

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/S011

Date of FDA Notice of Approval: October 11, 2023

The original Premarket Approval (PMA) (P190032) 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 F1LCDx 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)	<i>BRAF</i> V600E	BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib)

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 genomic alterations in 311 genes. These include substitutions, insertions and deletions (indels) in 311 genes, rearrangements in 8 genes, and copy number alterations in 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)	<i>ALK</i> Rearrangements	ALECENSA® (alectinib)
	<i>BRAF</i> V600E	BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib)
	<i>EGFR</i> Exon 19 deletions and <i>EGFR</i> Exon 21 L858R substitution	<i>EGFR</i> tyrosine kinase inhibitors approved by FDA*
	<i>EGFR</i> Exon 20 insertions	EXKIVITY® (mobocertinib)
	<i>MET</i> single nucleotide variants (SNVs) and indels that lead to <i>MET</i> exon 14 skipping	TABRECTA® (capmatinib)
	<i>ROS1</i> fusions**	ROZLYTREK® (entrectinib)
Prostate cancer	<i>BRCA1</i> , <i>BRCA2</i> , and <i>ATM</i> alterations	LYNPARZA® (olaparib)
	<i>BRCA1</i> , <i>BRCA2</i> alterations	RUBRACA® (rucaparib)
Breast Cancer	<i>PIK3CA</i> mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y	PIQRAY® (alpelisib)
Solid Tumors	<i>NTRK1/2/3</i> fusions**	ROZLYTREK® (entrectinib)
Colorectal cancer (CRC)	<i>BRAF</i> V600E	BRAFTOVI® (encorafenib) in combination with cetuximab

*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 F1LCDx labeling.

V. DEVICE DESCRIPTION

The 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 targeted 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 in 311 genes. These include base substitutions and indels in 311 genes, copy number variants in 3 genes, and genomic rearrangements in 8 genes. A subset of targeted regions in 75 genes is baited for increased sensitivity.

Table 2: Genomic Regions in which Variants are Targeted by F1LCDx¹

<i>ABL1</i> [Exons 4-9]	<i>ACVR1B</i>	<i>AKT1</i> [Exon 3]	<i>AKT2</i>	<i>AKT3</i>	<i>ALK</i> [Exons 20-29, Introns 18, 19]	<i>ALOX12B</i>	<i>AMER1</i> (<i>FAM123B</i>)	<i>APC</i>	<i>AR</i>
<i>ARAF</i> [Exons 4, 5, 7, 11, 13, 15, 16]	<i>ARFRP1</i>	<i>ARID1A</i>	<i>ASXL1</i>	<i>ATM</i>	<i>ATR</i>	<i>ATRX</i>	<i>AURKA</i>	<i>AURKB</i>	<i>AXIN1</i>
<i>AXL</i>	<i>BAP1</i>	<i>BARD1</i>	<i>BCL2</i>	<i>BCL2L1</i>	<i>BCL2L2</i>	<i>BCL6</i>	<i>BCOR</i>	<i>BCORL1</i>	<i>BCR*</i> [Introns 8, 13, 14]
<i>BRAF</i> [Exons 11-13]	<i>BRCA1</i> [Introns 2, 7, 11]	<i>BRCA2</i> [Intron 2]	<i>BRD4</i>	<i>BRIP1</i>	<i>BTG1</i>	<i>BTG2</i>	<i>BTK</i> [Exons 2, 15]	<i>C11orf30</i> (<i>EMSY</i>)	<i>C17orf39</i> (<i>GID4</i>)

18, Introns 7-10]	8, 12, 16, 19, 20]								
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	GNAI1 [Exons 4, 5]	GNA13	GNAQ [Exons 4, 5]	GNAS [Exons 1, 8]	GRM3
GSK3B	H3F3A	HDAC1	HGF	HNF1A	HRAS [Exons 2, 3]	HSD3B1	ID3	IDH1 [Exon 4]	IDH2 [Exon 4]
IGF1R	IKBKE	IKZF1	INPP4B	IRF2	IRF4	IRS2	JAK1	JAK2 [Exon 14]	JAK3 [Exons 5, 11, 12, 13, 15, 16]
JUN	KDM5A	KDM5C	KDM6A	KDR	KEAP1	KEL	KIT [Exons 8, 9, 11, 12, 13, 17, Intron 16]	KLHL6	KMT2A (MLL) [Introns 6, 8-11, Intron 7]
KMT2D (MLL2)	KRAS	LTK	LYN	MAF	MAP2K1 (MEK1) [Exons 2, 3]	MAP2K2 (MEK2) [Exons 2-4, 6, 7]	MAP2K4	MAP3K1	MAP3K13
MAPK1	MCL1	MDM2	MDM4	MED12	MEF2B	MEN1	MERTK	MET	MITF
MKNK1	MLH1	MPL [Exon 10]	MRE11A	MSH2 [Intron 5]	MSH3	MSH6	MST1R	MTAP	MTOR [Exons 19, 30, 39, 40, 43-45, 47, 48, 53, 56]
MUTYH	MYB* [Intron 14]	MYC [Intron 1]	MYCL (MYCL1)	MYCN	MYD88 [Exon 4]	NBN	NF1	NF2	NFE2L2
NFKBIA	NKX2-1 (TTF-1)	NOTCH1	NOTCH2 [Intron 26]	NOTCH3	NPM1 [Exons 4-6, 8, 10]	NRAS [Exons 2, 3]	NSD3 (WHSC1L1)	NT5C2	NTRK1 [Exons 14, 15, Introns 8-11]
NTRK2 [Intron 12]	NTRK3 [Exons 16, 17]	NUTM1* [Intron 1]	P2RY8	PALB2	PARK2	PARP1	PARP2	PARP3	PAX5

