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/S016
Date of FDA Notice of Approval:	June 16, 2020

The original PMA (P170019) for FoundationOne®CDx (F1CDx) was approved on November 30, 2017 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, and ovarian cancer. Subsequently, eight PMA supplements were approved for expanding the indications for use of F1CDx since its original approval. PMA supplement (P170019/S005) for adding genomic loss of heterozygosity (LOH) was approved on April 10, 2019. 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. 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. PMA supplement (P170019/S006) for adding an indication for PIQRAY® (alpelisib) in breast cancer patients with *PIK3CA* alterations was approved on December 3, 2019. PMA supplement (P170019/S010) for adding a second site in Research Triangle Park, NC, where the F1CDx assay will be performed, was approved on December 16, 2019. PMA supplement (P170019/S013) for adding an indication for PEMZYRE® (pemigatinib) in cholangiocarcinoma patients with *FGFR2* fusions was approved on April 17, 2020. 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. 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.

The current supplement was submitted to expand the intended use of F1CDx to include a companion diagnostic indication for high tumor mutational burden (TMB) at the cut-off of 10 mutations per megabase (mut/Mb) 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 cancer 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

Indication	Biomarker	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), 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	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib)

Indication	Biomarker	Therapy
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)
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 $\geq$ 10 mutations per megabase	Keytruda® (pembrolizumab)

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 assay 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 ($\geq 20\%$ 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 SAMtools 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 microsatellite instability (MSI) status, 95 intronic homopolymer repeat loci (10-20 bp long in the human reference genome) with adequate coverage on the F1CDx assay are analyzed for length variability and compiled into an overall MSI score via principal components analysis (PCA). Using the 95 loci, for each sample the repeat length is calculated in each read that spans the locus. The means and variances of repeat lengths are recorded. PCA is used to project the 190-dimension data onto a single dimension (the first principal component) that maximizes the data separation, producing an MSI score. Each sample is assigned a qualitative status of MSI-High (MSI-H) or MSI-Stable (MSS); ranges of the MSI score are assigned MSI-H or MSS by manual unsupervised clustering. Samples with low coverage (< 250X median) are assigned a status of MSI-unknown.

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).

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. 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	

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. Physicians should consider the best method that suits their patients and that best meets their expectations.

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-V600E and V600K	THxID BRAF Kit	bioMerieux	PCR	MEKINIST (tramatenib)	Melanoma
	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

	Device	Company	Technology	Therapy	Indication
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)	Colorectal cancer
NRAS	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	Colorectal cancer
KRAS	**cobas** KRAS Mutation Test	Roche Molecular Systems, Inc.	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	Colorectal cancer
	therascreen KRAS RGQ PCR Kit	QIAGEN	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	Colorectal cancer
	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	Colorectal cancer
ALK – fusion	Vysis ALK Break Apart FISH Probe Kit	Abbott Molecular, Inc.	FISH	XALKORI (crizotinib)	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
	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

	Device	Company	Technology	Therapy	Indication
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.

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

The performance of F1CDx in detecting high TMB (TMB-H) as a qualitative pan tumor biomarker with respect to the 10 mut/Mb cut-off is supported by the data

presented using a broad range of tumor specimens across all validation studies. Over 400 unique samples comprising over 200 disease ontologies, including the rare cancers enrolled in the clinical validation study (please refer to Section X), were included in the analytical validation for TMB as a pan tumor biomarker. Samples had TMB scores ranging from 0 to 375 mut/Mb across a spectrum of tumor purities, from 4.8% to 99.9% (please refer to Section IX.A.2(a), below, for information on the detection limits of the TMB biomarker), as well as sufficient pre-analytical (e.g., percent tumor nuclei, DNA input) and post-sequencing (e.g., tumor purity, $\geq$ 100X coverage, median coverage) QC metrics to support robust F1CDx TMB calling across the intended use population. Analytical accuracy/concordance, limit of detection (LoD), and precision studies as well as analyses of DNA extraction and interfering substances data were conducted to support the indication for TMB as a pan tumor biomarker at the cut-off of $\geq$ 10 mut/Mb for TMB-H tumors.

For the F1CDx platform validation (P170019), device performance characteristics were established using DNA derived from a wide range of FFPE tissue types, and tissue types associated with CDx indications were included in each study. Each study included CDx variants as well as a broad range of representative alteration types, including substitution and insertion and deletion variants, in various genomic contexts across several genes. Analyses of genomic signatures including MSI and TMB were also conducted. TMB was previously analyzed as a score in mutations per megabase (mut/Mb) to support tumor profiling for the F1CDx platform (P170019). Results from the platform-level validation (P170019) have been leveraged, including precision, interfering substances, and DNA extraction studies, to support F1CDx detection of TMB as a qualitative biomarker with respect to the 10 mut/Mb cut-off for TMB-H samples. A post-market study will be conducted to support quantitative TMB score reporting for the CDx biomarker. For information regarding the F1CDx platform-level validation, please see Summary of Safety and Effectiveness Data P170019.

1. Analytical Accuracy/Concordance

a. Comparison to Whole Exome Sequencing for TMB Calling

An analytical accuracy study was performed to demonstrate the concordance between F1CDx and an externally validated whole exome sequencing (WES) assay for the detection of TMB-H as a qualitative biomarker. The WES assay sequenced matched tumor-normal samples to determine germline mutations in sample pairs, and germline mutations were filtered from the tumor sample results prior to the TMB calculation. The WES TMB algorithm included a variant calling threshold of 5% allele frequency or greater for SNVs and indels. Only mutations in coding regions were included in the TMB WES score. The final alteration count was converted to mut/Mb by dividing by 34.7 Mb, which was the total length of the coding regions in the WES assay.

A total of 218 samples with valid F1CDx and WES TMB scores were evaluated that represented prevalent tumor types as well as the rare tumors from the clinical validation study. 64 samples obtained from rare tumors were assessed, including 45 samples that were screened for patient enrollment into

the KEYNOTE-158 clinical study, which supports the clinical validation of the F1CDx assay for TMB (please refer to Section X, below). 154 samples represented common tumor types including NSCLC, breast cancer, CRC, ovarian cancer, and melanoma. Of the 218 samples in the concordance analysis, 89 were selected by a non-FMI assay, and the remaining 129 samples were selected by F1CDx. TMB measurements were dichotomized using 10 mut/Mb as the TMB-H cut-off for both the F1CDx and WES assays. The positive percent agreements (PPA) and negative percent agreements (NPA) with 95% confidence intervals (CI) derived through bootstrapping are provided for the sample set selected by a non-FMI assay (unadjusted for prevalence) and samples selected by F1CDx (adjusted for prevalence of TMB-H estimated as 19%) in Tables 8 and 9, respectively.

Table 8. TMB concordance summary for samples selected by a non-FMI assay (unadjusted for prevalence)

TMB Cut-off	F1CDx+/ WES+	F1CDx-/ WES+	F1CDx+/ WES-	F1CDx-/ WES-	Unadjusted PPA (95% CI)	Unadjusted NPA (95% CI)
10 mut/Mb	28	7	4	50	80.0% (62.5%, 90.62%)	92.59% (82.62%, 98.04%)

Table 9. TMB concordance summary for samples selected by F1CDx (adjusted for prevalence)

TMB Cut-off	F1CDx+/ WES+	F1CDx-/ WES+	F1CDx+/ WES-	F1CDx-/ WES-	Adjusted PPA (95% CI)	Adjusted NPA (95% CI)
10 mut/Mb	23	1	17	88	92.31% (65.74%, 100%)	90.84% (87.76%, 93.99%)

The overall PPA and NPA based on a weighted average of the results (unadjusted and adjusted for TMB-H prevalence) in the TMB concordance analysis are provided in Table 10, below, with 95% bootstrap CI.

