SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next generation sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	FoundationOne®CDx (F1CDx)
Device Procode:	PQP
Applicant's Name and Address:	Foundation Medicine, Inc. 150 Second Street Cambridge, MA 02141
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P170019/S048
Date of FDA Notice of Approval:	November 16, 2023

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

The current supplement was submitted to expand the indication for F1CDx to include a companion diagnostic (CDx) claim for the detection of *PIK3CA/AKT1/PTEN*-alterations in patients with breast cancer who may benefit from treatment with TRUQAP™ (capivasertib) in combination with FASLODEX® (fulvestrant)**.**

II. INDICATIONS FOR USE

FoundationOne®CDx (F1CDx) is a qualitative next-generation sequencing based *in vitro* diagnostic test that uses targeted high throughput hybridization-based capture technology for detection of substitutions, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 324 genes and select gene rearrangements, as well as genomic signatures including microsatellite instability (MSI) and tumor mutational burden (TMB) using DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens when using the DNAx extraction method. The test is intended for detection of substitutions and indels in 324 genes, CNAs in 16 genes and select gene rearrangements,

as well as genomic signatures including MSI and TMB using DNA isolated from FFPE tumor tissue specimens when using the CoExtraction method for DNA isolation. The test is intended as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling. Additionally, F1CDx is intended to provide tumor mutation profiling to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. Genomic findings other than those listed in Table 1 are not prescriptive or conclusive for labeled use of any specific therapeutic product.

Table 1. Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	EGFR Tyrosine Kinase Inhibitors (TKI) approved by FDA*
	EGFR exon 20 T790M alterations	TAGRISSO® (osimertinib)
	ALK rearrangements	ALECENSA® (alectinib), ALUNBRIG® (brigatinib) XALKORI® (crizotinib), or ZYKADIA® (ceritinib)
	BRAF V600E	BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib)
		TAFINLAR® (dabrafenib) in combination with MEKINIST® (trametinib)
	MET single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	TABRECTA™ (capmatinib)
	ROS1 Fusions	ROZLYTREK® (entrectinib)
Melanoma	*BRAF* V600E	BRAF Inhibitors approved by FDA*
	BRAF V600E and V600K	MEKINIST® (trametinib) or BRAF/MEK Inhibitor Combinations approved by FDA*
	BRAF V600 mutation-positive	TECENTRIQ® (atezolizumab) in combination with COTELLIC®

		(cobimetinib) and ZELBORAF® (vemurafenib)
Breast cancer	*ERBB2* (HER2) amplification	HERCEPTIN® (trastuzumab), KADCYLA® (ado-trastuzumabemtansine), or PERJETA® (pertuzumab)
	PIK3CA C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y alterations	PIQRAY® (alpelisib)
	AKT1 E17K, *PIK3CA* R88Q, N345K, C420R, E542K, E545A, E545D, E545Q, E545K, E545G, Q546E, Q546K, Q546R, Q546P, M1043V, M1043I, H1047Y, H1047R, H1047L, and G1049R; and *PTEN* alterations	TRUQAP™ (capivasertib) in combination with FASLODEX® (fulvestrant)
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)
Cholangiocarcinoma	*FGFR2* fusions and select rearrangements	PEMAZYRE® (pemigatinib) or TRUSELTIQ™ (infigratinib)
Prostate cancer	Homologous Recombination Repair (HRR) gene (*BRCA1, BRCA2, ATM, BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, RAD51B, RAD51C, RAD51D* and *RAD54L*) alterations	LYNPARZA® (olaparib)
	BRCA1, BRCA2 alterations	AKEEGA® (niraparib + abiraterone acetate)
Solid tumors	MSI-High	KEYTRUDA® (pembrolizumab)

	TMB ≥ 10 mutations per megabase	KEYTRUDA® (pembrolizumab)
	NTRK1/2/3 fusions	ROZLYTREK® (entrectinib) or VITRAKVI® (larotrectinib)
	RET fusions	RETEVMO® (selpercatinib)

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

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

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the FoundationOneCDx assay labeling.

