SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name: Next generation sequencing oncology panel, somatic or germline variant detection system

Device Trade Name: FoundationOne® Liquid CDx

Device Procode: PQP

Applicant's Name and Address: Foundation Medicine, Inc.
150 Second Street
Cambridge, MA 02141

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190032/S005

Date of FDA Notice of Approval: May 3, 2023

The original Premarket Approval (PMA) (190032) for FoundationOne® Liquid CDx (F1LCDx) was approved on August 26, 2020 as a companion diagnostic for *BRCA1* and *BRCA2* alterations in metastatic castration-resistant prostate cancer (mCRPC) patients who may benefit from treatment with RUBRACA® (rucaparib) and *EGFR* activating mutations (Exon 19 deletions and L858R substitution mutation) in patients with advanced and metastatic non-small cell lung cancer (NSCLC) who may benefit from treatment with IRESSA® (gefitinib), TAGRISSO® (osimertinib), and TARCEVA® (erlotinib). Subsequently, additional PMA supplements were approved for expanding the indications for use of F1LCDx since its original approval. See Section VII for more details.

The current supplement was submitted to expand the indication for the FoundationOne Liquid CDx test as a companion diagnostic for the indication listed in the table below.

New Indication Being Sought in this PMA supplement submission.

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* Exon 20 insertions	EXKIVITY® (mobocertinib)

II. INDICATIONS FOR USE

FoundationOne Liquid CDx is a qualitative next generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions, insertions and deletions (indels) in 311 genes, rearrangements in eight (8) genes, and copy number alterations in three (3) genes.

FoundationOne Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne Liquid CDx cfDNA blood collection tubes included in the FoundationOne Liquid CDx Blood Sample Collection Kit. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1: Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*ALK* Rearrangements	ALECENSA® (alectinib)
	EGFR Exon 19 deletions and *EGFR* Exon 21 L858R substitution	*EGFR* tyrosine kinase inhibitors approved by FDA*
	EGFR Exon 20 insertions	EXKIVITY® (mobocertinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	TABRECTA® (capmatinib)
	ROS1 fusions**	ROZLYTREK® (entrectinib)
Prostate cancer	*BRCA1*, *BRCA2*, and *ATM* alterations	LYNPARZA® (olaparib)
	BRCA1, *BRCA2* alterations	RUBRACA® (rucaparib)
Breast Cancer	*PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y	PIQRAY® (alpelisib)
Solid Tumors	*NTRK1/2/3* fusions**	ROZLYTREK® (entrectinib)

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

Additionally, FoundationOne Liquid CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms.

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients who are negative for the mutations listed in Table 1 (see **Note for *NTRK1/2/3* and *ROS1* fusions) should be reflexed to routine biopsy and

their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

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

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

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

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

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

V. DEVICE DESCRIPTION

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

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

Table 2: Genomic Regions in which Variants are Reported by F1LCDx[1]

ABL1 [Exons 4-9]	*ACVR1B*	***AKT1 [Exon 3]***	*AKT2*	*AKT3*	***ALK [Exons 20-29, Introns 18,19]***	*ALOX12B*	*AMER1 (FAM123B)*	***APC***	***AR***
ARAF [Exons 4, 5, 7, 11, 13, 15, 16]	*ARFRP1*	*ARID1A*	*ASXL1*	***ATM***	***ATR***	*ATRX*	*AURKA*	*AURKB*	*AXIN1*
AXL	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BCR* [Introns 8, 13,

									14]
***BRAF [Exons 11-18**, Introns 7-10]*	***BRCA1** [Introns 2, 7, 8, 12, 16, 19, 20]*	***BRCA2** [Intron 2]*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*	***BTK [Exons 2, 15]***	*C11orf30 (EMSY)*	*C17orf39 (GID4)*
CALR	*CARD11*	*CASP8*	*CBFB*	*CBL*	***CCND1***	*CCND2*	*CCND3*	*CCNE1*	*CD22*
CD70	*CD74* [Introns 6-8]*	*CD79A*	*CD79B*	***CD274 (PD-L1)***	*CDC73*	***CDH1***	***CDK12***	***CDK4***	***CDK6***
CDK8	*CDKN1A*	*CDKN1B*	***CDKN2A***	*CDKN2B*	*CDKN2C*	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*
CREBBP	***CRKL***	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*	***CTNNB1 [Exon 3]***	*CUL3*	*CUL4A*	*CXCR4*
CYP17A1	*DAXX*	*DDR1*	***DDR2 [Exons 5, 17, 18]***	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	***EGFR [Introns 7**, 15, **24-27]***	*EP300*
EPHA3	*EPHB1*	*EPHB4*	***ERBB2***	***ERBB3 [Exons 3, 6,7,8, 10, 12,20, 21, 23,24, 25]***	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1 [Exons 4-8]***
ETV4 [Intron 8]*	*ETV5* [Introns 6,7]*	***ETV6* [Introns 5,6]***	*EWSR1* [Introns 7-13]*	***EZH2 [Exons 4,16, 17, 18]***	*EZR* [Introns 9 - 11]*	*FAM46C*	*FANCA*	*FANCC*	*FANCG*
FANCL	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*	*FGF4*
FGF6	***FGFR1** [Introns 1,5, **Intron17]***	***FGFR2** [Intron 1, **Intron 17]***	***FGFR3 [Exons 7, 9 (alternative designation exon 10),14, 18, Intron 17]***	*FGFR4*	*FH*	*FLCN*	*FLT1*	***FLT3 [Exons 14, 15, 20]***	***FOXL2***
FUBP1	*GABRA6*	*GATA3*	*GATA4*	*GATA6*	***GNA11 [Exons 4, 5]***	*GNA13*	***GNAQ [Exons 4, 5]***	***GNAS [Exons 1, 8]***	*GRM3*
GSK3B	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	***HRAS [Exons 2, 3]***	*HSD3B1*	*ID3*	***IDH1 [Exon 4]***	***IDH2 [Exon 4]***
IGF1R	*IKBKE*	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	***JAK2 [Exon 14]***	***JAK3 [Exons 5, 11, 12, 13, 15, 16]***
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	***KIT [Exons 8, 9, 11, 12, 13, 17, Intron 16]***	*KLHL6*	*KMT2A (MLL) [Introns 6, 8-11, Intron 7]*
KMT2D (MLL2)	***KRAS***	*LTK*	*LYN*	*MAF*	***MAP2K1 (MEK1) [Exons 2, 3]***	***MAP2K2 (MEK2) [Exons 2-4, 6, 7]***	*MAP2K4*	*MAP3K1*	*MAP3K13*
MAPK1	*MCL1*	***MDM2***	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***	*MITF*
MKNK1	*MLH1*	***MPL [Exon 10]***	*MRE11A*	*MSH2 [Intron 5]*	*MSH3*	*MSH6*	*MST1R*	*MTAP*	***MTOR [Exons 19, 30, 39 40, 43-45, 47, 48, 53, 56]***
MUTYH	*MYB* [Intron 14]*	***MYC [Intron 1]***	*MYCL (MYCL1)*	***MYCN***	***MYD88 [Exon 4]***	*NBN*	***NF1***	*NF2*	*NFE2L2*
NFKBIA	*NKX2-1 (TTF-1)*	*NOTCH1*	*NOTCH2 [Intron 26]*	*NOTCH3*	***NPM1 [Exons 4-6, 8, 10]***	***NRAS [Exons 2, 3]***	*NSD3 (WHSC1L1)*	*NT5C2*	***NTRK1 [Exons 14,15, Introns 8-11]***
NTRK2 [Intron 12]	***NTRK3 [Exons 16,***	*NUTM1* [Intron 1]*	*P2RY8*	***PALB2***	*PARK2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*

