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/S043
Date of FDA Notice of Approval:	October 6, 2023

The original PMA (P170019) was approved on November 30, 2017, for the detection of genetic alterations in patients who may benefit from one of eighteen FDA-approved therapies for non-small cell lung cancer (NSCLC), melanoma, breast cancer, colorectal cancer (CRC), and ovarian cancer. Subsequently, additional PMA supplements were approved for expanding the indications for use of F1CDx since the original approval. See Section VII for more details.

The current supplement was submitted to expand the indication for F1CDx to include a companion diagnostic (CDx) indication for the detection of *RET* fusions in patients with solid tumors who may benefit from treatment with RETEVMO® (selpercatinib).

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 when using the DNAx extraction method. The test is intended for detection of

substitutions and indels in 324 genes, CNAs in 15 genes and select gene rearrangements, as well as genomic signatures including MSI and TMB using DNA isolated from FFPE tumor tissue specimens when using the CoExtraction method for DNA isolation. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Table 1. Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	EGFR Tyrosine Kinase Inhibitors (TKI) approved by FDA*
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Alunbrig® (brigatinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Tabrecta® (capmatinib)
	ROS1 fusions	Rozlytrek® (entrectinib)
Melanoma	*BRAF* V600E	BRAF Inhibitors approved by FDA*
	BRAF V600E and V600K	Mekinist® (trametinib) or BRAF/MEK Inhibitor Combinations approved by FDA*
	BRAF V600 mutation-positive	Tecentriq® (atezolizumab) in combination with Cotellic® (cobimetinib) and Zelboraf® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	Piqray® (alpelisib)

Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and NRAS wild type (absence of mutations in exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian Cancer	*BRCA1/2* alterations	Lynparza® (olaparib)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre® (pemigatinib) or Truseltiq™ (infigratinib)
Prostate cancer	Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations	Lynparza® (olaparib)
	BRCA1, BRCA2 alterations	Akeega® (niraparib + abiraterone acetate)
Solid Tumors	MSI-High	Keytruda® (pembrolizumab)
	TMB ≥ 10 mutations per megabase	Keytruda® (pembrolizumab)
	NTRK1/2/3 fusions	Rozlytrek® (entrectinib) Vitrakvi® (larotrectinib)
	RET fusions	Retevmo® (selpercatinib)

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

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

The F1CDx assay is 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, qualified by FMI and procedures for testing DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples.

The assay employs two extraction methods (either DNAx or CoExtraction, an automated DNA/RNA co-extraction methodology) for DNA extraction from routine FFPE biopsy or surgical resection specimens; 50-1000 ng of DNA will undergo 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 detects alterations in a total of 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 is then 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 select genomic rearrangements (e.g., gene fusions). Rearrangements in one of the targeted genes included in Table 3 may be reported along with their uniquely identified genomic partners, which can be any gene in the genome even if not explicitly targeted by the assay. Additionally, genomic signatures including microsatellite instability (MSI), tumor mutational burden (TMB), and positive homologous recombination deficiency (HRD) status (tBRCA-positive and/or LOH high) are 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*	*PRKARA*	*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*	*SMARC A4*	*WHSC1*
ASXL1	*CBL*	*CTCF*	*FANCC*	*GNA13*	*KEAP1*	*MSH2*	*PARP1*	*QKI*	*SMARC B1*	*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*	*PDCD1L G2*	*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*	

*Genes with copy number alteration reporting are limited to CDx variants when using the CoExtraction method

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 8*	*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*

*ETV6 is a common rearrangement partner for NTRK3

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

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

DNAx Extraction Method

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.

CoEx Extraction Method

Specimens passing pathology review are queued for nucleic acid extraction which begins with placement of the FFPE samples into an AutoLys tube, where using a pre-programmed automated method, the AutoLys STAR adds RNA digestion and proteinase K solutions. The RNA containing lysate is removed for downstream RNA extraction using the KingFisher RNA extraction process.

The AutoLys Tubes containing partially digested tissue then receive DNA Lysis solution and are placed into a Vortemp for digestion. The sample is then centrifuged to separate sample-associated paraffin from the lysate, and the lysate is transferred to a KingFisher dKF plate. The dKF plate is loaded onto the Hamilton STAR for automated addition of DNA binding buffer and magnetic beads, and DNA isolation is performed using the KingFisher Flex. The DNA samples are then transferred to matrix tubes on DNAE plates using Hamilton STAR before proceeding to DNA quantification.

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. The 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 New England Biolabs), including mixes for end repair, dA addition and ligation, are performed in 96-well plates (Eppendorf) on the Bravo Benchbot (Agilent) or Hamilton Microlab STAR/STARLet Liquid Handling Workstation (Hamilton) using the "withbead" 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, F1CDx employs a fraction based (FB) MSI algorithm to categorize a tumor specimen as MSI-High (MSI-H) or microsatellite stable (MSS). The FB-MSI algorithm calculates the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score) based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The final fraction unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci. Two FB-MSI score thresholds are applied to classify a tumor specimen as having MSI-H or MSS status. MSI-H status is reported for patients with solid tumors whose samples have FB-MSI scores ≥ 0.0124 while MSS status is reported for patients with solid tumors whose samples have FB-MSI scores ≤ 0.0041. Per the F1CDx assay, a patient whose tumor has an MSI-H score ≥ 0.0124 is reported as eligible for treatment with KEYTRUDA.

For patients with solid tumors whose samples have FB-MSI scores >0.0041 and <0.0124, an MSI "Cannot be Determined" result is reported. Patients with this result should be re-tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. Patients with solid tumors may also receive an MSI status reported as MSI-Cannot Be Determined due to a quality control (QC) failure. Patients with this result should consider re-testing with FoundationOne CDx or an orthogonal (alternative) method, if clinically appropriate.

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

To compute the percentage of genomic LOH for each tumor, LOH segments are inferred across the 22 autosomal chromosomes using the genome-wide aneuploidy/copy number profile and minor allele frequencies of the more than 3500 SNPs sequenced in the Foundation Medicine's next-generation sequencing (NGS)-based platform. A comparative genomic hybridization (i.e., log-ratio profile of the sample) is obtained from the NGS sequencing data by normalizing the sequence coverage obtained at all exons and genome-wide SNPs against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate copy number (Ci) and minor allele count (Mi) at each segment (i). A segment is determined to have LOH if $Ci \neq 0$ and $Mi = 0$. Two types of LOH segments are excluded from the calculation of percent genomic LOH: (1) LOH segments spanning ≥ 90% of a whole chromosome or chromosome arm, as these LOH events usually arise through non-homologous recombination deficiency (HRD) mechanisms (e.g., mitotic nondisjunction), and (2) regions in which LOH inference is ambiguous (e.g., some small genomic regions that do not have sufficient heterozygous SNPs to support LOH calling).

After completion of the Analysis Pipeline, variant data are displayed in the FMI custom developed CATi software applications with sequence QC metrics. As part of data analysis QC for every sample, the F1CDx assay assesses cross-contamination through the use of a SNP profile algorithm, reducing the risk of false-positive calls that could occur as a result of an unexpected contamination event. Sequence data are

reviewed by trained bioinformatics personnel. Samples failing any QC metrics are automatically held and not released.

G. Report Generation

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

H. Internal Process Controls Related to the System

Positive Control

Each assay run includes a control sample run in duplicate. The control sample contains a pool of ten HapMap cell lines and is used as a positive mutation detection control. 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 Tables 6-8, 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* gene (for olaparib CDx claim only)

M1I	Y2470D	R2832C
M1L	R2547_S2549del	S2855_V2856>RI
M1T	A2622V	D2913Y
P292L	D2625_A2626>EP	R3008C
D2016G	D2708N	R3008H
R2032K	V2716A	splice site 331+5G>A
A2067D	G2765S	splice site 8418+5_8418+8delGTGA
R2227C	F2827C	

Table 7. List of short variants in *BRCA1*

M1R	C44S	R1495T	D1692Y*	G1738E	Y1853C
M1I	C44Y	E1559K	C1697R	G1738R	C1787_G1788>SD
M1V	C47F	E1559Q	R1699Q	L1764P	splice site 212+3A>G
M1T	C61G	A1623G	R1699W	I1766S	splice site 213-11T>G
M18T	C61Y*	S1655F	L1705P	G1770V	splice site 213-12A>G
L22S	C64G	T1685A	G1706E	M1775K	splice site 302-3C>G
C24R	C64R	T1685I	G1706R	M1775R	splice site 4986+3G>C
T37K	C64W*	H1686R	A1708E	L1780P	splice site 4986+5G>A
C39G*	C64Y	V1688del	S1715N	G1788V	splice site 4986+6T>G
C39R	R71K	M1689R	S1715R	P1812A	splice site 4986+6T>C
C39W*	R71G	T1691I	W1718C	V1833M	splice site 5074+3A>G
C39Y	R170Q*	T1691K	S1722F	W1837R	splice site 5194-12G>A
H41R	R1495K	D1692N	V1736A	W1837C	splice site 5406+4A>G
C44F	R1495M	D1692H	V1736G	V1838E	

*variants are part of the biomarker definition for the niraparib CDx claim only

Table 8. List of short variants in *BRCA2*

M1R	R2336L*	R2659G	L2686P	Y2726C	N3124I
M1I	R2336P	R2659K	L2688P	G2748D	splice site 316+4delA
S142I*	L2510P	R2659T	T2722R	G2793R	splice site 316+5G>A
V159M	H2623R	Y2660D	D2723A	E3002K	splice site 8487+3A>G
V211I	W2626C	E2663V	D2723G	R3052W	splice site 8754+4A>G

V211L	I2627F	S2670L	D2723H	G3076V	splice site 8754+5G>A
R2336H	L2653P	I2675V	D2723V	D3095E	splice site 8754+3G>C

*variants are part of the biomarker definition for the niraparib CDx claim only

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

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

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

Biomarker Rules for *ALK* Rearrangements:
Rearrangements in *ALK* shall be considered CDx biomarker positive if the following criterion is met:

- Any oncogenic *ALK* rearrangement whose breakpoint occurs within ALK intron 19 or whose partner gene is EML4

Biomarker Rules for *FGFR2* Fusions and Select Rearrangements:
Rearrangements in *FGFR2* shall be considered CDx biomarker positive if the following criteria are met:

- The rearrangement event involved *FGFR2* and a literature-derived known partner gene regardless of strand or frame,
- The rearrangement event involved *FGFR2* and a novel partner gene that is both in-frame and in-strand,
- Any *FGFR2* rearrangement with one breakpoint in the hotspot region (intron 17-18) and the other breakpoint in the intergenic region or within another gene. This rule excludes 3’ duplications of only exon 18,
- Intragenic duplication of kinase domain (exon 9-17).

