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® Liquid CDx (F1 Liquid CDx)
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: P190032/S004

Date of FDA Notice of Approval: December 22, 2022

The FoundationOne® Liquid CDx was approved on August 26, 2020 as a companion diagnostic for *BRCA1* and *BRCA2* alterations in metastatic castration-resistant prostate cancer (mCRPC) patients who may benefit from treatment with RUBRACA® (rucaparib) and *EGFR* activating mutations (Exon 19 deletions and L858R substitution mutation) in patients with advanced and metastatic non-small cell lung cancer (NSCLC) who may benefit from treatment with IRESSA® (gefitinib), TAGRISSO® (osimertinib), and TARCEVA® (erlotinib). On October 26, 2020 the FoundationOne® Liquid CDx test was approved as a companion diagnostic for *BRCA1* and *BRCA2* alterations in epithelial ovarian cancer for patients who may benefit from treatment with RUBRACA® (rucaparib), *ALK* rearrangements in non-small cell lung cancer for patients who may benefit from treatment with ALECENSA® (alectinib), and *PIK3CA* mutations patients with breast cancer who may benefit from treatment with PIQRAY® (alpelisib). On November 6, 2020, the FoundationOne Liquid CDx test was approved as a companion diagnostic for *BRCA1, BRCA2* and *ATM* alterations in mCRPC patients who may benefit from treatment with LYNPARZA® (olaparib). On July 15, 2021, the FoundationOne® Liquid CDx test was approved as a companion diagnostic for *MET* exon 14 skipping alterations in patients with NSCLC who may benefit from treatment with TABRECTA® (capmatinib). On September 21, 2022, the companion diagnostic indication for FoundationOne Liquid CDx to identify patients with ovarian cancer harboring *BRCA1* or *BRCA2* alterations for treatment with RUBRACA® (rucaparib) was removed. On December 19, 2022, FoundationOne Liquid CDx was approved for a companion diagnostic group labeling claim to identify patients with advanced and metastatic NSCLC harboring *EGFR* activating mutations (Exon 19 deletions and L858R substitution mutation) for treatment with any one of the FDA-approved *EGFR* tyrosine kinase inhibitors.

The SSEDs to support the previously approved indications are available on the CDRH website. The current supplement was submitted to expand the indication for the FoundationOne Liquid CDx test as a companion diagnostic for the indication listed in the table below.

New Indication Being Sought in this PMA supplement submission.

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*ROS1* fusions	ROZLYTREK® (entrectinib)
Solid Tumors	*NTRK1/2/3* fusions	ROZLYTREK® (entrectinib)

II. INDICATIONS FOR USE

FoundationOne Liquid CDx is a qualitative next generation sequencing based in vitro diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions, insertions and deletions (indels) in 311 genes, rearrangements in eight (8) genes, and copy number alterations in three (3) genes. FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne Liquid CDx cfDNA blood collection tubes included in the FoundationOne Liquid CDx Blood Sample Collection Kit. The test is intended to be used 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.

Table 1: Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*ALK* Rearrangements	ALECENSA® (alectinib)
	EGFR Exon 19 deletions and *EGFR* Exon 21 L858R alteration	*EGFR* tyrosine kinase inhibitors approved by FDA*
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	TABRECTA® (capmatinib)
	ROS1 fusions**	ROZLYTREK® (entrectinib)
Prostate cancer	*BRCA1*, *BRCA2*, and *ATM* alterations	LYNPARZA® (olaparib)
	BRCA1, *BRCA2* alterations	RUBRACA® (rucaparib)
Breast Cancer	*PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y	PIQRAY® (alpelisib)

Tumor Type	Biomarker(s) Detected	Therapy
Solid Tumors	*NTRK1/2/3* fusions**	ROZLYTREK® (entrectinib)

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

Additionally, FoundationOne Liquid CDx 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.

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients who are negative for the mutations listed in Table 1 (see **Note for *NTRK1/2/3* and *ROS1* fusions) should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA- approved tumor tissue test, if feasible.

**Note: when considering eligibility for ROZLYTREK® based on the detection of *NTRK1/2/3* and *ROS1* fusions, testing using plasma specimens is only appropriate for patients for whom tumor tissue is not available for testing.

Genomic findings other than those listed in Table 1 of the intended use statement are not prescriptive or conclusive for labeled use of any specific therapeutic product.

FoundationOne Liquid CDx is a single-site assay performed at Foundation Medicine, Inc. in Cambridge, MA.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
- The test is not intended to replace germline testing or to provide information about cancer predisposition.
- Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved tumor tissue test, if possible.

V. DEVICE DESCRIPTION

The FoundationOne Liquid CDx (F1LCDx) assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes. All coding exons of 309 genes are targeted; select intronic or non-coding regions are targeted in three genes (refer to Table 2 for the complete list of genes reported by F1LCDx).

Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq 6000 platform. Sequence data are processed using a custom analysis pipeline designed to detect genomic alterations, including base substitutions and indels in 311 genes, copy number variants in three genes, and genomic rearrangements in eight genes. A subset of targeted regions in 75 genes is baited for increased sensitivity.

Table 2: Genomic Regions in which Variants are Reported by FoundationOne® Liquid[1]

ABL1 [Exons 4-9]	*ACVR1B*	***AKT1 [Exon 3]***	*AKT2*	*AKT3*	***ALK [Exons 20-29, Introns 18,19]***	*ALOX12B*	*AMER1 (FAM123B)*	***APC***	***AR***
ARAF [Exons 4, 5, 7, 11, 13, 15, 16]	*ARFRP1*	*ARID1A*	*ASXL1*	***ATM***	***ATR***	*ATRX*	*AURKA*	*AURKB*	*AXIN1*
AXL	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BCR* [Introns 8, 13, 14]*
***BRAF [Exons 11-18**, Introns 7-10]*	***BRCA1** [Introns 2, 7, 8, 12, 16, 19, 20]*	***BRCA2** [Intron 2]*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*	***BTK [Exons 2, 15]***	*C11orf30 (EMSY)*	*C17orf39 (GID4)*
CALR	*CARD11*	*CASP8*	*CBFB*	*CBL*	***CCND1***	*CCND2*	*CCND3*	*CCNE1*	*CD22*
CD70	*CD74* [Introns 6-8]*	*CD79A*	*CD79B*	***CD274 (PD-L1)***	*CDC73*	***CDH1***	***CDK12***	***CDK4***	***CDK6***
CDK8	*CDKN1A*	*CDKN1B*	***CDKN2A***	*CDKN2B*	*CDKN2C*	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*
CREBBP	***CRKL***	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*	***CTNNB1 [Exon 3]***	*CUL3*	*CUL4A*	*CXCR4*
CYP17A1	*DAXX*	*DDR1*	***DDR2 [Exons 5, 17, 18]***	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	***EGFR [Introns 7**, 15, 24-27]***	*EP300*
EPHA3	*EPHB1*	*EPHB4*	***ERBB2***	***ERBB3 [Exons 3, 6,7,8, 10, 12,20, 21, 23,24, 25]***	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1 [Exons 4-8]***
ETV4 [Intron 8]*	*ETV5* [Introns 6,7]*	***ETV6* [Introns 5,6]***	*EWSR1* [Introns 7-13]*	***EZH2 [Exons 4,16, 17, 18]***	*EZR* [Introns 9 - 11]*	*FAM46C*	*FANCA*	*FANCC*	*FANCG*
FANCL	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*	*FGF4*
FGF6	***FGFR1** [Introns 1,5,*	***FGFR2** [Intron 1,*	***FGFR3 [Exons 7, 9***	*FGFR4*	*FH*	*FLCN*	*FLT1*	***FLT3 [Exons 14, 15,***	***FOXL2***

	Intron17]	***Intron 17]***	***(alternative designation exon 10),14, 18, Intron 17]***					***20]***	
FUBP1	*GABRA6*	*GATA3*	*GATA4*	*GATA6*	***GNA11 [Exons 4, 5]***	*GNA13*	***GNAQ [Exons 4, 5]***	***GNAS [Exons 1, 8]***	*GRM3*
GSK3B	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	***HRAS [Exons 2, 3]***	*HSD3B1*	*ID3*	***IDH1 [Exon 4]***	***IDH2 [Exon 4]***
IGF1R	*IKBKE*	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	***JAK2 [Exon 14]***	***JAK3 [Exons 5, 11, 12, 13, 15, 16]***
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	***KIT [Exons 8, 9, 11, 12, 13, 17,*** *Intron 16]*	*KLHL6*	*KMT2A (MLL) [Introns 6, 8-11, Intron 7]*
KMT2D (MLL2)	***KRAS***	*LTK*	*LYN*	*MAF*	***MAP2K1 (MEK1) [Exons 2, 3]***	***MAP2K2 (MEK2) [Exons 2-4, 6, 7]***	*MAP2K4*	*MAP3K1*	*MAP3K13*
MAPK1	*MCL1*	***MDM2***	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***	*MITF*
MKNK1	*MLH1*	***MPL [Exon 10]***	*MRE11A*	*MSH2 [Intron 5]*	*MSH3*	*MSH6*	*MST1R*	*MTAP*	***MTOR [Exons 19, 30, 39 40, 43-45, 47, 48, 53, 56]***
MUTYH	*MYB* [Intron 14]*	***MYC [Intron 1]***	*MYCL (MYCL1)*	***MYCN***	***MYD88 [Exon 4]***	*NBN*	***NF1***	*NF2*	*NFE2L2*
NFKBIA	*NKX2-1 (TTF-1)*	*NOTCH1*	*NOTCH2 [Intron 26]*	*NOTCH3*	***NPM1 [Exons 4-6, 8, 10]***	***NRAS [Exons 2, 3]***	*NSD3 (WHSC1L1)*	*NT5C2*	***NTRK1 [Exons 14,15, Introns 8-11]***
NTRK2 [Intron 12]	***NTRK3 [Exons 16, 17]***	*NUTM1* [Intron 1]*	*P2RY8*	***PALB2***	*PARK2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*
PBRM1	*PDCD1 (PD-1)*	***PDCD1L G2 (PD-L2)***	***PDGFRA [Exons 12, 18, Introns 7, 9, 11]***	***PDGFRB [Exons 12-21, 23]***	*PDK1*	*PIK3C2B*	*PIK3C2G*	***PIK3CA [Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)]***	*PIK3CB*
PIK3R1	*PIM1*	*PMS2*	*POLD1*	*POLE*	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*
PRKCI	*PTCH1*	***PTEN***	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*
RAD51C	*RAD51D*	*RAD52*	*RAD54L*	***RAF1 [Exons 3, 4, 6, 7, 10, 14, 15, 17, Introns 4-8]***	*RARA [Intron 2]*	***RB1***	*RBM10*	*REL*	***RET [****Introns 7, 8,* ***Exons 11, 13-16, Introns 9-11]***
RICTOR	*RNF43*	***ROS1 [Exons 31, 36-38, 40, Introns 31-35]***	*RPTOR*	*RSPO2* [Intron 1]*	*SDC4* [Intron 2]*	*SDHA*	*SDHB*	*SDHC*	*SDHD*

SETD2	*SF3B1*	*SGK1*	*SLC34A2* [Intron 4]*	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	***SMO***	*SNCAIP*
SOCS1	*SOX2*	*SOX9*	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	***STK11 (LKB1)***	*SUFU*
SYK	*TBX3*	*TEK*	*TERC* {ncRNA}*	***TERT* {Promoter}***	*TET2*	*TGFBR2*	*TIPARP*	*TMPRSS2* [Introns 1-3]*	*TNFAIP3*
TNFRSF14	***TP53***	*TSC1*	*TSC2*	*TYRO3*	*U2AF1*	***VEGFA***	*VHL*	*WHSC1*	*WTI*
XPO1	*XRCC2*	*ZNF217*	*ZNF703*						

[1] As part of its FDA-approved intended use, the F1LCDx assay interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *). Select genes and select exons (indicated in bold) are captured with increased sensitivity.

