# SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

## I. GENERAL INFORMATION

| | |
|---|---|
| Device Generic Name: | Next generation sequencing oncology panel, somatic or germline variant detection system |
| Device Trade Name: | FoundationOne®CDx (F1CDx) |
| Device Procode: | PQP |
| Applicant's Name and Address: | Foundation Medicine, Inc.<br>150 Second Street,<br>Cambridge, MA 02141 |
| Date(s) of Panel Recommendation: | None |
| Premarket Approval Application (PMA) Number: | P170019/S014 |
| Date of FDA Notice of Approval: | [June 7, 2022] |

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

The current supplement was submitted to expand the intended use of F1CDx to include companion diagnostic indications for *ROS1* fusions in NSCLC patients and *NTRK1*, *NTRK2*, *NTRK3* fusions in solid tumor patients who may benefit from treatment with Rozlytrek® (entrectinib).

## II. INDICATIONS FOR USE

FoundationOne®CDx (F1CDx) is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology for detection of substitutions, insertion and deletion alterations (indels) and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may

benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

**Table 1. Companion diagnostic indications**

| **Indication** | **Biomarker** | **Therapy** |
|---|---|---|
| Non-small cell lung cancer (NSCLC) | *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations | EGFR tyrosine kinase inhibitors (TKI) approved by FDA* |
| | *EGFR* exon 20 T790M alterations | TAGRISSO® (osimertinib) |
| | *ALK* rearrangements | ALECENSA® (alectinib), ALUNBRIG® (brigatinib) XALKORI® (crizotinib), or ZYKADIA® (ceritinib) |
| | *BRAF* V600E | TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib) |
| | *MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping | TABRECTA™ (capmatinib) |
| | *ROS1* fusions | ROZLYTREK® (entrectinib) |
| Melanoma | *BRAF* V600E | BRAF Inhibitors approved by FDA* |
| | *BRAF* V600E and V600K | MEKINIST® (trametinib) or<br><br>BRAF/MEK Inhibitor Combinations approved by FDA* |
| | *BRAF* V600 mutation-positive | TECENTRIQ® (atezolizumab) in combination with COTELLIC® (cobimetinib) and ZELBORAF® (vemurafenib) |
| Breast cancer | *ERBB2* (HER2) amplification | HERCEPTIN® (trastuzumab), KADCYLA® (ado-trastuzumab-emtansine), or<br>PERJETA® (pertuzumab) |
| | *PIK3CA* C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations | PIQRAY® (alpelisib) |
| Colorectal cancer | *KRAS* wild-type (absence of mutations in codons 12 and 13) | ERBITUX® (cetuximab) |

| Indication | Biomarker | Therapy |
|---|---|---|
| | *KRAS* wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild-type (absence of mutations in exons 2, 3, and 4) | VECTIBIX® (panitumumab) |
| Ovarian cancer | *BRCA1/2* alterations | LYNPARZA® (olaparib) or RUBRACA® (rucaparib) |
| Cholangiocarcinoma | *FGFR2* fusions and select rearrangements | PEMAZYRE® (pemigatinib) or TRUSELTIQ™ (infigratinib) |
| Prostate cancer | Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations | LYNPARZA® (olaparib) |
| Solid tumors | TMB $\geq$ 10 mutations per megabase | KEYTRUDA® (pembrolizumab) |
| | *NTRK1/2/3* fusions | ROZLYTREK® (entrectinib) or VITRAKVI® (larotrectinib) |
| | MSI-High | KEYTRUDA® (pembrolizumab) |

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

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

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

## III. CONTRAINDICATIONS

There are no known contraindications.

## IV. WARNINGS AND PRECAUTIONS

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

## V. DEVICE DESCRIPTION

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

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

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

| ABL1 | BRAF | CDKN1A | EPHA3 | FGFR4 | IKZF1 | MCL1 | NKX2-1 | PMS2 | RNF43 | TET2 |
|---|---|---|---|---|---|---|---|---|---|---|
| ACVR1B | BRCA1 | CDKN1B | EPHB1 | FH | INPP4B | MDM2 | NOTCH1 | POLD1 | ROS1 | TGFBR2 |
| AKT1 | BRCA2 | CDKN2A | EPHB4 | FLCN | IRF2 | MDM4 | NOTCH2 | POLE | RPTOR | TIPARP |
| AKT2 | BRD4 | CDKN2B | ERBB2 | FLT1 | IRF4 | MED12 | NOTCH3 | PPARG | SDHA | TNFAIP3 |
| AKT3 | BRIP1 | CDKN2C | ERBB3 | FLT3 | IRS2 | MEF2B | NPM1 | PPP2R1A | SDHB | TNFRSF14 |
| ALK | BTG1 | CEBPA | ERBB4 | FOXL2 | JAK1 | MEN1 | NRAS | PPP2R2A | SDHC | TP53 |
| ALOX12B | BTG2 | CHEK1 | ERCC4 | FUBP1 | JAK2 | MERTK | NT5C2 | PRDM1 | SDHD | TSC1 |
| AMER1 | BTK | CHEK2 | ERG | GABRA6 | JAK3 | MET | NTRK1 | PRKAR1A | SETD2 | TSC2 |
| APC | C11orf30 | CIC | ERRFI1 | GATA3 | JUN | MITF | NTRK2 | PRKCI | SF3B1 | TYRO3 |
| AR | CALR | CREBBP | ESR1 | GATA4 | KDM5A | MKNK1 | NTRK3 | PTCH1 | SGK1 | U2AF1 |
| ARAF | CARD11 | CRKL | EZH2 | GATA6 | KDM5C | MLH1 | P2RY8 | PTEN | SMAD2 | VEGFA |
| ARFRP1 | CASP8 | CSF1R | FAM46C | GID4 (C17orf39) | KDM6A | MPL | PALB2 | PTPN11 | SMAD4 | VHL |
| ARID1A | CBFB | CSF3R | FANCA | GNA11 | KDR | MRE11A | PARK2 | PTPRO | SMARCA4 | WHSC1 |
| ASXL1 | CBL | CTCF | FANCC | GNA13 | KEAP1 | MSH2 | PARP1 | QKI | SMARCB1 | WHSC1L1 |

| *ATM* | *CCND1* | *CTNNA1* | *FANCG* | *GNAQ* | *KEL* | *MSH3* | *PARP2* | *RAC1* | *SMO* | *WT1* |
|---|---|---|---|---|---|---|---|---|---|---|
| *ATR* | *CCND2* | *CTNNB1* | *FANCL* | *GNAS* | *KIT* | *MSH6* | *PARP3* | *RAD21* | *SNCAIP* | *XPO1* |
| *ATRX* | *CCND3* | *CUL3* | *FAS* | *GRM3* | *KLHL6* | *MST1R* | *PAX5* | *RAD51* | *SOCS1* | *XRCC2* |
| *AURKA* | *CCNE1* | *CUL4A* | *FBXW7* | *GSK3B* | *KMT2A (MLL)* | *MTAP* | *PBRM1* | *RAD51B* | *SOX2* | *ZNF217* |
| *AURKB* | *CD22* | *CXCR4* | *FGF10* | *H3F3A* | *KMT2D (MLL2)* | *MTOR* | *PDCD1* | *RAD51C* | *SOX9* | *ZNF703* |
| *AXIN1* | *CD274* | *CYP17A1* | *FGF12* | *HDAC1* | *KRAS* | *MUTYH* | *PDCD1LG2* | *RAD51D* | *SPEN* | |
| *AXL* | *CD70* | *DAXX* | *FGF14* | *HGF* | *LTK* | *MYC* | *PDGFRA* | *RAD52* | *SPOP* | |
| *BAP1* | *CD79A* | *DDR1* | *FGF19* | *HNF1A* | *LYN* | *MYCL* | *PDGFRB* | *RAD54L* | *SRC* | |
| *BARD1* | *CD79B* | *DDR2* | *FGF23* | *HRAS* | *MAF* | *MYCN* | *PDK1* | *RAF1* | *STAG2* | |
| *BCL2* | *CDC73* | *DIS3* | *FGF3* | *HSD3B1* | *MAP2K1* | *MYD88* | *PIK3C2B* | *RARA* | *STAT3* | |
| *BCL2L1* | *CDH1* | *DNMT3A* | *FGF4* | *ID3* | *MAP2K2* | *NBN* | *PIK3C2G* | *RB1* | *STK11* | |
| *BCL2L2* | *CDK12* | *DOT1L* | *FGF6* | *IDH1* | *MAP2K4* | *NF1* | *PIK3CA* | *RBM10* | *SUFU* | |
| *BCL6* | *CDK4* | *EED* | *FGFR1* | *IDH2* | *MAP3K1* | *NF2* | *PIK3CB* | *REL* | *SYK* | |
| *BCOR* | *CDK6* | *EGFR* | *FGFR2* | *IGF1R* | *MAP3K13* | *NFE2L2* | *PIK3R1* | *RET* | *TBX3* | |
| *BCORL1* | *CDK8* | *EP300* | *FGFR3* | *IKBKE* | *MAPK1* | *NFKBIA* | *PIM1* | *RICTOR* | *TEK* | |

