SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	FoundationOne®CDx
Device Procode:	PQP
Applicant's Name and Address:	Foundation Medicine, Inc. 150 Second Street Cambridge, MA 02141
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P170019/S006
Date of FDA Notice of Approval:	December 3, 2019

The original PMA (P170019) for FoundationOne®CDx (F1CDx) was approved on November 30, 2017 for the detection of genetic alterations in patients who may benefit from one of fifteen FDA-approved therapies for non-small cell lung cancer (NSCLC), melanoma, breast cancer, colorectal cancer, and ovarian cancer. Subsequently, three PMA supplements were approved for expanding the intended use of F1CDx since its original approval. PMA supplement (P170019/S005) for adding genomic loss of heterozygosity (LOH) was approved on April 10, 2019. PMA supplement (P170019/S004) for adding an indication for Lynparza® (olaparib) in ovarian cancer patients with *BRCA1/2* alterations was approved on July 1, 2019. PMA supplement (P170019/S008) for adding an indication for Tagrisso® (osimertinib) in NSCLC patients with *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations was approved on July 1, 2019.

The current supplement was submitted to expand the intended use of F1CDx to include a companion diagnostic indication for *PIK3CA* alterations in breast cancer patients who may benefit from treatment with PIQRAY® (alpelisib).

II. INDICATIONS FOR USE

FoundationOne®CDx (F1CDx) is a next generation sequencing based *in vitro* diagnostic device for detection of substitutions, insertion and deletion alterations (indels) and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational

burden (TMB) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for cancer patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Table 1. Companion diagnostic indications

Indication	**Biomarker**	**Therapy**
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Gilotrif® (afatinib), Iressa® (gefitinib), Tagrisso® (osimertinib), or Tarceva® (erlotinib)
	EGFR exon 20 T790M alterations	Tagrisso® (osimertinib)
	ALK rearrangements	Alecensa® (alectinib), Xalkori® (crizotinib), or Zykadia® (ceritinib)
	BRAF V600E	Tafinlar® (dabrafenib) in combination with Mekinist® (trametinib)
Melanoma	*BRAF* V600E	Tafinlar® (dabrafenib) or Zelboraf® (vemurafenib)
	BRAF V600E and V600K	Mekinist® (trametinib) or Cotellic® (cobimetinib) in combination with Zelboraf® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	Herceptin® (trastuzumab), Kadcyla® (ado-trastuzumab-emtansine), or Perjeta® (pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	Piqray® (alpelisib)
Colorectal cancer	*KRAS* wild-type (absence of mutations in codons 12 and 13)	Erbitux® (cetuximab)
	KRAS wild-type (absence of mutations in exons 2, 3, and 4) and *NRAS* wild-type (absence of mutations in exons 2, 3, and 4)	Vectibix® (panitumumab)
Ovarian cancer	*BRCA1/2* alterations	Lynparza® (olaparib) or Rubraca® (rucaparib)

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

The F1CDx assay is a single-site assay performed at Foundation Medicine, Inc.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS/PRECAUTIONS AND LIMITATIONS

The warnings/precautions and limitations are included in the FoundationOne®CDx assay labeling.

V. DEVICE DESCRIPTION

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

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

ABL1	*BRAF*	*CDKN1A*	*EPHA3*	*FGFR4*	*IKZF1*	*MCL1*	*NKX2-1*	*PMS2*	*RNF43*	*TET2*
ACVR1B	*BRCA1*	*CDKN1B*	*EPHB1*	*FH*	*INPP4B*	*MDM2*	*NOTCH1*	*POLD1*	*ROS1*	*TGFBR2*
AKT1	*BRCA2*	*CDKN2A*	*EPHB4*	*FLCN*	*IRF2*	*MDM4*	*NOTCH2*	*POLE*	*RPTOR*	*TIPARP*
AKT2	*BRD4*	*CDKN2B*	*ERBB2*	*FLT1*	*IRF4*	*MED12*	*NOTCH3*	*PPARG*	*SDHA*	*TNFAIP3*
AKT3	*BRIP1*	*CDKN2C*	*ERBB3*	*FLT3*	*IRS2*	*MEF2B*	*NPM1*	*PPP2R1A*	*SDHB*	*TNFRSF14*
ALK	*BTG1*	*CEBPA*	*ERBB4*	*FOXL2*	*JAK1*	*MEN1*	*NRAS*	*PPP2R2A*	*SDHC*	*TP53*
ALOX12B	*BTG2*	*CHEK1*	*ERCC4*	*FUBP1*	*JAK2*	*MERTK*	*NT5C2*	*PRDM1*	*SDHD*	*TSC1*
AMER1	*BTK*	*CHEK2*	*ERG*	*GABRA6*	*JAK3*	*MET*	*NTRK1*	*PRKAR1A*	*SETD2*	*TSC2*
APC	*C11orf30*	*CIC*	*ERRFI1*	*GATA3*	*JUN*	*MITF*	*NTRK2*	*PRKCI*	*SF3B1*	*TYRO3*
AR	*CALR*	*CREBBP*	*ESR1*	*GATA4*	*KDM5A*	*MKNK1*	*NTRK3*	*PTCH1*	*SGK1*	*U2AF1*
ARAF	*CARD11*	*CRKL*	*EZH2*	*GATA6*	*KDM5C*	*MLH1*	*P2RY8*	*PTEN*	*SMAD2*	*VEGFA*
ARFRP1	*CASP8*	*CSF1R*	*FAM46C*	*GID4 (C17orf39)*	*KDM6A*	*MPL*	*PALB2*	*PTPN11*	*SMAD4*	*VHL*
ARID1A	*CBFB*	*CSF3R*	*FANCA*	*GNA11*	*KDR*	*MRE11A*	*PARK2*	*PTPRO*	*SMARCA4*	*WHSC1*
ASXL1	*CBL*	*CTCF*	*FANCC*	*GNA13*	*KEAP1*	*MSH2*	*PARP1*	*QKI*	*SMARCB1*	*WHSC1L1*
ATM	*CCND1*	*CTNNA1*	*FANCG*	*GNAQ*	*KEL*	*MSH3*	*PARP2*	*RAC1*	*SMO*	*WT1*
ATR	*CCND2*	*CTNNB1*	*FANCL*	*GNAS*	*KIT*	*MSH6*	*PARP3*	*RAD21*	*SNCAIP*	*XPO1*
ATRX	*CCND3*	*CUL3*	*FAS*	*GRM3*	*KLHL6*	*MST1R*	*PAX5*	*RAD51*	*SOCS1*	*XRCC2*
AURKA	*CCNE1*	*CUL4A*	*FBXW7*	*GSK3B*	*KMT2A (MLL)*	*MTAP*	*PBRM1*	*RAD51B*	*SOX2*	*ZNF217*
AURKB	*CD22*	*CXCR4*	*FGF10*	*H3F3A*	*KMT2D (MLL2)*	*MTOR*	*PDCD1*	*RAD51C*	*SOX9*	*ZNF703*
AXIN1	*CD274*	*CYP17A1*	*FGF12*	*HDAC1*	*KRAS*	*MUTYH*	*PDCD1LG2*	*RAD51D*	*SPEN*	
AXL	*CD70*	*DAXX*	*FGF14*	*HGF*	*LTK*	*MYC*	*PDGFRA*	*RAD52*	*SPOP*	
BAP1	*CD79A*	*DDR1*	*FGF19*	*HNF1A*	*LYN*	*MYCL*	*PDGFRB*	*RAD54L*	*SRC*	
BARD1	*CD79B*	*DDR2*	*FGF23*	*HRAS*	*MAF*	*MYCN*	*PDK1*	*RAF1*	*STAG2*	
BCL2	*CDC73*	*DIS3*	*FGF3*	*HSD3B1*	*MAP2K1*	*MYD88*	*PIK3C2B*	*RARA*	*STAT3*	
BCL2L1	*CDH1*	*DNMT3A*	*FGF4*	*ID3*	*MAP2K2*	*NBN*	*PIK3C2G*	*RB1*	*STK11*	
BCL2L2	*CDK12*	*DOT1L*	*FGF6*	*IDH1*	*MAP2K4*	*NF1*	*PIK3CA*	*RBM10*	*SUFU*	
BCL6	*CDK4*	*EED*	*FGFR1*	*IDH2*	*MAP3K1*	*NF2*	*PIK3CB*	*REL*	*SYK*	
BCOR	*CDK6*	*EGFR*	*FGFR2*	*IGF1R*	*MAP3K13*	*NFE2L2*	*PIK3R1*	*RET*	*TBX3*	
BCORL1	*CDK8*	*EP300*	*FGFR3*	*IKBKE*	*MAPK1*	*NFKBIA*	*PIM1*	*RICTOR*	*TEK*	