<i>PBRM1</i>	<i>PDCD1</i> (PD-1)	<i>PDCD1L G2</i> (PD-L2)	<i>PDGFRA</i> [Exons 12, 18, Introns 7, 9, 11]	<i>PDGFRB</i> [Exons 12- 21, 23]	<i>PDK1</i>	<i>PIK3C2B</i>	<i>PIK3C2G</i>	<i>PIK3CA</i> [Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4- 7, 9, 13, 18, 20)]	<i>PIK3CB</i>
<i>PIK3R1</i>	<i>PIM1</i>	<i>PMS2</i>	<i>POLD1</i>	<i>POLE</i>	<i>PPARG</i>	<i>PPP2R1A</i>	<i>PPP2R2A</i>	<i>PRDM1</i>	<i>PRKARIA</i>
<i>PRKCI</i>	<i>PTCH1</i>	<i>PTEN</i>	<i>PTPN11</i>	<i>PTPRO</i>	<i>QKI</i>	<i>RAC1</i>	<i>RAD21</i>	<i>RAD51</i>	<i>RAD51B</i>
<i>RAD51C</i>	<i>RAD51D</i>	<i>RAD52</i>	<i>RAD54L</i>	<i>RAF1</i> [Exons 3, 4, 6, 7, 10, 14, 15, 17, Introns 4-8]	<i>RARA</i> [Intron 2]	<i>RB1</i>	<i>RBM10</i>	<i>REL</i>	<i>RET</i> [Introns 7, 8, Exons 11, 13-16, Introns 9-11]
<i>RICTOR</i>	<i>RNF43</i>	<i>ROS1</i> [Exons 31, 36-38, 40, Introns 31- 35]	<i>RPTOR</i>	<i>RSPO2*</i> [Intron 1]	<i>SDC4*</i> [Intron 2]	<i>SDHA</i>	<i>SDHB</i>	<i>SDHC</i>	<i>SDHD</i>
<i>SETD2</i>	<i>SF3B1</i>	<i>SGK1</i>	<i>SLC34A2*</i> [Intron 4]	<i>SMAD2</i>	<i>SMAD4</i>	<i>SMARCA4</i>	<i>SMARCB1</i>	<i>SMO</i>	<i>SNCAIP</i>
<i>SOCS1</i>	<i>SOX2</i>	<i>SOX9</i>	<i>SPEN</i>	<i>SPOP</i>	<i>SRC</i>	<i>STAG2</i>	<i>STAT3</i>	<i>STK11</i> (<i>LKB1</i>)	<i>SUFU</i>
<i>SYK</i>	<i>TBX3</i>	<i>TEK</i>	<i>TERC*</i> {ncRNA}	<i>TERT*</i> {Promoter}	<i>TET2</i>	<i>TGFBR2</i>	<i>TIPARP</i>	<i>TMPRSS2*</i> [Introns 1-3]	<i>TNFAIP3</i>
<i>TNFRSF14</i>	<i>TP53</i>	<i>TSC1</i>	<i>TSC2</i>	<i>TYRO3</i>	<i>U2AF1</i>	<i>VEGFA</i>	<i>VHL</i>	<i>WHSC1</i>	<i>WT1</i>
<i>XPO1</i>	<i>XRCC2</i>	<i>ZNF217</i>	<i>ZNF703</i>						

¹While 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 *), F1LCDx reports alterations only in 311 genes (309 genes with coding coverage and 2 genes with non-coding coverage). F1LCDx also reports Rearrangements in 13 genes from non-coding region, only when the partner gene is approved for reporting, e.g., CD74 in a ROS1-CD74 fusion. 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	<i>BRCA1, BRCA2, ERBB2</i>
Rearrangements	<i>ALK, BRCA1, BRCA2, NTRK1, NTRK2, NTRK3</i>

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 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
Thermo Fisher Scientific Kingfisher Flex DW 96
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 liquid handling workstation (Hamilton STAR) using the “with-bead” protocol to maximize reproducibility and library yield. Dual-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 pre-specifications 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 <i>BRCA1</i> or <i>BRCA2</i> 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 <i>BRCA2</i> K3326*
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions $\pm$ 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 trial

<i>BRCA1</i> Alterations (Protein Change)					<i>BRCA2</i> 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 <i>BRCA2</i> K3326*	Does not include VUS. Includes mutations on the canonical transcript only for genes <i>ATM</i> , <i>BRCA1</i> , and <i>BRCA2</i> .
	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 $\pm$ 2bp from the intron/exon junction. Includes mutations on the canonical transcript only for genes <i>ATM</i> , <i>BRCA1</i> , and <i>BRCA2</i> .
	Homozygous deletions	Sequence analysis identifies deletions in both gene alleles of ≥ 1 exon in size	Does not include VUS Only reported for <i>BRCA1</i> and <i>BRCA2</i> . Not reported for <i>ATM</i> .
	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 <i>ATM</i> , <i>BRCA1</i> , and <i>BRCA2</i> .

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.

<i>BRCA1</i>		<i>BRCA2</i>		<i>ATM</i>	
Protein Effect (PE)	FMI Annotated PE	Protein Effect (PE)	FMI Annotated PE	Protein Effect (PE)	FMI Annotated PE
M1V	M1V	M1R	M1R	M1T	M1T
MII	MII	MII	MII	R2032K	R2032K
C61G	C61G	V159M	V159M	R2227C	R2227C
C64Y	C64Y	V211L	V211L	R2547- S2549del	R2547- S2549del

R71G	R71G	V211I	V211I	G2765S	G2765S
R71K	R71K	R2336P	R2336P	R2832C	R2832C
R1495M	R1495M	R2336H	R2336H	S2855- V2856delinsR1	S2855- V2856delinsR1 S2855- V2856>R1
E1559K	E1559K			R3008C	R3008C
D1692N	D1692N			R3008H	R3008H
D1692H	D1692H			[VUS from Jan 2016 HRR* List to be Excluded]	
R1699W	R1699W			V2424G	V2424G
A1708E	A1708E			[Excluded from Jan 2016 HRR List]	
G1788V	G1788V			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:*
 1. Base substitutions resulting in *EGFR* L858R
 2. 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

9. *BRAF V600E to identify metastatic CRC patients eligible for treatment with BRAFTOVI (encorafenib) in combination with cetuximab and*

NSCLC patients for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib)

CDx classification criteria for BRAF substitutions:

- Base substitutions resulting in BRAF V600E

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of *BRAF* V600E to identify patients with NSCLC eligible for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib).