V. DEVICE DESCRIPTION

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

FoundationOneCDx (F1CDx) is performed exclusively as a laboratory service using DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples. The assay employs two extraction methods (either DNAx or CoExtraction, an automated DNA/RNA co-extraction methodology) for DNA extraction from routine FFPE biopsy or surgical resection specimens; 50-1000 ng of DNA will undergo whole-genome shotgun library construction and hybridization-based capture of all coding exons from 309 cancer-related genes, one promoter region, one non-coding (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 for the complete list of genes included in F1CDx). In total, the assay therefore detects alterations in 324 genes. Using the Illumina® HiSeq 4000 platform, hybrid-capture selected libraries are sequenced to high uniform depth (targeting > 500X median coverage with > 99% of exons at coverage > 100X). Sequence data is then 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). Rearrangements in one of the targeted genes included in Table 2 may be reported along with their uniquely identified genomic partners, which can be any gene in

the genome even if not explicitly targeted by the assay. 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 FoundationOneCDx 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*	*PRKARA*	*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*	*SMARC A4*	*WHSC1*
ASXL1	*CBL*	*CTCF*	*FANCC*	*GNA13*	*KEAP1*	*MSH2*	*PARP1*	*QKI*	*SMARC B1*	*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*	*PDCD1L G2*	*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*	

*Genes with copy number alteration reporting are limited to CDx variants when using the CoExtraction method.

Table 3. Genes with select intronic regions for the detection of gene rearrangements, and select non-coding regions that include a 3'UTR, a promoter region, and a ncRNA gene.

ALK introns 18, 19	*BRCA1 introns 2, 7, 8, 12, 16, 19, 20*	*ETV4 introns 8*	*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*

**ETV6* is a common rearrangement partner for *NTRK3*

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

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 (≥ 20% tumor), and sufficient nucleated cells are present to proceed with the assay.

B. DNA Extraction

DNAx Extraction Method

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.

CoEx Extraction Method

Specimens passing pathology review are queued for nucleic acid extraction which begins with placement of the FFPE samples into an AutoLys tube, where using a pre-programmed automated method, the AutoLys STAR adds RNA digestion and proteinase K solutions. The RNA containing lysate is removed for downstream RNA extraction using the KingFisher RNA extraction process.

The AutoLys Tubes containing partially digested tissue then receive DNA Lysis solution and are placed into a Vortemp for digestion. The sample is then centrifuged to separate sample-associated paraffin from the lysate, and the lysate is transferred to a KingFisher dKF plate. The dKF plate is loaded onto the Hamilton STAR for automated addition of DNA binding buffer and magnetic beads, and DNA isolation is performed using the KingFisher Flex. The DNA samples are then transferred to matrix tubes on DNAE plates using Hamilton STAR before proceeding to DNA quantification.

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

C. Library Construction

Library Construction (LC) begins with normalization of DNA to 50-1000 ng. The normalized DNA samples are randomly sheared (fragmented) to ~200 bp by adaptive focused acoustic sonication using the Covaris LE220-Plus before purification with a 1.8X volume of AMPure® XP Beads (Agencourt®). Solid-phase reversible immobilization (SPRI) purification and subsequent library construction with the NEBNext® reagents (custom-filled kits by New England Biolabs), including mixes for end repair, dA addition and ligation, are performed in 96-well plates (Eppendorf) on the Bravo Benchbot (Agilent) or Hamilton Microlab STAR/STARLet Liquid Handling Workstation (Hamilton) using the "withbead" 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, F1CDx employs a fraction based (FB) MSI algorithm to categorize a tumor specimen as MSI-High (MSI-H) or microsatellite stable (MSS). The FB-MSI algorithm calculates the fraction of microsatellite loci determined to be altered or unstable (i.e., the fraction unstable loci score) based on a genome-wide analysis across >2000 microsatellite loci. For a given microsatellite locus, non-somatic alleles are discarded, and the microsatellite is categorized as unstable if remaining alleles differ from the reference genome. The

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

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

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

G. Report Generation

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

H. Internal Process Controls Related to the System

Positive Control

Each assay run includes a control sample run in duplicate. The control sample contains a pool of ten HapMap cell lines and is used as a positive mutation detection control. 100 different germline SNPs present across the entire targeted region are

required to be detected by the analysis pipeline. If SNPs are not detected as expected, this results in a QC failure, as it indicates a potential processing error.

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

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

I. Variant Classification

Biomarker Rules for SNVs and indels that lead to *MET* exon 14 skipping
An SNV or indel in *MET* shall be considered to result in skipping of exon 14 if one or more of the following criteria are met:

1. Deletions greater than or equal to 5 bp that affect positions -3 to -30 in the intronic region immediately adjacent to the splice acceptor site at the 5′ boundary of *MET* exon 14.
2. Indels affecting positions -1 or -2 at the splice acceptor site of the 5′ boundary of *MET* exon 14.
3. Base substitutions and indels affecting positions 0, +1, +2, or +3 at the splice donor site of the 3′ boundary of *MET* exon 14.