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

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

Test Output

The output of the test includes:

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

Category 2: Cancer Mutations with Evidence of Clinical Significance

Category 3: Cancer Mutations with Potential Clinical Significance

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

Test Kit Contents

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

- Specimen Preparation Instructions
- Shipping Instructions
- Return Shipping Label

Instruments

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

Table 4. Instruments for use with the F1CDx assay

Instrument
Illumina HiSeq 4000
Illumina cBot
Beckman Biomek NXP Span-8 Liquid Handler
Thermo Scientific Kingfisher Flex DW 96

Test Process

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

A. Specimen Collection and Preparation

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

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

B. DNA Extraction

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

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

C. Library Construction

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

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

D. Hybrid Capture

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

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

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

E. Sequencing

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

F. Sequence Analysis

Sequence data are analyzed using proprietary software developed by FMI. Sequence data are mapped to the human genome (hg19) using Burrows-Wheeler Aligner

(BWA) v0.5.9.[4] PCR duplicate read removal and sequence metric collection are performed using Picard 1.47 (http://picard.sourceforge.net) and SAMtools 0.1.12a.[5] Local alignment optimization is performed using Genome Analysis Toolkit (GATK) 1.0.4705.[6] Variant calling is performed only in genomic regions targeted by the test.

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

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

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

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

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

Genomic rearrangements are identified by analyzing chimeric read pairs. Chimeric read pairs are defined as read pairs for which reads map to separate chromosomes, or at a distance of over 10 megabase (Mb). Pairs are clustered by genomic coordinate of the pairs, and clusters containing at least five chimeric pairs (three for known fusions)

are identified as rearrangement candidates. Filtering of candidates is performed by mapping quality (average read mapping quality in the cluster must be 30 or above) and distribution of alignment positions. Rearrangements are annotated for predicted function (e.g., creation of fusion gene).

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

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

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

G. Report Generation

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

H. Internal Process Controls Related to the System

Positive Control

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

Sensitivity Control

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

Negative Control

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using FFPE tumor specimens, as listed in Table 1 of the F1CDx intended use statement. The approved CDx tests are listed in Table 5, below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools.

Table 5. List of FDA approved CDx assays for genes targeted by F1CDx

	Device	Company	Technology	Therapy	Indication
HER2-Amplification	PathVysion HER-2 DNA Probe Kit	Abbott Molecular, Inc.	FISH	Herceptin (trastuzumab)	Breast cancer
	PATHWAY Anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody	Ventana Medical Systems, Inc.	IHC	Herceptin (trastuzumab)	Breast cancer
	InSite HER-2/neu Kit	Biogenex Laboratories, Inc.	IHC	Herceptin (trastuzumab)	Breast cancer
	SPOT-Light HER2 CISH Kit	Life Technologies, Inc.	CISH	Herceptin (trastuzumab)	Breast cancer
	Bond Oracle HER2 IHC System	Leica Biosystems	IHC	Herceptin (trastuzumab)	Breast cancer
	HER2 CISH pharmDx Kit	Dako Denmark A/S	CISH	Herceptin (trastuzumab)	Breast cancer
	INFORM HER2 Dual ISH DNA Probe Cocktail	Ventana Medical Systems, Inc.	Dual ISH	Herceptin (trastuzumab)	Breast cancer
	HercepTest	Dako Denmark A/S	IHC	Herceptin (trastuzumab) Perjeta (pertuzumab) Kadcyla (ado-trastuzumab emtansine)	Breast cancer Gastric or Gastroesophageal junction adenocarcinoma
	HER2 FISH pharmDx Kit	Dako Denmark A/S	FISH	Herceptin (trastuzumab) Perjeta (pertuzumab) Kadcyla (ado-trastuzumab emtansine)	Breast cancer Gastric or Gastroesophageal junction adenocarcinoma
BRAF-V600	THxID BRAF Kit	bioMerieux	PCR	Mekinist (tramatenib)	Melanoma
	cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems, Inc.	PCR	Zelboraf (vemurafenib)	Melanoma
BRAF-600E	THxID BRAF Kit	bioMerieux	PCR	Tafinlar (dabrafenib)	Melanoma
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	Tafinlar (dabrafenib) Mekinist (trametinib)	Non-small cell lung cancer
NRAS	Praxis Extended RAS Panel	Illumina, Inc.	NGS	Vectibix (panitumumab)	Colorectal cancer

	Device	Company	Technology	Therapy	Indication
KRAS	**cobas** KRAS Mutation Test	Roche Molecular Systems, Inc.	PCR	Erbitux (cetuximab) Vectibix (panitumumab)	Colorectal cancer
	therascreen KRAS RGQ PCR Kit	QIAGEN	PCR	Erbitux (cetuximab) Vectibix (panitumumab)	Colorectal cancer
	Praxis Extended RAS Panel	Illumina, Inc.	NGS	Vectibix (panitumumab)	Colorectal cancer
***ALK* - fusion**	Vysis ALK Break Apart FISH Probe Kit	Abbott Molecular, Inc.	FISH	Xalkori (crizotinib)	Non-small cell lung cancer
	ALK (D5F3) CDx Assay	Ventana Medical Systems, Inc.	IHC	Xalkori (crizotinib)	Non-small cell lung cancer
***EGFR* – Exon 19 deletions & L858R**	**cobas** EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	Tarceva (erlotinib) Tagrisso (osimertinib) Iressa (gefitinib)	Non-small cell lung cancer
	therascreen EGFR RGQ PCR Kit	QIAGEN	PCR	Gilotrif (afatinib) Iressa (gefitinib)	Non-small cell lung cancer
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	Iressa (gefitinib)	Non-small cell lung cancer
***EGFR* T790M**	**cobas** EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	Tagrisso (osimertinib)	Non-small cell lung cancer
BRCA1/2	FoundationFocus CDx$_{BRCA}$	Foundation Medicine, Inc.	NGS	Rubraca (rucaparib)	Advanced ovarian cancer
PIK3CA	*therascreen* PIK3CA RGQ PCR Kit	QIAGEN	PCR	PIQRAY (alpelisib)	Breast cancer

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

VII. MARKETING HISTORY

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

The F1CDx Premarket Approval (PMA) was originally approved on November 30, 2017 by FDA (P170019) and is commercially available in the U.S. since March 30, 2018. The following PMA supplements were approved for expanding the intended use of F1CDx since its original approval: PMA supplement (P170019/S005) for adding LOH was approved by FDA on April 10, 2019; PMA supplement (P170019/S004) for adding an indication for Lynparza® (olaparib) in ovarian cancer patients with *BRCA1/2* alterations was approved on July 1, 2019; and PMA supplement (P170019/S008) for adding an indication for Tagrisso® (osimertinib) in non-small cell lung cancer (NSCLC) patients with *EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations was approved on July 1, 2019.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

The primary evidence for supporting the performance of F1CDx in detecting *PIK3CA* alterations was from the data presented using intended use specimens across all validation studies. In addition to the existing platform-level *PIK3CA* validation results (P170019), analytical accuracy/concordance, and precision at the limit of detection (LoD) studies as well as *in silico* analyses of existing DNA extraction, guard band, and FFPE slide stability studies were conducted to support the indication for *PIK3CA* alterations.

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

conducted. The platform validation studies included samples with *PIK3CA* biomarker alterations in breast cancer and non-breast FFPE tissue specimens. These results from the platform-level validation (P170019) have been leveraged to support F1CDx detection of *PIK3CA* biomarker alterations. Results from non-breast samples carrying *PIK3CA* alterations were acceptable following successful demonstration of comparability of assay performance across tumor tissue types (please see Section IX.A.10, Table 24 in Summary of Safety and Effectiveness Data P170019).

In addition to the alteration and tissue-specific analytical validation studies described below, real-world data from the Foundation Medicine clinical database demonstrate robust sequencing quality metrics across the *PIK3CA* gene (please see Section IX.A.10, below).

