targeting > 500X median coverage with > 99% of exons at coverage > 100X). Sequence data are processed using a customized analysis pipeline designed to detect all classes of genomic alterations, including base substitutions, indels, copy number alterations (amplifications and homozygous deletions), and selected genomic rearrangements (e.g., gene fusions). Additionally, genomic signatures including microsatellite instability (MSI), tumor mutational burden (TMB), and positive homologous recombination deficiency (HRD) status (tBRCA-positive and/or LOH high) will be reported.

Table 2. Genes with full coding exonic regions included in F1CDx for the detection of substitutions, insertions and deletions (indels), and copy number alterations (CNAs)

ABL1	*BRAF*	*CDKN1A*	*EPHA3*	*FGFR4*	*IKZF1*	*MCL1*	*NKX2-1*	*PMS2*	*RNF43*	*TET2*
ACVR1B	*BRCA1*	*CDKN1B*	*EPHB1*	*FH*	*INPP4B*	*MDM2*	*NOTCH1*	*POLD1*	*ROS1*	*TGFBR2*
AKT1	*BRCA2*	*CDKN2A*	*EPHB4*	*FLCN*	*IRF2*	*MDM4*	*NOTCH2*	*POLE*	*RPTOR*	*TIPARP*
AKT2	*BRD4*	*CDKN2B*	*ERBB2*	*FLT1*	*IRF4*	*MED12*	*NOTCH3*	*PPARG*	*SDHA*	*TNFAIP3*
AKT3	*BRIP1*	*CDKN2C*	*ERBB3*	*FLT3*	*IRS2*	*MEF2B*	*NPM1*	*PPP2R1A*	*SDHB*	*TNFRSF14*
ALK	*BTG1*	*CEBPA*	*ERBB4*	*FOXL2*	*JAK1*	*MEN1*	*NRAS*	*PPP2R2A*	*SDHC*	*TP53*
ALOX12B	*BTG2*	*CHEK1*	*ERCC4*	*FUBP1*	*JAK2*	*MERTK*	*NT5C2*	*PRDM1*	*SDHD*	*TSC1*
AMER1	*BTK*	*CHEK2*	*ERG*	*GABRA6*	*JAK3*	*MET*	*NTRK1*	*PRKAR1A*	*SETD2*	*TSC2*
APC	*C11orf30*	*CIC*	*ERRFI1*	*GATA3*	*JUN*	*MITF*	*NTRK2*	*PRKCI*	*SF3B1*	*TYRO3*
AR	*CALR*	*CREBBP*	*ESR1*	*GATA4*	*KDM5A*	*MKNK1*	*NTRK3*	*PTCH1*	*SGK1*	*U2AF1*
ARAF	*CARD11*	*CRKL*	*EZH2*	*GATA6*	*KDM5C*	*MLH1*	*P2RY8*	*PTEN*	*SMAD2*	*VEGFA*
ARFRP1	*CASP8*	*CSF1R*	*FAM46C*	*GID4 (C17orf39)*	*KDM6A*	*MPL*	*PALB2*	*PTPN11*	*SMAD4*	*VHL*
ARID1A	*CBFB*	*CSF3R*	*FANCA*	*GNA11*	*KDR*	*MRE11A*	*PARK2*	*PTPRO*	*SMARCA4*	*WHSC1*
ASXL1	*CBL*	*CTCF*	*FANCC*	*GNA13*	*KEAP1*	*MSH2*	*PARP1*	*QKI*	*SMARCB1*	*WHSC1L1*
ATM	*CCND1*	*CTNNA1*	*FANCG*	*GNAQ*	*KEL*	*MSH3*	*PARP2*	*RAC1*	*SMO*	*WT1*
ATR	*CCND2*	*CTNNB1*	*FANCL*	*GNAS*	*KIT*	*MSH6*	*PARP3*	*RAD21*	*SNCAIP*	*XPO1*
ATRX	*CCND3*	*CUL3*	*FAS*	*GRM3*	*KLHL6*	*MST1R*	*PAX5*	*RAD51*	*SOCS1*	*XRCC2*
AURKA	*CCNE1*	*CUL4A*	*FBXW7*	*GSK3B*	*KMT2A (MLL)*	*MTAP*	*PBRM1*	*RAD51B*	*SOX2*	*ZNF217*
AURKB	*CD22*	*CXCR4*	*FGF10*	*H3F3A*	*KMT2D (MLL2)*	*MTOR*	*PDCD1*	*RAD51C*	*SOX9*	*ZNF703*
AXIN1	*CD274*	*CYP17A1*	*FGF12*	*HDAC1*	*KRAS*	*MUTYH*	*PDCD1LG2*	*RAD51D*	*SPEN*	
AXL	*CD70*	*DAXX*	*FGF14*	*HGF*	*LTK*	*MYC*	*PDGFRA*	*RAD52*	*SPOP*	
BAP1	*CD79A*	*DDR1*	*FGF19*	*HNF1A*	*LYN*	*MYCL*	*PDGFRB*	*RAD54L*	*SRC*	
BARD1	*CD79B*	*DDR2*	*FGF23*	*HRAS*	*MAF*	*MYCN*	*PDK1*	*RAF1*	*STAG2*	
BCL2	*CDC73*	*DIS3*	*FGF3*	*HSD3B1*	*MAP2K1*	*MYD88*	*PIK3C2B*	*RARA*	*STAT3*	
BCL2L1	*CDH1*	*DNMT3A*	*FGF4*	*ID3*	*MAP2K2*	*NBN*	*PIK3C2G*	*RB1*	*STK11*	
BCL2L2	*CDK12*	*DOT1L*	*FGF6*	*IDH1*	*MAP2K4*	*NF1*	*PIK3CA*	*RBM10*	*SUFU*	
BCL6	*CDK4*	*EED*	*FGFR1*	*IDH2*	*MAP3K1*	*NF2*	*PIK3CB*	*REL*	*SYK*	
BCOR	*CDK6*	*EGFR*	*FGFR2*	*IGF1R*	*MAP3K13*	*NFE2L2*	*PIK3R1*	*RET*	*TBX3*	
BCORL1	*CDK8*	*EP300*	*FGFR3*	*IKBKE*	*MAPK1*	*NFKBIA*	*PIM1*	*RICTOR*	*TEK*	

Table 3. Genes with select intronic regions for the detection of gene rearrangements, a promoter region, and an ncRNA gene

ALK introns 18, 19	*BRCA1 introns 2, 7, 8, 12, 16, 19, 20*	*ETV4 introns 5, 6*	*EZR introns 9-11*	*KIT intron 16*	*MYC intron 1*	*NUTM1 intron 1*	*RET introns 7-11*	*SLC34A2 intron 4*

BCL2 3'UTR	*BRCA2 intron 2*	*ETV5 introns 6, 7*	*FGFR1 intron 1, 5, 17*	*KMT2A (MLL) introns 6-11*	*NOTCH2 intron 26*	*PDGFRA introns 7, 9, 11*	*ROS1 introns 31-35*	*TERC ncRNA*
BCR introns 8, 13, 14	*CD74 introns 6- 8*	*ETV6 introns 5, 6*	*FGFR2 intron 1, 17*	*MSH2 intron 5*	*NTRK1 introns 8-10*	*RAF1 introns 4-8*	*RSPO2 intron 1*	*TERT Promoter*
BRAF introns 7-10	*EGFR introns 7, 15, 24-27*	*EWSR1 introns 7-13*	*FGFR3 intron 17*	*MYB intron 14*	*NTRK2 Intron 12*	*RARA intron 2*	*SDC4 intron 2*	*TMPRSS2 introns 1- 3*

Test Output
The output of the test includes:

Category 1: CDx Claims noted in Table 1 of the Intended Use

Category 2: Cancer Mutations with Evidence of Clinical Significance

Category 3: Cancer Mutations with Potential Clinical Significance

Genomic findings other than those listed in Table 1 of the intended use statement (i.e., Categories 2 and 3) are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Test Kit Contents
The test includes a sample shipping kit, which is sent to ordering laboratories. The shipping kit contains the following components:

- Specimen Preparation Instructions
- Shipping Instructions
- Return Shipping Label

Instruments
The F1CDx assay is intended to be performed with serial number-controlled instruments as indicated in Table 4, below. All instruments are qualified by Foundation Medicine, Inc. (FMI) under FMI's Quality System.

Table 4. Instruments for use with the F1CDx assay

Instrument
Illumina® HiSeq 4000
Illumina cBot® System
Agilent Technologies Benchbot Workstation with Integrated Bravo Automated Liquid Handler
Beckman Biomek NX^{P} Span-8 Liquid Handler
Hamilton Microlab STAR/STARlet Liquid Handling Workstation
Thermo Fisher Scientific KingFisher™ Flex with 96 Deep-well Head
Covaris LE220-Plus Focused-ultrasonicator

Test Process
All assay reagents included in the F1CDx assay process are qualified by FMI and are compliant with the medical device Quality System Regulation (QSR).

A. Specimen Collection and Preparation

Formalin-fixed, paraffin-embedded (FFPE) tumor specimens are collected and prepared following standard pathology practices. FFPE specimens may be received either as unstained slides or as an FFPE block.

Prior to starting the assay, a Hematoxylin and Eosin (H&E) stained slide is prepared, and then reviewed by a board-certified pathologist to confirm disease ontology and to ensure that adequate tissue (≥ 0.6 mm^3), tumor content (≥ 20% tumor), and sufficient nucleated cells are present to proceed with the assay.

B. DNA Extraction

Specimens passing pathology review are queued for DNA extraction which begins with lysis of cells from FFPE tissue by digestion with a proteinase K buffer followed by automated purification using the 96-well KingFisher™ Flex Magnetic Particle Processor.

After completion of DNA extraction, double-stranded DNA (dsDNA) is quantified by the Quant-iT™ PicoGreen® fluorescence assay using the provided lambda DNA standards (Invitrogen) prior to Library Construction (LC). The sample must yield a minimum of 55 ng of genomic DNA to ensure sufficient DNA for quality control (QC) and to proceed with LC.

C. Library Construction

Library Construction (LC) begins with normalization of DNA to 50-1000 ng. Normalized DNA samples are randomly sheared (fragmented) to ~200 bp by adaptive focused acoustic sonication using the Covaris LE220-Plus before purification with a 1.8X volume of AMPure® XP Beads (Agencourt®). Solid-phase reversible immobilization (SPRI) purification and subsequent library construction with the NEBNext® reagents (custom-filled kits by NEB), including mixes for end repair, dA addition and ligation, are performed in 96-well plates (Eppendorf) on the Bravo Benchbot (Agilent) or Microlab STAR (Hamilton) using the "with-bead" protocol[1] to maximize reproducibility and library yield. Indexed (6 bp barcodes) sequencing libraries are PCR amplified with HiFi™ (Kapa) for 10 cycles and subsequently 1.8X SPRI purified. Purification and dilution for QC are performed.

Following LC, a QC procedure is performed by quantifying single-stranded DNA (ssDNA) from purified libraries using the Quant-iT™ OliGreen® ssDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Libraries yielding insufficient sequencing library are failed.

D. Hybrid Capture

Hybrid Capture (HC) begins with normalization of each library to 500-2000 ng. Normalized samples then undergo solution hybridization which is performed using a > 50-fold molar excess of a pool of individually synthesized 5'-biotinylated DNA 120 bp oligonucleotides. The baits target ~1.8 Mb of the human genome including all coding exons of 309 cancer-related genes, introns or non-coding regions of 35 genes, plus > 3,500 single nucleotide polymorphisms (SNPs) located throughout the genome. Baits are designed by tiling overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; SNP targets are allocated one bait each. Intronic baits are filtered for repetitive elements[2] as defined by the UCSC Genome RepeatMasker track.

After hybridization, the library-bait duplexes are captured on paramagnetic MyOne™ streptavidin beads (Invitrogen), and off-target material is removed by washing one time with 1X SSC at 25°C and four times with 0.25X SSC at 55°C. The PCR master mix is added to directly amplify (12 cycles) the captured library from the washed beads.[3] After 12 cycles of amplification, the samples are 1.8X SPRI purified. Purification and dilution for QC are performed.

QC for HC is performed by measuring dsDNA yield using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Captured libraries yielding less than 140 ng of sequencing library are failed.

E. Sequencing

Sequencing is performed using off-board clustering on the Illumina cBot with patterned flow cell technology to generate monoclonal clusters from a single DNA template followed by sequencing using sequencing by synthesis (SBS) chemistry on the Illumina HiSeq 4000. Fluorescently labeled 3′-blocked dNTPs along with a polymerase are incorporated through the flow cell to create a growing nucleotide chain that is excited by a laser. A camera captures the emission color of the incorporated base and then is cleaved off. The terminator is then removed to allow the nucleotide to revert to its natural form and to allow the polymerase to add another base to the growing chain. A new pool of fluorescently labeled 3′-blocked dNTPs are added with each new sequencing cycle. The color changes for each new cycle as a new base is added to the growing chain. This method allows for millions of discrete clusters of clonal copies of DNA to be sequenced in parallel.

F. Sequence Analysis

Sequence data are analyzed using proprietary software developed by FMI. Sequence data are mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA) v0.5.9.[4] PCR duplicate read removal and sequence metric collection are performed using Picard 1.47 (http://picard.sourceforge.net) and SAM tools 0.1.12a.[5] Local alignment optimization is performed using Genome Analysis Toolkit (GATK) 1.0.4705.[6] Variant calling is performed only in genomic regions targeted by the test.

Base substitution detection is performed using a Bayesian methodology, which allows for the detection of novel somatic alterations at low mutant allele frequency (MAF) and increased sensitivity for alterations at hotspot sites through the incorporation of tissue-specific prior expectations.[7] Reads with low mapping (mapping quality < 25) or base calling quality (base calls with quality ≤ 2) are discarded. Final calls are made at MAF ≥ 5% (MAF ≥ 1% at hotspots).

To detect indels, *de novo* local assembly in each targeted exon is performed using the de-Bruijn approach.[8] Key steps are:

- Collecting all read-pairs for which at least one read maps to the target region.
- Decomposing each read into constituent k-mers and constructing an enumerable graph representation (de-Bruijn) of all candidate non-reference haplotypes present.
- Evaluating the support of each alternate haplotype with respect to the raw read data to generate mutational candidates. All reads are compared to each of the candidate haplotypes via ungapped alignment, and a read 'vote' for each read is assigned to the candidate with best match. Ties between candidates are resolved by splitting the read vote, weighted by the number of reads already supporting each haplotype. This process is iterated until a 'winning' haplotype is selected.
- Aligning candidates against the reference genome to report alteration calls.

Filtering of indel candidates is carried out similarly to base substitutions, with an empirically increased allele frequency threshold at repeats and adjacent sequence quality metrics as implemented in GATK: % of neighboring bases mismatches < 25%, average neighboring base quality > 25, average number of supporting read mismatches ≤ 2. Final calls are made at MAF ≥ 5% (MAF ≥ 3% at hotspots).

Copy number alterations (CNAs) are detected using a comparative genomic hybridization (CGH)-like method. First, a log-ratio profile of the sample is acquired by normalizing the sequence coverage obtained at all exons and genome-wide SNPs (~3,500) against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate tumor purity and copy number at each segment. Amplifications are called at segments with ≥ 6 copies (or ≥ 7 for triploid/≥ 8 for tetraploid tumors) and homozygous deletions at 0 copies, in samples with tumor purity ≥ 20%. Amplifications in *ERBB2* are called positive at segments with ≥ 5 copies for diploid tumors.

Genomic rearrangements are identified by analyzing chimeric read pairs. Chimeric read pairs are defined as read pairs for which reads map to separate chromosomes, or at a distance of over 10 megabase (Mb). Pairs are clustered by genomic coordinate of the pairs, and clusters containing at least five chimeric pairs (three for known fusions) are identified as rearrangement candidates. Filtering of candidates is performed by mapping quality (average read mapping quality in the cluster must be 30 or above) and distribution of alignment positions. Rearrangements are annotated for predicted function (e.g., creation of fusion gene).

To determine a patient's MSI status, F1CDx employs a fraction based (FB) MSI algorithm to categorize a tumor specimen as MSI-High (MSI-H) or microsatellite stable (MSS). The FB-MSI algorithm calculates the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score) based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The final fraction unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci. Two FB-MSI score thresholds are applied to classify a tumor specimen as having MSI-H or MSS status. MSI-H status is reported for patients with solid tumors whose samples have FB-MSI scores ≥ 0.0124 while MSS status is reported for patients with solid tumors whose samples have FB-MSI scores ≤ 0.0041. Per the F1CDx assay, a patient whose tumor has an MSI-H score ≥ 0.0124 is reported as eligible for treatment with KEYTRUDA. For patients with solid tumors whose samples have FB-MSI scores >0.0041 and <0.0124, an MSI "Cannot be Determined" result is reported. Patients with this result should be re-tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. Patients with solid tumors may also receive an MSI status reported as MSI-Cannot Be Determined due to a quality control (QC) failure. Patients with this result should consider re-testing with FoundationOneCDx or an orthogonal (alternative) method, if clinically appropriate.

Tumor mutational burden (TMB) is measured by counting all synonymous and non-synonymous substitution and indel variants present at 5% allele frequency or greater and filtering out potential germline variants according to published databases of known germline polymorphisms including Single Nucleotide Polymorphism database (dbSNP) and Exome Aggregation Consortium (ExAC). Additional germline alterations still present after database querying are assessed for potential germline status and filtered out using a somatic-germline/zygosity (SGZ) algorithm. Furthermore, known and likely driver mutations are filtered out to exclude bias of the data set. The resulting mutation number is then divided by the coding region corresponding to the number of total variants counted, or 793 kb. The resulting number is communicated as mutations per Mb unit (mut/Mb). Per the F1CDx assay, a patient whose tumor has a TMB ≥ 10 mut/Mb is reported as eligible for treatment with KEYTRUDA:

To compute the percentage of genomic LOH for each tumor, LOH segments are inferred across the 22 autosomal chromosomes using the genome-wide aneuploidy/copy number profile and minor allele frequencies of the more than 3500 SNPs sequenced in the Foundation Medicine's next-generation sequencing (NGS)-based platform. A comparative genomic hybridization (i.e., log-ratio profile of the sample) is obtained from the NGS sequencing data by normalizing the sequence coverage obtained at all exons and genome-wide SNPs against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate copy number (Ci) and minor allele count (Mi) at each

segment (i). A segment is determined to have LOH if $Ci \neq 0$ and $Mi = 0$. Two types of LOH segments are excluded from the calculation of percent genomic LOH: (1) LOH segments spanning ≥ 90% of a whole chromosome or chromosome arm, as these LOH events usually arise through non-homologous recombination deficiency (HRD) mechanisms (e.g., mitotic nondisjunction), and (2) regions in which LOH inference is ambiguous (e.g., some small genomic regions that do not have sufficient heterozygous SNPs to support LOH calling).