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

COSMIC, they would be listed as deleterious mutations (or "known" status in FMI reporting).

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

Table 5. Mutation types identified in the HRR genes

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

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

The specific deleterious mutation (DM) and suspected deleterious mutation (SDM) missense mutations or non-frameshift mutations for *BRCA1, BRCA2,* and *ATM* are shown in Tables 6-8, below. However, any missense or non-frameshift mutations in the other 12 genes would not be considered HRR positive.

Table 6. Eligible deleterious mutations in the *ATM* gene (for olaparib CDx claim only)

M1I	Y2470D	R2832C
M1L	R2547_S2549del	S2855_V2856>RI
M1T	A2622V	D2913Y
P292L	D2625_A2626>EP	R3008C
D2016G	D2708N	R3008H
R2032K	V2716A	splice site 331+5G>A
A2067D	G2765S	splice site 8418+5_8418+8delGTGA
R2227C	F2827C	

Table 7. List of short variants in *BRCA1*

M1R	C44S	R1495T	D1692Y*	G1738E	Y1853C
M1I	C44Y	E1559K	C1697R	G1738R	C1787_G1788>SD
M1V	C47F	E1559Q	R1699Q	L1764P	splice site 212+3A>G
M1T	C61G	A1623G	R1699W	I1766S	splice site 213-11T>G
M18T	C61Y*	S1655F	L1705P	G1770V	splice site 213-12A>G
L22S	C64G	T1685A	G1706E	M1775K	splice site 302-3C>G

C24R	C64R	T1685I	G1706R	M1775R	splice site 4986+3G>C
T37K	C64W*	H1686R	A1708E	L1780P	splice site 4986+5G>A
C39G*	C64Y	V1688del	S1715N	G1788V	splice site 4986+6T>G
C39R	R71K	M1689R	S1715R	P1812A	splice site 4986+6T>C
C39W*	R71G	T1691I	W1718C	V1833M	splice site 5074+3A>G
C39Y	R170Q*	T1691K	S1722F	W1837R	splice site 5194-12G>A
H41R	R1495K	D1692N	V1736A	W1837C	splice site 5406+4A>G
C44F	R1495M	D1692H	V1736G	V1838E	

*variants are part of the biomarker definition for the niraparib CDx claim only

Table 8. List of short variants in *BRCA2*

M1R	R2336L*	R2659G	L2686P	Y2726C	N3124I
M1I	R2336P	R2659K	L2688P	G2748D	splice site 316+4delA
S142I*	L2510P	R2659T	T2722R	G2793R	splice site 316+5G>A
V159M	H2623R	Y2660D	D2723A	E3002K	splice site 8487+3A>G
V211I	W2626C	E2663V	D2723G	R3052W	splice site 8754+4A>G
V211L	I2627F	S2670L	D2723H	G3076V	splice site 8754+5G>A
R2336H	L2653P	I2675V	D2723V	D3095E	splice site 8754+3G>C

*variants are part of the biomarker definition for the niraparib CDx claim only

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

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

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

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

biomarker negative. Unidentified partners (encoded as N/A) or LINC non-coding partners are also considered CDx biomarker negative.

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

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

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

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

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

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

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

Biomarker Rules for *RET* Fusions:
Fusions in *RET* shall be considered CDx biomarker positive if the following criteria are met:

- Any fusion event involving *RET* and another protein-coding gene
- *RET* and the partner gene must be in the same 5'-3' orientation
- *RET* must be on the 3' end of the detected rearrangement
- The *RET* breakpoint must occur before the start of the kinase domain (amino acids 724-1016)

Biomarker Rules for *AKT1*, *PIK3CA,* and *PTEN* Alterations:
Alterations in *AKT1*, *PIK3CA,* and/or *PTEN* are considered Companion biomarker positive if the following criteria are met:

Table 9. Biomarker definition for *AKT1*, *PIK3CA*, and *PTEN*

Gene (Transcript)	Alteration Type	Protein/Transcript Effect
AKT1 (NM_001014431)	SNV	E17K
PIK3CA (NM_006218)	SNV	R88Q, N345K, C420R, E542K, E545A, E545D, E545Q, E545K, E545G, Q546E, Q546K, Q546R, Q546P, M1043V, M1043I, H1047Y, H1047R, H1047L, and G1049R
PTEN (NM_000314)	SNV and indels	C124R, G129E, R130Q, C136R, S170R, R173C, C124S, G129V, R130G C136Y, G129R, R130L, R130P
		Any nonsense, frameshift, or splice site alteration • Missense mutations in the start codon and short variant deletions spanning from upstream of the start codon (annotated as M1?) are included in this category.
	Copy Number Alteration	Homozygous deletion (HD) represents a deletion of one or more exons regardless of transcript in both alleles
	Rearrangement (RE)	Any rearrangement that disrupts protein function, regardless of transcript • Intragenic events including duplications of only part of the gene, deletions, or inversions. • Translocations, deletions, or inversions where one breakpoint is in *PTEN* and the other breakpoint is in another gene or intergenic region.

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 10, below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices

at: https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

Table 10. 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-V600E and V600K	THxID BRAF Kit	bioMerieux	PCR	MEKINIST (tramatenib)	Melanoma
	cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems, Inc.	PCR	COTELLIC (cobimetinib) ZELBORAF (vemurafenib)	Melanoma
BRAF-V600E	cobas 4800 BRAF V600 Mutation Test	Roche Molecular Systems, Inc.	PCR	ZELBORAF (vemurafenib)	Melanoma
	THxID BRAF Kit	bioMerieux	PCR	TAFINLAR (dabrafenib)	Melanoma
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	TAFINLAR (dabrafenib) MEKINIST (trametinib)	NSCLC
	therascreen BRAF V600E RGQ PCR Kit	QIAGEN	PCR	BRAFTOVI (encorafenib) Erbitux (cetuximab)	CRC

NRAS	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	CRC
KRAS	cobas KRAS Mutation Test	Roche Molecular Systems, Inc.	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	CRC
	therascreen KRAS RGQ PCR Kit	QIAGEN	PCR	ERBITUX (cetuximab) VECTIBIX (panitumumab)	CRC
	Praxis Extended RAS Panel	Illumina, Inc.	NGS	VECTIBIX (panitumumab)	CRC
ALK –fusion	Vysis ALK Break Apart FISH Probe Kit	Abbott Molecular, Inc.	FISH	XALKORI (crizotinib)	NSCLC
	ALK (D5F3) CDx Assay	Ventana Medical Systems, Inc.	IHC	XALKORI (crizotinib)	NSCLC
EGFR – Exon 19 deletions & L858R	cobas EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	TARCEVA (erlotinib) TAGRISSO (osimertinib) IRESSA (gefitinib)	NSCLC
	therascreen EGFR RGQ PCR Kit	QIAGEN	PCR	GILOTRIF (afatinib) IRESSA (gefitinib)	NSCLC
	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	IRESSA (gefitinib)	NSCLC
EGFR T790M	cobas EGFR Mutation Test v2	Roche Molecular Systems, Inc.	PCR	TAGRISSO (osimertinib)	NSCLC
BRCA1/2	BRACAnalysis CDx	Myriad Genetic Laboratories, Inc.	Sanger and PCR	\ LYNPARZA (olaparib) - treatment/maintenance	Ovarian cancer
PIK3CA	therascreen PIK3CA RGQ PCR Kit	QIAGEN	PCR	PIQRAY (alpelisib)	Breast cancer
RET	Oncomine Dx Target Test	Life Technologies, Inc.	NGS	RETEVMO (selpercatinib)	NSCLC and Thyroid 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 approved PMA supplements that affected the Intended Use are listed in Table 11.