1. Analytical Accuracy/Concordance

a. Comparison to an Orthogonal Method for *PIK3CA* Alterations

An analytical accuracy study was performed with available FFPE clinical specimens (46 *PIK3CA* alteration positive patients and 48 *PIK3CA* alteration negative patients) from breast cancer patients randomized in the SOLAR-1 clinical trial (please see Section X.A for study details) to demonstrate the concordance between F1CDx and an externally validated NGS assay (evNGS) for the detection of *PIK3CA* alterations. This study evaluated a set of 101 breast cancer FFPE specimens, including 53 *PIK3CA* alteration positive patients with sufficient remaining DNA randomly selected according to the prevalence of each variant in the SOLAR-1 clinical trial. Due to the low prevalence of some *PIK3CA* alterations, samples from Foundation Medicine's clinical archive (7 *PIK3CA* alteration positive patients) were included to cover rare *PIK3CA* biomarker alterations. 48 *PIK3CA* alteration negative patients from the SOLAR-1 trial randomly selected with remaining DNA of sufficient quantity and quality were also included (Table 6). A summary of positive percent agreement (PPA) and negative percent agreement (NPA) and corresponding 95% two-sided exact confidence intervals (CIs) is provided in Table 7, below.

Table 6. Sample selection for concordance of *PIK3CA* alterations with SOLAR-1 clinical trial samples by variant

PIK3CA Variant	Number of Samples in SOLAR-1	Percent*	50**	Final Number Selected	Final Number from SOLAR-1	Final Number from FMI Clinical Database
H1047R	108	45%	22.59	23	22	1
E545K	58	24%	12.13	13	13	0
E542K	39	16%	8.16	7	7	0
H1047L	15	6%	3.14	3	3	0
C420R	5	2%	1.05	1	0	1
E545A	2	1%	0.42	1	0	1
Q546R	2	1%	0.42	1	1	0
E545G	5	2%	1.05	1	0	1
H1047Y	0	0%	0	1	0	1
Q546E	1	0%	0.21	1	0	1
E545D [1635G>T only]	4	2%	0.84	1	0	1
Total positives	239	100%		**53**	**46**	**7**
Total negatives				**48**	**48**	**0**

* Percent refers to the prevalence of each variant in the entire SOLAR-1 clinical trial
**50 refers to the proportional number of each variant when the sample size is 50

Table 7. Concordance summary for *PIK3CA* alterations with SOLAR-1 clinical trial samples

Variant	F1CDx+/ evNGS+	F1CDx- /evNGS+	F1CDx+ /evNGS-	F1CDx- /evNGS-	PPA (95%CI)	NPA (95%CI)
PIK3CA biomarker alterations	53	0	0	48	100.00% (93.3%, 100.0%)	100.00% (92.6%, 100.0%)

All positive and negative samples were in 100% agreement for the detection of *PIK3CA* biomarker alterations in the concordance analysis performed with SOLAR-1 clinical trial samples.

b. Comparison to an Orthogonal Method for F1CDx Platform Validation

For the F1CDx platform validation, the detection of alterations by F1CDx was compared to results of the evNGS. The comparison between short alterations, including base substitutions, detected by F1CDx and the orthogonal method included 188 samples from 46 different tumor tissue types, including breast carcinoma. A summary of PPA and NPA and corresponding 95% two-sided exact CIs is provided for substitutions in Table 8, below.

Table 8. Concordance summary for substitutions

Variant Type	F1CDx+/ evNGS+	F1CDx- /evNGS+	F1CDx+ /evNGS-	F1CDx- /evNGS-	PPA (95%CI)	NPA (95%CI)
Substitutions	1111	39	334	242540	96.6% (95.4%, 97.6%)	99.9% (99.8%, 99.9%)

For details on the F1CDx platform analytical accuracy, please refer to Section IX.A.1(a) of Summary of Safety and Effectiveness Data P170019.

F1CDx platform validation of analytical accuracy included 16 samples with *PIK3CA* biomarker alterations, including two breast cancer specimens. A summary of PPA and NPA is provided in Table 9, below.

Table 9. Concordance summary for *PIK3CA* alterations in F1CDx platform validation

Variant	F1CDx+/ evNGS+	F1CDx- /evNGS+	F1CDx+ /evNGS-	F1CDx- /evNGS-	PPA	NPA
PIK3CA biomarker alterations	15	0	1	172	100%	99.42%

One discordant case was observed in the F1CDx platform validation for *PIK3CA* alterations, which was detected by F1CDx but not the orthogonal method. The *PIK3CA* alteration was detected by F1CDx at an allele frequency below the detection limit of the evNGS assay.

2. Analytical Sensitivity

a. Limit of Detection (LoD) for F1CDx Platform Validation

The LoD of alteration types assessed by F1CDx were evaluated for the platform-level validation. A single FFPE tumor sample was selected for each of the variant categories. For each sample, six levels of MAF, with 13 replicates per level, were evaluated (total of 78 aliquots per sample). To meet the number of required replicates proposed in CLSI EP17-A2, multiple different pooled DNA extractions from single FFPE samples and banked DNA samples were used to meet the required variant characteristics. When estimating the LoD of platform substitutions, the analyses excluded certain variants that were fully detected at all dilution levels (N = 13 substitutions, MAF range 2.2%-15.2%) as they did not reach full dilution and were determined not to be representative of the remaining 166 alterations. Representative LoD for the range of substitution variants detected by the F1CDx platform are summarized in Table 10.

Table 10. Summary representative LoD for platform substitution alterations

Variant Category	Subcategory*	N	Range LoD** Allele Fraction (%)
Base Substitutions	known	21***	1.8-7.9
	other	166***	5.9-11.8

*Alterations classified as" known" are defined as those that are listed in COSMIC. Alterations classified as "other" include truncating events in tumor suppressor genes (splice, frameshift, and nonsense) as well as variants that appear in hotspot locations but do not have a specific COSMIC association or are considered variants of unknown significance (VUS) due to lack of reported evidence and conclusive change in function.

**LoD calculations for the platform variants were based on the hit rate approach for variants with less than three levels with hit rate between 10% and 90% and probit approach for variants with at least three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).

***Data includes an alteration in the *TERT* promoter 124C>T (LoD of 7.9%) *TERT* is the only promoter region interrogated by F1CDx and is highly enriched for repetitive context of poly-Gs, not present in coding regions.

The LoD of seven CDx biomarkers, including *PIK3CA* E542K, were determined in support of the platform-level validation. The LoD data for the *PIK3CA* biomarker alteration is summarized in Table 11, below.

Table 11. Summary of LoD for *PIK3CA* alterations

Alteration	LoD* Allele Fraction
PIK3CA E542K	4.9%

*LoD calculations were based on the hit rate approach, as there were less than three levels with hit rate between 10% and 90%. LoD from the hit rate approach is defined as the lowest level with 100% hit rate (worst scenario).

The LoD for *PIK3CA* alterations was established as 4.9% allele fraction based on detection of the E542K variant in breast cancer tissue. The LoD for *PIK3CA* alterations was confirmed by testing breast cancer samples near the established LoD level in the precision studies (please see Section IX.A.5, below).

Please refer to the Summary of Safety and Effectiveness Data P170019 (Section IX.A.2) for additional F1CDx platform-level analytical sensitivity data, including limit of blank (LoB) and tumor purity.

3. Analytical Specificity

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

4. Carryover/Cross-Contamination

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

5. Precision and Reproducibility

a. Intermediate Precision for *PIK3CA* Alterations

In addition to the platform-level precision data to support the F1CDx performance characteristics for detection of *PIK3CA* alterations (see Section IX.A.5(b), below), a precision study was conducted to assess one breast cancer sample at LoD (as the primary analysis) and five additional non-breast samples (as an exploratory analysis) with *PIK3CA* alterations. Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three different sequencers and two different reagent lots, across multiple days (typical assay workflow spans 10 days) of performance by multiple operators. A full factorial design for this study was carried out with four replicates per reagent lot/sequencer combination for samples with 24 replicates. The previous precision studies for F1CDx (P170019) and FoundationFocus CDx$_{BRCA}$ (P160018) were conducted with 36 replicates using a full factorial study design and yielded high agreement rates; thus, 24 replicates per sample to demonstrate F1CDx precision for *PIK3CA* substitution variants were deemed acceptable to support this PMA supplement.

The results for the primary analysis for the breast cancer sample at LoD are summarized in Table 12, below. There was one replicate that failed library construction (LC) QC, which is within the normal failure rate for F1CDx. Intra-run repeatability was evaluated across 12 duplicates per plate as percent agreement and was 100% concordant. Inter-run reproducibility was evaluated across 24 replicates as percent agreement, which is the fraction of calls consistent with the majority call. Reproducibility was 100% across all replicates. The corresponding two-sided exact 95% CIs are provided for repeatability and reproducibility positive call rates.