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

| *ALK*<br>*introns 18, 19* | *BRCA1*<br>*introns 2, 7, 8, 12, 16, 19, 20* | *ETV4*<br>*introns 5, 6* | *EZR*<br>*introns 9-11* | *KIT*<br>*intron 16* | *MYC*<br>*intron 1* | *NUTM1*<br>*intron 1* | *RET*<br>*introns 7-11* | *SLC34A2*<br>*intron 4* |
|---|---|---|---|---|---|---|---|---|
| *BCL2*<br>*3'UTR* | *BRCA2*<br>*intron 2* | *ETV5*<br>*introns 6, 7* | *FGFR1*<br>*intron 1, 5, 17* | *KMT2A (MLL)*<br>*introns 6-11* | *NOTCH2*<br>*intron 26* | *PDGFRA*<br>*introns 7, 9, 11* | *ROS1*<br>*introns 31-35* | *TERC*<br>*ncRNA* |
| *BCR*<br>*introns 8, 13, 14* | *CD74*<br>*introns 6- 8* | *ETV6*<br>*introns 5, 6* | *FGFR2*<br>*intron 1, 17* | *MSH2*<br>*intron 5* | *NTRK1*<br>*introns 8-10* | *RAF1*<br>*introns 4-8* | *RSPO2*<br>*intron 1* | *TERT*<br>*Promoter* |
| *BRAF*<br>*introns 7-10* | *EGFR*<br>*introns 7, 15, 24-27* | *EWSR1*<br>*introns 7-13* | *FGFR3*<br>*intron 17* | *MYB*<br>*intron 14* | *NTRK2*<br>*Intron 12* | *RARA*<br>*intron 2* | *SDC4*<br>*intron 2* | *TMPRSS2*<br>*introns 1- 3* |

**Test Output**

The output of the test includes:

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

Category 2: Cancer Mutations with Evidence of Clinical Significance

Category 3: Cancer Mutations with Potential Clinical Significance

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

**Test Kit Contents**

The test includes a sample shipping kit, which is sent to ordering laboratories. The shipping kit contains the following components:

- Specimen Preparation Instructions
- Shipping Instructions
- Return Shipping Label

**Instruments**

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

**Table 4. Instruments for use with the F1CDx assay**

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

**Test Process**

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

**A. Specimen Collection and Preparation**

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

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

**B. DNA Extraction**

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

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

**C. Library Construction**

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

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

**D. Hybrid Capture**

Hybrid Capture (HC) begins with normalization of each library to 500-2000 ng. Normalized samples then undergo solution hybridization which is performed using a > 50-fold molar excess of a pool of individually synthesized 5′-biotinylated DNA 120 bp oligonucleotides. The baits target ~1.8 Mb of the human genome including all coding exons of 309 cancer-related genes, introns or non-coding regions of 35 genes, plus > 3,500 single nucleotide polymorphisms (SNPs) located throughout the genome. Baits are designed by tiling overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; SNP targets are allocated one bait each. Intronic baits are filtered for repetitive elements[2] as defined by the UCSC Genome RepeatMasker track.

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

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

**E. Sequencing**

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

**F. Sequence Analysis**

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

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

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

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

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

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

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

To determine a patient's MSI status, F1CDx employs a fraction based (FB) MSI algorithm to categorize a tumor specimen as MSI-High (MSI-H) or microsatellite stable (MSS). The FB-MSI algorithm calculates the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score) based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The final fraction

unstable loci score is calculated as the number of unstable microsatellite loci divided by the number of evaluable microsatellite loci. Two FB-MSI score thresholds are applied to classify a tumor specimen as having MSI-H or MSS status. MSI-H status is reported for patients with solid tumors whose samples have FB-MSI scores ≥ 0.0124 while MSS status is reported for patients with solid tumors whose samples have FB-MSI scores ≤ 0.0041. Per the F1CDx assay, a patient whose tumor has an MSI-H score ≥ 0.0124 is reported as eligible for treatment with KEYTRUDA. For patients with solid tumors whose samples have FB-MSI scores >0.0041 and <0.0124, an MSI "Cannot be Determined" result is reported. Patients with this result should be re-tested with a validated orthogonal (alternative) method as these MSI scores represent a range of scores with low reliability. Patients with solid tumors may also receive an MSI status reported as MSI-Cannot Be Determined due to a quality control (QC) failure. Patients with this result should consider re-testing with FoundationOneCDx or an orthogonal (alternative) method, if clinically appropriate.

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

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

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

## G. Report Generation

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

## H. Internal Process Controls Related to the System

**Positive Control**

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

**Sensitivity Control**

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

**Negative Control**

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

## I. Variant Classification

**Biomarker Rules for SNVs and indels that lead to *MET* exon 14 skipping**

An SNV or indel in *MET* shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:

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

3. Base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3′ boundary of *MET* exon 14.

**Homologous Recombination Repair (HRR) Genes**

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

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

**Table 5. Mutation types identified in the HRR genes**

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

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

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

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

| *ATM* | *BRCA1* | *BRCA2* |
|---|---|---|
| M1T | M1V | M1R |
| R2032K | M1I | M1I |
| R2227C | C61G | V159M |
| R2547_S2549del | C64Y | V211L |
| G2765S | R71G | V211I |
| R2832C | R71K | R2336P |
| S2855_V2856delinsRI (annotated as S2855_V2856>RI) | R1495M | R2336H |
| R3008C | E1559K | |
| R3008H | D1692N | |
| 8418+5_8418+8delGTGA or 8418+1_8418+4delGTGA | D1692H | |
| | R1699W | |
| | A1708E | |
| | G1788V | |

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

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

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

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

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

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

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

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

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

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

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

## VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in Table 1 of the F1CDx intended use statement. The approved CDx tests are listed in Table 7, below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-

companion-diagnostic-devices-vitro-and-imaging-tools. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

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

| | Device | Company | Technology | Therapy | Indication |
|---|---|---|---|---|---|
| ***HER2-Amplification*** | PathVysion HER-2 DNA Probe Kit | Abbott Molecular, Inc. | FISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | PATHWAY Anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody | Ventana Medical Systems, Inc. | IHC | HERCEPTIN (trastuzumab) | Breast cancer |
| | InSite HER-2/neu Kit | Biogenex Laboratories, Inc. | IHC | HERCEPTIN (trastuzumab) | Breast cancer |
| | SPOT-Light HER2 CISH Kit | Life Technologies, Inc. | CISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | Bond Oracle HER2 IHC System | Leica Biosystems | IHC | HERCEPTIN (trastuzumab) | Breast cancer |
| | HER2 CISH pharmDx Kit | Dako Denmark A/S | CISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | INFORM HER2 Dual ISH DNA Probe Cocktail | Ventana Medical Systems, Inc. | Dual ISH | HERCEPTIN (trastuzumab) | Breast cancer |
| | HercepTest | Dako Denmark A/S | IHC | HERCEPTIN (trastuzumab)<br>PERJETA (pertuzumab)<br>KADCYLA (ado-trastuzumab emtansine) | Breast cancer<br>Gastric or Gastroesophageal junction adenocarcinoma |
| | HER2 FISH pharmDx Kit | Dako Denmark A/S | FISH | HERCEPTIN (trastuzumab)<br>PERJETA (pertuzumab)<br>KADCYLA (ado-trastuzumab emtansine) | Breast cancer<br>Gastric or Gastroesophageal junction adenocarcinoma |
| ***BRAF-V600E and V600K*** | THxID BRAF Kit | bioMerieux | PCR | MEKINIST (tramatenib) | Melanoma |
| | cobas 4800 BRAF V600 Mutation Test | Roche Molecular Systems, Inc. | PCR | COTELLIC (cobimetinib)<br>ZELBORAF (vemurafenib) | Melanoma |
| ***BRAF-V600E*** | cobas 4800 BRAF V600 Mutation Test | Roche Molecular Systems, Inc. | PCR | ZELBORAF (vemurafenib) | Melanoma |
| | THxID BRAF Kit | bioMerieux | PCR | TAFINLAR (dabrafenib) | Melanoma |
| | Oncomine Dx Target Test | Life Technologies, Inc. | NGS | TAFINLAR (dabrafenib)<br>MEKINIST (trametinib) | NSCLC |
| | *therascreen* BRAF V600E RGQ PCR Kit | QIAGEN | PCR | BRAFTOVI (encorafenib)<br>Erbitux (cetuximab) | CRC |
| ***NRAS*** | Praxis Extended RAS Panel | Illumina, Inc. | NGS | VECTIBIX (panitumumab) | CRC |