Biomarker Rules for Rearrangements that Lead to *ROS1* Fusions:
Rearrangements in *ROS1* shall be considered CDx biomarker positive, i.e., to lead to *ROS1* RNA fusion, if the following condition is met:

- In-strand rearrangement events that may lead to a *ROS1* RNA fusion with another protein coding gene in which the *ROS1* kinase domain is not disrupted. *ROS1* must be on the 3′ end of the detected fusion.

In this regard, out-of-strand events are considered as non-fusion rearrangements and are classified as CDx biomarker negative. Intragenic fusions in which genomic rearrangement events are wholly internal to the *ROS1* (i.e., *ROS1-ROS1* events) are also considered biomarker negative. Unidentified partners (encoded as N/A) or LINC non-coding partners are also considered CDx biomarker negative. ROS1 fusions with novel partners are required to be in frame.

Biomarker Rules for *RET* Fusions:
Fusions in *RET* shall be considered CDx biomarker positive if the following criteria are met:

- Any fusion event involving *RET* and another protein-coding gene
- *RET* and the partner gene must be in the same 5'-3' orientation
- *RET* must be on the 3' end of the detected rearrangement
- The *RET* breakpoint must occur before the start of the kinase domain (amino acids 724-1016)

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 9, below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

Table 9. 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
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	TAFINLAR (dabrafenib) MEKINIST (trametinib)	NSCLC
	therascreen BRAF V600E RGQ PCR Kit	QIAGEN	PCR	BRAFTOVI (encorafenib) Erbitux (cetuximab)	CRC
NRAS	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	CRC
KRAS	cobas KRAS Mutation Test	Roche Molecular Systems, Inc.	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	CRC
	therascreen KRAS RGQ PCR Kit	QIAGEN	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	CRC
	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	CRC

ALK –fusion	Vysis ALK Break Apart FISH Probe Kit	Abbott Molecular, Inc.	FISH	XALKORI (crizotinib)	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 CDxBRCA	Foundation Medicine, Inc.	NGS	RUBRACA (rucaparib)	Advanced ovarian cancer
	BRACAnalysis CDx	Myriad Genetic Laboratories, Inc.	NGS	LYNPARZA (olaparib) LYNPARZA (olaparib) - treatment/maintenance TALZENNA (talazoparib)	Breast, pancreatic, and prostate cancers Ovarian cancer Breast cancer
	Myriad myChoice® CDx	Myriad Genetic Laboratories, Inc.	NGS	ZEJULA (niraparib) or Lynparza (olaparib)	Ovarian cancer
PIK3CA	therascreen PIK3CA RGQ PCR Kit	QIAGEN	PCR	PIQRAY (alpelisib)	Breast cancer
ROS1	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	XALKORI (crizotinib)	NSCLC
RET	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	RETEVMO (selpercatinib) GAVRETO (pralsetinib)	NSCLC and Thyroid Cancer NSCLC

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 approved PMA supplements that affected the Intended Use are listed in Table 10.

Table 10. Marketing History

Submission No.	Date of Approval	Biomarker/Update	Patient Population	Drug
P170019/S004	July 1, 2019	*BRCA1/2* alterations	Ovarian Cancer	LYNPARZA® (olaparib)
P170019/S005	April 10, 2019	genomic loss of heterozygosity (LOH)	Ovarian Cancer	N/A
P170019/S006	December 3, 2019	*PIK3CA* alterations	Breast Cancer	PIQRAY® (alpelisib)
P170019/S008	July 1, 2019	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Non-Small Cell Lung Cancer	TAGRISSO® (osimertinib)
P170019/S011	May 6, 2020	*MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Non-Small Cell Lung Cancer	TABRECTA® (capmatinib)
P170019/S013	April 17, 2020	*FGFR2* fusions	Cholangiocarcinoma	PEMZYRE® (pemigatinib)
P170019/S015	May 19, 2020	mutations in homologous recombination repair (HRR) genes	metastatic castration resistant prostate cancer (mCRPC)	LYNPARZA® (olaparib)
P170019/S016	June 16, 2020	high tumor mutational burden (TMB) at the cut- off of 10 mutations per megabase (mut/Mb)	Solid Tumors	KEYTRUDA® (pembrolizumab)
P170019/S017	October 23, 2020	*NTRK1, NTRK2, or NTRK3* fusions	Solid Tumors	VITRAKVI ® (larotrectinib)
P170019/S021	May 28, 2021	*FGFR2* Fusion/Rearrangements	Cholangiocarcinoma	Truseltiq (infigratinib)
P170019/S022	July 21, 2021	Additional variants to *BRCA1* and *BRCA2*	Ovarian Cancer	LYNPARZA® (olaparib)
		Additional variants to *BRCA1*, *BRCA2* and *ATM*	Prostate Cancer	LYNPARZA® (olaparib)
P170019/S023	June 30, 2021	*ALK* Rearrangements	Non-Small Cell Lung Cancer	Alunbrig® (brigatinib)

P170019/S025	November 10, 2021	*BRAF* V600E	Melanoma	BRAF Inhibitor Monotherapy Group Claim
		BRAF V600E or V600K Alterations	Melanoma	BRAF/MEK Inhibitor Combination Group Claim
P170019/S029	February 18, 2022	Microsatellite Instability High (MSI-H) Status	Solid Tumors	KEYTRUDA® (Pembrolizumab)
P170019/S030	January 19, 2022	*BRAF* V600 Mutation- Positive	Unresectable Or Metastatic Melanoma	Atezolizumab (Tecentriq) In Combination with Cobimetinib and Vemurafenib
P170019/S033	March 16, 2022	*EGFR* Exon 19 Deletions or *EGFR* Exon 21 L858R Mutations	Non-Small Cell Lung Cancer	Any One of The FDA-Approved EGFR Tyrosine Kinase Inhibitors (TKI)
P170019/S014	June 7, 2022	NTRK1, NTRK2, or NTRK3 fusions	Solid Tumors	ROZLYTREK® (entrectinib)
		ROS1 fusions	NSCLC	
P170019/S042	August 11, 2023	*BRCA1, BRCA2* alterations	Prostate Cancer	Akeega® (niraparib + abiraterone acetate)

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 primary evidence for supporting the performance of F1CDx in the detection of *RET* fusions in solid tumor patients was from data using intended use specimens across the validation studies. In addition to the existing platform-level validation results (P170019), refer to Section IX.A. in P170019 Summary of Safety and Effectiveness Data, analytical concordance, limit of blank (LoB), limit of detection (LoD), and site-to-site precision

studies were conducted to support the indication for *RET* fusions. Table 11 shows the distribution of tumor types evaluated in the studies.

The F1CDx device was modified after the analytical validation studies were completed. The update included addition of an automated DNA/RNA CoExtraction methodology (CoExtraction method) to enable isolation of DNA and RNA from the same FFPE tumor specimens. Addition of the new CoEx method for DNA isolation which can be used in lieu of the DNA extraction method for F1CDx approved under the original PMA, was supported by validation studies demonstrating comparability between the two nucleic acid extraction methods, and precision studies at and above the LoD levels of the *RET*-positive fusion samples. The comparability study between the DNAx and CoEx DNA extraction methods assessed a total of two (2) clinical samples harboring *RET* fusions from patients with thyroid papillary carcinoma and observed a PPA of 100%. The precision study assessed two specimens positive for *RET* fusions with chimeric read levels at 17x and 21x LoD (151.5 and 185.9 chimeric reads respectively) from two patients with thyroid cancer. Six (6) curls were cut from each source block and processed with the CoExtraction method using a combination of two unique CoExtraction reagent lots and two unique CoExtraction instrument lines. The extracted DNA from each curl was subdivided into four (4) DNA sub-aliquots, for a total of 24 total replicates per source block entering precision testing (6 curl extractions x 4 extracted DNA subaliquots each). The agreement for reproducibility and repeatability of two *RET* fusion positive samples was 100%. An additional precision study with two *RET* fusion positive specimens from patients with thyroid cancer was conducted with chimeric read levels closer to LoD (3.2x and 2.8xLoD). For this study, previously extracted DNA derived from FFPE specimens using the CoExtraction method was used. Selected DNA samples were diluted to varying levels using biomarker-negative DNA (diluent DNA) to achieve levels closer to LoD. A total of 24 replicates (2 reagent lots x 3 sequencers x 2 plates x 2 replicates per sample) were assessed and a 100% agreement for reproducibility and repeatability was achieved for these two samples.

Further, the F1CDx analytical pipeline was modified after the analytical and clinical validation studies were completed. The analytical and clinical validation provided to support the detection of *RET* fusions in solid tumors by F1CDx were performed using previous versions of the analytical pipeline that has undergone further iteration with modification in the final device design. Additionally, during review, it was identified that a NSCLC RET fusion-positive sample evaluated in the clinical concordance study failed the tumor purity QC metric, but was reported as passing the QC metric and reported as *RET* fusion-negative. This was a curation error and the sample should have been considered invalid per the F1CDx Genomic Analysis and Review protocol. To address the source of the error, the analytical pipeline was updated to include an automatic QC flag for low tumor purity, a process that was previously performed manually, and implemented as a mitigation. To demonstrate the F1CDx analytical pipeline changes continue to support the performance for which the test is approved, regression testing using the most current analytical pipeline version will be performed

post-market to confirm the robust performance for F1CDx for the detection of *RET* fusions (see section XIII).

Table 11. Distribution of disease ontologies (DO) in the analytical validation studies.