However, FoundationOne CDx (F1LCDx) test from Foundation Medicine, Inc. (FMI) is an FDA-approved CDx (P170019/S039) using formalin-fixed, paraffin-embedded (FFPE) tissue specimens 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 9.

Table 9. Marketing History

Submission No.	Date of Approval	Biomarker/Update	Indication	Drug
P200006	October 26, 2020	<i>ALK</i> Rearrangements	NSCLC	ALECENSA [®] (alectinib)
		<i>PIK3CA</i> alterations	Breast Cancer	PIQRAY [®] (alpelisib)
P200016	November 6, 2020	<i>BRCA1</i> , <i>BRCA2</i> , and <i>ATM</i> alterations	Prostate Cancer	LYNPARZA [®] (olaparib)
P190032/S001	July 15, 2021	<i>MET</i> single nucleotide variants (SNVs) and indels that lead to <i>MET</i> exon 14 skipping	NSCLC	TABRECTA [®] (capmatinib)
P190032/S004	December 22,	<i>NTRK1/2/3</i> fusions	Solid Tumors	ROZLYTREK [®]

Submission No.	Date of Approval	Biomarker/Update	Indication	Drug
	2022	<i>ROS1</i> fusions	NSCLC	(entrectinib)
P190032/S008	December 19, 2022	<i>EGFR</i> Exon 19 deletions and <i>EGFR</i> Exon 21 L858R alteration	NSCLC	<i>EGFR</i> tyrosine kinase inhibitors approved by FDA
P190032/S005	May 3, 2023	<i>EGFR</i> Exon 20 insertions	NSCLC	EXKIVITY® (mobocertinib)
P190032/S010	June 8, 2023	<i>BRAF</i> V600E alteration	CRC	BRAFTOVI® (encorafenib) in combination with cetuximab

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 BRAFTOVI (encorafenib) and MEKTOVI (binimetinib) 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 *BRAF* V600E is presented in this section. Analytical accuracy/concordance and precision near the limit of detection (LoD) studies were conducted to support the indication for *BRAF* V600E using clinical samples.

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 *BRAF* V600E.

For this study, a total of 318 biomarker-positive and biomarker-negative samples from residual banked cfDNA derived from clinical colorectal cancer (CRC, n=203) and NSCLC (n=115) samples, as well as clinical samples from the BEACON trial (CRC samples) were identified for testing. Of these samples, 14 CRC samples were excluded due to F1LCDx or evNGS quality control (QC) failures.

Analytical concordance of F1LCDx for detecting *BRAF* V600E was determined with 304 samples tested by F1LCDx assay (Table 10). 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% (91/91) with two-sided 95% confidence interval (CI) (95.95%, 100%), and NPV as 99.53% (212/213) with two-sided 95% CI (97.39%, 99.92%), as shown in Table 10, below. For informational purposes, unadjusted positive percent agreement (PPA) and negative percent agreement (NPA) are also displayed. Analytical concordance of F1LCDx for detecting *BRAF* V600E in NSCLC is displayed in Table 11.

Table 10. Concordance summary for *BRAF* V600E by F1LCDx and the evNGS in CRC and NSCLC

		evNGS			
		<i>BRAF</i> V600E positive	<i>BRAF</i> V600E negative	Total	PPV/NPV (95% CI ¹)
F1LCDx	<i>BRAF</i> V600E positive	91	0	91	PPV: 100% (95.95%, 100%)
	<i>BRAF</i> V600E negative	1	212	213	NPV: 99.53% (97.39%, 99.92%)
	Total	92	212	304	
	PPA/NPA (Unadjusted) (95% CI ¹)	PPA: 98.91% (94.1%, 99.81%)	NPA: 100% (98.22%, 100%)		

¹Calculated with Wilson 2-sided 95% CI

In the one (1) discordant sample that was F1LCDx-negative/evNGS-positive, there were low supporting reads (4) and a percent variant allele frequency (VAF) of 0.5% for F1LCDx. This discordance may be due to the low percent VAF, which did not pass F1LCDx calling threshold for *BRAF* V600E (refer to Section IX.A.2.b. below)

Table 11. Concordance summary for *BRAF* V600E by F1LCDx and the evNGS in NSCLC

		evNGS			
		<i>BRAF</i> V600E positive	<i>BRAF</i> V600E negative	Total	PPV/NPV (95% CI ¹)
F1LCDx	<i>BRAF</i> V600E positive	10	0	10	PPV: 100% (72.5%, 100%)
	<i>BRAF</i> V600E negative	0	105	105	NPV: 100% (96.47%, 100%)
	Total	10	105	115	
	PPA/NPA (Unadjusted) (95% CI ¹)	PPA: 100% (72.25%, 100%)	NPA: 100% (96.47%, 100%)		

¹Calculated with Wilson 2-sided 95% CI

The lowest VAF for *BRAF* V600E positive samples from patients with NSCLC used in the analytical accuracy study was 1.0%. However, the lowest VAF of the sample in the clinical trial (refer to section X below) evaluated in the clinical bridging study was 0.099%, with 14/48 (29%) *BRAF* V600E positive samples having a VAF < 0.5%. To demonstrate accuracy for *BRAF* V600E variants below 1%, FMI provided supplementary evidence in the form of positive concordance data from the F1LCDx orthogonal platform concordance study with *BRAF* V600E samples with VAFs between 0.11% and 0.43% by F1LCDx from patients with other cancers (n=5). Four out of 5 samples tested were concordant, i.e., determined to be positive by F1LCDx and the NGS comparator method.