After completion of the Analysis Pipeline, variant data are displayed in the FMI custom-developed CATi software applications with sequence QC metrics. As part of data analysis QC for every sample, the F1CDx assay assesses cross-contamination through the use of a SNP profile algorithm, reducing the risk of false-positive calls that could occur as a result of an unexpected contamination event. Sequence data are reviewed by trained bioinformatics personnel. Samples failing any QC metrics are automatically held and not released.

G. Report Generation

Approved results are annotated by automated software with CDx relevant information and are merged with patient demographic information and any additional information provided by FMI as a professional service prior to approval and release by the laboratory director or designee.

H. Internal Process Controls Related to the System

Positive Control

Each assay run includes a control sample run in duplicate. The control sample contains a pool of ten HapMap cell lines and is used as a positive mutation detection control. One hundred (100) different germline SNPs present across the entire targeted region are required to be detected by the analysis pipeline. If SNPs are not detected as expected, this results in a QC failure, as it indicates a potential processing error.

Sensitivity Control

The HapMap control pool used as the positive control is prepared to contain variants at 5%-10% MAF which must be detected by the analysis pipeline to ensure the expected sensitivity for each run.

Negative Control

Samples are barcoded molecularly at the LC stage. Only reads with a perfect molecular barcode sequence are incorporated into the analysis. The Analysis Pipeline includes an algorithm that analyzes the SNP profile of each specimen to identify potential contamination that may have occurred prior to molecular barcoding and can detect contamination lower than 1%.

I. Variant Classification

Biomarker Rules for SNVs and indels that lead to *MET* exon 14 skipping

An SNV or indel in *MET* shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:

1. Deletions greater than or equal to 5 bp that affect positions -3 to -30 in the intronic region immediately adjacent to the splice acceptor site at the 5′ boundary of *MET* exon 14.
2. Indels affecting positions -1 or -2 at the splice acceptor site of the 5′ boundary of *MET* exon 14.
3. Base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3′ boundary of *MET* exon 14.

Homologous Recombination Repair (HRR) Genes

A clinical report is provided to the ordering physician for each F1CDx test performed at Foundation Medicine, Inc. Each report is generated and reviewed by an internal team consisting of clinical bioinformatics analysts, scientists, curators, and pathologists for mutations positive for the therapies identified. Each sample is assessed for mutations in the 14 HRR genes, *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D,* and *RAD54L* (Table 5). For these genes, both deleterious and suspected deleterious mutations in short variant, copy number alteration, and rearrangement variant classes are determined by an in-house software pipeline. Alterations listed in the COSMIC database and homozygous deletions are considered deleterious. Suspected deleterious mutations include truncating events (i.e., splice, frameshift, and nonsense alterations), as well as large rearrangements that disrupt the coding sequence. The COSMIC check is a second layer of check for HRR positive suspected deleterious alterations. All splice, nonsense, and frameshift alterations in HRR genes are considered biomarker positive and would be considered as suspected deleterious mutations (or "likely" status in FMI reporting rules). If these mutations are additionally reported in COSMIC, they would be listed as deleterious mutations (or "known" status in FMI reporting).

The F1CDx assay is intended as an aid in selecting prostate cancer patients with deleterious or suspected deleterious HRR variants, identified by the rules below, and who may be eligible for treatment with Lynparza® (olaparib).

Table 5. Mutation types identified in the HRR genes

Variant Class	Alteration type	Description*
Short Variant	Nonsense, frameshift, or splice site	Any deleterious nonsense, frameshift, or splicing event that spans or occurs within ±2 bases of the intron/exon junction
	Missense or non-frameshift	Any of the mutations listed in Table 6 for *ATM*, *BRCA1*, and *BRCA2*
Copy Number Alteration	Homozygous copy number loss	Deleterious homozygous copy number loss of one or more exons
Rearrangement	Rearrangement	Any rearrangement that disrupts protein function

*For *BRCA2*, truncating mutations must occur upstream of bases encoding amino acid 3326. Additionally, the frameshift mutation T367fs*13 in *FANCL* is ineligible. All short variants must occur in the canonical transcript.

The specific deleterious mutation (DM) and suspected deleterious mutation (SDM) missense mutations or non-frameshift mutations for *BRCA1, BRCA2,* and *ATM* are shown in Table 6, below. However, any missense or non-frameshift mutations in the other 12 genes would not be considered HRR positive.

Table 6. Eligible deleterious mutations in the *ATM, BRCA1,* and *BRCA2* genes

ATM	***BRCA1***	***BRCA2***
M1T	M1V	M1R
R2032K	M1I	M1I
R2227C	C61G	V159M
R2547_S2549del	C64Y	V211L
G2765S	R71G	V211I
R2832C	R71K	R2336P
S2855_V2856delinsRI (annotated as S2855_V2856>RI)	R1495M	R2336H
R3008C	E1559K	
R3008H	D1692N	
8418+5_8418+8delGTGA or 8418+1_8418+4delGTGA	D1692H	
	R1699W	
	A1708E	
	G1788V	

Biomarker Rules for Rearrangements that Lead to *NTRK1, NTRK2,* or *NTRK3* Fusions:

Rearrangements in *NTRK1*, *NTRK2*, or *NTRK3* shall be considered CDx biomarker positive, that is, to lead to a *NTRK1, NTRK2,* or *NTRK3* RNA fusion, if the following criterion is met:

- In-strand rearrangement events that may lead to an *NTRK1, NTRK2* or *NTRK3* RNA fusion with a previously reported or novel partner gene in which the kinase domain is not disrupted. This also includes rearrangement events that result in reciprocal fusions (*NTRK*-3' and 5'-*NTRK* events).

In this regard out-of-strand events are considered as non-fusion rearrangements and are classified as CDx biomarker negative. Intragenic fusions in which genomic rearrangement events are wholly internal to the *NTRK1, NTRK2,* or *NTRK3* genes (i.e., *NTRK1-NTRK1, NTRK2-NTRK2, NTRK3-NTRK3* events) are also considered biomarker negative. Unidentified partners (encoded as N/A) or LINC non-coding partners are also considered CDx biomarker negative.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in Table 1 of the F1CDx intended use statement. The approved CDx tests are listed in Table 7, below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

Table 7. List of FDA approved CDx assays for genes targeted by F1CDx

	Device	Company	Technology	Therapy	Indication
HER2-Amplification	PathVysion HER-2 DNA Probe Kit	Abbott Molecular, Inc.	FISH	HERCEPTIN (trastuzumab)	Breast cancer
	PATHWAY Anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody	Ventana Medical Systems, Inc.	IHC	HERCEPTIN (trastuzumab)	Breast cancer
	InSite HER-2/neu Kit	Biogenex Laboratories, Inc.	IHC	HERCEPTIN (trastuzumab)	Breast cancer
	SPOT-Light HER2 CISH Kit	Life Technologies, Inc.	CISH	HERCEPTIN (trastuzumab)	Breast cancer
	Bond Oracle HER2 IHC System	Leica Biosystems	IHC	HERCEPTIN (trastuzumab)	Breast cancer
	HER2 CISH pharmDx Kit	Dako Denmark A/S	CISH	HERCEPTIN (trastuzumab)	Breast cancer
	INFORM HER2 Dual ISH DNA Probe Cocktail	Ventana Medical Systems, Inc.	Dual ISH	HERCEPTIN (trastuzumab)	Breast cancer
	HercepTest	Dako Denmark A/S	IHC	HERCEPTIN (trastuzumab) PERJETA (pertuzumab) KADCYLA (ado-trastuzumab emtansine)	Breast cancer Gastric or Gastroesophageal junction adenocarcinoma
	HER2 FISH pharmDx Kit	Dako Denmark A/S	FISH	HERCEPTIN (trastuzumab) PERJETA (pertuzumab) KADCYLA (ado-trastuzumab emtansine)	Breast cancer Gastric or Gastroesophageal junction adenocarcinoma
BRAF-	THxID BRAF Kit	bioMerieux	PCR	MEKINIST (tramatenib)	Melanoma

Table 7. List of FDA approved CDx assays for genes targeted by F1CDx

	Device	Company	Technology	Therapy	Indication
	cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems, Inc.	PCR	COTELLIC (cobimetinib) ZELBORAF (vemurafenib)	Melanoma
BRAF-V600E	cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems, Inc.	PCR	ZELBORAF (vemurafenib)	Melanoma
	THxID BRAF Kit	bioMerieux	PCR	TAFINLAR (dabrafenib)	Melanoma
BRAF-V600E	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	TAFINLAR (dabrafenib) MEKINIST (trametinib)	NSCLC
	therascreen BRAF V600E RGQ PCR Kit	QIAGEN	PCR	BRAFTOVI (encorafenib) Erbitux (cetuximab)	CRC
NRAS	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	CRC
KRAS	**cobas** KRAS Mutation Test	Roche Molecular Systems, Inc.	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	CRC
	therascreen KRAS RGQ PCR Kit	QIAGEN	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	CRC
	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	CRC
ALK – fusion	Vysis ALK Break Apart FISH Probe Kit	Abbott Molecular, Inc.	FISH	XALKORI (crizotinib) ALUNBRIG (brigatinib)	NSCLC
	ALK (D5F3) CDx Assay	Ventana Medical Systems, Inc.	IHC	XALKORI (crizotinib)	NSCLC
EGFR – Exon 19 deletions & L858R	**cobas** EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	TARCEVA (erlotinib) TAGRISSO (osimertinib) IRESSA (gefitinib)	NSCLC
	therascreen EGFR RGQ PCR Kit	QIAGEN	PCR	GILOTRIF (afatinib) IRESSA (gefitinib)	NSCLC

Table 7. List of FDA approved CDx assays for genes targeted by F1CDx

	Device	Company	Technology	Therapy	Indication
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	IRESSA (gefitinib)	NSCLC
EGFR T790M	**cobas** EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	TAGRISSO (osimertinib)	NSCLC
BRCA1/2	FoundationFocus CDx$_{BRCA}$	Foundation Medicine, Inc.	NGS	RUBRACA (rucaparib)	Advanced ovarian cancer
PIK3CA	*therascreen* PIK3CA RGQ PCR Kit	QIAGEN	PCR	PIQRAY (alpelisib)	Breast cancer

Abbreviations: FISH – fluorescence *in situ* hybridization; IHC – immunohistochemistry; CISH – chromogenic *in situ* hybridization; ISH – *in situ* hybridization; PCR – polymerase chain reaction; NGS – next generation sequencing.

VII. MARKETING HISTORY

Foundation Medicine, Inc. initially designed and developed the FoundationOne® laboratory developed test (F1 LDT), and the first commercial sample was tested in 2012. The F1 LDT has been used to detect the presence of genomic alterations in FFPE tumor tissue specimens. The F1 LDT is not FDA-cleared or -approved.

The F1CDx Premarket Approval (PMA) was originally approved on November 30, 2017 by FDA (P170019) and is commercially available in the U.S. since March 30, 2018. The following PMA supplements affecting the Intended Use were approved by FDA.

- P170019/S005 was approved on April 10, 2019.
- P170019/S004 and P170019/S008 were approved on July 1, 2019.
- P170019/S009 was approved on August 21, 2019.
- P170019/S006 was approved on December 3, 2019.
- P170019/S010 was approved on December 16, 2019.
- P170019/S013 was approved on April 17, 2020.
- P170019/S011 was approved on May 6, 2020.
- P170019/S015 was approved on May 19, 2020.
- P170019/S016 was approved on June 16, 2020.
- P170019/S017 was approved on October 23, 2020.
- P170019/S021 was approved on May 28, 2021.
- P170019/S023 was approved on June 30, 2021.
- P170019/S026 was approved on July 30, 2021.
- P170019/S025 was approved on November 10, 2021
- P170019/S030 was approved on January 19, 2022

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 undergo treatment with one of the therapies listed in the above 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.

For the specific adverse events related to the approved therapeutics, please see the approved drug product labels.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Analytical validation for F1CDx platform-level validation (P170019), performance characteristics were established using DNA derived from a wide range of FFPE tumor tissue types in support of companion diagnostic (CDx) indications and have been described previously (P170019). The validation studies performed to gain approval for the fraction-based MSI (FB-MSI) caller on the F1CDx platform included samples across multiple tumor types representing a pan-solid tumor intended use population, and across the range of MSI scores, including samples with MSI-H status. Analytical validation studies conducted to gain platform and CDx approval for the FB-MSI caller included limit of detection (LoD), limit of blank (LoB), precision, and analytical accuracy.

This section summarizes the analytical studies conducted to support the identification of MSI-H status in patients with solid tumors using the FB-MSI caller.

1. Analytical Accuracy/Concordance

To demonstrate the analytical accuracy of MSI calling three independent studies were conducted. Two studies evaluated the concordance of F1CDx MSI calling to a PCR based comparator. A third study was conducted to evaluate concordance between F1CDx MSI calling and mismatch repair (MMR) immunohistochemistry (IHC) assays through a retrospective chart review of a randomly selected set of specimens from colorectal and endometrial cancer patients.

1. **Comparison to an PCR-based Orthogonal Method for Detecting MSI – Study I**

   To demonstrate the analytical accuracy of the MSI calling, an analysis to assess concordance of F1CDx MSI calling was performed using a PCR-based comparator assay. The study included 186 FFPE tumor specimens representing various disease ontologies (see Table 8), of which 86 were

selected with the PCR-based comparator assay, while 100 were selected with F1CDx.

Table 8. Distribution of Organ System and Disease Ontologies in Analytical Accuracy Study I

Organ System	Disease Ontology	Number of Samples Tested		
		PCR Comparator Enrolled	F1CDx Enrolled	Total (n)
Gastrointestinal	Colon adenocarcinoma (CRC)	1	12	13
	Rectum adenocarcinoma (CRC)	-	8	8
	Esophagus squamous cell carcinoma (SCC)	-	1	1
	Appendix adenocarcinoma	-	3	3
	Ampullary adenocarcinoma	-	2	2
	Small intestine adenocarcinoma	4	-	4
	Stomach adenocarcinoma (NOS)	10	-	10
	Esophagus adenocarcinoma	2	-	2
	Esophagus carcinoma (NOS)	8	-	8
Reproductive	Breast invasive ductal carcinoma (IDC)	-	1	1
	Vagina adenocarcinoma	-	2	2
	Breast carcinoma (NOS)	-	5	5
	Prostate acinar adenocarcinoma	-	7	7
	Ovary endometrioid adenocarcinoma	-	2	2
	Testis germ cell tumor (non-seminoma)	-	2	2
	Cervix adenocarcinoma	-	1	1
	Cervix squamous cell carcinoma (SCC)	-	1	1
	Penis squamous cell carcinoma (SCC)	-	2	2
	Uterus endometrial adenocarcinoma (NOS)	15	-	15

	Ovary cancer (NOS)	2	-	2
	Ovary epithelial carcinoma (NOS)	4	-	4
	Ovary serous carcinoma	1	-	1
	Vulva squamous cell carcinoma (SCC)	-	1	1
	Uterus endometrial adenocarcinoma endometrioid	-	4	4
	Peritoneum serous carcinoma	-	1	1
Hepato-Pancreatobiliary	Gallbladder adenocarcinoma	6	1	7
	Liver cholangiocarcinoma	1	1	2
	Bile duct adenocarcinoma		1	1
	Gallbladder carcinoma	7	-	7
	Liver hepatocellular carcinoma (HCC)	-	2	2
	Pancreas ductal adenocarcinoma	-	4	4
Endocrine	Pancreas neuroendocrine tumor (pNET)	-	1	1
	Adrenal gland cortical carcinoma	-	2	2
	Thyroid anaplastic carcinoma	-	1	1
	Thyroid papillary carcinoma	2	1	3
	Thyroid follicular carcinoma	1	-	1
	Thyroid medullary carcinoma	1	-	1
	Thyroid carcinoma (NOS)	8	-	8
	Lung large cell neuroendocrine carcinoma	-	1	1
Urinary	Kidney chromophobe carcinoma	-	1	1
	Bladder urothelial (transitional cell) carcinoma	-	6	6
	Kidney clear cell carcinoma	-	1	1
	Kidney urothelial carcinoma	-	2	2

Thoracic	Lung adenocarcinoma	-	5	5
	Lung small cell undifferentiated carcinoma	-	2	2
	Lung squamous cell carcinoma (SCC)	-	2	2
Skin/Soft Tissue	Skin squamous cell carcinoma (SCC)	-	3	3
	Pleura mesothelioma	-	2	2
	Soft tissue sarcoma (NOS)	3	-	3
Other	Brain glioblastoma (GBM)	-	4	4
	Brain meningioma	-	1	1
	Head and neck squamous cell carcinoma (HNSCC)	-	1	1
	Unknown	1	-	1
	Unknown primary adenocarcinoma	1	-	1
	Unknown primary carcinoma (NOS)	5	-	5
	Unknown primary squamous cell carcinoma (SCC)	1	-	1
	Unknown primary malignant neoplasm (NOS)	2	-	2
Total		**86**	**100**	**186**

Of the 186 FFPE tumor specimens, 159 were evaluable with valid MSI results from both F1CDx and the comparator assay. Of the 86 samples selected with the PCR comparator assay, 24 failed to provide evaluable or valid F1CDx results due to QC failures, i.e., a 28% failure rate was observed primarily due to contamination for the samples that were procured externally using the PCR-based comparator. Of the 100 samples selected with F1CDx, i.e., internally procured, three (3) failed to provide evaluable or valid F1CDx results, i.e., a 3% failure rate was observed.

The PCR-based assay-selected subset contained 62 samples and the F1CDx assay-selected subset contained 97 samples that were available for concordance analysis. For concordance analysis, samples with F1CDx MSI scores ≥ 0.0124 were treated as MSI-H/positive in the concordance analysis. Samples with F1CDx FB- MSI scores <0.0124

were treated as non-MSI-H or negative results. The PCR assay includes MSI-High (MSI-H), MSI-Low (MSI-L), and microsatellite stable (MSS) results, which were further dichotomized into MSI-H or positive and non-MSI-H (MSI-L and MSS) or negative in the concordance analysis.

Concordance results between F1CDx and PCR for MSI-H calling within the combined datasets, i.e., samples selected with the PCR comparator assay and F1CDx assay are summarized in Table 10 below. Positive percent agreement (PPA) and negative percent agreement (NPA) are estimates of agreement between the assays, calculated by determining the proportion of comparator positive and negative samples that F1CDx was able to call concordantly. PPA and NPA were calculated using the observed, unadjusted data from the sample subset enrolled with the F1CDx assay, therefore, results may be biased due to how samples were selected.

The combined PPA and NPA using the ≥ 0.0124 threshold for MSI-H calling was 98.46% (95% CI [91.79% - 99.73%]) and 96.81% (95% CI [91.03% - 98.91%]) respectively. A total of four (4) discordant cases were observed (Table 9). A F1CDx-/PCR+ sample was from a patient with kidney chromophobe carcinoma and had an FB-MSI score of 0.0105. Three (3) F1CDx+/PCR- results were from patients with uterus endometrial carcinoma, thyroid anaplastic carcinoma, or brain glioblastoma, and had the following FB-MSI scores 0.0135, 0.0163 and 0.0174 respectively. Therefore, the four discordant samples had FB-MSI scores near the 0.0124 threshold.

