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/S013
Date of FDA Notice of Approval:	April 17, 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, four PMA supplements were approved for expanding the intended use of F1CDx since its original approval: PMA supplement (P170019/S005) for adding a claim to detect genomic loss of heterozygosity (LOH) in ovarian cancer patients 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.

The current supplement was submitted to expand the intended use of F1CDx t include a companion diagnostic indication for *FGFR2* fusions and select rearrangements in cholangiocarcinoma patients who may benefit from treatment with PEMAZYRE® (pemigatinib).

II. INDICATIONS FOR USE

FoundationOne®CDx (F1CDx) is a next generation sequencing based in vitro diagnostic device 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)
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS (exons 2, 3, and 4) and *NRAS* (exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Lynparza® (olaparib) or Rubraca® (rucaparib)

Indication	Biomarker	Therapy
Breast cancer	*PIK3CA* C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	PIQRAY® (alpelisib)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre® (pemigatinib)

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 (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 is be performed at Foundation Medicine, Inc. sites located in Cambridge, MA and Morrisville, NC.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS/PRECAUTIONS AND LIMITATIONS

The warnings/precautions and limitations are included in the F1CDx assay labeling.

V. DEVICE DESCRIPTION

FoundationOne CDx (F1CDx) is performed at Foundation Medicine, Inc. 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 complete list of genes included in F1CDx). In total, the assay therefore detects alterations in a total of 324 genes. Using the Illumina® HiSeq 4000 platform, hybrid-capture–selected libraries will be sequenced to high uniform depth (targeting > 500X median coverage with > 99% of exons at coverage > 100X). Sequence data is 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) and tumor mutational burden (TMB) will be reported.

Table 2. Genes with full coding exonic regions included in F1CDx for the detection of substitutions, insertion-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 fusions/gene rearrangements, a promoter region and a ncRNA gene.

ALK	*BRCA1*	*ETV4 introns 5, 6*	*EZR*	*KIT intron 16*	*MYC intron 1*	*NUTM1 intron 1*	*RET*	*SLC34A2 intron 4*

introns 18, 19	*introns 2, 7, 8, 12, 16, 19, 20*		*introns 9-11*				*introns 7-11*	
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

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 qualitied by FMI

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

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

A. Specimen Collection and Preparation

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

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

B. DNA Extraction

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

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

C. Library Construction

Library Construction (LC) begins with the normalization of DNA to 50-1000 ng. The normalized DNA samples are randomly sheared (fragmented) to ~200 bp by adaptive focused acoustic sonication using a Covaris LE220 before purification using 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 a Bravo Benchbot (Agilent) 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.

Quality Control for Hybrid Capture is performed by measuring dsDNA yield using a 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 dNTP's 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 is analyzed using proprietary software developed by FMI. Sequence data is mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA) v0.5.9.[4] PCR duplicate read removal and sequence metric collection is 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 megabases (Mb). Pairs are clustered by genomic coordinate of the pairs, and clusters containing at least five (5) chimeric pairs [three (3) 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 assessed 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 F1CDx Assay are analyzed for length variability and compiled into an overall MSI score via principal components analysis. 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. Principal component analysis (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 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 is displayed in the FMI custom-developed CATi software applications with sequence quality control 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 is reviewed by trained bioinformatics personnel. Samples failing any QC metrics are automatically held and not released.

G. Report Generation

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

H. Internal Process Controls Related to the System

Positive Control

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

Sensitivity Control

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

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 5, 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.

Table 5. 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

	Device	Company	Technology	Therapy	Indication
BRAF-V600	THxID BRAF Kit	bioMerieux	PCR	Mekinist (tramatenib)	Melanoma
	cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems, Inc.	PCR	Zelboraf (vemurafenib)	Melanoma
BRAF-600E	THxID BRAF Kit	bioMerieux	PCR	Tafinlar (dabrafenib)	Melanoma
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	Tafinlar (dabrafenib) Mekinist (trametinib)	Non-small cell lung 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)	Non-small cell lung cancer
	ALK (D5F3) CDx Assay	Ventana Medical Systems, Inc.	IHC	Xalkori (crizotinib)	Non-small cell lung cancer
***EGFR* – Exon 19**	**cobas** EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	Tarceva (erlotinib) Tagrisso (osimertinib) Iressa (gefitinib)	Non-small cell lung cancer
***EGFR* T790M**	*therascreen* EGFR RGQ PCR Kit	QIAGEN	PCR	Gilotrif (afatinib) Iressa (gefitinib)	Non-small cell lung cancer
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	Iressa (gefitinib)	Non-small cell lung cancer
	cobas EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	Tagrisso (osimertinib)	Non-small cell lung cancer
BRCA1/2	FoundationFocus CDx*BRCA*	Foundation Medicine, Inc.	NGS	Rubraca (rucaparib)	Advanced ovarian cancer
PIK3CA	*therascreen* PIK3CA RGQ PCR Kit	QIAGEN	PCR	PIQRAY (alpelisib)	Breast cancer

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 US since March 30, 2018. The following PMA supplements affecting the Intended Use were approved by FDA:

- P170019/S005 for adding LOH was approved on April 10, 2019 in ovarian cancer patients
- P170019/S004 for adding an indication for LYNPARZA® (olaparib) in ovarian cancer patients with *BRCA1/2* alterations was approved on July 1, 2019
- P170019/S008 for adding an indication for TAGRISSO® (osimertinib) in non-small cell lung cancer (NSCLC) patients with *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations was approved on July 1, 2019.
- P170019/S006 for adding an indication for PIQRAY® (alpelisib) in breast cancer patients with *PIK3CA* alterations was approved on December 3, 2019