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 (20-60 ng) 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 0.5%. Further *BRAF* V600E was not observed in any of the replicates.

b. Limit of Detection (LoD)

The LoD for *BRAF* V600E was estimated panel-wide (for short variants) as 0.4% VAF 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). To confirm the LoD, FMI performed a LoD confirmation study using two (2) clinical specimens

[one (1) from a patient with CRC and one (1) from patient with NSCLC] positive for *BRAF* V600E. These two samples were tested near the platform estimated LoD of 0.4% VAF for short variants to assess the VAF necessary for the accurate detection and sensitivity of *BRAF* V600E. The LoDs were confirmed using 24 replicates for CRC sample and 22 replicates for NSCLC sample. The LoD for *BRAF* V600E is confirmed to be 0.86% VAF in patients with NSCLC as the lowest VAF level tested in the confirmation of LoD study for which 100% hit rate was observed across the two samples, as summarized in Table 13, below. This LoD is similar to the LoD observed when testing patient samples from CRC patients, 0.70% VAF as shown in Table 12.

Table 12: Confirmation of LoD Results for Targeted *BRAF* V600E

Disease Ontology	Target Alteration	# Agree	# Valid Total	Observed Average VAF (%)	Hit Rate (%) 95% CI
CRC	1799T>A	24	24	0.70	100 [86.2, 100]
NSCLC	1799T>A	22	22	0.86	100 [85.13, 100]

3. Precision and Reproducibility

Within-Laboratory (Intermediate) Precision

A precision study was conducted using two clinical samples harboring *BRAF* V600E, one (1) sample from a patient with metastatic CRC, and one (1) sample from a patient with NSCLC.

Reproducibility including inter-run performance (run on different plates under different conditions) and repeatability including intra-run performance (run on the same plate under the same conditions) were assessed and compared in duplicates across two different sequencers and three different reagent lots, across multiple days of performance by multiple operators. The results for the precision study for the clinical samples near LoD are summarized in Table 13 and Table 14 below.

Table 13. Reproducibility results for *BRAF* V600E variant

Disease Ontology	Targeted Alteration	# Agree	# Valid Total	Reproducibility (%) [95% CI]	Observed Average VAF (%)	Fold LoD ¹
CRC	1799T>A	24	24	100 [86.2, 100]	0.7	1.76x
NSCLC	1799T>A	22	22	100 [85.1, 100]	0.86	2.17x

¹Fold LoD value = observed average %VAF / 1x LoD (%VAF)

Table 14. Repeatability results for *BRAF* V600E variant

Disease Ontology	Targeted Alteration	# Agree	# Valid Total	Reproducibility (%) [95% CI]
CRC	1799T>A	12	12	100 [75.75, 100]
NSCLC	1799T>A	10	10	100 [72.25, 100]

Inter-run reproducibility was evaluated across 24 CRC and 22 NSCLC sample replicates and intra-run repeatability was evaluated across 12 (CRC) and 10 (NSCLC) duplicates per plate. No discordances were observed. Overall, both reproducibility and repeatability were 100%. The corresponding two-sided Wilson score 95% CIs are provided for the reproducibility and repeatability.

B. Animal Studies

No animal studies were conducted using the F1LCDx assay.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The reasonable assurance of safety and effectiveness for F1LCDx for detection of *BRAF* V600E in patients with NSCLC who may benefit from treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib) was established through retrospective analysis of banked plasma samples from patients enrolled in the clinical trial, the PHAROS (ARRAY-818-202) trial, as well as *BRAF* V600E-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. PHAROS Study Design

The PHAROS trial is an open-label, multicenter, single-arm study in patients with *BRAF* V600E-positive metastatic NSCLC. Eligible patients were either treatment-naïve or had received treatment with chemotherapy and/or immunotherapy (previously treated). Prior use of *BRAF* inhibitors or MEK inhibitors was not permitted. The major efficacy outcome measure was confirmed objective response rate (ORR) by an independent review committee (IRC) per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria to determine whether treatment with BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib) is effective. A key secondary efficacy outcome was duration of response (DoR) as assessed by an IRC.

The PHAROS clinical study population comprises *BRAF* V600E-positive subjects from treatment naïve and previously treated patient Cohorts. Ninety-eight (98) subjects were enrolled based on the presence of *BRAF* V600E by local tissue testing or clinical trial assays (CTA).

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 *BRAF* V600E, and 2) the clinical validity of F1LCDx in identifying patients with NSCLC with *BRAF*

V600E who may be eligible for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib).

F1LCDx testing was performed on patients with available plasma samples from the PHAROS trial that tested positive for *BRAF* V600E by local tissue testing or clinical trial assays (CTA). The PHAROS clinical study did not include patients negative for *BRAF* V600E and therefore did not represent the F1LCDx (+), tissue-based CTA (-) [F1LCDx+ CTA-] subgroup of the F1LCDx intended use population. As such, supplemental matched tissue and plasma samples were obtained from subjects available through commercial sources and only plasma samples from the FMI archives, and a sensitivity analysis was performed to evaluate the potential impact of the F1LCDx(+)/CTA (-) population on the efficacy in the F1LCDx intended use population.