Table 9: Concordance Results for FB-MSI Detection Using PCR-based Comparator Assay

	F1CDx+/ PCR+	F1CDx- /PCR +	F1CDx+ /PCR -	F1CDx- /PCR -	PPA (95% CI)	NPA (95% CI)
Combined datasets (PCR and F1CDx enrolled)	64	1	3	91	98.46% (91.79% - 99.73%)	96.81% (91.03% - 98.91%)

While the concordance was high for samples from colorectal cancer (CRC) patients for the combined data sets, both PPA and NPA were 100.00%, concordance was lower for other disease ontologies (non-CRC). For non-CRC patient samples, the observed combined PPA and NPA using the ≥ 0.0124 threshold for MSI-H calling were 98.04% and 97.53% respectively. Although the number of samples from patients with uterus endometrial adenocarcinoma that had valid F1CDx results was low, 8 out of 19 were valid per F1CDx, the observed combined PPA and NPA using the ≥ 0.0124 threshold for MSI-H calling were 100% and 66.67%, respectively.

In this study there were 10 samples with MSI-Cannot be Determined status due to FB-MSI scores >0.0041 and <0.0124, one (1) was MSI-H, seven (7) were MSS, while the remaining two (2) samples had an MSI-L status per the PCR-based comparator. All samples were from non-CRC tumors. The MSI-H sample per the PCR comparator was from a patient with kidney chromophobe carcinoma. The two (2) MSI-L samples per the PCR comparator were from patients with uterus endometrial adenocarcinoma, or brain glioblastoma. The seven (7) samples that were MSS per the PCR comparator were from patients with adrenal gland cortical carcinoma (1), appendix adenocarcinoma (1), kidney urothelial carcinoma (1), adrenal gland cortical carcinoma (1), liver cholangiocarcinoma (1), ampullary adenocarcinoma (1), or thyroid follicular carcinoma (1).

2. **Comparison to an PCR-based Orthogonal Method for Detecting MSI – Study II**

An additional study to assess concordance of MSI-H calling to the PCR-based comparator assay was conducted to demonstrate the analytical validity of F1CDx. This study included the evaluation of a test set of 56 screen failure samples from Merck's clinical trial study KEYNOTE-158, which supported the clinical validity of the F1CDx, see Section X below. An additional set of 161 commercially procured samples were included to support the analytical accuracy F1CDx. In total 217 specimens across multiple tumor types were evaluated in the study. Table 10 shows the distribution of tumor types in the second analytical accuracy study.

Of the 161 commercially procured samples, 154 were selected by an external vendor who coordinated mismatch repair (MMR) immunohistochemistry (IHC) testing to enrich the population of potential MSI-H samples for procurement prior to testing with the PCR comparator method and F1CDx. To support the pan-tumor assessment, seven (7) additional commercially procured samples with MSI status previously determined by F1CDx were selected by FMI and subsequently tested with the PCR comparator assay.

Table 10: Distribution of Disease Ontologies in Analytical Accuracy Study II

Organ System	Disease Ontology	Number of Samples Tested			
		PCR Comparator-Enrolled, Commercially Procured	PCR Comparator Enrolled, Clinical Trial Screen Failure	F1CDx-Enrolled, Commercially Procured	Total (n)
Endocrine	Thyroid carcinoma (NOS)	1	7	-	8
	Unknown primary neuroendocrine tumor (NET)	-	5	-	5
	Unknown primary undifferentiated neuroendocrine carcinoma	2	-	-	2
Gastrointestinal	Anus squamous cell carcinoma	-	3	-	3
	Bile duct adenocarcinoma	-	5	-	5
	Colon adenocarcinoma (CRC)	53	-	-	53
	Esophagus carcinoma (NOS)	1	-	-	1
	Stomach adenocarcinoma (NOS)	9	-	-	9
	Stomach carcinoma (NOS)	18	-	-	18
Hepato-Pancreatobiliary	Unknown primary adenocarcinoma	-	-	1	1
Reproductive	Breast invasive ductal carcinoma (IDC)	8	-	-	8
	Cervix squamous cell carcinoma (SCC)	2	6	-	8
	Ovary epithelial carcinoma (NOS)	2	-	-	2
	Prostate acinar adenocarcinoma	2	-	-	2
	Uterus endometrial adenocarcinoma (NOS)	50	8	-	58
	Vulva squamous cell carcinoma (SCC)	-	14	-	14
Soft Tissue/Skin	Skin melanoma	-	-	1	1
Thoracic	Lung adenocarcinoma	-	-	1	1

	Lung small cell undifferentiated carcinoma	2	7	-	9
	Lung squamous cell carcinoma (SCC)	-	-	4	4
	Unknown primary mesothelioma	-	1	-	1
Urinary	Kidney renal cell carcinoma (NOS)	4	-	-	4
Total		**154**	**56**	**7**	**217**

After sample processing and screening externally at the vendor and internally at FMI, a total of 111 samples failed to yield an evaluable or valid F1CDx result, while one (1) sample that underwent F1CDx sample processing was not evaluable by the PCR assay due to poor amplification. Of the 111 samples that failed to yield F1CDx evaluable results, three (3) samples failed for having less than 20% tumor nuclei, two (2) samples failed for having an insufficient quantity of slides. In addition, two (2) samples failed DNA extraction specifications due to low yield and four (4) samples failed HC QC specifications due to insufficient DNA mass. One hundred (100) samples failed F1CDx Post-Sequencing QC. Among the 100 sample failures identified, 91 samples displayed low-level contamination (≥1.01%, ≤3.57%) which was above the F1CDx specification of 1% and were removed from the primary analysis. Of note, 89 of the 91 contaminated samples were commercially procured. Of these 91 samples, 3 samples in addition to failing to meet the contamination QC also failed other F1CDx post-sequencing QC. An exploratory analysis including these samples was performed and is discussed below.

105 samples that met all sample selection and testing QC criteria and were included in the primary analysis. MSI assessment was dichotomized to MSI-H or non-MSI-H for both F1CDx, and the PCR assay results. Samples with F1CDx MSI scores ≥ 0.0124 were treated as MSI-H/positive in the concordance analysis. Samples with F1CDx FB-MSI scores <0.0124 were treated as non-MSI-H or negative results. As for the accuracy Study I, the PCR assay includes MSI-H, MSI-L, and MSS, which was further dichotomized into MSI-H or positive and non-MSI-H (MSI-L and MSS) or negative in the concordance analysis.

Among the 105 samples, 98 were PCR-enrolled and seven (7) were F1CDx-enrolled. Because concordance in the F1CDx-enrolled set was 100%, a prevalence adjustment does not change estimates of PPA or NPA for the dataset. As such, the analysis was simplified, and the two (2) datasets (PCR-enrolled and F1CDx-enrolled) were combined

directly. Point estimates for PPA and NPA were calculated directly, along with 95% two-sided Wilson Score confidence intervals (CIs).

The combined PPA was 100.00% with 2-sided 95% CI of [87.54% - 100.00%], see Table 11. The combined NPA was 97.37% with 2-sided 95% CI of [91.12% - 99.29%], see Table 11. Two (2) samples, both from endometrial cancer patients, exhibited discordant MSI status results between the assays. Both discordant samples were PCR-enrolled and exhibited F1CDx MSI-H results (FB-MSI score of 0.0216 and 0.0192) and PCR non-MSI-H results (MSS).

Table 11. Concordance Results MSI Detection Using PCR-based Comparator Assay

	F1CDx+/ PCR+	F1CDx-/PCR +	F1CDx+ /PCR -	F1CDx-/PCR -	PPA (95% CI)	NPA (95% CI)
Combined data sets (PCR and F1CDx enrolled)	27	0	2	76	100.00% (87.54% - 100.00%)	97.44% (91.12% - 99.29%)

For CRC patients in the combined data sets, for samples that yielded evaluable results (21 out of 53, 39.6%), PPA was 100% (10/10) and NPA was 100.00% (11/11) using the ≥ 0.0124 threshold for MSI-H calling. For non-CRC patient samples with evaluable results, the observed PPA and NPA in the combined data sets using the ≥ 0.0124 threshold for MSI-H calling was 100.00% (17/17) and 97.01% (63/65).

For the samples from patients with uterus endometrial adenocarcinoma that had evaluable results (25 out of 58, 43.10%) the observed PPA and NPA in the combined data sets using the ≥ 0.0124 threshold for MSI-H calling was 100% (9/9) and 87.50% (14/16) respectively.

There were two (2) samples, both from patients with thyroid carcinoma, with MSI-Cannot be Determined status due to FB-MSI scores >0.0041 and <0.0124; both were MSS per the PCR based comparator.

An exploratory analysis was conducted where the MSI QC rule for contamination was not imposed, enabling 88 samples that were removed from the primary analysis to be included for analysis. This exploratory analysis increases the number of evaluable samples from 105 to 193. All sample failures, other than exclusively for contamination, remained excluded from the analysis. Among the 193 samples, 191 samples had concordant MSI status results between the F1CDx and PCR assays. All 88 additional samples included in the exploratory analysis produced

100% concordant MSI results between F1CDx and PCR assay. The same two (2) samples, that were discordant in the primary analysis remained discordant in the exploratory analysis. The combined PPA was 100.00% with 95% two-sided CI of (94.58% - 100.00%). The combined NPA was 98.41% with 95% two-sided Wilson Score CI of (94.40% - 99.56%). In the exploratory analysis, there were five (5) samples with MSI-Cannot be Determined status due to FB-MSI scores >0.0041 and <0.0124, the same two samples in the primary analysis and three additional ones, from patients with uterus endometrial adenocarcinoma (1), bile duct adenocarcinoma (1) or cervix squamous cell carcinoma (1). All five patient samples had MSS status per the PCR comparator. Although the study showed a high failure rate by F1CDx due to the strict contamination QC metric, exclusion of the samples that failed the contamination QC metric does not impact concordance results.

The presence of contamination in commercially procured samples often occurs at an elevated rate compared to clinical F1CDx samples and may be caused by FPPE block processing techniques used by outside FFPE tissue procurement vendors as well as the age of the specimen. This contamination level is low (~0-3%), but above the specified threshold of 1%. A retrospective analysis of the F1CDx commercial tests suggests that the high failure rate observed in the commercial lab (21.47%) is not as high as that seen in the accuracy study. A post market study will be conducted to confirm the failure rate of the F1CDx in the commercial setting, refer for Section XIII.

3. **Retrospective Chart Review Concordance Results of FB-MSI Caller and IHC**

To demonstrate the analytical accuracy of the MSI calling, an additional evaluation of the concordance between the MSI classification (MSI-High (MSI-H)/deficient mismatch repair (dMMR) versus non-MSI-H/proficient mismatch repair (pMMR)) determined by the FB-MSI caller and mismatch repair (MMR) immunohistochemistry (IHC) assays was performed through a retrospective chart review of a randomly selected set of 134 colorectal cancer (CRC) and 52 uterus endometrial cancer patients from the FMI clinical commercial database. Overall, a total of 178 samples (including 128 CRC and 50 uterus endometrial cases) passed the F1CDx QC assessment, i.e., provided evaluable or valid F1CDx results and were used as the analysis dataset. Samples with FB-MSI scores ≥ 0.0124 were treated as MSI-H/positive in the concordance analysis. Samples with F1CDx FB- MSI scores <0.0124 were treated as non-MSI-H or negative results. The IHC assays provide two outcomes, dMMR and pMMR. dMMR are considered positive results and pMMR are considered negative results for the analyses.

The PPA and NPA for the overall sample set and CRC and uterus endometrial cancer datasets are shown in Table 12. Point estimates and 95% two-sided CI for PPA and NPA for the overall sample set were PPA 85.19% (95%CI [67.52, 94.08%]) and NPA 100.00% (95% CI [97.52%, 100.00%]).

Table 12: Retrospective Chart Review Concordance Results Using IHC based Comparator Assays

Dataset	F1CDx+/ IHC+	F1CDx-/IHC+	F1CDx+/IHC-	F1CDx-/IHC-	PPA (95% CI)	NPA (95% CI)
Combined CRC and Endometrial	23	4	0	151	85.19% (67.52, 94.08%)	100.00% (97.52%, 100.00%)
CRC Only	11	3	0	114	78.57% (52.41%, 92.43%)	100.00% (96.74%, 100.00%)
Endometrial Only	12	1	0	37	92.31% (66.69%, 98.63%)	100.00% (90.59%, 100.00%)

Four (4) discordant samples were identified in this study, three (3) within the CRC subset and one (1) within the uterus endometrial cancer subset. All discordant samples were F1CDx non-MSI-H/dMMR.

MSI-Cannot be determined status for MSI cases with FB MSI >0.0041 and <0.0124 was observed in 11 samples, four CRC and seven uterus endometrial cancer samples. Of the 11 samples, 1 (9%) uterus endometrial cancer sample was dMMR while the remaining 10, (91%) had pMMR status.

2. Analytical Sensitivity

1. Limit of Blank (LoB)

To assess LoB for MSI, 111 test replicates from 10 individual tumor FFPE-derived DNA samples as well as a pool of biomarker-negative DNA derived from 30 unique FFPE tumor DNA samples were evaluated. The LoB was confirmed to be zero as 0 out of 111 test replicates yielded MSI-H results. The average, median, minimum, and maximum MSI scores observed were 0.0006, 0.0004, 0 and 0.0032 respectively.

2. Limit of Detection (LoD)

The LoD for MSI-H calling using the FB-MSI caller in the F1CDx assay was assessed through evaluation of eight samples (see Table 14). The eight samples included five (5) disease ontologies that represent the organ systems where MSI-H cases are the most prevalent in the Intended Use (IU) population.

To establish LoD, five (5) to six (6) levels of varying computational tumor purity (TP) and 13 to 20 replicates per level were evaluated. To achieve the targeted tumor purity, biomarker-positive (MSI-H) FFPE-derived tumor DNA were mixed with unmatched biomarker-negative (microsatellite stable (MSS)) FFPE-derived tumor DNA from the same disease ontology. The hit rate for each dilution was computed as the number of replicates with MSI-H calls (MSI score was ≥ 0.0124) per the total number of replicates tested at each level. The empirical hit rate approach was used since there were less than three hit rate levels with less than 95% detection rate. The LoD was defined as the lowest computational tumor purity with at least 95% detection.

Of the replicate measurements processed, 838 replicates across all eight (8) samples, 807 yielded F1CDx evaluable results. Twenty-five (25) replicates were unevaluable due to failure at the library construction (LC) quality control (QC), two (2) replicates, were unevaluable due to failure at the hybrid construction (HC) QC, two (2) replicates failed to meet MSI QC metrics, one (1) replicate failed to meet coverage requirements and one (1) replicate failed to meet both coverage requirements and MSI QC metrics.

Given that not all replicates were evaluable, LoD was established using 12 to 20 evaluable replicates per level. For each sample, the lowest computational tumor purity level measured at which MSI-H hit rates with at least 95% hit rate is shown in Table 13.

As 15.67% tumor purity is the maximal LoD value observed, the established MSI-H LoD is 15.67% tumor purity.

Table 13: MSI-H Sample LoD Results

Sample	Organ System	Disease Ontology/Tissue Type	MSI-H LoD (Mean % Tumor Purity)
1	Gastrointestinal	Colon adenocarcinoma (CRC)	8.25%
2	Gastrointestinal	Colon adenocarcinoma (CRC)	8.33%
3	Reproductive	Uterus endometrial adenocarcinoma	8.85%
4	Hepato-pancreatobiliary	Liver cholangiocarcinoma	9.56%
5	Thoracic	Lung squamous cell carcinoma	9.96%
6	Gastrointestinal	Colon adenocarcinoma (CRC)	12.01%
7	Urinary	Kidney urothelial carcinoma	15.50%
8	Gastrointestinal	Colon adenocarcinoma (CRC)	15.67%

3. Analytical Specificity

1. **Interference**

In an inference study for other biomarkers, eighteen (18) FFPE samples with MSS status were evaluated with four potentially interfering substances (hemoglobin, triglycerides, conjugated bilirubin, and unconjugated bilirubin) to evaluate whether these interfering substances impact the F1CDx MSI status calling. However, since MSI-H samples from various solid tumors were not included in this study, a post-market interfering substance study will be performed to evaluate the effects of endogenous interfering substances including necrotic tissue, melanin, and hemoglobin, and exogenous interference substances including molecular barcodes, proteinase K, and ethanol including MSI-H samples representing a range of solid tumors across the intended use population.

4. Precision (Within-Laboratory Precision)and Reproducibility (Site to Site Precision)

1. **Within-Laboratory Precision**

To evaluate the performance of MSI detection using the FB-MSI caller, a prospectively designed retrospective analysis was performed using samples from various tumor types. FFPE-derived DNA samples were selected from banked Foundation Medicine DNA samples. From the 67 samples originally tested in the prospective study, 66 were considered in the retrospective analysis. One (1) unknown primary melanoma sample and associated replicates were excluded from the retrospective analysis study as the result of high laboratory failure rates observed in the original prospective study due to contamination present in the source sample material. Therefore, in total 66 samples were considered in the retrospective analysis. These samples consisted of a set of seven (7) MSI-H samples, six (6) from CRC patients and one (1) from a patient with uterus endometrial adenocarcinoma and 59 non-

MSI-H samples from eight tumor types, ten (10) from patients with breast carcinoma, eight (8) from CRC patients, two (2) from patients with liver cholangiocarcinoma, eighteen (18) from patients with lung adenocarcinoma, four (4) from patients with lung non-small cell lung carcinoma, six (6) from patients with rectum adenocarcinoma, seven (7) patients with skin melanoma, three (3) with unknown primary melanoma, one (1) with thyroid carcinoma. Each sample was tested in two or three replicates in two (2) separate runs, with three (3) sequencers, and two (2) or three (3) reagent lots in a factorial design. A total of 36 replicates per sample were processed.

Of the 2,409 sample aliquots processed, 2,377 (98.7%) were successfully processed from LC to HC; 32 process failures were observed. Seven (7) failures were observed at LC QC step of the workflow, whereas additional 25 failures were observed at HC QC step. As noted above an unknown primary melanoma sample and associated replicates were excluded from retrospective analysis study due to high laboratory failure rates observed. Of the 2,344 replicates that underwent pipeline re-analysis to evaluate the precision of MSI calling, 2,273 (97.0%) passed post-sequencing MSI biomarker QC; 71 MSI biomarker QC failures were observed in replicates from five (5) source samples. Two (2) failures were observed due to low coverage; 68 were observed due to contamination, whereas an additional one was observed due to both low coverage and contamination. Out of all replicates from the 66 source samples (N=2373), a total of 100 replicates (4.2%) failed to meet F1CDx QC metrics at laboratory processing or post sequencing MSI biomarker QC. These replicates were removed from concordance data analysis. All 36 replicates of a skin melanoma sample failed to meet F1CDx QC metrics for MSI. Therefore, there were 65 evaluable samples for this study, 7 MSI-H samples and 58 non-MSI samples.