The reporting of rearrangements and copy number alterations are restricted to those genes included in Table 3, below.

Table 3: Genes for which copy number alterations and rearrangements are reported for tumor profiling by F1LCDx

Alteration Type	Genes
Copy Number Alterations	*BRCA1, BRCA2, ERBB2*
Rearrangements	*ALK, BRCA1, BRCA2, NTRK1, NTRK2, NTRK3*

The test report includes variants reported in the following levels:

Level 1: Companion Diagnostics (CDx)
Clinical evidence should be presented from a prospectively designed clinical trial. Results can also be presented from a retrospective clinical bridging study demonstrating that the clinical endpoints are preserved using plasma samples in trials where enrollment was based on tissue test results. For follow-on markers, a clinical concordance study demonstrating non-inferiority to the original FDA-approved cfDNA-based companion diagnostic device (refer to Li, Meijuan. Statistical Methods for Clinical Validation of Follow-On Companion Diagnostic Devices via an External Concordance Study. Statistics in Biopharmaceutical Research. 8: 35-363, 2016) is required. In addition to the clinical validation, analytical validation for each specific Level 1 CDx biomarker should be presented.

Level 2: cfDNA Biomarkers with Strong Evidence of Clinical Significance in cfDNA
For a Level 2 claim of cfDNA biomarkers with strong evidence of clinical significance, clinical validation needs to be from evidence presented with FDA-approved liquid biopsy companion diagnostic biomarkers for the specific tumor type at the biomarker or variant level. Such claims should also be supported by analytical performance for each biomarker from at least LoD, precision/ reproducibility, and accuracy studies.

Level 3A: Biomarkers with Evidence of Clinical Significance in Tissue Supported by Strong Analytical Validation Using cfDNA and Concordance Between cfDNA and Tissue.

Clinical evidence can be provided from tissue-based companion diagnostics. This should also be supported by analytical validation (LoD, precision, analytical accuracy, and concordance study to a tissue-based test) for the specific tumor type at the biomarker or variant level, using a representative approach for SNVs and indels. Evidence evaluating concordance between cfDNA- and tissue-samples for FDA-approved tissue markers should be demonstrated using an FDA-approved tissue test or a validated tissue test.

Level 3B: Biomarkers with Evidence of Clinical Significance in Tissue Supported by Analytical Validation Using cfDNA
Clinical evidence can be provided from tissue-based companion diagnostics, with analytical validation supported by a representative approach for SNVs and indels from key analytical studies (such as LoD, accuracy, and precision).

Level 4: Other Biomarkers with Potential Clinical Significance
Biomarkers not categorized into Levels 1, 2, or 3 can be included under Level 4 for informational purposes or to be used to direct patients toward clinical trials for which they may be eligible. Such claims can be supported by clinical rationale for inclusion in the panel. Such rationale could also include peer-reviewed publications for genes/ variants in tissue, variant information from well curated public databases, or in vitro pre-clinical models. Analytical validation should be supported by a representative approach for SNVs and indels from key analytical studies (such as LoD, accuracy, and precision).

FoundationOne® Liquid CDx cfDNA Blood Specimen Collection Kit Contents
The test includes a blood specimen collection kit, which is sent to ordering laboratories. The shipping kit contains the following components:

- Specimen preparation and shipping instructions
- Two FoundationOne® Liquid CDx cfDNA Blood Collection Tubes (8.5 mL nominal fill volume per tube)
- Return shipping label

Instruments
The F1LCDx assay is intended to be performed with the serial number-controlled instruments indicated in Table 4, below. All instruments are qualified by Foundation Medicine, Inc. (Foundation Medicine or FMI) under Foundation Medicine's Quality System.

Table 4: Instruments for use with the F1LCDx assay

Instrument
Illumina NovaSeq 6000
Beckman Biomek NXP Span-8 Liquid Handler
Thermo Scientific Kingfisher Flex DW 96
Bravo Benchbot
Hamilton STARlet STAR Liquid Handling Workstation

Test Process

All assay reagents including blood collection tubes included in the F1LCDx assay process are qualified by Foundation Medicine and are compliant with the medical device Quality System Regulation (QSR).

A. Specimen Collection and Preparation

Whole blood specimens are collected in F1LCDx cfDNA Blood Collection Tubes (BCT) provided as a component of the F1LCDx specimen collection kit. Prior to cfDNA isolation, the plasma is separated from whole blood by centrifugation, which separates the plasma from the buffy coat (white blood cells) and red blood cells. The plasma layer is removed from the buffy coat to avoid contamination of cellular DNA into the plasma sample. A residual volume of plasma remains in the tube to avoid disturbing the buffy coat. A second spin of the separated plasma at high-speed further pellets cell debris and protein.

B. DNA Extraction

Following the separation of plasma from whole blood, cfDNA is isolated from plasma using the KingFisher™ Flex Magnetic Particle Processor, which uses an efficient and automated method to purify cfDNA. The KingFisher™ Instrument uses magnetic rods to move nucleic acid through purification phases of binding, washing, and elution to yield high purity cfDNA. After isolating cfDNA, the Agilent 4200 TapeStation is used to quantify cfDNA.

C. Library Construction

Library Construction (LC) begins with the normalization of cfDNA. The samples are purified, using AMPure XP Beads (Agencourt). Solid-phase reversible immobilization (SPRI) purification is used subsequent to library construction with the NEBNext kits (NEB), including mixes for end repair with blunt-end and 5'- phosphorylate the cfDNA fragments using T4 Polynucleotide Kinase and T4 DNA Polymerase. This step prepares the 3'-end for dA-addition while also preparing the 5'-end of the DNA fragment for ligation. Second, dA-addition will incorporate a single dAMP to the 3'-end of the End-Repaired material. After dA-addition, a universal Y-adaptor is ligated onto each end of the DNA fragment using a DNA ligase. These steps are performed in 96-well plates (Eppendorf) on a Bravo Benchbot (Agilent) using the "with-bead" protocol to maximize reproducibility and library yield. Indexed (Foundation Medicine customized six base pair barcodes) sequencing libraries are PCR amplified with a high-fidelity DNA polymerase (HiFi™, Kapa) for ten cycles, SPRI purified and quantified by PicoGreen fluorescence assay (Invitrogen). Process matched control (PMC) is prepared and added to the plate with other cfDNA samples at the beginning of LC.

D. Hybrid Capture

Hybrid Capture begins with the normalization of each library from 500 ng to 2000 ng. Solution hybridization is performed using a >50-fold molar excess of

a pool of individually synthesized 5'-biotinylated DNA 120 base pair oligonucleotides (Integrated DNA Technology) for baits. The baits target regions from 324 cancer- related genes including all coding exons of 309 genes and only select introns or non- coding regions in 15 genes. Baits were designed by appointing 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; single nucleotide polymorphism (SNP) targets were allocated one bait each. Intronic baits were filtered for repetitive elements as defined by the University of California at Santa Cruz (UCSC) Genome Repeat Masker track. Hybrid selection of targets demonstrating reproducibly low coverage was boosted by increasing the number of baits for these targets.

Upon completion of the pre-capture normalization, blocking DNA (adaptor block, Cot, Salmon Sperm DNA) is added to the sequencing library and the mixture is lyophilized in a 96-well plate. The library is then re-suspended in nuclease-free water, heat denatured at 95°C for 5 minutes, temperature ramps from 95°C to 68°C to anneal blocking DNA, and then the samples are incubated at 68°C for a minimum of 5 minutes before the addition of the bait set reagent. After a 20-24-hour incubation, the library-bait duplexes are captured on paramagnetic MyOne™ streptavidin beads (Invitrogen) and off-target library is removed by washing one time with Saline Sodium Citrate (SSC) at 25°C and four times with SSC at 55°C. The PCR master mix is added to directly amplify the captured library from the washed beads. After amplification, the samples are SPRI purified and quantified by PicoGreen.

E. Sequencing

Sequencing on the Illumina NovaSeq 6000 platform employs on-board cluster generation (OBCG) using patterned flow cell (FC) technology to generate monoclonal clusters via ExAmp from a single DNA template. The clusters are then sequenced using sequencing by synthesis (SBS) chemistry. The NovaSeq system is capable of sequencing up to two flow cells at a time. During OBCG, a single DNA template is introduced into each of the primer substrate layered nanowells of the flow cell, where the template is immediately and rapidly amplified by ExAmp. This rapid amplification prevents other DNA templates from binding, ensuring a monoclonal cluster is formed in each nanowell. The procedure allows for fixed size and spacing of the clusters which results in improved and more accurate resolution.

A growing nucleotide chain is created on the flow cell by incorporating fluorescently labeled, 3'-blocked dNTPs. After excitation by a laser, the camera captures the emission color of the incorporated, fluorescently labeled nucleotide. The 3'-block is then removed, reverting the nucleotide to its natural form, which allows the polymerase to add another base to the growing double strand of DNA. With each successive SBS cycle, a new fluorescently labeled 3'- blocked dNTP is added. SBS allows for millions of discrete clusters of clonal copies of DNA to be sequenced in parallel.

F. Sequence Analysis

Sequence data are analyzed using mainly proprietary software developed by Foundation Medicine. External tools used include: 1) BWA (Burrows-Wheeler Aligner) v0.7.17, for aligning sequence reads to the genomic reference, 2) SAMtools v1.6 for utility operations, 3) Picard tools v1.56 for metrics calculations, and 4) Biopython for the pairwise2 sequence alignment module.

Reads from each Illumina flow cell are demultiplexed (sorted into sets of reads deriving from distinct samples), and their fragment barcodes (FBCs) are extracted and encoded into the read names. For each sample, read pairs with matching, valid FBCs are aligned and processed together to: 1) identify clusters of reads originating from the same original fragment; 2) merge overlapping read pairs into single reads, where possible; and 3) generate consensus reads representing all information in the set of reads for each cluster, encoding positions with mismatches (errors) with base quality 20. The consensus reads are then aligned to the reference genome to generate the 'consensus' BAM.

For the detection of short variants (e.g., substitutions and small indels) in each target region of interest, a de novo assembly is performed. This is done using proprietary software to generate a de Bruijn graph including all k-mers in reads mapping to a particular locus. The graph is parsed to identify paths that originate and terminate in reference nodes from the locus. Increased k-mer sizes may be used to account for ambiguities, cycles, and other problematic regions within the graph. The result of the graph traversal is a set of candidate variants. For each variant, there is a set of k-mers supporting the variant and a set of k-mers that would support the reference or another variant at the location.

Each candidate variant is then scanned against reads in the locus to identify which reads support either the candidate variant or a different variant or reference at the location. The cluster membership of the supporting reads is then assessed to determine which clusters show unambiguous support for the variant and which have conflicting assignments, indicating that the variant may have arisen as an error in sequencing or library preparation. The final variant calls are made based on a model that takes into account the coverage at the location, the number of supporting read clusters and their redundancy level, and the number of error-containing clusters.

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 Foundation Medicine as a professional service prior to approval and release by the laboratory director or designee.

H. Internal Process Controls

Process Control
Each assay run includes a control sample run in duplicate. The control sample contains a pool of eleven 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.

Sensitivity Control
The HapMap control pool used as the positive control is prepared to contain variants at 0.1%, 10% mutant allele frequency (MAF) which must be detected by the analysis pipeline to ensure expected sensitivity for each run.

Negative Control
Samples are barcoded molecularly at the library construction (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.

I. CDx Classification Criteria

1. *BRCA1 and BRCA2 alterations to identify patients eligible for rucaparib in prostate cancer:*
   The CDx classification criteria and the list of *BRCA1/BRCA2* missense mutations for rucaparib, based on the trial prespecifications are described in Table 5 and Table 6; however, not all the missense mutations listed below were observed in the TRITON2, and PROfound clinical studies.

