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/S017
Date of FDA Notice of Approval:	October 23, 2020

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

PMA supplement (P170019/S015) for adding an indication for LYNPARZA® (olaparib) in metastatic castration resistant prostate cancer (mCRPC) patients with mutations in homologous recombination repair (HRR) genes was approved on May 19, 2020. PMA supplement (P170019/S016) for adding an indication for KEYTRUDA® (pembrolizumab) in patients with solid tumors high tumor mutational burden (TMB) at the cut-off of 10 mutations per megabase (mut/Mb) was approved on June 16, 2020.

The current supplement was submitted to expand the indication for the F1CDx to include a companion diagnostic indication for fusions of neurotrophic tyrosine receptor kinases genes *NTRK1, NTRK2*, or *NTRK3* fusions in patients with solid tumors who may benefit from treatment with VITRAKVI® (larotrectinib).

II.   **INDICATIONS FOR USE**

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

Table 1. Companion diagnostic indications

Indication	Biomarker	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (afatinib), Iressa® (gefitinib), Tagrisso® (osimertinib), or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Tabrecta™ (capmatinib)

Indication	Biomarker	Therapy
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	Piqray® (alpelisib)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild-type (absence of mutations in exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Lynparza® (olaparib) or Rubraca® (rucaparib)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	Pemazyre™ (pemigatinib)
Prostate cancer	Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations	Lynparza® (olaparib)
Solid tumors	TMB $\geq$ 10 mutations per megabase	Keytruda® (pembrolizumab)
	NTRK1/2/3 fusions	Vitrakvi® (larotrectinib)

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

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

III. **CONTRAINDICATIONS**

There are no known contraindications.

IV. **WARNINGS AND PRECAUTIONS**

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

V. **DEVICE DESCRIPTION**

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

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

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

ABL1	BRAF	CDKN1A	EPHA3	FGFR4	IKZF1	MCL1	NKX2-1	PMS2	RNF43	TET2
ACVR1B	BRCA1	CDKN1B	EPHB1	FH	INPP4B	MDM2	NOTCH1	POLD1	ROS1	TGFBR2
AKT1	BRCA2	CDKN2A	EPHB4	FLCN	IRF2	MDM4	NOTCH2	POLE	RPTOR	TIPARP
AKT2	BRD4	CDKN2B	ERBB2	FLT1	IRF4	MED12	NOTCH3	PPARG	SDHA	TNFAIP3
AKT3	BRIP1	CDKN2C	ERBB3	FLT3	IRS2	MEF2B	NPM1	PPP2R1A	SDHB	TNFRSF14
ALK	BTG1	CEBPA	ERBB4	FOXL2	JAK1	MEN1	NRAS	PPP2R2A	SDHC	TP53
ALOX12B	BTG2	CHEK1	ERCC4	FUBP1	JAK2	MERTK	NT5C2	PRDM1	SDHD	TSC1
AMER1	BTK	CHEK2	ERG	GABRA6	JAK3	MET	NTRK1	PRKAR1A	SETD2	TSC2
APC	C11orf30	CIC	ERRFI1	GATA3	JUN	MITF	NTRK2	PRKCI	SF3B1	TYRO3
AR	CALR	CREBBP	ESR1	GATA4	KDM5A	MKNK1	NTRK3	PTCH1	SGK1	U2AF1

ARAF	CARD11	CRKL	EZH2	GATA6	KDM5C	MLH1	P2RY8	PTEN	SMAD2	VEGFA
ARFRP1	CASP8	CSF1R	FAM46C	GID4 (C17orf39)	KDM6A	MPL	PALB2	PTPN11	SMAD4	VHL
ARID1A	CBFB	CSF3R	FANCA	GNA11	KDR	MRE11A	PARK2	PTPRO	SMARCA4	WHSC1
ASXL1	CBL	CTCF	FANCC	GNA13	KEAP1	MSH2	PARP1	QKI	SMARCB1	WHSC1L1
ATM	CCND1	CTNNA1	FANCG	GNAQ	KEL	MSH3	PARP2	RAC1	SMO	WT1
ATR	CCND2	CTNNB1	FANCL	GNAS	KIT	MSH6	PARP3	RAD21	SNCAIP	XPO1
ATRX	CCND3	CUL3	FAS	GRM3	KLHL6	MST1R	PAX5	RAD51	SOCS1	XRCC2
AURKA	CCNE1	CUL4A	FBXW7	GSK3B	KMT2A (MLL)	MTAP	PBRM1	RAD51B	SOX2	ZNF217
AURKB	CD22	CXCR4	FGF10	H3F3A	KMT2D (MLL2)	MTOR	PDCD1	RAD51C	SOX9	ZNF703
AXIN1	CD274	CYP17A1	FGF12	HDAC1	KRAS	MUTYH	PDCD1LG2	RAD51D	SPEN	
AXL	CD70	DAXX	FGF14	HGF	LTK	MYC	PDGFRA	RAD52	SPOP	
BAP1	CD79A	DDR1	FGF19	HNF1A	LYN	MYCL	PDGFRB	RAD54L	SRC	
BARD1	CD79B	DDR2	FGF23	HRAS	MAF	MYCN	PDK1	RAF1	STAG2	
BCL2	CDC73	DIS3	FGF3	HSD3B1	MAP2K1	MYD88	PIK3C2B	RARA	STAT3	
BCL2L1	CDH1	DNMT3A	FGF4	ID3	MAP2K2	NBN	PIK3C2G	RB1	STK11	
BCL2L2	CDK12	DOT1L	FGF6	IDH1	MAP2K4	NF1	PIK3CA	RBM10	SUFU	
BCL6	CDK4	EED	FGFR1	IDH2	MAP3K1	NF2	PIK3CB	REL	SYK	
BCOR	CDK6	EGFR	FGFR2	IGF1R	MAP3K13	NFE2L2	PIK3R1	RET	TBX3	
BCORL1	CDK8	EP300	FGFR3	IKBKE	MAPK1	NFKBIA	PIM1	RICTOR	TEK	

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

ALK introns 18, 19	BRCA1 introns 2, 7, 8, 12, 16, 19, 20	ETV4 introns 5, 6	EZR introns 9-11	KIT intron 16	MYC intron 1	NUTM1 intron 1	RET introns 7-11	SLC34A2 intron 4
BCL2 3'UTR	BRCA2 intron 2	ETV5 introns 6, 7	FGFR1 intron 1, 5, 17	KMT2A (MLL) introns 6-11	NOTCH2 intron 26	PDGFRA introns 7, 9, 11	ROS1 introns 31-35	TERC ncRNA
BCR introns 8, 13, 14	CD74 introns 6-8	ETV6 introns 5, 6	FGFR2 intron 1, 17	MSH2 intron 5	NTRK1 introns 8-10	RAF1 introns 4-8	RSPO2 intron 1	TERT Promoter

BRAF introns 7-10	EGFR introns 7, 15, 24-27	EWSR1 introns 7-13	FGFR3 intron 17	MYB intron 14	NTRK2 Intron 12	RARA intron 2	SDC4 intron 2	TMPRSS2 introns 1- 3

Test Output
The output of the test includes:

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

Category 2: Cancer Mutations with Evidence of Clinical Significance

Category 3: Cancer Mutations with Potential Clinical Significance

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

Test Kit Contents
The test includes a sample shipping kit, which is sent to ordering laboratories. The shipping kit contains the following components:
- Specimen Preparation Instructions
- Shipping Instructions
- Return Shipping Label

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

Table 4. Instruments for use with the F1CDx assay

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

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

A. Specimen Collection and Preparation

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

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

B. DNA Extraction

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

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

C. Library Construction

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

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

D. Hybrid Capture

Hybrid Capture (HC) begins with normalization of each library to 500-2000 ng. Normalized samples then undergo solution hybridization which is performed using a > 50-fold molar excess of a pool of individually synthesized 5'-biotinylated DNA 120

bp oligonucleotides. The baits target ~1.8 Mb of the human genome including all coding exons of 309 cancer-related genes, introns or non-coding regions of 35 genes, plus > 3,500 single nucleotide polymorphisms (SNPs) located throughout the genome. Baits are designed by tiling overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; SNP targets are allocated one bait each. Intronic baits are filtered for repetitive elements[2] as defined by the UCSC Genome RepeatMasker track.

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

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

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

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

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

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

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

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

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

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

and distribution of alignment positions. Rearrangements are annotated for predicted function (e.g., creation of fusion gene).

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

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

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

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

H. Internal Process Controls Related to the System
Positive Control

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

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

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

I. **Variant Classification**
Biomarker Rules for SNVs and indels that lead to *MET* exon 14 skipping
An SNV or indel in *MET* shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:

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

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

is a second layer of check for HRR positive suspected deleterious alterations. All splice, nonsense, and frameshift alterations in HRR genes are considered biomarker positive and would be considered as suspected deleterious mutations (or "likely" status in FMI reporting rules). If these mutations are additionally reported in COSMIC, they would be listed as deleterious mutations (or "known" status in FMI reporting).

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

Table 5. Mutation types identified in the HRR genes

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

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

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

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

ATM	*BRCA1*	*BRCA2*
M1T	M1V	M1R
R2032K	M1I	M1I
R2227C	C61G	V159M
R2547_S2549del	C64Y	V211L
G2765S	R71G	V211I
R2832C	R71K	R2336P
S2855_V2856delinsRI (annotated as S2855_V2856>RI)	R1495M	R2336H
R3008C	E1559K	

ATM	BRCA1	BRCA2
R3008H	D1692N	
8418+5_8418+8delGTGA or 8418+1_8418+4delGTGA	D1692H	
	R1699W	
	A1708E	
	G1788V	

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

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

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

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

VI.   ALTERNATIVE PRACTICES AND PROCEDURES

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

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

	Device	Company	Technology	Therapy	Indication
HER-2	PathVysion HER-2 DNA Probe Kit	Abbott Molecular, Inc.	FISH	HERCEPTIN (trastuzumab)	Breast cancer

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

	Device	Company	Technology	Therapy	Indication
	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
BRAF-V600E	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	TAFINLAR (dabrafenib) MEKINIST (trametinib)	NSCLC

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

	Device	Company	Technology	Therapy	Indication
NRAS	*therascreen* BRAF V600E RGQ PCR Kit	QIAGEN	PCR	BRAFTOVI (encorafenib) Erbitux (cetuximab)	CRC
	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 CDx$_{BRCA}$	Foundation Medicine, Inc.	NGS	RUBRACA (rucaparib)	Advanced ovarian cancer
PIK3CA	*therascreen* PIK3CA RGQ PCR Kit	QIAGEN	PCR	PIQRAY (alpelisib)	Breast cancer

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

VII. MARKETING HISTORY

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

The F1CDx Premarket Approval (PMA) was originally approved on November 30, 2017 by FDA (P170019) and is commercially available in the U.S. since March 30, 2018. The following PMA supplements affecting the Intended Use were approved by FDA.
- P170019/S005 was approved on April 10, 2019.
- P170019/S004 and P170019/S008 were approved on July 1, 2019.
- P170019/S009 was approved on August 21, 2019.
- P170019/S006 was approved on December 3, 2019.
- P170019/S010 was approved on December 16, 2019.
- P170019/S013 was approved on April 17, 2020.
- P170019/S011 was approved on May 6, 2020.
- P170019/S015 was approved on May 19, 2020.
- P170019/S016 was approved on June 16, 2020.

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 detecting *NTRK1, NTRK2*, and *NTRK3* fusions, hereafter refered to as *NTRK1/2/3* fusions was from the data presented using intended use specimens across all validation studies. In addition to the existing platform-level validation results (P170019), analytical accuracy, intermediate precision, and limit of detection (LoD) studies as well as *in silico* analyses

of real-world data from the Foundation Medicine clinical database were conducted to support the indication for *NTRK1/2/3* fusions. Table 8 shows the distribution of tumor types in analytical studies. The counts corresponding to *NTRK*-positive or *NTRK*-negative status are with respect to the CDx rules for *NTRK1/2/3* (restricting to fusions only). Table 9 shows the distribution of F1CDx *NTRK1/2/3* fusions per *NTRK* gene evaluated in the analytical validation studies.

For F1CDx platform-level validation (P170019), performance characteristics were established using DNA derived from a wide range of FFPE tissue types; tissue types associated with CDx indications were included in each study. Each study included CDx variants as well as a broad range of representative alteration types (substitution, insertion and deletion, copy number alterations, rearrangements) in various genomic contexts across several genes. Analyses of genomic signatures including MSI and TMB were also conducted. Precision data from a Conditions of Approval (CoA) study for P170019 included two *NTRK* samples with additional precision data to support the *NTRK1/2/3* fusion CDx indication.