Cancer Type	Disease Ontology	LoB	LoD	Precision	Concordance
Biliary	Ampullary adenocarcinoma	0	0	0	1
Breast	Breast carcinoma (NOS)	0	0	0	17
	Breast invasive ductal carcinoma (IDC)	2	1	0	3
Cervix	Cervix squamous cell carcinoma (SCC)	0	0	0	1
Colorectal	Colon adenocarcinoma (CRC)	1	1	6	50
	Colon neuroendocrine carcinoma	0	0	0	1
	Rectum adenocarcinoma (CRC)	0	0	0	1
Neuroendocrine	Adrenal gland neuroblastoma	0	0	0	1
Endometrial	Uterus endometrial adenocarcinoma	1	0	0	0
Glioma	Brain glioblastoma (GBM)	0	0	0	1
	Brain gliosarcoma	0	0	0	1
Kidney	Kidney clear cell carcinoma	1	0	0	0
Lung	Lung adenocarcinoma	6	2	3	121
	Lung non-small cell lung carcinoma (NOS)	3	0	0	37
	Lung squamous cell carcinoma	5	0	0	2
Melanoma	Eye intraocular melanoma	0	0	0	1
	Skin melanoma	0	0	0	1
Ovary	Ovary carcinoma mixed histology	0	0	0	1
	Ovary clear cell carcinoma	0	0	0	2
	Ovary epithelial carcinoma (NOS)	0	0	0	5
	Ovary high grade serous carcinoma	0	0	0	7
	Ovary mucinous carcinoma	0	0	0	1
	Ovary serous carcinoma	1	0	1	8
Pancreas	Pancreas carcinoma (NOS)	0	0	0	3
	Pancreas ductal adenocarcinoma	0	0	0	9

	Pancreatobiliary carcinoma (NOS)	0	0	0	1
	Pancreas neuroendocrine carcinoma	0	0	1	1
Parathyroid	Parathyroid carcinoma	0	0	0	1
Prostate	Prostate acinar adenocarcinoma	0	0	0	8
Salivary Gland	Salivary gland adenocarcinoma	0	0	0	2
	Salivary gland carcinoma (NOS)	0	0	0	4
	Salivary gland mammary analogue secretory carcinoma (MASC)	0	0	0	2
Skin	Skin basal cell carcinoma	0	0	0	2
Skin sarcoma	Skin sarcoma	1	0	0	0
Small intestine	Small intestine adenocarcinoma	0	0	0	3
Soft tissue sarcoma	Soft tissue sarcoma (NOS)	0	0	0	1
	Unknown primary sarcoma (NOS)	0	0	0	1
Thyroid	Medullary Thyroid Cancer (MTC)	1	0	0	0
	Thyroid anaplastic carcinoma	0	0	0	12
	Thyroid carcinoma (NOS)	0	0	2	33
	Thyroid follicular carcinoma	4	0	0	2
	Thyroid follicular oncocytic carcinoma	0	0	0	1
	Thyroid papillary carcinoma	1	2	1	51
Unknown	Unknown primary malignant neoplasm (NOS)	0	0	0	1
	Unknown primary adenocarcinoma	0	0	0	5
	Unknown primary carcinoma (NOS)	0	0	0	2
	Unknown primary urothelial carcinoma	0	0	0	1
	Unknown primary neuroendocrine tumor (NET)	0	0	0	1
	Total	**27**	**6**	**14**	**410**

1. **Analytical Accuracy/Concordance**
   a. **Comparison to Orthogonal Method for *RET* fusions**

An analytical accuracy study was performed to demonstrate the concordance between F1CDx and an externally validated NGS assay (evNGS) for the detection of *RET* fusions in solid tumors.

The analytical accuracy study was performed with available residual DNA previously extracted from FFPE clinical specimens (31 samples with sufficient remaining material) from patients with solid tumors enrolled in the clinical study, LOXO-RET-17001 (LIBRETTO-001, NCT03157128) that supported the RETEVMO (selpercatinib) approval (refer to Section X for study details). Due to the limited number of available clinical trial samples, the study also included *RET* fusion-positive samples (N=125) and *RET* fusion-negative (N=154) from the FMI clinical archives that were previously evaluated at FMI. The study also included 100 *RET* fusion-negative samples from patients with NSCLC previously processed and tested by the evNGS in prior concordance validation studies; these previously executed concordance studies were newly evaluated for *RET* fusions.

In total, 410 samples were processed in the analytical accuracy study: 160 NSCLC samples, 99 thyroid cancer (TC) samples, and 151 solid tumor samples. The solid tumor samples included samples from biliary tract (1), breast (20), cervix (1), colorectal (52), neuroendocrine (1), glioma (2), kidney (1), melanoma (2), ovary (24), pancreas (14), prostate (8), salivary gland (8), small intestine (3), soft tissue sarcoma (2) and unknown (10) cancer types. Table 12 presents a breakdown of the sample selection based on *RET* fusions.

Table 12. Samples evaluated for *RET* fusion concordance

Source material	Biomarker Status	Disease Ontology			Total
		NSCLC	TC	Solid tumors	
FMI clinical archives	POSITIVE	49	38	38	125
	NEGATIVE	1	51	102	154
LIBRETTO-001	POSITIVE	10	10	11	31
Samples for evNGS re-analysis	NEGATIVE	100	-	-	100
Total		160	99	151	410

There were three invalid samples: one LIBRETTO-001 sample had insufficient remaining material after F1CDx testing and two solid tumor samples failed to meet QC metrics during F1CDx sequencing and post-sequencing steps.

The F1CDx and evNGS results for the detection of *RET* fusions, using evNGS as the reference method, are provided in the contingency table below (Table 13).

Table 13. Contingency table comparing F1CDx and evNGS results for the detection of *RET* fusions

		evNGS			
		***RET*+**	***RET*-**	**Invalid**	**Total**
F1CDx	***RET*+**	146	7	1	154
	***RET*-**	0	254	0	254
	Invalid	0	2	0	2
	Total	146	263	1	410

The positive percent agreements (PPA), negative percent agreements (NPA), positive predictive value (PPV), and negative predictive value (NPV) with 95% confidence intervals (CI) are presented in Table 14 below.

Table 14. Summary of agreement measures

Agreement Statistic	Estimate [95% CI]
PPA	100% [97.44%, 100%]
NPA	97.32% [94.57%, 98.69%]
PPV	95.42% [90.86%, 97.77%]
NPV	100% [98.51%, 100%]

Since the PPA, NPA, PPV and NPV were calculated without adjusting for the distribution of samples enrolled, i.e., from the clinical trial or from FMI's clinical sample archives, the estimates of PPA, NPA, PPV, and NPV may be subject to potential bias.

There were seven samples (all from FMI clinical archives) that were discordant between the F1CDx and evNGS test results. All discordant samples were determined to be *RET* fusion-positive by F1CDx and *RET* fusion-negative by evNGS. Of the seven discordant calls, there were four samples that were not reported by evNGS due to low tumor content or low sample quality, one sample that had a fusion detected in a biomarker-negative orientation by evNGS, and two samples that were not detected by evNGS.

2. Analytical Sensitivity

a. Limit of Blank (LoB)

The limit of blank was evaluated by testing matched normal samples from patients with solid tumors. The solid tumor samples comprised of the following diseases and specimen types: lung squamous cell carcinoma and lung adenocarcinoma (lung), ovary serous carcinoma (ovary), breast invasive ductal carcinoma (breast), kidney clear cell carcinoma (kidney), colon adenocarcinoma (colon), skin sarcoma (skin), and uterus endometrial adenocarcinoma (uterus).

Each sample was assessed in replicates of four to 15, resulting in a total of 136 replicates. Of the 136 replicates, 135 were successfully sequenced and 126 passed the post-sequencing quality control (QC) criteria and were included in the analysis. Of the 126 valid sample replicates, there were no *RET* fusions detected, resulting in a false positive rate of 0%, and confirming the LoB to be zero.

The limit of blank was also evaluated by testing FFPE tumor specimens from patients with NSCLC and TC. For the 6 NSCLC samples, a total of 60 replicates were assessed, and 58 replicates were valid. For the 6 TC samples, a total of 66 replicates were assessed; 64 replicates were valid. The false positive rate was 0%, and LoB of zero was confirmed in both studies.

b. Limit of Detection (LoD)

The limit of detection (LoD) for the detection of *RET* fusions by F1CDx was determined by assessing six (6) samples. Selection of specimens for assessment of *RET* fusions represented various tumor types as shown in Table 15.

Table 15. Samples assessed in LoD study for the detection of *RET* fusions

Sample	Target Gene	Partner Gene	Fusion Partner or Alteration Description	Disease Indication (Specimen Site)
1	*RET*	*TRIM24*	5'-*TRIM24(*ex1-17 NM_003852*)*-*RET(*ex12-19 NM_020630)	Lung adenocarcinoma
2	*RET*	*ERC1*	5'-*ERC1(*ex1-7 NM_178039*)*-*RET(*ex12-19 NM_020630)	Lung adenocarcinoma
3	*RET*	*NCOA4*	5'-*NCOA4(*ex1-8 NM_005437*)*-*RET(*ex12-19 NM_020630)	Thyroid papillary carcinoma
4	*RET*	*CCDC6*	5'-*CCDC6*(ex1-1 NM_005436)-*RET*(ex12-19 NM_020630)	Thyroid papillary carcinoma
5	*RET*	*KIF5B*	5'-*KIF5B*(ex1-15 NM_004521)-*RET*(ex12-19 NM_020630)	Breast invasive ductal carcinoma (IDC)
6	*RET*	*CCDC6*	5'-*CCDC6*(ex1-1 NM_005436)-*RET*(ex11-19 NM_020630)	Colon adenocarcinoma (CRC)

Each sample was assessed at five targeted chimeric read levels (4, 12, 18, 24, and 30) with 20 replicates tested for each dilution level, except the dilution level of 30 chimeric reads, where 14 replicates were assessed. In total, 94 replicates were tested per sample, and each specimen was evaluated close to the minimum input requirements of the assay (50 ng). The LoD for each of the samples was determined based on chimeric reads using the hit rate method.

A summary of the LoD results based on chimeric reads using the hit rate method are shown in Table 16.

Table 16. Summary of LoD analysis for *RET* fusions

Sample	Target Gene	Partner Gene	*RET* Fusion LoD (chimeric reads)[1]
1	*RET*	*CCDC6*	4.90
2	*RET*	*NCOA4*	N/A[2]
3	*RET*	*TRIM24*	8.50
4	*RET*	*ERC1*	10.85
5	*RET*	*CCDC6*	8.75
6	*RET*	*KIF5B*	10.80

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

[2]The LoD was not estimated because the criteria to determine LoD by hit rate were not met.

The LoD of one sample was not estimated due to not meeting the hit rate method criteria, therefore, the LoD for *RET* fusions was determined using the median LoD from the five remaining samples. The LoD was determined to be 8.75 chimeric reads.

3. Precision and Reproducibility

a. Site-to-site Precision and Reproducibility

A site-to-site precision study was conducted to evaluate the inter-run reproducibility and intra-run repeatability of *RET* fusion detection. The study evaluated 14 different solid tumor samples at challenging DNA input (close to 50 ng) across different sites (Cambridge, MA and Morrisville, NC), reagent lots, and library construction start days. For each sample, 24 replicates were processed.

Table 17 summarizes the disease ontologies (DO) and *RET* fusion status for the 14 samples included in the precision study. Each sample was mixed with *RET* fusion-negative, DO-matched DNA to dilute the samples to 1-3x LoD.