Table 5: Classification Criteria for Deleterious Tumor *BRCA* Variants

Qualification Criteria	Sequence Classification	Methodology
A *BRCA1* or *BRCA2* alteration that includes any of the sequence classifications	Protein truncating mutations	Sequence analysis identifies premature stop codons anywhere in the gene coding region, except, 3' of and including *BRCA2* K3326*
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions -/+ 2bp of exon starts/ends
	Homozygous deletions	Sequence analysis identifies deletions in both gene alleles of ≥1 exon in size
	Large protein truncating rearrangements	Sequence analysis identifies protein truncating rearrangements
	Deleterious missense mutations	Curated list

Table 6: Deleterious *BRCA* Missense Alterations in rucaparib

***BRCA1* Alterations (Protein Change)**					***BRCA2* Alterations (Protein Change)**		
M1V	C44Y	R71T	R1699W	G1770V	M1V	R2336P	T2722R
M1T	C44F	R71M	R1699Q	M1775K	M1T	R2336L	D2723H
M1R	C47S	S770L	G1706R	M1775R	M1R	R2336H	D2723G
M1I	C47Y	R1495T	G1706E	C1787S	M1I	T2412I	G2724W
M18T	C47F	R1495M	A1708E	G1788V	D23N	R2602T	G2748D
L22S	C61S	R1495K	S1715R	P1812A	D23Y	W2626C	A2911E
I26N	C61G	E1559K	S1722F	A1823T	S142N	I2627F	E3002K
T37K	C61Y	E1559Q	V1736A	V1833M	S142I	R2659T	R3052W
C39R	C64R	T1685A	G1738R	W1837R	V159M	R2659K	D3095G
C39G	C64G	T1685I	G1738E	V1838E	V211I	E2663V	D3095E
C39Y	C64Y	D1692N	K1759N		V211L	S2670L	N3124I
C39W	C64W	M1689R	L1764P		Y600C	I2675V	N3187K
H41R	R71G	D1692H	I1766N		K1530N	T2722K	
C44S	R71K	D1692Y	I1766S				

2. *ATM, BRCA1 and BRCA2 alterations to identify patients eligible for olaparib in mCRPC:*

Table 7: Rules Applied to the Aforementioned Genes:

Qualification Criteria	Sequence Classification	Methodology	Comments
A gene alteration that includes any of the sequence classifications	Protein truncating mutations	Sequence analysis identifies premature stop codons anywhere in the gene coding region, except 3' of and including *BRCA2* K3326*	Does not include VUS. Includes mutations on the canonical transcript only for genes *ATM*, *BRCA1,* and *BRCA2.*
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions -/+ 2bp of exon starts/ends	Does not include VUS. Includes indels that extend through ±2bp from the intron/exon junction. Includes mutations on the canonical transcript only for genes *ATM*, *BRCA1,* and *BRCA2.*
	Homozygous deletions	Sequence analysis identifies deletions in both gene alleles of ≥1 exon in size	Does not include VUS Only reported for *BRCA1* and *BRCA2*. Not reported for *ATM*.
	Large protein truncating rearrangements	Sequence analysis identifies protein truncating rearrangements	Does not include VUS

Qualification Criteria	Sequence Classification	Methodology	Comments
	Deleterious missense mutations	Curated list	Protein effects from list of missense mutations on the canonical transcript only for genes *ATM*, *BRCA1,* and *BRCA2.*

Alterations reported are limited to those within the alteration-calling capabilities of FMI as of March 2, 2020. ATM missense mutations were identified from the ClinVar database. Should the calling capabilities expand, additional alterations that meet the above criteria may also be reported, per FDA approval.

Table 8. List of Deleterious Missense Mutations by Protein Effect, Implemented on the Respective Canonical Transcript.

BRCA1		***BRCA2***		***ATM***	
Protein Effect (PE)	**FMI Annotated PE**	**Protein Effect (PE)**	**FMI Annotated PE**	**Protein Effect (PE)**	**FMI Annotated PE**
MIV	MIV	MIR	MIR	MIT	MIT
MlI	MlI	MlI	MlI	R2032K	R2032K
C6IG	C6IG	VI59M	VI59M	R2227C	R2227C
C64Y	C64Y	V211L	V211L	R2547 S2549del	R2547 S2549del
R7IG	R7IG	V211I	V211I	G2765S	G2765S
R7IK	R7IK	R2336P	R2336P	R2832C	R2832C
RI495M	RI495M	R2336H	R2336H	S2855 V2856delinsR1	S2855 V2856delinsRl S2855 V2856>Rl
EI559K	EI559K			R3008C	R3008C
DI692N	DI692N			R3008H	R3008H
DI692H	DI692H			[VUS from Jan 2016 HRR* List to be Excluded]	
RI699W	RI699W			V2424G	V2424G
AI708E	AI708E			[Excluded from Jan 2016 HRR List]	
G1788V	GI788V			K750K	splice site 2250G>A

HRR = Homologous Recombination Repair genes

Intronic Variants

Gene	Chr	Position	Ref	Alt	dbSNP	FMI Protein Effect
ATM	*chr11*	*108128198*	*T*	*G*	*rs730881346*	**[Variant Not Called by FMI]**
ATM	*chr11*	*108214102*	AGTGA	A	rs730881295	splice site 8418+5_8418+8delGTGA or splice site 8418+1_8418+4delGTGA

3. *CDx classification criteria for EGFR alterations:*
   - Base substitutions resulting in *EGFR* L858R
   - In-frame deletions occurring within *EGFR* Exon 19

4. *ALK rearrangements to identify patients eligible for treatment with ALECENSA® (alectinib):*
   CDx positivity for an *ALK* rearrangement is based on the following variant classification criteria:
   - The *ALK* rearrangement must have pathogenic driver status (FMI driver status of "known" or "likely")
   - AND the disease type must be NSCLC
   - AND one of the following two conditions must hold:
     1. The partner gene is *EML4*, or
     2. The *ALK* breakpoint occurs within *ALK* intron 19

5. *SNVs and indels that lead to MET exon 14 skipping to identify patients eligible for treatment with TABRECTA® (capmatinib):*
   A 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.

6. *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* may be on either the 5' or the 3' end of the detected fusion).

   In this regard out-of-strand events are considered as non-fusion rearrangements and are classified as CDx biomarker negative. Intragenic fusions in which genomic rearrangement events are wholly internal to the *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.

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

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

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using cfDNA isolated from plasma samples, as listed in Table 1 of the F1LCDx intended use statement. The approved CDx tests are listed in Table 9, below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/media/119249/download. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

Table 9: FDA-approved companion diagnostic (CDx) alternatives

Biomarker(s) Detected	Device	Company	Technology	Therapy	Indication
EGFR Exon 19 deletions and L858R Substitution Mutation	**cobas** *EGFR* Mutation Test v2	Roche Molecular Systems, Inc.	Polymerase Chain Reaction (PCR)	TARCEVA® (erlotinib), TAGRISSO® (osimertinib), and IRESSA® (gefitinib)	NSCLC
	Guardant360 CDx	Guardant Health, Inc.	NGS		
				TAGRISSO® (osimertinib)	
PIK3CA: C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y	*therascreen PIK3CA* RGQ PCR test	QIAGEN, Inc.	PCR	PIQRAY® (alpelisib)	Breast Cancer

There are no FDA-approved CDx alternatives for the detection of genomic alterations of *BRCA1* or *BRCA2* for the identification of patients with prostate cancer eligible for treatment with RUBRACA® (rucaparib).

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of genomic alterations of *BRCA1*, *BRCA2*, and *ATM* for the identification of patients with prostate cancer eligible for treatment with LYNPARZA® (olaparib).

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of genomic alterations of SNVs and indels that lead to *MET* exon 14 skipping to identify patients with NSCLC eligible for treatment with TABRECTA® (capmatinib).

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of rearrangements that lead to *NTRK1*, *NTRK2*, *NTRK3* fusions to identify patients with solid tumors eligible for treatment with ROZLYTREK® (entrectinib).

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of rearrangements that lead to *ROS1* fusions to identify patients with NSCLC eligible for treatment with ROZLYTREK® (entrectinib).

VII. MARKETING HISTORY

Foundation Medicine designed and developed F1LCDx based on previous versions of the assay, including the FoundationACT (FACT) and FoundationOne® Liquid laboratory developed test (LDT), a revised version of FACT. The first commercial sample was tested in 2016. The FACT and FoundationOne® Liquid LDT have been used to detect the presence of genomic alterations in blood and plasma specimens. Neither the FACT nor FoundationOne® Liquid LDT were FDA-cleared or -approved.

The F1LCDx assay was FDA-approved on August 7, 2020, and subsequently commercialized in the USA. On September 21, 2022, the companion diagnostic indication for F1LCDx to identify patients with ovarian cancer harboring *BRCA1* or *BRCA2* alterations for treatment with RUBRACA® (rucaparib) was removed.

The F1LCDx assay has been marketed in the United States, the European Union, and in several other foreign countries since August 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 F1LCDx assay results, and subsequently, inappropriate patient management decisions. Patients with false positive CDx biomarker results may undergo treatment with one of the therapies listed in 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 targeted 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 approved drug product labels.

For the specific adverse events that occurred in the clinical study, please see the FDA approved package inserts for ROZLYTREK® (entrectinib) which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The evidence in support of the analytical performance of F1LCDx in detecting *NTRK1/2/3* and *ROS1* fusions is presented in this section. Analytical accuracy/concordance and precision near the limit of detection (LoD) studies were conducted to support the indication for *NTRK1/2/3* and *ROS1* fusions using clinical samples. The analytical validation studies did not include evaluation of *NTRK2* fusions due to their low prevalence. Therefore, additional post-market studies are planned to demonstrate the detection of *NTRK2* fusions by F1LCDx (See Section XIII). A limitation is included in the device labeling noting that *NTRK2* fusions per the F1LCDx biomarker calling rules were not represented in the analytical validation studies (refer to Section XII.C. below).

The primer set used for library construction as part of the F1LCDx assay process was changed since the analytical validation studies, described below, were conducted. The change to the primer reagent design and associated lowering of the minimum cfDNA input level required for library construction to 20ng, was supported by comparability, precision, limit of blank (LoB), cfDNA input guard banding, and reagent stability studies. The comparability study between the prior and updated primer sets assessed a total of three (3) NSCLC clinical samples harboring *ROS1* fusions and observed a PPA of 100%. In addition, the cfDNA input guard banding study assessed a contrived sample harboring an *NTRK1* fusion at 10-80ng cfDNA input and observed 100% agreement across replicates. The post-market studies described in Section XIII, below, will be conducted using the most current version of F1LCDx to provide additional data to support the updated primer set performance.

For F1LCDx platform-level validation (P190032), due to the lack of sufficient volume of clinical specimens, analytical performance characteristics were established for some of the studies using contrived samples, which consisted of enzymatically sheared cell line DNA spiked into human plasma and diluted with cfDNA isolated from healthy donor plasma. A contrived sample functional characterization (CSFC) study was conducted to demonstrate comparable performance of sheared cell line DNA samples as compared to cfDNA isolated from plasma specimens obtained from cancer positive patient specimens. Clinical specimens were used to assess analytical accuracy, precision, and confirmation of the estimated LoD, and to evaluate sample stability. For information regarding the platform-level validation, refer to Section IX.A. in Summary of Safety and Effectiveness Data P190032.

1. Analytical Accuracy/Concordance

a. Comparison to an Orthogonal Method for *NTRK1/2/3* fusions

An analytical accuracy/concordance study was performed to demonstrate the concordance between F1LCDx and an externally validated NGS assay (evNGS) for the detection of *NTRK1/2/3* fusions. For this study, seven (7) residual cfDNA samples were selected from patients enrolled in the STARTRK-2 trial used to support the effectiveness of the device (refer to Section X below), seven (7) residual cfDNA clinical samples were

externally sourced, and 102 residual cfDNA samples were sourced from FMI's clinical archives.