Table 8. Distribution of Tumor Types in Analytical Studies. The counts corresponding to *NTRK*-positive or *NTRK*- negative status are with respect to the CDx rules for *NTRK1/2/3* (restricting to fusions only)

Tumor Type \ Study	Accuracy		Precision (N=7)	LoD (N=7)
	NTRK positive (N =83)	*NTRK* negative (N =543)		
Appendix	0	0	0	0
Breast	6	17	0	1
Bone Sarcoma	0	2	0	0
Cholangiocarcinoma[1]	2	2	1	0
CRC[2]	19	51	3	1
GIST	2	6	0	1
Head and Neck	11	10	0	0
IFS	2	0	0	0
Lung	7	246	0	1
Skin (includes Melanoma)	1	1	0	0
Neuroendocrine[3]	1	2	0	0
Oral	0	1	0	0
Other[4]	0	0	0	1
Pancreas	0	2	0	0
Primary CNS	0	1	0	0
Reproductive	5	171	1	0
Salivary Gland	11	11	1	1
Soft Tissue Sarcoma	4	8	0	0
Thyroid	6	2	1	1
Unknown	5	1	0	0
Urinary[5]	1	9	0	0

[1] Cholangiocarcinoma includes hepatic cancer

[2] CRC includes colon and anal cancers
[3] Neuroendocrine includes thymus cancers
[4] Other includes malignant neoplasm with neuroendocrine features and unspecified primary serous carcinoma
[5] Urinary includes congenital mesoblastic nephroma

Table 9. Distribution of CDx *NTRK1/2/3* Fusions (as determined by F1CDx per F1CDx's *NTRK1/2/3* Fusion Rules)

Study	*NTRK1*	*NTRK2*	*NTRK3*
Accuracy	46	6	31
Precision	2	1	4
LoD	2	2	3

1. **Analytical Accuracy/Concordance**
   a. **Comparison to an Orthogonal Method for *NTRK1/2/3* rearrangements**
      Analytical accuracy was evaluated by demonstrating concordance between F1CDx and an externally validated NGS assay (evNGS) for the detection of *NTRK1/2/3* rearrangements.

      The analytical accuracy study was performed with available residual DNA previously extracted from FFPE clinical specimens (38 samples with sufficient remaining material) from patients with solid tumors enrolled in the clinical studies LOXO-TRK - 14001 (NCT02122913), -15002 (NAVIGATE, NCT02576431), and -15003 (SCOUT, NCT02637687) that supported VITRAKVI (larotrectinib) approval, refer to Section X for study details. The 38 samples from the clinical studies LOXO-TRK - 14001, - 15002, and -15003 included the following cancer types: bone sarcoma (1), breast (1), cholangiocarcinoma (2), colorectal cancer (7), infantile fibrosarcoma (2), lung (4), melanoma (1), oral cancer (1), primary CNS (1), salivary gland (3), soft tissue sarcoma (10), neuroendocrine (2), thyroid (2), and urinary (1) cancer.

      Due to the limited number of available clinical trial samples, the study also included *NTRK1/2/3* rearrangement positive (N=94) samples from the FMI clinical archives which were previously evaluated at FMI. These 94 samples included samples from breast (14), cholagiocarcinoma (1), colorectal cancer (15), gastrointestinal stromal tumor GIST (2), head and neck (14), lung (10), neuroendocrine (1), reproductive (11), salivary gland (9), thyroid (6), and urinary (6) cancer. For five (5) samples, the cancer type was not known.

      The study also included *NTRK1/2/3* rearrangement negative samples previously processed and tested by the evNGS in prior concordance validation studies. The previously executed concordance studies were newly evaluated for the presence or absence of *NTRK1/2/3* rearrangements. This evaluation included 474 samples that were tested in previous concordance analyses.

NTRK1/2/3 rearrangement positive and negative sample sets included disease ontologies that were representative of the intended use population. However, an additional ten (10) pediatric samples and ten (10) salivary gland samples were also included as part of the *NTRK1/2/3* rearrangement negative sample set to better align with the intended use population. The ten (10) pediatric samples included the following cancer types: bone sarcoma (1), GIST (1), head and neck (1), pancreas (1), soft tissue sarcoma (1), and urinary cancer (1). For one sample the cancer type was unknown.

In total, 626 clinical FFPE samples (543 *NTRK* fusion negative and 83 *NTRK* positive fusion samples) from patients with solid tumors were evaluated in this analytical accuracy study.

Table 10 shows a breakdown of the sample selection based on *NTRK* rearrangements (includes fusion and non-fusion rearrangements).

Table 10. Samples Evaluated for *NTRK1/2/3* Rearrangement Concordance.

Sample Subset	Disease Cohort Represented	Number of Samples
NTRK1/2/3 Rearrangement Positive[a]	Pan-tumor	94
NTRK1/2/3 Rearrangement Negative[a]	Pediatric	10
	Salivary Gland	10
NTRK1/2/3 Rearrangement Positive and Rearrangement Negative (from LOXO 14001, 15002, and 15003) [a]	Pan-tumor	38
Total Samples Processed by F1CDx and evNGS		
NTRK1/2/3 Rearrangement Negative for evNGS Re-analysis[b]	Reproductive	152
	Reproductive	123 (121[c])
	Lung, Neuroendocrine	34
	Pan-tumor	34
	Breast	74
	Lung	15
	Lung	187
Total Samples for evNGS Re-analysis Only		474
Total Samples Evaluated for Concordance		626

[a] Samples processed by F1CDx and evNGS assays.
[b] Samples previously processed as part of prior concordance validations with evNGS; only reanalysis by evNGS performed for this validation.
[c] Two (2) samples were considered positive per the primary analysis approach. These two samples would be F1CDx biomarker negative per the F1CDx biomarker rules for *NTRK 1/2/3* fusions

There were no invalid results from either F1CDx or evNGS. Two analyses were conducted to assess concordance.

Primary Analysis
The primary analysis focused on the concordance of *NTRK1/2/3* rearrangement detection by F1CDx and evNGS assay by considering a sample to be positive if any *NTRK1/2/3* rearrangements was present, otherwise it was considered as negative. This analysis was conducted to determine the analytical accuracy of *NTRK1/2/3* rearrangement detection as fusions are a subset of rearrangements, and the methodology to detect *NTRK1/2/3* rearrangement (fusions and non-fusion rearrangements) is the same.

The samples were categorized into two subsets based on the origin of the sample: 588 samples from the FMI clinical archives whose status was initially determined and ascertained by an F1CDx device, and 38 clinical samples from the larotrectinib clinical trial patients whose *NTRK* fusion status was initially determined and ascertained by the local clinical trial assays (LCTAs) prior to enrollment in the VITRAKVI clinical trials. As a result of the two distinct sample selection methods by assay, each subset of samples was evaluated separately for the calculation of percent positive agreement (PPA) and negative percent agreement (NPA) and adjusted according to a prevalence (0.32%) of *NTRK1*, *NTRK2*, and *NTRK3* in the intended use population[9].

Table 11 and Table 12 summarize the results for the primary analysis for Subset 1, while Table 13 and Table 14 summarize the results for the primary analysis for Subset 2. Subset 1 refers to the 588 samples from the FMI database and Subset 2 refers to the 38 VITRAKVI clinical trial samples.

Table 11. Contingency Table Comparing F1CDx and evNGS Results for the Detection of *NTRK1/2/3* Rearrangements Based on the Primary Analysis - Sample Subset 1

NTRK1/2/3		evNGS		
		NTRK +	*NTRK -*	**Total**
F1CDx	*NTRK +*	78	18	96
	NTRK -	0	492	492
	Total	78	510	588

Table 12. Summary of Agreement Statistics Based on the Primary Analysis -Sample Subset 1

Agreement Statistic	Estimate (95%CI)[a]
PPV	81.25% (72.30%, 87.80%)
NPV	100.00% (99.23%, 100.00%)
Adjusted PPA	100.00% (95.31%, 100.00%)[b]
Adjusted NPA	99.94% (99.91%, 99.96%)
Unadjusted PPA	100.00% (95.31%, 100.00%)
Unadjusted NPA	96.47% (94.49%, 97.76%)

[a] For unadjusted value, 95% 2-sided score CI was computed; for adjusted value, 95% 2-sided Cl was calculated by selecting 2.5% and 97.5% percentile of bootstrapped (10000 times) samples.
[b] 95% 2-sided score CI of unadjusted PPA was computed given adjusted PPA was 100%.

Table 13. Contingency Table Comparing F1CDx and evNGS Results for the Detection of *NTRK1/2/3* Rearrangements Based on the Primary Analysis - Sample Subset 2

NTRK1/2/3		UW-OncoPlex		
		NTRK +	*NTRK -*	Total
F1CDx	*NTRK +*	16	0	16
	NTRK -	2	20	22
	Total	18	20	38

Table 14. Summary of Agreement Statisitics Based on the Primary Analysis - Sample Subset 2

Agreement Statistic	Estimate (95%CI)[a]
PPA	88.89% (67.20%, 96.90%)
NPA	100.00% (83.89%, 100.00%)
Adjusted PPV	100.00% (80.64%, 100.00%)[b]
Adjusted NPV	99.96% (99.91%, 100.00%)
Unadjusted PPV	100.00% (80.64%, 100.00%)
Unadjusted NPV	90.91% (72.19%, 97.47%)

[a] For unadjusted value, 95% 2-sided score CI was computed; for adjusted value, 95% 2-sided Cl was calculated by selecting 2.5% and 97.5% percentile of bootstrapped (10000 times) samples.
[b] 95% 2-sided score CI of unadjusted PPV was computed given adjusted PPV was 100%.

The entire data set (n=626 samples) was evaluated by combining both sample subsets together to calculate the weighted PPA, NPA, positive predictive value (PPV), and negative predictive value (NPV), and bootstrapping the 626 samples 10000 times. Table 15 presents the weighted PPA, PPV, NPA, and NPV based on the bootstrapping the combined data sets 10000 times. The weighted PPA was 90.00% (75.00%, 100.00%) and the weighted NPA was 99.94% (99.92%, 99.97%).

Table 15. Weighted PPA, PPV, NPA, and NPV for the Detection of *NTRK1/2/3* Rearrangements Combining Sample Subsets 1 and 2 Based on the Primary Analysis.

Weighted Agreement Statistic	Estimate (95%CI)[a]
Weighted PPA	90.00% (75.00%,100.00%)
Weighted PPV	98.13% (97.53%, 99.08%)
Weighted NPA	99.94% (99.92%, 99.97%)
Weighted NPV	100.00% (100.00%, 100.00%)

In total there were 20 samples that were discordant based on the primary analysis. In 18 of the discordant samples, the F1 CDx assay detected *NTRK1/2/3* rearrangements, while the evNGS assay did not detect the rearrangements. In the remaining 2 discordant samples, *NTRK1/2/3* rearrangements were detected by the evNGS assay but not the F1CDx assay.

Of the 18 discordant calls that are F1CDx positive (present) and evNGS negative (absent), based on the primary analysis:

- Nine (9) samples had breakpoints residing outside of evNGS baited regions
- Five (5) samples had the event observed but were not reported due to being below the evNGS assay limit of detection
- One (1) sample had an intragenic *NTRK3-NTRK3* rearrangement that was intentionally not reported by evNGS due to being intragenic
- Two (2) samples were not reported by evNGS likely due to low sample quality as reported by the evNGS assay
- One sample (1) (*NTRK3-KDM2A*) was not detected by evNGS

Secondary Analysis

The secondary analysis focused on the concordance of *NTRK1/2/3* rearrangement detection that results in an *NTRK1/2/3* fusion event per the F1CDx biomarker rule for the *NTRK1/2/3* fusion CDx biomaker. In the secondary analysis, a sample was considered F1CDx positive only if it met the *NTRK1/2/3* biomarker rule, otherwise it was considered as F1CDx negative.

Table 16 and Table 17 summarize the results for the secondary analysis for Subset 1, while Table 18 and Table 19 summarize the results for the secondary analysis for Subset 2. Subset 1 refers to the 588 samples from the FMI database and Subset 2 refers to the 38 VITRAKVI clinical trial samples.

Table 16. Contingency Table Comparing F1CDx and evNGS Results for the Detection of *NTRK1/2/3* Rearrangements Based on the Secondary Analysis - Sample Subset 1

NTRK1/2/3		EvNGS		
		NTRK +	*NTRK -*	**Total**
F1CDx	*NTRK +*	64	4	68
	NTRK -	10	510	520
	Total	74	514	588

Table 17. Summary of Agreement Statistics Based on the Secondary Analysis - Sample Subset 1

Agreement Statistic	Estimate (95%CI)[a]
PPV	94.12% (85.83%, 97.69%)
NPV	98.08% (96.50%, 98.95%)
Adjusted PPA	13.58% (8.66%, 25.25%)
Adjusted NPA	99.98% (99.96%, 100.00%)
Unadjusted PPA	86.49 % (76.88%, 92.49%)
Unadjusted NPA	99.22% (98.02%. 99.70%)

[a] For unadjusted value, 95% 2-sided score CI was computed; for adjusted value, 95% 2-sided Cl was calculated by selecting 2.5% and 97.5% percentile of bootstrapped (10000 times) samples.

Table 18. Contingency Table Comparing F1CDx and evNGS Results for the Detection of NTRK1/2/3 Rearrangements based on the Secondary Analysis - Sample Subset 2

NTRK1/2/3		EvNGS		
		NTRK +	*NTRK -*	**Total**
F1CDx	*NTRK +*	15	0	15
	NTRK -	3	20	23
	Total	18	20	38

Table 19. Summary of Agreement Statistics Based on the Secondary Analysis - Sample Subset 2

Agreement Statistic	Estimate (95%CI)[a]
PPA	83.33% (60.78%, 94.16%)
NPA	100.00% (83.89%, 100.00%)
Adjusted PPV	100.00%(79.61%, 100.00%)[b]
Adjusted NPV	99.95% (99.88%, 100.00%)
Unadjusted PPV	100.00% (79.61%, 100.00%)
Unadjusted NPV	86.96% (67.87%, 95.46%)

[a] For unadjusted value, 95% 2-sided score Cl was computed; for adjusted value, 95% 2-sided Cl was calculated by selecting 2.5% and 97.5% percentile of bootstrapped (10000 times) samples.
[b] 95% 2-sided score Cl of unadjusted PPV was computed given adjusted PPV was 100%.