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

| | Device | Company | Technology | Therapy | Indication |
|---|---|---|---|---|---|
| ***KRAS*** | **cobas** KRAS Mutation Test | Roche Molecular Systems, Inc. | PCR | ERBITUX (cetuximab)<br>VECTIBIX (panitumumab) | CRC |
| | *therascreen* KRAS RGQ PCR Kit | QIAGEN | PCR | ERBITUX (cetuximab)<br>VECTIBIX (panitumumab) | CRC |
| | Praxis Extended RAS Panel | Illumina, Inc. | NGS | VECTIBIX (panitumumab) | CRC |
| ***ALK – fusion*** | Vysis ALK Break Apart FISH Probe Kit | Abbott Molecular, Inc. | FISH | XALKORI (crizotinib) | NSCLC |
| | ALK (D5F3) CDx Assay | Ventana Medical Systems, Inc. | IHC | XALKORI (crizotinib) | NSCLC |
| ***EGFR – Exon 19 deletions & L858R*** | **cobas** EGFR Mutation Test v2 | Roche Molecular Systems, Inc. | PCR | TARCEVA (erlotinib)<br>TAGRISSO (osimertinib)<br>IRESSA (gefitinib) | NSCLC |
| | *therascreen* EGFR RGQ PCR Kit | QIAGEN | PCR | GILOTRIF (afatinib)<br>IRESSA (gefitinib) | NSCLC |
| | Oncomine Dx Target Test | Life Technologies, Inc. | NGS | IRESSA (gefitinib) | NSCLC |
| ***EGFR T790M*** | **cobas** EGFR Mutation Test v2 | Roche Molecular Systems, Inc. | PCR | TAGRISSO (osimertinib) | NSCLC |
| ***BRCA1/2*** | FoundationFocus CDx*BRCA* | Foundation Medicine, Inc. | NGS | RUBRACA (rucaparib) | Advanced ovarian cancer |
| | BRACAnalysis CDx | Myriad Genetic Laboratories, Inc. | NGS | LYNPARZA (olaparib)<br><br>LYNPARZA (olaparib) - treatment/maintenance<br><br>TALZENNA (talazoparib) | Breast, pancreatic, and prostate cancers<br><br>Ovarian cancer<br><br>Breast cancer |
| | Myriad myChoice® CDx | Myriad Genetic Laboratories, Inc. | NGS | ZEJULA (niraparib) or Lynparza (olaparib) | Ovarian cancer |
| ***PIK3CA*** | *therascreen* PIK3CA RGQ PCR Kit | QIAGEN | PCR | PIQRAY (alpelisib) | Breast cancer |
| ***ROS1*** | Oncomine Dx Target Test | Life Technologies, Inc. | NGS | XALKORI (crizotinib) | NSCLC |

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

## VII. MARKETING HISTORY

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

The F1CDx Premarket Approval (PMA) was originally approved on November 30, 2017 by FDA (P170019) and is commercially available in the U.S. since March 30, 2018. The approved PMA supplements that affected the Intended Use are listed in Table 8.

**Table 8: Marketing History**

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

| Submission No. | Date of Approval | Biomarker/Update | Patient Population | Drug |
|---|---|---|---|---|
| P170019/S022 | July 21, 2021 | Additional variants to *BRCA1* and *BRCA2* | Ovarian Cancer | LYNPARZA® (olaparib) or RUBRACA® (rucaparib) |
| | | Additional variants to *BRCA1*, *BRCA2* and *ATM* | Prostate Cancer | LYNPARZA® (olaparib) |
| P170019/S023 | June 30, 2021 | *ALK* Rearrangements | Non-Small Cell Lung Cancer | Alunbrig® (brigatinib) |
| P170019/S025 | November 10, 2021 | *BRAF* V600E Alterations | Melanoma | BRAF Inhibitor Monotherapy Group Claim |
| | | *BRAF* V600E or V600K Alterations | Melanoma | BRAF/MEK Inhibitor Combination Group Claim |
| P170019/S029 | February 18, 2022 | Microsatellite Instability High (MSI-H) Status | Solid Tumors | KEYTRUDA® (Pembrolizumab) |
| P170019/S030 | January 19, 2022 | *BRAF* V600 Mutation-Positive | Unresectable Or Metastatic Melanoma | Atezolizumab (Tecentriq) In Combination with Cobimetinib and Vemurafenib |
| P170019/S033 | March 16, 2022 | *EGFR* Exon 19 Deletions or *EGFR* Exon 21 L858R Mutations | Non-Small Cell Lung Cancer | Any One of The FDA-Approved EGFR Tyrosine Kinase Inhibitors (TKI) |

## VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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

## IX. SUMMARY OF NONCLINICAL STUDIES

### A. Laboratory Studies

The primary evidence for supporting the performance of F1CDx in detecting *NTRK1, NTRK2*, and *NTRK3* fusions in solid tumor patients, hereafter referred to as *NTRK1/2/3* fusions and *ROS1* fusions in NSCLC patients was from the data presented using intended use specimens across all validation studies. In addition to the existing platform-level validation results (P170019), analytical accuracy, within-laboratory (intermediate) precision, and limit of detection (LoD) studies, as well as *in silico* analyses of real-world data from the Foundation Medicine clinical database were conducted to support the indication for *NTRK1/2/3* fusions and *ROS1* fusions.

For F1CDx platform-level validation (P170019), performance characteristics were established using DNA derived from a wide range of FFPE tissue types; tissue types associated with CDx indications were included in each study. Each study included CDx variants as well as a broad range of representative alteration types (substitution, insertion and deletion, copy number alterations, rearrangements) in various genomic contexts across several genes.

1. **Analytical Accuracy/Concordance**
   a. **Comparison to an Orthogonal Method for *NTRK1/2/3* fusions**
   The premarket data to support the analytical accuracy of *NTRK1/2/3* fusions was provided in P170019/S017. Refer to the Summary of Safety and Effectiveness Data P170019/S017 (Section IX.A.1.a) for F1CDx analytical accuracy determination of *NTRK1/2/3* fusions.

   b. **Comparison to an Orthogonal Method for *ROS1* fusions**
   Analytical concordance of F1CDx for detecting *ROS1* fusion was determined with 188 NSCLC samples (84 putative *ROS1*-positive and 103 putative *ROS1*-negative) tested by the F1CDx assay versus an externally-validated next generation sequencing assay (evNGS). One sample processing failure was observed due to failed LC QC by F1CDx. Fourteen (14) samples were determined to be of low quality by the evNGS.

   A contingency table reporting the results of the study is presented in Table 9.

**Table 9 Concordance summary for *ROS1* fusions by F1CDx and the evNGS**

| | | evNGS | | | |
|---|---|---|---|---|---|
| | | ***ROS1* positive** | ***ROS1* negative** | **Invalid** | **Total** |
| **F1CDx** | ***ROS1* positive** | 79 | 2 | 3 | 84 |
| | ***ROS1* negative** | 8 | 84 | 11 | 103 |
| | **Invalid** | 0 | 1 | 0 | 1 |
| | **Total** | 87 | 87 | 14 | 188 |

Measures of analytical concordance were calculated and presented in Table 10.

**Table 10. Agreement measures of analytical comparison between the detection of *ROS1* fusions by F1CDx and the evNGS excluding invalid calls**

| Agreement Measures | % Agreement | Two-Sided 95% CI |
|---|---|---|
| PPA | 90.80% (79/87) | [82.89%, 95.27%][1] |
| NPA | 97.67% (84/86) | [91.91%, 99.36%][1] |
| PPV | 97.53% (79/81) | [91.44%, 99.32%][2] |
| NPV | 91.30% (84/92) | [83.77%, 95.53%][2] |

[1] Two-sided 95% CI is calculated by the Wilson Score Method.
[2] Predicted values are calculated for the analytical comparison study prevalence of 50.3%, (79+8)/(79+8+2+84)=87/173.

The positive percent agreement (PPA) and negative percent agreement (NPA) were observed to be 90.8% and 97.7%, respectively, as summarized in Table 10.

Since the PPA and NPA were calculated without adjusting for the distribution of samples enrolled, the estimate of PPA and NPA may be subject to potential bias. The positive predictive value (PPV) and negative predictive value (NPV) were also estimated using a study prevalence of 50.3%.

2. **Analytical Sensitivity**
   a. **Limit of Blank (LoB)**
   The LoB was confirmed using DNA from nine (9) samples from patients with solid tumors that were biomarker negative. These comprised samples from the following diseases and specimen types in parenthesis: breast invasive ductal carcinoma (breast), colon adenocarcinoma (colon), stomach adenocarcinoma (stomach), pediatric brain medulloblastoma (brain), pleura mesothelioma (pleura), small intestine neuroendocrine carcinoma (small intestine), pancreas carcinoma (Whipple resection), salivary gland adenoid cystic carcinoma (lung), and thyroid papillary carcinoma (thyroid). Six additional NSCLC samples known to be biomarker negative for fusions in *ROS1* were also tested.

   Each biomarker-negative sample was assessed in replicates of seven (7), resulting in a total of 63 sample aliquots to assess LoB. The number of replicate samples with incorrect calls was counted and converted into a percentage with respect to the number of all replicate LoB samples and reported as the percentage of false-positive results. If the percentage of false-positive results did not exceed 5% (type I error risk a=0.05), then at least 95% of the result was zero and LoB = zero was confirmed. For sample aliquots evaluated to assess LoB, fusions in *ROS1* were not observed in any of the 60 replicates from NSCLC samples and the *NTRK1/2/3* genes were not reported in any of the 63 sample aliquots. The percent of samples with incorrect calls was zero and confirmed the LoB = zero.

   b. **Limit of Detection (LoD) for *NTRK1/2/3 Fusions***
   A combination of premarket data and issuance of a condition of approval study to support the LoD of *NTRK1/2/3* fusions was provided in P170019/S017. Refer to the Summary of Safety and Effectiveness Data P170019/S017 (Sections IX.A.2.b and XIII) for F1CDx LoD determination of *NTRK1/2/3* fusions.

   c. **Limit of Detection (LoD) for *ROS1 Fusions***
   F1CDx LoD for the detection of *ROS1* fusions was investigated by assessing three (3) samples listed in Table 11.