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

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

Analytical validation for F1CDx platform-level validation (P170019), performance characteristics were established using DNA derived from a wide range of FFPE tumor tissue types in support of companion diagnostic (CDx) indications and have been described previously (P170019). Each study included CDx variants as well as a broad range of representative alteration types (substitution, insertion and deletion, copy number alterations, fusions and rearrangements) in various genomic contexts across several genes. The platform validation studies included samples with *FGFR2* fusions and a rearrangement and other gene rearrangement in FFPE tissue specimens other than cholangiocarcinoma which have been leveraged to support F1CDx detection of *FGFR2* rearrangement and were not repeated (i.e., cross-contamination, reagent stability, DNA

stability, and guard band studies). Cholangiocarcinoma is a rare tumor type (0.001%) and *FGFR2* fusions and rearrangements are found in approximately 10% of cholangiocarcinoma studies. Results from non-cholangiocarcinoma samples carrying *FGFR2* fusion or rearrangement and other gene rearrangement were acceptable following successful demonstration of comparability of assay performance across tumor tissue types.

This section summarizes the analytical studies conducted to support the identification of *FGFR2* rearrangements for cholangiocarcinoma (CCA) patients.

1. Analytical Accuracy/Concordance

a. Comparison to an Orthogonal Method

An analytical accuracy study was performed specifically to evaluate the concordance between the F1CDx assay and an externally validated NGS (evNGS). This study evaluated a set of 26 *FGFR2*-postive CCA samples and 133 *FGFR2*-negative CCA samples. FFPE CCA samples were commercially procured. Due to the very limited availability of CCA specimens, patient samples from the clinical trial INCB 54828-202 were prioritized to include in the clinical bridging study and were not included in this study. Due to prevalence of *FGFR2*-fusions and rearrangements (9.6%), testing with the evNGS was performed on samples selected based on test results by the F1CDx assay and, and therefore prevalence adjusted PPA, adjusted NPA, adjusted OPA, PPV and NPV statistics with corresponding 95% 2-sided Cls were calculated and summarized in Table 6 below.

Table 6. Contingency Table Comparing the *FGFR2* Biomarker Status by F1CDx and the evNGS

	evNGS				
	FGFR2-positive	***FGFR2-negative***	***Invalid***	***Total***	**Predictive Value**
FGFR2-positive	25	1	0	26	PPV: 96.15% [80.36%, 99.90%]
FGFR2-negative	2	130	1	133	NPV: 98.48% [94.63%, 99.82%]
Invalid	0	0	0	0	PPV: 96.15% [80.36%, 99.90%]
Total	27	131	1	159	NPV: 98.48% [94.63%, 99.82%]
Adjusted Agreement	87.08% [61.40%,98.30%]	99.59% [92.87%, 100.00%]		98.26% [93.26%, 99.82%]	

The comparison between the F1CDx and evNGS for the detection of *FGFR2* rearrangements showed adjusted PPA 87.08% and adjusted NPA 99.59%. Three (3) discordant cases were observed (2 false negatives and 1 false positive), and 1 invalid. Due to the limited availability of positive cases, testing with additional *FGFR2*-postive CCA samples will be performed as part of a post-market study. The *FGFR2* fusions and rearrangements evaluated in this study are shown below in Table 7:

Table 7. *FGFR2* fusions and rearrangements in the accuracy study.

Target Gene	Other Gene	N	Description
FGFR2	N/A	3	FGFR2(NM_000141) rearrangement exon 17
FGFR2	BICC1	4	BICC1(NM_001080512)-FGFR2(NM_000141) fusion (B2; F18)
FGFR2	BICC1	3	FGFR2(NM_000141)-BICC1(NM_001080512) fusion (F17; B3)
FGFR2	SPECC1	2	FGFR2(NM_000141)-SPECC1(NM_001243439) fusion (F18*; S2)
FGFR2	PHLDB2	1	FGFR2(NM_000141)-PHLDB2(NM_145753) fusion (F17; P3)
FGFR2	FAM81A	1	FGFR2(NM_000141)-FAM81A(NM_152450) fusion (F17; F4)
FGFR2	CCDC6	1	FGFR2(NM_000141)-CCDC6(NM_005436) fusion (F17; C2)
FGFR2	SLC31A1	1	FGFR2(NM_000141) rearrangement intron 17
FGFR2	POC1B	2	FGFR2(NM_000141)-POC1B(NM_172240) fusion (F17; P8)
FGFR2	SORBS3	1	SORBS3(NM_005775)-FGFR2(NM_000141) fusion (S4; F18)
FGFR2	AFF4	1	AFF4(NM_014423)-FGFR2(NM_000141) fusion (A5; F18)
FGFR2	AHCYL1	2	FGFR2(NM_000141)-AHCYL1(NM_006621) fusion (F17; A2)
FGFR2	NRL	2	FGFR2(NM_000141)-NRL(NM_006177) fusion (F17; N2)
FGFR2	STAU2	1	FGFR2(NM_000141)-STAU2(NM_014393) fusion (F18*; S4*)
FGFR2	C10orf85	1	FGFR2(NM_000141) rearrangement exon 9
FGFR2	RIMS2	2	RIMS2(NM_001100117)-FGFR2(NM_000141) fusion (R2; F18)

2. Analytical Sensitivity

a. Limit of Detection (LoD) for *FGFR2* fusions and rearrangement

The minimum tumor fraction required to support the LoD of F1CDx for *FGFR2* rearrangement detection was evaluated with 2 *FGFR2* rearrangement positive (*FGFR2-BICC1* and *FGFR2-LRRFIP2*) cholangiocarcinoma specimens. For this study, each sample was assessed at 5 targeted tumor purity levels between 20% and 2.5% tumor purity with 14-20 replicates at each level. A total of 94 replicate mixtures of tumor and biomarker-negative DNA were tested including targeted tumor purity dilutions to represent levels both above and below the putative LoD. The analytical sensitivity of *FGFR2* fusion and rearrangement detection was calculated using the empirical hit rate and probit regression methods and is summarized in Table 8. The data show that the assay detected 10.75 chimera reads or 5.31% tumor purity based on the empirical statistical method, and 8.61 chimera reads or 5.38% tumor purity based on the probit regression method for *FGFR2* rearrangements.