Table 17. Samples selected for site-to-site precision study

Sample	Partner Gene	Target Gene	Fusion Partner or Alteration Description	Disease Ontology
1	*CCDC6*	*RET*	5'-*CCDC6*(ex1-1 NM_005436)-*RET*(ex12-19 NM_020630)	Thyroid carcinoma
2	*CCDC6*	*RET*	5'-*CCDC6*(ex1-1 NM_005436)-*RET*(ex12-19 NM_020630)	Thyroid papillary carcinoma
3	*CCDC6*	*RET*	5'-*CCDC6*(ex1-1 NM_005436)-*RET*(ex12-19 NM_020630)	Thyroid carcinoma

4	*KIF5B*	*RET*	5'-*KIF5B*(ex1-15 NM_004521)-*RET*(ex12-19 NM_020630)	Lung adenocarcinoma
5	*KIF5B*	*RET*	5'-*KIF5B*(ex1-15 NM_004521)-*RET*(ex12-19 NM_020630)	Lung adenocarcinoma
6	*KIF5B*	*RET*	5'-*KIF5B*(ex1-15 NM_004521)-*RET*(ex12-19 NM_020630)	Lung adenocarcinoma
7	*NCOA4*	*RET*	5'-*NCOA4*(ex1-8 NM_005437)-*RET*(ex12-19 NM_020630)	Colon adenocarcinoma
8	*NCOA4*	*RET*	5'-*NCOA4*(ex1-9 NM_005437)-*RET*(ex12-19 NM_020630)	Colon adenocarcinoma
9	*NCOA4*	*RET*	5'-*NCOA4*(ex1-9 NM_005437)-*RET*(ex12-19 NM_020630)	Colon adenocarcinoma
10	*NCOA4*	*RET*	5'-*NCOA4*(ex1-9 NM_005437)-*RET*(ex12-19 NM_020630)	Colon adenocarcinoma
11	*TRIM24*	*RET*	5'-*TRIM24*(ex1-9 NM_003852)-*RET*(ex12-19 NM_020630)	Colon adenocarcinoma
12	*PRPF19*	*RET*	5'-*PRPF19*(ex1-10 NM_014502)-*RET*(ex12-19 NM_020630)	Colon adenocarcinoma
13	*ERC1*	*RET*	5'-*ERC1*(ex1-7 NM_178039)-*RET*(ex12-19 NM_020630)	Pancreas neuroendocrine carcinoma
14	*CCDC6*	*RET*	5'-*CCDC6*(ex1-2 NM_005436)-*RET*(ex11-19 NM_020630)	Ovary serous carcinoma

Repeatability was evaluated in the 14 samples by processing two replicates from the same source sample and plate within each site, reagent lot, and start day. The result was considered in agreement if the duplicate replicates processed under identical conditions had the same detection status for the targeted *RET* fusion. The point estimates and 95% CIs for repeatability of each sample are detailed in Table 18.

Table 18. Repeatability for *RET* fusions

Sample	Observed Average Chimeric Reads	Fold LoD	Partner Gene	Target Gene	# Positive Replicates	# Total Valid Replicates	Repeatability (95% CI*)
1	13.1	1.50x	*ERC1*	*RET*	11	11	100% [74.1%, 100%]
2	12.0	1.37x	*CCDC6*	*RET*	12	12	100% [75.8%, 100%]
3	18.3	2.09x	*CCDC6*	*RET*	10	10	100% [N/A**]
4	13.4	1.53x	*NCOA4*	*RET*	12	12	100% [75.8%, 100%]
5	15.4	1.76x	*KIF5B*	*RET*	11	11	100% [74.1%, 100%]
6	18.7	2.14x	*CCDC6*	*RET*	11	11	100% [74.1%, 100%]

7	19.0	2.18x	*KIF5B*	*RET*	12	12	100% [75.8%, 100%]
8	21.1	2.41x	*NCOA4*	*RET*	9	10	90.0% [N/A**]
9	16.6	1.90x	*CCDC6*	*RET*	12	12	100% [75.8%, 100%]
10	13.0	1.48x	*KIF5B*	*RET*	12	12	100% [75.8%, 100%]
11	18.4	2.10x	*TRIM24*	*RET*	12	12	100% [75.8%, 100%]
12	21.8	2.49x	*NCOA4*	*RET*	12	12	100% [75.8%, 100%]
13	21.4	2.45x	*NCOA4*	*RET*	12	12	100% [75.8%, 100%]
14	12.4	1.41x	*PRPF19*	*RET*	11	12	91.7% [64.6%, 98.5%]

*Two-sided 95% CI is calculated by the Wilson Score Method.
**CI not provided for sample sizes ≤10.

Reproducibility was evaluated by processing replicates from the same source sample under conditions where one factor was changed at a time (e.g., reagent lots, site, days). To be considered a positive call, the *RET* fusion had to be detected in each replicate of the source sample and meet the biomarker definition. The point estimates and 95% CIs for reproducibility of each sample are detailed in Table 19.

Table 19. Reproducibility for *RET* fusions

Sample	Observed Average Chimeric Reads	Fold LoD	Partner Gene	Target Gene	# Positive Replicates	# Total Valid Replicates	Reproducibility (95% CI*)
1	13.1	1.50x	*ERC1*	*RET*	23	23	100% [85.7%, 100%]
2	12.0	1.37x	*CCDC6*	*RET*	24	24	100% [86.2%, 100%]
3	18.3	2.09x	*CCDC6*	*RET*	22	22	100% [85.1%, 100%]
4	13.4	1.53x	*NCOA4*	*RET*	24	24	100% [86.2%, 100%]
5	15.4	1.76x	*KIF5B*	*RET*	23	23	100% [85.7%, 100%]
6	18.7	2.14x	*CCDC6*	*RET*	23	23	100% [85.7%, 100%]
7	19.0	2.18x	*KIF5B*	*RET*	24	24	100% [86.2%, 100%]
8	21.1	2.41x	*NCOA4*	*RET*	21	22	95.5% [78.2%, 99.2%]
9	16.6	1.90x	*CCDC6*	*RET*	24	24	100% [86.2%, 100%]
10	13.0	1.48x	*KIF5B*	*RET*	24	24	100% [86.2%, 100%]
11	18.4	2.10x	*TRIM24*	*RET*	24	24	100% [86.2%, 100%]
12	21.8	2.49x	*NCOA4*	*RET*	24	24	100% [86.2%, 100%]
13	21.4	2.45x	*NCOA4*	*RET*	24	24	100% [86.2%, 100%]
14	12.4	1.41x	*PRPF19*	*RET*	23	24	95.8% [79.8%, 99.3%]

*Two-sided 95% CI is calculated by the Wilson Score Method

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 *RET* fusions in patients with solid tumors was demonstrated in a retrospective analysis of specimens from patients enrolled in the LIBRETTO-001 clinical study of RETEVMO (selpercatinib). Data generated from the LIBRETTO-001 trial supported the clinical validation of the F1CDx assay for the identification of patients with *RET* fusion-positive solid tumors who may benefit from treatment with selpercatinib.

A summary of the clinical study is presented below.

A. FoundationOne CDx Retrospective Analysis of *RET* fusions in LIBRETTO-001

A reasonable assurance of safety and effectiveness for F1CDx for the detection of *RET* fusions in patients with solid tumors who may benefit from treatment with RETEVMO® (selpercatinib) was established through a clinical bridging study using tumor tissue FFPE specimens from patients enrolled in the LOXO-RET-17001 (LIBRETTO-001) clinical study with known *RET* fusion status, as well as *RET* fusion negative samples from the FMI archives. The clinical efficacy analysis was performed by analyzing concordance between F1CDx and the enrollment clinical trial assays (CTAs), followed by the imputation of the missing F1CDx results, and finally determining the clinical outcome of the *RET* fusion positive population identified with F1CDx.

1. LIBRETTO-001 Study Design

The LIBRETTO-001 clinical study is an open-label, multi-center Phase 1/2 study in patients with advanced solid tumors, including *RET* fusion-positive solid tumors (e.g., NSCLC, thyroid, pancreas, colorectal), *RET*-mutant MTC, and other tumors with *RET* activation (e.g., mutations in other tumor types or other evidence of *RET* activation). LIBRETTO-001 was initiated on May 2, 2017. This study included two parts: Phase 1 (dose escalation and dose expansion) and Phase 2 (dose expansion). The primary objective of the Phase 1 portion of the study was to determine the maximum tolerated dose (MTD)/recommended Phase 2 dose (RP2D) of RETEVMO. RP2D was determined to be 160 mg of RETEVMO orally twice daily (BID). Primary efficacy, the primary objective of Phase 2, was measured by the objective response rate (ORR) using Response Evaluation Criteria in Solid Tumors (RECIST 1.1) or Response Assessment in Neuro-Oncology (RANO), as appropriate for tumor type, as assessed by blinded independent review committee (BIRC). RETEVMO was approved by FDA for *RET* fusion positive non-small cell lung cancer (NSCLC), *RET* fusion positive thyroid

cancer, and *RET* mutation positive medullary thyroid cancer in May 2020 based on safety and efficacy data from patients in LIBRETTO-001 with *RET* alterations. Further, RETEVMO was approved by FDA for *RET* fusion positive solid tumors in September 2022 based on safety and efficacy data from patients in LIBRETTO-001 with *RET* fusions.

2. *RET* fusion Evaluation by F1CDx

The clinical effectiveness of F1CDx for detecting *RET* fusions in patients with solid tumors who may benefit from treatment with RETEVMO was demonstrated in a retrospective analysis of specimens from the LIBRETTO-001 clinical study. A bridging study was conducted to assess: (1) concordance between the clinical trial assays (CTAs) and F1CDx in identifying patients with *RET* fusions, (2) the efficacy of RETEVMO in patients from the LIBRETTO-001 clinical study who have *RET* fusions as determined by F1CDx, and (3) the robustness of the concordance analysis and efficacy analysis with a sensitivity analysis that accounts for the uncertainty due to missing data for *RET* fusion status as determined by F1CDx.

Clinical Bridging Study Design

The clinical bridging study evaluated the clinical validity of F1CDx as a companion diagnostic (CDx) to identify *RET* fusion-positive patients from the LIBRETTO-001 clinical study. F1CDx testing was performed on LIBRETTO-001 patients who had samples with sufficient tissue material remaining and who tested positive for *RET* fusions by the CTAs (CTA+). *RET* fusion negative samples from FMI's banked clinical samples archives were selected and tested with FoundationOne (F1) LDT, an NGS tissue assay, and subsequently tested by F1CDx for the CTA negative (CTA-) results.

i. Clinical Inclusion and Exclusion Criteria

Samples meeting the pre-defined criteria specified below were included in the clinical bridging study.