**Table 11. Samples assessed in LoD study for the detection of *ROS1* fusions**

| Sample[1] | Target Gene | Partner Gene | Fusion Partner or Alteration Description | Disease Indication (Specimen Site) |
|---|---|---|---|---|
| 1 | *ROS1* | *CD74* | 5′-CD74(ex1-6 NM_004355)-*ROS1*(ex34-43 NM_002944) | Lung Adenocarcinoma |
| 2 | *ROS1* | *EZR* | 5′-EZR(ex1-9 NM_003379)-*ROS1*(ex33-43 NM_002944) | Lung Adenocarcinoma |
| 3 | *ROS1* | *SLC34A2* | 5′-SLC34A2(ex1-13 UTR NM_006424)-*ROS1*(ex33-43 NM_002944) | Lung Adenocarcinoma |

[1] All samples were CDx positive for *ROS1* fusions.

Each sample was assessed at five targeted tumor purity levels (2.5%, 5%, 10%, 15%, and 20%). Twenty replicates were assessed for each dilution level other than the 20% level, where 14 replicates were run.

A summary of the LoD results based on reads is summarized in Table 12.

**Table 12. Summary of LoD analysis for *ROS1* fusions**

| Sample[1] | Target *ROS1* Gene | Partner Gene | *ROS1* LoD (mean %Tumor Purity)[2] | *ROS1* LoD (# of chimeric reads)[2] |
|---|---|---|---|---|
| 1 | *ROS1* | *CD74* | 2.88% | 10.53 |
| 2 | *ROS1* | *EZR* | 5.71% | 11.85 |
| 3 | *ROS1* | *SLC34A2* | 5.79% | 9.10 |

[1] All samples were CDx positive for *ROS1* fusions.
[2] LoD calculations were based on the hit rate approach; defined as the lowest level with ≥ 95% hit rate (worst scenario).

The final LoD for *ROS1* fusions presented was determined as the highest LoD observed per gene. The *ROS1* LoD was determined to be 5.8% tumor purity and 11.85 for chimeric reads.

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

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

5. **Precision and Reproducibility**
   a. **Within-Laboratory (Intermediate) Precision of *NTRK* Fusions**
      Refer to the Summary of Safety and Effectiveness Data P170019/S017 (Section IX.A.5.a) for F1CDx precision determination for *NTRK1/2/3* fusions.

b. **Within-Laboratory (Intermediate) Precision for *ROS1* Fusions**
To support the F1CDx performance characteristics for the detection of *ROS1* fusions, the within-laboratory (intermediate) precision of two (2) samples from patients with lung adenocarcinomas were evaluated; refer to Table 13 for the samples evaluated in this study. These samples evaluated had computational tumor purity ranging from 13.5% to 23.9%. The cut-off for a passing sample based on computational tumor purity is 20% and samples evaluated in the precision study included samples near the computational tumor purity input specification of 20% tumor purity for the F1CDx assay (see Table 13 and Table 14).

**Table 13**. **Samples Evaluated in the Within-Laboratory (Intermediate) Precision Study.**

| Sample[1] | Target Gene | Partner Gene | Fusion Partner or Alteration Description | Disease Ontology |
|---|---|---|---|---|
| 1 | *ROS1* | *CD74* | 5′-CD74(ex1-6 NM_004355)-*ROS1*(ex34-43 NM_002944) | Lung Adenocarcinoma |
| 2 | *ROS1* | *CD74* | 5′-CD74(ex1-6 NM_004355)-*ROS1*(ex34-43 NM_002944) | Lung Adenocarcinoma |

[1] All samples were CDx positive for *ROS1* fusions.

For the assessment of repeatability, each sample was divided into either 24 or 36 aliquots, with 12 aliquots processed in duplicate or triplicate under the same conditions. The conditions were applied on a plate-level and included the same operator, same day, same reagent lot and same sequencer. The result was considered concordant if all duplicates or triplicates matched the majority call for all aliquots of that sample. Table 14 summarizes the repeatability statistics across samples evaluated.

**Table 14. Repeatability of variant calling.**

| Sample | Mean TP | Mean Reads | Fold LoD based on TP | Target *NTRK* Gene | Partner Gene | # Agree | Total # | Agreement (95% CI*) |
|---|---|---|---|---|---|---|---|---|
| 1 | 13.5% | 31.31 | 2.33x | *ROS1* | *CD74* | 12 | 12 | 100.00% (75.75%, 100.00%) |
| 2 | 23.9% | 42.33 | 4.13x | *ROS1* | *CD74* | 12 | 12 | 100.00% (75.75%, 100.00%) |

Abbreviation: TP: tumor purity
* Two-sided 95% CI is calculated by the Wilson Score Method.

Reproducibility in the two (2) samples was evaluated by processing aliquots originating from the same source DNA sample, under conditions where one factor was changed at a time (e.g., reagent lot and sequencers). The result was

considered concordant when the aliquot matched the targeted and majority call of all 36 replicates. Table 15 summarizes the reproducibility statistics across the two (2) samples evaluated.

**Table 15. Reproducibility of variant calling**

| Sample | Mean TP | Mean Reads | Fold LoD based on TP | Target *NTRK* Gene | Partner Gene | # Agree | Total # | Agreement (95% CI*) |
|---|---|---|---|---|---|---|---|---|
| 1 | 13.5% | 31.31 | 2.33x | *ROS1* | *CD74* | 36 | 36 | 100.00% (90.36%, 100.00%) |
| 2 | 23.9% | 42.33 | 4.13x | *ROS1* | *CD74* | 36 | 36 | 100.00% (90.36%, 100.00%) |

Abbreviations: TP: tumor purity
* Two-sided 95% CI is calculated by the Wilson Score Method.

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

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

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

**8. General Laboratory Equipment and Reagent Evaluation**

**a. DNA Amplification**
Identical reagents with the same specifications are used following the same protocols for both the FoundationFocus $CDx_{BRCA}$ assay and F1CDx. For DNA amplification performance data, see the Summary of Safety and Effectiveness Data for P160018.

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

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

### B. Animal Studies
No animal studies were conducted using the F1CDx assay.

### C. Additional Studies
No additional studies were conducted using the F1CDx assay.

## X. SUMMARY OF PRIMARY CLINICAL STUDY

The reasonable assurance of safety and effectiveness for F1CDx for detection of *NTRK1*, *NTRK2* and *NTRK3* fusions in patients with solid tumors who may benefit from treatment with ROZLYTREK® (entrectinib), was established through a clinical bridging study using clinical specimens from patients enrolled in the ALKA-372-001 (ALKA), RXDX-101-01 (STARTRK-1), and RXDX-101-02 (STARTRK-2), as well as *NTRK* fusion negative samples from the FMI archives. The clinical efficacy analysis was performed by analyzing the concordance between F1CDx and the enrollment clinical trial assays (CTAs), followed by the imputation of the missing F1CDx result, and finally determining the clinical outcome of the *ROS1* or *NTRK1/2/3*-positive population identified with F1CDx.

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* molecular alterations. 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 NSCLC patients with fusions in the *ROS1* gene.

A summary of the clinical study is presented below.

### A. FoundationOne CDx Clinical Bridging Studies for *ROS1*
The clinical effectiveness of F1CDx for detecting *ROS1* fusions in NSCLC patients who may benefit from treatment with entrectinib was demonstrated in a retrospective analysis of specimens from patients enrolled in clinical trials ALKA-372-001 (ALKA), RXDX-101-01 (STARTRK-1), and RXDX-101-02 (STARTRK-2).

A bridging study was conducted to assess: 1) concordance between the local clinical trial assays (CTAs) and F1CDx; and 2) estimate the overall response rate (ORR) in

the efficacy population (CDx-positive population) for entrectinib treatment among clinical study participants whose tumor samples met the biomarker criteria outlined in Section I, as determined by retrospective testing with the F1CDx.

1. **Clinical Bridging Study Design for *ROS1***
   The clinical efficacy analysis was performed by analyzing the concordance between F1CDx and the enrollment CTAs, followed by the imputation of the missing F1CDx result to determine the clinical efficacy of entrectinib treatment for the *ROS1*-fusion positive population identified with F1CDx.

   The *ROS1* clinical efficacy population (n=51) consisted of nine (9) patients from ALKA, seven (7) from STARTRK-1, and 35 patients from STARTRK-2. *ROS1* 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* positivity using the study clinical trial assay (CTA). The ORR of the *ROS1*-positive patient population used to support approval of ROZLYTREK® (entrectinib) was 78%. The 95% Confidence Interval (CI) was [65%, 89%].