Table 10. Overall TMB concordance summary (weighted average)

TMB Cut-off	Overall PPA (95% CI)	Overall NPA (95% CI)
10 mut/Mb	87.28% (64.42%, 96.17%)	91.56% (85.66%, 95.64%)

Overall PPA was 87.28% with 95% CI (64.42%, 96.17%) and overall NPA was 91.56% with 95% CI (85.66%, 95.64%). Passing-Bablok regression was performed to assess the relationship between F1CDx and WES using the underlying continuous TMB score. The estimated slope was 0.93 with 95% CI (0.87, 1.03), and the estimated offset was -0.08 mut/Mb with 95% CI (-0.46, 0.2).

29 discordant results were observed in the TMB concordance analysis. An investigation of the discordances determined that 10 of the 29 discordant samples had F1CDx TMB scores that were close to the cut-off of 10 mut/Mb for TMB-H samples. Further, an analysis of the alteration level agreements was performed to explore the differences in the underlying component variants included in the TMB scores determined by the F1CDx and WES TMB algorithms. The alteration level assessment identified discordances in variant calls between the F1CDx and WES assays due to driver mutation exclusions, differences in germline variant designation, variants detected at low allele frequencies (i.e., below the F1CDx or WES TMB algorithm threshold of 5% MAF), and mutations in non-coding regions. The alteration level evaluation determined that exclusion of driver mutations by the F1CDx TMB algorithm and not by the WES TMB algorithm as well as differences in germline variant designation to be the greatest sources of TMB component variant discordances between F1CDx and WES.

2. Analytical Sensitivity

a. Limit of Detection (LoD)

The limit of detection (LoD) for TMB calling by F1CDx based on the cut-off of 10 mut/Mb was estimated with respect to computational tumor purity. Computational tumor purity is calculated by fitting the observed log-ratio and minor allele frequency data with statistical models that predict a genome-wide copy number profile, tumor ploidy, and tumor purity (i.e., computational tumor purity). The log-ratio profile is obtained by normalizing aligned tumor sequence reads by dividing read depth by that of a process-matched normal control, followed by a GC-content bias correction using Loess regression. The minor allele frequency profile is obtained from the heterozygous genome-wide SNPs.

11 TMB-H ($\geq$ 10 mut/Mb) FFPE samples were included in the analysis representing lung, colon, breast, bladder, and skin cancers as well as rare tumor types in the clinical validation study including anal and endometrial tissues. LoD was assessed at 5 levels of computational tumor purity, ranging from 2.5% to 50%, with 20 replicates per level, except for the highest tumor purity level at which 14 replicates were tested. The LoD for TMB calling based on computational tumor purity was determined empirically by the hit rate method, defined as the lowest level with 95% hit rate.

The LoD for TMB calling based on computational tumor purity is 28.16%. The LoD may be driven by the proximity of the TMB score to the cut-off, as the sample with an average TMB score of 11.83 mut/Mb (at the highest level of tumor purity evaluated) had the highest hit rate LoD at 28.16% tumor purity for the cut-off of 10 mut/Mb.

Several samples were tested at or near the TMB LoD based on computational tumor purity in the precision study (please see Section IX.A.5). Please also

refer to Section IX.A.2 of Summary of Safety and Effectiveness Data for P170019 for additional analytical sensitivity data.

b. Limit of Blank (LoB)

The limit of blank (LoB) of zero was confirmed by demonstrating that the percentage of false positive results did not exceed 5% (type I error risk α=0.05). 21 biomarker-negative samples were processed for a total of 220 replicates. Of the 220 aliquots, one failed prior to sequencing, and 219 replicates were available for the LoB analysis. 16 of the 21 biomarker-negative samples had TMB scores of 0 mut/Mb, and the mean TMB score for all 21 samples evaluated was 0.14 mut/Mb. All 219 replicates were below the TMB cut-off of 10 mut/Mb (non-TMB-H), resulting in a false positive rate of 0% and thus confirming the LoB of zero.

3. Analytical Specificity

a. Interfering Substances

To evaluate the potential impact of endogenous and exogenous interfering substances on the performance of the F1CDx assay for TMB calling, an *in silico* assessment of interfering substances data for the F1CDx platform (P170019) was conducted. A total of 19 FFPE specimens were included in the evaluation representing a range of tumor types including colon, breast, lung, ovary, skin, liver, gastroesophageal, kidney, and prostate tissues. Two TMB-H samples, including one sample close to the 10 mut/Mb threshold for TMB-H, were analyzed. The addition of interfering substances, including melanin (endogenous), ethanol (exogenous), proteinase K (exogenous), and molecular index barcodes (MIB) (exogenous), was evaluated to determine if they impacted TMB calling with respect to the qualitative cut-off of 10 mut/Mb (Table 11).

Table 11. Interfering substances evaluated

Substance	Level	# Samples	# Replicates/Sample
No interferent	-	19	2 (4 controls for MIB)
Melanin	0.025 µg/mL	5	2
Melanin	0.05 µg/mL	5	2
Melanin	0.1 µg/mL	5	2
Melanin	0.2 µg/mL	5	2
Proteinase K	0.04 mg/mL	18	2
Proteinase K	0.08 mg/mL	18	2
Ethanol	2.5%	18	2
Ethanol	5%	18	2
MIB	0	18	2 or 4
MIB	5%	5	4
MIB	15%	18	2 or 4
MIB	30%	18	2 or 4

Each interfering substance was evaluated by the concordance of TMB status with the corresponding control sample with respect to the 10 mut/Mb cut-off. A summary of the overall concordance results for each interferent with corresponding 95% CI using score method is provided in Table 12, below.

Table 12. TMB concordance across each interfering substance

Substance	# Concordant	# Total	Concordance (95% score CI)
No interferent	55	56	98.2% (90.6%, 99.7%)
Melanin	38	40	95% (83.5%, 98.6%)
Proteinase K	72	72	100% (94.9%, 100%)
Ethanol	72	72	100% (94.9%, 100%)
MIB	110	111	99.1% (95.1%, 99.8%)

The concordance for TMB calling was 100% for the proteinase K and ethanol evaluations, 99.1% for the MIB evaluation, and 95% for the melanin evaluation.

A post-market interfering substances study will be performed to evaluate the effects of endogenous interfering substances, including necrotic tissue and hemoglobin, on TMB calling (≥ 10 mut/Mb) in solid tumors across the intended use population.

4. Carryover/Cross-Contamination

Please see Section IX.A.4 of Summary of Safety and Effectiveness Data for P170019.

5. Precision and Reproducibility

a. Intermediate Precision for TMB-H (≥ 10 mut/Mb)

A precision study was conducted to evaluate the intermediate precision for TMB-H calling with respect to the 10 mut/Mb cut-off in FFPE specimens across solid tumors. Samples were enriched for TMB-H status, and an additional analysis was performed to support F1CDx precision for TMB calling in non-TMB-H samples (please see Section IX.A.5(b), below). 46 samples were analyzed that represented prevalent solid tumors as well as the rare tumors included in the clinical validation study (please see Section X) covering 7 major organ systems: gastrointestinal, hepato-pancreatobiliary, urinary, endocrine, skin, thoracic, and reproductive. The study focused on the evaluation of F1CDx precision for TMB-H calling across challenging samples at low DNA input (i.e., close to the minimum requirement of 50 ng) with a range of TMB scores, including samples with TMB scores close to the 10

mut/Mb cut-off, and a range of tumor purities. Of the 46 samples, 22 were tested at challenging tumor purity levels near or below the established TMB LoD of 28.16% computational purity (please refer to Section IX.A.2(a), above).

Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three different sequencers and two different reagent lots, over multiple days (typical assay workflow spans 10 days). A full factorial study design was executed with four replicates per reagent lot/sequencer combination for a total of 24 replicates per sample. The previous precision studies for F1CDx (P170019) and FoundationFocus CDx$_{BRCA}$ (P160018) were conducted with 36 replicates using a full factorial study design and yielded high agreement rates; thus, 24 replicates per sample to demonstrate F1CDx precision for TMB calling were deemed acceptable to support this PMA supplement due to adequate F1CDx platform precision in the previous studies.

Based on the cut-off of 10 mut/Mb for TMB-H, there were 44 TMB-H and 2 non-TMB-H samples in this precision analysis. The TMB scores for the selected samples ranged from 6.25 mut/Mb to 156.4 mut/Mb (based on the mean TMB score for valid replicates per sample), and 6 samples had TMB scores near the TMB-H threshold of 10 mut/Mb. Repeatability and reproducibility results with the corresponding two-sided exact 95% CI are summarized in Table 13, below.

Table 13. Precision results for TMB-H (≥ 10 mut/Mb)

TMB Cut-off	Repeatability Positive Call Rate (95% exact CI)	Repeatability Negative Call Rate (95% exact CI)	Reproducibility Positive Call Rate (95% exact CI)	Reproducibility Negative Call Rate (95% exact CI)
10 mut/Mb	99.61% (98.61%, 99.95%)	95.83% (78.88%, 99.89%)	99.81% (99.3%, 99.98%)	97.83% (88.47%, 99.94%)

For repeatability, the PPA for 44 TMB-H samples was 99.61% with 95% CI (98.61%, 99.95%), and the NPA for 2 non-TMB-H samples was 95.83% with 95% CI (78.88%, 99.89%). For reproducibility, the PPA was 99.81% with 95% CI (99.3%, 99.98%), and the NPA was 97.83% with 95% CI (88.47%, 99.94%). The overall repeatability agreement for samples tested was 99.54% with 95% CI (98.39%, 99.98%), and overall reproducibility was 99.72% with 95% CI (99.18%, 99.94%).

For repeatability and reproducibility of the TMB score as a continuous variable, the coefficient of variance (%CV) from variance component analysis was estimated for each sample. All samples had %CV < 30% for repeatability and reproducibility.

b. Intermediate Precision for non-TMB-H (< 10 mut/Mb)

To provide additional precision data for F1CDx TMB calling in non-TMB-H samples (< 10 mut/Mb), an analysis was conducted in 20 FFPE specimens across multiple tumor types including lung, colon, skin, thyroid, salivary gland, liver, and uterus cancers. The study included challenging samples with low DNA input (i.e., close to the minimum requirement of 50 ng) and a range of tumor purities. Repeatability including intra-run performance and reproducibility including inter-run performance were assessed and compared across three different sequencers, two different reagent lots, and multiple days (typical assay workflow spans 10 days). A full factorial study design was executed with three replicates per two runs for a total of 36 replicates across the paired reagent lot/sequencer combinations.

Of the 20 samples tested in the additional precision analysis to support F1CDx TMB calling in non-TMB-H samples, there were 1 TMB-H and 19 non-TMB-H samples based on the cut-off of $\geq$ 10 mut/Mb for TMB-H. Overall repeatability and reproducibility results with the corresponding two-sided exact 95% CI are summarized in Table 14, below.

Table 14. Precision results for non-TMB-H (< 10 mut/Mb)

TMB Cut-off	Overall Repeatability (95% exact CI)	Overall Reproducibility (95% exact CI)
10 mut/Mb	100% (98.4%, 100%)	100% (99.5%, 100%)

Repeatability and reproducibility of TMB status with respect to the 10 mut/Mb cut-off was 100% for all samples. The overall repeatability agreement for samples tested was 100% with 95% CI (98.4%, 100%), and overall reproducibility was 100% with 95% CI (99.5%, 100%).

c. Site-to-site Precision for TMB

A site-to-site precision study was performed to support F1CDx TMB calling at the second site in Morrisville, NC. 46 FFPE samples representing ovarian, breast, lung, colorectal, and skin cancers across a range of tumor purities were included. A total of 9 TMB-H samples and 37 non-TMB-H samples were evaluated for the site-to-site precision analysis. Repeatability including intra-run performance and reproducibility including inter-run performance were assessed. Each of the 46 samples was tested at two sites (Cambridge, MA and Morrisville, NC) with two replicates, two reagent lots, and on three non-consecutive days by multiple operators. A full factorial study design was conducted with a total of 24 replicates across the paired reagent lot/sequencer combination. For the evaluation of TMB with respect to the 10 mut/Mb cut-off, repeatability and reproducibility was 100% for all replicates for 45 of 46 samples. Only one sample demonstrated 79.17% reproducibility and 58.33%

repeatability with 5 discordant replicates near the 10 mut/Mb threshold; this sample had an average computational tumor purity of 11.02%, which was below the TMB LoD level of 28.16% tumor purity (see Section IX.A.2(a), above). The overall repeatability agreement for samples tested was 99.05% with 95% CI (97.79%, 99.69%), and overall reproducibility was 99.53% with 95% CI (98.91%, 99.85%).

Additional post-market data will be provided for the intermediate precision of the TMB component alterations, including repeatability and reproducibility.

6. Reagent Lot Interchangeability

There were no changes to the reagents and specifications between the FoundationFocus™ CDx$_{BRCA}$ assay and F1CDx. Therefore, for reagent lot interchangeability results, please see Section IX.A(g) of Summary of Safety and Effectiveness Data for P160018.

7. 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. Additional post-market data for DNA and FFPE slide stability with respect to TMB biomarker ($\geq$ 10 mut/Mb) calling will be provided based on the re-analysis of the existing F1CDx platform-level data with the updated bioinformatics pipeline (v3.3.x).

8. General Lab Equipment and Reagent Evaluation

a. DNA Amplification

There were no changes to the reagents and specifications between the FoundationFocus™ CDx$_{BRCA}$ assay and F1CDx. For equipment and reagent interchangeability results, please see Section IX.A.h(a) of Summary of Safety and Effectiveness Data for P160018.

b. DNA Extraction

The performance of DNA extraction from FFPE tumor specimens was measured by the concordance of TMB status based on the qualitative TMB-H cut-off of 10 mut/Mb. 35 FFPE specimens represented a range of tissue types including lung, breast, ovarian, colorectal, bladder, brain, liver, pancreas, thyroid, prostate, and skin cancers. The study included 7 TMB-H samples, including one sample near the threshold of 10 mut/Mb, as well as challenging samples at low tumor purities. Samples were run in duplicate employing two different KingFisher Flex Magnetic Particle Processors and comparing across two or three extraction reagent lots. Concordance of TMB calling was analyzed across replicates for each sample, and the overall results with respect to TMB status are summarized in Table 15, below.

Table 15. Summary of TMB concordance across replicates in DNA extraction study

TMB Status	# of Concordant Replicates	# of Total Replicates	Concordance Rate (95% 2-sided score CI)
TMB-H	63	63	100% (94.25%, 100%)
non-TMB-H	285	290	98.28% (96.03%, 99.26%)

For additional details on the F1CDx platform DNA extraction study, please refer to Section IX.A.8(b) of Summary of Safety and Effectiveness Data P170019.

9. Guard banding/Robustness

Please see Section IX.A.9 of Summary of Safety and Effectiveness Data for P170019. Additional post-market data for guard banding with respect to TMB biomarker (≥ 10 mut/Mb) calling will be provided based on the re-analysis of the existing F1CDx platform-level data with the updated bioinformatics pipeline (v3.3.x).

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 clinical performance of FoundationOne®CDx (F1CDx) for detecting TMB-H (defined as TMB ≥ 10 mut/Mb) in patients with solid tumors was demonstrated in a prospectively-planned retrospective analysis of specimens from patients enrolled in the KEYNOTE-158 clinical study of pembrolizumab. Data generated from the KEYNOTE-158 trial supported the clinical validation of the F1CDx assay for the identification of TMB-H subjects (≥ 10 mut/Mb) with solid cancers who may benefit from pembrolizumab treatment.