The entire data set (n=626 samples) was evaluated by combining both sample subsets together to calculate the weighted PPA, NPA, PPV, and NPV, and

bootstrapping the 626 samples 10000 times. Table 20 presents the weighted PPA, PPV, NPA, and NPV based on the bootstrapping the combined data sets 10000 times. The weighted PPA was 54.08% (37.94%, 71.37%) and the weighted NPA was 99.98% (99.96%, 100.00%).

Table 20. Weighted PPA, PPV, NPA, and NPV for the Detection of *NTRK1/2/3* Rearrangements Combining Sample Subsets 1 and 2 Based on the Secondary Analysis

Weighted Agreement Statistic	Estimate (95%CI)
Weighted PPA	54.08% (37.94%, 71.37%)
Weighted PPV	97.53% (94.57%, 99.54%)
Weighted NPA	99.98% (99.96%, 100.00%)
Weighted NPV	98.14% (96.90%, 99.24%)

In total there were 17 samples that were discordant based on the secondary analysis. Of 17 discordant samples, 13 were F1CDx biomarker negative but evNGS biomarker positive. Of the 13 samples, 10 were non-fusion rearrangements and thus considered as F1CDx biomarker negative per the F1CDx biomarker rules.

However, these 10 were considered evNGS biomarker positive as the evNGS assay cannot differentiate between fusions and rearrangements. Thus, these 10 were not considered as discordant per the primary analysis.

The three clinical trial samples that were positive by the evNGS assay but negative by F1CDx were from patients enrolled in the clinical trials that did not respond to VITRAKVI, (two patients had stable disease and one had progressive disease).

2. Analytical Sensitivity
a. Limit of Blank (LoB)
The LoB was confirmed using DNA from nine (9) samples from patients with solid tumors that were biomarker negative. These comprised samples from the following diseases and specimen types in parenthesis: breast invasive ductal carcinoma (breast), colon adenocarcinoma (colon), stomach adenocarcinoma (stomach), pediatric brain medulloblastoma (brain), pleura mesothelioma (pleura),small intestine neuroendocrine carcinoma (small intestine), pancreas carcinoma (whipple resection), salivary gland adenoid cystic carcinoma (lung), and thyroid papillary carcinoma (thyroid).

Each biomarker-negative sample was assessed in replicates of seven (7), resulting in a total of 63 sample aliquots to assess LoB. The number of replicate samples with incorrect calls was counted and converted into a percentage with respect to the number of all replicate LoB samples and reported as the percentage of false-positive results. If the percentage of false-positive results did

not exceed 5% (type I error risk a=0.05), then at least 95% of the result was zero and LoB = zero was confirmed. For sample aliquots evaluated to assess LoB, rearrangements in the *NTRK1/2/3* genes were not reported in any of the 63 sample aliquots. The percent of samples with incorrect calls was zero and confirmed the LoB = zero.

b. Limit of Detection (LoD)
The F1CDx LoD for the detection of *NTRK1, NTRK2,* and *NTRK3* fusions was investigated by assessing seven (7) samples (see Table 21). Selection of specimens for assessment of *NTRK* fusions represented various tumor types as shown in Table 21.

Table 21. Samples assessed in the LoD study for *NTRK* fusions

Sample	Target NTRK Gene	Partner Gene	Alteration Type	Fusion Partner or Alteration Description	Disease Indication (Specimen Site)
1	*NTRK1*	*TPR*	Fusion (CDx - positive)	5'-TPR(x1-21)-NTRK1(x10-17)	NSCLC (Lung)
2	*NTRK1*	*LMNA*	Fusion (CDx - positive)	5'-LMNA(ex1-2 NM_005572)-NTRK1(ex10-17 NM_002529)	CRC (Colon)
3	*NTRK2*	*BCR*	Fusion (CDx - positive)	5'-BCR(ex1-14 NM_004327)-NTRK2(ex12-21 NM_006180)	Malignant neoplasm w/ neuroendocrine feature (Brain)
4	*NTRK2*	*GARNL3*	Fusion (CDx - positive)	5'-GARNL3 (NM_032293 exons 1-2) – NTRK2 (NM_006180 exons 16-21)	Breast carcinoma (NOS) (Adrenal Gland)
5	*NTRK3*	*ETV6*	Fusion (CDx - positive)	5'-ETV6 (x1-5) – NTRK3 (x14-19 NM_002530)	Small intestine GIST Sarcoma (Small Intestine)
6	*NTRK3*	*ETV6*	Fusion (CDx - positive)	5'-ETV6(ex1-5 NM_001987)-NTRK3(ex14-19 NM_002530)	Thyroid papillary carcinoma (Lymph Node)
7	*NTRK3*	*ETV6*	Fusion (CDx - positive)	5'-ETV6(ex1-5 NM_001987)-NTRK3(ex15-19 NM_002530)	Salivary gland carcinoma (Parotid Gland)

To establish the LoD each sample was assessed at five (5) targeted computational tumor purity levels (2.5%, 5%, 10%, 15%, and 20%). Computational tumor purity is calculated by fitting the observed log-ratio and minor allele frequency data with statistical models that predict a genome-wide copy number profile, tumor ploidy, and tumor purity (i.e., computational tumor purity). The log-ratio profile is obtained by normalizing aligned tumor sequence reads by dividing read depth by that of a process-matched normal control, followed by a GC-content bias correction using Loess regression. The minor

allele frequency profile is obtained from the heterozygous genome-wide SNPs. Twenty (20) replicates were assessed for each dilution level, except 20%, which examined fourteen (14) replicates. Ninety-four (94) replicates were tested per sample. Each specimen was evaluated close to the minimum input requirements of the assay (50 ng), representing the most challenging evaluation of rearrangement detection. LoDs were determined based on tumor purity and reads using the empirical hit rate method for *NTRK1, NTRK2,* and *NTRK3*:

- Empirical hit rate approach was used when there were less than three hit rate levels. The LoD is defined as the lowest level (tumor purity or reads) with at least 95% detection.

A summary of the LoD results based on reads and tumor purity using the empirical hit rate method for *NTRK* gene alteration in each sample evaluated are shown in Table 22.

Table 22. LoD of *NTRK1/2/3* Rearrangements Based on the Empirical Hit rate Method by Sample

Sample	Target *NTRK* Gene	Partner Gene	Alteration Type	*NTRK1/2/3* LoD (mean %Tumor Purity)	*NTRK1/2/3* LoD (# of chimeric reads)
1	*NTRK1*	*TPR*	Fusion (CDx - positive)	**12.10%[1]**	9.55
2	*NTRK1*	*LMNA*	Fusion (CDx - positive)	11.90%	**24.55[1]**
3	*NTRK2*	*BCR*	Fusion (CDx - positive)	**11.50%[1]**	19.75
4	*NTRK2*	*GARNL3*	Fusion (CDx - positive)	6.70%	**24.16[1]**
5	*NTRK3*	*ETV6*	Fusion (CDx - positive)	4.10%	9.05
6	*NTRK3*	*ETV6*	Fusion (CDx - positive)	5.70%	**14.65[1]**
7	*NTRK3*	*ETV6*	Fusion (CDx - positive)	**6.10%[1]**	8.65

[1]Final LoD for each *NTRK* gene fusion.

The final LoD for each *NTRK* gene fusion presented was determined as the highest LoD observed per gene. The LoD for each gene are:

- *NTRK1* fusion: 12.10% tumor purity and 24.55 for chimeric reads
- *NTRK2* fusion: 11.50% tumor purity and 24.16 for chimeric reads
- *NTRK3* fusion: 6.10 % tumor purity and 14.65 for chimeric reads

Given the limited number of samples with *NTRK1* and *NTRK2* fusions evaluated to determine the assay LoD, a post-market study is planned with additional *NTRK1* and *NTRK2* fusions samples (see section XIII).

3. **Analytical Specificity**
   Refer to the Summary of Safety and Effectiveness Data P710019 (Section IX.A.3) for F1CDx platform validation of analytical specificity, including interfering substances and *in silico* hybrid capture bait specificity.

4. **Carryover/Cross-Contamination**

   Refer to the Summary of Safety and Effectiveness Data P170019 (Section IX.A.4) for F1CDx platform validation of carryover/cross-contamination.

5. **Precision and Reproducibility**

   a. **Intermediate Precision**

      To support the F1CDx performance characteristics for the detection of *NTRK1, NTRK2,* and *NTRK3* fusions, the precision of seven (7) samples from patients with solid tumors were evaluated at a challenging DNA input (close to 50 ng); refer to Table 23 for the samples evaluated in this study. These samples evaluated had computational tumor purity ranging from 17.2% to 40.1%. The cut-off for a passing sample based on computational tumor purity is 20% and samples evaluated in the precision study included samples near the computational tumor purity input specification of 20% tumor purity for the F1CDx assay (see Table 23 and Table 24). The mean chimeric read count for these samples ranged from 8.48 to 159.04. The fold LoD based on chimeric reads ranged from 0.58 xLoD to 10.86 xLoD

Table 23. **Samples Evaluated in the Intermediate Precision Study.**

Sample	Target *NTRK Gene*	Partner Gene	Alteration Type	Fusion Partner or Alteration Description	Disease Ontology
1	*NTRK3-*	*ETV6*	Fusion (CDx-Positive)	5'-NTRK3(ex1-13 NM_002530)-ETV6(ex6-8 NM_001987)	Thyroid carcinoma
2	*NTRK3*	*ETV6*	Fusion (CDx-Positive)	5'-ETV6(ex1-5 NM_001987)-NTRK3(ex15-19 NM_002530)	Salivary gland carcinoma
3	*NTRK1*	*TPM3*	Fusion (CDx-Positive)	5'-TPM3(NM_153649)-NTRK1(NM_002529) fusion (T7; N12)	CRC
4	*NTRK3*	*ETV6*	Fusion (CDx-Positive)	5'-ETV6(ex1-5 NM_001987)-NTRK3(ex15-19 NM_002530)	CRC
5	*NTRK1*	*LMNA*	Fusion (CDx-Positive)	5'-LMNA(NM_005572)-NTRK1(NM_002529) fusion (L4; N11)	CRC
6	*NTRK2*	*DSTYK*	Fusion (CDx-Positive)	5'-NTRK2(NM_006180)-DSTYK(NM_015375) fusion (N13*; D11)	Ovary Serous Carcinoma
7	*NTRK3*	*ETV6*	Fusion (CDx-Positive)	5'-NTRK3(NM_002530)-ETV6(NM_001987) fusion (N14; E6)	Liver cholangiocarcinoma

For the assessment of repeatability, each sample was divided into either 24 or 36 aliquots, with 12 aliquots processed in duplicate or triplicate under the same conditions. The conditions were applied on a plate-level and included the same operator, same day, same reagent lot and same sequencer. The result was

considered concordant if all duplicates or triplicates matched the majority call for all aliquots of that sample. Table 24 summarizes the repeatability statistics across samples evaluated.

Table 24. Repeatability of variant calling.

Sample	Mean TP	Mean Reads	Fold LoD based on Reads	Target *NTRK* Gene	Partner Gene	# Agree	Total #	Agreement (95% CI)
1	40.1%	39.25	2.68	*NTRK3*	*ETV6*	12	12	100.00% (73.50%, 100.00%)
2	20.0%	35.39	2.42	*NTRK3*	*ETV6*	12	12	100.00% (73.50%, 100.00%)
3	17.2%	22.71	0.93	*NTRK1*	*TPM3*	12	12	100.00% (73.50%, 100.00%)
4	34.4%	159.04	10.86	*NTRK3*	*ETV6*	12	12	100.00% (73.50%, 100.00%)
5	18.2%	41.52	1.69	*NTRK1*	*LMNA*	11	11	100.00% (71.50%, 100.00%)
6	35.2%	35.04	1.45	*NTRK2*	*DSTYK*	11	11	100.00% (71.50%, 100.00%)
7	20.0%	8.48	0.58	*NTRK3*	*ETV6*	11	12	91.70%, (61.50%, 99.80%)

Abbreviation: TP: tumor purity

Reproducibility in the seven (7) samples was evaluated by processing aliquots originating from the same source DNA sample, under conditions where one factor was changed at a time (e.g., reagent lot and sequencers). The result was considered concordant when the aliquot matched the targeted and majority call of all 24 or 36 replicates. Table 25 summarizes the reproducibility statistics across the seven (7) samples evaluated.