3. Clinical Inclusion and Exclusion Criteria

The sample inclusion and exclusion criteria for the retrospective testing of the clinical bridging study were:

Sample inclusion criteria:

- Specimens in frozen plasma.
- Samples meet minimum criteria for F1LCDx operational testing requirements
- For F1LCDx: Samples must have cfDNA input, as assessed by the TapeStation assay, ≥ 20 ng, for primary analysis

Sample exclusion criteria:

- Tissue, other liquid samples are excluded
- Samples that do not meet minimum F1LCDx operational testing requirements
- For F1LCDx: Samples with <20 ng DNA as assessed by the TapeStation assay

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

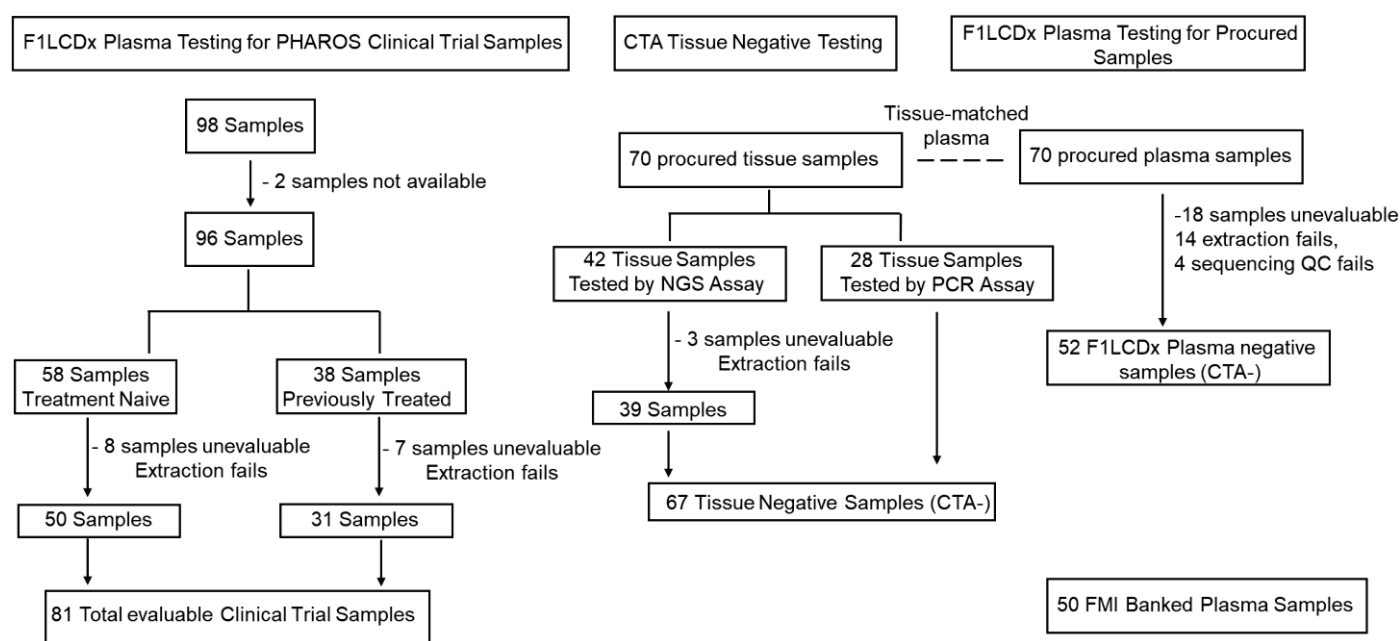
The primary endpoint of the PHAROS trial is the ORR defined as the proportion of patients who have achieved a confirmed best ORR (CR or PR) as determined by IRC per RECIST v1.1 in both the treatment naïve and previously treated settings.

B. Accountability of PMA Cohort

The F1LCDx clinical bridging study included a total of 98 patient samples from PHAROS trial, which consists of 59 treatment-naïve and 39 previously treated patient samples. Of these, 48 subjects (49%) tested *BRAF* V600E positive by F1LCDx and were included in the primary objective analysis set, while 33 (34%) tested negative, and 17 (17%) subjects enrolled in the PHAROS trial were not evaluable due to extraction failures and lack of available plasma (Figure 1).

For biomarker-negative (negative for *BRAF* V600E) samples, matched tissue and plasma samples were procured from commercial sources and plasma only samples from the FMI archives. The 120 negative samples consisted of 70 procured commercial tissue samples with matched plasma and 50 negative plasma samples from FMI archives. The samples procured from commercial vendors were tested using PCR and NGS based tests, which were representative of the CTAs used to enroll subjects into the PHAROS clinical study. Three procured samples did not have enough material for the external testing and were unevaluable for CTA. As a result, these 3 samples were not included in the analysis. Therefore, a total of 117 (120 less 3) CTA (-) samples were included in the analysis. Among the 70 procured plasma samples, 18 samples were unevaluable by F1LCDx (14 extraction failures and 4 QC failures).

Figure 1: Sample Processing and Accountability Flow Chart



C. Study Population Demographics and Baseline Parameters

The key demographic and clinical characteristics for F1LCDx-evaluable patients and F1LCDx-unevaluable patients are summarized in Table 15 (treatment naïve) and Table 16 (previously treated), below. An analysis comparing the two groups was performed as part of the sensitivity analysis in the clinical bridging study to identify those characteristics that were imbalanced.

Table 15. Demographics and Clinical Characteristics in the F1LCDx Evaluable-and F1LCDx-Unevaluable Subsets (treatment naïve Cohort)

Covariate	F1LCDx Evaluable/CTA +	F1LCDx Unevaluable/CTA+	Difference in mean/proportion (95% CI)	p-value comparing the two subsets*
Age				0.94
Mean	66.62	66.00	0.62 (-8, 7)	
Minimum	51.00	47.00		
Q1	60.25	60.00		
Median	68.00	70.00		
Q3	73.00	72.00		
Maximum	83.00	82.00		
SD	8.15	10.82		
Sex				0.49
Male	21 (42%)	5 (55.46%)	-13.56% (-42.07%, 18.45%)	
Female	29 (58%)	4 (44.44%)	13.56% (-18.45%, 42.07%)	
ECOG status				1.00
0	16 (32%)	3 (33.33%)	-1.33% [-34.54%, 24.03%]	
1	34 (68%)	6 (66.67%)	1.33% [-24.03%, 34.54%]	
Race				1.00
Asian	3 (6%)	0 (0%)	6% [-24.17%, 16.22%]	
Black/African American	1 (2%)	0 (0%)	2% [-27.96%, 10.5%]	
White	44 (88%)	9 (100%)	-12% [-23.8%, 18.59%]	
Unknown	1 (2%)	0 (0%)	2% [-27.96%, 10.5%]	
American Indian or Alaska Native	1 (2%)	0 (0%)	2% [-27.96%, 10.5%]	
Ethnicity				1.00
Not Hispanic or Latino	49 (98%)	9 (100%)	-2% [-10.5%, 27.96%]	
Unknown	1 (2%)	0 (0%)	2% [-27.96%, 10.5%]	
Height BL [#]				0.96
Mean	167.19	166.60	0.59 [-8.1, 10]	
Min	143.00	149.90		
Q1	159.25	160.75		
Median	165.10	166.50		
Q3	175.23	172.73		
Max	210.50	182.00		
SD	11.90	11.44		
N/A	0	1		
Weight BL [#]				0.77
Mean	74.01	78.12	-4.11 [-17.65, 17.5]	
Minimum	39.80	50.10		
Q1	55.67	60.70		
Median	71.34	70.00		