Inclusion Criteria:

- FFPE tissue samples (blocks or slides)
- DNA derived from FFPE samples
- Samples that meet F1CDx processing requirements
- Samples that meet minimum criteria for F1CDx testing requirements
- Samples from the LIBRETTO-001 clinical trial with proper informed consent

Exclusion Criteria:

- Samples failing to meet any of the inclusion criteria
- LIBRETTO-001 clinical trial samples that lack clear identification or labeling

- Samples not obtained in accordance with Institutional Review Board (IRB) approval

ii. Follow-up Schedule

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

iii. Clinical Endpoints

The objectives of the F1CDx clinical bridging study were to:

- Establish the clinical validity of F1CDx in identifying *RET* fusion-positive solid tumors for treatment with RETEVMO
- Assess concordance of results for the *RET* fusion status between the F1CDx assay and the CTAs used for enrollment onto the LIBRETTO-001 clinical trial

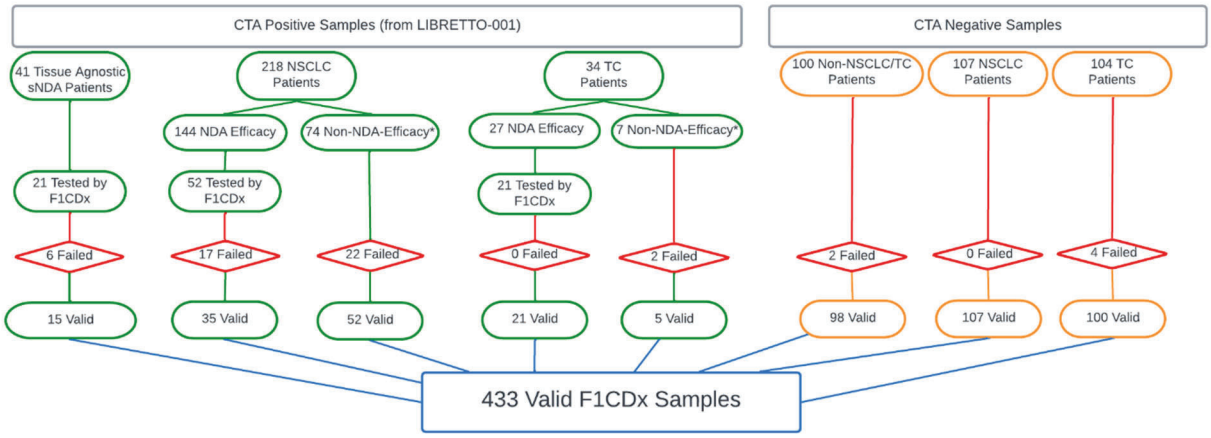
The efficacy analysis was performed using the primary efficacy outcome measure of the LIBRETTO-001 clinical study: Objective Response Rate (ORR) using RECIST 1.1, as appropriate for tumor type, as assessed by BIRC. Endpoints for the concordance analysis included PPA, NPA, and prevalence adjusted PPV and NPV.

B. Accountability of PMA Cohort

There were 175 patients from the LIBRETTO-001 clinical trial with sufficient tissue samples available for testing with F1CDx. Of the 41 patients in the tissue agnostic (TA) supplemental new drug application (sNDA) population, 21 had samples available. Of the 144 patients in the non-small cell lung cancer (NSCLC) new drug application (NDA) population, 52 had samples available, and 74 additional samples were also available from the non-NDA efficacy patients for the concordance assessment. Additionally, of the 27 patients in the thyroid cancer (TC) NDA population, 21 had samples available, and 7 additional samples were also available from non-NDA efficacy patients for the concordance assessment. After sample processing, there were 15 TA sNDA samples, 87 NSCLC samples (35 NDA efficacy, 52 non-NDA efficacy), and 26 TC samples (21 NDA efficacy, 5 non-NDA efficacy) with valid F1CDx results. The pooled pan-tumor NDA efficacy population (PPT) consisted of the TA sNDA population, the NSCLC NDA population, and the TC NDA population, which yielded a total of 71 samples with valid F1CDx test results.

In addition to the clinical study samples, 311 samples (100 pan-tumor, 107 NSCLC, and 104 TC) from FMI's clinical archives were processed by F1CDx for the *RET* fusion-negative population. There were six sample failures and 305 samples with valid F1CDx results; 138 samples (98 solid tumor, 20 NSCLC, and 20 TC) with valid F1CDx results were used in the PPT concordance analysis.

See Figure 1 for a schematic of the sample accountability in the F1CDx clinical bridging study.



*Non-NDA efficacy patients are included for concordance analysis only.
Figure 1: Clinical Bridging Study Sample Accountability

C. Study Population Demographics and Baseline Parameters

In the clinical bridging study, baseline characteristics were compared between CDx-evaluable and CDx-unevaluable populations of the PPT population. The unevaluable population included patients who did not have a sample tested by F1CDx or those whose sample failed F1CDx testing (invalid). The demographics and disease characteristics for the CDx-evaluable and CDx-unevaluable PPT patients are provided in Table 20. Differences in characteristics of the CDx-evaluable and CDx-unevaluable populations were identified by covariate analysis. The covariates that showed significant differences between the two groups at a significance level of α=0.2 were considered to be imbalanced. The number of patients without information collected or available are listed under NA (Missing).

Table 20. Comparison of PPT demographics and clinical characteristics in the F1CDx evaluable-and F1CDx-unevaluable groups

Covariates	F1CDx-evaluable| CTA+	F1CDx-unevaluable| CTA+	Difference	P-value**
Clinical Outcome (Response)				0.166
NO	26.8% (19)	36.9% (52)	-10.1%	
YES	73.2% (52)	63.1% (89)	10.1%	
Age				0.607
Min	20.0	21.0	-1.0	
Q1	47.5	48.0	-0.5	

Median	57.0	60.0	-3.0	
Mean	56.1	57.5	-1.5	
Q3	68.0	69.0	-1.0	
Max	86.0	88.0	-2.0	
SD	15.2	13.6	1.6	
Race				0.349
Asian	28.2% (20)	27.7% (39)	0.5%	
Black or African American	5.6% (4)	5.0% (7)	0.7%	
Mative Hawaiian or other Pacific Islander	1.4% (1)	0.0% (0)	1.4%	
Other	5.6% (4)	2.1% (3)	3.5%	
White	56.3% (40)	64.5% (91)	-8.2%	
NA (missing)	2.8% (2)	0.7% (1)	2.1%	
Ethnicity				0.733
Hispanic or Latino	5.6% (4)	4.3% (6)	1.4%	
not hispanic or latino	90.1% (64)	93.6% (132)	-3.5%	
NA (missing)	4.2% (3)	2.1% (3)	2.1%	
Sex				0.464
Female	52.1% (37)	58.2% (82)	-6.0%	
Male	47.9% (34)	41.8% (59)	6.0%	
Tumor Stage (Grouped*)				1.000
STAGE I/II/III	5.6% (4)	5.0% (7)	0.7%	
STAGE IV	91.5% (65)	92.9% (131)	-1.4%	
NA (missing)	2.8% (2)	2.1% (3)	0.7%	
Smoking History				0.332
Current smoker	1.4% (1)	1.4% (2)	0.0%	
Former smoker	21.1% (15)	29.8% (42)	-8.7%	
Never a smoker	77.5% (55)	68.1% (96)	9.4%	
NA (missing)	0.0% (0)	0.7% (1)	-0.7%	
Geographic Region				0.460
Asia Pacific (APAC)	25.4% (18)	21.3% (30)	4.1%	
European Union (EU)	7.0% (5)	12.1% (17)	-5.0%	
Middle East (ME)	0.0% (0)	2.1% (3)	-2.1%	
North America	67.6% (48)	64.5% (91)	3.1%	
ECOG Status				0.107
0	33.8% (24)	33.3% (47)	0.5%	
1	59.1% (42)	65.2% (92)	-6.1%	
2	7.0% (5)	1.4% (2)	5.6%	
Tumor Grade				0.288
Moderately differentiated	4.2% (3)	7.8% (11)	-3.6%	
Not applicable	9.9% (7)	13.5% (19)	-3.6%	
Poorly differentiated	32.4% (23)	19.1% (27)	13.2%	
Undifferentiated	1.4% (1)	0.7% (1)	0.7%	
Well differentiated	5.6% (4)	3.5% (5)	2.1%	
NA (missing)	46.5% (33)	55.3% (78)	-8.8%	

Diagnosis of Primary Tumor				<0.001
Anaplastic thyroid cancer	1.4% (1)	0.0% (0)	1.4%	
Anaplastic thyroid carcinoma	1.4% (1)	0.0% (0)	1.4%	
Atypical carcinoid tumor	1.4% (1)	0.0% (0)	1.4%	
Biliary tract	0.0% (0)	0.7% (1)	-0.7%	
Breast	1.4% (1)	0.7% (1)	0.7%	
Cancer of parotid gland	0.0% (0)	0.7% (1)	-0.7%	
Colon	2.8% (2)	5.7% (8)	-2.9%	
Cutaneous juvenile xanthogranuloma	0.0% (0)	0.7% (1)	-0.7%	
Disseminated cutaneous juvenile xanthogranulomatosis	0.0% (0)	0.7% (1)	-0.7%	
Hurthle cell	1.4% (1)	0.0% (0)	1.4%	
Neuroendocrine-rectal	1.4% (1)	0.0% (0)	1.4%	
Non-small cell lung cancer (nsclc)	49.3% (35)	77.3% (109)	-28.0%	
Ovary	1.4% (1)	0.0% (0)	1.4%	
Pancreas	5.6% (4)	5.0% (7)	0.7%	
Papillary thyroid cancer	23.9% (17)	2.8% (4)	21.1%	
Parotid gland	1.4% (1)	0.0% (0)	1.4%	
Parotid gland cancer	0.0% (0)	0.7% (1)	-0.7%	
Poorly differentiated thyroid cancer	1.4% (1)	1.4% (2)	0.0%	
Pulmonary carcinosarcoma	0.0% (0)	0.7% (1)	-0.7%	
Salivary gland adenocarcinoma	1.4% (1)	0.0% (0)	1.4%	
Sarcoma	2.8% (2)	0.0% (0)	2.8%	
Skin-non-melanoma	0.0% (0)	0.7% (1)	-0.7%	
Small bowel adenocarcinoma	1.4% (1)	0.0% (0)	1.4%	
Unknown primary	0.0% (0)	0.7% (1)	-0.7%	
Unknown primary (urothelial or renal source suspected)	0.0% (0)	0.7% (1)	-0.7%	
Unknown primary malignant	0.0% (0)	0.7% (1)	-0.7%	
Primary Tumor Type (Grouped*)				<0.001
NSCLC	49.3% (35)	77.3% (109)	-28.0%	
Other	21.1% (15)	18.4% (26)	2.7%	
Thyroid	29.6% (21)	4.3% (6)	25.3%	
Tumor Subtype				<0.001

Adenocarcinoma	43.7% (31)	66.0% (93)	-22.3%	
Anaplastic thyroid cancer	2.8% (2)	0.0% (0)	2.8%	
Hurthle cell thyroid cancer	1.4% (1)	0.0% (0)	1.4%	
Large cell neuroendocrine carcinoma	0.0% (0)	1.4% (2)	-1.4%	
Papillary thyroid cancer	23.9% (17)	2.8% (4)	21.1%	
Poorly differentiated thyroid cancer	1.4% (1)	1.4% (2)	0.0%	
Squamous cell carcinoma	0.0% (0)	0.7% (1)	-0.7%	
NA (missing)	26.8% (19)	27.7% (39)	-0.9%	
Cancer Surgery				<0.001
N	28.2% (20)	54.6% (77)	-26.4%	
Y	71.8% (51)	45.4% (64)	26.4%	

*Factor Levels were aggregated to increase number of data points within groups
** p-value was from nonparametric Mann-Whitney Test for continuous measures, and Fisher-Freeman-Halton Test for categorical measures between the CDx-evaluable and CDx-unevaluable sets

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with RETEVMO (selpercatinib) was addressed during the review of the NDA and is not addressed in detail in this Summary of Safety and Effectiveness Data. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs. Please refer to Drugs@FDA for complete safety information on selpercatinib.