Data analysis was performed for each sample separately with replicates that passed QC, and yielded evaluable results. According to their MSI scores, samples were classified as MSI-H (≥ 0.0124) or non-MSI-H (<0.0124). An evaluation of within-laboratory (intermediate) precision for MSI status was performed by evaluation of within-laboratory precision and repeatability estimated as the percent agreement for each sample. The seven (7) MSI-H samples had MSI scores ranging from 0.0226 to 0.0682 and the 58 non-MSI-H samples had MSI scores ranging from 0.0043 to 0.0001. The agreement for within-laboratory precision and repeatability were 100% for the 65 evaluable samples. For 64 of 65 samples, the lower bound the two-sided 95% score CI ranged from 89.57% to 90.36% for within-laboratory precision and from 74.12% to 82.41% for repeatability. One sample with an average MSI score of 0.0009 had 7 evaluable replicates and agreement for within-laboratory precision (7/7) was 100%; the lower bound of the two-sided 95% score CI was 64.57%. For the same sample, the repeatability was 100%; the lower bound of the two-sided 95% score CI was 34.24% (see Table 14 for agreement results).

Table 14: MSI-H vs non- MSI-H Within-Laboratory (Intermediate) Precision results

#	Disease Ontology	MSI-H status	Mean MSI Score	Mean Tumor Purity	Within-laboratory Precision			Repeatability		
					Agree	Total	Agreement (95% 2-sided score CI)	Agree Pairs*	Total Pairs*	Agreement (95% 2-sided score CI)
1	Colon adenocarcinoma (CRC)	MSI-H	0.0682	30.19%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
2	Colon adenocarcinoma (CRC)	MSI-H	0.0588	46.63%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
3	Colon adenocarcinoma (CRC)	MSI-H	0.0579	30.97%	30	30	100.00% (88.65%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
4	Colon adenocarcinoma (CRC)	MSI-H	0.0458	17.40%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
5	Colon adenocarcinoma (CRC)	MSI-H	0.0428	31.38%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
6	Colon adenocarcinoma (CRC)	MSI-H	0.0328	38.85%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
7	Lung adenocarcinoma	non MSI-H	0.0033	42.40%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
8	Colon adenocarcinoma (CRC)	non MSI-H	0.0024	44.34%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
9	Breast invasive ductal carcinoma (IDC)	non MSI-H	0.0022	48.28%	34	34	100.00% (89.85%, 100.00%)	16	16	100.00% (80.64%, 100.00%)
10	Skin melanoma	non MSI-H	0.0021	86.71%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
11	Breast invasive ductal carcinoma (IDC)	non MSI-H	0.0019	51.85%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
12	Breast invasive ductal carcinoma (IDC)	non MSI-H	0.0019	66.53%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
13	Rectum adenocarcinoma (CRC)	non MSI-H	0.0018	13.94%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
14	Skin melanoma	non MSI-H	0.0016	37.70%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
15	Skin melanoma	non MSI-H	0.0016	81.63%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)

16	Lung adenocarcinoma	non MSI-H	0.0015	34.84%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
17	Skin melanoma	non MSI-H	0.0014	89.64%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
18	Breast carcinoma (NOS)	non MSI-H	0.0014	83.69%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
19	Breast invasive ductal carcinoma (IDC)	non MSI-H	0.0013	49.85%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
20	Lung adenocarcinoma	non MSI-H	0.0012	65.51%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
21	Lung adenocarcinoma	non MSI-H	0.0012	31.67%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
22	Breast invasive ductal carcinoma (IDC)	non MSI-H	0.0012	32.18%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
23	Rectum adenocarcinoma (CRC)	non MSI-H	0.0011	71.63%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
24	Rectum adenocarcinoma (CRC)	non MSI-H	0.0011	72.58%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)100.00%
25	Lung adenocarcinoma	non MSI-H	0.0010	23.98%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
26	Skin melanoma	non MSI-H	0.0010	60.81%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
27	Lung adenocarcinoma	non MSI-H	0.0009	46.94%	7	7	100.00% (64.57%, 100.00)	2	2	100.00% (34.24%, 100.00%)
28	Rectum adenocarcinoma (CRC)	non MSI-H	0.0009	33.06%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
29	Lung adenocarcinoma	non MSI-H	0.0008	33.21%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
30	Rectum adenocarcinoma (CRC)	non MSI-H	0.0008	58.89%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
31	Lung adenocarcinoma	non MSI-H	0.0008	27.87%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
32	Unknown primary melanoma	non MSI-H	0.0007	69.23%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
33	Breast invasive ductal carcinoma (IDC)	non MSI-H	0.0007	71.12%	34	34	100.00% (89.85%, 100.00%)	16	16	100.00% (80.64%, 100.00%)

34	Breast carcinoma (NOS)	non MSI-H	0.0006	45.30%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
35	Colon adenocarcinoma (CRC)	non MSI-H	0.0006	40.82%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
36	Lung squamous cell carcinoma (SCC)	non MSI-H	0.0005	59.92%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
37	Lung non-small cell lung carcinoma (NOS)	non MSI-H	0.0004	30.74%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
38	Breast invasive lobular carcinoma (ILC)	non MSI-H	0.0003	48.87%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
39	Colon adenocarcinoma (CRC)	non MSI-H	0.0003	33.46%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
40	Lung adenocarcinoma	non MSI-H	0.0003	26.45%	34	34	100.00% (90.36%, 100.00%)	16	16	100.00% (80.64%, 100.00%)
41	Colon adenocarcinoma (CRC)	non MSI-H	0.0003	10.14%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
42	Breast carcinoma (NOS)	non MSI-H	0.0003	36.56%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
43	Skin melanoma	non MSI-H	0.0002	23.83%	36	36	100.00% (90.36%, 100.00%)	18	18	100.00% (82.41%, 100.00%)
44	Lung squamous cell carcinoma (SCC)	non MSI-H	0.0002	57.30%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
45	Lung non-small cell lung carcinoma (NOS)	non MSI-H	0.0001	43.09%	35	35	100.00% (90.11%, 100.00%)	17	17	100.00% (81.57%, 100.00%)
46	Skin melanoma	non MSI-H	NA	NA	NA	NA	NA	NA	NA	NA
#	**Disease Ontology**	**MSI-H status**	**Mean MSI Score**	**Mean Tumor Purity**	**Within-laboratory Precision**			**Repeatability**		
					Pass	**Total**	**Agreement (95% 2-sided score CI)**	**Pass Triples***	**Total Triples ***	**Agreement (95% 2-sided score CI)**
47	Uterus endometrial adenocarcinoma endometrioid	MSI-H	0.0226	24.33%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
48	Colon adenocarcinoma (CRC)	non MSI-H	0.0043	11.71%	35	35	100.00% (90.11%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
49	Liver cholangiocarcinoma	non MSI-H	0.0031	14.60%	33	33	100.00%' (89.57%, 100.00%)	11	11	100.00% (74.12%, 100.00%)

50	Salivary gland carcinoma (NOS)	non MSI-H	0.0020	17.39%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
51	Lung adenocarcinoma	non MSI-H	0.0019	10.59%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
52	Lung adenocarcinoma	non MSI-H	0.0017	24.04%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
53	Thyroid carcinoma (NOS)	non MSI-H	0.0016	37.99%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
54	Lung adenocarcinoma	non MSI-H	0.0016	41.54%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
55	Liver cholangiocarcinoma	non MSI-H	0.0014	21.52%	35	35	100.00% (90.11%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
56	Unknown primary melanoma	non MSI-H	0.0010	13.77%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
57	Lung adenocarcinoma	non MSI-H	0.0010	16.16%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
58	Lung adenocarcinoma	non MSI-H	0.0008	16.94%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
59	Lung adenocarcinoma	non MSI-H	0.0008	11.59%	35	35	100.00% (90.11%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
60	Colon adenocarcinoma (CRC)	non MSI-H	0.0007	36.91%	35	35	100.00% (90.11%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
61	Lung adenocarcinoma	non MSI-H	0.0007	16.72%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
62	Colon adenocarcinoma (CRC)	non MSI-H	0.0007	13.56%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
63	Colon adenocarcinoma (CRC)	non MSI-H	0.0005	41.58%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
64	Lung adenocarcinoma	non MSI-H	0.0004	14.64%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
65	Lung adenocarcinoma	non MSI-H	0.0004	15.09%	35	35	100.00% (90.11%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
66	Unknown primary melanoma	non MSI-H	0.0004	10.82%	36	36	100.00% (90.36%, 100.00%)	12	12	100.00% (75.75%, 100.00%)

*Agree Pass Pairs/Triples: number of replicate pairs/triples matching target MSI status for repeatability assessment. Total Pairs/Triples: total number of replicate pairs/triples available for repeatability assessment.

A second precision study was conducted to evaluate within-run precision (repeatability) and within-laboratory precision for MSI calling by the F1CDx assay in tumors derived from 7 major organ systems (i.e., gastrointestinal, hepato-pancreatobiliary, urinary, endocrine, skin, thoracic, and reproductive) to support pan-tumor testing. This study was comprised of 44 MSI-H, and 2 non-MSI samples (see Table 15). FFPE-derived DNA samples were selected from banked Foundation Medicine DNA samples. The study examined reagent lots and instruments as factors in the within-laboratory precision study by assessing samples with two (2) replicates in each of two (2) separate runs, using two (2) reagent lots, and three (3) sequencers.

In total, twelve (12) plates, composed of 46 unique FFPE DNA sample aliquots and two (2) HapMap Controls, were processed for a total of 1,104 sample replicates. Of the 1,104 sample aliquots processed, 1,072 (97.1%) were successfully processed from LC to HC; 32 process failures were observed. Ten (10) failures were observed at LC QC, whereas an additional 22 were observed at HCQC. Of note, 20 out of 24 replicates for a lung adenocarcinoma sample failed to meet LC QC and HC QC specifications, suggesting that the DNA quality for the sample stock DNA may have been poor. Only four (4) replicates were sequenced, and each resulted in a qualified sequence result that did not meet specification for median coverage (i.e., ≥250 observed median depth of coverage). These four (4) samples resulted in a qualified status. Only two (2) replicates, out of a total of 1072 replicates, were removed due to the FB-MSI QC filter. One (1) replicate from a uterus endometrial adenocarcinoma endometrioid sample was removed due to high contamination, and one (1) replicate from a lung adenocarcinoma samples was removed as no FB-MSI score was able to be computed due to insufficient coverage.

According to their MSI scores, samples were classified as MSI-H (≥ 0.0124) or non-MSI-H (<0.0124). Of the 46 samples, 42 samples had agreement for within-laboratory precision ranging from 90.48% to 100%, with the lower bound the two-sided 95% score CI ranging from 71.09% to 86.20%. For 4 samples with MSI scores close to the 0.0124 cut-off, ranging from 0.0116 to 0.0133, agreement for within-laboratory precision ranged from 56.52% to 82.61%, with the lower bound of the two-sided 95% score CI ranging from 36.81% to 62.86%. Of the 46 samples, 39 samples had agreement for repeatability ranging from 91.67% to 100%, with the lower bound of the two-sided 95% score CI ranging from 64.61% to 74.74%. For six (6) samples, agreement for repeatability ranged from 54.55% to 83.83%, with the lower bound the two-sided 95% score CI ranging from 28.01% to 55.20%. The observed MSI scores of these six samples were close to the cut-off of 0.0124, ranged from 0.0116 to 0.0154, thus explaining the disagreement of MSI status results near the threshold. The repeatability value of the 7th sample could not be calculated because 21 of 24 replicates were removed from the analysis due to failures in F1CDx laboratory process QC or MSI QC rules.

Table 15: MSI-H vs non- MSI-H Within-Laboratory (Intermediate) Precision Results

					Within-Laboratory Precision			Repeatability		
#	Disease Ontology	MSI-H Status	Mean MSI score	Mean Tumor Purity	Agree	Total	Agreement (95% 2-sided score CI)	Agree Pairs*	Total Pairs	Agreement (95% 2-sided score CI)
1	Pancreatobiliary carcinoma (NOS)	MSI-H	0.0255	21.44%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
2	Breast carcinoma (NOS)	MSI-H	0.0189	59.49%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
3	Lung squamous cell carcinoma (SCC)	MSI-H	0.0345	34.20%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
4	Lung non-small cell lung carcinoma (NOS)	MSI-H	0.0298	51.72%	23	23	100.00% (85.69%, 100.00%)	11	11	100% (74.12%, 100.00%)
5	Uterus endometrial adenocarcinoma endometrioid	MSI-H	0.0173	54.99%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
6	Lung squamous cell carcinoma (SCC)	MSI-H	0.0220	25.18%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
7	Colon adenocarcinoma (CRC)	MSI-H	0.0359	34.40%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
8	Unknown primary undifferentiated small cell carcinoma	MSI-H	0.0182	96.01%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
9	Bladder urothelial (transitional cell) carcinoma	MSI-H	0.0272	44.38%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
10	Uterus endometrial adenocarcinoma endometrioid	MSI-H	0.0174	68.09%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
11	Unknown primary undifferentiated neuroendocrine carcinoma	MSI-H	0.0128	62.87%	13	23	56.52% (36.81%, 74.37%)	6	11	54.55% (28.01%, 78.73%)
12	Pancreatobiliary carcinoma (NOS)	MSI-H	0.0154	36.04%	19	21	90.48% (71.09%, 97.35%)	7	9	77.78% (45.26%, 93.68%)

13	Prostate acinar adenocarcinoma	MSI-H	0.0345	23.92%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
14	Uterus endometrial adenocarcinoma endometrioid	MSI-H	0.0133	16.87%	19	23	82.61% (62.86%, 93.02%)	7	11	63.64% (35.38%, 84.83%)
15	Liver cholangiocarcinoma	MSI-H	0.0184	58.87%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
16	Skin adnexal carcinoma	MSI-H	0.0507	71.93%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
17	Uterus neuroendocrine carcinoma	MSI-H	0.0364	37.50%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
18	Kidney urothelial carcinoma	MSI-H	0.0256	31.88%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
19	Small intestine neuroendocrine carcinoma	MSI-H	0.0585	37.91%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
20	Ampullary adenocarcinoma	MSI-H	0.0448	27.30%	24	24	100% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
21	Ureter urothelial carcinoma	MSI-H	0.0283	56.19%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
22	Skin adnexal carcinoma	MSI-H	0.0332	90.35%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
23	Lung squamous cell carcinoma (SCC)	MSI-H	0.0245	97.40%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
24	Breast carcinoma (NOS)	MSI-H	0.0154	54.20%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
25	Liver cholangiocarcinoma	MSI-H	0.0177	67.24%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
26	Ampullary adenocarcinoma	MSI-H	0.0217	21.53%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
27	Colon adenocarcinoma (CRC)	MSI-H	0.0250	17.19%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)

28	Ureter urothelial carcinoma	MSI-H	0.0173	34.56%	23	24	95.83% (79.76%, 99.26%)	11	12	91.67% (64.61%, 98.51%)
29	Bladder urothelial (transitional cell) carcinoma	MSI-H	0.0130	79.36%	19	24	79.17% (59.53%, 90.76%)	9	12	75.00% (46.77%, 91.11%)
30	Anus squamous cell carcinoma	MSI-H	0.0245	33.27%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
31	Unknown primary undifferentiated neuroendocrine carcinoma	MSI-H	0.0281	64.24%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
32	Skin squamous cell carcinoma (SCC)	MSI-H	0.0140	79.60%	22	24	91.67% (74.15%, 97.68%)	10	12	83.33% (55.20%, 95.30%)
33	Stomach adenocarcinoma (NOS)	MSI-H	0.0232	23.30%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
34	Liver cholangiocarcinoma	MSI-H	0.0152	63.53%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
35	Lung non-small cell lung carcinoma (NOS)	MSI-H	0.0140	94.54%	23	24	95.83% (79.76%, 99.26%)	11	12	91.67% (64.61%, 98.51%)
36	Unknown primary undifferentiated neuroendocrine carcinoma	MSI-H	0.0258	12.21%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
37	Prostate acinar adenocarcinoma	MSI-H	0.0207	25.61%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
38	Prostate acinar adenocarcinoma	MSI-H	0.0171	65.04%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
39	Ureter urothelial carcinoma	MSI-H	0.0222	22.79%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
40	Lung non-small cell lung carcinoma (NOS)	MSI-H	0.0154	34.43%	24	24	100.00% (86.20%, 100.00%)	12	12	100% (75.75%, 100.00%)
41	Skin squamous cell carcinoma (SCC)	MSI-H	0.0307	69.78%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)

42	Small intestine adenocarcinoma	MSI-H	0.0269	16.07%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
43	Ovary clear cell carcinoma	MSI-H	0.0196	11.06%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
44	Liver cholangiocarcinoma	MSI-H	0.0210	43.69%	24	24	100.00% (86.20%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
45	Lung adenocarcinoma	non MSI-H	0.0082	65.03%	3	3	100.00% (43.85%, 100.00%)	0	0	NA
46	Uterus neuroendocrine carcinoma	non MSI-H	0.0116	96.98%	17	24	70.83% (50.83%, 85.09%)	9	12	75.00% (46.77%, 91.11%)

*Agree Pairs: number of replicate pairs matching target MSI status for repeatability assessment. Total Pairs: total number of replicate pairs available for repeatability assessment.

To provide for a more robust assessment of the impact of sequencing reagent lots on the within-laboratory (intermediate) precision of MSI calling by F1CDx, a supplemental evaluation was conducted using the 44 MSI-H samples and 2 non-MSI-H sample described in the second intermediate precision study above representing 7 major organ systems with a total of three (3) sequencing reagent lots. MSI within-laboratory precision and repeatability were assessed by testing each of the 46 samples with two (2) replicates, per each of two (2) separate runs (plates), using three (3) sequencing reagent lots, and three (3) HiSeq 4000 sequencers using a full factorial design with a total of 36 sample replicates across the paired reagent lot/sequencer combination.

Since data from the previous study were used to support the precision assessment by comparing three (3) lots of reagents, processing failures noted in previous study were integrated into this validation study. Samples with less than 36 replicates could be evaluated, however, any sample that resulted in sequencing data for ≤12 evaluable replicates (e.g., wherein ≥24 replicates failed during processing) was not evaluated for repeatability or within-laboratory precision. In total 1656 sample replicates were evaluated in the study. Of the 1656 sample replicates, 1518 replicates were successfully sequenced, and provided F1CDx evaluable results. Among the 46 samples, one lung adenocarcinoma sample was excluded from the analysis as all sample replicates failed LC or HC QC leaving no evaluable replicates for evaluation. Therefore, 45 of 46 samples provided F1CDx evaluable replicates that were considered in the data analysis.