Table 25. Reproducibility of variant calling

Sample	Mean TP	Mean Reads	Fold LoD based on Reads	Target *NTRK* Gene	Partner Gene	# Agree	Total #	Agreement (95% CI)
1	40.1%	39.25	2.68	*NTRK3*	*ETV6*	36	36	100.00% (90.26%, 100.00%)
2	20.0%	35.39	2.42	*NTRK3*	*ETV6*	36	36	100.00% (90.26%, 100.00%)
3	17.2%	22.71	0.93	*NTRK1*	*TPM3*	24	24	100.0% (85.75%, 100.00%)
4	34.4%	159.04	10.86	*NTRK3*	*ETV6*	23	23	100.0% (85.18%, 100.00%)
5	18.2%	41.52	1.69	*NTRK1*	*LMNA*	23	23	100.0% (85.18%, 100.00%)
6	35.2%	35.04	1.45	*NTRK2*	*DSTYK*	23	23	100.0%

								(85.18%, 100.00%)
7	20.0%	8.48	0.58	*NTRK3*	*ETV6*	23	24	95.8% (78.90%, 99.90%)

Abbreviations: TP: tumor purity

Given the limited number of samples with *NTRK2* fusions evaluated in the precision study, a post-market study is planned to supplement the precision study with additional samples (see section XIII).

b. Site-to-Site reproducibility
A reproducibility study to include the second site in Morrisville, North Carolina was not conducted to support the *NTRK* indication. Study results from a site-to-site reproducibility will be provided as a post-market study (see section XIII).

6. Reagent Lot Interchangeability
Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus CDxBRCA assay and F1CDx. For reagent lot interchangeability performance data, please see the Summary of Safety and Effectiveness Data for P160018.

7. Stability
Please refer to the Summary of Safety and Effectiveness Data P170019 [Section IX.A.7(a,b)] for F1CDx platform validation of reagent, DNA, and FFPE slide stability.

8. General Laboratory Equipment and Reagent Evaluation
a. DNA Amplification
Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus CDxBRCA assay and F1CDx. For DNA amplification performance data, see the Summary of Safety and Effectiveness Data for P160018.

b. DNA Extraction
For F1CDx platform-level validation, the performance of DNA extraction from FFPE tumor specimens was evaluated. For details, refer to Section IX.A.8(b) of Summary of Safety and Effectiveness Data P170019.

c. Guard banding/Robustness
Guard banding study results were leveraged from the F1CDx platform validation to evaluate the performance of the F1CDx assay and the impact of process variation with regard to uncertainty in the measurement of DNA concentration at various stages of the process. For details, refer to Section IX.A.9 in Summary of Safety and Effectiveness Data P170019.

9. **Real-World Evidence Demonstrating Sequencing Quality Metrics for the Detection of *NTRK* Alterations**

   A study analyzing F1CDx assay data was conducted to provide coverage analysis based on real-world data to support the pan-tumor indication for *NTRK1/2/3* rearrangements. Since F1CDx's approval, 76,597 samples have been processed by the DX1 baitset used by F1CDx for the most prevalent *NTRK* tumor specimen sites listed in Figure 1. An *in-silico* analysis was performed to present median exon coverage across these tumor specimen sites. Figure 1 and Table 26 show median exon coverage for all tumor specimen sites to be around 900x, which is above the quality control (QC) threshold of 250x.
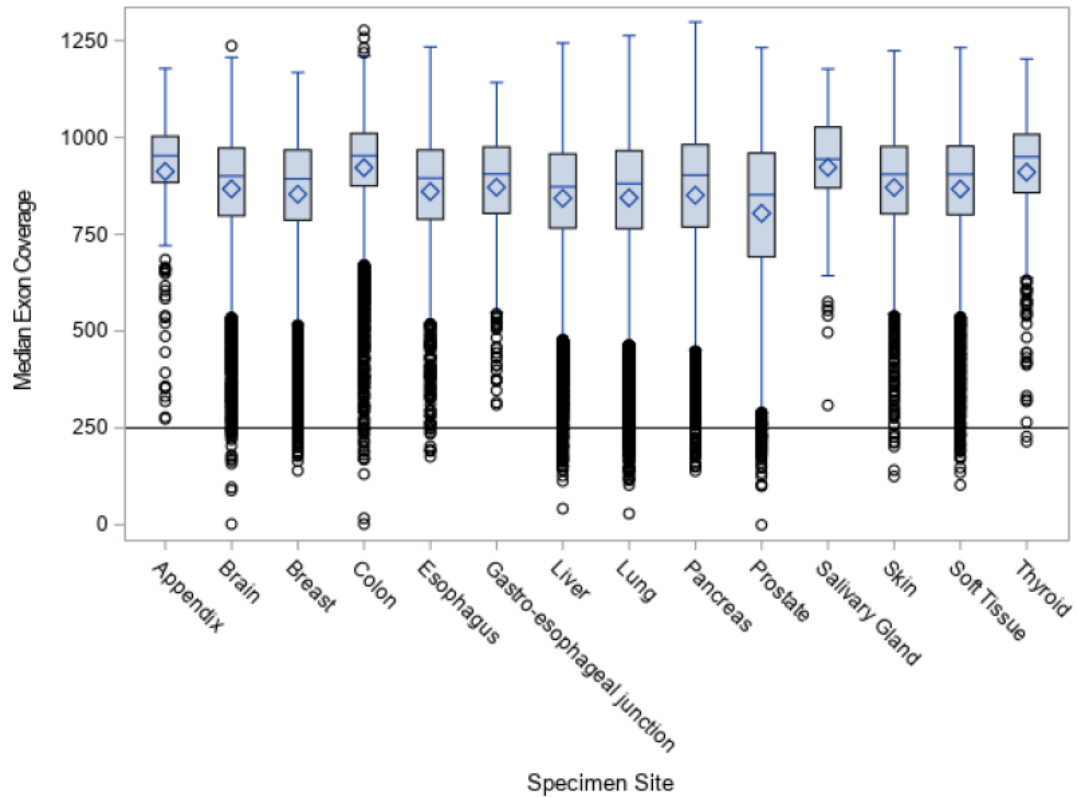
Figure 1. Median exon coverage for multiple tumor types in the F1CDx clinical database.

Table 26 summarizes critical post-DNA extraction QC metrics, namely, the mean of LC DNA yield, HC DNA yield, median exon coverage, % target 100X coverage, and the pass rate for the 15 different biopsy specimen sites.

Table 26. Critical Post-DNA Extraction QC Metrics

Specimen Site	Mean LC Yield (ng)	Mean HC Yield (ng)	Mean Median Exon Coverage	% Target 100X Coverage	% Pass
Appendix	7805.52	517.50	827	97.74	91.73

Specimen Site	Mean LC Yield (ng)	Mean HC Yield (ng)	Mean Median Exon Coverage	% Target 100X Coverage	% Pass
Brain	8118.94	537.61	778	95.48	98.90
Breast	7578.56	526.12	778	95.52	99.06
Colon	7763.74	505.53	828	96.24	99.05
Esophagus	7568.68	524.17	765.5	97.16	98.97
Gastro-esophageal junction	7050.72	487.46	767	96.02	96.81
Liver	7341.55	538.11	750	96.77	97.79
Lung	7483.32	527.89	765	96.96	97.79
Pancreas	7139.99	521.69	764	95.63	96.90
Pancreatobiliary	7079.14	503.75	782	94.31	91.87
Prostate	5652.69	434.15	753	91.93	92.82
Salivary Gland	7753.89	518.89	822	95.22	99.20
Skin	7526.18	514.82	785	96.02	99.04
Soft Tissue	7724.26	533.95	768	95.69	98.32
Thyroid	8173.36	506.99	827	96.67	97.00

10. *In-silico* **Assessment of *NTRK* Bait set Coverage Analyses**

The DX1 baitset covers coding regions of *NTRK1, NTRK2*, and *NTRK3*, as well as select introns in *NTRK1* (introns 8, 9, 10, and 11) and *NTRK2* (intron 12). However *NTRK1* intron 8, and *NTRK2* intron 12 are not fully baited.Though *NTRK3* introns are not baited, *ETV6*, the most common fusion partner for *NTRK3*, has introns that are baited allowing for detection of *ETV6-NTRK3* fusions (*ETV6* introns 5 and 6). However, *ETV6* intron 5 is not fully baited. Further, a portion of fusion events between these two genes are likely undetected by F1CDx as a result of DX1 not having baits for intron 4 of *ETV6*.

An *in-silico* analysis was performed with F1CDx test results of 12,464 clinical specimens across solid tumors to assess the baitset coverage levels of *NTRK*. Data from 12,464 clinical samples evaluated by F1CDx showed median exon coverage for the target regions in *NTRK1, NTRK2*, and *NTRK3* >250X in >99% of specimens. A decrease in coverage, albeit to a small degree, was seen in *NTRK1* intron 8 due that region being partially baited.

B. **Animal Studies**

No animal studies were conducted using the F1CDx assay.

C. **Additional Studies**

No additional studies were conducted using the F1CDx assay.

X. **SUMMARY OF PRIMARY CLINICAL STUDY**

The reasonable assurance of safety and effectiveness for F1CDx for detection of *NTRK1*, *NTRK2* and *NTRK3* fusions in patients with solid tumors who may benefit from treatment

with VITRAKVI® (larotrectinib), was established through a clinical bridging study using tumor tissue FFPE specimens from patients enrolled in the LOXO-TRK-14001 (Bayer 20288, NCT02122913), -15002 (Bayer 20289, NAVIGATE, NCT02576431), and -15003 (Bayer 20290, SCOUT, NCT02637687), 14001, 15002, and 15003 clinical trials respectively, with known *NTRK* fusion status and an additional set of *NTRK* fusion negative samples from the FMI archives.

A summary of the clinical study is presented below.

A. **Study Design**

The clinical effectiveness of F1CDx for detecting *NTRK1, NTRK2,* and *NTRK3* fusions in patients with solid tumors who may benefit from treatment with VITRAKVI was demonstrated in a retrospective analysis of tumor tissue FFPE specimens from patients enrolled in studies 14001, 15002, and 15003 and an additional set of *NTRK* fusion negative tumor tissue FFPE specimens from the FMI archives. A bridging study was conducted to assess: 1) concordance of results for the *NTRK* gene fusion status between the F1CDx assay and the local clinical trial assays (LCTAs) used to determine patient eligibility for enrollment, and 2) the clinical utility of F1CDx in identifying patients with *NTRK* fusion positive status for treatment with VITRAKVI.

Below is a summary of the clinical studies 140001, 15002 and 150003.

Therapeutic Clinical Study TRK-14001 (Study 14001)

Study 14001 is an ongoing multicenter open-label Phase 1 dose escalation study in adult patients with advanced solid tumors (all comers) unselected for the presence of *NTRK* gene fusions. Study objectives include characterization of safety, the identification of a maximum tolerated dose (MTD) and/or an appropriate dose for further study, and the characterization of pharmacokinetics. Planned dose levels ranged from 50 mg once daily (QD) to 200 mg twice daily (BID). Following the dose escalation portion of the study, a dose expansion was initiated for patients with solid

tumors with documented *NTRK* fusions and for patients who the Investigator believed might benefit from a highly selective tropomyosin-related kinase (TRK) inhibitor. Diagnoses and main criteria for inclusion for Study LOXO-TRK-14001included:

- Adult patients with a locally advanced or metastatic solid tumor that has progressed or is nonresponsive to available therapies, are unfit for standard chemotherapy or for which no standard or available curative therapy exists.
- Proof of a malignancy harboring an *NTRK* fusion as previously determined with prior testing from a Clinical Laboratory Improvement Amendments (CLIA)-certified or equivalent-certified laboratory.
- At least 18 years of age.
- Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 and life expectancy of at least 3 months.
- Adequate hematologic, hepatic and renal status, as defined in the individual study protocols.

Therapeutic Clinical Study TRK-15002 (Study 15002)
Study 15002 is an ongoing multicenter open-label Phase 2 "basket" study in patients age 12 and older with recurrent advanced solid tumors with locally-advanced or metastatic malignancy with an *NTRK1, NTRK2* or *NTRK3* gene fusion as identified through molecular assays performed at CLIA or other similarly-certified laboratories. Study objectives include the characterization of antitumor activity, specifically best overall response, overall response rate (ORR), duration of response, progression-free survival (PFS), and overall survival (OS). Patients are enrolled into 1 of 8 tumor-defined cohorts consisting of non-small cell lung, thyroid, sarcoma, colorectal, salivary gland, biliary, primary central nervous system (CNS), and "all other"/ non-measurable cancers. All patients receive a starting dose of 100 mg BID, with dose modifications as needed for toxicity.

Diagnoses and main criteria for inclusion for Study LOXO-TRK-15002 included:

- Locally-advanced or metastatic malignancy with an *NTRK1, NTRK2* or *NTRK3* gene fusion, identified through molecular assays as routinely performed at CLIA or other similarly-certified laboratories.
- Patients must have received prior standard therapy appropriate for their tumor type and stage of disease, or in the opinion of the investigator, would be unlikely to tolerate or derive clinically meaningful benefit from appropriate standard of care therapy.
- Patients must have had at least 1 measurable lesion as defined by Response Evaluation Criteria in Solid Tumors, RECIST v1.1. Patients without RECIST v1.1 measurable disease (e.g., evaluable disease only) were eligible for enrollment to Cohort 8, regardless of tumor type. Patients in Cohort 7 (primary CNS tumors) should have met the following criteria:

- o Had received prior treatment including radiation and/or chemotherapy, with radiation completed >12 weeks prior to Cycle 1 Day 1 (C1D1) of therapy, as recommended or appropriate for that CNS tumor type
  - o Had ≥1 site of bi-dimensionally measurable disease (confirmed by magnetic resonance imaging [MRI] and evaluable by Response Assessment in Neuro- Oncology Criteria [RANO] criteria), with the size of at least 1 of the measurable lesions ≥1 cm in each dimension and noted on more than 1 imaging slice
  - o Imaging study performed within 28 days before enrollment while on stable dose steroid medication for at least 5 days immediately before and during the imaging study.
- At least 12 years of age.
- ECOG score of ≤3. For those entered into Cohort 7, Karnofsky Performance Status of 50.
- Adequate organ function as defined in the individual study protocols.