Table 11. Marketing History

Submission No.	Date of Approval	Biomarker/Update	Patient Population	Drug
P170019/S004	July 1, 2019	*BRCA1/2* alterations	Ovarian Cancer	LYNPARZA® (olaparib)
P170019/S006	December 3, 2019	*PIK3CA* alterations	Breast Cancer	PIQRAY® (alpelisib)
P170019/S008	July 1, 2019	*EGFR* exon 19 deletions and *EGFR* exon 21 L858R alterations	Non-Small Cell Lung Cancer	TAGRISSO® (osimertinib)
P170019/S011	May 6, 2020	*MET* single nucleotide variants (SNVs) and indels that lead to *MET* exon 14 skipping	Non-Small Cell Lung Cancer	TABRECTA® (capmatinib)
P170019/S013	April 17, 2020	*FGFR2* fusions	Cholangiocarcinoma	PEMZYRE® (pemigatinib)
P170019/S015	May 19, 2020	mutations in homologous recombination repair (HRR) genes	metastatic castration resistant prostate cancer (mCRPC)	LYNPARZA® (olaparib)
P170019/S016	June 16, 2020	high tumor mutational burden (TMB) at the cut- off of 10 mutations per megabase (mut/Mb)	Solid Tumors	KEYTRUDA® (pembrolizumab)
P170019/S017	October 23, 2020	*NTRK1, NTRK2, or NTRK3* fusions	Solid Tumors	VITRAKVI ® (larotrectinib)
P170019/S021	May 28, 2021	*FGFR2* Fusion/Rearrangements	Cholangiocarcinoma	TRUSELTIQ (infigratinib)
P170019/S023	June 30, 2021	*ALK* Rearrangements	Non-Small Cell Lung Cancer	ALUNBRIG® (brigatinib)
P170019/S025	November 10, 2021	*BRAF* V600E	Melanoma	BRAF Inhibitor Monotherapy Group Claim

Submission No.	Date of Approval	Biomarker/Update	Patient Population	Drug
		BRAF V600E or V600K Alterations	Melanoma	BRAF/MEK Inhibitor Combination Group Claim
P170019/S029	February 18, 2022	Microsatellite Instability High (MSI-H) Status	Solid Tumors	KEYTRUDA® (Pembrolizumab)
P170019/S030	January 19, 2022	*BRAF* V600 Mutation- Positive	Unresectable Or Metastatic Melanoma	Atezolizumab (TECENTRIQ®) In Combination with Cobimetinib and Vemurafenib
P170019/S033	March 16, 2022	*EGFR* Exon 19 Deletions or *EGFR* Exon 21 L858R Mutations	Non-Small Cell Lung Cancer	Any one of The FDA-Approved EGFR Tyrosine Kinase Inhibitors (TKI)
P170019/S014	June 7, 2022	NTRK1, NTRK2, or NTRK3 fusions	Solid Tumors	ROZLYTREK® (entrectinib)
		ROS1 fusions	NSCLC	
P170019/S042	August 11, 2023	*BRCA1, BRCA2* alterations	Prostate Cancer	AKEEGA® (niraparib + abiraterone acetate)
P170019/S043	October 6, 2023	*RET* fusions	Solid Tumors	RETEVMO® (selpercatinib)
P170019/S039	October 11, 2023	*BRAF V600E*	NSCLC	BRAFTOVI® (encorafenib) in combination with MEKTOVI® (binimetinib)

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The primary evidence for supporting the performance of F1CDx in detecting *AKT1*, *PIK3CA,* and *PTEN* alterations in breast cancer patients was from the data presented

using intended use specimens across all validation studies. In addition to the existing platform-level validation results (P170019), refer to Section IX.A. in P170019 Summary of Safety and Effectiveness Data, analytical accuracy/concordance, limit of blank (LoB), limit of detection (LoD), intermediate precision, and site-to-site precision studies were conducted to support the indication for *AKT1*, *PIK3CA,* and *PTEN* alterations. Table 12 shows the distribution of genes and alteration types that were represented in all the analytical validation studies to support performance of the assay for detection of all the variant types in the *AKT1*, *PIK3CA,* and *PTEN* genes.

The analytical studies were performed using the DNAx extraction method. The F1CDx device was updated to include addition of an automated DNA/RNA CoExtraction methodology (CoEx method) to enable isolation of DNA and RNA from the same FFPE tumor specimens. The F1CDx was authorized for DNA only when using the CoEx method. Use of the CoEx method for the new intended use in this supplement was supported by validation studies demonstrating comparability between the two nucleic acid extraction methods, and precision studies at and above the LoD levels of the *PIK3CA* and *PTEN* positive samples. The comparability study between the DNAx and CoEx DNA extraction methods assessed a total of 11 clinical specimens from patients with breast cancer harboring 10 *PIK3CA* SNVs and one *PTEN* HD. The observed PPA was 100% when using the DNAx method as reference, while the NPA ranged from 90% to 100% since one *PIK3CA* SNV was detected by the CoEx method but not the DNAx method for one of the specimens. To assess precision, 6 curls were cut from each source block and processed with the CoExtraction method using a combination of two unique CoExtraction reagent lots and two unique CoExtraction instrument lines. The extracted DNA from each curl was subdivided into four (4) DNA sub-aliquots, for a total of 24 total replicates per source block entering precision testing (6 curl extractions x 4 extracted DNA subaliquots each). The study evaluated 6 specimens from breast cancer patients with *PIK3CA* SNVs with variant allele frequencies ranging from 3.1% to 45.3 %. The precision study also included one specimen with a *PTEN* homozygous deletion with tumor purity of 60%. One replicate from on specimen with a *PIK3CA* SNV failed to yield a valid result. The agreement for reproducibility and repeatability of the *PIK3CA* and *PTEN* positive specimens from patients with breast cancer that yielded valid results was 100%.