According to their MSI scores, samples were classified as MSI-H (≥ 0.0124) or non-MSI-H (< 0.0124). Of the 45 samples, 41 samples had agreement for reproducibility ranging from 91.18% to 100%, with the lower bound the two-sided 95% score CI ranging from 77.04% to 90.36%. For 4 samples with MSI

scores close to the 0.0124 cut-off, ranging from 0.011535 to 0.013764, agreement for reproducibility ranged from 61.29% to 88.89%, with the lower bound of the two-sided 95% score CI ranging from 43.82% to 74.69%. Of the 45 samples, 39 samples had agreement for repeatability ranging from 92.86% to 100%, with the lower bound of the two-sided 95% score CI ranging from 68.53% to 80.64%. For 6 samples, agreement for repeatability ranged from 61.54% to 81.25%, with the lower bound the two-sided 95% score CI ranging from 35.52% to 56.99%. The observed MSI scores of these six samples were close to the cut-off of 0.0124, ranging from 0.011535 to 0.015729, thus explaining the disagreement of MSI status results near the threshold.

Together the results of these studies demonstrate that while the within-laboratory precision is high for samples with MSI scores well above and below the FB-MSI score threshold 0.0124 which was applied to classify a tumor specimen as having MSI-H or non-MSI-H status, there may be imprecision as shown by within-laboratory and repeatability results for samples near the threshold for MSI-H calling.

2. **Reproducibility (Site-to-Site Precision)**
A reproducibility (site-to-site precision) study was conducted to assess the F1CDx performance at the two locations where F1CDx is run, Cambridge, MA and Morrisville, NC.

The site-to-site precision of the F1CDx assay was assessed by testing 46 samples: 12 NSCLC, 4 melanoma, 6 breast cancer, 8 CRC, 6 ovarian cancer and 10 unknown samples. Seven (7) samples had MSI-H status, while 38 samples had non-MSI-H status. DNA derived from FFPE samples was selected for this evaluation from a collection of banked Foundation Medicine DNA samples or procured FFPE tumor tissue samples.

The 46 samples were tested with two replicates per run, using two sites, two reagent lots, and three non-consecutive days by multiple operators. A full factorial design for this study was carried out with a total of 24 replicates across the paired reagent lot/sequencer combination

In total, the 46 unique FFPE DNA samples tested in 24 replicates each were processed for a total of 1,104 sample replicates. Fifteen (15) replicates out of 1,104 failed either LC or HC, leaving 1,089 replicates passing F1CDx sample processing. There were an additional 19 failures due to low coverage (general post-sequencing QC and MSI QC), leaving 1,070 valid replicates considered in the precision analysis.

For the evaluation of the FB-MSI caller, the reproducibility and repeatability results for all 46 samples are presented in Table 17. Reproducibility was calculated with the missing replicates excluded from reproducibility

calculations. For repeatability, if one or more members of a pair was missing, the entire pair was removed from repeatability calculations.
In total, 45 of 46 samples exhibited 100% repeatability and 100% reproducibility. One sample demonstrated 62.50% (15/24) reproducibility and 58.33% (7/12) repeatability. The MSI scores for the replicates of the source sample were close to the MSI-H threshold (≥ 0.0124), with an observed mean value of 0.0127. A small standard deviation (0.0015) and %CV (11.76%) across replicates support precise calling, with the low reproducibility attributed to the proximity of the scores to the MSI-H threshold.

Repeatability and reproducibility of MSI calling 100% agreement was observed for 45 samples for MSI status. One sample had a repeatability agreement of 58.33%. The MSI scores for replicates of this sample, were at the threshold for MSI- H calling (mean MSI score was 0.0127).

Table 16: MSI-H vs non- MSI-H Site to Site Results

					Reproducibility			Repeatability		
#	MSI-H Status	Tumor Type	Mean MSI score	Mean Tumor Purity	Agree	Total	Agreement (95% 2-sided score CI)	Agree Pairs	Total Pairs	Agreement (95% 2-sided score CI)
1	MSI-H	Unknown	0.0372	64.03%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
2	MSI-H	Ovarian cancer	0.0497	34.68%	22	22	100.00% (85.13%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
3	MSI-H	NSCLC	0.0127	39.46%	15	24	62.50% (42.71%, 78.84%)	7	12	58.33% (31.95%, 80.67%)
4	MSI-H	CRC	0.0457	47.41%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
5	MSI-H	CRC	0.0243	15.04%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
6	MSI-H	Unknown	0.0423	13.15%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
7	MSI-H	Unknown	0.0312	16.87%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
8	non MSI-H	Breast cancer	0.0007	29.64%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
9	non MSI-H	NSCLC	0.0013	18.87%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
10	non MSI-H	Breast cancer	0.0012	14.93%	24	24	100.00% (86.2%,	12	12	100.00%

							100.00%)			(75.75%, 100.00%)
11	non MSI-H	NSCLC	0.0003	17.77%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
12	non MSI-H	NSCLC	0.0029	11.68%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
13	non MSI-H	Ovarian cancer	0.0011	12.55%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
14	non MSI-H	Unknown	0.0004	43.26%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
15	non MSI-H	CRC	0.0014	19.44%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
16	non MSI-H	Breast cancer	0.0024	35.83%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
17	non MSI-H	CRC	0.0007	42.02%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
18	non MSI-H	Unknown	0.0008	30.78%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
19	non MSI-H	NSCLC	0.0007	11.96%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
20	non MSI-H	Melanoma	0.0010	9.95%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
21	non MSI-H	CRC	0.0002	15.74%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
22	non MSI-H	NSCLC	0.0007	27.37%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
23	non MSI-H	Breast cancer	0.0005	16.80%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
24	non MSI-H	NSCLC	0.0006	20.52%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
25	non MSI-H	CRC	0.0018	42.05%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
26	non MSI-H	Melanoma	0.0008	13.69%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
27	non MSI-H	Unknown	0.0009	35.80%	8	8	100.00% (67.56%, 100.00%)	3	3	100.00% (43.85%, 100.00%)

28	non MSI-H	Unknown	0.0009	81.74%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
29	non MSI-H	Ovarian cancer	0.0003	15.61%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
30	non MSI-H	Unknown	0.0006	30.58%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
31	non MSI-H	Breast cancer	0.0007	21.66%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
32	non MSI-H	CRC	0.0005	18.76%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
33	non MSI-H	Unknown	0.0009	23.09%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
34	non MSI-H	CRC	0.0017	13.73%	21	21	100.00% (84.54%, 100.00%)	9	9	100.00% (70.09%, 100.00%)
35	non MSI-H	Breast cancer	0.0010	50.81%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
36	non MSI-H	NSCLC	0.0028	20.30%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
37	non MSI-H	Unknown	0.0006	18.95%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
38	non MSI-H	Ovarian cancer	0.0008	32.56%	18	18	100.00% (82.41%, 100.00%)	7	7	100.00% (64.57%, 100.00%)
39	non MSI-H	NSCLC	0.0001	71.68%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
40	non MSI-H	Ovarian cancer	0.0007	71.00%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
41	non MSI-H	NSCLC	0.0005	20.99%	23	23	100.00% (85.69%, 100.00%)	11	11	100.00% (74.12%, 100.00%)
42	non MSI-H	NSCLC	0.0001	43.32%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
43	non MSI-H	Ovarian cancer	0.0011	20.00%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
44	non MSI-H	Melanoma	0.0007	11.04%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)
45	non MSI-H	Melanoma	0.0014	10.40%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)

46	non MSI-H	NSCLC	0.0006	53.58%	24	24	100.00% (86.2%, 100.00%)	12	12	100.00% (75.75%, 100.00%)

*Agree Pairs: number of replicate pairs matching target MSI status for repeatability assessment. Total Pairs: total number of replicate pairs available for repeatability assessment.

Together, the results of this study demonstrate that reproducibility (site-to site precision) is high for samples that have MSI scores well above or below the MSI-H threshold (≥0.0124), there is imprecision as shown by reproducibility and repeatability results near the threshold for MSI-H calling. As noted earlier, patients with FB-MSI scores >0.0041 and <0.0124, or MSI "Cannot be Determined" result should consider re-testing with FoundationOneCDx or an orthogonal (alternative) method, if clinically appropriate.

5. Stability

Please refer to the Summary of Safety and Effectiveness Data P170019 [Section IX.A.7(a,b)] for F1CDx platform validation of reagent, DNA, and FFPE slide stability. A post-market study will be conducted in the post-mark setting to support robust MSI calling within the F1CDx stability claims for FFPE slide stability.

B. Animal Studies

No animal studies were conducted using the F1CDx assay.

C. Additional Studies

No additional studies were conducted using the F1CDx assay.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The reasonable assurance of safety and effectiveness for F1CDx for detection of MSI-H status in patients with solid tumors who may benefit from treatment with KEYTRUDA® (pembrolizumab), was established through a clinical device bridging study using tumor tissue FFPE specimens from patients enrolled in the Merck clinical studies KEYNOTE-158 and KEYNOTE-164, and an additional set of commercially procured (tumor bank) specimens evaluated as a putative, supplementary source of specimens from the biomarker negative population to estimate negative percent agreement. A summary of the clinical study is presented below.

A. Study Design

The clinical effectiveness of F1CDx for detecting MSI-H status in patients with solid tumors who may benefit from treatment with KEYTRUDA was demonstrated in a retrospective analysis of tumor tissue FFPE specimens from patients enrolled in studies KEYNOTE-158, KEYNOTE-164 and an additional set of tumor tissue FFPE specimens commercially procured (tumor bank). A bridging study was conducted to assess: 1) concordance between the local clinical trial assays (CTAs) and the F1CDx for the MSI test outcomes; and 2) estimate the overall response rate (ORR) in the efficacy population (CTA-positive population) for KEYTRUDA treatment among

clinical study participants whose archived solid tumor samples were MSI-H as determined by retrospective testing with the F1CDx.

Below is a summary of the clinical studies KEYNOTE 158 and KEYNOTE 164.

Therapeutic Clinical Study KEYNOTE 158

KEYNOTE-158 is an ongoing multicenter, global, open-label trial of KEYTRUDA in participants with multiple types of advanced (unresectable and/or metastatic) cancers who have failed prior therapy. All participants enrolled in this study had a histologically or cytologically documented, advanced solid tumor that was incurable and for which prior standard first-line treatment had failed. Participants had progressed on or were intolerant to therapies that are known to provide clinical benefit. All participants received pembrolizumab 200 mg every 3 weeks (Q3W).

Participants with any of 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, i.e., irrespective of their biomarker status.

- Cohort K: participants with any advanced solid tumor (except CRC) that is MSI- H/dMMR.

Participants were eligible to enroll if they had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, life expectancy of >3 months, and previous treatment with standard therapies; had no known CNS metastases, autoimmune disease, immunosuppressive therapy, or prior treatment with anticancer monoclonal antibody (mAb) within 4 weeks prior to trial Day 1; and had adequate tumor tissue to test for PD-L1, gene expression profiling (GEP), and MSI-H.

A primary objective of the study was to evaluate the ORR based on response evaluation criteria in solid tumors (RECIST) 1.1 as assessed by independent central review (ICR) to KEYTRUDA in Cohort A-K participants with MSI-H tumors. ORR was defined as the proportion of participants in the analysis population who had a confirmed response, complete response (CR) or partial response (PR), as measured by ICR per RECIST 1.1. Additionally, secondary objectives included assessment of duration of response (DOR), progression free survival (PFS) and overall survival (OS) in pembrolizumab-treated Cohort A-K participants with MSI-H tumors. DOR was defined as time from the first documented evidence of CR or PR until disease progression or death due to any cause, whichever occurred first. PFS was defined as time from allocation/the first dose of study treatment to documented progressive

disease or death due to any cause, whichever occurred first; and OS was defined as time from allocation/the first dose of study treatment to death due to any cause.

Local testing was used to determine MSI/MMR tumor status of Cohort K participants to assess eligibility for the study.

Study enrollment is complete (last participant enrolled October 2020) for KEYNOTE- 158 Cohorts A-K. A total of 1066 participants were enrolled and treated with at least 1 dose of pembrolizumab in the combined KEYNOTE-158 Cohorts A-J. A total of 355 participants were enrolled and treated with at least 1 dose of pembrolizumab in KEYNOTE-158 Cohort K. Of these, 351 participants were enrolled before the data cut off (DCO) date of October 5, 2020. Further, 321 participants had at least 6 months of follow-up by the DCO date and were considered for clinical efficacy analyses.

Therapeutic Clinical Study KEYNOTE-164

KEYNOTE-164 is a single-arm, multisite, multicohort, Phase 2 study designed to evaluate the efficacy of pembrolizumab in participants with locally advanced unresectable or metastatic (Stage IV) MSI-H/dMMR CRC. Participants who met eligibility criteria were enrolled into one of 2 cohorts:

- Cohort A: participants with locally advanced unresectable or metastatic MSI-H/dMMR CRC who had been previously treated with at least 2 lines of standard therapies, which included fluoropyrimidine, oxaliplatin, and irinotecan.

- Cohort B: participants with locally advanced unresectable or metastatic MSI-H/dMMR CRC who had been previously treated with at least 1 line of systemic SOC therapy (fluoropyrimidine + oxaliplatin or fluoropyrimidine + irinotecan +/- anti-VEGF/EGFR mAb)

All participants received pembrolizumab 200 mg Q3W. Participants were eligible to enroll if they had an ECOG PS of 0 or 1, life expectancy of >3 months and had histologically proven locally advanced unresectable or metastatic (Stage IV) CRC. Tumor response assessments were performed every 9 weeks. Participants continued to be treated with pembrolizumab until progressive disease (PD), unacceptable adverse events (AEs), or the participant had received 35 trial treatments (approximately 2 years) with pembrolizumab or met other criteria for discontinuation of trial intervention as specified in the trial protocol. All participants were followed up for OS. AEs were monitored throughout the trial and graded in severity according to the guidelines outlined in the NCI CTCAE Version 4.0.

The primary objective of the study was to evaluate the ORR per RECIST 1.1 assessed by central imaging of pembrolizumab. Additionally, secondary objectives included assessment of DOR, Disease Control Rate (DCR), and PFS per RECIST 1.1 assessed

by central imaging, and OS in pembrolizumab-treated participants. Definitions of these endpoints are provided above.

Local testing was used to determine MSI/MMR tumor status of participants to assess eligibility for the study.

Study enrollment is complete (last participant enrolled September 2016). In KEYNOTE-164, a total of 124 participants were treated with pembrolizumab, including 61 in Cohort A and 63 in Cohort B. One patient out of 124 participants treated with pembroizumab was not MSI-H/dMMR, therefore 123 were considered in the clinical bridging, see below. The data cut-off date for the clinical efficacy analyses for KEYNOTE 164 was September 9, 2019.

Clinical Bridging Study Design

The bridging study is based on Merck clinical studies KEYNOTE-158 and KEYNOTE-164 and tumor bank samples. As noted above, KEYNOTE-158 Cohorts A-J enrolled patients with specific tumors irrespective of their biomarker (e.g., MSI/MMR) status, while Cohort K enrolled patients with non-CRC solid tumors that were MSI-H/dMMR by local testing. KEYNOTE-164 enrolled patients with CRC that were MSI-H/dMMR by local testing. KEYNOTE-158 Cohort K enrolled patients with MSI-H/dMMR non-CRC, and KEYNOTE-164 enrolled patients with MSI-H/dMMR CRC, to inform the intended use of the F1CDx as a CDx in identifying patients with MSI-H solid tumors for treatment with pembrolizumab. It should be noted that data presented in this summary of safety and effectiveness data is not fully aligned with the Post Marketing Requirement (PMR #3213-1) issued at the time of BLA-125514/S014 approval and therefore the latest drug United States Prescribing Information (USPI) should be referred to for more information.

Eligible patients from KEYNOTE-158 Cohort K and KEYNOTE-164, enrolled using local testing, are collectively addressed as "CTA- positive" in the bridging study. At the time of KEYNOTE-158 Cohort K initiation, there was no requirement for tumor sample submission for enrolled patients, and hence, there was incomplete and non-sequential sample availability for F1CDx MSI testing. However, tumor sample submission was made a requirement and included as an eligibility criterion in Amendment-7 of the protocol. KEYNOTE-164 was initiated in August 2015 and completed enrollment in September 2016. The KEYNOTE-164 study protocol did not mandate collection of tumor samples from the enrolled patients and as a result, there was incomplete sample availability for F1CDx MSI testing.

While clinical outcome data (e.g., tumor objective response) from KEYNOTE-158 Cohorts A-J was not used for primary efficacy analyses, MSI/MMR tumor status from a subset of these patients was used for concordance analyses.

KEYNOTE-158 Cohort K and KEYNOTE-164 utilized local MSI/MMR testing for screening and enrolled only patients who were MSI-H/dMMR by these tests, and such an enrolled population is distinct from the F1CDx MSI-H/dMMR population.

For clarity, tumors that are not MSI-H or dMMR (not MSI-H/dMMR) by local testing could be identified as MSI-H by the F1CDx; however, such cases were not enrolled into KEYNOTE-158 Cohort K and KEYNOTE-164 and hence, pembrolizumab response data is not available for these cases. In addition, some local testing MSI-H/dMMR tumors can be identified as not MSI-H or dMMR by F1CDx. Statistical bridging methodology was be used to conduct analyses to understand efficacy for F1CDx positive patients in the intent to diagnose (ITD) population and utilizes information on the PPA, NPA, and clinical efficacy data on CTA MSI-H/dMMR patients in the key statistical analyses conducted.

Testing of samples from enrolled patients with the F1CDx allowed for an assessment of the PPA between the local MSI/MMR and F1CDx tests. An assessment of the NPA between the local MSI/MMR tests and the F1CDx was also needed. Testing patients with negative local screening MSI-H/dMMR results from KEYNOTE-158 Cohort K and KEYNOTE-164 with F1CDx to assess the NPA was not possible as tumor samples from patients who were CTA-negative at the time of study screening were not available for testing with the F1CDx. To support the NPA analysis of CDx vs CTA, a simulated local-testing negative population, referred to henceforth as "CTA-negative", was assembled to evaluate the NPA. This CTA-negative population includes patients from KEYNOTE-158 Cohorts A-J, as well as tumor bank samples. Further details for the CTA-negative population used in the sensitivity and concordance analyses are included below.

1. Clinical Inclusion and Exclusion Criteria

The inclusion/exclusion criteria for selection into the clinical bridging study are

Sample Inclusion Criteria

- Samples must be FFPE blocks or slides.
- Samples that meet F1CDx processing requirements.
- Samples obtained following HIPAA guideline with patients' consent in compliance with 21 CFR Part 50 "Protection of Human Subjects" and 21 CFR Part 56 "Institutional Review Boards"

Exclusion Criteria

- Lack of clear identification or label on stored patient sample.
- Physical damage of stored patient sample.
- Samples that do not meet F1CDx processing requirements
- Samples not obtained in accordance with 21 CFR Part 50 "Protection of Human Subjects" and 21 CFR Part 56 "Institutional Review Boards.

2. Follow-up Schedule

The F1CDx clinical bridging study involved only retrospective testing of tissue tumor FFPE samples; as such, no additional patient follow-up was conducted.