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

   Sample Inclusion Criteria
   - Samples must be FFPE blocks or slides, or DNA or TNA derived from NSCLC FFPE blocks or slides.
   - Samples that meet F1CDx processing requirements.

   Exclusion Criteria
   - Lack of clear identification or label on stored patient sample.
   - Blood, other liquid, and fresh-frozen samples were excluded.
   - Any sample that was not derived from NSCLC FFPE.
   - Samples that do not meet F1CDx processing requirements

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

4. **Clinical Endpoints**
   The objectives of the F1CDx clinical study were to:
   1. To estimate agreement between the CTAs and the F1CDx for the detection of *ROS1* fusions in NSCLC fitting the biomarker criteria.
   2. To estimate the ORR for entrectinib treatment among clinical study patients who met *ROS1* fusion biomarker criteria by retrospective testing with the CDx. Clinical efficacy analysis was performed based on patients

with CDx results. Sensitivity analysis included subjects with and without CDx results and evaluated the impact on clinical efficacy for the proportion of subjects who are local CTA negative but CDx positive and therefore not enrolled by the clinical trial. The missing CDx results were imputed in the sensitivity analysis.

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

A total 395 unique samples were evaluated, including 85 clinical trial samples and 310 procured samples. Initially, the clinical bridging study included 51 *ROS1* NSCLC efficacy evaluable samples, as well as 41 additional *ROS1*-positive, *ROS1* inhibitor-naive NSCLC patients with measurable disease who had insufficient follow-up (<12 months) at time of the NDA submission and an additional 67 *ROS1* NSCLC patients, who were enrolled prior to October 31, 2018. In total, clinical outcome data from 159 patients enrolled before October 31, 2018 (based on the May 1, 2019 clinical data cutoff date) were planned for use in the bridging analysis. There were 104 invalid samples due to either quality control failures, insufficient DNA, or lack of informed consent. Ultimately, 85 of these clinical trial samples were available to support the clinical bridging analysis; 16 *ROS1* NSCLC efficacy evaluable samples, 21 additional *ROS1*-positive, *ROS1* inhibitor-naive NSCLC patients with measurable disease who had insufficient follow-up (<12 months) at time of the NDA submission and an additional 48 *ROS1* NSCLC patients who were enrolled prior to October 31, 2018. Of these 85 samples, 55 samples had samples of sufficient quality for testing with F1CDx.

A detailed breakdown of the clinical samples is provided in Table 16. Additional outside clinical trial CTA negative (310 in total samples were collected and retested by F1CDx. Out of the 310 supplemental samples, 245 had valid F1CDx results.

**Table 16. Samples evaluated in clinical bridging study**

| Biomarker Status | Sample Type | Sample Number |
|---|---|---|
| *ROS1* Positive | NDA Population from ALKA, STARTRK-1, and STARTRK-2 | 51 |
| | Consistency Cohort | 41 |
| | Additional Cohort | 67 |
| *ROS1* Negative | Procured FFPE NSCLC tumor tissue | 310 |
| Total | | 469 |

6. **Study Population Demographics and Baseline Parameters**
   Demographics and baseline disease characteristics were well balanced for the 6 evaluated clinical and baseline covariates between the Entrectinib clinical efficacy analysis population, CDx-evaluable and CDx-unevaluable populations. In general, the demographics and disease characteristics for the CDx-evaluable and CDx-unevaluable patients were similar (Table 17).

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

| Population | CTA+ | F1CDx evaluable (CTA+ with valid F1CDx results) | F1CDx non- evaluable (CTA+ without F1CDx results) | p-value comparing the two subsets |
|---|---|---|---|---|
| n | 159 | 46 | 113 | |
| ORR | 67.3% | 63.0% | 69.0% | 0.46 |
| 1. AGE (Mean) | 54.6 | 54.6 | 54.6 | 1.00 |
| Minimum | 20 | 34 | 20 | |
| Q1 | 46 | 45 | 47 | |
| Median | 54 | 53 | 54 | |
| Q3 | 64 | 64 | 63 | |
| Maximum | 86 | 79 | 86 | |
| 2. Sex | | | | 0.72 |
| Male | 55 (34.6%) | 17 (37.0%) | 38 (33.6%) | |
| Female | 104 (65.4%) | 29 (63.0%) | 75 (66.4%) | |
| 3. ECOG | | | | 0.31 |
| 0 | 65 (40.9%) | 19 (41.3%) | 46 (40.7%) | |
| 1 | 78 (49.0%) | 25 (54.3%) | 53 (46.9%) | |
| 2 | 16 (10.1%) | 2 (4.3%) | 14 (12.4%) | |
| 4. RACE | | | | 0.26 |
| Asian | 73 (45.9%) | 22 (47.8%) | 51 (45.1%) | |
| White | 69 (43.4%) | 18 (39.1%) | 51 (45.1%) | |
| Black or African American | 7 (4.4%) | 0 (0%) | 7 (6.2%) | |
| NR* | 10 (6.3%) | 6 (13.1%) | 4 (3.6%) | |
| 5. Smoking History | | | | 0.45 |
| Current | 7 (4.4%) | 1 (2.2%) | 6 (5.3%) | |
| Former | 53 (33.3%) | 13 (28.3%) | 40 (35.4%) | |
| NR* | 99 (62.3%) | 32 (69.6%) | 67 (59.3%) | |
| 6. Any CNS** lesion at baseline | | | | 0.72 |
| Yes | 55 (34.6%) | 17 (37.0%) | 38 (33.6%) | |
| No | 104 (65.4%) | 29 (63.0%) | 75 (66.4%) | |

*NR – Not reported; will not be used in p-value calculation.
**Central nervous system

### 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

There were 306 NSCLC samples available (55 *ROS1*-positive clinical trial samples and 251 *ROS1*-negative procured samples) for the concordance analysis between F1CDx and the enrollment assays. The results of the analysis between CTAs and F1CDx for the detection of *ROS1* fusion is summarized in Table 18.

**Table 18. Concordance for *ROS1* fusion between F1CDx and the CTAs**

| | | CTAs | | |
|---|---|---|---|---|
| | | **Detected** | **Not Detected** | **Total** |
| **F1CDx** | **Detected** | 34 | 2 | 36 |
| | **Not Detected** | 12 | 243 | 255 |
| | **Invalid** | 39 | 65 | 104 |
| | **Total** | 85 | 310 | 395 |
| **Agreement Statistics Excluding Invalid Results** | | PPA<br>73.9% (34/46)<br>95% CI*: (59.7%, 84.4%) | NPA<br>99.2% (243/245)<br>95% CI*: (97.1%, 99.8%) | |
| **Percent Invalid** | | 45.9% (39/85)<br>95% CI*: (35.7%, 56.4%) | 21.0% (65/310)<br>95% CI*: (16.8%, 25.8%) | |

*Calculated with Wilson 2-sided 95% CI.

The PPA was 73.9% (34/46) with 95% two-sided confidence interval [59.7%, 84.4%] and NPA was 99.2% (243/245) with 95% two-sided CI of [97.1%, 99.8%] after excluding invalid results.

##### ii. Bridging clinical outcome from CTA to F1CDx

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 67.3% (107/159), (95% CI: 59.4, 74.5). Thirty-four (34) patients (34/46) were CTA+ and exhibited F1CDx *ROS1*-positive results. The ORR for this population was 64.7% (22/34) with Exact 2-sided 95% CI [46.5, 80.3]. Twelve (12) patients were CTA+ but F1CDx *ROS1*-negative. The ORR for this population was 58.3% (7/12) with the Exact 2-sided 95% CI [27.7, 84.8].

One-hundred thirteen (113) patients were CTA+ but without a F1CDx *ROS1* result. The ORR for this population was 69.0% (78/113) with Exact 2-sided 95% CI [59.6, 77.4], as summarized in Table 19.

**Table 19. Efficacy by *ROS1* Status in Biomarker Subgroups**

| **Clinical outcome** | **Total CTA positive population* (N=159)** | **F1CDx positive and CTA positive (N=34)** | **F1CDx negative and CTA positive (N=12)** | **F1CDx result missing and CTA positive (N=113)** |
|---|---|---|---|---|
| **ORR%** [95% CI**] | 67.3% [59.4, 74.5] | 64.7% [46.5, 80.3] | 58.3% [27.7, 84.8] | 69.0% [59.6, 77.4] |
| Complete response | 14 (8.8%) | 3 (8.8%) | 0 (0%) | 11 (9.7%) |
| Partial response | 93 (58.5%) | 19 (55.9%) | 7 (58.3%) | 67 (59.3%) |
| Number of responders | **N=107** | **N=22** | **N=7** | **N=78** |
| **Duration of Response** | | | | |
| Median in months (range) | 9.5 (1.8-42.3) | 10.1 (1.9-24.6) | 9.5 (3.5-24.6) | 9.5 (1.8-42.3) |
| % with duration ≥ 9 months | 61.7% (66/107) | 72.7% (16/22) | 57.1% (4/7) | 59.0% (46/78) |
| % with duration ≥ 12 months | 41.1% (44/107) | 36.4% (8/22) | 42.9% (3/7) | 42.3% (33/78) |
| % with duration ≥ 18 months | 19.6% (21/107) | 4.5% (1/22) | 14.3% (1/7) | 24.4% (19/78) |

***See Table 16 for a description of the total population**
****Exact 2-sided 95% CI reported**

There were 29 CTA-positive participants who also had F1CDx results with partial or complete responses (22 F1CDx+ and 7 F1CDx-). Among them 75.9% (22/29) were positive by F1CDx (95% CI: 56.5, 89.7). There were 17 CTA-positive participants who also had F1CDx results with no responses (34-22=12 and 12-7=5). Among the 17 CTA positive patients who did not respond to entrectinib, only 70.6% (12/17) were positive by F1CDx (95% CI: 44.0, 89.7).