Further, the F1CDx analytical pipeline was modified after the analytical and clinical validation studies were completed. The analytical and clinical validation provided to support the detection of *PIK3CA/AKT1/PTEN* alterations in breast cancer by F1CDx were performed using previous versions of the analytical pipeline that has undergone further iteration with modification in the final device design. To demonstrate the F1CDx analytical pipeline changes continue to support the performance for which the test is approved, regression testing using the most current analytical pipeline version will be performed post-market to confirm the robust performance for F1CDx for the detection of *PIK3CA/AKT1/PTEN* alterations (see section XIII).

Table 12: Alterations evaluated in the Analytical Validation Studies

Analytical Validation Study	Gene	Alteration Type	Number of Alterations
LoD	*PTEN*	HD	1
	PTEN	RE	3
Intermediate Precision	*AKT1*	SNV	2
	PIK3CA	SNV*	12
	PTEN	HD	3
		Indel*	1
		SNV	4
Site-to-Site Reproducibility	*AKT1*	SNV	2
	PIK3CA	SNV	2
	PTEN	RE	3
Accuracy**	*AKT1*	SNV	12
	PIK3CA	SNV	87
	PTEN	SNV	22
		Indel	17
		HD	16
		RE	5

*There are 4 non-target alterations in the intermediate precision study, *PIK3CA C420R, PIK3CA* E545Q, and PTEN C124S and *PTEN*_77_78insTG AC. Non-target variants are defined as variants that meet the biomarker definition but were not specified in the initial analytical validation protocol as being a target of the analysis.

** The number of alterations evaluated in the accuracy study correspond to alterations detected as positive by F1CDx, or externally validated orthogonal method, or both.

1. **Analytical Accuracy/Concordance**
   a. **Comparison to an Orthogonal Method for *PIK3CA/AKT1/PTEN* alterations**

   To demonstrate analytical accuracy, comparisons between F1CDx and an externally validated NGS assay (evNGS) to detect *AKT1*, *PIK3CA,* and *PTEN* alterations was performed.

   The concordance study was conducted with available residual DNA previously extracted from 188 FFPE tissue from breast cancer patients enrolled in the CAPItello-290 (NCT03997123) and CAPItello-291 (NCT04305496) clinical studies that supported TRUQAP (capivasertib) approval (refer to Section X Summary of Primary Clinical Study for study details). The study was supplemented with 49 samples selected from the FMI clinical archives. In total the available samples for the analytical accuracy

study included 127 *PIK3CA/AKT1/PTEN* -altered (biomarker positive) and 110 non-altered (biomarker negative) samples.

In total, 237 samples were processed in the concordance study with representation from all genes (*PIK3CA*, *AKT1* and *PTEN*) and variant types (SNVs, indels, RE, and HD) included in the capivasertib CDx biomarker definition. Of the 49 samples selected from FMI's archives, all were processed successfully from LC to sequencing by F1CDx, including two that were processed conditionally as they did not meet the required minimum DNA input for library construction, i.e., DNA levels less than 50ng. These were included in the concordance analysis because they harbored rare alterations.

All but 1 of the 237 samples completed lab processing and passed the sample QC metrics for the evNGS assay; however, 8 samples were identified as having low tumor content (ranging from 13.2-20%). One sample failed library construction for multiple repeats due to low sample quality.

A total of 236 samples were included in the concordance analysis. The F1CDx and externally validated NGS (evNGS) results for the detection of *AKT1*, *PIK3CA,* and *PTEN* alterations and stratified by alteration type, using evNGS as the comparator assay, are provided in the contingency table in Table 13. Variant-level concordance was assessed and the CDx biomarker definition was applied to the F1CDx and evNGS test results. The positive predictive value (PPV) and negative predictive value (NPV) were calculated along with positive percent agreement (PPA) and negative percent agreement (NPA) unadjusted for prevalence for all three genes and stratified by gene.