	17]								
PBRM1	*PDCD1 (PD-1)*	***PDCD1L G2 (PD-L2)***	***PDGFRA [Exons 12, 18, Introns 7, 9, 11]***	***PDGFRB [Exons 12-21, 23]***	*PDK1*	*PIK3C2B*	*PIK3C2G*	***PIK3CA [Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)]***	*PIK3CB*
PIK3R1	*PIM1*	*PMS2*	*POLD1*	*POLE*	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*
PRKCI	*PTCH1*	***PTEN***	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*
RAD51C	*RAD51D*	*RAD52*	*RAD54L*	***RAF1 [Exons 3, 4, 6, 7, 10, 14, 15, 17, Introns 4-8]***	*RARA [Intron 2]*	***RB1***	*RBM10*	*REL*	***RET [****Introns 7, 8****, Exons 11, 13-16, Introns 9-11]***
RICTOR	*RNF43*	***ROS1 [Exons 31, 36-38, 40, Introns 31-35]***	*RPTOR*	*RSPO2* [Intron 1]*	*SDC4* [Intron 2]*	*SDHA*	*SDHB*	*SDHC*	*SDHD*
SETD2	*SF3B1*	*SGK1*	*SLC34A2* [Intron 4]*	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	***SMO***	*SNCAIP*
SOCS1	*SOX2*	*SOX9*	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	***STK11 (LKB1)***	*SUFU*
SYK	*TBX3*	*TEK*	*TERC* {ncRNA}*	***TERT* {Promoter}***	*TET2*	*TGFBR2*	*TIPARP*	*TMPRSS2* [Introns 1-3]*	*TNFAIP3*
TNFRSF14	***TP53***	*TSC1*	*TSC2*	*TYRO3*	*U2AF1*	***VEGFA***	*VHL*	*WHSC1*	*WTI*
XPO1	*XRCC2*	*ZNF217*	*ZNF703*						

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

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

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

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

The test report includes variants reported in the following levels:

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

in Biopharmaceutical Research. 8: 35-363, 2016) is required. In addition to the clinical validation, analytical validation for each specific Level 1 CDx biomarker should be presented.

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

Level 3A: Biomarkers with Evidence of Clinical Significance in Tissue Supported by Strong Analytical Validation Using cfDNA and Concordance Between cfDNA and Tissue.
Clinical evidence can be provided from tissue-based companion diagnostics. This should also be supported by analytical validation (LoD, precision, analytical accuracy, and concordance study to a tissue-based test) for the specific tumor type at the biomarker or variant level, using a representative approach for SNVs and indels. Evidence evaluating concordance between cfDNA- and tissue-samples for FDA-approved tissue markers should be demonstrated using an FDA-approved tissue test or a validated tissue test.

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

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

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

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

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

Table 4: Instruments for use with the F1LCDx assay

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

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

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

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

C. Library Construction
Library Construction (LC) begins with the normalization of cfDNA. The samples are purified, using AMPure XP Beads (Agencourt). Solid-phase reversible immobilization (SPRI) purification is used subsequent to library construction with the NEBNext kits (NEB), including mixes for end repair with blunt-end and 5'- phosphorylate the cfDNA fragments using T4 Polynucleotide Kinase and T4 DNA Polymerase. This step prepares the 3'-end for dA-addition while also preparing the 5'-end of the DNA fragment for

ligation. Second, dA-addition will incorporate a single dAMP to the 3'-end of the End-Repaired material. After dA-addition, a universal Y-adaptor is ligated onto each end of the DNA fragment using a DNA ligase. These steps are performed in 96-well plates (Eppendorf) on a Bravo Benchbot (Agilent) using the "with-bead" protocol to maximize reproducibility and library yield. Indexed (Foundation Medicine customized six base pair barcodes) sequencing libraries are PCR amplified with a high-fidelity DNA polymerase (HiFi™, Kapa) for ten cycles, SPRI purified and quantified by PicoGreen fluorescence assay (Invitrogen). Process matched control (PMC) is prepared and added to the plate with other cfDNA samples at the beginning of LC.

On May 25, 2022, F1LCDx was approved for a change in the design of the primers used for LC. Specifically, a change was made to the regions of the primer that hybridize to the universal Y-adaptors during the PCR amplification process, which resulted in increased amplification efficiency and reduced the minimum recommended cfDNA input level to 20ng.

D. Hybrid Capture

Hybrid Capture begins with the normalization of each library from 500 ng to 2000 ng. Solution hybridization is performed using a >50-fold molar excess of a pool of individually synthesized 5'-biotinylated DNA 120 base pair oligonucleotides (Integrated DNA Technology) for baits. The baits target regions from 324 cancer- related genes including all coding exons of 309 genes and only select introns or non- coding regions in 15 genes. Baits were designed by appointing overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; single nucleotide polymorphism (SNP) targets were allocated one bait each. Intronic baits were filtered for repetitive elements as defined by the University of California at Santa Cruz (UCSC) Genome Repeat Masker track. Hybrid selection of targets demonstrating reproducibly low coverage was boosted by increasing the number of baits for these targets.