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

The effectiveness of F1CDx to identify patients with *RET* fusions who may benefit from treatment with selpercatinib is supported by the evaluation of a pooled pan-tumor patient population enrolled onto the LIBRETTO-001 clinical trial including a tissue agnostic, NSCLC, and TC patient population. The concordance results, efficacy analysis and sensitivity analysis of missing results are presented for the PPT population (2.i.a-c), and also separately for the TA population (2.ii.a-c), NSCLC population (2.iii.a-c), and the thyroid cancer population (2.iv.a-c).

i. Pooled Pan-Tumor (PPT) Population

a. Concordance Results

A total of 71 *RET* fusion-positive and 138 *RET* fusion-negative samples with valid F1CDx results in the PPT population were evaluated in the

concordance assessment between F1CDx and the CTAs used for patient enrollment into the LIBRETTO-001 clinical trial. A contingency table with the concordance results is provided in Table 21. The PPA and NPA were established as 90.1% (95% CI [81.0%, 95.1%]) and 100% (95% CI [97.3%, 100%]), respectively after excluding invalid results. However, the NPA estimate between F1CDx and CTA could be subject to bias and the invalid rate may be underestimated given that the *RET* fusion negative population was selected from the FMI clinical archives and had been previously tested using other FoundationOne NGS methods.

Table 21. PPT contingency table comparing *RET* fusions between the CTAs and F1CDx

		CTA		
		Detected (+)	**Not Detected (-)**	**Total**
F1CDx	**Detected (+)**	64	0	64
	Not Detected (-)	7	138	145
	Invalid[†]	23	2	25
	Total	94	140	234
Agreement statistics excluding invalid[†] results [2-sided 95% CI]*		PPA 90.1% [81.0%, 95.1%]	NPA 100% [97.3%, 100%]	
Percent invalids[†]		24.5% (23/94)	1.43% (2/140)	

[†]Invalid describes samples that failed F1CDx testing and does not include CDx- unevaluable CTA+ patients whose samples were not tested by F1CDx. The percent F1CDx-unevaluable for the CTA+ patients was 66.5% (141 out of the 212 total patients). F1CDx-unevaluable CTA- patients only include those whose sample failed F1CDx testing (1.43%; 2 out of the 140 patients tested by F1CDx).
*Two-sided 95% CI is calculated by the Wilson Score Method

Of the seven discordant patients in the PPT population, four patients had complete or partial response, supporting that these four samples were most likely true positives. Investigation findings concluded that of the four patients who responded to selpercatinib, two were biomarker-negative by the CDx rules, one was biomarker-negative by the CDx rules but the fusion event was removed due to contamination, and one was biomarker-positive, but the fusion event was removed due to contamination. One of the samples that was biomarker-negative by the CDx rules had low-tumor purity, which many have led to a false negative result. Additionally, three of the four patients (75%) who responded to treatment were enrolled into the trial using RNA-based NGS assays. Further, of the seven discordant patients, three patients did not respond to selpercatinib. Of the three samples, two were biomarker-negative (one of which was due to CDx biomarker rules) and one was biomarker-positive, but the fusion event was removed due to contamination.

b. Clinical Efficacy Results

The clinical validity of F1CDx for the detection of *RET* fusions in patients with solid tumors for the PPT population was based on estimation of clinical efficacy in the F1CDx-positive population and subgroups of the CTA+ population by F1CDx status. The primary efficacy endpoint was measured by the ORR using RECIST 1.1 as assessed by BIRC. The ORR was defined as the observed proportion of patients whose best overall response is confirmed complete response (CR) or partial response (PR) as determined by independent review committee and by the treating investigator. The ORR and two-sided 95% CI, calculated using the Wilson-Score method, for each population in the PPT population is presented in Table 22.

Table 22. PPT efficacy in the bridging study subpopulations

	CTA+	F1CDx+|CTA+	F1CDx-|CTA+	F1CDx unevaluable|CTA+
# Total	212	64	7	141
Responders (CR or PR)	141	48	4	89
ORR (%)	66.5	75.0	57.1	63.1
Two-sided 95% Score CI (%)	[59.9, 72.5]	[63.2, 84.0]	N/A*	[54.9, 70.6]

* The CI is not provided for sample sizes ≤10.

The ORR estimated for the F1CDx-positive population was 75% (95% CI [64.4%, 85.6%]). The ORR for the CDx+ PPT population is presented in Table 23.

Table 23. PPT efficacy for the F1CDx-positive population

	F1CDx+
ORR (%)	75.0
Two-sided 95% CI (%)*	[64.4, 85.6]

*Calculated using normal approximation CI based on $Var(\delta_{CDx+})$/Wald method.

c. Sensitivity Analysis for Missing CDx results

A sensitivity analysis with regard to missing values was conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with unevaluable F1CDx results. Samples were considered

unevaluable if the samples were not tested or if they were tested but returned as invalid result.

Amongst the CTA-positive PPT population, 66.5% (141/212) did not have a F1CDx result, either due to unavailable tissue or invalid result.

The sensitivity analysis employed the multiple imputation method to impute missing F1CDx results and used the imputed and observed datasets to estimate the ORR for F1CDx positive patients. The point estimate of ORR for the F1CDx+ population in the sensitivity analysis was 68.8% (95% CI [67.3%, 70.4%]). The CI was estimated using the variance from 100 bootstrap datasets within each of the 50 imputed data sets and applying Rubin's rules.

ii. Tissue Agnostic (TA) Population

a. Concordance Results

A total of 15 *RET* fusion-positive and 98 *RET* fusion-negative samples with valid F1CDx results in the TA population were evaluated in the concordance assessment between F1CDx and the CTAs used for patient enrollment into the LIBRETTO-001 clinical trial. A contingency table with the concordance results is provided in Table 24.

Table 24. Contingency table for concordance analysis with TA sNDA patients

		CTA		
		Detected (+)	**Not Detected (-)**	**Total**
F1CDx	**Detected (+)**	13	0	13
	Not Detected (-)	2	98	100
	Invalid[†]	6	2	2
	Total	21	100	121
Agreement statistics excluding Invalid[†] results [2-sided 95% CI]*		PPA: 86.7% [62.1%, 96.3%]	NPA: 100% [96.2%, 100%]	
Percent Invalid[†]		28.6% (6/21)	2.0% (2/100)	

[†]Invalid describes samples that failed F1CDx testing and does not include CDx-unevaluable CTA+ patients whose samples were not tested by F1CDx. The percent F1CDx-unevaluable for the CTA+ patients was 63.4% (26 out of the 41 total patients). F1CDx-unevaluable CTA- patients only include those whose sample failed F1CDx testing (2.0%; 2 out of the 100 patients tested by F1CDx).
*Two-sided 95% CI is calculated by the Wilson Score Method

The PPA and NPA were established as 86.67% (95% CI [62.12%, 96.26%]) and 100% (95% CI [96.23%, 100%]), respectively, after excluding invalid results. However, the NPA estimate between F1CDx and CTA could be subject to bias and the invalid rate may be

underestimated given that the *RET* fusion negative population was selected from the FMI clinical archives and had been previously tested using other FoundationOne NGS methods.

There were two discordant patients in the TA population; both patients did not respond to selpercatinib treatment. Upon investigation, one sample was biomarker-positive, but the fusion event was removed due to contamination, and one sample did not have a *RET* fusion detected by F1CDx.

b. Clinical Efficacy Results

The clinical validity of F1CDx for the detection of *RET* fusions in patients with solid tumors for the TA population was based on the estimation of clinical efficacy in the F1CDx-positive population and subgroups of the CTA+ population by F1CDx status. The efficacy outcome measure was ORR using RECIST 1.1 as assessed by BIRC. The ORR and two-sided 95% CI, calculated using the Wilson-Score method, for each population in the TA population is presented in Table 25.

Table 25. TA efficacy in the bridging study subpopulations

	CTA+	F1CDx+|CTA+	F1CDx-|CTA+	F1CDx unevaluable|CTA+
# Total	41	13	2	26
# Responders (CR or PR)	18	8	0	10
ORR (%)	43.9	61.5	0	38.5
Two-sided 95% Score CI (%)	[29.9, 59.0]	[35.5, 82.3]	N/A*	[22.4, 57.5]

* The CI is not provided for sample sizes ≤10.

The ORR estimated for the F1CDx-positive population was 61.5% (95% CI [35.1%, 88%]). The ORR for the CDx+ TA population is presented in Table 26.

Table 26. TA efficacy for the F1CDx-positive population

	F1CDx+
ORR (%)	61.5
Two-sided 95% CI (%)*	[35.1, 88.0]

*Calculated using normal approximation CI based on $Var(\delta_{CDx+})$/Wald method.

ORR was also assessed by tumor type, and the subgroup analysis is depicted in Table 27 below.