Table 13. Contingency Table Comparing the Detection of *AKT1*, *PIK3CA*, and *PTEN* Alterations by the F1CDx and evNGS

***AKT1/PIK3CA/PTEN* alterations**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	
F1CDx +	144	9	1	154	PPV:94.12% [95%CI*: 89.20%, 96.87%]
F1CDx -	6	13,529	57	13,592	NPV:99.96% [95%CI* :99.90%, 99.98%]
Invalid	0	0	0	0	
Total			58	13,746	
	PPA: 96.00% [95%CI*: 91.55%, 98.15%]	NPA: 99.93% [95%CI*: 99.87%, 99.97%]			

***AKT1* E17K**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	
F1CDx +	12	0	0	12	PPV:100.00% [95%CI*: 75.75%, 100.00%]
F1CDx -	0	224	1	225	NPV:100.00% [95%CI*: 98.31%, 100.00%]
Invalid	0	0	0	0	
Total	0	0	1	237	
	PPA: 100.00% [95%CI*: 75.75%, 100.00%]	NPA: 100.00% [95%CI*: 98.31%, 100.00%]			
***PIK3CA* Alterations**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	
F1CDx +	84	2	1	87	PPV: 97.67% [95%CI*: 91.91%, 99.36%]
F1CDx -	0	4398	18	4416	NPV:100.00% [95%CI*: 99.91%, 100.00%]
Invalid	0	0	0	0	
Total	84	4400	19	4503	
	PPA: 100.00% [95%CI*: 95.63%, 100.00%]	NPA: 99.95% [95%CI*: 99.83%, 99.99%]			
***PTEN* Alterations**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	
F1CDx +	48	7	0	55	PPV:96.67% [95%CI*: 91.74%, 98.70%]
F1CDx -	6	8907	38	8951	NPV:100.00% [95%CI*: 99.96%, 100.00%]
Invalid	0	0	0	0	
Total	0	8914	38	9606	
	PPA: 88.89% [95%CI*: 77.81%, 94.81%]	NPA: 99.92% [95%CI*: 99.84%, 99.96%]			
***PTEN* SNVs**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	
F1CDx +	116	4	1	121	PPV:96.67% [95%CI*: 91.74%, 98.70%]
F1CDx -	0	9084	38	9122	NPV: 100.00% [95%CI*: 99.96%, 100.00%]
Invalid	0	0	0	0	
Total	116	9088	39	9243	
	PPA: 100.00% [95%CI*: 96.79%, 100%]	NPA: 99.96% [95%CI*: 99.89 %, 99.98%]			
***PTEN* indels**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	

F1CDx +	11	1	0	0	PPV: 91.67% [95%CI*: %, %]
F1CDx -	5	3759	16	16	NPV: 99.87% [95%CI*: 99.69%, 99.94%]
Invalid	0	0	0	0	
Total	16	3760	16	3792	
	PPA: 68.75% [95%CI*: 44.40%, 85.84%]	NPA: 99.97% [95%CI*: 99.85%, 100%]			
***PTEN* RE**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	
F1CDx +	2	3	0	5	PPV: 40% [95%CI*: 11.76%, 76.93 %]
F1CDx -	0	467	2	469	NPV 100%: % [95%CI*: 99.18%, 100.00%]
Invalid	0	0	0	0	
Total	2	470	2	474	
	PPA: 100.00% [95%CI*: 34.24%, 100%]	NPA: 99.36% [95%CI*: 98.14%, 99.78%]			
***PTEN* HD**					
	evNGS +	**evNGS -**	**Invalid**	**Total**	
F1CDx +	15	1	0	16	PPV: 93.75% [95%CI*: 71.67%, 98.89%]
F1CDx -	1	219	1	221	NPV: 99.55% [95%CI*: 97.47 %, 99.20%]
Invalid	0	0	0	0	
Total	16	220	1	237	
	PPA: 93.75% [95%CI*: 71.67%, 98.89%]	NPA: 99.55% [95%CI*: 97.47%, 99.92%]			

*95% 2-sided confidence intervals (CI) were calculated using the Wilson score method.