Analytical concordance of F1LCDx for detecting *NTRK1/2/3* fusions was determined with 116 samples tested by the F1LCDx assay. However, one (1) *NTRK1/2/3* fusion negative sample was not tested by the evNGS assay due to limitations in processing batch size and two (2) *NTRK1/2/3* fusion positive samples were not tested by the evNGS assay due to the breakpoints falling in regions that the evNGS assay does not bait for. Of the 113 samples tested by both assays, one (1) *NTRK1/2/3* fusion negative sample had a F1LCDx post-sequencing QC failure, while 10 *NTRK1/2/3* fusion negative samples had an evNGS post-sequencing QC failure. In total there were 102 samples with valid results by both F1LCDx and the evNGS assay.

Measures of analytical concordance for the 102 samples that passed QC with both assays were determined. Since specimens were selected based on F1LCDx and confirmed by the evNGS agreement, positive predictive value (PPV) and negative predictive value (NPV) are estimated conditional on F1LCDx. PPV was estimated as 40% (4/10) with two-sided 95% CI (16.8%, 68.7%), and NPV as 100% (92/92) with two-sided 95% CI (95.99%, 100.00%), as shown in Table 10, below. For informational purposes, unadjusted positive percent agreement (PPA) and negative percent agreement (NPA) are also displayed.

Table 10. Concordance summary for *NTRK1/2/3* fusions by F1LCDx and the evNGS

		evNGS			
		***NTRK1/2/3* fusion positive**	***NTRK1/2/3* fusion negative**	**Total**	**PPV/NPV (95% CI[3])**
F1LCDx	***NTRK1/2/3* fusion positive[1]**	4	6[2]	10	PPV: 40.0% (16.8%, 68.7%)
	***NTRK1/2/3* fusion negative**	0	92	92	NPV: 100% (95.99%, 100%)
	Total	4	98	102	
	PPA/NPA (Unadjusted) (95% CI[3])	PPA: 100% (51.01%, 100%)	NPA: 93.9% (87.3%, 97.2%)		

[1]No *NTRK2* fusion positive samples were evaluated in this study
[2]These six samples were discordant due to the fusion breakpoints falling into regions that the evNGS did not bait for.
[3]Calculated with Wilson 2-sided 95% CI

The six (6) samples that were *NTRK1/2/3* fusion positive by F1LCDx and *NTRK1/2/3* fusion negative by the evNGS were discordant due to the fusion breakpoints falling in regions that the evNGS assay does not bait for. Specifically, the evNGS assay did not claim to generate coverage in certain regions of interest (e.g., intron 8 of *NTRK1* and intron 5 of *ETV6*), and thus were negative by the evNGS comparator assay.

Given the very limited number of *NTRK1/3,* and no *NTRK2*, fusion positive samples that were evaluated in the analytical accuracy study, a limitation is included in the device labeling to indicate that a study evaluating the concordance to a second method demonstrated that the agreement between F1LCDx positive results and a comparator method for *NTRK1/3* was ≤ 50% (i.e., whether these are potential F1LCDx false positives or false negatives by the comparator is unknown) (refer to Section XII.C. below).

b. Comparison to an Orthogonal Method for *ROS1* fusions

The analytical concordance of *ROS1* fusions was assessed using clinical samples in the F1LCDx platform analytical concordance study (see Summary of Safety and Effectiveness Data for P190032), which consisted of 278 samples across solid tumors, including two (2) clinical samples with *ROS1* fusions. Among the two (2) clinical samples with *ROS1* fusions, one (1) sample was discordant between F1LCDx and the evNGS assay. This discordant sample had low VAF, below the LoD of both assays. Since specimens were selected based on F1LCDx and confirmed by the evNGS agreement, positive predictive value (PPV) and negative predictive value (NPV) are estimated conditional on F1LCDx. PPV was estimated as 50.0% (1/2), with two-sided 95% CI (9.5%, 90.6%), and NPV as 100% (276/276) with two-sided 95% CI (98.6%, 100.0%), as shown in Table 11, below. For informational purposes, unadjusted positive percent agreement (PPA) and negative percent agreement (NPA) are also displayed.

Table 11. Concordance summary for *ROS1* fusions by F1LCDx and the evNGS

		evNGS			
		***ROS1* fusion positive**	***ROS1* fusion negative**	**Total**	**PPV/NPV (95% CI[1])**
F1LCDx	***ROS1* fusion positive**	1	1	2	PPV: 50% (9.5%, 90.6%)
	***ROS1* fusion negative**	0	276	276	NPV: 100% (98.6%, 100%)
	Total	1	277	278	
	PPA/NPA (Unadjusted) (95% CI[1])	PPA: 100% (20.7%, 100%)	NPA: 99.6% (98.0%, 99.9%)		

[1]Calculated with Wilson 2-sided 95% CI

Given the very limited number of *ROS1* fusion positive samples that were evaluated in the analytical accuracy study, a post-market study is planned to demonstrate the analytical accuracy for the detection of *ROS1* fusions in samples from patients with NSCLC (See Section XIII). Additionally, a limitation is included in the device labeling to indicate that a study evaluating the concordance to a second method demonstrated that the agreement between F1LCDx positive results and a comparator method for

ROS1 was ≤ 50% (i.e., whether these are potential F1LCDx false positives or false negatives by the comparator is unknown) (refer to Section XII.C. below).

2. **Analytical Sensitivity**

   a. **Limit of Blank (LoB)**

   See Summary of Safety and Effectiveness Data for P190032.

   b. **Limit of Detection (LoD) for *NTRK1/2/3* Fusions**

   The LoD of *NTRK1* and *NTRK3* fusions in two (2) contrived samples was evaluated as part of the LoD study for PMA P190032 (see Summary of Safety and Effectiveness Data for P190032). The *TPM3-NTRK1* fusion was determined to have an estimated LoD of 0.44% using a probit analysis. The *ETV6-NTRK3* fusion was determined to have an estimated LoD of 0.27% VAF using a probit analysis.

   c. **Limit of Detection (LoD) for *ROS1* Fusions**

   The LoD of *ROS1* fusions in two contrived samples was evaluated as part of the LoD study for PMA P190032 (see Summary of Safety and Effectiveness Data for P190032). The *GOPC-ROS1* fusion was determined to have an estimated LoD of 0.75% VAF using the empirical hit rate approach. The *SLC34A2-ROS1* fusion was determined to have an estimated LoD of 0.28% VAF using the empirical hit rate approach.

3. **Precision**

   a. **Within-Laboratory (Intermediate) Precision of *NTRK1/2/3* Fusions**

   A precision study was conducted using four (4) clinical samples containing *NTRK1* and *NTRK3* fusions. No samples containing *NTRK2* fusions were evaluated in this precision study.

   Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three different sequencers and two different reagent lots, across multiple days of performance by multiple operators.

   The results for the precision study for the clinical samples are summarized in Tables 12 and 13, below.

Table 12. Reproducibility results for *NTRK1/2/3* fusions

Alteration Subtype	Targeted Alteration	Previously Established LoD VAF[1]	Mean VAF Calculated	Concordant /Total (n/N)	Reproducibility (%) 95%CI[2]	Fold LoD
NTRK1 Fusion	TPR-*NTRK1*	0.44%	0.75%	23/23	100% (85.69, 100)	x1.70
NTRK1 Fusion	TPM3-*NTRK1*	0.44%	0.83%	23/23	100% (85.69, 100)	x1.88

Alteration Subtype	Targeted Alteration	Previously Established LoD VAF[1]	Mean VAF Calculated	Concordant /Total (n/N)	Reproducibility (%) 95%CI[2]	Fold LoD
NTRK3 Fusion	ETV6-*NTRK3*	0.44%	0.82%	24/24	100% (86.20, 100)	x1.86
NTRK3 Fusion	ETV6-*NTRK3*	0.44%	0.68%	23/23	100% (85.69, 100)	x1.54

[1]0.44% VAF represents the higher LoD VAF of the two contrived samples evaluated in the LoD establishment study (See Section IX.A.2.b)
[2]Calculated with Wilson 2-sided 95% CI

Table 13. Repeatability results for *NTRK1/2/3* fusions

Alteration Subtype	Targeted Alteration	Previously Established LoD VAF[1]	Mean VAF Calculated	Concordant /Total (n/N)	Repeatability (%) 95%CI[2]	Fold LoD
NTRK1 Fusion	TPR-*NTRK1*	0.44%	0.75%	11/11	100% (74.12, 100)	x1.70
NTRK1 Fusion	TPM3-*NTRK1*	0.44%	0.83%	11/11	100% (74.12, 100)	x1.88
NTRK3 Fusion	ETV6-*NTRK3*	0.44%	0.82%	12/12	100% (75.75, 100)	x1.86
NTRK3 Fusion	ETV6-*NTRK3*	0.44%	0.68%	11/11	100% (74.12, 100)	x1.54

[1]0.44% VAF represents the higher LoD D VAF of the two contrived samples evaluated in the LoD establishment study (See Section IX.A.2.b)
[2]Calculated with Wilson 2-sided 95% CI

b. Within-Laboratory (Intermediate) Precision of *ROS1* Fusions

A precision study was conducted using two (2) clinical NSCLC samples harboring *ROS1* fusions as part of the precision study for PMA P190032 (see Summary of Safety and Effectiveness Data for P190032).

Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three different sequencers and two different reagent lots, across multiple days of performance by multiple operators.

The results for the precision study for the clinical samples are summarized in Tables 14 and 15, below.

Table 14. Reproducibility results for *ROS1* fusions

Targeted Alteration	Previously Established LoD VAF[1]	Mean VAF Calculated	Concordant/ Total (n/N)	Reproducibility (%) 95%CI[2]	Fold LoD
EZR-*ROS1*	0.75%	1.3%	24/24	100% (85.75, 100)	x1.73
CD74-*ROS1*	0.75%	1.32%	24/24	100% (85.75, 100)	x1.76

[1]0.75% VAF represents the higher VAF of the two contrived samples evaluated in the LoD establishment study (See Section IX.A.2.c)
[2]Calculated with Wilson 2-sided 95% CI

Table 15. Repeatability results for *ROS1* fusions

Targeted Alteration	Previously Established LoD VAF[1]	Mean VAF Calculated	Concordant/ Total (n/N)	Repeatability (%) 95%CI[2]	Fold LoD
EZR-*ROS1*	0.75%	1.3%	12/12	100% (75.75, 100)	x1.73
CD74-*ROS1*	0.75%	1.32%	12/12	100% (75.75, 100)	x.1.76

[1]0.75% VAF represents the higher VAF of the two contrived samples evaluated in the LoD establishment study (See Section IX.A.2.c)
[2]Calculated with Wilson 2-sided 95% CI

B. Animal Studies

No animal studies were conducted using the F1LCDx assay.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The reasonable assurance of safety and effectiveness for F1LCDx for detection of *NTRK1*, *NTRK2* and *NTRK3* fusions in patients with solid tumors and *ROS1* fusions in patients with NSCLC who may benefit from treatment with ROZLYTREK® (entrectinib), was established through a clinical bridging study using clinical plasma specimens from patients enrolled in the ALKA-372-001 (ALKA), RXDX- 101-01 (STARTRK-1), and RXDX-101-02 (STARTRK-2) trials, as well as commercially procured *NTRK1/2/3* fusion- and *ROS1* fusion-negative tissue-matched plasma samples. The clinical efficacy analysis was performed by analyzing the concordance between F1LCDx and the enrollment clinical trial assays (CTAs), followed by the imputation of the missing F1LCDx result, and finally determining the clinical outcome of the *ROS1* or *NTRK1/2/3* fusion positive population identified with F1LCDx.

ALKA was a Phase 1 dose-escalation study of entrectinib in adult patients with advanced/metastatic solid tumors. STARTRK-1 was a Phase 1, multicenter, open-label study of entrectinib in adult patients with locally advanced or metastatic cancer confirmed to be positive for *NTRK1*, *NTRK2*, *NTRK3*, or *ROS1* fusions. STARTRK-2 was an open-label, multicenter basket study of entrectinib for the treatment of patients with solid tumors that harbor an *NTRK1*, *NTRK2*, *NTRK3* fusions, or patients with NSCLC with fusions in the *ROS1* gene.