3. Clinical Endpoints

The objectives of the F1CDx clinical study were to:

1. To estimate agreement between the CTA and the F1CDx for the MSI test outcomes.

2. To estimate the ORR for KEYTRUDA treatment among clinical study patients whose archived solid tumor specimens are MSI-H as determined by retrospective testing with the CDx. Primary analysis was based on patients with complete CDx status. Sensitivity analysis included subjects with and without CDx results and evaluated the impact on clinical efficacy for the proportion of subjects who are local CTA negative but CDx positive (MSI-H/dMMR) and therefore not enrolled by the clinical trial. The missing CDx results were imputed in the sensitivity analysis.

B. Accountability of PMA Cohort

Overall, 1664 tumor samples were tested by the F1CDx assay and 1189 were F1CDx-evaluable. Sample accounting by study is shown in Table 17. Only treated patients in the clinical studies (KEYNOTE-158 and KEYNOTE-164) are included in the table. A total of 3 untreated (screen failure) patient's tumor samples were sent for F1CDx testing, and thus are not included in the table.

Of the 1664 samples tested by F1CDx, 28.5% failed to yield F1CDx evaluable results. Of the 28.5% failed samples, 7.94% failed to meet MSI QC. Of the remaining failed samples, 11.7% failed pathology review (i.e., failed to meet required viable nucleated tissue volume and/or tumor nuclei), 4.3% failed to meet required DNA input for tests, 0.5% failed to meet library (LC) QC, 1.4% failed to meet required hybrid capture QC, and 2.6% failed to meet genomic analysis QC.

Table 17: Sample Accounting

STUDY	N	F1CDx Tested	F1CDx-Evaluable	F1CDx-Positive	F1CDx-Negative
All	2343	1661	1186	141	1045
KEYNOTE 158 COHORT K Pre-Amendment 7†	193	79	47	27	20
KEYNOTE 158 Cohort K Amendment 7 Onwards†	128	89	57	32	25
KEYNOTE 158 Cohort A to J *‡	1066	1004	714	23	691
KN164 †**,***	123	80	61	48	13
Tumor Bank‡	833	409	307	11	296

† Samples from KN158 Cohort K and KN164 participants that were not tested by F1CDx were not available to be sent to FMI.
* Two untreated subjects are not included: both are F1CDx-negative. One is CTA-negative and the other is missing CTA status.
** One untreated subject is not included: F1CDx-positive, CTA status is not available.

*** One CTA-negative treated subject is not included (this subject's tumor sample was not tested by F1CDx).
‡ Samples used to assemble the CTA-negative population.

Patients treated with KEYTRUDA as part of KEYNOTE-158 Cohort K Amendment 7 onwards and through March 2020 (n=128) and participants enrolled prior to Amendment 7 of the protocol (n=193) are considered part of the CTA-positive efficacy population. From KEYNOTE-164, all enrolled and KEYTRUDA treated patients (n=123) were included in the CTA-positive efficacy population for the bridging study. In total, the efficacy analysis considered 444 patients, including 321 patients enrolled in KEYNOTE-158 Cohort K and 123 patients enrolled in KEYNOTE-164.

Of the 444 patients, 248 patients were tested with F1CDx, and 165 had F1CDx valid or evaluable results. The efficacy analysis was conducted for the 165 patients with F1CDx evaluable results. Sensitivity analyses with regard to missing values were conducted to evaluate the impact on clinical efficacy for the 279 patients without F1CDx results as well as the clinical efficacy of F1CDx in the F1CDx intended use population including both clinical trials evaluated CTA positive, F1CDx positive population and the unenrolled CTA negative, F1CDx positive population.

Tumor samples from 196 of the 444 patients, (153 KEYNOTE-158 Cohort K and 43 KEYNOTE-164) were not available to be tested by F1CDx. Of the 248 samples tested with F1CDx, 83 (64 KEYNOTE-158 Cohort K and 19 KEYNOTE-164 samples) had invalid or non-evaluable F1CDx MSI tumor status.

In addition, 1006 samples from KEYNOTE-158 Cohorts A-J participants (patients in rare tumor non-CRC cohorts who had failed prior therapy, 2 of which were not treated with KEYTRUDA) and 409 tumor bank samples were tested with F1CDx and utilized to determine the NPA of the F1CDx vs. CTA. Among the 1413 samples from KEYNOTE-158 Cohorts A-J patients and from the tumor bank combined, 1023 had evaluable F1CDx results (716 from KEYNOTE-158 Cohorts A-J and 307 from the tumor bank). Together with samples from 166 patients in KEYNOTE-158 Cohort K (104) and KEYNOTE-164 patients (62, one which was not treated with KEYTRUDA), with evaluable F1CDx MSI tumor results were used to determine PPA and NPA in the concordance evaluation.

C. Study Population Demographics and Baseline Parameters

For the clinical device bridging study, baseline characteristics were compared between the CTA positive, F1CDx evaluable and F1CDx non-evaluable populations. The extended CTA-positive population consists of 444 patients, 321 from KEYNOTE-158 Cohort K and 123 from KEYNOTE-164. Among these 444 patients, the baseline characteristics were: median age of 59 years, 36% ≥ 65 years of age; 46% male; 78% White, 13% Asian, and 4% Black; and 44% had an ECOG PS of 0 and 56% had an ECOG PS of 1. Ninety-three percent (93%) of patients had metastatic disease. Sixty-two percent (62%) of patients received 2 or more prior lines of therapy (see Table 18 below).

Table 18: Patient Characteristics in CTA-Positive Population, and by F1CDx Evaluability in CTA-Positive Population (KEYNOTE-158 Cohort K and KEYNOTE-164)

	CTA-positive Population		F1CDx Evaluable		F1CDx Missing	
	n	(%)	n	(%)	n	(%)
Patients in population	444		165		279	
Sex						
Male	204	45.9	81	49.1	123	44.1
Female	240	54.1	84	50.9	156	55.9
Age (Years)						
< 65	284	64	105	63.6	179	64.2
>= 65	160	36	60	36.4	100	35.8
Mean	58.1		58.6		57.9	
SD	13.8		13.6		14	
Median	59		58		60	
Range	20 to 89		23 to 89		20 to 86	
Race						
American Indian Or Alaska Native	16	3.6	6	3.6	10	3.6
Asian	59	13.3	33	20	26	9.3
Black Or African American	16	3.6	8	4.8	8	2.9
Multiple	7	1.6	3	1.8	4	1.4
Black Or African American, White	5	1.1	3	1.8	2	0.7
White, Asian	2	0.5	0	0	2	0.7
White	345	77.7	115	69.7	230	82.4
Missing	1	0.2	0	0	1	0.4
Ethnicity						
Hispanic Or Latino	57	12.8	11	6.7	46	16.5
Not Hispanic Or Latino	352	79.3	139	84.2	213	76.3

Not Reported	35	7.9	15	9.1	20	7.2
ECOG						
0	194	43.7	68	41.2	126	45.2
1	250	56.3	97	58.8	153	54.8
Metastatic Staging						
MX	17	3.8	2	1.2	15	5.4
M0	15	3.4	5	3	10	3.6
M1	412	92.8	158	95.8	254	91
Cancer Staging						
III	3	0.7	1	0.6	2	0.7
IIIA	1	0.2	1	0.6	0	0
IIIB	4	0.9	0	0	4	1.4
IIIC	2	0.5	0	0	2	0.7
IV	394	88.7	147	89.1	247	88.5
IVA	1	0.2	0	0	1	0.4
IVB	30	6.8	12	7.3	18	6.5
IVC	9	2	4	2.4	5	1.8
Brain Metastasis						
Yes	6	1.4	2	1.2	4	1.4
No	438	98.6	163	98.8	275	98.6
Prior Line of Therapy						
0	8	1.8	2	1.2	6	2.2
Adjuvant/Neoadjuvant/Definitive	3	0.7	1	0.6	2	0.7
1	156	35.1	54	32.7	102	36.6
2	130	29.3	56	33.9	74	26.5
3	76	17.1	25	15.2	51	18.3
4	31	7	14	8.5	17	6.1
5 or more	40	9	13	7.9	27	9.7
Database Cutoff Date: KEYNOTE 164: September 9, 2019, KEYNOTE 158: October 5, 2020						

The distribution of patients based on tumor organ system and tumor types is similar between the CTA-positive population and the CTA-positive/F1CDx-evaluable subpopulation, see Table 19 and Table 20.

Table 19: Organ system distribution in CTA-Positive Population, and by F1CDx Evaluability in CTA-Positive Population (KEYNOTE-158 Cohort K and KEYNOTE-164)

Organ System	CTA-Positive (N=444)		CTA-Positive/F1CDx-Evaluable (N=165)		CTA-Positive/F1CDx-Missing (N=279)	
	n	%	n	%	n	%

Endocrine	6	1.4	4	2.4	2	0.7
GI	225	50.7	95	57.6	130	46.6
Hepatic	22	5.0	7	4.2	15	5.4
Reproductive	121	27.3	38	23.0	83	29.7
Soft tissue	14	3.2	7	4.2	7	2.5
Thoracic	12	2.7	3	1.8	9	3.2
Urinary	10	2.3	4	2.4	6	2.2
Other	34	7.7	7	4.2	27	9.7
Total	444		165		279	

Table 20: Tumor Type distribution in CTA-Positive Population, and by F1CDx Evaluability in CTA-Positive Population (KEYNOTE-158 Cohort K and KEYNOTE-164)

Tumor Type	CTA-Positive (N=444)		CTA-Evaluable (N=165)		CTA-Positive/F1CDx-missing (N=279)	
	n	%	n	%	n	%
CRC	123	27.7	61	37.0	62	22.2
Endometrial	68	15.3	21	12.7	47	16.8
Gastric	42	9.5	14	8.5	28	10.0
Ovarian	24	5.4	10	6.1	14	5.0
Small Intestine	25	5.6	9	5.5	16	5.7
Prostate	8	1.8	3	1.8	5	1.8
Cholangiocarcinoma	22	5.0	7	4.2	15	5.4
Breast	11	2.5	2	1.2	9	3.2
Pancreatic	22	5.0	4	2.4	18	6.5
Cervical	8	1.8	2	1.2	6	2.2
Adrenocortical	7	1.6	3	1.8	4	1.4
Salivary	4	0.9	1	0.6	3	1.1
Brain	17	3.8	2	1.2	15	5.4
Sarcoma	14	3.2	7	4.2	7	2.5
Neuro endocrine	12	2.7	6	3.6	6	2.2
Thyroid	6	1.4	4	2.4	2	0.7
Mesothelioma	6	1.4	3	1.8	3	1.1
Other	3	0.7	1	0.6	2	0.7
Renal	4	0.9	1	0.6	3	1.1
SCLC	6	1.4	0	0.0	6	2.2
Urothelial	6	1.4	3	1.8	3	1.1
Vaginal	1	0.2	0	0.0	1	0.4
Anal	1	0.2	1	0.6	0	0.0
HNSCC	1	0.2	0	0.0	1	0.4
Nasopharyngeal	1	0.2	0	0.0	1	0.4
Retroperitoneal	1	0.2	0	0.0	1	0.4
Testicular	1	0.2	0	0.0	1	0.4

Total	444	165	279

The distribution of patient characteristics is generally similar between the KEYNOTE-158 (KN158) Cohort A to J and the F1CDx-evaluable subpopulation, see Table 21.

Table 21: Patient Characteristics by F1CDx Status (KN158 Cohort A to J)

	KN158 Cohort A to J		KN158 Cohort A to J F1CDx Tested		KN158 Cohort A to J F1CDx Evaluable		KN158 Cohort A to J F1CDx Missing	
	n	(%)	n	(%)	n	(%)	n	(%)
Participants in population	1,066		1,004		714		290	
Sex								
Male	395	(37.1)	373	(37.2)	251	(35.2)	122	(42.1)
Female	671	(62.9)	631	(62.8)	463	(64.8)	168	(57.9)
Age (Years)								
< 65	638	(59.8)	603	(60.1)	442	(61.9)	161	(55.5)
>= 65	428	(40.2)	401	(39.9)	272	(38.1)	129	(44.5)
Mean	60.3		60.3		59.9		61.2	
SD	11.7		11.7		11.8		11.5	
Median	62.0		61.5		61.0		63.0	
Range	22 - 87		22 - 87		22 - 85		25 - 87	
Race								
American Indian Or Alaska Native	6	(0.6)	6	(0.6)	3	(0.4)	3	(1.0)
Asian	208	(19.5)	201	(20.0)	137	(19.2)	64	(22.1)
Black Or African American	25	(2.3)	25	(2.5)	16	(2.2)	9	(3.1)
Multiple	6	(0.6)	6	(0.6)	5	(0.7)	1	(0.3)
American Indian Or Alaska Native, Black Or African American	1	(0.1)	1	(0.1)	1	(0.1)	0	(0.0)
Black Or African American, White	5	(0.5)	5	(0.5)	4	(0.6)	1	(0.3)
Native Hawaiian Or Other Pacific Islander	3	(0.3)	3	(0.3)	2	(0.3)	1	(0.3)
White	818	(76.7)	763	(76.0)	551	(77.2)	212	(73.1)
Ethnicity								
Hispanic Or Latino	64	(6.0)	60	(6.0)	36	(5.0)	24	(8.3)
Not Hispanic Or Latino	921	(86.4)	867	(86.4)	624	(87.4)	243	(83.8)
Not Reported	81	(7.6)	77	(7.7)	54	(7.6)	23	(7.9)

ECOG								
0	430	(40.3)	404	(40.2)	287	(40.2)	117	(40.3)
1	633	(59.4)	597	(59.5)	424	(59.4)	173	(59.7)
2	3	(0.3)	3	(0.3)	3	(0.4)	0	(0.0)

The distribution of tumor organ system and tumor types in KEYNOTE-158 (KN158) Cohort A to J and the F1CDx-evaluable subpopulation, see Table 22 and 23.

Table 22: Tumor Organ System Distribution by F1CDx Status, KEYNOTE-158 Cohort A to J

	KN158 Cohort A to J All n	KN158 Cohort A to J F1CDx Tested n (%) [a]	KN158 Cohort A to J F1CDx Evaluable n (%) [b]	KN158 Cohort A to J F1CDx Missing n (%) [b]
Participants in population	1066	1004 (94.2)	714 (71.1)	290 (28.9)
Endocrine	103	100 (97.1)	72 (72.0)	28 (28.0)
GI	219	206 (94.1)	158 (76.7)	48 (23.3)
Hepatic	104	94 (90.4)	65 (69.1)	29 (30.9)
Other	109	104 (95.4)	68 (65.4)	36 (34.6)
Reproductive	306	288 (94.1)	218 (75.7)	70 (24.3)
Thoracic	225	212 (94.2)	133 (62.7)	79 (37.3)
[a] Denominator is KN158 Cohort A to J All [b] Denominator is KN158 Cohort A to J F1CDx Tested				

Table 23: Tumor Type Distribution by F1CDx Status KEYNOTE-158 Cohort A to J

	KN158 Cohort A to J All n	KN158 Cohort A to J F1CDx Tested n (%) [a]	KN158 Cohort A to J F1CDx Evaluable n (%) [b]	KN158 Cohort A to J F1CDx Missing n (%) [b]
Participants in population	1066	1004 (94.2)	714 (71.1)	290 (28.9)
Anal carcinoma	112	100 (89.3)	87 (87.0)	13 (13.0)
Cervical cancer	98	91 (92.9)	69 (75.8)	22 (24.2)
Cholangiocarcinoma	104	94 (90.4)	65 (69.1)	29 (30.9)
Endometrial carcinoma	107	99 (92.5)	81 (81.8)	18 (18.2)
Mesothelioma	118	116 (98.3)	78 (67.2)	38 (32.8)
NET	107	106 (99.1)	71 (67.0)	35 (33.0)
Salivary cancer	109	104 (95.4)	68 (65.4)	36 (34.6)
Small Cell lung cancer	107	96 (89.7)	55 (57.3)	41 (42.7)
Thyroid carcinoma	103	100 (97.1)	72 (72.0)	28 (28.0)
Vulvar cancer	101	98 (97.0)	68 (69.4)	30 (30.6)
[a] Denominator is KN158 Cohort A to J All				

[b]Denominator is KN158 Cohort A to J F1CDx Tested

The tumor bank samples and KEYNOTE-158 Cohorts A-J samples, were included in the bridging analyses to construct the CTA-negative population for the purposes for negative percent agreement (NPA) assessment. Tumor bank samples were used to supplement any shortfalls from KEYNOTE-158 Cohorts A-J in matching the tumor organ system representation in the efficacy population. Based on these reasons, while there was an initial pool of 833 tumor bank samples available (as noted in Table 17 above), 429 samples were considered for CTA or F1CDx testing. Therefore, the tumor bank information included in Tables 24, 25 and 26 are based on the 429 samples. Further, demographic information is available for only a subset of the samples, as shown in the Table 24 below.

Table 24: Patient Characteristics by F1CDx Status, Tumor Bank Samples

	Tumor Bank All		Tumor Bank F1CDx Tested		Tumor Bank F1CDx Evaluable		Tumor Bank F1CDx Missing	
	n	(%)	n	(%)	n	(%)	n	(%)
Participants in population	429		409		307		102	
Sex								
Male	82	(19.1)	82	(20.0)	63	(20.5)	19	(18.6)
Female	60	(14.0)	60	(14.7)	49	(16.0)	11	(10.8)
Missing	287	(66.9)	267	(65.3)	195	(63.5)	72	(70.6)
Age (Years)								
< 65	43	(10.0)	43	(10.5)	34	(11.1)	9	(8.8)
>= 65	66	(15.4)	66	(16.1)	54	(17.6)	12	(11.8)
Unknown[a]	320	(74.6)	300	(73.3)	219	(71.3)	81	(79.4)
Mean	67.1		67.1		67.1		66.8	
SD	11.5		11.5		11.8		10.5	
Median	67.0		67.0		68.0		66.0	
Range	35 - 85		35 - 85		35 - 85		35 - 83	
Race								
Asian	11	(2.6)	11	(2.7)	1	(0.3)	10	(9.8)
White	109	(25.4)	109	(26.7)	88	(28.7)	21	(20.6)
Missing	309	(72.0)	289	(70.7)	218	(71.0)	71	(69.6)

[a] Not included in summary statistics for age.