Table 8. Summary of LoD analysis for *FGFR2* fusions and rearrangements

LoD Analysis Method	*FGFR2* LoD % Tumor Purity	*FGFR2* LoD # of Reads
Probit Regression[1]	5.38%	8.61
Empirical Method[2]	5.31%	10.75

[1] LoD calculations were based on the Probit approach with 95% hit rate.
[2] LoD calculations were based on the hit rate approach; defined as the lowest level with > 95% hit rate

b. Limit of Blank (LoB)

The limit of blank (LoB) was confirmed using the mutation calls from two variant-negative cholangiocarcinoma samples for a total of 60 replicates with a percentage of false-positive results less than 5% (type I error risk α=0.05). Fifty-six (56) samples were used for the assessment of LoB. Two replicates failed during processing and two replicates failed post-sequencing QC metrics. The analysis confirmed that each replicate of LoB sample was negative for variants included in the LoD analysis and therefore confirmed the LoB was equal to zero.

3. Precision and Reproducibility

a. Intermediate Precision of *FGFR2* fusion and rearrangement

To support the F1CDx performance characteristics for detection of *FGFR2* rearrangement, the precision of 5 *FGFR2* rearranged cholangiocarcinoma specimens was evaluated with specimens near the tumor purity tissue input specification of 20% for the F1CDx assay and at a challenging DNA input amount (i.e., approximately 50ng). Three (specimens) were evaluated to supplement precision data obtained previously with data submitted in P170019. The representative rearrangements are listed in Table 9. The mean chimera read count of these samples ranges from 16.7 to 120.6. The samples were tested in 2 or 3 replicates by 2 separate runs (plates), using 2 reagent lots and 3 HiSeq 4000 sequencers, resulting in a total of 24 or 36 replicates per sample. Two (2) replicates of sample *FGFR2_TFCP2* failed the Library Construction (LC) and Post-Sequencing QC metrics due to low yield and low median coverage, respectively. Similarly, 1 replicate of *FGFR2-CCDC6* and 3 replicates of *FGFR2-BICC1* failed QC and were removed from subsequent repeatability and reproducibility analysis. The reproducibility and repeatability were determined to be 95.45% and 90% for sample *FGFR2_TFCP2*(TRF089077) while the other samples demonstrated 100% reproducibility and repeatability, as shown in Table 10.

Table 9. Summary of specimens for two precision studies in Study 1.

Read count			**Average Tumor Purity**	**LC DNA Input Mass (ng)**	**Aberration**
Mean	**Min**	**Max**			
120.6	89	160	17.9%	52	*FGFR2_BICC1*
71.2	48	92	18.4%	52	*FGFR2_TFCP2*
16.7	7	24	12.6%	52	*FGFR2* rearrangement
79.7	53	100	22.1%	52	*FGFR2_ CCDC6*
47.5	36	66	13.4%	52	*FGFR2_BICC1*

LC= Library Construction

Table 10. Summary of reproducibility and repeatability results (N=5) Excluding Invalids Study 2

Target Gene	Target Alteration	# pass	# total	Repro[1] (%)	# pass pair	# total pair	Within-run (%)
FGFR2	BICC1 fusion	24	24	100.00	12	12	100.00
FGFR2	TFCP2 fusion	21	22	95.45	9	10	90.00
FGFR2	*FGFR2* rearrangement	24	24	100.00	12	12	100.00
FGFR2	CCDC6	35	35	100.00	12	12	100.00
FGFR2	BICC1	33	33	100.00	11	11	100.00

[1] Repro refers to Reproducibility

b. Site-to-site reproducibility (*FGFR2* fusions and rearrangements)

A reproducibility study to include the new second site in Research Triangle Park (RTP), North Carolina was not conducted. Site-to-site reproducibility is being provided as a post-market study.

4. Analytical Specificity-Interfering Substances

The original PMA evaluated five FFPE specimens representing five tumor types (ovary, lung, colorectal, breast cancer and melanoma). No CCA specimens were evaluated. Interfering substances tested in original PMA included melanin, ethanol, proteinase K, and molecular index barcodes. A post-market analytical specificity will be conducted to evaluate CCA tissue specific interfering substance: Hemoglobin, Triglycerides, Bilirubin (conjugated and unconjugated).

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 STUDIES

The clinical performance of FoundationOne® CDx (F1CDx) for detecting *FGFR2* rearrangements in cholangiocarcinoma (CCA) patients who may benefit from treatment with pemigatinib, was established with clinical data generated from the Incyte Corporation (Incyte) trial INCB 54828-202 (FIGHT-202), and a clinical bridging study to demonstrate concordance between the enrollment assay and the F1CDx assay to establish the clinical efficacy of the F1CDx assay. The study enrolled 107 patients with *FGFR2* fusions or rearrangements. The major efficacy outcome measures were overall response rate (ORR) and duration of response (9.1 months; 95% CI 6.0, 14.5; data not shown) as determined by an independent review committee (IRC) according to Resist v1.1. ORR was 36% and the median time to response was 2.7 months (range 0.7 – 6.9 months). A bridging study between the

FM1CDx and the CTA demonstrated 100% concordance to the evaluable patient specimen set supporting the use of the test as a companion diagnostic for this indication.