A summary of the clinical study is presented below.

A. FoundationOne Liquid CDx Clinical Bridging Studies for *ROS1* fusions

The *ROS1* clinical efficacy population (n=51) that supported the entrectinib approval consisted of nine (9) patients from ALKA, seven (7) from STARTRK-1, and 35 patients from STARTRK-2, enrolled based on tissue testing. *ROS1* fusion positivity was determined by NGS in 71% and by FISH in 29% of the study patient population. Fifty-five percent (55%) had central laboratory confirmation of *ROS1* fusion

positivity using the study CTAs. The ORR of the *ROS1* fusion positive patient population used to support approval of entrectinib was 78% with a two-sided 95% CI (65%, 89%).

1. **Clinical Bridging Study Design for *ROS1* fusions**
   A clinical bridging study was conducted to evaluate: 1) the concordance between the local CTAs and F1LCDx; and 2) the clinical validity of F1LCDx in identifying patients with NSCLC with *ROS1* fusions who may be eligible for treatment with entrectinib.

   Plasma samples from ALKA, STARTRK-1, and STARTRK-2 patients were collected by the therapeutic investigational sites per the study protocol and study documents and shipped to the central testing laboratories. Only samples from STARTRK-2 were available for testing by F1LCDx, with blood collected on Cycle 1 Day 1 prior to treatment. Plasma samples were shipped to Foundation Medicine for retrospective testing with F1LCDx assay.

2. **Clinical Inclusion and Exclusion Criteria**
   The inclusion/exclusion criteria for selection into the clinical bridging study are:

   Inclusion Criteria:
   - Specimens in frozen plasma
   - Samples must meet F1LCDx operational testing requirements

   Exclusion Criteria:
   - Tissue, other liquid samples
   - Samples that do not meet F1LCDx operational testing requirements

   Specimens included in the clinical bridging study were tested according to the standard testing protocol for the F1LCDx assay test with a minimum recommended cfDNA input of ≥ 30 ng for the library construction step. A subset of patient specimens was also tested at lower cfDNA inputs of ≥ 20 ng and <30 ng cfDNA input based on pre-specified assay procedures and processed only if the samples passed pre-specified in-process quality criteria. In the instances where a sample had ≥20 and <30ng of DNA following extraction, the sample was tested. This reflects FMI's current practice of processing samples with <30 ng cfDNA for input into the assay in cases where the clinician is contacted and there is not sufficient sample to be re-run.

3. **Follow-up Schedule**
   The F1LCDx clinical bridging study involved only retrospective testing of plasma samples; as such, no additional patient follow-up was conducted.

4. **Clinical Endpoints**

The primary endpoint of ALKA, STARTRK-1, and STARTRK-2 trials was the overall response rate (ORR) by Blinded Independent Review Committee (BIRC) assessment by cohort to determine whether treatment with entrectinib is effective. Duration of response (DOR) as assessed by BICR was the key secondary endpoint.

5. **Accountability of PMA Cohort for *ROS1* fusions**

A total of 255 patients were included in the clinical bridging study. Of these 255 patients, 161 were determined as *ROS1* fusion positive based on testing by the CTAs. Initially, the clinical bridging study included 51 *ROS1* fusion positive NSCLC patients from the new drug application (NDA) efficacy population, 41 *ROS1* fusion positive, *ROS1* inhibitor-naive NSCLC patients with measurable disease who had insufficient follow-up (<12 months) at the time of the NDA submission, 67 *ROS1* fusion positive NSCLC patients who were enrolled prior to October 31, 2018, and two (2) NSCLC patients with prior *ROS1* inhibitor treatment and used only for the concordance evaluation. In total, clinical outcome data from 161 *ROS1* fusion positive patients enrolled before October 31, 2018 (based on the May 1, 2019 clinical data cutoff date) were planned for use in the bridging analysis. Of the 94 *ROS1* fusion negative samples, 73 were patients enrolled in the clinical trial by the CTAs as *NTRK1/2/3*-positive. The remaining 21 *ROS1* fusions negative samples were FFPE tissue-matched plasma samples procured from a commercial source, with tissue testing by one of the CTAs used for clinical trial enrollment.

A detailed breakdown of the clinical samples is provided in Table 16.

Table 16. Samples evaluated in clinical bridging study

Biomarker Status	Sample Type	Sample Number
ROS1 Fusion Positive	NDA population from ALKA, STARTRK-1, and STARTRK-2	51
	Consistency Cohort	41
	Additional Cohort	67
	Pre-treated	2
ROS1 Fusion Negative	*NTRK* Positive from ALKA, STARTRK-1, and STARTRK-2	73
	Procured plasma	21
Total		255

6. **Study Population Demographics and Baseline Parameters**

Demographics and baseline disease characteristics for the CDx-evaluable and CDx-unevaluable patients (cfDNA input ≥30 ng) were similar (Table 17).

Table 17. Comparison of baseline demographic and clinical characteristics between the CDx-evaluable patients and the CDx-unevaluable patients

Baseline characteristic	CTA+	CDx-evaluable	CDx-unevaluable	p-value comparing the two subsets*
N	159	105	54	
ORR	67.3%	66.7%	68.5%	0.86
Age				
Mean (SD)	54.6 (12.5)	56 (12.5)	51.9 (11.9)	
Minimum	20	28	20	
Q1	46	48	44.2	
Median	54	57	51.5	0.07
Q3	64	65	60	
Maximum	86	86	73	
Sex				0.03
Male	55 (34.6%)	30 (28.6%)	25 (46.3%)	
Female	104 (65.4%)	75 (71.4%)	29 (53.7%)	
ECOG status				0.11
0	65 (40.9%)	39 (37.1%)	26 (48.1%)	
1	78 (49.1%)	52 (49.5%)	26 (48.1%)	
2	16 (10.1%)	14 (13.3%)	2 (3.7%)	
Race				0.2001
Asian	73 (45.9%)	48 (45.7%)	25 (46.3%)	
Black/African American	7 (4.4%)	7 (6.7%)	0 (0%)	
White	69 (43.4%)	43 (41.0%)	26 (48.1%)	
Other	2 (1.3%)	1 (1.0%)	1 (1.9%)	
NR[±]	8 (5.0%)	6 (5.7%)	2 (3.7%)	
Smoking History				0.69
Current	7 (4.4%)	4 (3.8%)	3 (5.6%)	
Former	53 (33.3%)	35 (33.3%)	18 (33.3%)	
NR[±]	99 (62.3%)	66 (62.9%)	33 (61.1%)	
Any CNS lesion at baseline				0.38
Yes	55 (34.6%)	39 (37.1%)	16 (29.6%)	
No	104 (65.4%)	66 (62.9%)	38 (70.4%)	
Histology				0.0001[#]
Adenocarcinoma	140 (88.1%)	103 (98.1%)	37 (68.5%)	
Adenosquamous Carcinoma	1 (0.6%)	0 (0%)	1 (1.9%)	
Bronchioloalveolar Carcinoma	1 (0.6%)	1 (1%)	0 (0%)	
Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements	1 (0.6%)	1 (1%)	0 (0%)	
Others[§]	9 (5.7%)	0 (0%)	9 (16.7%)	
NR[±]	7 (4.4%)	0 (0%)	7 (13.0%)	

*p-value was from nonparametric Mann-Whitney Test for age, and Fisher-Freeman-Halton Test for categorical factors between the CDx-evaluable and CDx-unevaluable sets.

[±]NR – Not reported

[#]p-value was from the comparison between Adenocarcinoma and non-Adenocarcinoma

[§]Others category included seven histological and two cytological in the CTA+ population

7. Safety and Effectiveness

a. Safety Results

The safety with respect to treatment with entrectinib was addressed during the review of the entrectinib 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 ROZLYTREK® (entrectinib).

b. Efficacy Results

i. Concordance Analysis with enrollment CTAs

As described above, 255 samples were included in the analysis. Only samples from STARTRK-2 were available for testing by F1LCDx and, thus, 218 of the 255 samples were evaluated by retrospective F1LCDx testing. Among them, 203 samples met the F1LCDx quality control metrics, and 175 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 28 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng.

Concordance of the F1LCDx assay with the enrolling CTAs was demonstrated with the CDx-evaluable population. The primary concordance analysis was performed with 175 patient samples (107 *ROS1* fusion positive patient samples, and 68 *ROS1* fusion negative patient samples) that had DNA content ≥30 ng and met all F1LCDx QC metrics and summarized in Table 18, below.

Table 18. Concordance for *ROS1* fusions between F1LCDx and the CTAs in samples with DNA content ≥30 ng

		CTAs		
		Detected	**Not Detected**	**Total**
F1LCDx	**Detected**	55	0	55
	Not Detected	52	68	120
	Unevaluable	54	26	80
	Total	161	94	255
Agreement Statistics Excluding CDx-Unevaluable Results		PPA: 51.4% (55/107) 95% CI*: (42.05%, 60.66%)	NPA: 100% (68/68) 95% CI*: (94.65%, 100%)	
Percent Unevaluable		33.5% (54/161) 95% CI*: (26.7%, 41.1%)	27.7% (26/94) 95% CI*: (19.6%, 37.4%)	

*Calculated with Wilson 2-sided 95% CI

The PPA was 51.4% (55/107) with 95% two-sided CI (42.05%, 60.66%) and NPA was 100% (68/68) with 95% two-sided CI (94.65%, 100%) after excluding CDx-unevaluable results. The

discordances between the CTAs and F1LCDx among *ROS1* fusion positive patients was evaluated by stratifying the PPA into two subgroups, DNA-based NGS CTAs and RNA-based NGS CTAs. The PPA between F1LCDx and DNA-based NGS CTAs was 55.6% (10/18) with 95% two-sided CI (33.7%, 75.4%). The PPA between F1LCDx and RNA-based NGS CTAs was 50.6% (40/79) with 95% two-sided CI (39.8%, 61.4%). Of the 52 CTA positive patients who were F1LCDx negative, 92.3% (48/52) did not have detectable tumor fraction as determined by F1LCDx, suggesting that the ctDNA content in these samples was low.

Therefore, F1LCDx may miss approximately half of patients with NSCLC with *ROS1* fusions who may derive benefit from entrectinib. A restriction is included in the intended use to address the risk of a false negative results by indicating that testing for the presence of *ROS1* fusions using plasma specimens is only appropriate for patients for whom tumor tissue is not available for testing (refer to Section II).

Due to the small number of *ROS1* fusion negative samples to support the concordance analysis given the low prevalence of *ROS1* fusions in NSCLC patients (i.e., ~1-2%), the estimates of NPA and PPV are not reliable. Therefore, a limitation is included in the device labeling to address the risk of false positive results for patients with NSCLC by indicating that, due to the low prevalence of *ROS1* fusions, the positive predictive value of the test (F1LCDx positive, tissue negative) may be lower than reported in test labeling (refer to Section XII.C. below).

ii. Bridging clinical outcome from CTA to F1LCDx

The clinical efficacy of entrectinib in the clinical trials was measured in ORR with either confirmed complete response (CR) or partial response (PR) based on blinded independent centralized review (BICR). Only clinical samples with clinical outcome data were used in this part of the study analysis.

The ORR in the CTA positive population was 67.3% (107/159) with 95% two-sided CI (59.7%, 74.1%). Fifty-four (54) patients were CTA positive and had F1LCDx *ROS1* fusion positive results. The ORR for this population was 66.7% (36/54) with 95% two-sided CI (53.4%, 77.8%). Fifty-one (51) patients were CTA positive but had F1LCDx *ROS1* negative results. The ORR for this population was 66.7% (34/51) with 95% two-sided CI (53.0%, 78.0%).

Fifty-four (54) patients were CTA positive but were unevaluable by F1LCDx. The ORR for this population was 68.5% (37/54) with 95% two-sided CI (55.3%, 79.3%), as summarized in Table 19.