Table 25: Tumor Type Distribution by F1CDx Status Tumor Bank Samples

	Tumor Bank All n	Tumor Bank F1CDx Tested n (%)[a]	Tumor Bank F1CDx Evaluable n (%)[b]	Tumor Bank F1CDx Missing n (%)[b]

Participants in population	429	409 (95.3)	307 (75.1)	102 (24.9)
CRC	130	120 (92.3)	89 (74.2)	31 (25.8)
Non-CRC	299	289 (96.7)	218 (75.4)	71 (24.6)
Anal carcinoma	1	0 (0.0)	NA	NA
Cervical cancer	1	0 (0.0)	NA	NA
Cholangiocarcinoma	23	21 (91.3)	18 (85.7)	3 (14.3)
Colorectal Carcinoma	130	120 (92.3)	89 (74.2)	31 (25.8)
Endometrial carcinoma	2	0 (0.0)	NA	NA
Esophageal carcinoma	110	110 (100.0)	97 (88.2)	13 (11.8)
Gastric carcinoma	64	62 (96.9)	52 (83.9)	10 (16.1)
HNSCC	24	24 (100.0)	9 (37.5)	15 (62.5)
Liver Cancer	2	2 (100.0)	1 (50.0)	1 (50.0)
Pancreatic cancer	13	13 (100.0)	8 (61.5)	5 (38.5)
Salivary cancer	16	16 (100.0)	6 (37.5)	10 (62.5)
Sarcoma	20	20 (100.0)	12 (60.0)	8 (40.0)
Small intestine adenocarcinoma	8	6 (75.0)	5 (83.3)	1 (16.7)
Urothelial carcinoma	15	15 (100.0)	10 (66.7)	5 (33.3)
[a]Denominator is Tumor Bank All [b]Denominator is Tumor Bank F1CDx Tested				

Table 26: Tumor Organ System Distribution by F1CDx Status, Tumor Bank Samples

	Tumor Bank All n	Tumor Bank F1CDx Tested n (%)[a]	Tumor Bank F1CDx Evaluable n (%)[b]	Tumor Bank F1CDx Missing n (%)[b]
Participants in population	429	409 (95.3)	307 (75.1)	102 (24.9)
GI	336	319 (94.9)	261 (81.8)	58 (18.2)
Hepatic	15	15 (100.0)	9 (60.0)	6 (40.0)
Other	40	40 (100.0)	15 (37.5)	25 (62.5)
Reproductive	3	0 (0.0)	NA	NA
Soft Tissue	20	20 (100.0)	12 (60.0)	8 (40.0)
Urinary	15	15 (100.0)	10 (66.7)	5 (33.3)
[a]Denominator is Tumor Bank All [b]Denominator is Tumor Bank F1CDx Tested				

D. Safety and Effectiveness Results

1. Safety Results

The overall safety profile of pembrolizumab in patients with MSI-H/dMMR solid tumors was generally consistent with the established safety profile of pembrolizumab. No new or unexpected safety signals for pembrolizumab were identified.

No adverse events were reported in connection with the bridging study used to support this PMA supplement, as the study was performed retrospectively using banked 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 patients with MSI-H/dMMR solid tumors. Patients' tumors determined to be not MSI-H/dMMR by local testing were not enrolled and their samples were not retained for further testing, as it was not a protocol requirement. Therefore, KEYNOTE-158 Cohorts A-J and tumor bank samples were used to assemble a simulated-local CTA-negative population.

For the purposes of generating simulated-local MSI/MMR tumor results, PCR testing for MSI status and IHC testing for MMR status were performed at external laboratories to simulate PCR and IHC local tests.

The CTA status was derived based on available PCR, IHC or other (e.g., NGS – only applies to KEYNOTE-158 Cohort K samples) results for the samples. CTA status was considered positive if any of the following conditions was satisfied:

- CTA PCR was MSI-H or
- CTA IHC was dMMR or
- CTA Other was MSI-H

CTA status was considered Negative if none of the above conditions was satisfied and at least one of following was true:

- CTA PCR was not-MSI-H
- CTA IHC was pMMR
- CTA Other was not-MSI-H

CTA status was considered Non-Evaluable if none of the CTA PCR, CTA IHC and CTA Other was evaluable.

As stated above in Section X.B., overall, 1664 tumor samples were tested by F1CDx assay and 1189 were F1CDx- evaluable. There were 15 CTA non-evaluable samples (14 from KEYNOTE-158 Cohort A-J and 1 tumor bank) and these samples were therefore excluded from the concordance analyses, leaving 1,174 for the analyses.

Concordance between the CTA and F1CDx for MSI/MMR test outcomes was assessed in the concordance analysis population. There were a total of 1174 samples having both F1CDx and CTA results (192 CTA-positive, which included samples from KEYNOTE-158 Cohorts A-K, KENYNOTE-164 and Tumor Bank,

and 982 CTA-negative samples, which included samples KEYNOTE-158 Cohorts A-J and Tumor Bank). Concordance was evaluated for these 1174 samples and results are shown below.

The results of the concordance analysis demonstrated agreement between the F1CDx assay and the CTAs. Agreement measures, PPA, NPA and overall percent agreement (OPA), between the F1CDx and CTAs, with valid F1CDx results and CTA results, were calculated using the CTA results as reference (Table 27). The point estimates of PPA, NPA and OPA were 69.8%, 99.3% and 94.5% respectively.

Table 27: Agreement between CTA and F1CDx with CTA as Reference (Clinical Trial Subjects and Tumor Bank samples with CTA and F1CDx evaluable results)

	CTA-Positive	CTA-Negative	Total
F1CDx-Positive	134	7	141
F1CDx-Negative	58	975	1033
Total	192	982	1174
PPA (95% CI)	69.8 (63.0, 75.8)		
NPA (95% CI)	99.3 (98.5, 99.7)		
OPA (95% CI)	94.5 (93.0, 95.6)		

CTA-Positive includes samples from KEYNOTE-158 Cohorts A-K, KEYNOTE 164 and Tumor Bank
CTA-Negative includes sample from KEYNOTE-158 Cohorts A-J and Tumor Bank

Agreement measures, PPA, NPA and OPA, between the F1CDx and CTAs, with valid F1CDx results and CTA results, were also calculated per tumor type using the CTA results as the reference and shown in Table 28. The point estimates of PPA, NPA and OPA per tumor type ranged from 0% to 100%, from 92.8% to 100% and from 0% to 100% respectively. The reason from the wide ranges observed is generally due to the small sample sizes for some of the tumor types evaluated.

Table 28: Agreement between CTA and F1CDx with CTA as Reference (Clinical Trial Subjects and Tumor Bank samples with CTA and F1CDx evaluable results) per Tumor Type

Tumor Type	N	CTA Positive F1CDx Positive	CTA Positive F1CDx Negative	CTA Negative F1CDx Positive	CTA Negative F1CDx Negative	PPA % (95% CI)	NPA % (95% CI)	OPA % (95% CI)
All	1174	134	58	7	975	69.8 (63.0, 75.8)	99.3 (98.5, 99.7)	94.5 (93.0, 95.6)
Colorectal	151	60	13	0	78	82.2 (71.9, 89.3)	100.0 (95.3, 100.0)	91.4 (85.8, 94.9)
Endometrial	101	28	4	5	64	87.5 (71.9, 95.0)	92.8 (84.1, 96.9)	91.1 (83.9, 95.2)
Gastric	66	11	3	0	52	78.6 (52.4, 92.4)	100.0 (93.1, 100.0)	95.5 (87.5, 98.4)
Ovarian	10	7	3	0	0	70.0 (39.7, 89.2)	NA	70.0 (39.7, 89.2)
Others	846	28	35	2	781	44.4 (32.8, 56.7)	99.7 (99.1, 99.9)	95.6 (94.0, 96.8)

N/A: Not applicable.

For the tumor profiling claim, the F1CDx test provides three potential MSI statuses using the FB-MSI algorithm: MSI-H (fraction-based score ≥0.0124), MSI-Cannot be determined (fraction-based score >0.0041 and <0.0124), and MSS (fraction based score ≤0.0041). In the context of the CDx indication for KEYTRUDA, MSI-H is considered F1CDx-positive, and both MSI Cannot Be Determined and MSS are considered F1CDx-negative for MSI CDx status. All CTA+/F1CDx discordances, a total of 58, included 45 patient samples from patients enrolled in KEYNOTE 158 Cohort K, and 13 patient samples from KEYNOTE-164, respectively. Of the 45 discordances in KEYNOTE-158 Cohort K primary analysis population, nine (20.0%) are MSI-Cannot be determined (fraction-based score >0.0041 and <0.0124), indicating an intermediate MSI score that is near the cutoff point for MSI-H. Seven of these nine (77.8%) MSI-Cannot be determined (fraction-based score >0.0041 and <0.0124) participants were non-responders to KEYTRUDA, and two subjects (22.2%) were partial responders. None of the discordant samples (n=13) in KEYNOTE-164 were classified as MSI-Cannot be determined (fraction-based score >0.0041 and <0.0124).

The F1CDx assay also generates a TMB score as part of the test results. Previous studies suggest that samples that are MSI-H are typically TMB-High (TMB-H). All F1CDx-positive (MSI-H) samples were TMB-H, as defined by a cut point of >10 mut/Mb, except one sample in KEYNOTE-158 Cohort K (endometrial, 6 mut/Mb). In addition, the median TMB score in KEYNOTE-158 Cohort K was 33 mut/Mb in the F1CDx positive (MSI-H) samples. The median TMB score in the F1CDx-positive (MSI-H) population in KEYNOTE-164 was 41 mut/Mb. In contrast, the TMB score in the F1CDx-negative (non-MSI-H) population was 3 mut/Mb in KEYNOTE-158 cohort K, as well as in KEYNOTE-164. This supplementary genomic information from the F1CDx assay suggest the likely accuracy of the F1CDx MSI results.

KEYNOTE-164 and KEYNOTE-158 cohort K enrolled patients primarily using local PCR and/or IHC CTA results. Some enrolled patients had both PCR and

IHC CTA results, hence the total of the denominators for each study is greater than the total number of discordant samples. One patient that was F1CDx-negative had a CTA-positive test result that was NGS based. It should be noted that two patients enrolled in KEYNOTE-164 were F1CDx-positive and had two available CTA results that were discordant: pMMR (IHC-negative) and MSI-H (PCR-positive). This patient was considered CTA-positive and was enrolled and was not considered discordant from the F1CDx-positive result.

Agreement measures, PPA, NPA and OPA, between the F1CDx and CTAs, with valid F1CDx results and CTA results, were also calculated using CTA IHC assays (n=659) and CTA PCR assays (n= 1036) as reference and shown below.

The point estimates of PPA, NPA and OPA were 66.4%, 99.2% and 92.9% respectively when using CTA IHC assays as reference as shown in Table 29 below.

Table 29: Agreement between CTA (IHC) and F1CDx with CTA (IHC) as Reference (Clinical Trial Subjects and Tumor Bank samples with CTA (IHC) and F1CDx evaluable results)

	CTA (IHC)-Positive	CTA (IHC)-Negative	Total
F1CDx-Positive	85	4	89
F1CDx-Negative	43	527	570
Total	128	531	659
PPA (95% CI)	66.4 (57.9, 74.0)		
NPA (95% CI)	99.2 (98.1, 99.7)		
OPA (95% CI)	92.9 (90.6, 94.6)		

Agreement measures, PPA, NPA and OPA, between the F1CDx and CTA IHC assays, with valid F1CDx results and CTA IHC results, were also calculated for CRC (n=126) and non-CRC patients (n=533). The point estimates of PPA, NPA and OPA for CRC patients were 85.4% (41/48, 95% CI:72.8%, 92.8%), 94.9 (74/78, 95%CI: 87.5%, 98.0%), and 91.3% (115/126, 95%CI: 85.0%, 95.1%). The point estimates of PPA, NPA and OPA for non-CRC patients were 55.0% (44/80; 95% CI: 44.1%, 65.4%), 100.0% (453/453; 95% CI: 99.2%, 100.0%), and 93.2% (497/533; 95% CI: 90.8%, 95.1%) respectively.

There were 43 patients with F1CDx negative/CTA IHC positive status. MSI-Cannot be determined status for MSI cases with FB MSI >0.0041 and <0.0124 was observed in seven (7) patient samples. Seven (7) of the 43 patients with F1CDx negative/CTA IHC positive status responded to KEYTRUDA (7/43 responders, 16.3% response rate).

There were also four (4) patients with F1CDx positive/CTA IHC negative status. Two out of four patient samples were from the tumor bank and therefore efficacy information is not relevant. Of the two patients with F1CDx positive/CTA IHC

negative status, none responded to KEYTRUDA (0/2 responders, 0.0% response rate).

The point estimates of PPA, NPA and OPA were 81.6%, 99.3% and 98.0% respectively when using CTA PCR assays as reference as shown in Table 30 below.

Table 30: Agreement between CTA (PCR) and F1CDx with CTA (PCR) as Reference (Clinical Trial Subjects and Tumor Bank samples with CTA (PCR) and F1CDx evaluable results)

	CTA (PCR) - Positive	CTA (PCR)- Negative	Total
F1CDx-Positive	62	7	69
F1CDx-Negative	14	953	967
Total	76	960	1036
PPA (95% CI)	81.6 (71.4, 88.7)		
NPA (95% CI)	99.3 (98.5, 99.6)		
OPA (95% CI)	98.0 (96.9, 98.7)		

Agreement measures, PPA, NPA and OPA, between the F1CDx and CTA PCR assays, with valid F1CDx results and CTA IHC results, were also calculated for CRC (n=113) and non-CRC patients (n=923). The point estimates of PPA, NPA and OPA for CRC patients were 85.7% (36/42, 95%CI: 72.2%, 93.3%), 100.0% (71/71, 95%CI: 94.9%, 100.0%) and 94.7% (107/113, 95%CI: 88.9%, 97.5%). The point estimates of PPA, NPA and OPA for non-CRC patients were 76.5% (26/34; 95% CI: 60.0, 87.6), 99.2% (882/889; 95% CI: 98.4, 99.6), and 98.4(908/923; 95% CI: 97.3, 99.0) respectively.

There were 14 patients with F1CDx negative/CTA PCR positive status. MSI-Cannot be determined status for MSI cases with FB MSI >0.0041 and <0.0124 was observed in two (2) patient samples. None of the 14 patients with F1CDx negative/CTA PCR positive status responded to KEYTRUDA (0/14 responders, 0.0% response rate).
There were also seven (7) patients with F1CDx positive/CTA PCR negative status. Three out of seven patients (3/7) responded to KEYTRUDA therapy (42.9% response rate).

In general, there was an unexpectedly high proportion of CTA-positive samples from KEYNOTE-158 and KEYNOTE-164 that were F1CDx-negative, leading to further investigation to understand the likely correct MSI status of these samples. One of the primary reasons for the observed discordances may be due to heterogeneity (e.g., differences in reagents, instrumentation, varying levels of assay characterization) in MSI/MMR local testing, or regional differences in testing consistency given KEYNOTE-158 was conducted in 18 different countries. The observed discordances were more prevalent in non-CRC indications as compared to CRC.

Clinical Efficacy Results

The clinical validity of F1CDx for the detection of MSI-H status in patients with solid tumors was based on estimation of clinical efficacy in the F1CDx- positive, CTA-positive population. The major efficacy outcome measure was ORR per RECIST 1.1 (assessed by central imaging). ORR outcomes for the CTA positive, F1CDx positive/CTA positive, F1CDx negative/CTA positive and F1CDx missing/CTA positive are presented in Tables 31, 32 and 33.

Table 31: Efficacy Results in KEYNOTE-164 and KEYNOTE-158 Cohort K Combined

Clinical outcome	**CTA positive (N=444)**	**F1CDx positive and CTA positive (N=107)**	**F1CDx negative and CTA positive (N=58)**	**F1CDx result missing and CTA positive (N=279)**
ORR% (95% CI*)	31.8% (27.4, 36.3)	43.0% (33.5, 52.9)	12.1% (5.0, 23.3)	31.5% (26.1, 37.3)
Complete response	38 (8.6%)	13 (12.1%)	2 (3.4%)	23 (8.2%)
Partial response	103 (23.2%)	33 (30.8%)	5 (8.6%)	65 (23.3%)
Duration of Response	**N=141**	**N=46**	**N=7**	**N=88**
Median in months (range)	NR (2.1+ - 51.1+)	NR (3.7+ - 46.2+)	15.1 (6.1+ - 32.2+)	NR (2.1+ - 51.1+)
% with duration ≥ 6 months	129 (95.6)	43 (95.6)	7 (100.0)	79 (95.2)
% with duration ≥ 12 months	104 (90.1)	36 (88.6)	4 (83.3)	64 (91.4)

*Based on binomial exact confidence interval method.
Database Cutoff Date:
KEYNOTE 164: September 9, 2019,
KEYNOTE 158: October 5, 2020

Table 32: Efficacy Results in KEYNOTE-164

Clinical outcome	**CTA positive (N=123)**	**F1CDx positive and CTA positive (N=48)**	**F1CDx negative and CTA positive (N=13)**	**F1CDx result missing and CTA positive (N=62)**
ORR% (95% CI*)	34.1 (25.8, 43.2)	39.6 (25.8, 54.7)	7.7 (0.2, 36.0)	35.5 (23.7, 48.7)
Complete response	11 (8.9%)	6 (12.5%)	0 (0%)	5 (8.1%)
Partial response	31 (25.2%)	13 (27.1%)	1 (7.7%)	17 (27.4%)
Duration of Response	**N=42**	**N=19**	**N=1**	**N=22**
Median in months (range)	NR (3.9+ - 41.2+)	NR (4.4 - 40.3+)	NR (6.1+ - 6.1+)	NR (3.9+ - 41.2+)

Clinical outcome	CTA positive (N=123)	F1CDx positive and CTA positive (N=48)	F1CDx negative and CTA positive (N=13)	F1CDx result missing and CTA positive (N=62)
% with duration ≥ 6 months	39 (97.6)	18 (94.7)	1 (100.0)	20 (100.0)
% with duration ≥ 12 months	34 (95.0)	17 (94.7)	0 (NR)	17 (95.0)

*Based on binomial exact confidence interval method.
Database Cutoff Date:
KEYNOTE 164: September 9, 2019

Table 33: Efficacy Results in KEYNOTE-158 Cohort K

Clinical outcome	CTA positive (N=321)	F1CDx positive and CTA positive (N=59)	F1CDx negative and CTA positive (N=45)	F1CDx result missing and CTA positive (N=217)
ORR% (95% CI*)	30.8 (25.8, 36.2)	45.8 (32.7, 59.2)	13.3 (5.1, 26.8)	30.4 (24.4, 37.0)
Complete response	27 (8.4%)	7 (11.9%)	2 (4.4%)	18 (8.3%)
Partial response	72 (22.4%)	20 (33.9%)	4 (8.9%)	48 (22.1%)
Duration of Response	**N=99**	**N=27**	**N=6**	**N=66**
Median in months (range)	47.5 (2.1+ - 51.1+)	NR (3.7+ - 46.2+)	15.1 (6.5 - 32.2+)	47.5 (2.1+ - 51.1+)
% with duration ≥ 6 months	90 (94.8)	25 (96.2)	6 (100.0)	59 (93.7)
% with duration ≥ 12 months	70 (88.0)	19 (84.0)	4 (83.3)	47 (90.3)

*Based on binomial exact confidence interval method.
Database Cutoff Date:
KEYNOTE 158: October 5, 2020

The ORR in the CTA-positive population was 31.8% (141/444), (95% CI: 27.4, 36.3). There were 53 CTA-positive participants who also had F1CDx results with partial or complete responses. Among them 86.8% (46/53) were positive by F1CDx (95% CI: 74.7, 94.5). There were 112 CTA-positive participants who also had F1CDx results with no responses. Among the 112 CTA positive patients who did not respond to KEYTRUDA, only 54.5% (61/112) were positive by F1CDx (95% CI: 44.8, 63.9). Taken together, F1CDx has a higher percent of positive results among participants with responses than among participants without responses [difference between 86.8% (46/53) and 54.5% (61/112) was 32.3% with 95% CI: (17.6, 44.6)].