A. FoundationOne®CDx Retrospective Analysis of TMB in KEYNOTE-158

The safety and effectiveness of F1CDx for detecting TMB-H patients with respect to the cut-point of 10 mut/Mb was demonstrated in a prospectively-planned retrospective analysis of patients enrolled in the KEYNOTE-158 study. TMB biomarker analysis was pre-specified in the clinical trial protocol and retrospectively analyzed by the F1CDx assay.

1. Study Design

KEYNOTE-158 is an ongoing, Phase 2, multicenter, non-randomized, open-label, multi-cohort study designed to evaluate the safety and efficacy of pembrolizumab monotherapy (200 mg intravenously [IV] every 3 weeks [Q3W]) in multiple types of advanced (unresectable or metastatic) solid cancers that have progressed

following prior treatment and who have no satisfactory alternative treatment options. The study included participants with solid tumors grouped into 10 different cohorts, A to J. The aim of KEYNOTE-158 is to evaluate predictive biomarkers for response to pembrolizumab monotherapy treatment across multiple tumor types, regardless of specific tumor histology.

Participants with any of the tumor types in cohorts A to J listed below for whom previous first-line treatment failed were eligible to enroll in KEYNOTE-158.

Cohorts:

A. Anal squamous cell carcinoma
B. Biliary adenocarcinoma (gallbladder or biliary tree [intrahepatic or extrahepatic] cholangiocarcinoma) except Ampulla of Vater cancers
C. Neuroendocrine tumors (well- and moderately-differentiated) of the lung, appendix, small intestine, colon, rectum, or pancreas
D. Endometrial carcinoma (sarcomas and mesenchymal tumors are excluded)
E. Cervical squamous cell carcinoma
F. Vulvar squamous cell carcinoma
G. Small cell lung carcinoma
H. Mesothelioma
I. Thyroid carcinoma
J. Salivary gland carcinoma (sarcomas and mesenchymal tumors are excluded)

In addition to having any of the advanced solid tumors noted above, participants were eligible for study enrollment 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; no known active central nervous system (CNS) metastasis, autoimmune disease, immunosuppressive therapy, or prior treatment with anticancer monoclonal antibody (mAb) within 4 weeks prior to study Day 1; and had adequate tumor tissue to test programmed death ligand-1 (PD-L1), gene expression profile (GEP), and microsatellite instability-high (MSI-H). The TMB biomarker was pre-specified and retrospectively analyzed by the F1CDx assay.

The primary endpoint was objective response rate (ORR), defined as the proportion of participants in the analysis population who had a response (complete response [CR] or partial response [PR]) as measured by blinded independent central review (BICR) per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1. Secondary endpoints were as follows:

- Duration of response (DOR), defined as the time from the first documented evidence of CR or PR until disease progression or death due to any cause, whichever occurred first;
- Progression free survival (PFS), defined as the time from randomization/the first dose of study treatment to documented progressive disease or death due to any cause, whichever occurred first; and

- Overall survival (OS), defined as the time from randomization/the first dose of study treatment to death due to any cause.

2. TMB Evaluation by F1CDx

Evaluation of the TMB biomarker was an objective for KEYNOTE-158. The interim analysis (IA)10 investigated the relationship between response to pembrolizumab treatment and TMB as determined by the F1CDx assay using a cut-point of 10 mut/Mb. Selection of 10 mut/Mb as the TMB score cut-point for TMB-H considered three key elements: 1) the ability to reliably enrich for objective response to pembrolizumab while preserving the percentage of responders (i.e., sensitivity) based on training data; 2) the relationship between TMB and inflammation in the tumor microenvironment as measured by gene expression; and 3) an effort to harmonize on a common TMB cut-off to define TMB-H across different sponsors working with TMB as an immunotherapy biomarker.

Pre-defined sample quality control (QC) testing parameters were used throughout the retrospective testing of KEYNOTE-158 clinical trial samples including, but not limited to, minimum DNA input, tumor purity, tissue volume, and average read depth to ensure adequate F1CDx performance of TMB calling.

The therapeutic efficacy (TE) population was the primary efficacy population to support the drug indication. For investigational testing and analysis of the TE population, a TMB result was valid if the following pre-specified sample inclusion criteria were met:

- Tissue volume $\geq$ 0.2 mm^3;
- Tumor nuclei content $\geq$ 10%; and
- Extracted DNA content > 55 ng.

For testing and analysis of the device validation (DV) population, a TMB result was considered valid if the following pre-specified sample inclusion criteria consistent with the F1CDx device specifications were met:

- Tissue volume $\geq$ 0.6 mm^3;
- Tumor nuclei content $\geq$ 20%; and
- Extracted DNA content > 55 ng.

In addition, all TE and DV population samples had sufficient DNA quantity and quality following extraction, in-process QC metrics, and acceptable post-sequencing metrics to be considered valid.

B. Study Population Demographics and Baseline Parameters

Baseline demographics and disease characteristics were generally similar in the TMB-H and non-TMB-H populations for both the TE and DV analysis populations (Table 16). At the time of IA10, the trial was being conducted globally in 21 countries, including the U.S., of which 81 sites had enrolled participants to the study treatment. Compared to the non-TMB-H population, the TMB-H population had a

higher percentage of participants whose tumors were PD-L1 positive (CPS ≥ 1). This is consistent with the understanding of the biological mechanisms by which tumors with increased neoantigens up-regulate PD-L1 to evade immune recognition. As expected, all participants with MSI-H tumors were in the TMB-H group, as MSI-H is a subset of the overall TMB-H population.

Table 16. Comparison of demographic and disease characteristics between TMB-H and non-TMB-H subjects evaluated by F1CDx for TE and DV populations

	TE Population				DV Population			
	TMB >=10 mut/Mb		TMB <10 mut/Mb		TMB >=10 mut/Mb		TMB <10 mut/Mb	
	n	(%)	n	(%)	n	(%)	n	(%)
Subjects in population	102		688		91		628	
Gender								
Male	35	(34.3)	253	(36.8)	31	(34.1)	226	(36.0)
Female	67	(65.7)	435	(63.2)	60	(65.9)	402	(64.0)
Age (Years)								
< 65	67	(65.7)	414	(60.2)	59	(64.8)	383	(61.0)
>= 65	35	(34.3)	274	(39.8)	32	(35.2)	245	(39.0)
Mean	60.0		60.2		59.7		60.1	
SD	10.7		12.0		11.2		12.0	
Median	61.0		61.0		60.0		61.0	
Range	27 to 80		22 to 87		27 to 80		22 to 85	
Race								
American Indian Or Alaska Native	0	(0.0)	4	(0.6)	0	(0.0)	3	(0.5)
Asian	17	(16.7)	133	(19.3)	15	(16.5)	118	(18.8)
Black Or African American	1	(1.0)	18	(2.6)	1	(1.1)	15	(2.4)
Multiple	0	(0.0)	6	(0.9)	0	(0.0)	5	(0.8)
American Indian Or Alaska Native, Black Or African American	0	(0.0)	1	(0.1)	0	(0.0)	1	(0.2)
Black Or African American, White	0	(0.0)	5	(0.7)	0	(0.0)	4	(0.6)
Native Hawaiian Or Other Pacific Islander	1	(1.0)	1	(0.1)	1	(1.1)	1	(0.2)
White	83	(81.4)	526	(76.5)	74	(81.3)	486	(77.4)
Ethnicity								
Hispanic Or Latino	2	(2.0)	45	(6.5)	1	(1.1)	40	(6.4)
Not Hispanic Or Latino	92	(90.2)	587	(85.3)	83	(91.2)	538	(85.7)
Not Reported	8	(7.8)	56	(8.1)	7	(7.7)	50	(8.0)