The ORR in F1CDx-positive/CTA-positive participants was 64.7% (22/34), (95% CI: 46.5, 80.3). The ORR in F1CDx-negative/CTA-positive participants was 58.3% (7/12), (95% CI: 27.7, 84.8). The difference in ORR between F1CDx-positive/CTA-positive participants [64.7% (22/34)] and F1CDx-negative/CTA-positive participants [58.3% (7/12)] was 6.4% with 95% CI: (-24.2, 38.2). The small sample size makes it difficult to interpret the efficacy of the F1CDx-/CTA+ population; however, this is being addressed in a post-approval study (See Section XIII).

The similarity of the ORR for the CTA-positive population (n=159) overall (67.3%, 95% CI: 59.4, 74.5) and for those missing a valid F1CDx result (n=113; 69.0%, 95% CI: 59.6, 77.4) suggests no overt imbalance in efficacy effect of entrectinib between patients with or without a valid F1CDx result.

**c. Sensitivity Analysis**

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with missing/invalid CDx results and the missing F1CDx-positive, CTA-negative population that was not enrolled and evaluated by ALKA, STARTRK-1, and STARTRK-2 clinical trial.

Amongst all CTA-positive patients, 71.1% did not have a F1CDx result (113/159).

To evaluate the impact of missing/invalid F1CDx results, the distribution of patients for baseline covariates and disease characteristics was compared among the CTA-positive population, the F1CDx-evaluable/CTA-positive subpopulation, and F1CDx-missing CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable F1CDx (n=113).

The clinical efficacy (ORR) for the F1CDx-positive subjects in the device intended use population was estimated under different assumed scenarios based on observed and imputed F1CDx results.

Sensitivity analysis considering the NPA and assuming different CTA positivity rates in the F1CDx intended use population, which ranged 1-2%, were investigated to assess influence on the efficacy estimated for the intended use, i.e., F1CDx positive subjects. These sensitivity analyses demonstrated the robustness of the clinical efficacy estimate.

Due to the large proportion of missing data additional clinical data will be evaluated post-market to confirm the clinical effectiveness of F1CDx, see section XIII below.

Subgroup Analysis
Response to entrectinib for the F1CDx fusion positive patients was analyzed by *ROS1* fusion partner (Table 20).

**Table 20. The overall response rate for CDx *ROS1* fusion positive patients in the efficacy analysis set by different subgroups**

| Subgroup | Number of Patients (N=34) | Number of patients with CR or PR (N=22) | ORR (%) (95% CI*) |
|---|---|---|---|
| ***ROS1* fusion partner** | | | |
| *CD74-ROS1* | 18 | 14 | 77.8% (52.4%, 93.6%) |
| *EZR-ROS1* | 4 | 4 | 100% |
| *FGD6-ROS1* | 1 | 1 | 100% |
| *LRIG3-ROS1* | 1 | 1 | 100% |
| *SDC4-ROS1* | 4 | 0 | 0% |
| *SLC34A2-ROS1* | 3 | 0 | 0% |
| *TPM3-ROS1* | 1 | 1 | 100% |
| *WNK1-ROS1* | 1 | 1 | 100% |
| *ZCCHC8-ROS1* | 1 | 0 | 0% |

* 95% 2-sided exact CIs were reported when sample size >10

### 8. Pediatric Extrapolation

In this premarket application for *ROS1* 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 CDx Clinical Bridging Studies for *NTRK1, NTRK2, NTRK3*

The clinical effectiveness of F1CDx for detecting *NTRK1, NTRK2,* and *NTRK3* fusions in patients with solid tumors who may benefit from treatment with ROZLYTREK was demonstrated in a retrospective analysis of specimens from patients enrolled in studies ALKA, STARTRK-1, and STARTRK-2 and an additional set of *NTRK1/2/3*- fusion negative tumor tissue FFPE specimens from the FMI archives.

A bridging study was conducted to assess: 1) concordance of results for the *NTRK* gene fusion status between the F1CDx assay and the CTAs used to determine patient

eligibility for enrollment, and 2) the clinical validity of F1CDx in identifying solid tumor patients with *NTRK*1/2/3 fusion positive status for treatment with ROZLYTREK.

1. **Clinical Bridging Study Design**
   The clinical efficacy analysis was performed by analyzing the concordance between F1CDx and the enrollment CTAs, followed by the imputation of the missing F1CDx result to then determine the clinical efficacy of the treatment with ROZLYTREK for the *NTRK* population identified with F1CDx.

   The *NTRK* clinical efficacy population (n=54) consisted of one (1) patient from ALKA, two (2) from STARTRK-1, and 51 patients from STARTRK-2. Patients were enrolled into one of the clinical studies, and testing was performed using one of a number of enrollment CTAs, including NGS assays and FISH. The ORR of the *NTRK*-positive NDA population was 57%. The 95% Confidence Interval (CI) was [43%, 71%].

   The clinical bridging study included 74 CTA-positive samples from patients enrolled across all the trials (ALKA, STARTRK-1, and STARTRK-2 clinical studies), supplemented with 20 additional samples non-NDA samples from the STARTRK-2 trial and 278 commercially procured samples to demonstrate the safety and effectiveness of F1CDx for identification of patients with solid tumors who may be eligible for treatment with ROZLYTREK. Of the 74 CTA *NTRK* fusion positive samples available, 42 samples were not available for retesting with F1CDx. Therefore, there were 32 patient samples from the three clinical trials included in the *NTRK* fusion positive CDx evaluable set. Of the 278 CTA *NTRK* fusion negative samples available, ten samples were not available for retesting.

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

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

The clinical efficacy analysis was performed by analyzing the concordance between F1CDx and the enrollment CTAs, followed by the imputation of the missing F1CDx result to determine the clinical efficacy of the treatment with ROZLYTREK for the *NTRK1/2/3*-positive population identified with F1CDx.

**2. Clinical Inclusion and Exclusion Criteria**

The inclusion/exclusion criteria for selection into the clinical bridging study are:

Sample Inclusion Criteria

- Samples must be FFPE blocks or slides, or DNA or TNA derived from pan-tumor FFPE blocks or slides
- Samples that meet F1CDx processing requirements.

Exclusion Criteria

- Lack of clear identification or label on stored patient sample.
- Blood, other liquid, and fresh-frozen samples were excluded.
- Any sample that was not derived from FFPE.
- Samples that do not meet F1CDx processing requirements

**3. Follow-up Schedule**

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

**4. Clinical Endpoints**

The objectives of the F1CDx clinical study were to:

1. To estimate agreement between the CTAs and the F1CDx for the detection of *NTRK1/2/3* fusions in solid tumor specimens.

2. To estimate the ORR for entrectinib treatment among clinical study patients who met *NTRK*1/2/3 fusion biomarker criteria by retrospective testing with the CDx. The clinical efficacy analysis was based on patients with complete CDx status. Sensitivity analysis included subjects with and without CDx results and evaluated the impact on clinical efficacy for the proportion of subjects who are local CTA negative but CDx positive and therefore not enrolled by the clinical trial. The missing CDx results were imputed in the sensitivity analysis.

**5. Accountability of the PMA Cohort for *NTRK1/2/3***

Of the 74 patients in the PMA cohort, which included 54 patients from the *NTRK* efficacy population and 20 additional patients who were enrolled after the data cutoff. There were 42 samples that were not available for retesting due to lack of consent, or insufficient material. An additional 278 FFPE

samples from procured from commercial sources to demonstrate concordance between F1CDx and the enrollment CTAs. Among the 278 commercially procured samples, a total of five (5) samples were excluded from the bridging analysis. Four (4) samples could not be linked to the CTA sample identification number and one (1) F1CDx sample ID contained two CTA IDs (both data rows were excluded to eliminate any bias in selecting the sample result). In total, 352 solid tumor samples (74 *NTRK*-positive clinical trial samples and 278 *NTRK* status unknown procured samples) were included in the analysis (Table 21).

**Table 21. Samples evaluated in clinical bridging study**

| Biomarker Status | Sample Type | Sample Number |
|---|---|---|
| *NTRK* Positive | NDA population from ALKA, STARTRK-1, STARTRK-2 | 54 |
| | Additional samples (non-NDA population) from STARTRK-2 | 20 |
| *NTRK* Status Unknown | Procured FFPE tumor tissue | 278 |
| Total | | 352 |

All of the 278 procured samples listed in Table 21 were processed first by the study CTA and then tested by the F1CDx assay.