A. Study Design

FIGHT-202 (INCB 54828-202; NCT02924376), a multicenter open-label single-arm trial, evaluated the efficacy of PEMAZYRE in 107 patients with locally advanced unresectable or metastatic cholangiocarcinoma whose disease had progressed on or after at least 1 prior therapy and who had an *FGFR2* gene fusion or rearrangement, as determined by a clinical trial assay performed at a central laboratory. The primary endpoint of the study was the objective response rate (ORR) in participants with *FGFR2*-fusions and rearrangements in tumor tissue from cholangiocarcinoma patients. ORR was defined as the proportion of participants who achieved a confirmed complete or partial response based on the RECIST v1.1 criteria.

Participants were enrolled at 67 sites across the United States and 11 in other countries. A total of 171 patients were screened for the study, of which 146 participants were enrolled in the INCB 54828-202 study at the time of data cutoff (22 MAR 2019), testing consisted of confirmatory tumor tissue-based testing using FMI F1 Clinical Trial Assay (CTA)(one specimen was tested with the FMI's F1 Heme LDT). These 145 patients were assigned to one of the following cohorts for statistical analyses:

- Cohort A: 107 participants with *FGFR2*-fusions or rearrangements detected in cholangiocarcinoma
- Cohort B: 20 participants with other *FGF/FGFR* alterations (patient specimens are negative for *FGFR2* fusions and rearrangements but may have other *FGFR2* mutation types)
- Cohort C: 18 participants with tumors negative for *FGF/FGFR* alterations

FGFR2 candidate fusions and select rearrangements were defined as having activated kinase domains as follows:

- An FGFR2 rearrangement predicted to be a fusion: Breakpoint is within the FGFR2 intron 17/ exon 18 hotspot and the gene partner is known in the literature or is a novel partner that is predicted to be in frame with FGFR2.
- An FGFR2 rearrangement, which cannot be predicted to be a fusion: Breakpoint is within the FGFR2 intron 17/exon 18 hotspot but the partner gene is out of frame or out of strand with exon 17 of FGFR2. Alternatively, the downstream end of the breakpoint may be in an intergenic region and not within another gene (designated as partner N/A).

FMI's definition for *FGFR2* fusions and select rearrangements includes events considered to be likely activating but may not previously have had definitive evidence to be considered an oncogenic fusion prior to the trial.

B. Accountability of PMA Cohort

A description of enrollment into the clinical trial is described in Section A above. This section describes the accountability for the bridging study that supported the PMA and is shown in Table 11: Patients with valid F1CDx results together with FMI archived samples were used to demonstrate concordance of F1CDx to the FMI F1 CTA. Additional test negative cases from the FMI archival database were used to represent test negatives. A total of 228 specimens were obtained (clinical trial samples, screen failure samples, and samples from the FMI archival database in this study). Of the 228 samples, a total of 186 samples were processed and tested at FMI**;** 42 samples were not processed due to insufficient DNA (18% of total samples). Retrospective testing with F1CDx yielded 181 CDx-evaluable results used for further analysis. For the clinical bridging study, the following sample cohorts were used:

- 80 CTA-patients with *FGFR2* fusions and select rearrangements positive patients enrolled in Cohort A; 74.8% (80/107) of *FGFR2*-fusion/rearrangement CTA-positive trial sample were tested by F1CDx assay.; 25.2% (27/107) were unevaluable.
- 14 CTA-negative patients enrolled in Cohort B; 63.2% (24/38) of *FGFR2*-fusion/rearrangement CTA-negative trial sample were tested by F1CDx assay. 36.8% (14/38) were unevaluable.
- 10 CTA-negative patients enrolled in Cohort C (56%)
- 4 screen failure samples
- 73* negative samples from the FMI archival database

*Five (5) of 78 originally selected samples were excluded in the concordance analysis as they didn't meet the inclusion criteria (they were *FGFR2*-positive samples).

The total percentage of missing samples is 28.3% (41/145). For these missing cases, the F1CDx results were imputed and the sensitivity analysis was performed to demonstrate the robustness of the results.

Table 11. Final Sample Accountability

	Number of Specimens
F1CDx evaluable	**186**
Cohort A	80
Cohort B	14
Cohort C	10
Screen failures	4
FMI archival	78[a]
F1CDx unevaluable	**42**
Cohort A	27
Cohort B	6
Cohort C	8
Other	1
Total	**228**

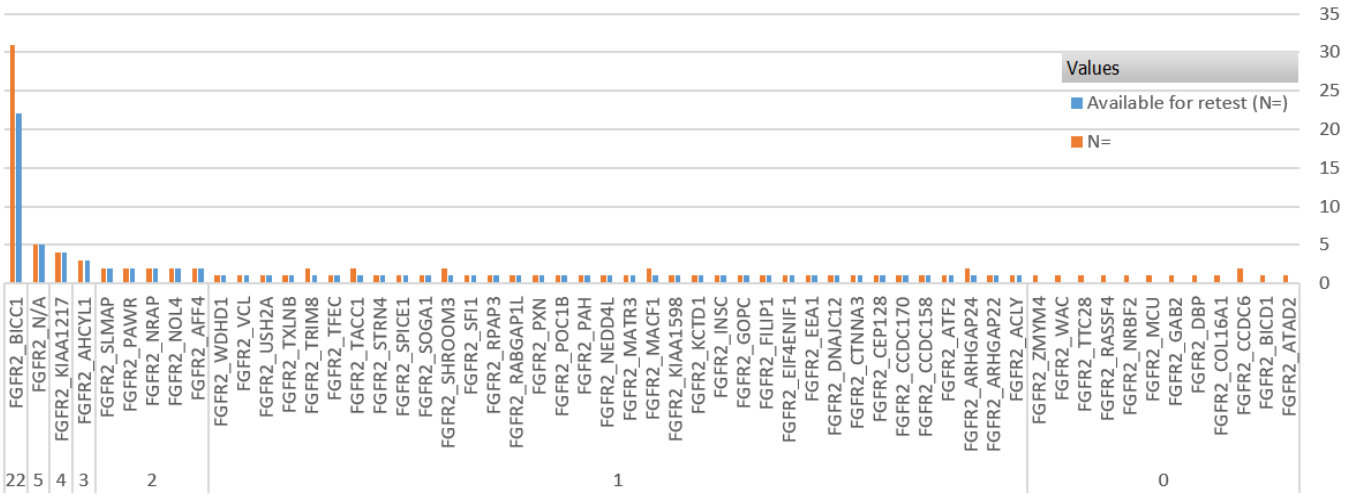
[a] Five (5) of 78 samples were inadvertently selected and processed; these were excluded in the concordance analysis as they didn't meet the inclusion criteria (they were *FGFR2*-positive

samples). Therefore, 73 FMI archival samples (181 total F1CDx evaluable) went on for analysis.