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

E. Sequencing

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

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

F. Sequence Analysis

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

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

For the detection of short variants (e.g., substitutions and small indels) in each target region of interest, a de novo assembly is performed. This is done using proprietary software to generate a de Bruijn graph including all k-mers in reads mapping to a particular locus. The graph is parsed to identify paths that

originate and terminate in reference nodes from the locus. Increased k-mer sizes may be used to account for ambiguities, cycles, and other problematic regions within the graph. The result of the graph traversal is a set of candidate variants. For each variant, there is a set of k-mers supporting the variant and a set of k-mers that would support the reference or another variant at the location.

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

G. Report Generation

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

H. Internal Process Controls

Process Control

Each assay run includes a control sample run in duplicate. The control sample contains a pool of eleven HapMap cell lines and is used as a positive mutation detection control. 100 different germline SNPs present across the entire targeted region are required to be detected by the analysis pipeline.

Sensitivity Control

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

Negative Control

Samples are barcoded molecularly at the library construction (LC) stage. Only reads with a perfect molecular barcode sequence are incorporated into the analysis. The Analysis Pipeline includes an algorithm that analyzes the SNP profile of each specimen to identify potential contamination that may have occurred prior to molecular barcoding.

I. Classification Criteria for CDx Biomarkers Detected by F1LCDx

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

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

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

Table 6: Deleterious *BRCA* Missense Alterations in rucaparib

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

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

Table 7: Rules Applied to the Aforementioned Genes:

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

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

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

BRCA1		***BRCA2***		***ATM***	
Protein Effect (PE)	**FMI Annotated PE**	**Protein Effect (PE)**	**FMI Annotated PE**	**Protein Effect (PE)**	**FMI Annotated PE**
MIV	MIV	MIR	MIR	MIT	MIT
MlI	MlI	MlI	MlI	R2032K	R2032K
C6IG	C6IG	VI59M	VI59M	R2227C	R2227C
C64Y	C64Y	V211L	V211L	R2547 S2549del	R2547 S2549del
R7IG	R7IG	V211I	V211I	G2765S	G2765S
R7IK	R7IK	R2336P	R2336P	R2832C	R2832C
RI495M	RI495M	R2336H	R2336H	S2855	S2855

				V2856delinsR1	V2856delinsRl S2855 V2856>Rl
EI559K	EI559K			R3008C	R3008C
DI692N	DI692N			R3008H	R3008H
DI692H	DI692H			[VUS from Jan 2016 HRR* List to be Excluded]	
RI699W	RI699W			V2424G	V2424G
AI708E	AI708E			[Excluded from Jan 2016 HRR List]	
G1788V	GI788V			K750K	splice site 2250G>A

HRR = Homologous Recombination Repair genes

Intronic Variants

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

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

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

5. *SNVs and indels that lead to MET exon 14 skipping to identify patients eligible for treatment with TABRECTA® (capmatinib):*
   A SNV or indel in *MET* shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:
   1. Deletions greater than or equal to 5 bp that affect positions -3 to -30 in the intronic region immediately adjacent to the splice acceptor site at the 5′ boundary of *MET* exon 14.
   2. Indels affecting positions -1 or -2 at the splice acceptor site of the 5′ boundary of *MET* exon 14.
   3. Base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3′ boundary of *MET* exon 14.

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

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

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

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

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

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

8. *EGFR exon 20 insertions to identify NSCLC patients eligible for treatment with EXKIVITY:*
   CDx positivity for *EGFR* exon 20 insertions is determined if the following criteria were met:
   - Any in-frame insertions affecting amino acids 762 – 775 (inclusive) in *EGFR* exon 20

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the

detection of *EGFR* exon 20 insertions to identify patients with NSCLC eligible for treatment with EXKIVITY.

However, Oncomine™ Dx Target Test (Life Technologies Corporation) is an FDA-approved CDx for the detection of *EGFR* exon 20 insertions in NSCLC to identify patients eligible for EXKIVITY with formalin-fixed, paraffin-embedded (FFPE) tissue specimens (P160045/S029) and RYBREVANT (amivantamab) (P160045/S027) using tissue for this indication.

There are FDA-approved alternatives for the detection of select CDx and tumor profiling genetic alterations using either cfDNA isolated from plasma samples or FFPE tissue specimens. For additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/media/119249/download. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

VII. MARKETING HISTORY

The F1LCDx assay was FDA-approved on August 26, 2020, and subsequently commercialized in the United States. The F1LCDx assay has been marketed in the United States, the European Union, and in several other foreign countries since the approval. On September 21, 2022, the companion diagnostic indication for F1LCDx to identify patients with ovarian cancer harboring *BRCA1* or *BRCA2* alterations for treatment with RUBRACA® (rucaparib) was removed. The approved PMA supplements that affected the intended use are listed in Table 10.

Table 10. Marketing History

Submission No.	Date of Approval	Biomarker/Update	Indication	Drug
P200006	October 26, 2020	*ALK* Rearrangements	NSCLC	ALECENSA® (alectinib)
		PIK3CA alterations	Breast Cancer	PIQRAY® (alpelisib)
P200016	November 6, 2020	*BRCA1, BRCA2,* and *ATM* alterations	Prostate Cancer	LYNPARZA® (olaparib)
P190032/S001	July 15, 2021	*MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	NSCLC	TABRECTA® (capmatinib)
P190032/S004	December 22, 2022	*NTRK1/2/3* fusions	Solid Tumors	ROZLYTREK® (entrectinib)
		ROS1 fusions	NSCLC	
P190032/S008	December 19, 2022	*EGFR* Exon 19 deletions and *EGFR* Exon 21 L858R alteration	NSCLC	*EGFR* tyrosine kinase inhibitors approved by FDA

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect F1LCDx assay results, and subsequently, inappropriate patient management decisions. Patients with false positive CDx biomarker results may undergo treatment with one of the therapies listed in the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated targeted therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy. For the specific adverse events related to the approved therapeutics, please see approved drug product labels.

For the specific adverse events that occurred in the clinical study, please see the FDA approved package insert for EXKIVITY which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The evidence in support of the analytical performance of F1LCDx in detecting *EGFR* exon 20 insertions is presented in this section. Analytical accuracy/concordance and precision near the LoD studies were conducted to support the indication for *EGFR* exon 20 insertions using clinical samples.

The F1LCDx was modified after the analytical validation studies were completed. The changes included (1) the primer set used for library construction as part of the F1LCDx assay process was modified, and (2) the minimum cfDNA input level required for library construction was lowered to 20ng. These changes were supported by comparability, precision, limit of blank (LoB), cfDNA input guard banding, and reagent stability studies. The comparability study between the prior and updated primer sets assessed a total of three (3) NSCLC clinical samples harboring *EGFR* exon 20 insertions (two 3bp insertions and one 9bp insertion) and observed a PPA of 100%.