6. **Study Population Demographics and Baseline Parameters**
Demographics and baseline disease characteristics were overall balanced between the entrectinib clinical efficacy analysis population, CDx-evaluable and CDx-unevaluable populations. In general, the demographics and disease characteristics for the CDx-evaluable and CDx-unevaluable patients were similar (Table 22).

**Table 22. Comparison of baseline demographic characteristics between the CDx-evaluable patients and the CDx-unevaluable patients**

| Population | CTA+ | F1CDx evaluable (CTA+ with valid F1CDx results) | F1CDx non-evaluable (CTA+ without F1CDx results) | p-values Comparing the Two Subsets |
|---|---|---|---|---|
| n | 74 | 32 | 42 | |
| ORR | 62.2% | 65.6% | 59.5% | 0.64 |
| AGE (Mean) | 56.5 | 56.3 | 56.7 | 0.94 |
| Minimum | 21 | 21 | 27 | |
| Q1 | 48 | 48.8 | 47.3 | |
| Median | 57 | 58 | 57 | |
| Q3 | 67 | 68.3 | 66 | |
| Maximum | 83 | 83 | 77 | |

| Population | CTA+ | F1CDx evaluable (CTA+ with valid F1CDx results) | F1CDx non-evaluable (CTA+ without F1CDx results) | p-values Comparing the Two Subsets |
|---|---|---|---|---|
| Sex | | | | 0.16 |
| Male | 35 (47.0%) | 12 (37.5%) | 23 (54.8%) | |
| Female | 39 (53.0%) | 20 (62.5%) | 19 (45.2%) | |
| ECOG | | | | 0.76 |
| 0 | 30 (41.0%) | 14 (43.8%) | 16 (38.1%) | |
| 1 | 34 (46.0%) | 13 (40.6%) | 21 (50.0%) | |
| 2 | 10 (14.0%) | 5 (15.6%) | 5 (11.9%) | |
| RACE | | | | 1.00 |
| Asian | 13 (18.0%) | 4 (12.5%) | 9 (21.4%) | |
| White | 52 (70.0%) | 25 (78.1%) | 27 (64.3%) | |
| NR* | 9 (12.0%) | 3 (9.4%) | 6 (14.3%) | |
| Smoking History | | | | 1.00 |
| Current | 8 (11.0%) | 3 (9.4%) | 5 (11.9%) | |
| Former | 21 (28.0%) | 12 (37.5%) | 9 (21.4%) | |
| NR* | 45 (61.0%) | 17 (53.1%) | 28 (66.7%) | |
| Any CNS lesion at baseline | | | | 0.60 |
| Yes | 19 (26.0%) | 9 (28.1%) | 10 (23.8%) | |
| No | 55 (74.0%) | 23 (71.9%) | 32 (76.2%) | |

*NR – Not reported.

## 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, 352 *NTRK* samples (74 *NTRK*-positive clinical trial samples and 278 procured samples) were included in the analysis. Eighty-eight (88) sample failures were observed

during sample processing. One (1) sample from the procured negative sample set was found to be biomarker positive by the CTA, but biomarker negative by F1CDx.

The concordance between CTAs and F1CDx for the detection of *NTRK* is summarized in Table 23.

**Table 23. Concordance for *NTRK* between F1CDx and CTAs excluding invalid results.**

| | | CTAs | | |
|---|---|---|---|---|
| | | **Detected** | **Not Detected** | **Total** |
| **F1CDx** | **Detected** | 21 | 0 | 21 |
| | **Not Detected** | 12 * | 232 | 244 |
| | **Invalid** | 42 | 45 | 87 |
| | **Total** | 75 | 277 | 352 |
| **Agreement Statistics Excluding Invalid Results** | | PPA<br>63.6% (21/33)<br>95% CI**: (46.6%,77.8%) | NPA<br>100.00% (232/232)<br>95% CI**: (98.4%,100.0%) | |
| **Percent Invalid** | | 56.0% (42/75)<br>95% CI**: (44.7%, 66.7%) | 16.2% (45/277)<br>95% CI**: (12.4%, 21.0%) | |

* Includes 1 sample from procured negative sample set that was CTA+/F1CDx-.
**Calculated with Wilson 2-sided 95% CI.

The PPA was 63.6% and NPA was 100.0%. as summarized in the table above, 42 CTA+ and 46 CTA- samples failed to generate a validate result due to processing failure.

ii. **Bridging clinical outcome from CTAs to F1CDx**

The clinical efficacy of entrectinib in the clinical trials was measured in ORR with either confirmed CR or PR based on BICR. Only clinical samples with clinical outcome data were used in this part of the study analysis.

The ORR for all 74 samples of this study (CTA+) population was 62.2% (46/74) with Exact 2-sided 95% CI [50.1, 73.2]. Seventeen (17) patients (17/21) were CTA+ and exhibited F1CDx *NTRK*-positive results. The ORR for this population was 81.0% with Exact 2-sided 95% CI [58.1, 94.6]. Eleven (11) patients were CTA+ but F1CDx *NTRK*-negative. The ORR for this population was 36.4% (4/11) with Exact 2-sided 95% CI [10.9, 69.2]. Forty-two (42) patients were CTA+ but without a F1CDx *NTRK* result. The ORR for this population was 59.5% (25/42) with Exact 2-sided 95% CI [43.3, 74.4], as summarized in Table 24.

**Table 24. Efficacy by *NTRK* Status in Biomarker Subgroups**

| Clinical outcome | Total CTA positive population* (N=74) | F1CDx positive and CTA positive (N=21) | F1CDx negative and CTA positive (N=11) | F1CDx result missing and CTA positive (N=42) |
|---|---|---|---|---|
| **ORR%** [95% CI**] | 62.2% [50.1, 73.2] | 81.0% [58.1, 94.6] | 36.4% [10.9, 69.2] | 59.5% [43.3, 74.4] |
| Complete response | 5 (6.8%) | 3 (14.3%) | 0 (0%) | 2 (4.8%) |
| Partial response | 41 (55.4%) | 14 (66.7%) | 4 (36.4%) | 23 (54.8%) |
| Number of responders | **N=46** | **N=17** | **N=4** | **N=25** |
| **Duration of Response** | | | | |
| Median in months (range) | 7.4 (1.4-26.0) | 9.2 (1.9-22.1) | 11.1 (1.4-26.0) | 7.1 (2.8-25.9) |
| % with duration ≥ 6 months | 54.3% (25/46) | 52.9% (9/17) | 75% (3/4) | 52% (13/25) |
| % with duration ≥ 9 months | 43.5% (20/46) | 52.9% (9/17) | 75% (3/4) | 32% (8/25) |
| % with duration ≥ 12 months | 30.4% (14/46) | 35.3% (6/17) | 50% (2/4) | 24% (6/25) |

***See Table 21 for details on the total population**
****Exact 2-sided 95% CI reported**

There were 21 CTA-positive participants who also had F1CDx results with partial or complete responses (17 F1CDx+ and 4 F1CDx-). Among them 81.0% (17/21) were positive by F1CDx (95% CI: 58.1, 94.6). There were 11 CTA-positive participants who also had F1CDx results with no responses (21-17=4 and 11-4=7). Among the 11 CTA positive patients who did not respond to entrectinib, only 36.4% (4/11) were positive by F1CDx (95% CI: 10.9, 69.2). Taken together, F1CDx has a higher percent of responders among participants with F1CDx positive results than the percent of responders among the participants with negative F1CDx results [difference between 81.0% (17/21) and 36.4% (4/11) was 44.6% with 95% CI: (9.2, 70.7)].

The similarity of the ORR for the CTA-positive population (n=74) overall (62.2%, 95% CI: 50.1, 73.2) and for those missing a valid F1CDx result (n=42; 59.5%, 95% CI: 43.3, 74.4) suggests no overt imbalance in efficacy effect of entrectinib between patients on whom the F1CDx was or was not obtained.

**c. Sensitivity Analysis**

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates in consideration of the subjects with missing/invalid CDx results and the missing F1CDx-positive, CTA-negative population that was not enrolled and evaluated by ALKA, STARTRK-1, and STARTRK-2 clinical trials.

Amongst all CTA-positive patients, 56.8% did not have a F1CDx result (42/74).

To evaluate the impact of missing/invalid F1CDx results, the distribution of patients for baseline covariates and disease characteristics was compared among the CTA-positive population, the F1CDx-evaluable/CTA-positive subpopulation, and F1CDx-missing CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable F1CDx (n=42).

The clinical efficacy (ORR) for the F1CDx-positive subjects in the device intended use population was estimated under different assumed scenarios based on observed and imputed F1CDx results. This sensitivity analyses demonstrated the robustness of the clinical efficacy estimate from the primary analysis.

Due to the large proportion of missing data, additional clinical data will be evaluated post-market to confirm the clinical effectiveness of F1CDx, see section XIII below.

Subgroup Analysis

Response to entrectinib for the F1CDx fusion positive patient population was analyzed by *NTRK* fusion gene, *NTRK* gene fusion partner and primary tumor type (Table 25).

Within the F1CDx positive patients for the efficacy set, 14 tumor types were represented. Response rates and sample counts by tumor type are shown in Table 25.