The ORR in F1CDx-positive/CTA-positive participants was 43.0% (46/107), (95% CI: 33.5, 52.9). The ORR in F1CDx-negative/CTA-positive participants was 12.1% (7/58), (95% CI: 5.0, 23.3). The ORR in F1CDx-positive/CTA-positive participants was higher than the ORR in F1CDx-negative/CTA-positive participants [difference between 43.0% (46/107) and 12.1% (7/58) was 30.9% with 95% CI: (16.7, 42.8)].

The similarity of the ORR for the CTA-positive population (n=444) overall (31.8%, 95% CI: 27.4, 36.3) and for those missing a valid F1CDx result (n=279; 31.5%, 95% CI: 26.1, 37.3) suggests no overt imbalance in efficacy effect of pembrolizumab between patients on whom the F1CDx was or was not obtained.

Sensitivity Analysis

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with missing/invalid CDx results and the missing F1CDx-positive, CTA-negative population that was not enrolled and evaluated by KEYNOTE-158 Cohort K and KEYNOTE-164 clinical studies.

To evaluate the impact of missing/invalid F1CDx results, the distribution of patients for baseline covariates, disease characteristics, tumor organ system, and tumor types was compared among the CTA-positive population, the F1CDx-evaluable/CTA-positive subpopulation, and F1CDx-missing CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable F1CDx MSI tumor status (n=279). The imputation model included the clinical outcome and covariates that are considered predictive of missingness of the F1CDx tumor status and showing some predictive value of the F1CDx tumor status.

The clinical efficacy (ORR) for the F1CDx-positive subjects in the device intended use population was estimated under different assumed scenarios based on observed and imputed F1CDx results.

For the F1CDx-positive, CTA-negative population that was not enrolled and evaluated by KEYNOTE-158 Cohort K and KEYNOTE-164 clinical studies, bridging equations that involved an ORR attenuation factor that ranges from 0 (assume full attenuation of the efficacy in CTA-negative/F1CDx-positive) to 1 (assume no attenuation of the efficacy in CTA-negative/F1CDx-positive compared to the observed ORR in F1CDx-positive patients in the efficacy population) were used for the clinical efficacy analysis in this missing population.

Sensitivity analysis considering the NPA and assuming different CTA positivity rates in the F1CDx intended use population, which ranged 2-5%, were investigated to assess influence on the efficacy estimated for the intended use, i.e., F1CDx positive subjects. These sensitivity analyses demonstrated the robustness of the clinical efficacy estimate from the primary analysis.

Due to the large proportion of missing data additional clinical data from KEYNOTE-158 cohort K will be evaluated post-market to confirm the clinical effectiveness of F1CDx, see section XIII below.

3. Subgroup Analyses
Response to KEYTRUDA for the CTA positive, F1CDx positive/CTA positive, F1CDx negative/CTA positive and F1CDx missing/CTA positive the F1CDx patients was analyzed by primary tumor type and by organ system type. Within the F1CDx positive patients for the efficacy set, 13 tumor types were represented. Response rates by tumor types are included in Table 34, and response per organ system type are included in Table 35.

Table 34: ORR Estimates per tumor type in Subpopulations by F1CDx Status

Tumor Type	Responder (n) / Subpopulation (N), ORR% (95% CI)			
	CTA-Positive (N=444)	**F1CDx-Positive and CTA-Positive (N=107)**	**F1CDx-Negative and CTA-Positive (N=58)**	**F1CDx-result missing and CTA-Positive (N=279)**
CRC	42/123, 34.1% (25.8, 43.2)	19/48, 39.6% (25.8 54.7)	1/13, 7.7% (0.2, 36.0)	22/62, 35.5% (23.7, 48.7)
Non-CRC	99/321, 30.8% (25.8, 36.2)	27/59, 45.8% (32.7, 59.2)	6/45, 13.3% (5.1, 26.8)	66/217, 30.4% (24.4, 37.0)
Endometrial	33/68, 48.5% (36.2, 61.0)	5/17, 29.4% (10.3, 56.0)	3/4, 75.0% (19.4, 99.4)	25/47, 53.2% (38.1, 67.9)
Gastric	13/42, 31.0% (17.6, 47.1)	6/11, 54.5% (23.4, 83.3)	0/3, 0.0% (0.0, 70.8)	7/28, 25.0% (10.7, 44.9)
Ovarian	8/24, 33.3% (15.6, 55.3)	5/7, 71.4% (29.0, 96.3)	0/3, 0.0% (0.0, 70.8)	3/14, 21.4% (4.7, 50.8)
Small Intestine	12/25, 48.0% (27.8, 68.7)	3/9, 33.3% (7.5, 70.1)	NA	9/16, 56.3% (29.9, 80.2)
Cholangio carcinoma	9/22, 40.9% (20.7, 63.6)	3/4, 75.0% (19.4, 99.4)	1/3, 33.3% (0.8, 90.6)	5/15, 33.3% (11.8, 61.6)
Breast	1/11, 9.1% (0.2, 41.3)	1/1, 100.0% (2.5, 100)	0/1, 0.0% (0.0, 97.5)	0/9, 0.0% (0.0, 33.6)

Pancreatic	4/22, 18.2% (5.2, 40.3)	2/2, 100.0% (15.8, 100.0)	0/2, 0.0% (0.0, 84.2)	2/18, 11.1% (1.4, 34.7)
Brain	1/17, 5.9% (0.1, 28.7)	NA	1/2, 50.0% (1.3, 98.7)	0/15, 0.0% (0.0, 21.8)
Sarcoma	3/14, 21.4% (4.7, 50.8)	NA	0/7, 0.0% (0.0, 41.0)	3/7, 42.9% (9.9, 81.6)
Neuro endocrine	2/12, 16.7% (2.1, 48.4)	NA	0/6, 0.0% (0.0, 45.9)	2/6, 33.3% (4.3, 77.7)
Other	13/64, 20.3% (11.3, 32.2)	2/8, 25.0% (3.2, 65.1)	1/14, 7.1% (0.2, 33.9)	10/42, 23.8% (12.1, 39.5)

NA: Not Applicable
*Based on binomial exact confidence interval method.
Database Cutoff Date:
KEYNOTE 164: September 9, 2019,
KEYNOTE 158: October 5, 2020

In the two (2) tumor types with the highest MSI-H prevalence across solid tumors, the ORR in F1CDx-positive/CTA-positive subpopulation CRC (n=48) and endometrial cancer (n=17) was 39.6% (95% CI: 25.8, 54.7) and 29.4% (95% CI: 10.3, 56.0) respectively. For non-CRC tumors combined, the ORR in F1CDx-positive/CTA-positive subpopulation (n=59) was 45.8% (95% CI: 2.7, 59.2)

While the point estimate for ORR in endometrial cancer patients with F1CDx positive/CTA positive status is lower than the point estimate for CTA positive patients, additional efficacy data from KEYNOTE 158 Cohort D, which enrolled endometrial cancer patients provides additional efficacy data for the F1CDx positive endometrial cancer population. There were 17 KEYNOTE-158 Cohort D patients that were determined to be F1CDx MSI-H, the CTA identified 11 out of 17 patients to be MSI-H. Seven out of the 17 F1CDx MSI-H (positive) patients were responders, and thus the ORR in these 17 F1CDx positive patients was 41.2% (95% CI: 18.4, 67.1). Of note five of the eleven F1CDx positive/CTA positive were responders, and the ORR in these eleven patients was 45.5% (16.7, 76.6).

Table 35: ORR Estimate per organ system in Subpopulations by F1CDx Status

Organ System	Responder (n) / Subpopulation (N), ORR% (95% CI*)			
	CTA-Positive (N=444)	F1CDx-Positive /CTA-Positive (N=107)	F1CDx-Negative/ CTA-Positive (N=58)	F1CDx-result missing/ CTA-Positive (N=279)
Endocrine	0/6, 0.0% (0.0, 45.9)	NA	0/4, 0.0% (0.0, 60.2)	0/2, 0.0% (0.0, 84.2)
GI	73/225, 32.4% (26.4, 39.0)	30/70, 42.9% (31.1, 55.3)	1/25, 4.0% (0.1, 20.4)	42/130, 32.3% (24.4, 41.1)
Hepatic	9/22, 40.9% (20.7, 63.6)	3/4, 75.0% (19.4, 99.4)	1/3, 33.3% (0.8, 90.6)	5/15, 33.3% (11.8, 61.6)
Reproductive	44/121, 36.4% (27.8, 45.6)	12/29, 41.4% (23.5, 61.1)	3/9, 33.3% (7.5, 70.1)	29/83, 34.9% (24.8, 46.2)
Soft tissue	3/14, 21.4% (4.7, 50.8)	NA	0/7, 0.0% (0.0, 41.0)	3/7, 42.9% (9.9, 81.6)
Thoracic	2/12, 16.7% (2.1, 48.4)	NA	0/3, 0.0% (0.0, 70.8)	2/9, 22.2% (2.8, 60.0)
Urinary	4/10, 40% (12.2, 73.8)	1/1, 100.0% (2.5, 100)	0/3, 0.0% (0.0, 70.8)	3/6, 50.0% (11.8, 88.2)
Other	6/34, 17.6% (6.8, 34.5)	0/3, 0.0% (0.0, 70.8)	2/4, 50.0% (6.8, 93.2)	4/27, 14.8% (4.2, 33.7)

*Based on binomial exact confidence interval method.
Database Cutoff Date:
KEYNOTE 164: September 9, 2019,
KEYNOTE 158: October 5, 2020

In the two (2) organ systems with the highest MSI-H prevalence across solid tumors, the ORR in CTA-positive/F1CDx-positive subpopulation GI (n=70) and reproductive (n=29) organ systems was 42.9% (95% CI: 31.1, 55.3) and 41.4% (95% CI: 23.5, 61.1) respectively.

4. 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 per BLA 125514/S-14 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.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator which was full-time employee of the sponsor and had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

- Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: [0]
- Significant payment of other sorts: [0]
- Proprietary interest in the product tested held by the investigator: [1]
- Significant equity interest held by investigator in sponsor of covered study: [0]

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. Statistical analyses were conducted by FDA to determine whether the financial interests/arrangements had any impact on the clinical study outcome. The information provided does not raise any questions about the reliability of the data.

XI. 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, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The data from the analytical validation and clinical device bridging studies support the reasonable assurance of safety and effectiveness of the F1CDx assay when used in accordance with the indications for use as an aid in identifying MSI-H status in patients with solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options who may benefit from treatment with KEYTRUDA. Data from the KEYNOTE-158 and KEYNOTE 164 trials with a data cut-off date of October 5, 2020 and September 9, 2019 demonstrate that patients who had MSI-H status received benefit from treatment with KEYTRUDA and support the addition of the proposed CDx indication to F1CDx. As noted above that data presented in this summary of safety and effectiveness data is not fully aligned with the PMR (#3213-1) issued at the time of BLA-125514/S014 approval and therefore the latest drug USPI should be referred to for more information.

B. Safety Conclusions

The F1CDx assay is an *in vitro* diagnostic test, which involves testing of DNA extracted from FFPE tumor tissue. The assay can be performed using DNA extracted from existing (archival) tissue samples routinely collected as part of the diagnosis and patient care. The risks of the device are based on data collected in the clinical study conducted to support PMA approval as described above. Risks of the F1CDx assay are associated with failure of the device to perform as expected or failure to correctly interpret test results and, subsequently, inappropriate patient management decisions in cancer treatment.
Patients with false positive results may undergo treatment with KEYTRUDA without clinical benefit and may experience adverse reactions associated with KEYTRUDA therapy. Patients with false negative results may not be considered for treatment with KEYTRUDA.

There is also a risk of delayed results, which may lead to delay of treatment with KEYTRUDA when patients receive an MSI “Cannot be Determined” result by the F1CDx assay. Patients with MSI “Cannot be Determined" result due an FB-MSI score >0.0041 and <0.0124, should be re-tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. The likelihood of a patient receiving this result is 3.29% within solid tumors. Patients with an MSI- “Cannot Be Determined" due to a quality control (QC) failure should consider re-testing with FoundationOneCDx or a validated orthogonal (alternative) method, if clinically appropriate. The likelihood of a patient receiving an invalid result for MSI status which takes into account samples failing to meet QC criteria defined within pathology review, DNA extraction, Library Construction, and Hybrid Capture, genomic analysis and MSI QC specific criteria is approximately 28.5% within solid tumors as observed in the device clinical bridging study, refer to Section X.B. Based on an assessment of the failure rates observed within a

consecutively selected sub-set of 10,000 samples within the F1CDx clinical commercial database in total the F1CDx failure to yield an MSI status result due to QC failure is estimated as 21.5%. The distribution of failures by F1CDx based on the assessment of consecutively selected sub-set of 10,000 samples within F1CDx clinical commercial database were as follows: 8.15% failed pathology review (i.e., failed to meet required viable nucleated tissue volume and/or tumor nuclei), 3.15% failed to meet required DNA input for tests, 0.04% failed to meet LC QC, 0.14% failed to meet required hybrid capture QC, 1.03 % failed to meet genomic analysis QC, and 8.96% failed to meet MSI QC.

Because a significant number of patients will receive an MSI Cannot be Determined result the following limitation statements are included in the device labeling:

- For patients with solid tumors whose samples have MSI scores >0.0041 and <0.0124, an MSI "Cannot Be Determined" result is reported. Patients with this result should be re-tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. The likelihood of a patient receiving this result is ~3.29% within solid tumors.
- Patients with solid tumors may also receive an MSI status reported as "Cannot Be Determined" due to a quality control (QC) failure. When all sample-level quality metrics are met, the rate of MSI "Cannot Be Determined" results due to a QC failure is 8.96%. Patients with this result should consider re-testing with FoundationOneCDx or an orthogonal (alternative) method, if clinically appropriate.

Further a post-market study to assess the failure rate for all possible failure modes for the F1CDx assay with regards to MSI status stratified by sample age over the course of one year of F1CDx clinical commercial testing will be conducted to provide an assessment of the performance of the F1CDx assay for MSI calling in the commercial/clinical setting with regards to evaluable results.

C. Benefit-Risk Determination

Clinical validation data from KEYNOTE 158 Cohort K and KEYNOTE 164 demonstrated a meaningful response to KEYTRUDA in subjects identified as MSI-H by the F1CDx assay. Of the 444 clinical trial assay (CTA) positive patients eligible for this clinical validation, using KEYNOTE 158 Cohort K and KEYNOTE 164, 165 patients had valid F1CDx results. Of these 165 patients, 107 patients were positive by F1CDx; in this population, there were 46 responders to KEYTRUDA resulting in a clinically meaningful ORR of 43.0% (46/107), which was higher than the 31.8% observed in the CTA positive population (n=444). Also, by contrast, the F1CDx negative/CTA positive population (n=58), had a lower ORR of 12.1 % (7/58). These results support the probable benefit from the use of the F1CDx assay to select MSI-high patients for treatment with KEYTRUDA, in the context of a patient harboring disease that has progressed following prior treatment and where no satisfactory alternative treatment options are available.

The probable risks associated with the use of this device are mainly due to: 1) false positives; 2) false negatives, and failure to provide a result; 3) incorrect interpretation of test results by the user; and 4) there is a possibility of delayed results. The probable risks of the F1CDx assay are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial, which may lead to adverse events. A false negative result may prevent a patient from accessing a potentially beneficial drug. However, the risk is mitigated by the clinical and analytical studies for F1CDx detection of MSI-H status in patients with solid tumors. The risks of potential false positive and false negative results are also partially mitigated by the analytical accuracy study, which showed an acceptable PPA and NPA, as described above, compared to PCR and IHC comparators.

The clinical and analytical performance of the device included in this submission demonstrate that the assay performance is expected to mitigate the probable risks associated with the use of this device. Although the overall clinical and analytical performance data were supportive of the indication, supplemental data for interfering substances as well as additional analyses for FFPE slide stability are needed in the post market setting along with other studies. An additional condition of approval clinical study is also needed (see Section XIII) to further confirm robust performance of the F1CDx assay to identify solid tumor patients with MSI-H status to be treated with KEYTRUDA.

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, treatment with KEYTRUDA provides meaningful clinical benefit to patients with MSI-H solid tumors, as measured by ORR demonstrated in the KEYNOTE-158 and KEYNOTE 164 trials. Given the available information, the data supports the conclusion that F1CDx has probable benefit is greater than the probable risk, in selecting patients with solid tumors with MSI-H status for treatment with KEYTRUDA.

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 clinical bridging study support the performance of F1CDx as an aid for the identification of MSI-H patients with solid tumors for whom KEYTRUDA may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on February 18, 2022. The final non-clinical and clinical conditions of approval cited in the approval order are described below.

The following data should be provided as separate reports, which may be followed by a PMA supplement, where applicable:

Interference

1. FMI will provide data evaluating the effects of endogenous interfering substances including necrotic tissue, melanin, and hemoglobin, and exogenous interference substances including molecular barcodes, proteinase K, and ethanol. The samples selected for this assessment will represent a range of solid tumors across the intended use population, including MSI-H samples. The data from this study must be adequate to support that potential endogenous interfering substances in solid tumors do not adversely impact F1CDx MSI calling.

   The study data and conclusions should be submitted within 3 months of the PMA approval date.

Stability

2. FMI must provide data from FFPE slide stability studies to support robust MSI calling for F1CDx. The samples included in the analyses to support FFPE slide stability with respect to MSI calling, respectively, must represent a range of solid tumors across the intended use population, including sufficient MSI-H samples. The data from this study must be adequate to support the F1CDx FFPE slide stability duration claims for MSI calling.

   The study protocol should be submitted within 60 days of the PMA approval date, and the study data and conclusions should be submitted within 2 years of the PMA approval date.

3. FMI will provide failure rate for all possible failure modes for the F1CDx assay with regards to MSI status stratified by sample age over the course of one year of F1CDx clinical commercial testing. The data from this report will provide an assessment of the performance of the F1CDx assay for MSI calling in the commercial/clinical setting.

   The study data and conclusions should be submitted within 18 months of the PMA approval date.

Clinical Effectiveness

4. FMI must provide clinical outcome data as assessed by overall response rate and duration of response from 41 additional patients enrolled and treated with KEYTRUDA in the clinical study KEYNOTE 158 Cohort K that were tested with F1CDx. This information must be provided to confirm the clinical effectiveness of F1CDx as a companion diagnostic (CDx) device for identification of patients with solid tumors with MSI-H status who may benefit from treatment with KEYTRUDA.

   The clinical study protocol should be submitted within 30 days of the PMA approval date, and the study data and conclusions should be submitted within 13 months of the PMA approval date.

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

XIV. 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.

XV. REFERENCES

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