For F1LCDx platform-level validation (P190032), due to the challenges with obtaining sufficient volume of clinical specimens, analytical performance characteristics were established for some of the studies using contrived samples, which consisted of enzymatically sheared cell line DNA spiked into human plasma from healthy donors, extracted according to the assay's standard procedure, and diluted with cfDNA isolated from healthy donor plasma. A contrived sample functional characterization (CSFC) study was conducted to demonstrate comparable performance of sheared cell line DNA samples spiked into plasma as compared to cfDNA isolated from plasma specimens obtained from cancer positive intended use patient specimens. These contrived samples were used to establish the LoD for insertion variants evaluated in the platform-level LoD establishment study. For information regarding the platform-level validation, refer to Section IX.A. in P190032 Summary of Safety and Effectiveness Data.

1. Analytical Accuracy/Concordance

An analytical accuracy/concordance study was performed to demonstrate the concordance between F1LCDx and an externally validated NGS assay (evNGS) for the detection of *EGFR* exon 20 insertions. For this study, 101 frozen plasma samples were identified from clinical trial assay-positive (CTA+) patients enrolled in the AP32788-15-101 trial (from non-new drug application [non-NDA] population) and 125 residual cfDNA samples were sourced from FMI's clinical archives. Of the 125 residual cfDNA samples, four (4) were excluded due to diluted DNA concentration being out of acceptable range or evNGS post-sequencing QC failure. Of the 101 frozen plasma samples from CTA+ patients, 71 were excluded from the analysis mainly due to insufficient cfDNA yield, while a minority were excluded due to diluted DNA concentration being out of acceptable range or evNGS post-sequencing QC failure.

Analytical concordance of F1LCDx for detecting *EGFR* exon 20 insertions was determined with 151 samples tested by F1LCDx assay (Table 11). Since specimens were selected based on F1LCDx and confirmed by the evNGS assay, positive predictive value (PPV) and negative predictive value (NPV) are estimated conditional on F1LCDx. PPV was estimated as 100% (49/49) with two-sided 95% CI (92.70%, 100%), and NPV as 99.02% (101/102) with two-sided 95% CI (94.65%, 99.83%), as shown in Table 11, below. For informational purposes, unadjusted positive percent agreement (PPA) and negative percent agreement (NPA) are also displayed.

Table 11. Concordance summary for *EGFR* exon 20 insertion by F1LCDx and the evNGS

		evNGS			
		***EGFR* exon 20 insertion positive**	***EGFR* exon 20 insertion negative**	**Total**	**PPV/NPV (95% CI[1])**
F1LCDx	***EGFR* exon 20 insertion positive**	49	0	49	PPV: 100% (92.70%, 100%)
	***EGFR* exon 20 insertion negative**	1	101	102	NPV: 99.02% (94.65%, 99.83%)
	Total	50	101	151	
	PPA/NPA (Unadjusted) (95% CI[1])	PPA: 98.00% (89.50%, 99.65%)	NPA: 100% (96.34%, 100%)		

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

In the one (1) discordant sample that was F1LCDx-negative/evNGS-positive, a 3 bp *EGFR* exon 20 insertion reported by the evNGS was not detected in the variant analysis pipeline of F1LCDx. This discordance may be due to the low variant allele frequency (VAF), 0.14% VAF, which is below the LoD range of F1LCDx for insertions evaluated in the platform LoD study (refer to Section IX.A.2.b. below)

The lowest VAF F1LCDx test result observed in the accuracy study was 0.18% VAF; therefore, accuracy of F1LCDx was not demonstrated for samples with variants below this VAF level. A limitation is included in the device labeling to

address the uncertainty of the accuracy of F1LCDx for detecting *EGFR* exon 20 insertions with VAFs below those evaluated in the analytical concordance study (refer to Section XII.C. below).

2. Analytical Sensitivity
   a. Limit of Blank (LoB)
      The LoB of F1LCDx was evaluated in the platform LoB study for PMA P190032 (refer to Section IX.A.3.a. in the Summary of Safety and Effectiveness Data for P190032).

      A supplemental LoB study was performed for F1LCDx to support the updated LC input range (20-60ng) by collecting whole blood samples from 44 healthy donors and preparing two plasma cfDNA replicates per donor for a total of 88 cfDNA sample replicates. Additionally, one matched gDNA replicate per donor was isolated from buffy coat and mechanically fragmented for F1LCDx testing to obtain non-tumor variant (e.g., germline) information to support the LoB analysis. One cfDNA replicate was excluded from the analysis due to failure at the DNA extraction step. All variants were determined to have an LoB of 0, based on the detection rate not significantly exceeding 5%. Further *EGFR* insertions/deletions (indels) were not observed in any of the replicates.

   b. Limit of Detection (LoD)
      The LoD of *EGFR* exon 20 insertions was not established as part of this PMA supplement. However, the LoD for insertion variants in contrived samples was established as part of the LoD study for PMA P190032 (refer to Section IX.A.3.b in the Summary of Safety and Effectiveness Data for P190032). In this study, 13 insertion variants were evaluated and ranged from 1 bp to 39 bp in length. The LoD for *EGFR* exon 20 insertions was extrapolated from the range of LoDs from all 13 insertion variants in the F1LCDx platform and was, thus, estimated as 0.308-1.711%. The LoD was confirmed with clinical samples in the precision study (refer to Section IX.A.3, below). The confirmed LoD range was 0.65-1.28%.

3. Precision and Reproducibility
   a. Within-Laboratory (Intermediate) Precision
      A precision study was conducted using three (3) clinical NSCLC samples harboring *EGFR* exon 20 insertions. Specifically, two (2) samples harbored 9 bp *EGFR* exon 20 insertions, while one (1) sample harbored a 3 bp insertion.

      Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three (3) different sequencers and two (2) different reagent lots, across multiple days of performance by multiple operators.