Table 12. Precision results for primary analysis of *PIK3CA* alterations

Gene	Tumor Type	Variant	Mutant Allele Fraction (MAF) (%)	# Valid Results	Repeatability Positive Call Rate (95% exact CI)	Reproducibility Positive Call Rate (95% exact CI)
PIK3CA	Breast	H1047L	4.65	23	100% (75.51%, 100%)	100% (85.18%, 100%)

The precision results for non-breast samples as supportive evidence demonstrated intra-run repeatability and inter-run reproducibility for the five non-breast samples were 100% concordant.

The overall repeatability for all samples tested in the additional precision studies (primary and supportive) for *PIK3CA* alterations validation was 100% with the

corresponding two-sided exact 95% CIs (93.94%, 100%), and the overall reproducibility for all samples was 100% (95% CI: 97.02%, 100%).

b. Intermediate Precision of F1CDx Platform

For F1CDx platform-level validation, precision was evaluated for alterations associated with CDx claims as well as representative alterations to support platform performance. The platform validation included a total of five samples with *PIK3CA* alterations: two samples were obtained from FFPE breast tissue with E545K and H1047R variants, respectively; and three samples were from non-breast tissue with E542K, E545K, and H1047R variants, respectively (Table 13).

Table 13. Sample set with *PIK3CA* alterations from platform-level validation

Gene	Tumor Type	Variant	Mutant Allele Fraction (MAF) (%)	Positive Call Rate
PIK3CA	Breast	H1047R	1	52.78%
PIK3CA	Breast	E545K	3	100%
PIK3CA	Non-breast	E545K	5	97.22%
PIK3CA	Non-breast	H1047R	23	100%
PIK3CA	Non-breast	E542K	53	100%

For the two samples with *PIK3CA* alterations near the established LoD of 4.9% allele fraction, the breast tissue sample with the E545K mutation at 3% MAF yielded a 100% positive call rate, and the non-breast sample with the E545K mutation at 5% MAF yielded a 97.22% positive call rate. The breast sample with the 52.78% positive call rate contained an H1047R variant at 1% MAF, which is below the established LoD of 4.9% allele fraction for *PIK3CA* alterations.

A post-market precision study will be performed with an intended use FFPE tissue sample with the *PIK3CA* C420R variant at 1.5X LoD to cover *PIK3CA* biomarker variants from all the positions that are identified by the F1CDx assay.

Please refer to the Summary of Safety and Effectiveness Data P170019 (Section IX.A.5(a,b)) for reagent lot-to-lot and instrument-to-instrument reproducibility data for F1CDx.

6. Reagent Lot Interchangeability

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

7. Stability

a. FFPE Slide Stability

The FFPE slide stability study is ongoing with data summarized for T_0, T_1 (30 days) and T_2 (6 months) time points. This study evaluated the stability of FFPE tumor tissue prepared as slides prior to DNA extraction for use with F1CDx. Five tumor samples were evaluated, including breast cancer, that contained a variety of DNA alterations. One non-breast sample contained two *PIK3CA* biomarker alterations, C420R and H1047Y. To assess stability of pre-cut FFPE tissue for *PIK3CA* alterations, the agreements between results from the defined time points were calculated by comparing the alteration call reported at each follow-up time point to the alteration call at baseline (T_0) (Table 14).

Table 14. Stability results for *PIK3CA* alterations

	Baseline Call (T_0)		Percent Agreement to T_0	Percent Agreement to T_0
Tissue	**Gene**	**Variant Effect**	**30 days (T_1)**	**6 months (T_2)**
Non-breast	*PIK3CA*	c.1258T>C, p.C420R	100.0% (2/2)	100.0% (2/2)
	PIK3CA	c.3139C>T, p.H1047Y	100.0% (2/2)	100.0% (2/2)

PIK3CA alterations at the 30-day time point and the 6-month time point were in 100% agreement with the day 0 baseline results (T_0). Therefore, FFPE slides stored in accordance with internal procedures can be considered stable for at least 6 months. Further assessment at months 12 and 15 will demonstrate stability of FFPE slides beyond 6 months.

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

8. General Lab Equipment and Reagent Evaluation

a. DNA Amplification

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

b. DNA Extraction

For F1CDx platform-level validation, the performance of DNA extraction from FFPE tumor specimens was evaluated. The DNA extraction procedure for the F1CDx assay was assessed by testing FFPE specimens with two samples per tissue type for ten different tissue types, including breast, with different representative types of alterations. Samples were run in duplicate for a total of 240 extractions, employing two different KingFisher Flex Magnetic Particle Processors (120 extractions per processor) and comparing across three extraction reagent lots (80 extractions per

reagent lot). The concordance across replicates for 1,685 total substitutions was 98.7% (95% CI: 98%, 99.1%). For details, please refer to Section IX.A.8(b) of Summary of Safety and Effectiveness Data P170019.

Two samples in the platform-level DNA extraction study contained *PIK3CA* biomarker alterations: E545K and H1047R, respectively. Concordance of *PIK3CA* alterations detected was 100% across 12 replicates for each sample (Table 15).

Table 15. Samples with *PIK3CA* alterations in DNA extraction study

Gene	Tumor Type	Variant	# Replicates	Concordance (95% CI)
PIK3CA	Non-breast	E545K	12	100% (71.8%, 100%)
PIK3CA	Non-breast	H1047R	12	100% (71.8%, 100%)

9. Guard banding/Robustness

Guard banding study results were leveraged from the F1CDx platform validation to evaluate the performance of the F1CDx assay and the impact of process variation with regard to uncertainty in the measurement of DNA concentration at various stages of the process. Concordance of detected substitution alterations was calculated for each condition to measure the variation for library construction (LC), hybrid capture (HC), and sequencing across various DNA input levels, and was found to be highly concordant across all successful replicates (please refer to Section IX.A.9 in Summary of Safety and Effectiveness Data P170019).

The samples included two FFPE tissue specimens with *PIK3CA* biomarker alterations, including one breast tissue sample with a C420R variant and one non-breast sample with a Q546R variant, processed in triplicate per condition (Table 16).

Table 16. Samples with *PIK3CA* alterations in guard band study

Gene	Tumor Type	Variant	# Replicates
PIK3CA	Breast	C420R	51
PIK3CA	Non-breast	Q546R	51

Consistent with the overall platform-level results, in samples with *PIK3CA* alterations the HC process was the most sensitive to low levels of DNA input (Table 17). The observed failures in the HC guard band test were at levels below normal input requirements. The guard band studies demonstrated reliable and robust performance at normal process DNA input levels for the samples with *PIK3CA* biomarker alterations.

Table 17. Summary of the success rate per process and per input level for *PIK3CA* alterations

Process	Input Level	# of sample failures	DNA input Success
LC	25 ng	1/6	83.3%
LC	40 ng	0/6	100%
LC	50 ng	0/6	100%
LC	1000 ng	0/6	100%
LC	1200 ng	0/6	100%
LC	1500 ng	0/6	100%
HC	0.25 µg	6/6	0%
HC	0.375 µg	6/6	0%
HC	0.5 µg	1/6	83.3%
HC	2.0 µg	0/6	100%
HC	2.5 µg	0/6	100%
HC	3.0 µg	0/6	100%
Seq	1.4 nM	0/6	100%
Seq	1.575 nM	0/6	100%
Seq	1.75 nM	1/6	83.3%
Seq	1.925 nM	0/6	100%
Seq	2.1 nM	0/6	100%

10. Real-World Evidence Demonstrating Sequencing Quality Metrics

Real-world data from the Foundation Medicine clinical database demonstrate robust sequencing quality metrics across the *PIK3CA* gene. The mean and median depths of coverage for all *PIK3CA* biomarker alterations from 661 breast carcinoma specimens are well above the QC threshold of 250X (Table 18).

Table 18. Depth of coverage for *PIK3CA* biomarker alterations

Gene	Alterations	Bait Target Region	GC Content	Target Depth Mean	Target Depth Median
PIK3CA	C420R	chr3: 178927950-178927999	0.33	893.85	831.76
PIK3CA	E542K	chr3: 178936035-178936084	0.41	1178.27	1087.92
PIK3CA	E545K, E545G, E545A, E545D [1635G>T only], Q546E, Q546R	chr3: 178936085-178936134	0.41	1208.2	1124.9
PIK3CA	H1047Y, H1047L, H1047R	chr3: 178952067-178952116	0.41	1412.4	1350.1

Furthermore, an analysis of the 415 FFPE clinical specimens from breast cancer patients randomized in the SOLAR-1 trial available for F1CDx testing show position-specific quality metrics for each of the *PIK3CA* biomarker alterations are of high quality (Table 19).