Geographic Region								
US	20	(19.6)	163	(23.7)	19	(20.9)	144	(22.9)
Non-US	82	(80.4)	525	(76.3)	72	(79.1)	484	(77.1)
ECOG								
[0] Normal Activity	42	(41.2)	277	(40.3)	37	(40.7)	254	(40.4)
[1] Symptoms, but ambulatory	59	(57.8)	409	(59.4)	53	(58.2)	372	(59.2)
[2] Ambulatory but unable to work	1	(1.0)	2	(0.3)	1	(1.1)	2	(0.3)
Metastatic Staging								
M0	9	(8.8)	72	(10.5)	7	(7.7)	65	(10.4)
M1	93	(91.2)	616	(89.5)	84	(92.3)	563	(89.6)
Brain Metastases Present								
Yes	6	(5.9)	17	(2.5)	3	(3.3)	16	(2.5)
No	96	(94.1)	671	(97.5)	88	(96.7)	612	(97.5)
Number of Prior Lines of Therapy								
0[†]	1	(1.0)	23	(3.3)	1	(1.1)	21	(3.3)
Adjuvant/Neoadjuvant/ Definitive[‡]	0	(0.0)	8	(1.2)	0	(0.0)	8	(1.3)
1	44	(43.1)	257	(37.4)	41	(45.1)	235	(37.4)
2	38	(37.3)	187	(27.2)	30	(33.0)	167	(26.6)
3	6	(5.9)	107	(15.6)	6	(6.6)	96	(15.3)
4	7	(6.9)	59	(8.6)	7	(7.7)	55	(8.8)
5 or more	6	(5.9)	47	(6.8)	6	(6.6)	46	(7.3)
Sum of Target Lesions Measurable at Baseline (mm)								
Subjects with data	102		684		91		624	
Mean	106.3		107.9		103.5		108.0	
SD	79.7		80.9		78.9		81.0	
Median	88.3		83.4		84.2		86.0	
Range	10.2 to 322.8		10.1 to 442.9		10.2 to 322.8		10.1 to 442.9	
Tumor Type								
ANAL	14	(13.7)	75	(10.9)	14	(15.4)	73	(11.6)
CERVICAL	16	(15.7)	59	(8.6)	15	(16.5)	52	(8.3)
CHOLANGIOCARCINOMA	0	(0.0)	63	(9.2)	0	(0.0)	55	(8.8)
ENDOMETRIAL	15	(14.7)	67	(9.7)	15	(16.5)	64	(10.2)
MESOTHELIOMA	1	(1.0)	84	(12.2)	1	(1.1)	80	(12.7)
NEUROENDOCRINE	5	(4.9)	82	(11.9)	5	(5.5)	73	(11.6)
SALIVARY	3	(2.9)	79	(11.5)	3	(3.3)	74	(11.8)
SMALL CELL LUNG	34	(33.3)	42	(6.1)	26	(28.6)	30	(4.8)
THYROID	2	(2.0)	78	(11.3)	2	(2.2)	75	(11.9)
VULVAR	12	(11.8)	59	(8.6)	10	(11.0)	52	(8.3)
Prior Radiation Therapy								

Yes	81	(79.4)	412	(59.9)	70	(76.9)	376	(59.9)
No	21	(20.6)	276	(40.1)	21	(23.1)	252	(40.1)
PD-L1 Status								
Positive	68	(66.7)	383	(55.7)	61	(67.0)	352	(56.1)
Negative	29	(28.4)	274	(39.8)	25	(27.5)	250	(39.8)
Not Evaluable	5	(4.9)	30	(4.4)	5	(5.5)	25	(4.0)
Missing	0	(0.0)	1	(0.1)	0	(0.0)	1	(0.2)
MSI-H Status								
MSI-High	14	(13.7)	0	(0.0)	14	(15.4)	0	(0.0)
non-MSI-High	81	(79.4)	672	(97.7)	73	(80.2)	614	(97.8)
Missing	7	(6.9)	16	(2.3)	4	(4.4)	14	(2.2)

† Participants did not receive systemic chemotherapy.

‡ Participants received adjuvant/neoadjuvant alone without recurrence < 12 months from completion of the therapy or received definitive therapy alone which cannot be considered as a line of therapy.

Subjects with unknown TMB status are not included.

(Database Cutoff Date: 27JUN2019).

The various tumor types in cohorts A through J were observed at different frequencies in the TMB-H and non-TMB-H populations. Small cell lung carcinoma (SCLC) was more common in the TMB-H population. Thyroid, neuroendocrine, mesothelioma, and salivary tumors each were more common in the non-TMB-H population. The differences in certain tumor types are based on small numbers, as 5 of the 10 tumor types were present in 5 or fewer participants in the TMB-H population. A high percentage of participants had received prior radiation therapy, which is consistent with the higher percentage of SCLC and other tumor types likely to be treated with radiation therapy.

C. Accountability of sPMA Cohort

At IA10 of KEYNOTE-158, a total of 1,072 participants were enrolled in the combined cohorts, A through J, of which 1,066 were treated with at least one dose of pembrolizumab (referred to as All Subjects as Treated [ASaT]) population). The safety analysis was conducted in the ASaT population of 1,066 participants, while the efficacy analysis population included 1,050 participants from the ASaT population who were enrolled 26 weeks prior to data cut-off.

From the enrolled population, 1,007 patients had samples that were accessioned at Foundation Medicine. One sample was lost in transit, resulting in a total of 1,006 sample test records.

Based on the F1CDx investigational sample specification requirements used to define the TE population, 808 subjects had a valid TMB result, and 199 subjects had samples that failed to meet the minimum test specifications: 5 participants did not pass pre-analytical QC due to insufficient samples for testing (n=1) or gender discordance (n=4); 36 did not pass pathology review based on TE specifications (i.e.,

≥ 10% tumor nuclei and ≥ 0.2 mm^3 viable nucleated tissue); 73 did not meet the minimum extracted DNA criterion (> 55 ng); and 86 yielded invalid results after sequencing and reporting. Among the 808 participants with a valid TMB result, 15 were enrolled < 26 weeks before the data cut-off for IA10, two were not treated, and one did not have a TMB score; as a result, the TE population consists of 790 patients with TMB data available. Of the 790 participants in the TE population with available TMB results, 102 were TMB-H (≥ 10 mut/Mb) and 688 were non-TMB-H (< 10 mut/Mb).

Based on the F1CDx final sample specification requirements used to define the DV population, 735 subjects had a valid TMB result, and 272 subjects had samples that failed to meet the minimum test specifications: 5 participants did not pass pre-analytical QC due to insufficient samples for testing (n=1) or gender discordance (n=4); 156 did not pass pathology review based on DV specifications (i.e., ≥ 20% tumor nuclei and ≥ 0.6 mm^3 of viable nucleated tissue); 40 did not meet the minimum extracted DNA criterion (> 55 ng); and 71 yielded invalid results after sequencing and reporting. Among the 735 patients with valid TMB results, 13 were enrolled < 26 weeks before the data cut-off for IA10, two were not treated, and one did not have a TMB score; as a result, the DV population consists of 719 patients with valid TMB scores. Of the 719 participants in the DV population with available TMB results, 91 were TMB-H (≥ 10 mut/Mb) and 628 were non-TMB-H (< 10 mut/Mb).

Information on the numbers of participants per cohort based on TMB-H versus non-TMB-H status are provided for the TE and DV populations, respectively, in Table 17 below.