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

Table 12. Reproducibility results for *EGFR* exon 20 insertions

EGFR exon 20 Insertion	Insertion Length	Previously Established LoD VAF[1]	Mean VAF Calculated	Concordant /Total (n/N)	Reproducibility (%) 95% CI[2]	Fold LoD
H773_V774insH	3 bp	0.308-1.711%	0.98%	24/24	100% (86.2, 100)	0.57-3.18x
V769_D770insASV	9 bp	0.308-1.711%	1.28%	24/24	100% (86.2, 100)	0.75-4.12x
D770_N771insSVD	9 bp	0.308-1.711%	0.65%	24/24	100% (86.2, 100)	0.38-2.11x

[1]0.308-1.711% VAF represents the range of insertion VAFs evaluated in the platform LoD establishment study using contrived samples (See Section IX.A.2.b)
[2]Calculated with Wilson 2-sided 95% CI

Table 13. Repeatability results for *EGFR* exon 20 insertions

EGFR exon 20 Insertion	Insertion Length	Previously Established LoD VAF[1]	Mean VAF Calculated	Concordant /Total (n/N)	Repeatability (%) 95%CI[2]	Fold LoD
H773_V774insH	3 bp	0.308-1.711%	0.98%	12/12	100% (75.75, 100)	0.57-3.18x
V769_D770insASV	9 bp	0.308-1.711%	1.28%	12/12	100% (75.75, 100)	0.75-4.12x
D770_N771insSVD	9 bp	0.308-1.711%	0.65%	12/12	100% (75.75, 100)	0.38-2.11x

[1]0.308-1.711% VAF represents the range of insertion VAFs evaluated in the platform LoD establishment study using contrived samples (See Section IX.A.2.b)
[2]Calculated with Wilson 2-sided 95% CI

B. Animal Studies

No animal studies were conducted using the F1LCDx assay.

C. Additional Studies

None

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The reasonable assurance of safety and effectiveness for F1LCDx for detection of *EGFR* exon 20 insertions in patients with NSCLC who may benefit from treatment with EXKIVITY was established through a clinical bridging study using clinical plasma specimens from patients enrolled in the Takeda AP32788-15-101 trial (i.e., Study 101), as well as *EGFR* exon 20 insertion-negative tissue-matched plasma samples from commercial sources and plasma samples from the FMI archives. Data from this clinical study were the basis for the PMA supplement approval decision.

A summary of the clinical study is presented below.

A. Study Design

1. AP32788-15-101 Study Design

   The AP32788-15-101 trial is a prospectively designed, international, open-label, multicohort clinical trial in adult patients with *EGFR* exon 20 insertion mutation-positive locally advanced or metastatic NSCLC whose disease had progressed on or after platinum-based chemotherapy. The primary objective was to assess the overall response rate (ORR) by blinded independent central review (BICR) assessment per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria to determine whether treatment with EXKIVITY is effective. Duration of response (DOR) as assessed by BICR was the key secondary endpoint.

   The majority of patients were screened for enrollment based on tumor tissue samples at Clinical Laboratory Improvement Amendments (CLIA)-certified (US sites) or accredited (outside of the US) local laboratories. Patients enrolled based on local laboratory testing results had their samples centrally retested with an analytically validated test, Life Technologies Oncomine Dx Target Test (ODxT Test), when residual tissue was available. The ODxT Test was approved as a companion diagnostic to identify patients with NSCLC whose tumors harbor *EGFR* exon 20 insertion mutations who may be eligible for treatment with EXKIVITY.

2. Clinical Bridging Study Design

   A clinical bridging study was conducted to evaluate: 1) the concordance between the F1LCDx assay and the CTAs for the detection of *EGFR* exon 20 insertions, and 2) the clinical validity of F1LCDx in identifying NSCLC patients with *EGFR* exon 20 insertions who may be eligible for treatment with EXKIVITY.

   Plasma samples from the AP32788-15-101 trial patients were collected by the therapeutic investigational sites per the study protocol and study documents and shipped to the central testing laboratories. All patients with available plasma samples from the NDA population (i.e., patient population that supported the EXKIVITY approval) from the AP32788-15-101 trial were tested by F1LCDx as part of this clinical bridging study. To further support the clinical validation of F1LCDx for the detection of *EGFR* exon 20 insertions, additional CTA-positive and CTA-negative patients from the non-NDA population (i.e., patient population that were included as part of the dose-escalation cohort or did not receive prior platinum treatment) of the AP32788-15-101 trial were included in the concordance analysis for a more robust estimate of the agreement between the CTAs and F1LCDx in identifying *EGFR* exon 20 insertions. Additionally, tissue and plasma-matched CTA-negative samples procured from commercial sources, and residual plasma samples (not tissue-matched) from the FMI clinical archive and processed in previous studies, were also included in the clinical bridging study.

3. Clinical Inclusion and Exclusion Criteria

   The inclusion/exclusion criteria for the retrospective testing of plasma samples in the clinical bridging study were:

Sample inclusion criteria:

- Samples from enrolled patients from Part 1 Dose Escalation, Part 2 Expansion, and Part 3 Extension cohorts with informed consent provided in the AP32788-15-101 trial
- Samples from commercial sources must have a valid CTA negative result from tissue
- Specimens in frozen plasma
- Samples must meet F1LCDx operational testing requirements

Sample exclusion criteria:

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

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

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

5. Clinical Endpoints
   The major efficacy outcome measure was ORR as assessed by BICR assessment according to RECIST version 1.1 to determine the effectiveness of EXKIVITY in patients with NSCLC. Additional efficacy outcome measures included DOR by BICR.

B. Accountability of PMA Cohort

A total of 358 samples were identified for the clinical bridging study. Among the 230 (114 NDA + 116 non-NDA) *EGFR* exon 20 insertion-positive samples, 31 NDA patients and 15 non-NDA patients did not have a plasma sample available for F1LCDx testing, and 3 NDA patient samples and 22 non-NDA patient samples failed the F1LCDx QC metrics, resulting in a total of 159 *EGFR* exon 20 insertion-positive samples that had F1LCDx evaluable results. Among the 159 *EGFR* exon 20 insertion-positive evaluable samples, 132 had cfDNA ≥30 ng for input to LC and were used for the primary analysis. Twenty-seven (27) *EGFR* exon 20 insertion-positive samples had cfDNA <30 ng and ≥20ng for input to LC, and these samples were included in the exploratory analysis.

Among the 128 (58 non-NDA + 47 procured + 23 retrospective) *EGFR* exon 20 insertion-negative samples, 3 non-NDA patient samples failed F1LCDx QC metrics, and 15 non-NDA patient samples and one (1) procured sample failed CTA QC metrics, resulting in a total of 109 *EGFR* exon 20 insertion-negative samples that were evaluated by F1LCDx. Among the 109 *EGFR* exon 20 insertion-negative evaluable samples, 100 had cfDNA ≥30 ng for input to LC and were used for the primary analysis. Nine (9) *EGFR* exon 20 insertion-negative samples had cfDNA <30 ng and ≥20ng for input to LC, and these samples were included in the exploratory analysis.