Therapeutic Clinical Study TRK-15003 (Study 15003)
Study 15003 is an ongoing multicenter open-label Phase 1/2 study in pediatric patients aged from birth to 21 years with advanced solid or primary CNS tumors. The study is divided into a Phase 1 dose escalation portion, a Phase 1 dose expansion portion, and a Phase 2 portion. In the Phase 2 portion, enrollment is restricted to patients with solid tumors with *NTRK* fusions, as documented by a CLIA or other similarly-certified laboratory.
Diagnoses and main criteria for inclusion for Study LOXO-TRK-15003 included:

- Phase 1 (dose escalation): Between 1 and 21 years of age with a locally advanced or metastatic solid tumor or primary CNS tumor that had relapsed, progressed, or was nonresponsive to available therapies and for which no standard or available systemic curative therapy exists; or infants from birth and older with a malignancy bearing a documented *NTRK* gene fusion that had progressed or was nonresponsive to available therapies, and for which no standard or available curative therapy exists; or locally advanced infantile fibrosarcoma that would otherwise require disfiguring surgery or limb amputation to achieve a complete surgical resection
  - o Phase 1 dose expansion: In addition to the above stated Inclusion Criteria, patients eligible for enrollment into this cohort must have a malignancy with a documented *NTRK* gene fusion with the exception of patients with Infantile Fibrosarcoma (IFS), Congenital Mesoblastic Nephroma tumors (CMN) or Secretory Breast Carcinoma (SBC). Patients with IFS, CMN or SBC may enroll into this cohort with documentation of an *ETV6* rearrangement by FISH or RT-PCR or a documented *NTRK* fusion by NGS.
- Phase 2: Infants from birth and older at C1D1 with either a locally advanced or metastatic infantile fibrosarcoma; patients with locally advanced infantile

fibrosarcoma must otherwise have required disfiguring surgery or limb amputation to achieve a complete surgical resection; or birth through 21 years of age with a locally advanced or metastatic solid tumor or primary CNS tumor that had relapsed, progressed, or was nonresponsive to available therapies and for which there was no standard or available systemic curative therapy and with documented *NTRK* gene fusion (or in the case of IFS, CMN or SBC with documented *ETV6* rearrangement by FISH or RT-PCR or a documented *NTRK* fusion by NGS)

- Histologic verification of malignancy at original diagnosis or relapse (with the exception of patients with intrinsic brain stem tumors or other rare CNS malignancies as outlined in the protocol)
- Measurable or evaluable disease as defined by RECIST or RANO:
  - Phase 1: patients may have evaluable disease only
  - Phase 2: patients must have at least 1 measurable lesion
- Karnofsky (16 years and older) or Lansky (less than 16 years) performance score of at least 50
- Recovered from the toxicities of prior anticancer therapies
- Adequate hematologic, hepatic and renal status, as defined in the individual study protocols.

The three (3) clinical studies with VITRAKVI, LOXO-TRK-14001, LOXO-TRK-15002, and LOXO-TRK-15003, were initiated between May 2014 and December 2015. These studies are being performed in adult and pediatric malignancies to evaluate the safety and efficacy of VITRAKVI across age groups and tumor types. VITRAKVI was approved by FDA in November 2018 based on efficacy data from the patients from Studies 14001, 15002, and 15003 with *NTRK1, NTRK2,* or *NTRK3* fusions. The assessment of efficacy of VITRAKVI was based on the first 55 patients with solid tumors with an *NTRK* gene fusion enrolled across the three clinical trials. This primary analysis set (PAS) for the NDA efficacy analysis had the following criteria:

- Documented *NTRK* fusion as determined by local testing
- Non-CNS primary tumor with 1 or more measurable lesions at baseline as assessed by RECIST 1.1.
- Received 1 or more doses of VITRAKVI

Clinical studies 14001, 15002, and 15003 enrolled a total of 144 patients prior to July 17, 2017. Of the 144 total patients, 105 had known *NTRK* fusion status and 39 had unknown *NTRK* fusion status. Patient eligibility into the trials was determined using LCTAs that included DNA next generation sequencing (NGS), RNA NGS, fluorescent in situ hybridization (FISH), and reverse transcriptase- polymerase chain reaction (RT-PCR) methods. The majority of the 105 clinical trial patients with known *NTRK* fusion status enrolled into the trials had been tested with NGS methods (92%); 51% of the 105 patients had been tested with DNA NGS methods and 41%

with RNA NGS methods. Of the 105 clinical trial patients, 78 patients were *NTRK* fusion positive and 27 were *NTRK* fusion negative. Of the 78 NTRK fusion positive patients, 55 made up the primary analysis set (PAS) for the new drug application (NDA) efficacy analysis, and 23 were additional patients enrolled prior to July 17, 2017 but not in the PAS. The breakdown of patients enrolled in studies 14001, 15002 and 15003 by *NTRK* status is summarized in Table 27 below.

Table 27. Patients in Studies 14001, 15002 and 15003 grouped by *NTRK* Status (enrolled as of July 17, 2017)

Studies	*NTRK* positive patients			*NTRK* negative samples	*NTRK* unknown samples
	PAS	Supplemental (Non-PAS)	Total		
14001	8	0	8	26	32
15002	35	12	47	0	0
15003	12	11	23	1	7
Total	55	23	78	27	39

Clinical Bridging Study Design

The objectives of the bridging study were to assess concordance of results for the *NTRK* gene fusion status between the F1CDx assay and the local clinical trial assays (LCTAs), and to establish the clinical validity of F1CDx in identifying *NTRK* fusion positive patients for treatment with larotrectinib. The clinical bridging study included 45 LCTA-positive samples and 24 LCTA-negative samples from patients enrolled in 14001, 15002, and 15003, supplemented with 206 NTRK fusion negative samples from FMI archives to demonstrate the safety and effectiveness of F1CDx for identification of patients with solid tumors who may be eligible for treatment with VITRAKVI. Of the 45 LCTA *NTRK* fusion positive samples available, one (1) sample was invalid due to failing the F1CDx input requirement for ≥20% tumor nuclei. Therefore, there were 44 patient samples from the three clinical trials included in the *NTRK* fusion positive CDx evaluable set. Of the 24 LCTA *NTRK* fusion negative samples available, one (1) sample was invalid due to failing the F1CDx quality control (QC) sequencing specification for computational tumor purity.

To supplement the *NTRK* negative clinical samples to assess the potential impact of the F1CDx positive/LCTA negative portion of the F1CDx positive intended use population on clinical effectiveness, 206 *NTRK* negative samples as determined by the FoundationOne laboratory developed test (LDT) were selected from the FMI clinical archive. Three (3) of the 206 samples had invalid results due to failing the F1CDx QC specification for hybrid capture input (one sample) or computational purity (two samples). The 23 negative patient samples from the three clinical trials and the 203 supplemental negative samples provided for a total of 226 samples in the *NTRK* fusion negative CDx evaluable set.

Concordance between F1CDx and the LCTAs was evaluated with clinical trial samples that met F1CDx sample testing criteria in the fusion positive and fusion negative analysis sets, as well as supplemental negatives from FMI archives. The clinical effectiveness of F1CDx was demonstrated by overall response rate of patients in the F1CDx positive population within the same patient set used in the VITRAKVI NDA efficacy analysis. The distribution of baseline demographics, disease, and treatment characteristics for the clinical trial patients was compared between the CDx evaluable and CDx non-evaluable sets within the fusion positive and fusion negative analysis populations to demonstrate the similarity.

Covariate and propensity analyses were conducted for the full fusion positive analysis set and for the efficacy analysis set. Sensitivity analyses were conducted to evaluate the impact of missing CDx results on concordance and efficacy.

1. Clinical Inclusion and Exclusion Criteria
   The inclusion/exclusion criteria for inclusion into the clinical bridging study are summarized below:

   Sample Inclusion Criteria
   - Clinical trial samples:
     Samples from *NTRK* fusion positive and *NTRK* fusion negative patients enrolled in studies LOXO-TRK-14001, -15002 and -15003, who started treatment on or prior to July 17, 2017.

   - Supplemental negative samples:
     Samples from *NTRK* fusion negative patients as determined by FoundationOne LDT.
   - Tissue samples: availability of adequate sample quality (≥20% tumor nuclei, ≥0.6mm^3 tumor volume).
   - DNA samples: availability of >50 ng DNA following requantification.

   Sample Exclusion Criteria
   - Lack of clear identification or label on stored patient sample.
   - Obvious physical damage of stored patient sample.
   - Samples not derived from FFPE.
   - Tissue samples: insufficient sample to meet CDx requirement for ≥20% tumor nuclei, ≥0.6mm^3 tumor volume.
   - DNA samples: insufficient DNA to meet CDx input requirement of >50 ng as assessed by the dsDNA PicoGreen assay.

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

3. Clinical Endpoints
   The objectives of the CDx device clinical study were to:
   - Establish the clinical validity of the F1CDx in identifying *NTRK* fusion positive patients for treatment with VITRAKVI
   - Assess concordance of results for the *NTRK* gene fusion status between the F1CDx assay and the local clinical trial assays

   Efficacy analysis was performed using the same efficacy population as the VITRAKVI NDA that supported drug approval (55 patients with solid tumors with *NTRK* fusion positive status in the PAS) using ORR as the primary endpoint. ORR was assessed according to Independent Review Committee (IRC) assessment using Response. Evaluation Criteria in Solid Tumors (RECIST) v1.1. The ORR and corresponding two-sided 95% exact CI using the Clopper-Pearson method were calculated for the F1CDx fusion positive patients in the *NTRK* fusion positive efficacy population and compared to the ORR in the VITRAKVI NDA efficacy population.

B. Accountability of PMA Cohort
A total of 144 patients were enrolled across the three clinical trials as of the original NDA cut-off of July 17, 2017. Of the 144 patients, 66 patients were enrolled in study 14001, 47 patients enrolled in 15002, and 31 patients enrolled in study 15003. *NTRK* fusion status was determined by the study investigators, and results provided to the study sponsor to assess patient eligibility for enrollment, Therefore, clinical trial patient/sample accountability relative to *NTRK* status is only available for the patients enrolled. As described above, supplemental samples from FMI's archives were used to supplement the LCTA *NTRK* fusion negative population.

Of the 66 patients enrolled in 14001, eight (8) were *NTRK* fusion positive, 26 were *NTRK* fusion negative, and 32 were *NTRK* fusion status unknown. Of the 47 patients enrolled in 15002, all 47 were *NTRK* fusion positive. Finally, of the 31 patients enrolled in 15003, 23 were *NTRK* fusion positive, one (1) was *NTRK* fusion negative, and seven (7) were *NTRK* fusion status unknown. Since *NTRK* testing was not required for patient eligibility in study 14001 and study 15003 dose escalation phases, 39 patients across those 2 studies had *NTRK* status unknown. See Table 28 for the accountability of the clinical trial patients by *NTRK* fusion status per the LCTAs.

Table 28. **VITRAKVI clinical trial patients by *NTRK* fusion status**

	LOXO-TRK-14001 (N=66)	LOXO-TRK-15002 (N=47)	LOXO-TRK-15003 (N=31)	Total (N=144)
NTRK gene fusion status, n (%)				
NTRK fusion negative	26 (39.4%)	0	1 (3.2%)	27 (18.8%)
NTRK fusion positive	8 (12.1%)	47 (100.0%)	23 (74.2%)	78 (54.2%)
NTRK fusion unknown	32 (48.5%)	0	7 (22.6%)	39 (27.1%)

All percentages were calculated using N as denominator. Patients were enrolled as of July 17, 2017.

Following testing by the LCTA, available tumor material from patients enrolled in clinical studies 14001, 15002, and 15003 were used for retrospective testing with the F1CDx assay to support the clinical bridging study analysis. Refer to Table 29 for full accountability of the *NTRK* fusion positive patients among the three clinical trials available for concordance analysis. A total of 45 *NTRK* fusion positive samples were available for testing with the F1CDx assay from the three studies.

Table 29. VITRAKVI clinical trial *NTRK* fusion positive patient accountability for the CDx device study

	LOXO-TRK-14001 (N=8)	LOXO-TRK-15002 (N=47)	LOXO-TRK-15003 (N=23)	Total (N=78)
Number (%) of patients				
NTRK fusion positive CDx evaluable set, n (%)	2 (25.0%)	28 (59.6%)	14 (60.9%)	44 (56.4%)
NTRK fusion positive CDx non-evaluable set, n (%)	6 (75.0%)	19 (40.4%)	9 (39.1%)	34 (43.6%)
Reasons for CDx non-evaluable				
Missing due to failing CDx input	0	0	1 (4.3%)	1 (1.3%)
Sample not available for CDx testing	6 (75.0%)	19 (40.4%)	8 (34.8%)	33 (42.3%)

From the three clinical trials, 24 *NTRK* fusion negative samples were available for testing with F1CDx (see Table 30). The fusion negative samples were supplemented with 206 *NTRK* negative samples from the FMI clinical archives (see Table 31).