Table 19. Efficacy by *ROS1* status in biomarker subgroups in samples with DNA content ≥30 ng

Clinical outcome	Total CTA+ population* (N=159)	CTA+/F1LCDx+ (N=54)	CTA+/F1LCDx– (N=51)	CTA+/F1LCDx unevaluable (N=54)
ORR% [95% CI**]	67.3%	66.7%	66.7%	68.5%
	[59.7%, 74.1%]	[53.4%, 77.8%]	[53.0%, 78.0%]	[55.3%, 79.3%]
Complete response	14 (8.8%)	5 (9.3%)	6 (11.8%)	3 (5.6%)
Partial response	93 (58.5%)	31 (57.4%)	28 (54.9%)	34 (63.0%)
Number of responders	**N=107**	**N=36**	**N=34**	**N=37**
Duration of response				
Median± in months (range)	9.5 (1.8, 42.3)	6.4 (1.8, 20.5)	13.4 (1.9, 27.6)	11.1 (4.6, 42.3)
% with duration ≥9 months	61.7%	38.9%	70.6%	75.7%
% with duration ≥12 months	41.1%	19.4%	55.9%	48.6%
% with duration ≥18 months	19.6%	5.6%	26.5%	27.0%

*See Table 13 for a description of the total population
**Two-sided 95% CI for each subgroup was based on the Wilson-score method
±Arithmetic median used (not Kaplan-Meier methods) since censoring data was not available

The similarity of the ORR for the CTA-positive population (n=159) overall (67.3%, 95% CI: 59.7, 74.1) and for the F1LCDx-unevaluable population (n=54; 68.5%, 95% CI: 55.3, 79.3) suggests no overt imbalance in efficacy effect of entrectinib between patients with or without a valid F1LCDx result.

There were 70 *ROS1* positive patients by the CTAs with partial or complete response to entrectinib, who also had a F1LCDx result. Among them, only 51.4% (36/70) were positive by F1LCDx (95% CI: 39.9, 62.8). There were 35 *ROS1* positive patients by the CTAs who did not respond to entrectinib, who also had a F1LCDx result (54-36=18 and 51-34=17). Among them, 51.4% (18/35) were positive by F1LCDx (95% CI: 35.6, 67.0).

There were nearly the same proportion of responders among patients with an F1LCDx positive or negative result. Therefore, a limitation is included in the device labeling to indicate that F1LCDx may miss a subset of patients with *ROS1* fusion positive tumors who may derive benefit from entrectinib.

iii. Sensitivity Analysis

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates considering F1LCDx unevaluable patients enrolled in the ALKA, STARTRK-1, and STARTRK-2 clinical trials. Samples were considered

missing if the samples were not tested, if they were tested but returned an invalid result, or if they did not satisfy the cfDNA minimum input requirement (i.e., ≥ 30ng).

Amongst all CTA-positive patients, 34.0% did not have a F1LCDx result (54/159).

To evaluate the impact of the F1LCDx unevaluable population, the distribution of patients for baseline covariates and disease characteristics was compared among the CTA-positive population, the F1LCDx-evaluable/CTA-positive subpopulation, and F1LCDx-unevaluable/CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable F1LCDx (n=54).

The imputed ORR by BIRC was estimated to be 67.1% (95% CI: 50.7, 78.9), which is similar to the estimated ORR for the CTA-positive population based on the observed data [67.3% (95% CI: 59.7%, 74.1%)]. Thus, the sensitivity analysis demonstrated the robustness of the clinical efficacy estimate.

8. Pediatric Extrapolation

In this premarket application for *ROS1* fusion indication, existing clinical data was not leveraged to support approval of a pediatric population since it is not applicable for the NSCLC indication.

B. FoundationOne Liquid CDx Clinical Bridging Studies for *NTRK1/2/3* fusions

The *NTRK/1/2/3* fusion clinical efficacy population (n=54) that supported the entrectinib approval consisted of one (1) patient from ALKA, two (2) from STARTRK-1, and 51 patients from STARTRK-2, enrolled largely based on tissue testing. *NTRK1/2/3* fusion positivity was determined by NGS in 96% and by other nucleic acid-based tests in 4% of the study patient population. Eighty-three percent (83%) had central laboratory confirmation of *NTRK1/2/3* fusion positivity using the study CTAs. The ORR of the *NTRK1/2/3* fusion positive patient population used to support approval of entrectinib was 57% with a 95% CI of 43%, 71%.

1. Clinical Bridging Study Design for *NTRK1/2/3* fusions

A clinical bridging study was conducted to evaluate: 1) the concordance between the local CTAs and F1LCDx; and 2) the clinical validity of F1LCDx in identifying solid tumor patients with *NTRK1/2/3* fusions who may be eligible for treatment with entrectinib.

Plasma samples from ALKA, STARTRK-1, and STARTRK-2 patients were collected by the therapeutic investigational sites per the study protocol and study documents and shipped to the central testing laboratories. Only samples from STARTRK-2 were available for testing by F1LCDx, with blood

collected on Cycle 1 Day 1 prior to treatment. Plasma samples were shipped to Foundation Medicine for retrospective testing with F1LCDx assay.

2. **Clinical Inclusion and Exclusion Criteria**
   The inclusion/exclusion criteria for selection into the clinical bridging study are:

   Inclusion Criteria:
   - Specimens in frozen plasma
   - Samples must meet F1LCDx operational testing requirements

   Exclusion Criteria:
   - Tissue, other liquid samples
   - Samples that do not meet F1LCDx operational testing requirements

   Specimens included in the clinical bridging study were tested according to the standard testing protocol for the F1LCDx assay test with a minimum recommended cfDNA input of ≥ 30 ng for the library construction step. A subset of patient specimens was also tested at lower cfDNA inputs of ≥ 20 ng and <30 ng cfDNA input based on pre-specified assay procedures and processed only if the samples passed pre-specified in-process quality criteria. In the instances where a sample had ≥20 and <30ng following DNA extraction, the sample was tested. This reflects FMI's current practice of processing samples with <30 ng cfDNA for input into the assay in cases where the clinician is contacted and there is not sufficient sample to be re-run.

3. **Follow-up Schedule**
   The F1LCDx clinical bridging study involved only retrospective testing of plasma samples; as such, no additional patient follow-up was conducted.

4. **Clinical Endpoints**
   The primary endpoint of ALKA, STARTRK-1, and STARTRK-2 trials was the ORR by BIRC assessment by cohort to determine whether treatment with entrectinib is effective. DOR as assessed by BIRC was the key secondary endpoint.

5. **Accountability of PMA Cohort for *NTRK1/2/3* fusions**
   A total of 256 patients were included in the clinical bridging study. Of these 256 patients, 74 were determined as *NTRK1/2/3* fusion positive based on testing by the CTAs. Initially, the clinical bridging study included 54 *NTRK1/2/3* fusion positive patients from the NDA efficacy population, as well as 20 *NTRK1/2/3* fusion positive patients who were enrolled after the data cutoff. Of the 182 *NTRK1/2/3* fusion negative samples, 161 were patients enrolled in the clinical trial by the CTAs as *ROS1* fusion positive. The remaining 21 *NTRK1/2/3* fusion negative samples were FFPE tissue-matched

plasma samples procured from a commercial source, with tissue testing by one of the CTAs used for clinical trial enrollment.

Only samples from STARTRK-2 were available for testing by F1LCDx and, thus, 218 of the 256 samples were included for retrospective F1LCDx testing. Among them, 203 samples met the F1LCDx quality control metrics, and 175 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 28 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng.

A detailed breakdown of the clinical samples is provided in Table 20.

Table 20. Samples evaluated in clinical bridging study

Biomarker Status	Sample Type	Sample Number – Total
NTRK Positive	NDA population from ALKA, STARTRK-1, and STARTRK-2	54
	Additional samples (non-NDA population) from STARTRK-2	20
NTRK Negative	*ROS1* Positive from ALKA, STARTRK-1, and STARTRK-2	161
	Procured plasma	21
Total		256

6. Study Population Demographics and Baseline Parameters

Demographics and baseline disease characteristics for the CDx-evaluable and CDx-unevaluable patients (cfDNA input ≥30 ng) were similar (Table 21).

Table 21. Comparison of baseline demographic and clinical characteristics between the CDx-evaluable patients and the CDx-unevaluable patients

Baseline characteristic	CTA+	CDx-evaluable	CDx-unevaluable	p-value comparing the two subsets*
N	74	51	23	
ORR	63.5%	64.7%	60.9%	0.79
Age				
Mean (SD)	56.5 (14.6)	55.6 (15.4)	58.6 (12.9)	
Minimum	21	21	31	
Q1	48	46.5	49.5	
Median	57	57	58	0.58
Q3	67	67	71	
Maximum	83	83	78	
Sex				0.80
Male	35 (47.3%)	25 (49.0%)	10 (43.5%)	
Female	39 (52.7%)	26 (51.0%)	13 (56.5%)	
ECOG status				0.11
0	30 (40.5%)	18 (35.3%)	12 (52.2%)	

Baseline characteristic	CTA+	CDx-evaluable	CDx-unevaluable	p-value comparing the two subsets*
1	34 (45.9%)	25 (49.0%)	9 (39.1%)	
2	10 (13.5%)	8 (15.7%)	2 (8.7%)	
Race				0.23
Asian	13 (17.6%)	7 (13.7%)	6 (26.1%)	
Black/African American	2 (2.7%)	2 (3.9%)	0 (0%)	
White	52 (70.3%)	39 (76.5%)	13 (56.5%)	
NR[±]	7 (9.5%)	3 (5.9%)	4 (17.4%)	
Smoking History				0.37
Current	8 (10.8%)	7 (13.7%)	1 (4.3%)	
Former	21 (28.4%)	13 (25.5%)	8 (34.8%)	
NR[±]	45 (60.8%)	31 (60.8%)	14 (60.9%)	
Any CNS lesion at baseline				0.39
Yes	19 (25.7%)	15 (29.4%)	4 (17.4%)	
No	55 (74.3%)	36 (70.6%)	19 (82.6%)	
Histology				0.29[#]
Adenocarcinoma	27 (36.5%)	17 (33.3%)	10 (43.5%)	
Carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements	2 (2.7%)	1 (2.0%)	1 (4.3%)	
Neuroendocrine	4 (5.4%)	4 (7.8%)	0 (0%)	
Spindle cell	7 (9.5%)	6 (11.8%)	1 (4.3%)	
Squamous-cell carcinoma	2 (2.7%)	1 (2.0%)	1 (4.3%)	
Others[§]	30 (40.5%)	22 (43.1%)	8 (34.8%)	
NR[±]	2 (2.7%)	0 (0%)	2 (8.7%)	

*p-value was from nonparametric Mann-Whitney Test for age, and Fisher-Freeman-Halton Test for categorical factors between the CDx-evaluable and CDx-unevaluable sets.

[±]NR – Not reported

[#]p-value was from the comparison between Adenocarcinoma and non-Adenocarcinoma

[§]Others category included one histological, three unclassified carcinoma/undifferentiated carcinoma, and 26 "Other" in the CTA+ population

7. Safety and Effectiveness

a. Safety Results

The safety with respect to treatment with entrectinib was addressed during the review of the Entrectinib 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 ROZLYTREK® (entrectinib).

b. Efficacy Results

i. Concordance Analysis with enrollment CTAs

As described above, 256 *NTRK* samples were included in the analysis. Only samples from STARTRK-2 were available for testing by F1LCDx and, thus, 218 of the 256 samples were

included for retrospective F1LCDx testing. Among them, 203 samples met the F1LCDx quality control metrics, and 175 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 28 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng.

Concordance of the F1LCDx assay with the enrolling CTA was demonstrated with the CDx-evaluable population. The primary concordance analysis was performed with 175 patients (51 *NTRK* fusion positive patients, and 124 *NTRK* fusion negative patients) that had DNA content ≥30 ng and met all F1LCDx QC metrics and summarized in Table 22, below.