Table 17. F1CDx TMB status availability by KEYNOTE-158 cohort

	TE Population		DV Population	
Cohort	**TMB Status Available**	**TMB Status Not Available**	**TMB Status Available**	**TMB Status Not Available**
A: Anal	89	23	87	25
B: Biliary	63	41	55	49
C: Neuroendocrine	87	20	78	29
D: Endometrial	82	25	79	28
E: Cervical	75	23	67	31
F: Vulvar	71	14	62	23
G: SCLC	76	31	56	51
H: Mesothelioma	85	33	81	37
I: Thyroid	80	23	77	26
J: Salivary Gland	82	27	77	32
Total	**790**	**260**	**719**	**331**

D. Safety and Effectiveness

1. Safety Results

The safety with respect to treatment with pembrolizumab was addressed during review of the sBLA and is not addressed in detail in this Summary of Safety and

Effectiveness Data. The safety profile of pembrolizumab in the TMB-H Safety Dataset is generally consistent with the established safety profile of pembrolizumab monotherapy. No new safety signals were identified in KEYNOTE-158. Please refer to Drugs@FDA for complete safety information on KEYTRUDA® (pembrolizumab).

2. Effectiveness Results

The effectiveness of F1CDx to identify TMB-H (≥ 10 mut/Mb) patients who may benefit from pembrolizumab treatment is supported by the efficacy results from IA10 of KEYNOTE-158. The efficacy results from KEYNOTE-158 show that pembrolizumab monotherapy provides a clinically meaningful benefit to participants with TMB-H advanced solid tumors that had progressed following prior treatment or who were intolerant to prior therapies. This was demonstrated by clinically meaningful ORR and DOR in the TE and DV populations.

The primary endpoint in KEYNOTE-158 was ORR, defined as the proportion of patients in the analysis population who had a response (CR or PR) as measured by central review (BIRC) per RECIST 1.1. For the pooled analysis across tumor types in cohorts A through J, response to pembrolizumab was enriched in TMB-H subjects, resulting in a clinically meaningful ORR of 29.4% in the TE population (Table 18) and 33% in the DV population (Table 19). By contrast, a lower ORR was observed in non-TMB-H subjects of 6.3% in the TE population and 6.5% in the DV population.

Table 18. Summary of best objective response based on RECIST 1.1 per central radiology assessment in TE population

Response Evaluation	TMB >=10 mut/Mb (N=102)			TMB <10 mut/Mb (N=688)		
	n	%	95% CI†	n	%	95% CI†
Complete Response (CR)	4	3.9	(1.1, 9.7)	11	1.6	(0.8, 2.8)
Partial Response (PR)	26	25.5	(17.4, 35.1)	32	4.7	(3.2, 6.5)
Objective Response (CR+PR)	**30**	**29.4**	**(20.8, 39.3)**	**43**	**6.3**	**(4.6, 8.3)**
Stable Disease (SD)	14	13.7	(7.7, 22.0)	227	33.0	(29.5, 36.6)
Non-CR/Non-PD (NN)	0	0.0	(0.0, 3.6)	3	0.4	(0.1, 1.3)
Progressive Disease (PD)	48	47.1	(37.1, 57.2)	349	50.7	(46.9, 54.5)
Non-evaluable (NE)	1	1.0	(0.0, 5.3)	13	1.9	(1.0, 3.2)
No Assessment	9	8.8	(4.1, 16.1)	53	7.7	(5.8, 10.0)

Central radiology assessed responses per RECIST 1.1 (confirmed) are included in this table.

† Based on binomial exact confidence interval method.

'No Assessment' (NA) counts subjects who had a baseline assessment evaluated by the central radiology assessment but no post-baseline assessment on the data cutoff date including missing, discontinuing or death before the first post-baseline scan.

Subjects with unknown TMB status are not included.

(Database Cutoff Date: 27JUN2019).

Table 19. Summary of best objective response based on RECIST 1.1 per central radiology assessment in DV population

Response Evaluation	TMB >=10 mut/Mb (N=91)			TMB <10 mut/Mb (N=628)		
	n	%	95% CI[†]	n	%	95% CI[†]
Complete Response (CR)	4	4.4	(1.2, 10.9)	11	1.8	(0.9, 3.1)
Partial Response (PR)	26	28.6	(19.6, 39.0)	30	4.8	(3.2, 6.7)
Objective Response (CR+PR)	**30**	**33.0**	**(23.5, 43.6)**	**41**	**6.5**	**(4.7, 8.8)**
Stable Disease (SD)	14	15.4	(8.7, 24.5)	210	33.4	(29.8, 37.3)
Non-CR/Non-PD (NN)	0	0.0	(0.0, 4.0)	3	0.5	(0.1, 1.4)
Progressive Disease (PD)	39	42.9	(32.5, 53.7)	313	49.8	(45.9, 53.8)
Non-evaluable (NE)	0	0.0	(0.0, 4.0)	12	1.9	(1.0, 3.3)
No Assessment	8	8.8	(3.9, 16.6)	49	7.8	(5.8, 10.2)

Central radiology assessed responses per RECIST 1.1 (confirmed) are included in this table.

[†] Based on binomial exact confidence interval method.

'No Assessment' (NA) counts subjects who had a baseline assessment evaluated by the central radiology assessment but no post-baseline assessment on the data cutoff date including missing, discontinuing or death before the first post-baseline scan.

Subjects with unknown TMB status are not included.

(Database Cutoff Date: 27JUN2019).

ORR was assessed by tumor type, and the results were similar in the TE and DV populations (Tables 20 and 21, respectively). ORR was generally higher in the TMB-H population for most tumor types than in the non-TMB-H population. Data from the biliary (cholangiocarcinoma) and mesothelioma tumor types were not informative, as there were no biliary participants and only one mesothelioma patient with TMB-H status. The enrichment of response in TMB-H anal cancer was not observed; however, participants with anal cancer, as well as participants with other tumor types, exhibited a spectrum of TMB.

Table 20. Summary of best objective response per tumor type based on RECIST 1.1 per central radiology assessment in TE population

Tumor Type	TMB >=10 mut/Mb				TMB <10 mut/Mb				ORR Ratio
	N	n	%	95% CI[†]	N	n	%	95% CI[†]	TMB >=10 mut/Mb vs. TMB <10 mut/Mb
Overall	102	30	29.4	(20.8, 39.3)	688	43	6.3	(4.6, 8.3)	4.7
Anal	14	1	7.1	(0.2, 33.9)	75	8	10.7	(4.7, 19.9)	0.7
Cholangiocarcinoma	0	0	--	--	63	2	3.2	(0.4, 11.0)	--
Neuroendocrine	5	2	40.0	(5.3, 85.3)	82	1	1.2	(0.0, 6.6)	32.8
Endometrial	15	7	46.7	(21.3, 73.4)	67	4	6.0	(1.7, 14.6)	7.8
Cervical	16	5	31.3	(11.0, 58.7)	59	7	11.9	(4.9, 22.9)	2.6
Vulvar	12	2	16.7	(2.1, 48.4)	59	2	3.4	(0.4, 11.7)	4.9
Small Cell Lung	34	10	29.4	(15.1, 47.5)	42	4	9.5	(2.7, 22.6)	3.1
Mesothelioma	1	0	0.0	(0.0, 97.5)	84	9	10.7	(5.0, 19.4)	0.0
Thyroid	2	2	100.0	(15.8, 100.0)	78	3	3.8	(0.8, 10.8)	26.0
Salivary	3	1	33.3	(0.8, 90.6)	79	3	3.8	(0.8, 10.7)	8.8

[†] Based on binomial exact confidence interval method.
Subjects with unknown TMB status are not included.
(Database Cutoff Date: 27JUN2019).