Among the 268 total F1LCDx evaluable samples, 232 samples had cfDNA ≥30 ng for input to LC and were used for the primary analysis.

The detailed sample accountability is shown in Table 14.

Table 14. Samples evaluated in clinical bridging study

***EGFR* exon 20 insertion Status**	**Sample Source**	**# of Patients**	**# of Failed or Unavail-able Samples**	**F1LCDx Evaluable**		
				# of total F1LCDx Samples	**# of F1LCDx Samples ≥30ng**	**# of F1LCDx Samples ≥20ng and <30ng**
Positive	NDA	114*	34	80	71	9
	Non-NDA	116	37	79	61	18
Negative	Non-NDA	58	18	40	34	6
	Procured	47	1	46	43	3
	Retrospective	23	0	23	23	0
Total		**358 (100%)**	**90 (25.1%)**	**268 (74.9%)**	**232 (64.8%)**	**36 (10.1%)**

* One patient had two sample replicates tested by F1LCDx. One of the F1LCDx replicates was blindly selected at random for inclusion in the analysis. After the replicate was selected for inclusion, it was confirmed that the F1LCDx *EGFR* exon 20 ins status was the same between the two replicates. Therefore, including either replicate would yield the same result and conclusions.

C. Study Population Demographics and Baseline Parameters

Demographics and baseline disease characteristics for the F1LCDx-evaluable and F1LCDx-unevaluable patients (cfDNA input ≥30 ng) were similar (Table 15).

Table 15. Comparison of baseline demographic and clinical characteristics between the CDx-evaluable patients and the CDx-unevaluable patients

Baseline characteristic	CTA-positive	F1LCDx-evaluable	F1LCDx-unevaluable	p-value comparing the two subsets*
Age				
Mean (SD)	59.6 (11.5)	60.8 (12.1)	57.5 (10.3)	
Minimum	27	27	34	
Q1	53	55	52	
Median	60	62	57	0.078
Q3	68	69	66.5	
Maximum	84	84	74	
Sex				0.685
Male	39 (34.2%)	23 (32.4%)	16 (37.2%)	
Female	75 (65.8%)	48 (67.6%)	27 (62.8%)	
ECOG status				0.825
0	29 (25.4%)	19 (26.8%)	10 (23.3%)	
1	85 (74.6%)	52 (73.2%)	33 (76.7%)	
Race				<0.001
Asian	68 (59.6%)	33 (46.5%)	35 (81.4%)	
Black/African American	3 (2.6%)	3 (4.2%)	0 (0.0%)	
White	42 (36.8%)	35 (49.3%)	7 (16.3%)	
Unknown	1 (0.9%)	0 (0.0%)	1 (2.3%)	
Ethnicity				1.000
Hispanic or Latino	1 (0.9%)	1 (1.4%)	0 (0.0%)	
Not Hispanic or Latino	113 (99.1%)	70 (98.6%)	43 (100%)	
Region				<0.001
Asia Pacific (China, Japan, other)	55 (48.2%)	23 (32.4%)	32 (74.4%)	
Europe	6 (5.3%)	5 (7.0%)	1 (2.3%)	
North America	53 (46.5%)	43 (60.6%)	10 (23.3.%)	
Smoking history				0.840
Current	2 (1.8%)	1 (1.4%)	1 (2.3%)	
Former	31 (27.2%)	18 (25.4%)	13 (30.2%)	
Never	81 (71.1%)	52 (73.2%)	29 (67.4%)	
Body Mass Index (BMI)				
Mean (SD)	24.8 (5.0)	25.4 (5.4)	23.9 (4.0)	
Minimum	15.1	15.1	16.8	
Q1	21.2	22.1	21.1	
Median	23.8	24.3	23.3	0.147
Q3	27.7	28.7	26.5	
Maximum	40.9	40.9	34.5	
Stage at study entry				0.377
IIIB	1 (0.9%)	0 (0.0%)	1 (2.3%)	
IV	113 (99.1%)	71 (100%)	42 (97.7%)	
Number of anatomical sites involved at study entry				
Mean (SD)	3.5 (1.7)	3.8 (1.8)	3.0 (1.4)	
Minimum	1.0	1.0	1.0	

Baseline characteristic	CTA-positive	F1LCDx-evaluable	F1LCDx-unevaluable	p-value comparing the two subsets*
Q1	2.0	3.0	2.0	
Median	3.0	4.0	3.0	0.032
Q3	4.0	5.0	4.0	
Maximum	9.0	9.0	7.0	
Brain involvement				0.045
Yes	40 (35.1%)	30 (42.3%)	10 (23.3%)	
N/A	74 (64.9%)	41 (57.7%)	33 (76.7%)	
Liver involvement				0.018
Yes	24 (21.1%)	20 (28.2%)	4 (9.3%)	
N/A	90 (78.9%)	51 (71.8%)	39 (90.7%)	
Histology				1.000
Adenocarcinoma	112 (98.2%)	69 (97.2%)	43 (100%)	
Large Cell	1 (0.9%)	1 (1.4%)	0 (0%)	
Squamous	1 (0.9%)	1 (1.4%)	0 (0%)	

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

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with EXKIVITY was addressed during the review of the EXKIVITY 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. The most common adverse reactions were QTc Prolongation and Torsades de Pointes, Interstitial Lung Disease/Pneumonitis, Cardiac Failure, and Diarrhea. In addition, the safety findings in this study are consistent with the known safety profile of EXKIVITY and no new or unexpected safety signals were identified. No adverse events were reported in connection with the bridging study used to support this PMA supplement, as the study was performed retrospectively using banked samples.

Please refer to Drugs@FDA for complete safety information on EXKIVITY.

2. Effectiveness Results

a. Concordance Analysis with enrollment CTAs

As described above, 358 *EGFR* exon 20 insertion samples were included in the analysis. Only 268 patients had a plasma sample available for testing by F1LCDx and met the F1LCDx QC metrics, and 232 samples met the recommended sample input of cfDNA ≥ 30ng. An additional 36 samples met the minimum F1LCDx sample input criteria of cfDNA ≥ 20ng.

Concordance of the F1LCDx assay with the enrolling CTAs was demonstrated with the CDx-evaluable population. The primary concordance analysis was performed with 232 samples (132 *EGFR* exon 20 insertion positive, and 100

EGFR exon 20 insertion negative) that had DNA content ≥30 ng and met all F1LCDx QC metrics and summarized in Table 16, below.