Table 30. VITRAKVI clinical trial *NTRK* fusion negative patient accountability for the CDx device study

	LOXO-TRK-14001 (N=26)	LOXO-TRK-15002 (N=0)	LOXO-TRK-15003 (N=1)	Total (N=27)
Number (%) of patients				
NTRK fusion negative CDx evaluable set, n (%)	22 (84.6%)	0	1 (100.0%)	23 (85.2%)
NTRK fusion negative CDx non-evaluable set, n (%)	4 (15.4%)	0	0	4 (14.8%)
Reasons for CDx non-evaluable				
Missing due to failing CDx input	0	0	0	0
Missing due to failing other CDx criteria	1 (3.8%)	0	0	1 (3.7%)

Sample not available for CDx testing	3 (11.5%)	0	0	3 (11.1%)

Table 31. Supplemental NTRK fusion negative patient accountability for the CDx device study

	Supplemental negatives (N=206)
Number (%) of patients	
NTRK fusion negative CDx evaluable set, n (%)	203 (98.5%)
NTRK fusion negative CDx non-evaluable set, n (%)	3 (1.5%)
Reasons for CDx non-evaluable	
Missing due to failing CDx input	0
Missing due to failing other CDx criteria	3 (1.5%)

All percentages were calculated using N as denominator.

The total number of samples available for the concordance analysis was 275 (45 NTRK positive clinical samples, 24 *NTRK* negative clinical samples, and 206 FMI banked NTRK negative samples). The F1CDx testing yielded 270 CDx-evaluable results and five (5) invalid results that were used for the CDx and LCTA concordance analysis. Included in the fusion positive analysis set detailed in Table 27 is the NDA PAS that consisted of 55 NTRK fusion positive patients from 14001, 15002, and 15003. Of the 55 samples, 32 had sufficient material to be tested by F1CDx (see Table 32).

Table 32. Patients available for CDx testing from the VITRAKVI efficacy primary analysis set

|	LOXO-TRK-14001 (N=8)	LOXO-TRK-15002 (N=35)	LOXO-TRK-15003 (N=12)	Total (N=55)
Number (%) of patients				
NTRK fusion positive CDx evaluable set, n (%)	2 (25.0%)	23 (65.7%)	6 (50.0%)	31 (56.4%)
NTRK fusion positive CDx non-evaluable set, n (%)	6 (75.0%)	12 (34.3%)	6 (50.0%)	24 (43.6%)
Reasons for CDx non-evaluable				
Missing due to failing CDx input	0	0	1 (8.3%)	1 (1.8%)
Sample not available for CDx testing	6 (75.0%)	12 (34.3%)	5 (41.7%)	23 (41.8%)

All percentages were calculated using N as denominator.

The efficacy analysis used the NDA PAS. Of the 55 patients in the PAS, 32 had samples available for testing with F1CDx. Of the 32 samples tested with F1CDx, one (1) sample was invalid. The efficacy analysis was conducted for the 31 patients with valid F1CDx

results. A sensitivity analysis to evaluate the impact of the 24 missing CDx results on overall response rate and a sensitivity analysis to estimate the clinical efficacy in the total F1CDx positive population were also performed.

Refer to Figure 2 for full accountability of the concordance and efficacy analysis sets.

Patients enrolled as of 17July2017
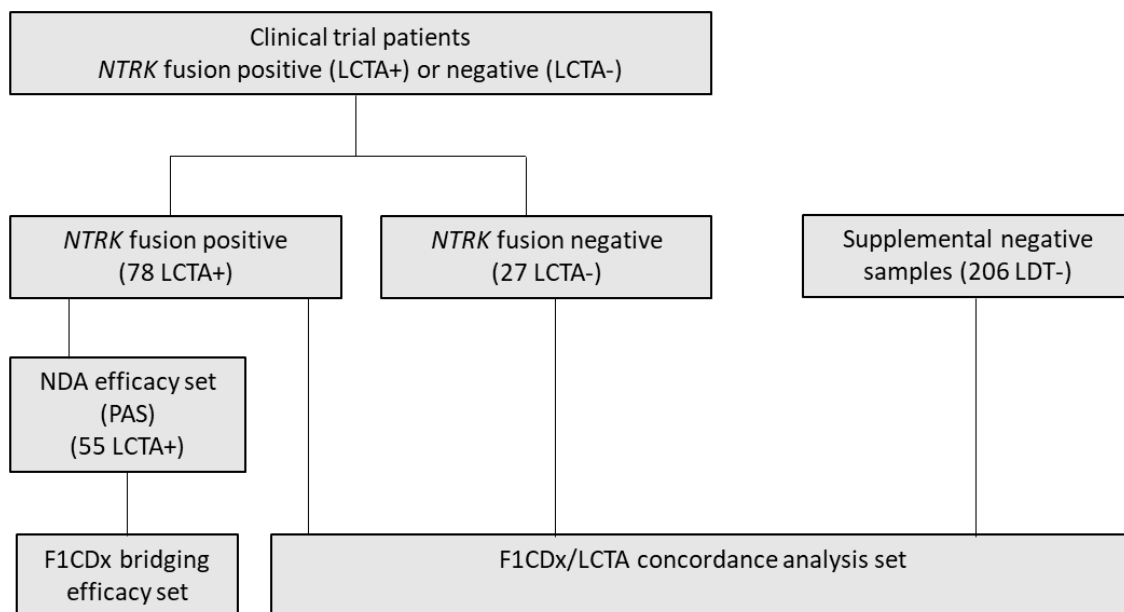
Abbreviations: CDx = Companion Diagnostic; LCTA = Local Clinical Trial Assay; *NTRK* = Neurotrophic Tyrosine Kinase; PAS = Primary Analysis Set

Figure 2: F1CDx clinical efficacy and concordance sets

C. Study Population Demographics and Baseline Parameters

For the bridging study, baseline characteristics were compared between CDx-evaluable and CDx-non-evaluable populations. The demographics and disease characteristics for the CDx-evaluable and CDx-nonevaluable patients are provided in Tables 33-36. Differences in characteristics of the CDx evaluable and CDx non-evaluable were identified by covariate analysis and included in the imputation models used in sensitivity analyses. Demographic and disease characteristics of the supplemental *NTRK* fusion negative patients are provided in Table 37.

Table 33. Comparison of baseline demographic characteristics between the CDx evaluable and CDx non-evaluable NTRK fusion positive patients

	CDx evaluable set (N=44)	CDx non-evaluable set (N=34)	Total (N=78)
Gender, n (%)			

Female	20 (45.5%)	16 (47.1%)	36 (46.2%)
Male	24 (54.5%)	18 (52.9%)	42 (53.8%)
Race, n (%)			
White	25 (56.8%)	28 (82.4%)	53 (67.9%)
Asian	3 (6.8%)	1 (2.9%)	4 (5.1%)
Black	3 (6.8%)	0	3 (3.8%)
Mixed/Other/Unknown	13 (29.5%)	5 (14.7%)	18 (23.1%)
Ethnicity, n (%)			
Not Hispanic or Latino	28 (63.6%)	27 (79.4%)	55 (70.5%)
Hispanic or Latino	6 (13.6%)	1 (2.9%)	7 (9.0%)
Unknown	10 (22.7%)	6 (17.6%)	16 (20.5%)
Age, years			
n	44	34	78
Mean	38.87	39.35	39.08
SD	25.25	26.57	25.66
Min	0.1	0.3	0.1
Median	45.00	40.00	42.00
Max	75.0	79.0	79.0
Age group, n (%)			
<18 years	12 (27.3%)	10 (29.4%)	22 (28.2%)
18+ years	32 (72.7%)	24 (70.6%)	56 (71.8%)

SD = Standard Deviation. All percentages were calculated using N as denominator

Table 34. Comparison of baseline disease characteristics between the CDx evaluable and CDx non-evaluable *NTRK* fusion positive patient

	CDx evaluable set (N=44)	CDx non-evaluable set (N=34)	Total (N=78)
Primary tumor type, n (%)			
Appendix	1 (2.3%)	0	1 (1.3%)
Bone sarcoma	2 (4.5%)	0	2 (2.6%)
Breast	1 (2.3%)	0	1 (1.3%)
Cholangiocarcinoma	1 (2.3%)	1 (2.9%)	2 (2.6%)
Colon	5 (11.4%)	1 (2.9%)	6 (7.7%)
Congenital mesoblastic nephroma	1 (2.3%)	0	1 (1.3%)
Gastrointestinal stroma	1 (2.3%)	4 (11.8%)	5 (6.4%)
Infantile fibrosarcoma	6 (13.6%)	4 (11.8%)	10 (12.8%)
Lung	3 (6.8%)	1 (2.9%)	4 (5.1%)
Melanoma	2 (4.5%)	2 (5.9%)	4 (5.1%)
Pancreas	0	1 (2.9%)	1 (1.3%)
Primary CNS	1 (2.3%)	1 (2.9%)	2 (2.6%)
Salivary gland	6 (13.6%)	8 (23.5%)	14 (17.9%)

	CDx evaluable set (N=44)	CDx non-evaluable set (N=34)	Total (N=78)
Soft tissue sarcoma	9 (20.5%)	8 (23.5%)	17 (21.8%)
Thyroid	5 (11.4%)	3 (8.8%)	8 (10.3%)
Extent of disease at enrollment, n (%)			
Locally advanced	9 (20.5%)	7 (20.6%)	16 (20.5%)
Metastatic	35 (79.5%)	27 (79.4%)	62 (79.5%)
Primary CNS	0	0	0
Stage at initial diagnosis, n (%)			
I	3 (6.8%)	4 (11.8%)	7 (9.0%)
II	7 (15.9%)	7 (20.6%)	14 (17.9%)
III	15 (34.1%)	8 (23.5%)	23 (29.5%)
IV	15 (34.1%)	6 (17.6%)	21 (26.9%)
Unknown	4 (9.1%)	9 (26.5%)	13 (16.7%)
ECOG performance status, n (%)			
0	23 (52.3%)	13 (38.2%)	36 (46.2%)
1	16 (36.4%)	18 (52.9%)	34 (43.6%)
2	5 (11.4%)	3 (8.8%)	8 (10.3%)

CNS = Central Nervous System; ECOG = Eastern Cooperative Oncology Group. All percentages were calculated using N as denominator.

For the *NTRK* fusion positive patients in the full analysis set, 44% of the patients had been tested using DNA NGS methods with the FoundationOne LDT test used most commonly (28%). RNA NGS methods had been used to test 49% of the *NTRK* fusion positive patients. Of the N*TRK* fusion positive patients, 5 had been tested by FISH and 1 had been tested by RT PCR methods. The *NTRK* fusion positive CDx evaluable set included patients that had been tested with DNA NGS methods (20 [45%] patients), RNA NGS methods (20 [45%] patients), FISH methods (3 [7%] patients) and the RT-PCR method (1 [2%] patients). The CDx evaluable and CDx nonevaluable sets were similar except the CDx evaluable set contained more patients tested by one of the RNA NGS methods (25%) compared to 15% for the CDx non-evaluable set, and no patients in the CDx evaluable set had been tested by one of the DNA NGS based methods compared to 15% for the CDx non-evaluable set.

Table 35. Comparison of baseline demographic characteristics between the CDx evaluable and CDx non-evaluable *NTRK* fusion negative patients from the VITRAKVI clinical studies

	CDx evaluable set (N=23)	CDx non-evaluable set (N=4)	Total (N=27)
Gender, n (%)			
Female	11 (47.8%)	4 (100.0%)	15 (55.6%)
Male	12 (52.2%)	0	12 (44.4%)
Race, n (%)			
White	21 (91.3%)	3 (75.0%)	24 (88.9%)
Black	1 (4.3%)	0	1 (3.7%)
Mixed/Other/Unknown	1 (4.3%)	1 (25.0%)	2 (7.4%)

	CDx evaluable set (N=23)	CDx non-evaluable set (N=4)	Total (N=27)
Ethnicity, n (%)			
Not Hispanic or Latino	20 (87.0%)	3 (75.0%)	23 (85.2%)
Hispanic or Latino	3 (13.0%)	1 (25.0%)	4 (14.8%)
Age, years			
n	23	4	27
Mean	59.03	55.75	58.54
SD	12.50	9.18	11.98
Min	17.6	44.0	17.6
Median	60.00	57.50	60.00
Max	76.0	64.0	76.0
Age group, n (%)			
<18 years	1 (4.3%)	0	1 (3.7%)
18+ years	22 (95.7%)	4 (100.0%)	26 (96.3%)

All percentages were calculated using N as denominator.