Of the evaluable specimens in cohort A (n=80), the most common finding was FGFR2-BICC1 [27% (22/80)] in the evaluable set. Patients also had rearrangements without an identifiable partner gene. All of the biomarker positive cases in the F1CDx FGFR2 CCA Clinical Bridging Study had breakpoints in the FGFR2 hotspot region, intron 17 – exon 18. (Figure 1)

The distribution of *FGFR2* fusions in the trial that were available for bridging is shown in Figure 1 below.

Figure 1. Distribution of *FGFR2* fusions and rearrangements in Cohort A in support of efficacy



C. Evaluation Imbalance of Covariates

Demographics of the population were as follows: The median age was 56 years (range: 26 to 77 years), 61% were female, 74% were white, and 95% had a baseline Eastern Cooperative Oncology Group (ECOG) performance status of 0 (42%) or 1 (53%). Ninety-eight percent (98%) of patients had intrahepatic cholangiocarcinoma. The majority of patients had fusions with BICC1 having the largest representation. All patients had received at least 1 prior line of systemic therapy, 27% had 2 prior lines of therapy, and 12% had 3 or more prior lines of therapy. Ninety-six percent of patients had received prior platinum-based therapy including 76% with prior gemcitabine/cisplatin

The clinical outcomes as well as baseline characteristics were compared between the CDx-evaluable population and the CDx-unevaluable population for the CTA-positive enrolled patients. The following covariates of patients from the NOA population were included in this analysis: age, BMI, sex, region, baseline ECOG performance status, initial diagnosis stage, time since initial diagnosis, number of prior anti-cancer therapies, metastasis site, phosphate as a surrogate of exposure and phosphate change from the baseline.

Continuous measures were summarized with mean, median, Q1, Q3, minimum and maximum; categorical measures were presented with the counts and percentages of subjects in each category. Covariate values were statistically compared between the F1CDx-evaluable and the F1CDx-unevaluable subset, using the nonparametric Mann-Whitney test for continuous measures and the Fisher-Freeman-Halton test (i.e., Fisher's Exact test) for categorical measures. The results are summarized in **Table 12** below. None of the covariates except for the covariate "Number of Prior System Treatment Regimen" (p-value=0.06) were significant at the a=0.10 level between the F1CDx-evaluable and the F1CDx-unevaluable groups.

Table 12. Covariate of F1cox-evaluable and F1COx-unevaluable Populations with P-values

Population	CTA+	F1CDx-evaluable (CTA+ with valid F1CDx results)	F1CDx-unevaluable (CTA+ without F1CDx results)	P-value comparing the two groups
n	107	80	27	
ORR	35.51%	37.50%	29.63%	0.50
Covariates				
1. Age (Mean)	55.31	54.76	56.93	0.35
Min	26.00	26.00	28.00	
Q1	47.50	47.00	48.50	
Med	56.00	55.00	59.00	
Q3	64.00	62.25	67.50	
Max	77.00	77.00	76.00	
2. BMI (Mean)	27.17	27.08	27.45	0.49
Min	17.81	17.81	19.18	
Q1	22.41	22.34	22.86	
Med	25.35	24.93	27.05	
Q3	29.92	29.84	30.81	
Max	58.16	58.16	41.30	
3. Sex				0.36
Male	42 (39.00%)	29 (36.00%)	13 (48.00%)	
Female	65 (61.00%)	51 (64.00%)	14 (52.00%)	
4. Race				0.21
Asian	11 (10.28%)	7 (8.75%)	4 (14.81%)	
Black or African American	7 (6.54%)	7 (8.75%)	0 (0.00%)	
White	79 (73.83%)	58 (72.50%)	21 (77.78)	
Other	4 (3.74%)	2 (2.50%)	2 (7.41%)	
NR[a]	6 (5.61%)	6 (7.50%)	0 (0.00%)	

Population	CTA+	F1CDx-evaluable (CTA+ with valid F1CDx results)	F1CDx-unevaluable (CTA+ without F1CDx results)	P-value comparing the two groups
5. Region				0.43
North America	64 (59.81%)	47 (58.75%)	17 (62.96%)	
Western Europe	32 (29.91%)	26 (32.50%)	6 (22.22%)	
Rest of World	11 (10.28%)	7 (8.75%)	4 (14.81%)	
6. ECOG				0.86
0	45 (42.06%)	35 (43.75%)	10 (37.04%)	
1	57 (53.27%)	41 (51.25%)	16 (59.26%)	
2	5 (4.67%)	4 (5.00%)	1 (3.70%)	
7. Initial Diagnosis Stage				0.14
1	11 (10.28%)	9 (11.25%)	2(7.41%)	
2	21 (19.63%)	19 (23.75%)	2(7.41%)	
3	7 (6.54%)	4 (5.00%)	3(1111%)	
4	66 (61.68%)	46 (57.50%)	20 (74.07%)	
NR[a]	2 (1.87%)	2 (2.50%)	0 (0.00%)	
8. Time Since Initial Diagnosis (Mean)	1.57	1.49	1.79	0.97
Min	0.03	0.03	0.19	
Q1	0.64	0.65	0.62	
Med	1.28	1.29	1.10	
Q3	1.93	1.89	2.20	
Max	11.09	9.06	11.09	
9. Number of Prior Systemic Treatment Regimen				0.06*
1	65(60.75%)	49 (61.25%)	16 (59.26%)	
2	29 (27.10%)	23 (28.75%)	6 (22.22%)	
3	8 (7.48%)	7 (8.75%)	1 (3.70%)	
4	2 (1.87%)	0 (0.00%)	2 (7.41%)	
5	3 (2.80%)	1 (1.25%)	2 (7.41%)	
10. Current Sites of Disease				0.18
Metastatic	84 (78.50%)	60 (75.00%)	24 (88.89%)	
Non-Metastatic	23 (21.50%)	20 (25.00%)	3(11.11%)	
11. Phosphate at Baseline (Mean)	3.26	3.30	3.17	0.28
Min	1.60	1.60	1.86	
Q1	2.94	3.00	2.80	
Med	3.30	3.34	3.10	
Q3	3.71	3.72	3.55	