Table 16. Concordance for *EGFR* exon 20 insertions between F1LCDx and the CTAs in samples with DNA content ≥30 ng

		CTAs		
		Detected	**Not Detected**	**Total**
F1LCDx	**Detected**	95	0	95
	Not Detected	37	100	137
	Unevaluable	98	28	126
	Total	230	128	358
Agreement Statistics Excluding CDx-Unevaluable Results		PPA: 72.0% (95/132) 95% CI[1]: (63.8%, 78.9%)	NPA: 100% (100/100) 95% CI[1]: (96.3%, 100%)	
Percent Unevaluable		42.6% (98/230)	21.9% (28/128)	

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

The PPA was 72.0% (95/132) with 95% two-sided CI (63.8%, 78.9%) and NPA was 100% (100/100) with 95% CI (96.3%, 100%) after excluding CDx-unevaluable results. Since patients were enrolled and initially tested by local CTAs, the PPV and NPV were calculated using the PPA and NPA, after adjusting for the prevalence of *EGFR* exon 20 insertions among the intention-to-treat (ITT) population. The prevalence estimate used in the adjusted agreement was 1.8%. In this analysis, F1LCDx demonstrated an adjusted PPV of 100% with 95% two-sided CI (96.1%, 100%) and NPV of 99.5% with 95% two-sided CI (99.3%, 100%).

An exploratory concordance analysis was conducted to include samples with cfDNA content of ≥20 ng for input into library construction. This analysis included an additional 27 and nine (9) *EGFR* exon 20 insertion-positive and negative samples, respectively. Concordance of the F1LCDx assay with the enrolling CTAs was maintained with a PPA of 68.9% (109/159) with 95% two-sided CI (61.0%, 75.3%) and NPA of 100% (109/109) with 95% two-sided CI (96.6%, 100%).

The discordance between the CTAs and F1LCDx among *EGFR* exon 20 insertion positive patients in the primary analysis were evaluated. Of the 37 CTA-positive/F1LCDx-negative samples, 27 had no *EGFR* exon 20 insertion detected by the F1LCDx pipeline. Ten (10) samples had an *EGFR* exon 20 insertion detected by F1LCDx but were filtered out for failing the pipeline's quality threshold. Among the 37 CTA-positive/F1LCDx-negative samples, 18 were from patients in the NDA population of the AP32788-15-101 trial and had clinical outcome data (refer to Section X.D.2.b, below).

Based on the low PPA between F1LCDx and the enrolling CTAs, which were predominately tissue-based tests, as shown in Table 16, F1LCDx may miss a large proportion of patients with NSCLC with *EGFR* exon 20 insertion who may

derive benefit from EXKIVITY. Therefore, reflex testing using tissue specimens to an FDA approved tissue test will be required, if feasible, if the plasma test is negative.

b. Bridging clinical outcome from CTA to F1LCDx
The clinical efficacy of EXKIVITY in the clinical trial was measured by ORR with either confirmed complete response (CR) or partial response (PR) based on 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 28.1% (32/114) with 95% two-sided CI (20.6%, 36.9%). Fifty-three (53) patients were CTA-positive and had F1LCDx *EGFR* exon 20 insertion positive results. The ORR for this population was 32.1% (17/53) with 95% two-sided CI (21.1%, 45.5%). Eighteen (18) patients were CTA-positive but had F1LCDx *EGFR* exon 20 insertion negative results. The ORR for this population was 16.7% (3/18) with 95% two-sided CI (5.8%, 39.2%). Forty-three (43) patients were CTA-positive but were unevaluable by F1LCDx. The ORR for this population was 27.9% (12/43) with 95% two-sided CI (16.7%, 42.7%), as summarized in Table 17.

Table 17. Efficacy by *EGFR* exon 20 insertion status in biomarker subgroups in samples with DNA content ≥30 ng

Clinical outcome	Total CTA+ population (N=114)	CTA+/F1LCDx+ (N=53)	CTA+/F1LCDx– (N=18)	CTA+/F1LCDx unevaluable (N=43)
ORR% [95% CI[1]]	28.1%	32.1%	16.7%	27.9%
	[20.6%, 36.9%]	[21.1%, 45.5%]	[5.8%, 39.2%]	[16.7%, 42.7%]
Number of responders[2]	**N=32**	**N=17**	**N=3**	**N=12**
Duration of response				
Median[3] in months (95% CI[1])	17.5 (7.4, 20.3)	7.4 (3.7, N/A[4])	N/A[5]	20.3 (8.3, N/A[4])
% with duration ≥6 months	59.4%	41.2%	66.7%	83.3%

[1]Calculated with Wilson 2-sided 95% CI
[2]All responses were partial response
[3]Median was determined using Kaplan-Meier estimate
[4]The upper bound of the 95% CI was not estimable
[5]The median could not be calculated for the CTA+/F1LCDx- subgroup due to the small sample size (the survival probability did not reach 50%)

An exploratory efficacy analysis was conducted to include samples with cfDNA content of ≥20 ng for input into library construction. This analysis included an additional nine (9) CTA-positive patients with F1LCDx results. Fifty-five (55) patients were CTA-positive and had F1LCDx *EGFR* exon 20 insertion positive results. The ORR for this population was 34.5% (19/55) with 95% two-sided CI (23.4%, 47.7%). Twenty-five (25) patients were CTA-positive but had F1LCDx *EGFR* exon 20 insertion negative results. The ORR for this population was 20.0% (5/25) with 95% two-sided CI (8.9%, 39.1%). Thirty-four (34) patients were CTA-positive but were unevaluable by F1LCDx. The

ORR for this population was 23.5% (8/34) with 95% two-sided CI (12.4%, 40.0%).

The median DOR in the CTA-positive population that responded to EXKIVITY (N=32) was 17.5 months with 95% two-sided CI (7.4, 20.3). Seventeen (17) patients that were CTA-positive and responded to EXKIVITY also had F1LCDx *EGFR* exon 20 insertion positive results. The median DOR for this population was 7.4 months with 95% two-sided CI (3.7, N/A). Twelve (12) patients that were CTA-positive and responded to EXKIVITY were not evaluable by F1LCDx. The median DOR for this population was 20.3 months with 95% two-sided CI (8.3, N/A). There are notable differences in the clinical characteristics between the F1LCDx-evaluable and unevaluable populations that may have contributed to differences in the median DOR. For example, F1LCDx-evaluable patients had a higher proportion of brain and liver involvement in their disease relative to F1LCDx-unevaluable patients, which may, in part, contribute to the shorter median DOR observed in the F1LCDx-positive subgroup. However, the size of this cohort (n=12) is small, and these results should be interpreted with caution.