Table 21. Summary of best objective response per tumor type based on RECIST 1.1 per central radiology assessment in DV population

Tumor Type	TMB >=10 mut/Mb				TMB <10 mut/Mb				ORR Ratio
	N	n	%	95% CI[†]	N	n	%	95% CI[†]	TMB >=10 mut/Mb vs. TMB <10 mut/Mb
Overall	91	30	33.0	(23.5, 43.6)	628	41	6.5	(4.7, 8.8)	5.0
Anal	14	1	7.1	(0.2, 33.9)	73	8	11.0	(4.9, 20.5)	0.7
Cholangiocarcinoma	0	0	--	--	55	2	3.6	(0.4, 12.5)	--
Neuroendocrine	5	2	40.0	(5.3, 85.3)	73	1	1.4	(0.0, 7.4)	29.2
Endometrial	15	7	46.7	(21.3, 73.4)	64	3	4.7	(1.0, 13.1)	10.0
Cervical	15	5	33.3	(11.8, 61.6)	52	6	11.5	(4.4, 23.4)	2.9
Vulvar	10	2	20.0	(2.5, 55.6)	52	2	3.8	(0.5, 13.2)	5.2
Small Cell Lung	26	10	38.5	(20.2, 59.4)	30	4	13.3	(3.8, 30.7)	2.9
Mesothelioma	1	0	0.0	(0.0, 97.5)	80	9	11.3	(5.3, 20.3)	0.0
Thyroid	2	2	100.0	(15.8, 100.0)	75	3	4.0	(0.8, 11.2)	25.0
Salivary	3	1	33.3	(0.8, 90.6)	74	3	4.1	(0.8, 11.4)	8.2

[†] Based on binomial exact confidence interval method.
Subjects with unknown TMB status are not included.
(Database Cutoff Date: 27JUN2019).

In the TE population, responses were durable, with the median DOR not reached in the TMB-H population at the time of the IA10 data cut-off based on Kaplan-Meier (KM) estimation. 66.6% of responders in the TMB-H population had a

DOR ≥ 24 months. In the non-TMB-H population, median DOR was 33.1 months, and 58.5% had a DOR ≥ 24 months. In the DV population, responses were consistent with those observed in the TE population: 66.6% of responders in the TMB-H population had a DOR ≥ 24 months; and in the non-TMB-H population, median DOR was 33.1 months, and 56.6% had a DOR ≥ 24 months.

Clinical response based on ORR was assessed for each biomarker category (i.e., TMB, PD-L1, and MSI-H) in the TE and DV populations (Tables 22 and 23, respectively). ORR was not driven by MSI-H status, as the pooled response rate in TMB-H subjects was similar when MSI-H patients (n=14) were excluded from the TMB-H group. In the TE population, the ORR was 26.1% with MSI-H patients excluded (n=88) compared to an overall ORR of 29.4% (n=102). In the DV population, the ORR was 29.9% with MSI-H patients excluded (n=77) compared to an overall ORR of 33% (n=91). In addition, TMB-H was associated with efficacy of pembrolizumab in participants with either PD-L1 positive (CPS ≥ 1) or PD-L1 negative (CPS < 1) tumors. In TMB-H subjects in the TE population, ORR was 35.3% in patients who were also PD-L1 positive, and ORR was 20.7% in PD-L1 negative patients. Similarly, in TMB-H subjects in the DV population, ORR was 39.3% in patients who were also PD-L1 positive, and ORR was 24% in PD-L1 negative patients. These results indicate that TMB is associated with the efficacy of pembrolizumab monotherapy regardless of PD-L1 expression or MSI-H status.

Table 22. Summary of best objective response based on RECIST 1.1 per central radiology assessment by biomarkers in TE population

TMB Status	Biomarker Category	N	Responders (n)	ORR (95% CI)[†]
	Participants with CPS	754		
TMB-H [‡]	CPS >=1	68	24	35.3 (24.1, 47.8)
	CPS <1	29	6	20.7 (8.0, 39.7)
Non-TMB-H [‡]	CPS >=1	383	33	8.6 (6.0, 11.9)
	CPS <1	274	9	3.3 (1.5, 6.1)
	Participants with MSI status	767		
TMB-H [§]	MSI-H	14	7	50.0 (23.0, 77.0)
	non-MSI-H	81	23	28.4 (18.9, 39.5)
Non-TMB-H [§]	MSI-H	0	-	-
	non-MSI-H	672	43	6.4 (4.7, 8.5)
	Participants with CPS and MSI Status	732		
TMB-H [‖]	CPS >=1 and MSI-H	9	4	44.4 (13.7, 78.8)
	CPS >=1 and non-MSI-H	55	20	36.4 (23.8, 50.4)
	CPS <1 and MSI-H	5	3	60.0 (14.7, 94.7)
	CPS <1 and non-MSI-H	22	3	13.6 (2.9, 34.9)
Non-TMB-H [‖]	CPS >=1 and MSI-H	0	-	-

	CPS >=1 and non-MSI-H	377	33	8.8 (6.1, 12.1)
	CPS <1 and MSI-H	0	-	-
	CPS <1 and non-MSI-H	264	9	3.4 (1.6, 6.4)

Subjects with unknown TMB status are not included.
† Based on binomial exact confidence interval method.
‡ Subjects with TMB status and PD-L1 status data available.
§ Subjects with TMB status and MSI-H status data available.
‖ Subjects with TMB status, PD-L1 status, and MSI-H status data available.
(Database Cutoff Date: 27JUN2019).

Table 23. Summary of best objective response based on RECIST 1.1 per central radiology assessment by biomarkers in DV population

TMB Status	Biomarker Category	N	Responders (n)	ORR (95% CI)†
	Participants with CPS	688		
TMB-H ‡	CPS >=1	61	24	39.3 (27.1, 52.7)
	CPS <1	25	6	24.0 (9.4, 45.1)
Non-TMB-H ‡	CPS >=1	352	32	9.1 (6.3, 12.6)
	CPS <1	250	8	3.2 (1.4, 6.2)
	Participants with MSI status	701		
TMB-H §	MSI-H	14	7	50.0 (23.0, 77.0)
	non-MSI-H	73	23	31.5 (21.1, 43.4)
Non-TMB-H §	MSI-H	0	-	-
	non-MSI-H	614	41	6.7 (4.8, 9.0)
	Participants with CPS and MSI Status	671		
TMB-H ‖	CPS >=1 and MSI-H	9	4	44.4 (13.7, 78.8)
	CPS >=1 and non-MSI-H	50	20	40.0 (26.4, 54.8)
	CPS <1 and MSI-H	5	3	60.0 (14.7, 94.7)
	CPS <1 and non-MSI-H	19	3	15.8 (3.4, 39.6)
Non-TMB-H ‖	CPS >=1 and MSI-H	0	-	-
	CPS >=1 and non-MSI-H	347	32	9.2 (6.4, 12.8)
	CPS <1 and MSI-H	0	-	-
	CPS <1 and non-MSI-H	241	8	3.3 (1.4, 6.4)

Subjects with unknown TMB status are not included.
† Based on binomial exact confidence interval method.
‡ Subjects with TMB status and PD-L1 status data available.
§ Subjects with TMB status and MSI-H status data available.
‖ Subjects with TMB status, PD-L1 status, and MSI-H status data available.
(Database Cutoff Date: 27JUN2019).

A sensitivity analysis was conducted to evaluate the robustness of the ORR estimates in TMB-H patients in the KEYNOTE-158 study to the approximately

25% of participants with missing data in the TE population (260 subjects had TMB status not available of 1,050 total subjects) and 30% of participants with missing data in the DV population (331 subjects had TMB status not available of 1,050 total subjects). The imputation model was driven by a hierarchical Bayesian logistic regression. An analysis of the association between baseline variables, missingness, and clinical outcome to support a missing at random proposition led to the selection of a final model for imputing TMB status containing cancer cohort and objective response. For the TE population, the imputation-based ORR was 29.7% with 95% CI (21.3%, 38.1%). For the DV population, the imputation-based ORR was 30.3% with 95% CI (21.7%, 39%). These results were similar to the to the ORRs for the 790 subjects with valid F1CDx TMB results in the TE population (29.4% [20.8%, 39.3%]) and for the 719 subjects with valid TMB results in the DV population (33% [23.5%, 43.6%]). Therefore, the observed enrichment of ORR in patients with TMB-H tumors is robust to the absence of valid F1CDx TMB scores in some patients enrolled in KEYNOTE-158.