Table 19. Position-specific quality metrics for *PIK3CA* alterations in SOLAR-1 clinical trial samples

Gene	Variant	Alteration Loci	Average Mapping Quality of the Reads*	Average Mapping Error Rate of the Reads	Average Base Quality (Phred score)
PIK3CA	C420R	chr3: 178927980	58.43	5.24 e-05	39.58
PIK3CA	E542K	chr3: 178936082	49.42	0.00 e-05	40.41
PIK3CA	E545K	chr3: 178936091	52.51	7.05 e-05	39.91
PIK3CA	E545G, E545A	chr3: 178936092	52.41	7.15 e-05	40.14
PIK3CA	E545D (1635G>T only)	chr3: 178936093	52.36	7.23 e-05	40.04
PIK3CA	Q546E	chr3: 178936094	52.45	7.27 e-05	40.19
PIK3CA	Q546R	chr3: 178936095	52.39	7.38 e-05	40.27
PIK3CA	H1047Y	chr3: 178952084	58.8	3.48 e-05	39.6
PIK3CA	H1047L, H1047R	chr3: 178952085	58.8	3.50 e-05	39.5

* The upper range for mapping quality is 60.

B. Animal Studies

No animal studies were conducted using the F1CDx assay.

C. Additional Studies

No additional studies were conducted using the F1CDx assay.

X. SUMMARY OF PRIMARY CLINICAL STUDY

For the intended use to identify *PIK3CA* alterations in breast cancer patients who may benefit from treatment with alpelisib (Table 1), the effectiveness of the F1CDx assay was demonstrated through a clinical bridging study using specimens from the patients screened for enrollment into the SOLAR-1 study.

A. FoundationOne®CDx Clinical Bridging Study for *PIK3CA*

The safety and effectiveness of F1CDx for detecting *PIK3CA* alterations in breast cancer patients who may benefit from treatment with alpelisib was demonstrated in a retrospective analysis of specimens from patients enrolled in the study CBYL719C2301 (SOLAR-1). A bridging study was conducted to assess: 1) the clinical efficacy of F1CDx in identifying *PIK3CA* alteration positive patients for treatment with alpelisib in combination with fulvestrant and 2) the concordance between *PIK3CA* status (alteration

positive or negative) tested with the clinical trial enrollment assays (referred to as clinical trial assay [CTA1] and [CTA2]) and F1CDx in the intent-to-test population.

1. Study Design

SOLAR-1 is a pivotal Phase III, randomized, double-blind, placebo-controlled study of alpelisib in combination with fulvestrant in postmenopausal women, and men, with hormone receptor positive (HR-positive), human epidermal growth factor receptor 2 negative (HER2-negative) locally advanced or metastatic breast cancer whose disease had progressed or recurred on or after an aromatase inhibitor (AI)-based treatment (with or without CDK4/6 combination) (SOLAR-1, NCT2437318).

A total of 572 breast cancer patients were enrolled into two cohorts, with or without a *PIK3CA* alteration. Patients and investigators were blinded to alteration status. Within each cohort, patients were randomized to receive either alpelisib 300 mg plus fulvestrant or placebo plus fulvestrant in a 1:1 ratio. Randomization was stratified by presence of lung and/or liver metastasis and previous treatment with Cyclin-Dependent Kinases 4 and 6 (CDK4/6) inhibitor(s).

The primary endpoint for the study was progression-free survival (PFS) using Response Evaluation Criteria in Solid Tumors (RECIST v1.1), based on investigator assessment in advanced or metastatic breast cancer patients enrolled with a *PIK3CA* alteration. Safety and tolerability were evaluated by assessment of type, frequency, and severity of adverse events and laboratory toxicities per Common Terminology Criteria for Adverse Events (CTCAE) v4.03.

2. Bridging Study

The aim of this bridging study was to determine the concordance between *PIK3CA* alteration results from the enrolling PCR assays generated at the time of patient screening for SOLAR-1 and the *PIK3CA* alteration results generated using F1CDx. The study was also conducted to establish the clinical utility of the F1CDx assay in identifying *PIK3CA* alteration positive patients for treatment with alpelisib in combination with fulvestrant.

PIK3CA alteration positive and negative patients were randomized in the SOLAR-1 trial based on their *PIK3CA* alteration status, initially performed with a *PIK3CA* mutation PCR CTA1 and then transitioned to the *PIK3CA* mutation PCR CTA2. Of the 572 randomized patients in SOLAR-1, 395 were enrolled based on the CTA1, and 177 were enrolled based on the CTA2. The F1CDx bridging study used the SOLAR-1 randomized population to demonstrate safety and effectiveness of the F1CDx assay for identification of HR-positive, HER2-negative advanced or metastatic breast cancer patients that may be eligible for treatment with alpelisib.

Retrospective testing with F1CDx was performed for 415 patients (see sPMA cohort accountability, below). Concordance between F1CDx and the enrolling assays was performed by separately evaluating the concordance between F1CDx and the CTA1 and the concordance between F1CDx and the CTA2. The F1CDx to CTA1

concordance analysis was conducted with 280 CTA1-enrolled patient samples that met the sample testing criteria and were tested by F1CDx (274 valid, 6 invalid). The F1CDx to CTA2 concordance analysis was conducted with 385 patient samples, including 280 CTA1-enrolled and CTA2-retested samples (274 valid, 6 invalid), and the 105 CTA2-enrolled patient samples (all valid) that met the sample testing criteria and were tested by F1CDx.

Clinical efficacy in the *PIK3CA* alteration positive and alteration negative patients as determined by the F1CDx assay was assessed on PFS by the local investigator assessment per RECIST v1.1 criteria on the 379 CDx-evaluable patient population, which included the patients who met F1CDx testing criteria and yielded valid CDx results. Sensitivity analyses were conducted to determine the impact of missing F1CDx results (193 patients, including not tested, tested on deviation, and invalid results) on concordance and efficacy results. The 379 CDx-evaluable patient population was representative of the CTA1-enrolled population and the CTA2-enrolled population with respect to the baseline demographics and disease characteristics.

B. <u>Accountability of sPMA Cohort</u>

A total of 1,442 patients were screened for SOLAR-1. As part of the screening process, *PIK3CA* alteration testing was performed on 1,326 of the 1,442 patients using the CTA1 (596 patients) and the CTA2 (730 patients). Of the 596 patients tested by the CTA1, 395 were randomized to the SOLAR-1 study based on *PIK3CA* status (169 *PIK3CA* alteration positive, 226 *PIK3CA* alteration negative). Of the 730 patients tested by the CTA2 during screening for SOLAR-1, 177 were randomized (172 *PIK3CA* alteration positive, 5 *PIK3CA* alteration negative). A total of 572 patients were enrolled into SOLAR-1. After testing by either the CTA1 or the CTA2, residual tumor material from each randomized patient was banked for retrospective testing. The 395 CTA1-enrolled patients were retrospectively retested with the CTA2 prior to the F1CDx bridging study and 389 yielded valid CTA2 results.

F1CDx was used to retrospectively test the stored patient samples from SOLAR-1 with sufficient residual tumor material (N = 415 of the total 572 enrolled patients). Samples from 296 patients of 395 enrolled with the CTA1 (119 *PIK3CA* alteration positive patients and 177 *PIK3CA* alteration negative patients), and 119 patients of 177 enrolled with the CTA2 (115 *PIK3CA* alteration positive patients and 4 *PIK3CA* alteration negative patients), were retrospectively tested with F1CDx, yielding 379 CDx-evaluable results (see Tables 21 and 22). 30 samples (16 CTA1-enrolled patients and 14 CTA2-enrolled patients) were tested on deviation and were not part of the primary analyses.

Full disposition of the patient samples from SOLAR-1 and those used for the F1CDx bridging study are shown in Tables 20, 21, and 22.