Samples that were positive by F1LCDx for *EGFR* exon 20 insertions in the clinical bridging study VAFs as low as 0.20%. Since F1LCDx does not have a pre-specified VAF cut-off for positivity, but rather is based on quality threshold based on select metrics for each variant position, it is possible that patients with VAFs below 0.20% can be reported as positive. The clinical effectiveness of F1LCDx has not been demonstrated for patients with NSCLC that have *EGFR* exon 20 insertions with VAF below 0.20%. A limitation addressing the uncertainty of the clinical effectiveness of the device for *EGFR* exon 20 insertions with VAFs below those evaluated in the clinical study is included as a limitation to the device (also refer to Section XII. C. below).

c. Sensitivity Analysis

Sensitivity analyses with regard to missing values were conducted to evaluate the robustness of the ORR estimates considering F1LCDx unevaluable patients enrolled in the AP32788-15-101 trial. Samples were considered missing if the samples were not tested, if they were tested but returned an invalid result, or if they did not satisfy the cfDNA minimum input requirement (i.e., ≥ 30ng).

Amongst all CTA-positive NDA patients, 37.7% did not have a F1LCDx result (43/114).

To evaluate the impact of the F1LCDx unevaluable population, the distribution of patients for baseline covariates and disease characteristics was compared among the CTA-positive population, the F1LCDx-evaluable/CTA-positive subpopulation, and F1LCDx-unevaluable/CTA-positive subpopulation. A multiple imputation method was utilized to account for patients with missing or non-evaluable F1LCDx results (n=43).

The imputed ORR by BICR was estimated to be 32.6% (95% CI: 17.0%, 48.2%), which is similar to the ORR for the CTA-positive population based on the observed data [28.1% (95% CI: 20.6%, 36.9%)]. Thus, the sensitivity analysis demonstrated the robustness of the clinical efficacy estimate.

3. Pediatric Extrapolation
   In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population.

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one (1) investigator which 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 F1LCDx to identify *EGFR* exon 20 insertions in patients with NSCLC who may benefit from treatment with EXKIVITY was demonstrated through clinical bridging studies using specimens from patients enrolled into the AP32788-15-101 trial. The data from the analytical validation and clinical bridging studies support

the reasonable assurance of safety and effectiveness of the F1LCDx assay when used in accordance with the indications for use. Data from the AP32788-15-101 trial show that patients with NSCLC harboring *EGFR* exon 20 insertions received benefit from treatment with EXKIVITY and support the addition of the CDx indication to F1LCDx.

B. Safety Conclusions

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

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

C. Benefit-Risk Determination

The probable benefit of the F1LCDx assay in identifying patients with NSCLC with *EGFR* exon 20 insertions for treatment with EXKIVITY was demonstrated through clinical bridging studies using specimens from patients enrolled into the AP32788-15-101 trial.

For patients with NSCLC with *EGFR* exon 20 insertion positive status, the ORR for the CTA-positive patients was 28.1% (32/114) with 95% CI (20.6%, 36.9%). Clinical outcome for patients with NSCLC with *EGFR* exon 20 insertion fusion positive status by the CTA and F1LCDx indicated an ORR of 32.1% (17/53) with 95% CI (21.1%, 45.5%), which was comparable to the ORR in the CTA-positive population and provides evidence of a meaningful clinical benefit in this population. Of note, in the concordance analysis, the NPA and PPV were 100%. The observed ORR for the F1LCDx *EGFR* exon 20 insertion positive patients supports probable benefit of F1LCDx in selecting *EGFR* exon 20 insertion positive NSCLC patients for treatment with EXKIVITY.

There is potential risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result, and 2) incorrect interpretation of test results by the user. The risks of the F1LCDx assay are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial which may lead to adverse events or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug.

The risks of false results are partially mitigated by the analytical and clinical performance of the device, as summarized above, including the analytical accuracy, and clinical concordance and bridging efficacy studies. In addition, the risks of false negative results are partially mitigated by a recommendation that those patients whose plasma generates a negative result for those alterations included in Table 1, including *EGFR* exon 20 insertions, should have their tumor mutation status verified by using an FDA-approved tumor tissue test, if feasible. Additional factors to consider in determining probable risks and benefits for F1LCDx included: the availability of alternative tests. Of note, there are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *EGFR* exon 20 insertions to identify patients with NSCLC for treatment with EXKIVITY, and this device may meet an unmet clinical need; however, there is an FDA-approved tissue test available for the identification of NSCLC patients with *EGFR* exon 20 insertions for treatment with EXKIVITY. The advantage of F1LCDx over the FDA-approved tissue test, is that it offers a non-invasive method to obtain DNA from the cancer, rather than a tissue biopsy and may meet an unmet need for patients who cannot otherwise provide a biopsy. Another factor to consider in the benefit-risk profile is that this device would provide additional potential benefit to the patient by profiling 324 cancer related genes. However, given uncertainties that remain based on the analytical and clinical validation data, the following limitations are included in the device labeling:

- The analytical accuracy for the FoundationOne Liquid CDx assay for detection of *EGFR* exon 20 insertions has not been demonstrated for samples with <0.18% VAF
- EXKIVITY® efficacy has not been established in patients with *EGFR* exon 20 insertions <0.20 % VAF tested with FoundationOne Liquid CDx

The overall clinical and analytical validation data support that for F1LCDx, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

1. Patient Perspective
   This submission did not include specific information on patient perspectives for this device.

In conclusion, given the available information above, the data support that for the selection of patients with NSCLC with *EGFR* exon 20 insertions for treatment with EXKIVITY, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical bridging study support the performance of F1LCDx as an aid for the identification of *EGFR* exon 20 insertions in patients with NSCLC for whom EXKIVITY may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on May 3, 2023. The final conditions of approval cited in the approval order are described below.

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

- FMI will submit a list of the cumulative changes and in sufficient detail acceptable to FDA, made between the currently deployed genomics platform, which includes analytical pipeline software version v3.21 (AP v3.21), and the AP versions used in the analytical and clinical validation studies in this supplement.

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

- FMI will provide evidence, acceptable to FDA, that performance expectations with the currently deployed genomics platform, including AP v3.21 are representative of the performance in the analytical and clinical validation studies in this supplement. Such evidence may include regression testing using the clinical and analytical datasets to perform *in silico* reanalysis of the results obtained in the analytical and clinical validation studies in this supplement and confirmation that there is little or no deviation in the quality metrics for each of the samples to support the accuracy and precision of the assay remains the same.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

None.