3. Pediatric Extrapolation

In this premarket application, existing clinical data was leveraged to support approval in the pediatric population. The drug indication is for adult and pediatric patients with unresectable or metastatic solid tumors with TMB-H ($\geq$ 10 mut/Mb) that have progressed following prior treatment and who have no satisfactory or 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 who is a 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:

- Proprietary interest in the product tested held by the investigator

The applicant has adequately disclosed the financial interest/arrangements with clinical investigators. 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 supplement 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 effectiveness of the F1CDx assay to identify solid tumor patients with high TMB (TMB-H) to be treated with pembrolizumab was demonstrated through a retrospective analysis of KEYNOTE-158 clinical trial specimens. The data from the analytical and clinical validation 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 selecting patients with TMB-H (≥ 10 mut/Mb) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. Data from the KEYNOTE-158 trial demonstrate that patients who had TMB-H status with respect to the ≥ 10 mut/Mb cut-off received benefit from treatment with pembrolizumab and support the addition of the proposed CDx indication to F1CDx.

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® (pembrolizumab) without clinical benefit and may experience adverse reactions associated with pembrolizumab therapy. Patients with false negative results may not be considered for treatment with Keytruda® (pembrolizumab). There is also a risk of delayed results, which may lead to delay of treatment with Keytruda® (pembrolizumab).

C. Benefit-Risk Determination

The probable benefits of the F1CDx assay for the TMB-H (≥ 10 mut/Mb) indication in patients with solid tumors were based on data collected in the KEYNOTE-158 clinical trial, which supports the clinical validation for PMA approval. The clinical benefit of F1CDx for the identification of patients with TMB-H solid tumors was demonstrated in a prospectively-planned retrospective analysis of efficacy and safety data obtained from the Phase 2 multicenter, non-randomized, open-label, multi-cohort study of pembrolizumab monotherapy in multiple types of advanced (unresectable or metastatic) solid cancers that have progressed following prior treatment and who have no satisfactory alternative treatment options.

Potential risk associated with the use of this device are mainly due to: 1) false positives; 2) false negatives, and failure to provide a result; and 3) incorrect interpretation of test results by the user. The 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 TMB as a pan tumor qualitative biomarker with respect to the 10 mut/Mb cut-point. The supporting clinical validation analyses demonstrated that response to pembrolizumab was enriched in TMB-H subjects as determined by the F1CDx assay, resulting in a clinically meaningful ORR of 29.4% in the TE population and 33% in the DV population; by contrast, a lower ORR was observed in non-TMB-H subjects of 6.3% in the TE population and 6.5% in the DV population. Therefore, these results support the use of the F1CDx assay as an aid in selecting patients with TMB-H ($\geq$ 10 mut/Mb) solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. The risks of potential false positive and false negative results are partially mitigated by the analytical accuracy study, which showed an acceptable PPA and NPA, as described above, compared to WES.

The clinical and analytical performance of the device included in this submission demonstrate that the assay performance is expected to mitigate the potential 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 and alteration level precision as well as additional *in silico* analyses for DNA and FFPE slide stability and guard banding studies are needed as conditions of approval to demonstrate robust performance of the F1CDx assay to identify solid tumor patients with TMB-H ($\geq$ 10 mut/Mb) status to be treated with pembrolizumab.

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

In conclusion, treatment with pembrolizumab provides meaningful clinical benefit to patients with TMB-H solid tumors, as measured by ORR demonstrated in the KEYNOTE-158 trial. Given the available information, the data supports the conclusion that F1CDx has probable benefit in selecting solid tumor patients with high TMB ($\geq$ 10 mut/Mb) for treatment with pembrolizumab.

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 validation study support the performance of F1CDx as an aid for the identification of TMB-H patients with solid tumors for whom Keytruda® (pembrolizumab) may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on June 16, 2020. The final conditions of approval cited in the approval order are described below.

The applicant will provide the following in a post-approval report:

1. You must provide data evaluating the effects of endogenous interfering substances including necrotic tissue and hemoglobin. The samples selected for this assessment will represent a range of solid tumors across the intended use population, including sufficient TMB-H (≥ 10 mut/Mb) samples. The data from this study must be adequate to support that potential endogenous interfering substances in solid tumors do not adversely impact F1CDx TMB calling.

2. You must provide data from an evaluation to support robust TMB calling within the F1CDx stability claims for DNA and FFPE slide stability. You may leverage the existing F1CDx platform DNA and FFPE slide stability data (P170019); however, you must provide the study results with the data generated by the updated F1CDx bioinformatics pipeline v3.3.x. The samples included in the analyses to support DNA and FFPE slide stability with respect to TMB calling, respectively, must represent a range of solid tumors across the intended use population, including sufficient TMB-H (≥ 10 mut/Mb) samples. The data from this study must be adequate to support the F1CDx DNA and FFPE slide stability duration claims for TMB calling.

3. You must provide data from a guard banding study to support the performance of F1CDx TMB calling and the impact of process variation with regard to uncertainty in the measurement of DNA concentration at various stages of the process. You may leverage the existing F1CDx platform guard banding data (P170019); however, you must provide the study results with the data generated by the updated F1CDx bioinformatics pipeline v3.3.x. The samples included in the guard banding analysis must represent a range of solid tumors across the intended use population, including sufficient TMB-H (≥ 10 mut/Mb) samples.

4. You must provide the F1CDx intermediate precision results, including repeatability and reproducibility, for the TMB component alterations, i.e., percentage agreement of variant calls among the replicates under repeatability or reproducibility conditions. The data from this supplemental analysis must provide information regarding the F1CDx precision for the underlying component variants included in the TMB score.

5. You must provide a robust and high-quality data set and analysis to support reporting the quantitative TMB score. To support quantitative TMB reporting, you must include clinical validation data from a well-conducted clinical study for the quantitative TMB score measurement (i.e., clinically meaningful efficacy for every unit of change of each continuous output) by an appropriate statistical analysis where patients' response status is the response variable and TMB scores and cancer types

are the predictor variables. Sensitivity analysis should also be conducted on the missing quantitative TMB scores, and quantitative TMB scores should be compared among baseline demographic characteristics for statistical differences. The samples selected for these studies will represent a range of solid tumors and TMB scores across the intended use population. The data from the clinical study must be adequate to support that TMB score is a significant variable to predict patients' response status and clinically meaningful efficacy. The data from the analytical studies must be adequate to support robust quantitative TMB score reporting in the intended use population.

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

1. Fisher S., Barry A., Abreu J., et al. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. Genome Biol 12, R1 (2011).
2. Karolchik, D., Hinrichs AS, Kent WJ., et al. The UCSC Table Browser data retrieval tool. Nucleic Acids Res 32, D493-496 (2004).
3. Gnirke A., Melnikov A., Maguire J., et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol 27, 182-189 (2009).
4. Li H. & Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26, 589-595 (2010).
5. Li H., Handsaker B., Wysoker A., et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078-2079 (2009).
6. DePristo MA., Banks E., Poplin R., et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43, 491-498 (2011).
7. Forbes SA, Bindal N., Bamford S., et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 39, D945-950 (2011).
8. Compeau PE., Pevzner PA., Tesler, G. How to apply de Bruijn graphs to genome assembly. Nat Biotechnol 29, 987-991 (2011).