Table 20. Disposition of all screened subjects (All screened subjects)

	Total patients	Tested by CDx
All screened	1442	415
Screened by CTA1	596	296
Tested as CTA1-positive	215	119
Randomized in Alpelisib arm	85	62
Randomized in Placebo arm	84	57
Not randomized (screen failure)	46	0
Tested as CTA1-negative	299	177
Randomized in Alpelisib arm	112	89
Randomized in Placebo arm	114	88
Not randomized (screen failure)	73	0
Tested as invalid	28	0
No test result	54	0
Screened by CTA2	730	119
Tested as CTA2-positive	277	115
Randomized in Alpelisib arm	84	54
Randomized in Placebo arm	88	61
Not randomized (screen failure)	105	0
Tested as CTA2-negative	382	4
Randomized in Alpelisib arm	3	3
Randomized in Placebo arm	2	1
Not randomized (screen failure)	377	0
Failed other clinical inclusion/exclusion criteria	57	0
Didn't fail other clinical inclusion/exclusion criteria	320	0
Tested as invalid	43	0
No test result	28	0
No test record	116	0

Table 21. Disposition of bridging subjects for CDx and CTA1 (Primary analysis set, CTA1-enrolled)

	CTA1	
CDx	**Positive N=169 (%)**	**Negative N=226 (%)**
Tested without deviation		
Positive	106 (62.7)	2 (0.9)
Negative	7 (4.1)	159 (70.4)

	CTA1	
CDx	**Positive N=169 (%)**	**Negative N=226 (%)**
Invalid	1 (0.6)	5 (2.2)
Tested with deviation		
Positive	3 (1.8)	1 (0.4)
Negative	0	9 (4.0)
Invalid	2 (1.2)	1 (0.4)
Not tested	50 (29.6)	49 (21.7)

Table 22. Disposition of bridging subjects for CDx and CTA2 (Concordance analysis set for CTA2)

	CTA2	
CDx	**Positive N=347 (%)**	**Negative N=219 (%)**
Tested without deviation		
Positive	197 (56.8)	2 (0.9)
Negative	18 (5.2)	162 (74.0)
Invalid	3 (0.9)	3 (1.4)
Tested with deviation		
Positive	9 (2.6)	0
Negative	9 (2.6)	7 (3.2)
Invalid	4 (1.2)	0
Not tested	107 (30.8)	45 (20.5)

For concordance between F1CDx and CTA1, the point estimates of PPA, NPA, and OPA were 93.8%, 98.8%, and 96.7%, respectively, and are detailed in Table 25, below. For concordance between F1CDx and CTA2, the point estimates of PPA, NPA, and OPA were 91.6%, 98.8%, and 94.7%, respectively, and are detailed in Table 26, below.

C. Study Population Demographics and Baseline Parameters

Demographics and baseline disease characteristics, tumor burden, and prior antineoplastic therapy were well balanced between the SOLAR-1 study arms and cohorts with or without a *PIK3CA* alteration. For the bridging study, baseline characteristics were compared between CDx-evaluable and CDx-unevaluable populations. In general, the demographics and disease characteristics for the CDx-evaluable and CDx-unevaluable patients were similar, except for differences in ECOG performance status, for CTA1- and CTA2-enrolled patients in the SOLAR-1 (Tables 23 and 24).

Table 23. Comparison of baseline demographic characteristics between the CDx-evaluable patients and the CDx-unevaluable patients (Primary analysis set, CTA1-enrolled)

	CTA1-positive			CTA1-negative		
Baseline characteristics	**CDx-evaluable N=113**	**CDx-unevaluable N=56**	**All N=169**	**CDx-evaluable N=161**	**CDx-unevaluable N=65**	**All N=226**
Age (years)						
n	113	56	169	161	65	226
Mean (SD)	63.7 (10.31)	64.4 (9.23)	63.9 (9.94)	62.0 (9.88)	62.8 (9.76)	62.2 (9.83)
Median	64.0	64.0	64.0	62.0	63.0	62.0
Q1-Q3	57.0-71.0	59.0-71.0	57.0-71.0	56.0-69.0	57.0-71.0	56.0-69.0
Min-Max	38-92	43-87	38-92	32-88	42-81	32-88
Race-n (%)						
White	74 (65.5)	37 (66.1)	111 (65.7)	102 (63.4)	45 (69.2)	147 (65.0)
Black or African American	3 (2.7)	0	3 (1.8)	4 (2.5)	0	4 (1.8)
Asian	27 (23.9)	11 (19.6)	38 (22.5)	37 (23.0)	13 (20.0)	50 (22.1)
American Indian or Alaska Native	1 (0.9)	0	1 (0.6)	1 (0.6)	1 (1.5)	2 (0.9)
Other	3 (2.7)	4 (7.1)	7 (4.1)	8 (5.0)	0	8 (3.5)
Unknown	5 (4.4)	4 (7.1)	9 (5.3)	9 (5.6)	6 (9.2)	15 (6.6)
Region-n (%)						
Europe	53 (46.9)	29 (51.8)	82 (48.5)	85 (52.8)	36 (55.4)	121 (53.5)
North America	19 (16.8)	11 (19.6)	30 (17.8)	18 (11.2)	5 (7.7)	23 (10.2)
Asia	26 (23.0)	10 (17.9)	36 (21.3)	37 (23.0)	12 (18.5)	49 (21.7)
Latin America	5 (4.4)	1 (1.8)	6 (3.6)	10 (6.2)	2 (3.1)	12 (5.3)
Other	10 (8.8)	5 (8.9)	15 (8.9)	11 (6.8)	10 (15.4)	21 (9.3)
ECOG performance status-n (%)						
0	80 (70.8)	30 (53.6)	110 (65.1)	115 (71.4)	45 (69.2)	160 (70.8)

Baseline characteristics	CTA1-positive CDx-evaluable N=113	CTA1-positive CDx-unevaluable N=56	CTA1-positive All N=169	CTA1-negative CDx-evaluable N=161	CTA1-negative CDx-unevaluable N=65	CTA1-negative All N=226
1	32 (28.3)	26 (46. 4)	58 (34.3)	45 (28.0)	20 (30.8)	65 (28.8)
Missing	1 (0.9)	0	1 (0.6)	1 (0.6)	0	1 (0.4)

- All percentages calculated using N as denominator.
- ECOG=Eastern Cooperative Oncology Group; SD=standard deviation.

Table 24. Comparison of baseline demographic characteristics between the CDx-evaluable patients and the CDx-unevaluable patients (Primary analysis set, CTA2-enrolled)

Baseline characteristics	CTA2-positive CDx-evaluable N=101	CTA2-positive CDx-unevaluable N=71	CTA2-positive All N=172	CTA2-negative CDx-evaluable N=4	CTA2-negative CDx-unevaluable N=1	CTA2-negative All N=5
Age (years)						
n	101	71	172	4	1	5
Mean (SD)	63.1 (10.82)	62.2 (9.49)	62.7 (10.27)	67.5 (8.66)	74.0 (NE)	68.8 (8.04)
Median	63.0	63.0	63.0	70.0	74.0	70.0
Q1-Q3	57.0-70.0	55.0-69.0	57.0-70.0	62.5-72.5	74.0-74.0	70.0-74.0
Min-Max	25-85	38-83	25-85	55-75	74-74	55-75
Race-n (%)						
White	68 (67.3)	47 (66.2)	115 (66.9)	3 (75.0)	1(100.0)	4 (80.0)
Black or African American	0	1 (1.4)	1 (0.6)	0	0	0
Asian	24 (23.8)	12 (16.9)	36 (20.9)	1 (25.0)	0	1 (20.0)
American Indian or Alaska Native	0	2 (2.8)	2 (1.2)	0	0	0
Other	5 (5.0)	6 (8.5)	11 (6.4)	0	0	0
Unknown	4 (4.0)	3 (4.2)	7 (4.1)	0	0	0
Region-n (%)						
Europe	54 (53.5)	37 (52.1)	91 (52.9)	3 (75.0)	1(100.0)	4 (80.0)
North America	6 (5.9)	7 (9.9)	13 (7.6)	0	0	0

Baseline characteristics	CTA2-positive CDx-evaluable N=101	CTA2-positive CDx-unevaluable N=71	CTA2-positive All N=172	CTA2-negative CDx-evaluable N=4	CTA2-negative CDx-unevaluable N=1	CTA2-negative All N=5
Asia	22 (21.8)	12 (16.9)	34 (19.8)	1 (25.0)	0	1 (20.0)
Latin America	14 (13.9)	11 (15.5)	25 (14.5)	0	0	0
Other	5 (5.0)	4 (5.6)	9 (5.2)	0	0	0
ECOG performance status-n (%)						
0	74 (73.3)	41 (57.7)	115 (66.9)	3 (75.0)	0	3 (60.0)
1	27 (26.7)	29 (40.8)	56 (32.6)	1 (25.0)	1(100.0)	2 (40.0)
Missing	0	1 (1.4)	1 (0.6)	0	0	0

- All percentages calculated using N as denominator.
- ECOG=Eastern Cooperative Oncology Group; SD=standard deviation.

D. Safety and Effectiveness

1. Safety Results

The safety with respect to treatment with alpelisib was addressed during the review of the NDA and is not addressed in detail in this Summary of Safety and Effectiveness Data. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs. Please refer to Drugs@FDA for complete safety information on PIQRAY® (alpelisib).