Table 36. Comparison of baseline disease characteristics between the CDx evaluable and CDx non-evaluable *NTRK* fusion negative patients from the VITRAKVI clinical studies

	CDx evaluable set (N=23)	CDx non-evaluable set (N=4)	Total (N=27)
Primary tumor type, n (%)			
Anal	1 (4.3%)	0	1 (3.7%)
Bone sarcoma	1 (4.3%)	0	1 (3.7%)
Breast	1 (4.3%)	0	1 (3.7%)
Cholangiocarcinoma	1 (4.3%)	0	1 (3.7%)
Colon	5 (21.7%)	0	5 (18.5%)
Hepatic	1 (4.3%)	0	1 (3.7%)
Lung	3 (13.0%)	0	3 (11.1%)
Melanoma	1 (4.3%)	0	1 (3.7%)
Oral	1 (4.3%)	0	1 (3.7%)
Ovarian	0	1 (25.0%)	1 (3.7%)
Pancreas	0	1 (25.0%)	1 (3.7%)
Primary CNS	1 (4.3%)	0	1 (3.7%)
Salivary gland	0	1 (25.0%)	1 (3.7%)
Soft tissue sarcoma	4 (17.4%)	1 (25.0%)	5 (18.5%)
Thymus	2 (8.7%)	0	2 (7.4%)
Thyroid	1 (4.3%)	0	1 (3.7%)
Extent of disease at enrollment, n (%)			
Locally advanced	0	0	0
Metastatic	22 (95.7%)	4 (100.0%)	26 (96.3%)
Primary CNS	1 (4.3%)	0	1 (3.7%)

	CDx evaluable set (N=23)	CDx non-evaluable set (N=4)	Total (N=27)
Stage at initial diagnosis, n (%)			
I	3 (13.0%)	0	3 (11.1%)
II	4 (17.4%)	0	4 (14.8%)
III	6 (26.1%)	1 (25.0%)	7 (25.9%)
IV	10 (43.5%)	3 (75.0%)	13 (48.1%)
ECOG performance status, n (%)			
0	8 (34.8%)	1 (25.0%)	9 (33.3%)
1	14 (60.9%)	3 (75.0%)	17 (63.0%)
2	1 (4.3%)	0	1 (3.7%)

CNS = Central Nervous System; ECOG = Eastern Cooperative Oncology Group; *NTRK* = Neurotrophic Tyrosine Kinase; SD = Standard Deviation. All percentages were calculated using N as denominator.

Table 37. Demographic and disease characteristics of the supplemental *NTRK* fusion negative patients

	Total (N=206)
Gender, n (%)	
Female	114 (55.3%)
Male	92 (44.7%)
Age, years	
Min	4
Median	60
Max	88
Age group, n (%)	
<18 years	10 (4.8%)
18+ years	196 (95.1%)
Disease ontology, n (%)	
Anal	2 (1.0%)
Appendix	1 (0.5%)
Bladder	3 (1.5%)
Bone, soft tissue sarcoma	4 (1.9%)
Breast	35 (17.0%)
Cervix	3 (1.4%)
CNS	11 (5.3%)
Colorectal	19 (9.2%)
Esophagus	5 (2.4%)
Head and neck (includes salivary gland)	14 (6.8%)
Gastrointestinal stroma	4 (1.9%)

	Total (N=206)
Kidney	3 (1.5%)
Liver	5 (2.3%)
Lung	35 (17.0%)
Melanoma	2 (1.0%)
Neoplasm/Cancer of unknown primary site	14 (6.8%)
Ovarian	13 (6.3%)
Pancreatobiliary	10 (4.9%)
Prostate	6 (2.9%)
Skin	1 (0.5%)
Stomach	3 (1.5%)
Thyroid	8 (3.9%)
Uterus	5 (2.3%)

Abbreviations: CNS = Central Nervous System; *NTRK* = Neurotrophic Tyrosine Kinase. All percentages were calculated using N as denominator.

For the *NTRK* fusion negative patients (Table 35, 36 and 37), 74% of patients (20 out of 27) had been tested using DNA NGS methods, with FoundationOne LDT used most commonly (18 out of 27, 67%). RNA NGS methods had been used to test 19% of the NTRK fusion negative patients (5 out of 27).

For the supplemental negatives from the FMI bank, all patients had been tested with the FoundationOne LDT assay.

D. Safety and Effectiveness Results

1. Safety Results
   The safety with respect to treatment with VITRAKVI 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 VITRAKVI® (larotrectinib).

   In brief, the most common adverse reactions ($\geq 20\%$) with VITRAKVI were fatigue, nausea, dizziness, vomiting, increased aspartate aminotransferase (AST), cough, increased alanine aminotransferase (ALT), constipation, and diarrhea. Adverse reactions were predominantly of grade 1 and 2.

   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
Concordance Results
The results of the concordance analysis demonstrated agreement between the F1CDx assay and the LCTAs. Of the 45 LCTA *NTRK* fusion positive samples, 37 were F1CDx positive and one (1) was invalid. Of the 230 LCTA *NTRK* fusion negative samples, 226 were F1CDx negative and four (4) were invalid. There were seven (7) discordant results (F1CDx negative, LCTA positive). See Table 38 for the contingency table of F1CDx and LCTA results.

Table 38. Contingency table comparing *NTRK* fusion detection results between the CDx and the LCTA for all CDx tested patients

F1CDx result	LCTA results		
	Positive	Negative	Total
Positive	37	0	37
Negative	7	226	233
Invalid	1	4	5
Total	45	230	275

The LCTA inferred *NTRK3* gene fusions were considered fusion positive.

Agreement measures, PPA, NPA and overall percent agreement (OPA), between the F1CDx and LCTAs, with and without invalid F1CDx results, were calculated using the LCTA results as reference (Table 39). The point estimates of PPA, NPA and OPA were 84.1%, 100% and 97.4% respectively, when excluding F1CDx invalid results. The point estimates of PPA, NPA, and OPA using valid and invalid results were 82.2%, 98.3%, and 95.6%, respectively.

Table 39. Concordance between the F1CDx and LCTA methods for detection of NTRK gene fusions based on the LCTA results (all patients tested by CDx)

Measure of Agreement	Excluding CDx Invalid Results		Including CDx invalid results	
	% Agreement (N)	95% CI [a]	% Agreement (N)	95% CI [a]
PPA	84.1% (37/44)	69.9% , 93.4%	82.2% (37/45)	67.9% , 92.0%
NPA	100.0% (226/226)	98.4% , 100.0%	98.3% (226/230)	95.6% , 99.5%
OPA	97.4% (263/270)	94.7% , 99.0%	95.6% (263/275)	92.5% , 97.7%

[a] The 95% CI was calculated using the Clopper-Pearson exact method.

A sensitivity analysis against the 34 missing CDx results was conducted to assess the robustness of the agreement analysis. Missing CDx results for the LCTA fusion positive patients were imputed using a logistic regression model including 10 covariates (race, ethnicity, age group, stage of disease at initial diagnosis, prior cancer systemic treatments, prior cancer related surgery, ECOG performance status, *NTRK* fusion gene, LCTA method sample substrate, and binary clinical response to larotrectinib). Agreement estimates, including the imputed values,

were PPA= 78.3%, 95% CI [64.4%, 89.9%] and NPA=100% (Table 40), and were similar to the agreement results of the primary concordance analysis (PPA = 84.1%, NPA = 100%). The method of calculation for the 95% confidence interval accounted for both within and between imputation variance.

Table 40. Concordance between the CDx and LCTA methods for detection of NTRK gene fusions including imputed values in LCTA fusion positive patients with missing CDx results

Measure of Agreement	% Agreement	95% CI (a)
PPA	78.3%	64.4%, 89.9%
NPA	100.0%	100.0%, 100.0%
OPA	94.4%	90.5%, 97.4%

[a] The 95% CI was calculated based on multiple imputation (MI) Boot pooled sample method.

The F1CDx assay showed high concordance with the DNA NGS LCTA methods with PPA = 95%, 95% CI [75%, 100%] and NPA = 100%, 95% CI [98%, 100%] (Table 41).

Table 41. Concordance between the CDx and DNA NGS LCTA methods for detection of *NTRK* gene fusions based on LCTA results and excluding invalid results

Measure of Agreement	% Agreement (N)	95% CI (a)
PPA	95.0% (19/20)	75.1%, 99.9%
NPA	100.0% (221/221)	98.3%, 100.0%
OPA	99.6% (240/241)	97.5%, 100.0%

[a] The 95% CI was calculated based on Clopper-Pearson exact method.

However, the positive concordance of F1CDx with RNA NGS methods was lower (PPA = 70%, 95% CI [46%, 88%]) (Table 42).

Table 42. Concordance between the CDx and RNA NGS LCTA methods for detection of NTRK gene fusions based on LCTA results and excluding invalid results

Measure of Agreement	% Agreement (N)	95% CI (a)
PPA	70.0% (14/20)	45.7%, 88.1%
NPA	100.0% (4/4)	39.8%, 100.0%
OPA	75.0% (18/24)	53.3%, 90.2%

[a] The 95% CI was calculated based on Clopper-Pearson exact method.

F1CDx was concordant with FISH and RT-PCR based on testing of 5 samples (Table 43). Due to the low sample counts, agreement measures were not calculated.

Table 43. Contingency table comparing NTRK fusion detection results between the CDx and the FISH and RT-PCR LCTA methods (all patients tested by CDx)

F1CDx result by test method	LCTA positive	LCTA negative
FISH		

CDx Positive	3	0
CDx Negative	0	1
Total	3	1
RT-PCR		
CDx Positive	1	0
CDx Negative	0	0
Total	1	0

FISH = Fluorescence in Situ Hybridization; RT-PCR = ReverseTranscriptase-Polymerase Chain Reaction.

A total of 7 of the 275 samples tested with the F1CDx assay showed discordant results between F1CDx and the LCTAs. All 7 discordant results were *NTRK* fusion positive by the LCTA and fusion negative by F1CDx. Of the seven (7) discordant results, six (6) had been tested with an RNA NGS LCTA method and one (1) with an DNA NGS LCTA method.

The discordances between the RNA NGS LCTA methods and F1CDx can be explained due to differences in technology used to detect *NTRK1/2/3* fusions, as well as expected degree of measurement error by the LCTAs and F1CDx. *NTRK* often presents complex genomic rearrangement events with a variety of breakpoints spanning multiple introns. This complexity of rearrangement events presents certain limitations for targeted DNA sequencing. F1CDx was designed to focus on hotspot introns that are repeatedly described in the literature, which means rare and complex breakpoints may not be captured by F1CDx baiting. As explained in Section IX.A.10., the DX1 baitset used by the F1CDx assay includes the coding regions of *NTRK1, NTRK2*, and *NTRK3* and select introns from these genes, however no introns within *NTRK3* are baited and the *NTRK1* intron 8, and *NTRK2* intron 12 are not fully baited. Further while the most common fusion partner of *NTRK3, ETV6*, has introns baited which allows for detection *ETV6-NTRK3* fusions, *EVT6* intron 5 is also not fully baited. A portion of fusion events between these two genes are likely being undetected as a result of DX1 not baiting intron 4 of *ETV6*.

Of the seven (7) discordant patients, four (4) patients had complete or partial response, supporting that these four (4) samples were most likely true positives. Investigation findings concluded that F1CDx did not detect the fusion events in six (6) of the discordances for one of two reasons: 1) F1CDx does not bait the intron where the breakpoint occurred, or 2) the rearrangement event was too complex to be fully baited by F1CDx, and therefore the full picture of the event was not captured. The remaining one (1) discordant case could have been explained by differences in sample collection, where the sample tested by F1CDx was from a sample collected at a different timepoint than used for the LCTA test.

<u>Clinical Efficacy Results</u>

Clinical utility of F1CDx was evaluated by estimation of clinical efficacy in the F1CDx- positive, LCTA-positive population. Clinical outcome was assessed by independent review committee using RECIST 1.1 criteria. Efficacy of VITRAKVI in the F1CDx positive, LCTA-positive population was 77% (95% CI: [56%, 91%]) overall response rate (see Table 44). This is comparable to the efficacy for the NDA filing, where VITRAKVI demonstrated an estimated 75% (95% CI: [61%, 85%]) overall response rate in the NDA efficacy population. Of the 26 F1CDx-positive patients in the efficacy set, six (6) (23%) patients had achieved a complete response and 14 (54%) had received a partial response with VITRAKVI therapy.

Efficacy results are summarized in Table 44.

Table 44. Primary efficacy results: the best overall response and overall response rate for NTRK fusion positive patients by LCTA and CDx results in the efficacy analysis set

Clinical outcome	LCTA fusion positive (N=55)	CDx fusion positive and LCTA fusion positive (N=26)	CDx fusion negative and LCTA fusion positive (N=5)	CDx fusion result missing and LCTA fusion positive (N=24)
ORR% (95% CI[a])	75% (61%, 85%)	77% (56%, 91%)	80% (28%, 99%)	71% (49%, 87%)
Complete response	12 (22%)	6 (23%)	2 (40%)	4 (17%)
Partial response	29 (53%)[b]	14 (54%)	2 (40%)	13 (54%)[b]
Duration of Response[c]	**N=41**	**N=20**	**N=4**	**N=17**
Range (months)	1.6, 33.2	1.6, 20.3	3.7, 23.6	2.7, 33.2
% with duration ≥ 6 months	73.2%	80.0%	50.0%	70.6%
% with duration ≥ 9 months	63.4%	65.0%	50.0%	64.7%
% with duration ≥ 12 months	39.0%	25.0%	50.0%	52.9%

[a] The 95% confidence interval was calculated using the Clopper-Pearson exact method.
[b] Includes one pediatric patient with unresectable infantile fibrosarcoma who underwent resection following partial response and who remained disease-free at data cutoff.
[c] Includes patients with ongoing response after data cutoff.