Table 22. Concordance for *NTRK1/2/3* fusions between F1LCDx and the CTAs in samples with DNA content ≥30 ng

		CTAs		
		Detected	**Not Detected**	**Total**
F1LCDx	**Detected**	25	0	25
	Not Detected	26	124	150
	Unevaluable	23	58	81
	Total	74	182	256
Agreement Statistics Excluding CDx-Unevaluable Results		PPA: 49.0% (25/51) 95% CI*: (35.9%, 62.3%)	NPA: 100% (124/124) 95% CI*: (97.0%, 100%)	
Percent Unevaluable		31.1% (23/74) 95% CI*: (21.7%, 42.3%)	31.9% (58/182) 95% CI*: (25.5%, 39.0%)	

*Calculated with Wilson 2-sided 95% CI

The PPA was 49.0% (25/51) with 95% two-sided CI (35.9%, 62.3%) and NPA was 100% (124/124) with 95% two-sided CI (97.0%, 100%) after excluding CDx-unevaluable results. The discordances between the CTAs and F1LCDx among *NTRK1/2/3* fusion positive patients was evaluated by stratifying the PPA into two subgroups, DNA-based NGS CTAs and RNA-based NGS CTAs. The PPA between F1LCDx and DNA-based NGS CTAs was 65.0% (13/20) with 95% two-sided CI (43.3%, 81.9%). The PPA between F1LCDx and RNA-based NGS CTAs was 38.7% (12/31) with 95% two-sided CI (23.7%, 56.2%).

Therefore, F1LCDx may miss approximately half of patients with solid tumors with *NTRK1*/2/3 fusions who may derive benefit from entrectinib. A restriction is included in the intended use to address the risk of a false negative results by indicating that testing for the presence of *NTRK1/2/3* fusions using plasma specimens is only appropriate for patients for whom tumor tissue is not available for testing (refer to Section II).

While there were two (2) *NTRK2* fusion positive patients as determined by the CTAs, these patients were negative by F1LCDx. Due to the rarity of the biomarker, the clinical concordance study did not demonstrate the ability of F1LCDx to detect *NTRK2* fusions. Therefore, a limitation is included in the device labeling indicating that ROZLYTREK® efficacy has not been established in patients with *NTRK2* fusions tested with F1LCDx (refer to Section XII.C. below).

Due to the small number of *NTRK1/2/3* fusion negative samples to support the concordance analysis given the low prevalence of *NTRK1/2/3* fusions in patients with solid tumors (i.e., ~0.32%), the estimates of NPA and PPV are not reliable. Therefore, a limitation is included in the device labeling to address the risk of false positive results for patients with solid tumors by indicating that, due to the low prevalence of *NTRK1/2/3* fusions, the positive predictive value of the test (F1LCDx positive, tissue negative) may be lower than reported in test labeling (refer to Section XII.C. below).

iv. Bridging clinical outcome from CTA to F1LCDx

The clinical efficacy of entrectinib in the clinical trials was measured in overall response rate (ORR) with either confirmed complete response (CR) or partial response (PR) based on blinded independent centralized review (BICR). Only clinical samples with clinical outcome data were used in this part of the study analysis.

The ORR in the CTA positive population was 63.5% (47/74) with 95% two-sided CI (52.1%, 73.6%). Twenty-five (25) patients were CTA positive and had F1LCDx *NTRK1/2/3* fusion positive results. The ORR for this population was 72.0% (18/25) with 95% two-sided CI (52.4%, 85.7%). Twenty-six (26) patients were CTA positive but had F1LCDx *NTRK1/2/3* fusion negative results. The ORR for this population was 57.7% (15/26) with 95% two-sided CI (38.9%, 74.5%).

Twenty-three (23) patients were CTA positive but were F1LCDx-unevaluable. The ORR for this population was 60.9% (14/23) with 95% two-sided CI (40.8%, 77.8%), as summarized in Table 23.

Table 23. Efficacy by *NTRK1/2/3* status in biomarker subgroups in samples with DNA content ≥30 ng

Clinical outcome	Total CTA+ population* (N=74)	CTA+/F1LCDx+ (N=25)	CTA+/ F1LCDx– (N=26)	CTA+/F1LCDx unevaluable (N=23)
ORR% [95% CI**]	63.5%	72.0%	57.7%	60.9%
	[52.1,73.6]	[52.4, 85.7]	[38.9, 74.5]	[40.8, 77.8]

Clinical outcome	Total CTA+ population* (N=74)	CTA+/F1LCDx+ (N=25)	CTA+/ F1LCDx– (N=26)	CTA+/F1LCDx unevaluable (N=23)
Complete response	5 (6.8%)	0 (0.0%)	1 (3.8%)	4 (17.4%)
Partial response	42 (56.8%)	18 (72.0%)	14 (53.8%)	10 (43.5%)
Number of responders	**N=47**	**N=18**	**N=15**	**N=14**
Duration of response				
Median[±] in months (range)	7.5 (1.4, 26.0)	5.9 (1.9, 16.6)	7.9 (1.4, 26.0)	8.3 (2.8, 25.9)
% with duration ≥9 months	44.7%	38.9%	46.7%	50.0%
% with duration ≥12 months	29.8%	22.2%	40.0%	28.6%
% with duration ≥18 months	10.6%	0.0%	13.3%	21.4%

*See Table 18 for a description of the total population

**Two-sided 95% CI for each subgroup was based on the Wilson-score method

[±]Arithmetic median used (not Kaplan-Meier methods) since censoring data was not available

The similarity of the ORR for the CTA positive population (n=74) overall (63.5%, 95% CI: 52.1, 73.6) and for the F1LCDx-unevaluable subpopulation (n=23; 60.9%, 95% CI: 40.8, 77.8) suggests no overt imbalance in efficacy effect of entrectinib between patients with or without a valid F1LCDx result.

There were 33 *NTRK1/2/3* positive patients by the CTAs with partial or complete response to entrectinib, who also had an F1LCDx result. Among them, only 54.5% (18/33) were positive by F1LCDx (95% CI: 38.0, 70.2). There were 18 CTA positive patients who did not respond to entrectinib, who also had a F1LCDx result (25-18=7 and 26-15=11). Among them, 38.9% (7/18) were positive by F1LCDx (95% CI: 20.3, 61.4).

There were nearly the same proportion of responders among patients with an F1LCDx positive or negative result. Therefore, a limitation is included in the device labeling to indicate that F1LCDx may miss a subset of patients with *NTRK1/2/3* fusion positive tumors who may derive benefit from entrectinib.

There were 25 patients positive for an *NTRK3* fusion in the entrectinib clinical studies. Among them, 68.0% (17/25) were negative for *NTRK3* fusions by F1LCDx. Among the 17 patients who were negative for *NTRK3* fusions by F1LCDx, 64.7% (11/17) had response to entrectinib. Further, F1LCDx detected only one (1) of seven (7) different *NTRK3* fusions that were detected by the CTAs.

Therefore, F1LCDx may miss a subset of patients with solid tumors with *NTRK3* fusions who may derive benefit from entrectinib. A limitation is included in the device labeling to

indicate that, due to the rarity of *NTRK3* fusions, the accuracy of F1LCDx for these fusions has not been adequately determined.

v. Sensitivity Analysis

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates considering F1LCDx unevaluable patients enrolled in the ALKA, STARTRK-1, and STARTRK-2 clinical trials. Samples were considered missing if the samples were not tested, if they were tested but returned an invalid result, or if they did not satisfy the cfDNA minimum input requirement (i.e., ≥ 30ng).

Amongst all CTA positive patients, 31.1% did not have a F1LCDx result (23/74).

To evaluate the impact of the F1LCDx unevaluable population, the distribution of patients for baseline covariates and disease characteristics was compared among the CTA-positive population, the F1LCDx-evaluable/CTA-positive subpopulation, and F1LCDx-unevaluable/CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable F1LCDx (n=23).

The imputed ORR by BIRC was estimated to be 67.5% (95% CI: 52.4, 87.2), which is similar to the estimated ORR for the CTA-positive population based on the observed data [63.5% (95% CI: 52.1, 73.6)]. Thus, the sensitivity analysis demonstrated the robustness of the clinical efficacy estimate.

vi. Subgroup Analysis

Response to entrectinib for the F1LCDx fusion positive patients was analyzed by *NTRK* fusion gene, *NTRK* gene fusion partner, and primary tumor type (Table 24).

Table 24. The overall response rate for F1LCDx *NTRK* fusion positive patients in the efficacy analysis set by different subgroups

Subgroup	Number of Patients (N=25)	Number of patients with CR or PR	ORR (%) (95% CI*)
***NTRK* fusion gene by CDx**			
NTRK1	17	11	64.7% (41.3, 82.7)
NTRK3	8	7	87.5% (52.9, 97.8)
***NTRK* fusion partner**			
CD74-NTRK1	1	1	100%
CDC42BPA-NTRK1	1	1	100%
CGN-NTRK1	1	0	0%
EFNA3-NTRK1[±]	1	1	100%

Subgroup	Number of Patients (N=25)	Number of patients with CR or PR	ORR (%) (95% CI*)
EPS15L1-NTRK1	1	1	100%
ETV6-NTRK3	8	7	87.5%
LMNA-NTRK1	1	1	100%
PEAR1-NTRK1	1	0	0%
PLEKHA6-NTRK1	1	1	100%
TPM3-NTRK1[±]	5	3	60%
TPR-NTRK1	4	3	75%
TRIM33-NTRK1	1	0	0%
Primary tumor type			
Breast (non-secretory)	2	1	50%
Cervical Adenocarcinoma	1	1	100%
Cholangiocarcinoma	1	1	100%
CRC	3	1	33.3%
MASC	4	4	100%
Neuroendocrine	2	2	100%
Non-CRC GI (NOS)	1	1	100%
NSCLC	5	4	80%
Pancreatic	2	2	100%
Papillary Thyroid	2	0	0%
Sarcoma (NOS)	2	1	50%

*****CIs were provided for sample sizes >10

[±]One patient had two biomarker positive *NTRK*-fusion events

9. Pediatric Extrapolation

Entrectinib was approved for the treatment of adult and pediatric patients 12 years of age and older with solid tumors that have an *NTRK* gene fusion without a known acquired resistance mutation, are metastatic or where surgical resection is likely to result in severe morbidity and have progressed following treatment or have no satisfactory alternative therapy. Per the FoundationCORE database, the overall prevalence of *NTRK* rearrangements in pediatric cancer $\leq$ 18 years of age) is 1.2%.

The safety of entrectinib in adolescent patients was established based on data from 30 pediatric patients enrolled in the STARTRK-NG (RXDX-101-03) clinical trial. The effectiveness of entrectinib in adolescent patients was established based on extrapolation of data from three open-label, single-arm clinical trials in adult patients with solid tumors harboring an *NTRK* gene fusion (ALKA, STARTRK-1, and STARTRK-2) and pharmacokinetic data in adolescents enrolled in STARTRK-NG. The STARTRK-NG clinical trial does not contribute to the clinical bridging studies.

The safety and effectiveness of ROZLYTREK in pediatric patients less than 12 years of age with solid tumors who have an *NTRK1/2/3* gene fusion have not been established.

The safety and effectiveness of ROZLYTREK in pediatric patients with *ROS1* fusion positive NSCLC have not been established.

C. 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 studies included six (6) investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). 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 F1LCDx to identify *ROS1* fusions in NSCLC patients and *NTRK1/2/3* fusions in solid tumor patients who may benefit from treatment with entrectinib was demonstrated through clinical bridging studies using specimens from patients enrolled into the ALKA, STARTRK-1, and STARTRK-2 studies. The data from the analytical validation and clinical bridging studies support the reasonable assurance of safety and effectiveness of the F1LCDx assay when used in accordance with the indications for use. Data from the ALKA, STARTRK-1, and STARTRK-2 studies show that patients with NSCLC harboring *ROS1* fusions and patients with solid tumors harboring *NTRK1/2/3* fusions received benefit from treatment with entrectinib and support the addition of the CDx indication to F1LCDx.