Covariate	F1LCDx Evaluable/CTA +	F1LCDx Unevaluable/CTA+	Difference in mean/proportion (95% CI)	p-value comparing the two subsets*
Q3	94.00	91.00		
Maximum	125.20	143.70		
SD	22.14	29.33		
TBSTAT [#]				0.10
Current smoker	5 (10%)	3 (33.33%)	-23.33% [-55.09%, 0.78%]	
Former smoker	28 (56%)	5 (55.56%)	0.44% [-28.56%, 32.06%]	
Never smoker	17 (34%)	1 (11.11%)	22.89% [-11.5%, 39.47%]	
Tissue Handling				0.84
Core Needle/Excisional Biopsy	24 (48%)	6 (66.67%)	-18.67% [-43.71%, 15.37%]	
Fine Needle Aspiration (FNA)	12 (24%)	1 (11.11%)	12.89% [-20.92%, 29.11%]	
Other	3 (6%)	0 (0%)	6% [-24.17%, 16.22%]	
Resection	9 (18%)	2 (22.22%)	-4.22% [-37.77%, 16.19%]	
N/A	2 (4%)	0 (0%)	4% [-26.05%, 13.46%]	
Sampling Site				0.66
Metastatic	18 (36%)	2 (22.22%)	13.78% [-20.84%, 34.87%]	
Primary	19 (38%)	5 (55.56%)	-17.56% [-45.86%, 14.48%]	
Plasma	13 (26%)	2 (22.22%)	3.78% [-30.28%, 24.67%]	

*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

Q1: first quartile, Q3: third quartile, SD: standard deviation, ECOG: Eastern Cooperative Oncology Group[#] Height BL: Height baseline, Weight BL: Weight baseline, TBSTAT: Tobacco use status

Table 16. Demographics and Clinical Characteristics in the F1LCDx Evaluable-and F1LCDx-Unevaluable Subsets (previously treated Cohort)

Covariate	F1LCDx Evaluable/CTA+	F1LCDx Unevaluable/CTA+	Difference in mean/proportion (95% CI)	p-value comparing the two subsets*
Age				0.41
Mean	69.00	72.38	-3.38 [-12, 4]	
Minimum	53.00	55.00		
Q1	62.00	69.00		
Median	71.00	72.00		
Q3	76.00	80.75		
Maximum	86.00	83.00		
SD	8.99	9.83		
Sex				0.04
Male	13 (41.94%)	7 (87.5%)	-45.56% [-64.17%, -6.89%]	
Female	18 (58.06%)	1 (12.5%)	45.56% [6.89%, 64.17%]	
ECOG status				0.62
0	5 (16.13%)	2 (25%)	-8.87% [-44.12%, 15.44%]	
1	26 (83.87%)	6 (75%)	8.87% [-15.44%, 44.12%]	

Covariate	F1LCDx Evaluable/CTA+	F1LCDx Unevaluable/CTA+	Difference in mean/proportion (95% CI)	p-value comparing the two subsets*
Race				0.72
Asian	4 (12.9%)	0 (0%)	12.90% [-20.45%, 28.85%]	
Black/African American	2 (6.45%)	0 (0%)	6.45% [-26.32%, 20.72%]	
White	25 (80.65%)	8 (100%)	-19.35% [-36.28%, 14.64%]	
Unknown	0 (0%)	0 (0%)	0% NA	
American Indian or Alaska Native	0 (0%)	0 (0%)	0% NA	
Ethnicity				NA*
Not Hispanic or Latino	31 (100%)	8 (100%)	0% [-11.03%, 32.44%]	
Unknown	0 (0%)	0 (0%)	0% NA	
Height BL [#]				0.06
Mean	165.75	173.34	-7.58 [-15, 0.3]	
Min	151.10	162.00		
Q1	158.50	165.68		
Median	165.10	169.70		
Q3	170.15	178.25		
Max	186.00	193.00		
SD	8.99	11.49		
N/A	1	0		
Weight BL [#]				0.28
Mean	70.44	78.73	-8.29 [-21.55, 6.45]	
Minimum	51.00	48.31		
Q1	59.76	70.55		
Median	72.30	77.20		
Q3	76.67	84.36		
Maximum	92.10	115.76		
SD	12.04	21.18		
TBSTAT [#]				0.02
Current smoker	2 (6.45%)	3 (37.50%)	-31.05% [-63.31%, -3.29%]	
Former smoker	18 (58.06%)	5 (62.50%)	-4.44% [-33.87%, 31.06%]	
Never smoker	11 (35.48%)	0 (0%)	35.48% [0%, 53.05%]	
Tissue Handling				1.00
Core Needle/Excisional Biopsy	21 (67.74%)	6 (75%)	-7.26% [-32.33%, 29.46%]	
Fine Needle Aspiration (FNA)	5 (16.13%)	1 (12.5%)	3.63% [-32.12%, 23.06%]	
Other	3 (9.68%)	1 (12.5%)	-2.82% [-37.99%, 15.53%]	
Resection	2 (6.45%)	0 (0%)	6.45% [-26.32%, 20.72%]	
Sampling Site				0.72
Metastatic	12 (38.71%)	2 (25%)	13.71% [-23.51%, 38.68%]	
Primary	14 (45.16%)	2 (25%)	20.16% [-17.48%, 44.86%]	