<u>Sensitivity Analyses</u>
<u>Sensitivity analysis for missing CDx results</u>
Twenty-four (24) patients have missing CDx results (43.64% of the PAS population have missing results). Sensitivity analysis against the 24 missing CDx results was conducted to assess the robustness of the clinical efficacy analysis for the F1CDx positive patients. Missing CDx results for the LCTA fusion positive patients in the efficacy set were imputed 100 times using a logistic regression model including 9

covariates based on the missing at random (MAR) assumption. Covariates identified included covariates imbalanced between the CDx evaluable and CDx non-evaluable sets, covariates associated with the F1CDx results and covariates associated with patient clinical response to larotrectinib. 9 covariates were used in the imputation model for the efficacy sensitivity analysis: race, ethnicity, age group, gender, stage of disease at initial diagnosis, ECOG performance status, *NTRK* fusion gene, LCTA method sample substrate, and binary clinical response to larotrectinib. Clinical efficacy, including the imputed CDx results, was ORR=74%, 95% CI [59%, 89%] (Table 45) and was similar to the results of the primary efficacy analysis (ORR=77%, 95% CI [56%, 91%]) (Table 44).

The clinical effectiveness of F1CDx to identify patients with solid tumors with *NTRK1, NTRK2* or *NTRK3* fusions who may benefit from VITRAKVI treatment is based on ~56% of the VITRAKVI efficacy population. To address the uncertainties due to the large proportion of missing data, a post market study to provide clinical outcome data (e.g., Real World Evidence, direct clinical data) will be provided to confirm the clinical effectiveness of F1CDx (see section XIII).

Table 45. Sensitivity analysis for overall response rate by CDx result for NTRK fusion positive patients including imputed values for missing CDx results in the efficacy analysis set

Clinical Outcome	CDx fusion positive and LCTA fusion positive	CDx fusion negative and LCTA fusion positive
ORR% (95% CI [a])	74% (59% - 89%)	78% (46% - 100%)

[a] The 95% confidence interval was calculated based on MI Boot pooled sample method.

<u>Sensitivity analysis for the missing F1CDx positive, LCTA negative population</u>
Sensitivity analysis was performed to estimate ORR in the total F1CDx positive population including the F1CDx positive, LCTA positive and the F1CDx positive, LCTA negative subpopulations. To assess the potential impact of the F1CDx positive, LCTA negative portion of the F1CDx positive intended use population on clinical effectiveness, 206 NTRK negative samples by the FoundationOne LDT were selected from the FMI clinical archive along with 24 *NTRK* negative clinical trial samples available for testing were used to obtain a NPA that was representative of the LCTAs used prior to enrolling patients in the VITRAKVI trials.   Since the estimated NPA (PPV) is 100%, the ORR for the F1CDx positive population is the same as the ORR for the F1CDx positive and LCTA positive population. However the NPA estimate between F1CDx and LCTA could be subject to bias given that the majority of the *NTRK* fusion negative in both the full population and those patients whose samples were available for testing with F1CDx had been tested using DNA NGS methods (>70%). FoundationOne LDT was the most commonly used DNA based NGS method for *NTRK* fusion status determination for patients in the VITRAKVI clinical trials. The same assay was also used to select the supplemental

negative samples in the clinical bridging study as the representative LCTA. Therefore the estimated NPA could be biased.

Sensitivity analysis to determine the minimum PPV that will lead to an ORR of 30% at the lower bound of the two-sided 95% confidence interval for the CDx positive population was performed. This analysis was conducted to determine the NPA corresponding to this tipping point PPV by assuming fixed prevalence of *NTRK* fusions (0.32%) and PPA (84%) observed from the concordance analysis to demonstrate the robustness of the study results.

For each value of c (the scaling factor for the assumed ORR (LCTA negative/F1CDx positive)), the tipping point PPV that led to an ORR of the F1CDx positive population with the lower bound of the two-sided 95% confidence interval at 30% was determined. When c is greater than or equal to 0.85, indicating the ORR in the LCTA negative/F1CDxpositive population is close to the ORR in the LCTA positive/F1CDxpositive population, the two-sided 95% lower confidence limit (LCL) of ORR is always greater than 30% so there is no tipping point of PPV. At all values of PPV (and NPA), the two-sided 95% LCL is > 30%. At c values between 0 and 0.8, a tipping point PPV ranges from 99.5% to 88.6% and NPA ranges from 100% to 99.97%.

3   Subgroup Analyses
Response to larotrectinib for the F1CDx fusion positive patients in the PAS population was analyzed by age, *NTRK* fusion gene, *NTRK* gene fusion partner and primary tumor type. For sub-groups containing less than 3 patients, ORR was not calculated (Table 46).

For pediatric patients (<18 years) ORR=100% (n=5) and ORR=71% (n=21) in adult patients (≥18 years). Response rate by *NTRK* gene fusion was ORR = 60% (n=15) for patients with *NTRK*1 gene fusions and ORR = 100% (n=11) for patients with *NTRK*3 gene fusions. For patients with *ETV6-NTRK3* fusions (n=11), the ORR was 100%.

Within the F1CDx positive patients for the efficacy set, 10 tumor types were represented. Response rates and sample counts by tumor type are shown in Table 46.

Table 46: The overall response rate for CDx *NTRK* fusion positive patients in the efficacy analysis set by different subgroups

Subgroup	Number of Patients (N=26)	Number of patients with CR + sCR + PR (N=20)	ORR % (95% CI[a])
Age			

Subgroup	Number of Patients (N=26)	Number of patients with CR + sCR + PR (N=20)	ORR % (95% CI[a])
<18 years	5	5	100% (48%, 100%)
18+ years	21	15	71% (48%, 89%)
NTRK fusion gene by CDx			
*NTRK*1	15	9	60% (32%, 84%)
*NTRK*3	11	11	100% (72%, 100%)
NTRK fusion partner			
ETV6-NTRK3	11	11	100% (72%, 100%)
IRF2BP2-NTRK1	1	1	Not calculated
LMNA-NTRK1	3	1	33% (1%, 91%)
PLEKHA6-NTRK1	1	0	Not calculated
PPL-NTRK1	1	1	Not calculated
SQSTM1-NTRK1	2	2	Not calculated
TPM3-NTRK1	5	2	40% (5%, 85%)
TPR-NTRK1	1	1	Not calculated
TRIM63-NTRK1	1	1	Not calculated
Primary tumor type			
Appendix	1	0	Not calculated
Breast	1	0	Not calculated
Cholangiocarcinoma	1	0	Not calculated
Colon	4	1	25% (1%, 81%)
Infantile fibrosarcoma	4	4	100% (40%, 100%)
Lung	3	3	100% (29%, 100%)
Melanoma	1	1	Not calculated
Salivary gland	5	5	100% (48%, 100%)
Soft tissue sarcoma	3	3	100% (29%, 100%)
Thyroid	3	3	100% (29%, 100%)

sCR = Surgical Complete Response. [a] The 95% confidence interval was calculated using the Clopper-Pearson exact method. Note: ORR only calculated for subgroups with three or more patients.

4. Pediatric Extrapolation

VITRAKVI® (larotrectinib) was approved for treatment of adult and pediatric patients with solid tumors that have *NTRK* gene fusion without a known acquired resistance mutation, are metastatic or where surgical resection is likely to result in severe morbidity, and have no satisfactory alternative treatments or that have progressed following treatment.

The safety and effectiveness of VITRAKVI in pediatric patients was established based upon data from three multicenter, open-label, single-arm clinical trials in adult or pediatric patients 28 days and older. The efficacy of VITRAKVI was

evaluated in 12 pediatric patients and the safety of VITRAKVI was evaluated in 44 pediatric patients who received VITRAKVI. Of these 44 patients, 27% were 1 month to < 2 years (n = 12), 43% were 2 years to < 12 years (n = 19), and 30% were 12 years to < 18 years (n = 13); 43% had metastatic disease and 57% had locally advanced disease; and 91% had received prior treatment for their cancer, including surgery, radiotherapy, or systemic therapy. The most common cancers were infantile fibrosarcoma (32%), soft tissue sarcoma (25%), primary CNS tumors (20%), and thyroid cancer (9%). The median duration of exposure was 5.4 months (range: 9 days to 1.9 years).

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 two investigators of which two were full-time or part-time employees of the sponsor and two of the investigators 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: [2]
- 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 F1CDx to identify patients with solid tumor with *NTRK1, NTRK2* or *NTRK3* fusions who may benefit from VITRAKVI (larotrectinib) treatment is supported by the results from the clinical bridging study. This study was performed using specimens from patients enrolled in the 14001, 15002, and 15003 studies with known *NTRK* fusion status and supplemented with *NTRK* 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 14001, 15002, and 15003 studies show that patients who had *NTRK* fusions received benefit from treatment with larotrectinib and support the addition of the CDx indication to F1CDx. The bridging study demonstrated the ability of F1CDx to detect *NTRK* fusion positive patients that benefit from VITRAKVI therapy.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical studies conducted to support PMA approval as described above. The F1CDx assay is an in vitro diagnostic test, which involves testing of DNA extracted from FFPE tumor tissue. The assay can be performed using DNA extracted from existing (archival) tissue samples routinely collected as part of the diagnosis and patient care.

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in Table 1 of the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy..

C. Benefit-Risk Determination

The probable benefits of F1CDx in identification for *NTRK1/2/3* fusions for treatment with VITRAKVI are based on data collected in the 14001, 15002, and 15003 studies and the bridging study. The clinical benefit of the F1CDx assay for the selection of solid tumor cancer patients with *NTRK* fusions was demonstrated in a retrospective bridging study using samples from patients enrolled in the 14001, 15002, and 15003 studies, and supplemented with additional *NTRK* fusion negative samples. Clinical outcome of *NTRK1/2/3* positive fusion positive patients by F1CDx positive patients, as assessed by independent review committee using RECIST 1.1 criteria, indicated an ORR of 77% (95% CI: [56%, 91%]), which provides a meaningful clinical benefit in

this population. This supports the probable benefit of F1CDx in selecting *NTRK1/2/3* fusion positive patients for treatment with VITRAKVI.

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 LCTAs, demonstrate a PPA of 84.1%, NPA of 100.0% and OPA of 97.4% indicating that a small subset of patients may be missed by F1CDx. In addition, an accuracy study of F1CDx for the detection of *NTRK* fusions with the externally validated NGS (evNGS) comparator method further supports this conclusion; however, there was an imperfect comparator method in terms of variant calling parameters. However, patients identified with the F1CDx assay show comparable overall response rate to larotrectinib as found in the 14001, 15002, and 15003 studies. Therefore, these results support the use of F1CDx as an aid in selecting patients with solid tumors harboring *NTRK1/2/3* fusions for larotrectinib treatment, albeit the risk that a small subset of patients with *NTRK1/2/3* fusion may be missed. The following limitation of F1CDx is included in the device labeling:

> While the overall positive percent agreement between trial enrollment assays and F1CDx was 84% (37/44), thirty percent (30%) (6/20) of patients enrolled in the VITRAKVI clinical studies using RNA-based NGS detection were negative for *NTRK* fusions by F1CDx. Four of the six patients (4/6 or 60%) that were negative for *NTRK* fusions by F1CDx had a response to larotrectinib. Therefore, F1CDx may miss a subset of patients with solid tumors with *NTRK1/2/3* fusions who may derive benefit from VITRAKVI.

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.

Additional factors to be considered in determining probable risks and benefits for the F1CDx assay include: analytical performance of the device in precision and limit of detection studies, and representation of *NTRK* fusion variants across the analytical

and clinical studies. 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 *NTRK* fusion solid tumor cancer patients for whom VITRAKVI may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on October 23, 2020. The final clinical conditions of approval cited in the approval order are described below.

1. Foundation Medicine, Inc. (FMI) must provide detailed protocols, including acceptance criteria where appropriate, for the studies that are noted below as conditions of approval. These studies must be adequate to confirm the safety and effectiveness of the FoundationOne CDx device and must include a detailed description of the numbers of sample to be tested, the type of samples to be tested, the tumor types for each sample, the complete testing protocol, and a robust statistical analysis plan, as applicable. These protocols must be submitted to FDA no later than 60 days after approval.

2. The limit of detection (LoD) and intermediate precision studies included a limited number of CDx positive samples that have *NTRK1* and NTRK2 fusions. To provide a more robust assessment of LoD and precision, FMI must provide LoD data for two additional CDx positive samples, specifically one additional *NTRK1* fusion positive and one additional *NTRK2* fusion positive sample. For precision, FMI must provide precision data for two additional CDx positive samples with *NTRK2* fusions.

3. Foundation Medicine, Inc. must provide the results of a site-to-site reproducibility study to include the second laboratory site in Morrisville, North Carolina using a

representative sample panel similar to that evaluated in support of the single site in Cambridge, Massachusetts. The study should include the same panel representation and testing strategy as was reviewed in the PMA.

4. FMI must provide clinical outcome data (e.g., Real World Evidence, direct clinical data) in the post-market setting in order to confirm the clinical effectiveness of F1CDx as a companion diagnostic (CDx) device for identification of patients with solid tumors with *NTRK1/2/3* fusions who may benefit from treatment with VITRAKVI.

The final study data, study conclusions, and labeling revisions should be submitted within 2 years of the PMA approval date.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

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