Briefly, the most common adverse reactions (ARs) in alpelisib plus fulvestrant treated patients (reported at a frequency > 20% and for which the rate for alpelisib plus fulvestrant exceeds the frequency for placebo plus fulvestrant) were hyperglycemia, diarrhea, rash, nausea, fatigue and asthenia, decreased appetite, stomatitis, vomiting, and weight decreased.

The most common Grade 3/4 ARs (reported at a frequency > 2% in the alpelisib plus fulvestrant arm and for which the frequency for alpelisib plus fulvestrant exceeds the frequency for placebo plus fulvestrant) were hyperglycemia, rash, diarrhea, lipase increased, hypertension, hypokalemia, weight decreased, anemia, fatigue and asthenia, nausea, stomatitis, alanine aminotransferase increased, lymphopenia, and mucosal inflammation.

Alpelisib dose reductions due to ARs, regardless of causality, occurred in 58% of patients receiving alpelisib plus fulvestrant and in 5% of patients receiving placebo plus fulvestrant. Permanent discontinuations of alpelisib and/or fulvestrant due to ARs were reported in 25% of patients compared to 5% with placebo and/or fulvestrant. The most common ARs leading to treatment discontinuation of both

alpelisib and fulvestrant were hyperglycemia (6%), rash (3%), diarrhea (3%), and fatigue (2%).

No adverse events were reported in connection with the bridging study used to support this PMA supplement, as the study was performed retrospectively using banked samples.

2. Effectiveness Results

a. Concordance Results

The concordance between F1CDx and the two enrollment assays (CTA1 and CTA2) was assessed. The point estimates of PPA, NPA, and OPA for F1CDx compared to the CTAs are provided in Tables 25 and 26, below.

Table 25. Agreement between CDx and CTA1 based on the CTA1 results (Primary analysis set, CTA1-enrolled)

	Without invalid CDx results		With invalid CDx results	
Measure of agreement	**Percent agreement (N)**	**95% CI (1)**	**Percent agreement (N)**	**95% CI (1)**
PPA	93.8% (106/113)	(87.7%, 97.5%)	93.0% (106/114)	(86.6%, 96.9%)
NPA	98.8% (159/161)	(95.6%, 99.8%)	95.8% (159/166)	(91.5%, 98.3%)
OPA	96.7% (265/274)	(93.9%, 98.5%)	94.6% (265/280)	(91.3%, 97.0%)

(1) The 95% CI calculated using the Clopper-Pearson Exact method.
- Samples not tested are excluded from the analysis.
- Samples tested on deviation are excluded from the analysis.

Table 26. Agreement between CDx and CTA2 based on the CTA2 results (Concordance analysis set for CTA2)

	Without invalid CDx results		With invalid CDx results	
Measure of Agreement	**Percent Agreement (N)**	**95% CI (1)**	**Percent Agreement (N)**	**95% CI (1)**
PPA	91.6% (197/215)	(87.1%, 95.0%)	90.4% (197/218)	(85.7%, 93.9%)
NPA	98.8% (162/164)	(95.7%, 99.9%)	97.0% (162/167)	(93.2%, 99.0%)
OPA	94.7% (359/379)	(92.0%, 96.7%)	93.2% (359/385)	(90.3%, 95.5%)

(1) The 95% CI calculated using the Clopper-Pearson Exact method.
- Samples not tested are excluded from the analysis.
- Samples tested on deviation are excluded from the analysis.

b. Clinical Efficacy Results in the SOLAR-1 *PIK3CA* Mutant Cohort

The SOLAR-1 clinical trial met its primary objective demonstrating a statistically significant improvement in PFS by investigator assessment in patients with *PIK3CA*

alteration positive tumors. Supportive analysis included PFS based on blinded independent review committee (BIRC).

Alpelisib in combination with fulvestrant demonstrated an estimated 35% risk reduction of disease progression or death compared to the placebo plus fulvestrant arm (HR = 0.65; 95% CI: 0.50, 0.85; p = 0.00065) in the *PIK3CA* alteration cohort. The median PFS was prolonged by a clinically relevant 5.3 months, from 5.7 months in the placebo plus fulvestrant arm to 11.0 months in the alpelisib plus fulvestrant arm (Table 27, Figure 1).

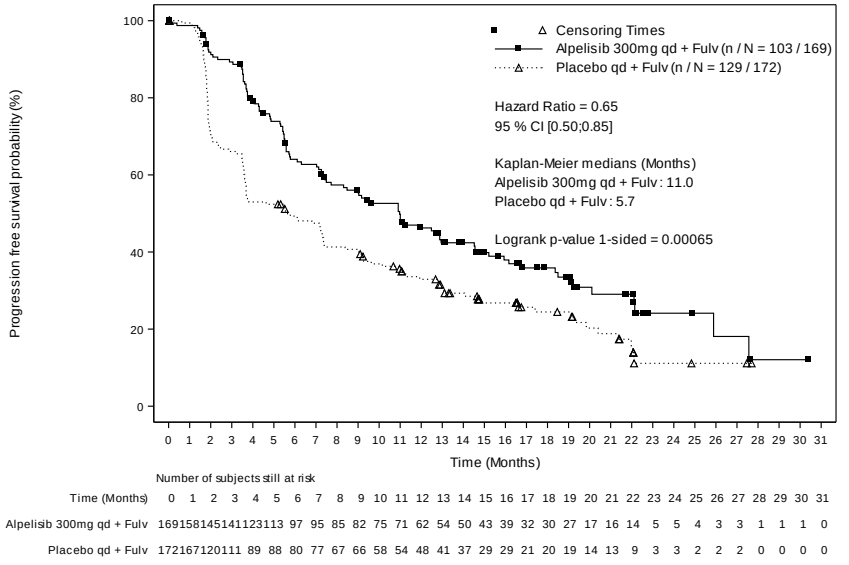
Table 27. Clinical efficacy on progression free survival in the mutant patients randomized in the original SOLAR-1 trial (Primary analysis set)

Progression free survival (months)	**Alpelisib 300mg qd + Fulv N=169**	**Placebo qd + Fulv N=172**	**HR (95% CI) Alpelisib 300mg qd + Fulv / Placebo qd + Fulv (1)**
No of events (%)	103 (60.9)	129 (75.0)	0.65 (0.50, 0.85)
PD (%)	99 (58.6)	120 (69.8)	
Death (%)	4 (2.4)	9 (5.2)	
No of censored (%)	66 (39.1)	43 (25.0)	
Median (95% CI) (2)	11.0 (7.5, 14.5)	5.7 (3.6, 7.4)	

(1) Hazard ratio (HR) estimated using Cox regression model stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor. CI: Wald Confidence Interval.

(2) The 95% CI calculated from PROC LIFETEST output using the method of Brookmeyer and Crowley (1982).

Figure 1. Kaplan-Meier plot of progression free survival by treatment in the mutant patients randomized in the original SOLAR-1 trial (Primary analysis set)



- Stratified Logrank test and stratified Cox model using strata defined by (i) prior CDK 4/6 inhibitor use. (ii) presence of liver and/or lung metastases.

c. Clinical Efficacy Results in the CDx-positive Population

Efficacy analyses were performed for patients determined to be CDx-positive (*PIK3CA* alteration detected by the F1CDx assay) and compared to the efficacy results in the SOLAR-1 *PIK3CA* mutant cohort. The clinical efficacy in the CDx-positive population was estimated by pooling the hazard ratios calculated for: 1) the CTA1-enrolled patients that were CDx-positive and 2) the CTA2-enrolled patients that were CDx-positive.

The efficacy in the CTA1-enrolled patients that were CDx-positive was estimated directly by performing stratified Cox regression analysis, adjusting for the 8 identified clinically relevant covariates, including age, race, region, ECOG performance status, patient population based on endocrine status and line of therapy, visceral disease, bone lesions only, and number of metastatic sites.

The CTA2-enrolled patients that were CDx-positive included (CTA2+, CDx+) patients and (CTA2-, CDx+) patients. The efficacy in (CTA2+, CDx+) was estimated from the stratified Cox regression, adjusting for the 7 identified clinically relevant covariates, including age, race, region, ECOG performance status, patient population based on endocrine status and line of therapy, visceral disease, and bone lesions only. The efficacy in (CTA2-, CDx+) was not estimable due to the small sample size (N = 1). Therefore, the sensitivity analysis was performed by assuming the efficacy in (CTA2-, CDx+) was a fraction (c) of that in the (CTA2+, CDx+) population with c ranging from 0 to 1.