Covariate	F1LCDx Evaluable/CTA+	F1LCDx Unevaluable/CTA+	Difference in mean/proportion (95% CI)	p-value comparing the two subsets*
Plasma	5 (16.13%)	2 (25%)	-8.87% [-44.12%, 15.44%]	
N/A	0 (0%)	2 (25%)	-25% [-59.07%, -4.02%]	

*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

Q1: first quartile, Q3: third quartile, SD: standard deviation, ECOG: Eastern Cooperative Oncology Group# Height

BL: Height baseline, Weight BL: Weight baseline, TBSTAT: Tobacco use status

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib) was addressed during the review of the supplemental New Drug Application (sNDA) and is not addressed in detail in this SSER. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs. No adverse events were reported in the conduct of the diagnostic studies used to support this sPMA as these involved retrospective testing of banked plasma specimens only.

Please refer to Drugs@FDA for complete safety information on BRAFTOVI (encorafenib) and MEKTOVI® (binimetinib).

2. Effectiveness Results

a. Concordance Between F1LCDx and enrolling CTAs

Concordance between F1LCDx and enrolling CTA testing using matched plasma and tissue samples from PHAROS clinical study, is shown in Table 17 below. While all samples from the PHAROS clinical study population were positive for *BRAF* V600E as a requirement for enrollment in the clinical study, biomarker negative subject samples were identified and commercially procured or from FMI archives to represent the *BRAF* V600E-negative population, and their plasma samples were tested by F1LCDx.

The PPA was 59.26% (48/81) with two-sided 95% confidence interval (48.38%, 69.3%) and NPA was 100% (99/99) with two-sided 95% confidence interval (96.26%, 100%) after excluding F1LCDx-unevaluable results when considering both patients that were treatment naïve. Since patients were enrolled and initially tested by local CTAs, the positive and negative predictive values (PPV and NPV) were calculated using the PPA and NPA, after adjusting for the prevalence of *BRAF* V600E among the intention-to-treat (ITT) population. The prevalence estimates used in the adjusted agreement were 0.5%, 1%, 2%, and 8%. In this analysis with 1% prevalence, F1LCDx demonstrated an adjusted PPV of 100% with two-sided 95% confidence interval (92.59%, 100%) and NPV of 99.59% with two-sided 95% confidence interval (99.48%, 99.69%).

PPAs of 62% (31/50) and 55% (17/31) were observed for patients that were from treatment naïve and previously treated patient Cohorts, respectively, indicating

comparable detection of the *BRAF* V600E in plasma by F1LCDx among the two patient populations.

Table 17. Concordance Between F1LCDx and CTA Testing

	CTA Testing		
	<i>BRAF</i> V600E +	<i>BRAF</i> V600E -	Total
F1LCDx			
<i>BRAF</i> V600E +	48	0	48
<i>BRAF</i> V600E -	33	99	132
Unevaluable	17	21	38
Total	98	120	218
PPA (95% CI*)	59.26% (48.38% - 69.3%)		
NPA (95% CI*)	100.0% (96.26% - 100.0%)		

*Two-sided 95% confidence intervals for PPA/NPA were calculated with Wilson score method.

The discordance between the CTA and F1LCDx among *BRAF* V600E positive patients in the primary analysis were evaluated. Of the 33 CTA-positive/F1LCDx-negative samples, 27 had no *BRAF* V600E detected by the F1LCDx pipeline. Six (6) samples had *BRAF* V600E detected by F1LCDx but were filtered out for failing the pipeline's quality threshold. All the 33 CTA-positive/F1LCDx-negative samples were from patients in the NDA population of the PHAROS 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 above, F1LCDx may miss a large proportion of patients with NSCLC with *BRAF* V600E who may derive benefit from BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib). Therefore, reflex testing using tissue specimens to an FDA approved tissue test will be required, if feasible, if the plasma test is negative.

b. ORR in Patients Positive by F1LCDx for *BRAF* V600E

The efficacy of encorafenib in combination with binimetinib was evaluated in the PHAROS clinical study in those subjects positive for *BRAF* V600E by F1LCDx for the treatment naïve and previously treated cohorts (Table 18 and Table 19). For the treatment naïve cohort, the efficacy results for the F1LCDx+|CTA+ population (74.19%) is comparable to that for the CTA+ patients (74.58%), indicating the clinical utility of F1LCDx in identifying *BRAF* V600E positive NSCLC patients. For the previously treated cohort, the efficacy results for the F1LCDx+|CTA+ population (35.29%) is lower than the CTA+ efficacy (46.15%), which may be attributable to the small sample size (n=17, F1LCDx+|CTA+). The median duration of response (DoR) for F1LCDx

clinical efficacy population for treatment naïve cohort was 23.1 months and for previously treated cohort was 16.72 months.

Table 18. Primary Efficacy in the Bridging Study Subpopulations (Treatment Naïve Cohort)

	CTA+ (n=59)	CTA+/F1LCDx+ (n=31)	CTA+/F1LCDx- (n=19)	CTA+/F1LCDx unevaluable (n=9)
ORR (95% CI)[#]	74.6% (61.6, 85.0)	74.2% (55.4, 88.1)	68.4% (43.4, 87.4)	88.9% (51.8, 99.7)
Complete response (CR)	9 (15%)	5 (16%)	3 (16%)	1 (11%)
Partial response (PR)	35 (59%)	18 (58%)	10 (53%)	7 (78%)
Duration of Response (DoR)				
Median DoR, months (95% CI)	NE* (23.1, NE ¹)	23.1 (12.0, NE ¹)	NE* (NE ¹ , NE ¹)	16.13 (NE ²)
% with DoR ≥6 months	75	65.2	92.3	75
% with DoR ≥12 months	59.1	47.8	84.6	50

NE*Median DOR is not estimable since the response rate did not fall below 50% in the Kaplan-Meier estimate.