Population	CTA+	F1CDx-evaluable (CTA+ with valid F1CDx results)	F1CDx-unevaluable (CTA+ without F1CDx results)	P-value comparing the two groups
Max	4.50	4.50	4.20	
12. Phosphate change from baseline (Mean)	2.78	2.80	2.74	0.96
Min	-1.00	-1.00	0.00	
Q1	2.10	2.12	1.80	
Med	2.90	2.89	2.90	
Q3	3.50	3.47	3.75	
Max	5.30	4.90	5.30	
13. Drug Compliance (%) (Mean)	100.40	100.60	99.79	0.30
Min	90.00	94.50	90.00	
Q1	100.00	100.00	99.50	
Med	100.00	100.00	100.00	
Q3	100.70	100.70	100.45	
Max	124.40	124.40	104.00	

*p<0.1

[a] NR- Not Reported.

D. Safety and Effectiveness

1. Safety Results

The safety with respect to treatment with pemigitinib was addressed during the review of the NDA and is not addressed in detail in this Summary of Effectiveness and Data. The evaluation of safety was based on the analysis of adverse events (AEs, clinical laboratory evaluations, physical examinations, and vital signs. Refer to the drug label available at Drugs@FDA for complete safety information on PEMAZYRE®

Briefly Serious adverse reactions occurred in 45% of patients receiving PEMAZYRE. Serious adverse reactions in ≥ 2% of patients who received PEMAZYRE included abdominal pain, pyrexia, cholangitis, pleural effusion, acute kidney injury, cholangitis infective, failure to thrive, hypercalcemia, hyponatremia, small intestinal obstruction, and urinary tract infection. Fatal adverse reactions occurred in 4.1% of patients, including failure to thrive, bile duct obstruction, cholangitis, sepsis, and pleural effusion.
Permanent discontinuation due to an adverse reaction occurred in 9% of patients who received PEMAZYRE. Adverse reactions requiring permanent discontinuation in ≥1% of patients included intestinal obstruction and acute kidney injury.

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

2. Effectiveness Results

a. Clinical Efficacy in the Intent to Treat Population

FIGHT-202 (NCT02924376), a multicenter open-label single-arm trial, evaluated the efficacy of PEMAZYRE in 107 patients with locally advanced unresectable or metastatic cholangiocarcinoma whose disease had progressed on or after at least 1 prior therapy and who had an *FGFR2* gene fusion or rearrangement, as determined by a clinical trial assay performed at a central laboratory.

Patients received PEMAZYRE in 21-day cycles at a dosage of 13.5 mg orally once daily for 14 consecutive days, followed by 7days off therapy. PEMAZYRE was administered until disease progression or unacceptable toxicity. The major efficacy outcome measures were overall response rate (ORR) and duration of response (DoR) as determined by an independent review committee (IRC) according to RECIST v1.1. The results of this study are shown in **Table 13** below.

Table 13. Efficacy Results in FIGHT 202 Trial

Efficacy Parameter	**PEMAZYRE N = 107**
ORR (95% CI)	36% (27, 45)
Complete response	2.8%
Partial response	33%

b. Clinical Efficacy in CTA and CDx double-positive Population

The FIGHT-202 study enrolled both *FGFR2* fusion and rearrangement CTA-positive and CTA-negative patients, and thus both were used for the efficacy analysis. Efficacy analysis was performed for patients determined to be CTA and CDx-positive and then compared to the efficacy results in the INCB 54828-202 Cohort A. There were 107 CTA positive (CTA+) samples (cohort A). Of the 107 samples, 80 were also
F1CDx *FGFR2* fusion/rearrangement-positive (F1CDx+) (concordant samples). Analysis was performed on the 80 patients enrolled in the INCB 54828-202 trial that were reported as CDx positive. There were no CTA-positive and F1CDx *FGFR2* fusion/rearrangement-negative samples (F1CDx-) (discordant samples). No CTA-negative patients (cohorts B and C) responded to the treatment.

Clinical utility of F1CDx was evaluated by estimation of clinical efficacy in the *FGFR2* rearranged, CTA-enrolled population based on the primary objective of ORR per central review per RECIST v1.1 criteria. The INCB 54828-202 clinical trial demonstrated a statistically significant improvement in the ORR, supported by investigator assessments and central review. Patients on pemigatinib demonstrated a confirmed tumor response of 36% (95% CI: 27, 45) among the 107 participants with *FGFR2*-rearranged cholangiocarcinoma (Cohort A) (Table 15). Among the subset of participants with *FGFR2* fusions and rearrangements

classified as fusions1, the ORR based on IRC-assessed, confirmed tumor responses was 34.8% (95% CI: 25.15, 45.43). Among the subset of participants with *FGFR2* rearrangements classified as non-fusion rearrangements, the ORR based on IRC-assessed, confirmed tumor responses was 40.0% (95% CI: 16.34, 67.71).
The objective response rate (ORR) for the CTA and F1CDx *FGFR2*-rearrangement-double positive population estimated by the bridging study was 37.50% and aligns with the ORR for the CTA *FGFR2*-fusion/rearrangement-positive population, which was 35.51%. The ORR of the different subgroups are summarized in Table 14 below.