Tables 28 and 29 show the efficacy results in the CTA1-enrolled CDx-positive patients (HR = 0.52; 95% CI: 0.29, 0.93) and the results in the CTA2-enrolled (CTA2+, CDx+) patients (HR = 0.35; 95% CI: 0.16, 0.77), respectively.

Table 30 shows the sensitivity analysis to c for the clinical efficacy of alpelisib in combination with fulvestrant for the *PIK3CA* CDx-positive population. The hazard ratio estimates ranged from 0.43 to 0.44. The upper bounds of the 95% CIs for the corresponding hazard ratios were all below 1.0. The improved efficacy results obtained with F1CDx compared to those observed in the SOLAR-1 clinical trial (HR = 0.65; 95% CI: 0.50, 0.85) may be due to discordant results between the enrolling assays (CTA1 and CTA2) and F1CDx, differences in the proportions of core needle biopsy and surgical resection samples tested by F1CDx, and differences in ECOG performance status between the CDx-evaluable and CDx-unevaluable patients (please refer to Tables 23 and 24).

Table 28. Clinical efficacy on progression free survival in the CTA1-enrolled CDx-positive patients (Primary analysis set, CTA1-enrolled)

Progression free survival (months)	**Alpelisib 300mg qd + Fulv N=56**	**Placebo qd + Fulv N=52**	**HR (95% CI) Alpelisib 300mg qd + Fulv / Placebo qd + Fulv (1)**
No of events (%)	41 (73.2)	41 (78.8)	0.52 (0.29, 0.93)
PD (%)	39 (69.6)	41 (78.8)	
Death (%)	2 (3.6)	0	
No of censored (%)	15 (26.8)	11 (21.2)	
Median (95% CI) (2)	11.2 (8.3, 18.5)	5.5 (1.9, 10.9)	

(1) Hazard ratio (HR) estimated using Cox regression model. The model is adjusted by the identified baseline clinical covariates, as well as the covariates that are imbalanced between treatment and control. The model is stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor.

CI: Wald Confidence Interval.

(2) The 95% CI calculated from PROC LIFETEST output using the method of Brookmeyer and Crowley (1982).

-CDx results obtained on deviation are treated as missing.

Table 29. Clinical efficacy on progression free survival in the CTA2-enrolled (CTA2+, CDx+) patients (Primary analysis set, CTA2-enrolled)

Progression free survival (months)	**Alpelisib 300mg qd + Fulv N=42**	**Placebo qd + Fulv N=48**	**HR (95% CI) Alpelisib 300mg qd + Fulv / Placebo qd + Fulv (1)**
No of events (%)	19 (45.2)	36 (75.0)	0.35 (0.16, 0.77)
PD (%)	18 (42.9)	31 (64.6)	

Progression free survival (months)	Alpelisib 300mg qd + Fulv N=42	Placebo qd + Fulv N=48	HR (95% CI) Alpelisib 300mg qd + Fulv / Placebo qd + Fulv (1)
Death (%)	1 (2.4)	5 (10.4)	
No of censored (%)	23 (54.8)	12 (25.0)	
Median (95% CI) (2)	10.9 (5.6, NE)	4.2 (2.1, 7.4)	

(1) Hazard ratio (HR) estimated using Cox regression model. The model is adjusted by the identified baseline clinical covariates, as well as the covariates that are imbalanced between treatment and control. The model is stratified by the two stratification factors: presence of lung and/or liver metastases, previous treatment with any CDK4/6 inhibitor.

CI: Wald Confidence Interval.

(2) The 95% CI calculated from PROC LIFETEST output using the method of Brookmeyer and Crowley (1982).

-CDx results obtained on deviation are treated as missing.

Table 30. Clinical efficacy on progression free survival in the CDx-positive patients (Primary analysis set)

Progression free survival (CDx-positive) C value	HR (95% CI) Alpelisib 300mg qd + Fulv / Placebo qd + Fulv (1)
0	0.44 (0.27, 0.70)
0.1	0.44 (0.27, 0.70)
0.2	0.44 (0.27, 0.70)
0.3	0.44 (0.27, 0.70)
0.4	0.44 (0.27, 0.70)
0.5	0.44 (0.27, 0.70)
0.6	0.44 (0.27, 0.70)
0.7	0.44 (0.27, 0.70)
0.8	0.43 (0.27, 0.70)
0.9	0.43 (0.27, 0.70)
1	0.43 (0.27, 0.70)

(1) Hazard ratio (HR) estimated by pooling together the HRs estimated for the CTA-enrolled CDx-positive patients and the CTA2-enrolled CDx-positive patients using sample size as the weight. CI: Wald Confidence Interval.

- When estimating HR in the CTA2-enrolled CDx-positive patients, the prevalence of CTA2-positive is calculated as Pr(CTA2+) in the FAS.

- CDx results obtained on deviation are treated as missing.

3. Pediatric Extrapolation

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

E. Financial Disclosure

The bridging study was conducted retrospectively at a single testing site in Cambridge, MA and exempt from the requirements for Investigational Device Exemption as defined in Title 21 of the Code of Federal Regulations (21 CFR), 812.2(c)(3). The investigational product was not used in the diagnosis or treatment of patients. The information provided does not raise any questions about the reliability of the data.

XI. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable.

XII. 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 supplement 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.

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

For the intended use to identify *PIK3CA* alterations in breast cancer patients to be treated with alpelisib, the effectiveness of the F1CDx assay was demonstrated through a clinical bridging study using specimens from patients screened for enrollment into the SOLAR-1 study. The data from the analytical validation and clinical bridging studies support the reasonable assurance of safety and effectiveness of the F1CDx assay when used in accordance with the indications for use. Data from the SOLAR-1 study show that patients who had qualifying *PIK3CA* alterations received benefit from treatment with alpelisib and support the addition of the CDx indication to F1CDx.

B. Safety Conclusions

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

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient

management decisions in cancer treatment. Patients with false positive results may undergo treatment with one of the therapies listed in Table 1 of the intended use statement without clinical benefit and may experience adverse reactions associated with the therapy. Patients with false negative results may not be considered for treatment with the indicated therapy. There is also a risk of delayed results, which may lead to delay of treatment with the indicated therapy.

C. Benefit-Risk Determination

For the *PIK3CA* alterations indication, the probable benefits of F1CDx are based on data collected in the SOLAR-1 study and the bridging study, which was conducted to support PMA approval. The clinical benefit of the F1CDx assay for the selection of breast cancer patients with *PIK3CA* alterations was demonstrated in a retrospective analysis of efficacy and safety data obtained from the Phase III, randomized, double-blind, placebo-controlled SOLAR-1 study. The risks of the F1CDx assay are associated with the potential mismanagement of patients resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial which may lead to adverse events or may have delayed access to treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial drug.

Risk associated with the F1CDx assay include the possibility of inaccurate results that may lead to mismanagement of patients. However, the risk is mitigated by the clinical and analytical concordance studies for F1CDx detection of *PIK3CA* biomarker alterations. The supporting clinical validation analyses demonstrate PPA (93.8% to CTA1, 91.6% to CTA2), NPA (98.8% to CTA1, 98.8% to CTA2) and OPA (96.7% to CTA1, 94.7% to CTA2) indicating the likelihood of patient misdiagnosis is low. An accuracy study of F1CDx for the detection of *PIK3CA* alterations with the externally validated NGS comparator method demonstrating PPA (100%) and NPA (100%) also supports such a conclusion. Use of the F1CDx assay shows comparable efficacy results as found in the SOLAR-1 study (HR 0.43-0.44, HR 0.65, respectively). Therefore, these results support the use of F1CDx as an aid in selecting patients with *PIK3CA* mutant breast cancer for PIQRAY® (alpelisib) treatment.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with high accuracy, mitigating the potential for false results.

Additional factors to be considered in determining probable risks and benefits for the F1CDx assay include: analytical performance of the device, representation of variants, additional and ongoing analytical testing. Additional analytical testing will be performed in the post-approval setting.

1. Patient Perspectives
   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 F1CDx assay, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical bridging study support the performance of F1CDx as an aid for the identification of *PIK3CA* alteration breast cancer patients for whom PIQRAY® (alpelisib) may be indicated.

XIV. CDRH DECISION

CDRH issued an approval order on December 3, 2019. The final conditions of approval cited in the approval order are described below.

1. Provide additional precision data with an intended use FFPE tissue sample containing the *PIK3CA* C420R variant at approximately 1.5X of the LoD according to the agreed upon study proposal.

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

XV. 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.

XVI. REFERENCES

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