Table 27. Summary of ORR per tumor type among tissue-agnostic sNDA patients

Tumor Type	CTA+			F1CDx+|CTA+			F1CDx-|CTA+			F1CDx-Unevaluable|CTA+		
	# Total	# Responders	ORR (%)	# Total	#Responders	ORR (%)	# Total	# Responders	ORR (%)	# Total	# Responders	ORR (%)
PANCREATIC	11	6	54.55	3	2	66.67	1	0	0.00	7	4	57.14
COLON	10	2	20.00	1	0	0.00	1	0	0.00	8	2	25.00
SALIVARY	4	2	50.00	2	1	50.00	0	N/A	N/A	2	1	50.00
UNKNOWN PRIMARY	3	1	33.33	0	N/A	N/A	0	N/A	N/A	3	1	33.33
BREAST	2	2	100	1	1	100	0	N/A	N/A	1	1	100
SARCOMA	2	1	50.00	2	1	50.00	0	N/A	N/A	0	N/A	N/A
XANTHOGRANULOMA	2	0	0.00	0	N/A	N/A	0	N/A	N/A	2	0	0.00
CARCINOID	1	1	100	1	1	100	0	N/A	N/A	0	N/A	N/A
CARCINOMA OF THE SKIN	1	0	0.00	0	N/A	N/A	0	N/A	N/A	1	0	0.00
CHOLANGIOCARCINOMA	1	1	100	0	N/A	N/A	0	N/A	N/A	1	1	100
OVARIAN	1	1	100	1	1	100	0	N/A	N/A	0	N/A	N/A
PULMONARY CARCINOSARCOMA	1	0	0.00	0	N/A	N/A	0	N/A	N/A	1	0	0.00
RECTAL NEUROENDOCRINE	1	0	0.00	1	0	0.00	0	NA	N/A	0	N/A	N/A
SMALL INTESTINE	1	1	100	1	1	100	0	N/A	N/A	0	N/A	N/A

c. Sensitivity Analysis for Missing CDx results

A sensitivity analysis with regard to missing values was conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with unevaluable F1CDx results. Samples were considered unevaluable if the samples were not tested or if they were tested but returned as invalid result.

Amongst the CTA-positive TA population, 63.4% (26/41) did not have a F1CDx result, either due to unavailable tissue or invalid result.

The sensitivity analysis employed the multiple imputation method to impute missing F1CDx results and used the imputed and observed datasets to estimate the ORR for F1CDx positive patients. The point estimate of ORR for the F1CDx+ population in the sensitivity analysis was 61.2% (95% CI [42.9%, 79.5%]). The CI was estimated using the variance from 100 bootstrap datasets within each of the 50 imputed data sets and applying Rubin's rules.

The clinical effectiveness of F1CDx to identify patients with solid tumors with *RET* fusions who may benefit from RETEVMO treatment is based on ~36.6% of the RETEVMO efficacy population. To address the uncertainties due to the large proportion of missing data, a post-market study to provide clinical outcome data will be provided to confirm the clinical effectiveness of F1CDx (see section XIII).

iii. NSCLC Population

a. Concordance Results

A total of 88 *RET* fusion-positive and 107 *RET* fusion-negative samples with valid F1CDx results in the NSCLC population were evaluated in the concordance assessment between F1CDx and the CTAs used for patient enrollment into the LIBRETTO-001 clinical trial. A contingency table with the concordance results is provided in Table 28.

Table 28. NSCLC contingency table comparing *RET* fusions between the CTAs and F1CDx

		CTA		
		Detected (+)	**Not Detected (-)**	**Total**
F1CDx	**Detected (+)**	80	0	80
	Not Detected (-)	7	107	115
	Invalid†	39	0	38
	Total	126	107	233
Agreement statistics excluding Invalid† results [2-sided 95% CI]*		PPA: 92.0% [84.3%, 96.0%]	NPA: 100% [96.5%, 100%]	
Percent Invalid†		31.0% (39/126)	0.0% (0/107)	

†Invalid describes samples that failed F1CDx testing and does not include CDx-unevaluable CTA+ patients whose samples were not tested by F1CDx. The percent F1CDx-unevaluable for the CTA+ patients was 60.1% (131 out of the 218 total patients). F1CDx-unevaluable CTA- patients only include those whose sample failed F1CDx testing (0.0%; 0 out of the 107 patients tested by F1CDx).
*Two-sided 95% CI is calculated by the Wilson Score Method

The PPA and NPA, presented in Table 28, were established as 92.0% (95% CI [84.3%, 96.0%]) and 100% (95% CI [96.5%, 100%]), respectively, after excluding invalid results. However, the NPA estimate between F1CDx and CTA could be subject to bias and the invalid rate

may be underestimated given that the *RET* fusion negative population was selected from the FMI clinical archives and had been previously tested using other FoundationOne NGS methods.

Of the 7 discordant patients in the NSCLC population, 6 patients had complete or partial response, supporting that these 6 samples were most likely true positives. Investigation findings concluded that of the 6 patients who responded to selpercatinib, 2 were biomarker-negative by the CDx rules and 4 were biomarker-positive but the fusion event was removed due to contamination. Further, 3 of the 6 patients who responded to treatment were enrolled onto the clinical trial using RNA-based NGS assays. The 1 remaining discordant sample came from a patient who did not respond to selpercatinib treatment. The sample was biomarker-positive, but the fusion event was removed due to contamination.

b. Clinical Efficacy Results

The clinical efficacy of F1CDx for the detection of *RET* fusions in patients with NSCLC was based on estimation of the NSCLC NDA clinical efficacy in the F1CDx-positive population, subgroups of the CTA+ population by F1CDx status and separated by treatment status: prior platinum treatment or treatment-naïve. The ORR and two-sided 95% CI, calculated using the Wilson-Score method, for each population in the NSCLC subgroups are presented in Tables 29 and 30. The ORR was 100% for the F1CDx negative, CTA positive patients in both subgroups, but the sample sizes were low (n=1).

Table 29. NSCLC efficacy for prior platinum-treated patients in the bridging study subpopulations

	CTA+	F1CD+|CTA+	F1CDx-|CTA+	F1CDx unevaluable|CTA+
# Total	105	26	1	78
# Responders (CR or PR)	67	19	1	47
ORR (%)	63.8	73.1	100	60.3
Two-sided 95% Score CI (%)	[54.3, 72.4]	[53.9, 86.3]	N/A*	[49.2, 70.4]

* The CI is not provided for sample sizes ≤10.

Table 30. NSCLC efficacy for treatment-naïve patients in the bridging study subpopulations

	CTA+	F1CDx+|CTA+	F1CDx-|CTA+	F1CDx unevaluable|CTA+
# Total	39	7	1	31
# Responders (CR or PR)	33	5	1	27
ORR (%)	84.6	71.4	100	87.1
Two-sided 95% Score CI (%)	[70.3, 92.8]	N/A*	N/A*	[71.1, 94.9]

* The CI is not provided for sample sizes ≤10.

The ORR estimated for the F1CDx-positive population was 73.1% (95% CI [56%, 90.1%]) for prior platinum-treated patients and 71.4% (95% CI [38%, 100%]) for treatment-naïve patients. The ORR for the NSCLC subgroups is presented in Tables 31 and 32.

Table 31. NSCLC efficacy for the prior platinum-treated F1CDx-positive population

	F1CDx+
ORR (%)	73.1
Two-sided 95% CI (%)*	[56.0, 90.1]

*Calculated using normal approximation CI based on $Var(\delta_{CDx+})$/Wald method.

Table 32. NSCLC efficacy for the treatment-naïve F1CDx-positive population

	F1CDx+
ORR (%)	71.4
Two-sided 95% CI (%)	[38.0, 100]*

*Calculated with low sample size using normal approximation CI based on $Var(\delta_{CDx+})$/Wald method.

c. Sensitivity Analysis for Missing CDx results

A sensitivity analysis with regard to missing values was conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with unevaluable F1CDx results. Samples were considered unevaluable if the samples were not tested or if they were tested but returned as invalid result.

Amongst the CTA-positive NSCLC population, 75.7% (109/144) did not have a F1CDx result, either due to unavailable tissue or invalid result.

The sensitivity analysis employed the multiple imputation method to impute missing F1CDx results and used the imputed and observed datasets to estimate the ORR for F1CDx positive patients. The point estimates of ORR for the F1CDx+ population in the sensitivity analysis was 62.5% (95% CI [53.7%, 71.2%]) for the patients with prior platinum treatment and was 82.6% (95% CI [70.5%, 94.8%]) for the treatment-naïve patients. The CI was estimated using the variance from 100 bootstrap datasets within each of the 50 imputed data sets and applying Rubin's rules.

iv. Thyroid Cancer (TC) Population

a. Concordance Results

A total of 26 *RET* fusion-positive and 100 *RET* fusion-negative samples with valid F1CDx results in the TC population were evaluated in the concordance assessment between F1CDx and the CTAs used for patient enrollment into the LIBRETTO-001 clinical trial. A contingency table with the concordance results is provided in Table 33.

Table 33. TC contingency table comparing *RET* fusions between the CTAs and F1CDx

		CTA		
		Detected (+)	**Not Detected (-)**	**Total**
F1CDx	**Detected (+)**	23	0	23
	Not Detected (-)	3	100	103
	Invalid†	2	4	12
	Total	28	104	138
Agreement statistics excluding invalid† results [2-sided 95% CI]*		PPA: 88.5% [71.0%, 96.0%]	NPA: 100% [96.3%, 100%]	
Percent invalid†		7.1% (2/28)	3.8% (4/104)	

†Invalid describes samples that failed F1CDx testing and does not include CDx-unevaluable CTA+ patients whose samples were not tested by F1CDx. The percent F1CDx-unevaluable for the CTA+ patients was 23.5% (8 out of the 34 total patients). F1CDx-unevaluable CTA- patients only include those whose sample failed F1CDx testing (3.8%; 4 out of the 104 patients tested by F1CDx).
*Two-sided 95% CI is calculated by the Wilson Score Method

The PPA, NPA and two-sided 95% Wilson-score CIs, presented in Table 34 were established as 88.5% (95% CI [71.0%, 96%]) and 100% (95% CI [96.3%, 100%]), respectively, after excluding invalid results. However, the NPA estimate between F1CDx and CTA could be subject to bias given that the *RET* fusion negative population was selected from

the FMI clinical archives and had been previously tested using other FoundationOne NGS methods.

Of the 3 discordant patients in the TC population, 2 patients had complete or partial response, supporting that these 2 samples were most likely true positives. Investigation findings concluded that of the 2 patients who responded to selpercatinib, 1 was biomarker-negative by the CDx rules and 1 was biomarker-negative but the fusion event was removed due to contamination. Further, both patients who responded to treatment were enrolled onto the clinical trial using RNA-based NGS assays. The remaining discordant patient did not respond to selpercatinib treatment. This1 sample was biomarker-negative by the CDx rules.

b. Clinical Efficacy Results

The clinical efficacy of F1CDx for the detection of *RET* fusions in patients with TC was based on estimation of the TC NDA clinical efficacy in the F1CDx-positive population and subgroups of the CTA+ population by F1CDx status and separated by treatment status: prior treatment or treatment-naïve. The ORR and two-sided 95% CI, calculated using the Wilson-Score method, for each population in the TC subgroups are presented in Tables 34 and 35. In the treatment-naïve subgroup, the ORR was 100% for the F1CDx negative, CTA positive patients, but the sample size was low (n=1).