NE¹ Not estimable in the upper 95% CI of median DOR due to lower (upper) 95% CI of the response rate did not fall below 50%.

NE² CI was not calculated since the sample size is less than 10.

[#]CI calculated based on exact method to align with the drug label.

Table 19. Primary Efficacy in the Bridging Study Subpopulations (Previously treated Cohort)

	CTA+ (n=39)	CTA+/F1LCDx+ (n=17)	CTA+/F1LCDx- (n=14)	CTA+/F1LCDx unevaluable (n=8)
ORR (95% CI)[#]	46.2% (30.1, 62.8)	35.3% (14.2, 61.7)	42.9% (17.7, 71.1)	75% (34.9, 96.8)
Complete response (CR)	4 (10%)	3 (18%)	0 (0%)	1 (12%)
Partial response (PR)	14 (36%)	3 (18%)	6 (43%)	5 (62%)
Duration of Response (DOR)				

	CTA+ (n=39)	CTA+/F1LCDx+ (n=17)	CTA+/F1LCDx- (n=14)	CTA+/F1LCDx unevaluable (n=8)
Median DoR, months (95% CI)	16.72 (11.93, NE ¹)	16.72 (NE ²)	11.93 (NE ²)	NE* (NE ²)
% with DoR ≥6 months	66.7	83.3	66.7	50.0
% with DoR ≥12 months	33.3	33.3	33.3	33.3

NE*Median DOR is not estimable since the response rate did not fall below 50% in the Kaplan-Meier estimate.

NE¹ Not estimable in the upper 95% CI of median DOR due to lower (upper) 95% CI of the response rate did not fall below 50%.

NE² CI was not calculated since the sample size is less than 10.

#CI calculated based on exact method to align with the drug label.

Samples that were positive by F1LCDx for *BRAF* V600E alteration in the clinical bridging study had VAFs as low as 0.0099%. Since F1LCDx does not have a pre-specified VAF cut-off for positivity, but rather uses an algorithm that is based on select metrics for each variant position, it is possible that patients with VAFs below the limit of detection (approximately 0.4 to 0.9 %) and below that which was enrolled in the trial (e.g., 0.0099%) are reported as positive. The clinical effectiveness of F1LCDx has not been demonstrated for patients with NSCLC who have *BRAF* V600E with VAF below 0.0099%. A limitation addressing the uncertainty of the clinical effectiveness of the device for *BRAF* V600E 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 PHAROS 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., ≥ 20ng).

Amongst all CTA-positive patients, 17.3% did not have a F1LCDx result (17/98).

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=17).

The imputed ORR by IRC was estimated to be 75.73% (95% CI: 61.73%, 89.74%) for treatment naïve cohort, which is comparable to the ORR for the CTA-positive population for treatment naïve cohort [74.58% (62.20, 83.94)]. Similarly, the imputed ORR for previously treated cohort [43.28% (22.31%, 64.26%)] was comparable to the ORR for the CTA-positive population for previously treated cohort [46.15% (31.57, 61.42)]. Thus, the sensitivity analysis demonstrated the robustness of the clinical efficacy estimate to the missing F1LCDx results.

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 the F1LCDx assay to identify a *BRAF* V600E in NSCLC patients to be treated with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib), was demonstrated through a clinical bridging study using specimens from patients enrolled into the PHAROS 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 PHAROS trial show that patients who had a qualifying *BRAF* V600E received benefit from treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib) 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 validation studies conducted to support PMA 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 *BRAF* V600E for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib) was demonstrated through clinical bridging studies using specimens from patients enrolled into the PHAROS trial.

For patients with NSCLC with *BRAF* V600E positive status, for the treatment naïve cohort, the ORR for the F1LCDx+|CTA+ population (74.58%) was comparable to ORR for the CTA+ patients (74.19%), indicating a meaningful clinical benefit of F1LCDx in identifying *BRAF* V600E positive NSCLC patients, for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib). For the previously treated cohort, the ORR for the F1LCDx+|CTA+ populations (35.29%) was lower than the CTA+ ORR (46.15%), which could be attributed to the small sample size; but nevertheless, provides evidence of a meaningful clinical benefit of this device, for the indicated use. Of note, in the concordance analysis, the NPA and PPV were 100%. The observed ORRs for the F1LCDx *BRAF* V600E-positive patients in the treatment naïve and previously treated cohorts, supports probable benefit of F1LCDx in selecting *BRAF* V600E positive patients with NSCLC for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib), in these patient populations.

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 falsely determined to be 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. Of note, in the analytical accuracy study performed (n=304), the PPA was 98.91% and the NPA was 100%. 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 *BRAF* V600E, 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 *BRAF* V600E to identify patients with NSCLC for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib), and this device may meet an unmet clinical need; however, there is an FDA-approved tissue test available for the identification of patients with NSCLC with *BRAF* V600E for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib). 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:

- BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib) efficacy has not been established in NSCLC patients with *BRAF* V600E <0.099 % 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 *BRAF* V600E for treatment with BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib), 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 FILCDx as an aid for the identification of *BRAF* V600E in patients with NSCLC who may be eligible for treatment with BRAFTOVI (encorafenib) in combination with MEKTOVI (binimetinib).

XIII. CDRH DECISION

CDRH issued an approval order on October 11, 2023. The final clinical 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:

1. 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.
2. 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.
3. 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), with a duration limited to one year. Subsequently, per the request from the sponsor, an extension of the variance for additional three years from August 26, 2021, to August 26, 2022, August 26, 2022 to August 26, 2023, and from August 26, 2023 to August 26, 2024, was granted to FMI.

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

None.