Table 14. Summary of ORR in Different Subpopulations for Completed Data

Population	**CTA+**	**CTA+ and F1CDx+**	**CTA+ and F1CDx-**
n	107	80	0
ORR	35.51%	37.50%	N/A
95% 2-sided exact CIs	[26.50%,45.35%]	[26.92%,49.04%]	N/A

Sensitivity analysis, using the multiple imputation method, was performed to evaluate the robustness of the clinical efficacy estimate against the 27 missing CDx results from Cohort A, and 14 missing results from cohorts B and C.

Given that the CTA result was a strong predictor of the F1CDx status, the missing data were separated into CTA-positive and CTA-negative groups. Instead of using the logistic regression model to impute the missing data, the binomial distribution (i.e., binomial(n, p)) was used for the imputation.

For the missing data imputation in the CTA-positive group, the lower bound of 95% CIs of PPA (i.e., 95.7%) was used as the probability of being F1c o x-positive following the binomial(n1, PPA_LB) distribution. For the missing data imputation of the CTA-negative group, the lower bound of 95% Cls of NPA (i.e., 96.27%) was used as the probability of being F1cox-negative following the binomial(nO, NPA_LB) distribution.

The F1CDx results for a total of 41 samples were imputed. For one (1) sample (TRF274323) in the 'Other' cohort, a sample failure occurred at FMI such that there

1 There were discordant designations in the study (e.g., fusion with CTA was later referred to as a rearrangement with CDx). During the process of manual curation, edits can be made to the rearrangement "type" (including "fusion" or "rearrangement") and their final assignment is performed by trained analysts according to SOPs and work instructions. The final curator assigned value to assess biomarker positivity or not. The binary biomarker decision (eligible/not eligible) remained the same.

was no F1 assay result, therefore it was not considered in the sensitivity of concordance analysis.

The robustness of the concordance analysis was assessed and a total of 50 imputed data sets were generated. The sensitivity of the agreement statistics, PPA, NPA, adjusted PPV and NPV were examined by the median and empirical 95% CIs. The results are summarized in **Table 15** below.

Table 15. Sensitivity Analysis Results for PPA, NPA, Adjusted PPV and NPV

Assessment	**Median and 95% empirical CIs** (%)
PPA	99.10 [97.30, 100.0]
NPA	100.00 [98.40, 100.0]
Adjusted PPV	100.00 [86.95, 100.0]
Adjusted NPV	99.90 [99.71, 100.0]

The sensitivity concordance analysis results were very close to the observed values of PPA (i.e., 100%), NPA (i.e., 100%), adjusted PPV (i.e., 100%) and adjusted NPV (i.e., 100%), which demonstrates the robustness of the concordance analysis.

The robustness of the clinical efficacy results was also assessed and a total of 50 imputed data sets were generated. The clinical efficacy results with the imputed data were examined by the median and empirical 95% CIs. The results are summarized in **Table 16** below.

Table 16. Sensitivity Analysis Results for Clinical Efficacy

ORR	**CTA+ and F1CDx+**	**CTA+ and F1CDx-**
Median	35.85%	0.00%
95% empirical CIs	[34.29%, 36.19%]	[0.00%, 100.00%]*

*The wide CIs were due to the very small size of imputed CTA+ and F1CDx population.

The observed ORR for the clinical efficacy of CTA+ and F1CDx+ population was 37.50%. The sensitivity analysis results of clinical efficacy of 35.85% were very close to the observed value, which demonstrates the robustness of the clinical efficacy analysis.

c. Clinical Concordance Analysis

The concordance between F1CDx and the confirmatory F1 CTA in conjunction with clinical outcome data were used to evaluate the efficacy of pemigatinib in patients with CCA. Using *FGFR2* rearrangement positive and negative samples, the concordance between F1CDx and the F1 CTA was evaluated using all available CDx results for patient samples that met the F1CDx testing criteria (N=80) from the 107 patients enrolled in Cohort A of the trial. The CDx and CTA concordance analysis was conducted with 73 negative archival samples and 108

clinical trial samples and screen failures, for a total of 181 positive and negative F1CDx evaluable samples included in the analysis.
The PPA, NPA, and OPA analyses were calculated with corresponding 95% 2-sided exact CIs using the F1 LDT as the reference assay. Additionally, the PPV and NPV, adjusted by the prevalence of *FGFR2* fusions and rearrangements in the CCA population (9.6% based on the FMI Clinical-T7 bait-set database), were provided as well. The PPA, NPA, OPA, PPV, and NPV all exhibited 100% agreement between the F1CDx assay and the F1 LDT. Both the PPA and NPA passed the acceptance criteria of 85% and 95%, respectively, while no acceptance criterion was imposed for OPA. The results, including the 95% CI, for samples that met F1CDx sample requirements are as follows:

- PPA 100.00% (95% CI: 95.70%, 100.00%)
- NPA 100.00% (95% CI: 96.27%, 100.00%)
- OPA 100.00% (95% CI: 97.98%, 100.00%)
- Adjusted PPV 100.00% (95% CI: 73.14%, 100.00%)
- Adjusted NPV 100.00% (95% CI: 99.53%, 100.00%)

The results of the agreement analysis demonstrated concordance between the F1CDx assay and the CTA. The concordance analysis results are summarized in the following 2x2 contingency table (**Table 17**).