Table 34. TC efficacy for prior-treated patients in the bridging study subpopulations

	CTA+	F1CDx+|CTA+	F1CDx-|CTA+	F1CDx unevaluable|CTA+
# Total	19	11	2	6
# Responders (CR or PR)	15	9	1	5
ORR (%)	78.9	81.8	50.0	83.3
Two-sided 95% Score CI (%)	[56.7, 91.5]	[52.3, 94.9]	N/A*	N/A*

* The CI is not provided for sample sizes ≤10.

Table 35. TC efficacy for treatment-naïve patients in the bridging study subpopulations

	CTA+	F1CDx+|CTA+	F1CDx-|CTA+	F1CDx unevaluable|CTA+
# Total	8	7	1	0

# Responders (CR or PR)	8	7	1	N/A
ORR (%)	100	100	100	N/A
Two-sided 95% Score CI (%)	N/A*	N/A*	N/A*	N/A

* The CI is not provided for sample sizes ≤10.

The ORR estimated for the F1CDx-positive population was 81.8%% (95% CI [59%, 100%]) for prior treated patients and 100% (95% CI [inestimable]) for treatment-naïve patients. The ORR for the TC subgroups are presented in Tables 36 and 37.

Table 36. TC efficacy for the prior-treated F1CDx-positive population

	F1CDx+
ORR (%)	81.8
Two-sided 95% CI (%)*	[59.0, 100]

*Calculated using normal approximation CI based on $Var(\delta_{CDx+})$/Wald method.

Table 37. TC efficacy for the treatment-naïve F1CDx-positive population

	F1CDx+
ORR (%)	100%
Two-sided 95% CI (%)	N/A*

* The variance to estimate the CI is inestimable using normal approximation methods because the ORR was 100%.

c. Sensitivity Analysis for Missing CDx results

A sensitivity analysis with regard to missing values was conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with unevaluable F1CDx results. Samples were considered unevaluable if the samples were not tested or if they were tested but returned as invalid result.

Amongst the CTA-positive TC population, 22.2% (6/27) did not have a F1CDx result, either due to unavailable tissue or invalid result.

The sensitivity analysis employed the multiple imputation method to impute missing F1CDx results and used the imputed and observed datasets to estimate the ORR for F1CDx positive patients. The point

estimates of ORR for the F1CDx+ population in the sensitivity analysis was 62.5% (95% CI [53.7%, 71.2%]) for the patients with prior platinum treatment and was 82.6% (95% CI [70.5%, 94.8%]) for the treatment-naïve patients. The CI was estimated using the variance from 100 bootstrap datasets within each of the 50 imputed data sets and applying Rubin's rules.

Overall, across the TA, NSCLC, and TC patient analyses (PPT population included a subset of these patients), there were 12 discordant patients. Of the 12 discordant patients, 8 patients had a complete or partial response to selpercatinib treatment, supporting that these 8 samples were likely true positive samples. Investigation findings concluded that of the 8 patients who responded to selpercatinib, 3 were biomarker-negative by the CDx rules, one was biomarker-negative, but the fusion event removed due to contamination, and 4 were biomarker-positive but the fusion event removed due to contamination. Further, of the patients who responded, 5 patients were enrolled onto the clinical trial using RNA-based next-generation sequencing (NGS) assays (62.5%), with the other 3 patients being enrolled using DNA-based NGS assays (37.5%). Of the 4 patients who did not have a response to selpercatinib, one was biomarker-negative by CDx rules, 2 were biomarker-positive but the fusion event was removed due to contamination, and 1 sample did not have a *RET* fusion detected by F1CDx. Two (2) patients were enrolled using RNA-based NGS assays and 2 patients were enrolled using DNA-based NGS assays. Overall, the number of discordant patients enrolled using DNA- and RNA-based NGS assays were 5 and 7 patients respectively, and 5 of the 7 discordant patients (71.4%) enrolled using RNA-based NGS assays responded to selpercatinib treatment compared to patients (3 out of 5; 60%) enrolled using DNA-based NGS assay. A limitation stating that F1CDx may miss a subset of patients with solid tumors with *RET* fusions who may derive benefit from selpercatinib due to technological differences in detection is included in the device labeling.

3. Pediatric Extrapolation

   The safety and effectiveness of RETEVMO have been established in pediatric patients aged 12 years and older for medullary thyroid cancer who require systemic therapy and for advanced *RET* fusion-positive thyroid cancer who require systemic therapy and are radioactive iodine-refractory (if radioactive iodine is appropriate). The safety and effectiveness of RETEVMO have not been established in these indications in patients less than 12 years of age or in pediatric patients for other indications.

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 was 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:

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

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

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

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

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of the F1CDx assay to identify patients with solid tumors with *RET* fusions who may benefit from RETEVMO (selpercatinib) treatment is supported by the results from the clinical bridging study. This study was performed using specimens from patients enrolled in the LIBRETTO-001 clinical trial with known *RET* fusion status and supplemented with *RET* fusion negative samples from the FMI archives. The data from the analytical validation and clinical bridging studies support the reasonable assurance of safety and effectiveness of the F1CDx assay when used in accordance with the indications for use. Data from the LIBRETTO-001 trial show that patients who had a *RET* fusion received benefit from treatment with RETEVMO and support the addition of the CDx indication to F1CDx. The bridging study demonstrated the ability of F1CDx to detect *RET* fusion positive patients that benefit from RETEVMO therapy.

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

C. Benefit-Risk Determination

The probable benefits of F1CDx in identification for *RET* fusions for treatment with RETEVMO are based on data collected in the LIBRETTO-001 clinical trial and the bridging study. The clinical benefit of the F1CDx assay for the selection of solid tumor cancer patients with *RET* fusions was demonstrated in a retrospective bridging study using samples from patients enrolled in LIBRETTO-001 and supplemented with additional *RET* fusion-negative samples. As assessed by independent review committee using RECIST 1.1 criteria, clinical efficacy of *RET* fusion-positive patients by F1CDx, indicated an ORR of 75.0% with Wald 2-sided 95% CI [64.4%, 85.6%], which was numerically higher than the ORR of 66.5% with Wilson-score 2-sided 95% CI [59.9%, 72.5%] in the CTA+ population for patients with solid tumors (including NSCLC and TC), in the combined drug applications, and provides a meaningful clinical benefit in this population. The ORR for the F1CDx-positive NSCLC population was 73.1% with 2-sided Wald 95% CI [56%, 90.1%] for prior platinum-treated patients and 71.4% with 2-sided Wald 95% CI [38%, 100%] for treatment-naïve patients. The ORR for the F1CDx-positive thyroid cancer population was 81.8% with 2-sided Wald 95% CI [59%, 100%] for prior treated patients and 100% (95% CI [inestimable]) for treatment-naïve patients. In addition, the ORR for the F1CDx-positive tissue agnostic population was 61.5% (8 out of 13 responded) with 2-sided Wald 95% CI [35.1%, 88%]. This supports the probable benefit of F1CDx in selecting *RET* fusion positive patients for treatment with RETEVMO, that is clinically meaningful considering the context of disease.

There is potential risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) 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 or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug.

The risk of false results is partially mitigated by clinical and analytical studies presented above. The supporting clinical validation analyses versus the CTAs demonstrate a PPA of 90.1%, NPA of 100% for the PPT population, i.e., NSCLC, TC, and other solid tumor patients combined from the drug applications, indicating that a small subset of patients may be missed by F1CDx. In addition, an accuracy study of F1CDx for the detection of *RET* fusions with the externally validated NGS (evNGS) comparator method further supports this conclusion. The accuracy study with an evNGS comparator method demonstrated supportive performance for *RET* fusions, which partially mitigates the risks of this test. In addition, patients identified with the F1CDx assay show higher overall response rate to RETEVMO as found in the LIBRETTO-001 trial. Therefore, the results support the use of F1CDx as an aid in selecting patients with solid tumors harboring *RET* fusions for RETEVMO treatment, albeit the risk that a small subset of patients with a *RET* fusion who may benefit from RETEVMO may be missed by F1CDx.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with reasonable accuracy, mitigating the potential for false results. In addition, to supplement the premarket data, some post-market studies are planned as summarized in Section XIII, below.

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

In conclusion, given the available information above, the data support that for the F1CDx assay, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical studies support the performance of F1CDx as an aid for the identification of patients with *RET* fusion positive solid tumor cancers for whom RETEVMO may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on October 6, 2023. The final clinical conditions of approval cited in the approval order are described below.

Clinical
Clinical Effectiveness:

1. FMI must provide clinical outcome data as assessed by overall response rate from additional tissue agnostic patients enrolled into the LIBRETTO-001 clinical trial in the post-market setting to confirm the clinical effectiveness of F1CDx as a companion diagnostic (CDx) device for identification of patients with solid tumors with *RET* fusions who may benefit from treatment with RETEVMO.

   The clinical study protocol should be submitted within 30 days of the PMA approval date, and the study data and conclusions should be submitted within 3 years of the PMA approval date.

Non-Clinical
Software:
FMI will provide the following software information in a post-approval report within 6 months of approval of this PMA supplement:

1. FMI must submit a list of the cumulative changes and in sufficient detail acceptable to FDA, made between the currently deployed genomics platform, which includes analytical pipeline software version v3.25.0 (AP v3.25), and the AP versions used in the clinical validation studies in this supplement.

2. FMI must submit a detailed description of the validation activity conducted to support the version change, including the associated risk assessments for each change, and the rationale, acceptable to FDA, that the validation performed supports reasonable assurance that the modification has not affected the performance or raised new concerns regarding the safety and effectiveness of the device.

3. FMI must provide evidence, acceptable to FDA, that performance expectations with the currently deployed genomics platform, including AP v3.25.0, are representative of the performance in the clinical validation study in this supplement and analytical validation studies that are leveraged. Such evidence may include regression testing using the clinical and analytical datasets to perform *in silico* reanalysis of the results obtained in the validation studies and confirmation that there is little or no deviation in the quality metrics for each of the samples to support that the performance of the assay remains the same.

Cybersecurity:
FMI will provide the following cybersecurity information in a post-approval report within 6 months of approval of this PMA supplement:

4. FMI must submit and complete documentation for cybersecurity, interoperability, risk assessment, risk management tests, traceability and validation, acceptable to FDA, including providing copies of associated documents (i.e., reference documents, letters, original reports, etc.), as required by section 524B of the Federal Food, Drug and Cosmetics Act, to ensure F1CDx and FMI's overall computational environment is cybersecure. The documentation should include a complete evaluation of all potential threats and vulnerabilities (e.g., software, instrumentation, software environment), validation of the use of third-party and Off-the-Shelf software, a complete anomaly assessment, security features installed on mobile devices, detailed protocols associated with the evaluation and review of software release candidates, and all relevant referenced documents.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

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