**Table 25. The overall response rate for CDx *NTRK* fusion positive patients in the efficacy analysis set by different subgroups**

| Subgroup | Number of Patients (N=21) | Number of patients with CR or PR (N=17) | ORR (%) (95% CI*) |
|---|---|---|---|
| ***NTRK* fusion gene by CDx** | | | |
| *NTRK1* | 10 | 8 | 80% |
| *NTRK3* | 11 | 9 | 81.8% (48.2%, 97.7%) |
| ***NTRK* fusion partner** | | | |
| *AKAP13 - NTRK3* | 1 | 0 | 0% |
| *ETV6 - NTRK3* | 10 | 9 | 90% |
| *CDC42BPA - NTRK1* | 1 | 1 | 100% |

| Subgroup | Number of Patients (N=21) | Number of patients with CR or PR (N=17) | ORR (%) (95% CI*) |
|---|---|---|---|
| *CGN - NTRK1* | 1 | 0 | 0% |
| *ERC1 - NTRK1* | 1 | 0 | 0% |
| *LMNA - NTRK1* | 1 | 1 | 100% |
| *PLEKHA6 - NTRK1* | 1 | 1 | 100% |
| *SQSTM1 - NTRK1* | 1 | 1 | 100% |
| *TPM3 - NTRK1* | 2 | 2 | 100% |
| *TPR - NTRK1* | 2 | 2 | 100% |
| **Primary Tumor Type** | | | |
| BREAST (NON-SECRETORY) | 2 | 1 | 50% |
| BREAST (SECRETORY) | 1 | 1 | 100% |
| CERVICAL ADENOSARCOMA | 1 | 1 | 100% |
| CHOLANGIOCARCINOMA | 1 | 1 | 100% |
| CRC | 1 | 1 | 100% |
| GIST | 1 | 1 | 100% |
| MASC | 4 | 4 | 100% |
| MPNST | 1 | 0 | 0% |
| NEUROENDOCRINE | 2 | 2 | 100% |
| NON-CRC GI (NOS) | 1 | 1 | 100% |
| NSCLC | 3 | 2 | 66.7% |
| PANCREATIC | 1 | 0 | 0% |
| SARCOMA (NOS) | 1 | 1 | 100% |
| UNDIFFERENITATED PLEOMORPHIC SARCOMA | 1 | 1 | 100% |

* 95% 2-sided exact CIs were reported when sample size >10

## 8. Pediatric Extrapolation

Rozlytrek (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* fusions in pediatric cancer (≤18 years of age) is 1.2%[9].

The safety of ROZLYTREK in adolescent patients was established based on data from 30 pediatric patients enrolled in the STARTRK-NG clinical trial. The effectiveness of ROZLYTREK 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 study.

The safety and effectiveness of ROZLYTREK in pediatric patients less than 12 years of age with solid tumors who have an *NTRK*1/2/3 gene fusion have not been established.

The safety and effectiveness of ROZLYTREK in pediatric patients with *ROS1*-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 study included one investigator that was a full-time employee of the sponsor and had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

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

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

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

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical

Genetics Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

## XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

### A. Effectiveness Conclusions

The effectiveness of F1CDx to identify *ROS1* fusions in NSCLC patients and *NTRK1, NTRK2, NTRK3* 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 F1CDx assay when used in accordance with the indications for use. Data from the ALKA, STARTRK-1, and STARTRK-2 studies show that NSCLC patients harboring *ROS1* fusions and solid tumor patients harboring *NTRK1, NTRK2, NTRK3* fusions received benefit from treatment with entrectinib and support the addition of the CDx indication to F1CDx.

### B. Safety Conclusions

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

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

### C. Benefit-Risk Determination

The probable benefit of the F1CDx assay in identifying solid tumor patients with *NTRK1, NTRK2* and *NTRK3* fusions for treatment with ROZLYTREK® (entrectinib) and NSCLC patients 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 *NTRK1, NTRK2* and *NTRK3* fusion positive solid tumor patients, the ORR for all 74 samples of this study (CTA+) population was 62.2% (46/74) with Exact 2-sided 95% CI [50.1, 73.2]. Clinical outcome of *NTRK1/2/3* positive fusion positive patients

by F1CDx, indicated an ORR of 81% (95% CI: [58.1, 94.6]), 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%, and the observed ORR for the F1CDx *NTRK* fusion positive patients supports the probable benefit of F1CDx in selecting *NTRK1/2/3* fusion positive patients for treatment with ROZLYTREK® (entrectinib).

For *ROS1* fusion positive NSCLC patients, the ORR for all 159 samples of this study (CTA+) was 67.3% (107/159) with Exact 2-sided 95% CI [59.4, 74.5]. Clinical outcome for *ROS1* positive fusion positive NSCLC patients by the CTA and F1CDx, indicated an ORR of 64.7% (22/34) (95% CI: [46.5, 80.3]), which was comparable to the ORR in the CTA+ population. Because the NPA in the concordance study was not 100%, a sensitivity analysis considering the NPA (99.2%) and assuming different CTA positivity rates in the F1CDx intended use population, which ranged 1-2%, were investigated to assess influence on the efficacy estimated for the intended use, i.e., F1CDx positive subjects. These sensitivity analyses demonstrated the robustness of the clinical efficacy estimate. This provides evidence of a meaningful clinical benefit in this population. This data supports the probable benefit of F1CDx in selecting *ROS1* fusion positive NSCLC patients for treatment with ROZLYTREK® (entrectinib).

There is potential risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. The risks of the F1CDx assay are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial which may lead to adverse events or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug.

The risk of false results is partially mitigated by clinical and analytical studies presented above. In addition, an accuracy study of F1CDx for the detection of *NTRK* fusions with the externally validated NGS (evNGS) comparator method further supports this conclusion. The accuracy study with an evNGS comparator method demonstrated supportive PPA and NPA values for *ROS1* fusions and for *NTRK* fusions, partially mitigating the risks of this test.

In addition, patients identified with the F1CDx assay as positive for *NTKR1, NTRK2* or *NTRK3* fusions in solid tumors or *ROS1* fusions in NSCLC, show comparable overall response rate to ROZLYTREK® (entrectinib), as found in the original ALKA, STARTRK-1, and STARTRK-2 studies. Therefore, these results support the use of F1CDx as an aid in selecting patients with solid tumors harboring *NTRK1/2/3* fusions for ROZLYTREK® (entrectinib), and patients with NSCLC harboring *ROS1* fusions for ROZLYTREK® (entrectinib). However, based on the data from the clinical trial,

there is a risk that a small subset of solid tumor patients with *NTRK1/2/3* fusions or NSCLC patients with *ROS1* fusions that may respond to the drug who may be missed by this device.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with reasonable accuracy, mitigating the potential for false results. Additional factors to be considered in determining probable risks and benefits for the F1CDx assay include: analytical performance of the device in precision and limit of detection studies, and representation of *NTRK* and *ROS1* fusion variants across the analytical and clinical studies. In addition, to supplement the premarket data, some post-market studies are planned as summarized in Section XIII, below.

1. Patient Perspective

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

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

### D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical bridging study support the performance of F1CDx as an aid for the identification of NSCLC patients with *ROS1* fusions and solid tumor patients with *NTRK1, NTRK2, NTRK3* fusions for ROZLYTREK (entrectinib) treatment.

## XIII. CDRH DECISION

CDRH issued an approval order on June 7, 2022. The final clinical conditions of approval cited in the approval order are described below.

1. Foundation Medicine, Inc. (FMI) must provide data from a site-to-site precision study and an intermediate precision study (using analyte levels at 1x-1.5x LoD) for at least two solid tumor samples that are biomarker positive by the biomarker calling rules for F1CDx for *NTRK1* and *NTRK2* rearrangements as conditions of approval, namely one additional sample for *NTRK1* fusion positive and one additional *NTRK2* fusion positive sample. These results must be adequate to confirm the safety and effectiveness of the FoundationOne CDx device.

2. Foundation Medicine, Inc. (FMI) must provide detailed protocols, including acceptance criteria where appropriate, for a site-to-site precision study and an intermediate precision study (using analyte levels at 1x-1.5x LoD) for at least two NSCLC samples biomarker positive by the biomarker calling rules for F1CDx for *ROS1* rearrangements as conditions of approval. These studies must be adequate to confirm the safety and effectiveness of the FoundationOne CDx device and must include a detailed description of the numbers of sample to be tested, the type of samples to be tested, the complete testing protocol, and a robust statistical analysis plan, as applicable. These protocols must be submitted to FDA no later than 60 days after approval.

3. FMI must provide clinical outcome data (e.g., Real World Evidence, direct clinical data) in the post-market setting in order to confirm the clinical effectiveness of F1CDx as a companion diagnostic (CDx) device for identification of patients with solid tumors with *NTRK1/2/3* fusions and NSCLC patients with *ROS1* fusions who may benefit from treatment with ROZLYTREK. A complete study protocol considered sufficient by FDA is required within 60 days of approval of P170019/S014. FMI must submit interim study reports every six months following approval of P170019/S014. The final study data, study conclusions, and labeling revisions should be submitted within 2 years of the PMA approval date.

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

## XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

## XV. REFERENCES

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