Table 17. 2x2 Contingency Table Comparing the F1CDx Assay with the F1 LDT Assay

		F1* LDT Assay (CTA)			
		FGFR2-positive	*FGFR2*-negative	Total	
F1CDx Assay	*FGFR2*-positive	84*	0	84	**PPV: 100.00%** [73.14%, 100.00%]
	FGFR2-negative	0	97	97	**NPV: 100.00%** [99.53%, 100.00%)
	Total	84	97	181	
		PPA: 100.00% [95.70%, 100.00%)	**NPA: 100.00%** [96.27%, 100.00%)		**OPA: 100.00%** [97.98 %, 100.00 %)

* One (1) sample was enrolled by the F1 Heme assay and was analyzed as an F1 result for the concordance analysis.

d. Clinical Efficacy in CDx positive Population

Since the NPA (Pr(F1CDx-|CTA-)) estimate is 100.00%, it means that all F1CDx-positive subjects will be CTA, F1CDx-double positive (since Pr(F1CDx+|CTA-) is 0%), therefore, The objective response rate (ORR) for the F1CDx FGFR2-rearrangement-positive population estimated by the bridging study was also 37.50% (95% CI, (26.92%,49.04%])). This result demonstrates the clinical efficacy of using the F1CDx assay as the CDx assay for FGFR2 rearrangement calling in CCA patients.

The common finding in the FGFR2 fusions and rearrangements where that the genomic breakpoints occur in the intron 17/exon 18 hotspot, downstream of the last kinase domain. Th genomic fusions and rearrangements resulted in sustained activation.

e. Pediatric Extrapolation

The safety and effectiveness of PEMAZYRE have not been established in pediatric patients.

E. Financial Disclosures

The Financial Disclosure by Clinical Investigators regulation (21CFR54) requires applicants who submi 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 X investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c) and (f).

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

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

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the F1CDx assay for the intended use in detection of *FGFR2* fusions and select rearrangements in CCA patients to determine eligibility for treatment with pemigatinib was demonstrated through a clinical bridging study using specimens screened for the INCB 54828-202 trial. The data from analytical and clinical bridging studies support the reasonable assurance of safety and effectiveness of the F1CDx assay when used in accordance with the indication for use. Data from the INCB 54828-202 trial demonstrate that patients who had qualifying *FGFR2* rearrangements received benefit from treatment with pemigatinib and supports the addition of the proposed CDx indication to F1CDx.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support PMA approval as described above. 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 an existing (archival) tissue samples routinely collected as part of the diagnosis and patient care.

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 in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in Table 1 of the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

C. Benefit-Risk Determination

Treatment with pemigatinib provides meaningful clinical benefit to cholangiocarcinoma patients with FGFR2 fusions/select rearrangements, as measured by ORR, was demonstrated in the FIGHT-202 (INCB 54828-202; NCT02924376) trial. Given the available information, the data supports the conclusion that FoundationOne CDx has probable benefit in selecting patients with FGFR2 fusions/select rearrangements for treatment with pemigatinib.

There is potential risk associated with the use of this device, mainly due to 1) false positives, false negatives, and failure to provide a result and 2) incorrect interpretation of test results by the user.

The risks of the F1CDx for selection of cholangiocarcinoma patients with FGFR2 fusions/select rearrangements for treatment with pemigatinib are associated with the potential mismanagement of patient's treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug combination that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen.

The likelihood of false results was assessed by an analytical accuracy study that performed specifically to evaluate the concordance between the F1CDx assay and an externally validated NGS (evNGS). This study evaluated a set of 26 FGFR2-postive CCA samples and 133 FGFR2-negative CCA samples. The comparison between the F1CDx and evNGS for the detection of FGFR2 fusions/rearrangements showed 2 false negatives out of the 27 FGFR2-positive patients (adjusted PPA 87.08% 95% CI: 61.4%-98.3%). In addition, this analysis revealed 1 false negative, with an adjusted NPA of 99.59% (95% CI: 92.87%-100%). Though, the overall data here was

supportive, this accuracy data was limited by the small number of FGFR2-positive samples included in the analysis, which has necessitated a condition of approval to provide a supplemental analytical accuracy data by testing an additional 20-25 cholangiocarcinoma samples that are FGFR2 fusion/select rearrangement positive.

The benefit-risk profile of this device remains undetermined and requires additional mitigating measures.

1. **Patient Perspectives**
   This submission did not include specific information on patient perspectives for this device. However, cholangiocarcinoma patient perspective was considered through interaction with CDER in support of the PEMAZYRA NDA. The patient perspective discussed the willingness to accept risks associated with treatment due to the paucity of treatment options available to this cancer type and the disease severity.

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 indication for use. Data from the clinical bridging study support the performance of F1CDx as an aid for the identification of *FGFR2* fusions and select rearrangements in cholangiocarcinoma patients for whom PEMZYRE® (pemigatinib) may be indicated.

XIV. CDRH DECISION

CDRH issued an approval order on April 17, 2019. The final conditions of approval cited in the approval order are described below.

1. Provide supplemental analytical accuracy data by testing an additional 20-25 cholangiocarcinoma samples which are *FGFR2* fusion or rearrangement positive and comparing the results to those obtained with an externally validated orthogonal method.
2. Provide data from a study evaluating the effect of interfering substances to include Hemoglobin, Triglycerides and Bilirubin (conjugated and unconjugated).
3. Provide the results of a site-to-site reproducibility study to include the second laboratory site in Research Triangle Park (RTP), North Carolina using the same representative sample panel as was evaluated in support of the single site in Cambridge, Massachusetts. The study should include the same panel representation and testing strategy as was reviewed in the PMA.
4. Submit a PMA Supplement that supports BIP updates to v3.3x and that BIP and supporting software components' migration to cloud service do not impact the safety and effectiveness of your device.

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

XV. 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.