B. Safety Conclusions

The risks of the device are based on data collected in the analytical and clinical validation studies conducted to support sPMA approval, as described above. The F1LCDx assay is an in vitro diagnostic test, which involves testing of cfDNA extracted from blood or plasma.

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

treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

C. Benefit-Risk Determination

The probable benefit of the F1LCDx assay in identifying patients with solid tumors with *NTRK1/2/3* fusions for treatment with ROZLYTREK® (entrectinib) and patients with NSCLC with *ROS1* fusions for treatment with ROZLYTREK® (entrectinib) was demonstrated through clinical bridging studies using specimens from patients enrolled into the ALKA, STARTRK-1, and STARTRK-2 studies.

For patients with NSCLC with *ROS1* fusion positive status, the ORR for all 159 samples of this study (CTA+) was 67.3% (107/159) with 95% CI [59.7%, 74.1%]. Clinical outcome for patients with NSCLC with *ROS1* fusion positive status by the CTA and F1LCDx indicated an ORR of 66.7% (36/54) with 95% CI: [53.4%, 77.8%], which was comparable to the ORR in the CTA+ population and provides evidence of a meaningful clinical benefit in this population. Of note, in the concordance analysis, the NPA and PPV were 100%. However, due to the small number of *ROS1* fusion negative samples to support the concordance analysis, there is some uncertainty around the estimate of NPA, given the low prevalence of *ROS1* fusions in patients with NSCLC (~1-2%). The observed ORR for the F1LCDx *ROS1* fusion positive patients supports probable benefit of F1LCDx in selecting *ROS1* fusion positive NSCLC patients for treatment with ROZLYTREK® (entrectinib). However, there is significant probable risk that this device may miss a substantial proportion of patients with NSCLC with *ROS1* fusion positive status; therefore, this test mainly has clinical value, in fulfilling an unmet clinical need, in the setting where tumor tissue is not available for testing.

For patients with solid tumors with *NTRK1/2/3* fusion positive status, the ORR for all 74 samples of this study (CTA+) population was 63.5% (47/74) with 95% CI [52.1%, 73.6%]. Clinical outcome of patients with *NTRK1/2/3* fusions by the CTA and F1LCDx, indicated an ORR of 72.0% with 95% CI: [52.4%, 85.7%], which was comparable to the ORR in the CTA+ population, and provides evidence of a meaningful clinical benefit in this population. Of note, in the concordance analysis, the NPA and PPV were 100%. However, due to the small number of *NTRK1/2/3* fusion negative samples to support the concordance analysis, there is some uncertainty around the estimate of NPA, given the low prevalence of *NTRK1/2/3* fusions in patients with solid tumors (~0.32%). The observed ORR for the F1LCDx *NTRK1/2/3* fusion positive patients supports the probable benefit of F1LCDx in selecting *NTRK1/2/3* fusion positive patients for treatment with ROZLYTREK® (entrectinib). However, there is significant probable risk that this device may miss a substantial proportion of patients with solid tumors with *NTRK1/2/3* fusion positive status; therefore, this test mainly has clinical value, in fulfilling an unmet clinical need, in a setting where tumor tissue is not available for testing.

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 F1LCDx 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.

Based on the data from the clinical trial, there is a risk that a substantial portion of solid tumor patients with *NTRK1/2/3* fusions or NSCLC patients with *ROS1* fusions that may respond to the drug may be missed by this device. However, using plasma specimens for F1LCDx to detect these biomarkers (*NTRK1/2/3* fusions and *ROS1* fusions) is only indicated for patients for whom tumor tissue is not available for testing; therefore, this device may fill an unmet need and have substantial probable benefit in this specific clinical setting.

Additional factors to be considered in determining probable risks and benefits for F1LCDx included: the availability of alternative tests and clinical use setting for entrectinib. Of note, there are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of rearrangements that lead to *NTRK1/2/3* or *ROS1* fusions to identify patients eligible for treatment with ROZLYTREK® (entrectinib), and this device may meet an unmet need for patients who do not have tumor tissue available for testing with a tissue based CDx test for these biomarkers. The F1LCDx assay has been analytically validated as summarized above; however, to supplement the premarket data, post-market studies are planned as summarized in Section XIII, below. It is key to recognize that:

- FoundationOne Liquid CDx may miss a substantial proportion of *NTRK1/2/3* and *ROS1* fusion positive patients; therefore, when considering eligibility for ROZYLTREK® based on the detection of *NTRK1/2/3* and *ROS1* fusions, testing using plasma specimens is only appropriate for patients for whom tumor tissue is not available for testing.

Further, given the uncertainties that remain based on the analytical and clinical validation data, the following limitations are included in the device labeling:

- Due to the low prevalence of *ROS1* fusions and *NTRK1/2/3* fusions, the positive predictive value of the test (F1LCDx positive, tissue negative) may be lower than reported in test labeling.
- FoundationOne Liquid CDx may miss a subset of patients with *NTRK1/2/3* fusion and *ROS1* fusion positive solid tumors who may derive benefit from ROZLYTREK®. In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, the data demonstrated that the FoundationOne Liquid CDx test did not detect approximately 46% of potential responders with *NTRK1/2/3* fusions and 49% of responders with *ROS1* fusions.

- ROZLYTREK® efficacy has not been established in patients with *NTRK2* fusions tested with FoundationOne Liquid CDx, given the low prevalence of the biomarker.
- In a retrospective-prospective clinical study assessing concordance between FoundationOne Liquid CDx test results in plasma and patients whose tumor tissue tested positive and was the basis for enrollment into a clinical trial, FoundationOne Liquid CDx detected 1 of 7 different *NTRK3* fusion partners. Due to the rarity of these fusions, the accuracy of FoundationOne Liquid CDx for *NTRK3* fusions has not been adequately determined.
- *NTRK2* fusions per the F1LCDx biomarker rules for *NTRK1/2/3* fusions were not represented in analytical validation studies.
- A study evaluating the concordance to a second method demonstrated that the agreement between FoundationOne Liquid CDx positive results and a comparator method for *NTRK1/3* and *ROS1* was ≤ 50% (i.e., whether these are potential FoundationOne Liquid CDx false positives or false negatives by the comparator is unknown).

However, it is noted that due to the limitations of shedding of ctDNA from the tumor into circulation, the maximum achievable PPA or a liquid biopsy relative to a tissue biopsy is likely to be less than 100%. The performance of this device should be considered in light of this biological limitation, in addition to the limitations of the assay.

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 F1LCDx assay, and the indications noted in the intended use statement, and in the setting where tumor tissue is not available for testing, 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 clinical bridging study support the performance of F1LCDx as an aid for the identification of patients with NSCLC with *ROS1* fusions and patients with solid tumors with *NTRK1*, *NTRK2*, *NTRK3* fusions for ROZLYTREK (entrectinib) treatment.

XIII. CDRH DECISION

CDRH issued an approval order on December 22, 2022. The final non-clinical conditions of approval cited in the approval order are described below. The final study protocols for the above studies, study data, study conclusions, and labeling revisions should be submitted within 1 year of the PMA approval date.

1. FMI must provide robust and high confidence data to demonstrate the analytical accuracy, limit of detection, and precision for the most current version of FoundationOne Liquid CDx for the detection of *NTRK2* fusions. These results must be adequate to confirm the safety and effectiveness of the FoundationOne Liquid CDx device.

2. FMI must provide robust and high confidence data to demonstrate the analytical accuracy with 10 *ROS1* fusion positive samples for the most current version of FoundationOne Liquid CDx for the detection of *ROS1* fusions to an externally validated comparator assay that has been accepted by FDA as suitable for this purpose. *ROS1* fusion positive samples can be procured from the intended use population. These results must be adequate to confirm the safety and effectiveness of the FoundationOne Liquid CDx device.

3. Blood Collection Tubes
    a. FMI must demonstrate clinically insignificant variability when different lots of the FoundationOne Liquid CDx Blood Collection tube are used with the FoundationOne Liquid CDx assay. FMI must provide data from a robust and high confidence precision study. This study must confirm the FoundationOne Liquid CDx assay's precision when the FoundationOne Liquid CDx cfDNA Blood Collection tubes are used and must use replicate samples from each of multiple different patients. Each patient who donates specimens for this study must have plasma collected in a total of four tubes, each from two tube lots; three lots are required to be represented in the study. This is important to assess variability between tube lots and across patient specimens. Each replicate must be run at or near the minimum standardized cfDNA input (i.e., at a target concentration of 30 ng). The samples must be collected from patients with at least 10 different tumor types and the study must include at least 10 pathogenic substitutions, 10 pathogenic indels, and rearrangements that are identified by the FoundationOne Liquid CDx assay. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne Liquid cfDNA Blood Collection tubes in the intended use population.

    b. FMI must provide robust and high confidence data from a well- designed and well-controlled study which is intended to confirm the shelf-life claims for the FoundationOne Liquid cfDNA Blood Collection tubes when used in conjunction with the FoundationOne Liquid CDx assay. FMI must provide evidence that when samples from the same patient collected in newly manufactured tubes, as well as in tubes that are at the end of their shelf life, are used in the FoundationOne® Liquid CDx assay, the FoundationOne Liquid CDx assay performance meets the

clinical and analytical performance claim in the FoundationOne Liquid CDx assay authorized labeling.

c. FMI must provide robust and high confidence data that the impact of preanalytical variables associated with the use of the FoundationOne Liquid CDx cfDNA Blood Collection tubes, such as hemolysis, has been validated for the FoundationOne Liquid CDx test system and that any impact of these factors on the FoundationOne Liquid CDx assay has been appropriately mitigated. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne Liquid CDx cfDNA Blood Collection tubes in the intended use population.

d. To support use of results submitted in FMI's clinical study generated from samples collected within 24 hours from cancer patients, FMI must provide robust and high confidence data from an appropriately designed study to confirm the claimed stability of cfDNA in the FoundationOne Liquid CDx cfDNA Blood Collection tubes. This study must compare FoundationOne® Liquid CDx results generated from freshly drawn blood specimens to FoundationOne Liquid CDx assay results generated from matched specimens (i.e., collected at the same time from the same patient) stored in the FoundationOne Liquid CDx cfDNA Blood Collection tube for a minimum of 24 hours. This study must be performed with replicate samples, when feasible, at each time point, and the samples tested must adequately represent all variant types across several tumor types at each tested time point. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens the intended use population.

e. FMI must provide robust and high confidence data from a stability study which demonstrates acceptable stability of whole blood collected from the CDx intended use patients and stored in the FoundationOne Liquid CDx cfDNA Blood Collection tubes. The study must confirm the claimed cfDNA storage stability and must confirm the suppression of white blood cells lysis across multiple lots. This study must also use the amount of cfDNA isolated and electropherogram data as a comparator method, in addition to sequencing results and quality metrics. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne Liquid CDx cfDNA Blood Collection tubes in the intended use population.

f. FMI must demonstrate clinically insignificant variability on the performance of the FoundationOne Liquid CDx assay when specimens collected in FoundationOne Liquid CDx cfDNA Blood Collection tubes are handled at different centrifugation conditions. The study must assess conditions that are below and above recommended relative centrifugal force and centrifugation time to account for potential performance issues that could occur due to centrifuge

malfunction or operator errors. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when expected handling conditions are used on specimens collected in the FoundationOne Liquid CDx cfDNA Blood Collection tubes in the intended use population.

In addition to the conditions of approval above, FMI agreed to implement alternative controls to address violations of the current good manufacturing practice requirements of the Quality System regulations found at Title 21, Code of Federal Regulations, Part 820 identified at the manufacturing facility of the cfDNA blood collection tubes used with the FoundationOne Liquid CDx assay. FDA subsequently approved a variance plan on August 26, 2020 that met the requirements set forth in 21 C.F.R. 820.1(e)(2).

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

None.