There were 13 samples with a total of 15 discordant calls between the F1CDx and evNGS test results (Two samples contained more than one alteration). The following was observed for the 15 discordant calls in the 13 samples:

- The 4 discordant SNVs, 2 *PIK3CA* SNVs and 2 *PTEN* SNVs (F1CDx-positive/evNGS-negative) had low VAFs ranging from 0.93% to 2.34% by F1CDx.
- One sample had a *PTEN* indel alteration detected at different splice sites by each assay
- Four samples with *PTEN* indel alterations were not detected by F1CDx; three of those samples were filtered out by the analysis pipeline because the alterations were below the threshold.
- One sample had a *PTEN* RE detected by F1CDx with low chimeric reads of 15. The evNGS assay called PTEN copy number alteration

but could not determine if mono-allelic or bi-allelic, i.e., the call did not meet biomarker criteria.

- Two additional discordant *PTEN* REs (F1CDx-positive/evNGS-negative) were called by F1CDx with 15 or fewer supporting chimeric read pairs.
- The only *PTEN* HD F1CDx-positive/evNGS negative was in a sample that the evNGS assay labelled as low tumor content (15%); F1CDx test results also revealed that this sample had a low computational tumor purity (TP) of 15%.

2. Analytical Sensitivity

a. Limit of Blank (LoB)

The LoB of zero was confirmed by testing 3 biomarker negative FFPE tissue samples (breast carcinoma or invasive ductal carcinoma). Of the 60 replicates, 100% produced valid results and no *AKT1, PIK3CA,* or *PTEN* alterations were detected across all sample replicates; therefore, the false positive rate for calling alterations was determined to be 0.00%. The study demonstrated that the F1CDx assay does not produce false-positive results in detecting *AKT1, PIK3CA,* and *PTEN* alterations in biomarker-negative FFPE tissue samples from patients with breast cancer.

b. Limit of Detection (LoD)

The F1CDx LoD for the detection of *PIK3CA, AKT1* and *PTEN* alterations was established through platform assessments of LoD and within CDx-specific LoD studies.

The LoD of *AKT1 E17K* was established through a previously conducted study for the PMA approval (P170019) as 6.13% VAF. The LoD of *PIK3CA* SNVs was established previously as 4.91% VAF (refer to SSED for P170019/S006). The LoD of *PTEN* SNVs and indels was taken as approximately 5% based on aggregated data analysis of LoD for SNVs from the original PMA. The LoD was confirmed through a precision study that used samples at or near 5% VAF. The lowest observed average VAF that achieved 100% reproducibility for *PTEN* SNVs via the intermediate precision study is the claimed LoD for *PTEN* SNVs. This value is 5.72% VAF, see intermediate precision study results below. Similarly, for *PTEN* indels, the average VAF that achieved 100% reproducibility for a *PTEN* insertion variant is taken as the confirmed LoD.

The LoDs of *PTEN* RE and HD were determined through a new study. DNA from three breast cancer samples harboring *PTEN* rearrangements were processed along with one breast cancer sample that had a *PTEN* HD. Each sample with a *PTEN* RE was assessed at five targeted chimeric read levels (30, 24, 18, 12, and 4). Twenty (20) replicates were assessed for each dilution level, except the 30 level, which examined 14 replicates. Ninety-four (94) replicates were tested per sample. Each sample with a *PTEN* homozygous

deletion was assessed at five targeted tumor purity dilution levels at 50, 40, 35, 30, 20% of tumor purity with 14, 20, 20, 19, and 18 replicates, respectively. Ninety-one (91) replicates were tested per sample. Each specimen was evaluated close to the minimum input requirements of the assay (50 ng), thus representing the most challenging evaluation of *PTEN* alteration detection.

The LoD for each sample was determined based on chimeric reads using hit rate method for *PTEN* RE and SNP-adjusted tumor purity (TP) using the probit method for *PTEN* homozygous deletions. The LoD for *PTEN* rearrangements was 20.45 chimeric reads and for *PTEN* HD was 42.28% tumor purity. The LoD for *PTEN* homozygous deletions was confirmed through an intermediate precision study where breast cancer samples targeted at or near platform LoD of 33.40% tumor purity were processed. The lowest observed tumor purity at 35.25% with >95% reproducibility was claimed as the LoD for *PTEN* homozygous deletions.

The summary of LoD values established for *AKT1* SNVs, *PIK3CA* SNVs, *PTEN* SNVs, *PTEN* rearrangements and *PTEN* homozygous deletions are captured in Table 14.

Table 14. Summary of LoD for *AKT1/PIK3CA/PTEN* alterations

Gene	Variant Type	LoD
AKT1	SNVs	6.13% VAF
PIK3CA	SNVs	4.91% VAF
PTEN	SNVs	5.72% VAF
PTEN	Indel	5.67% VAF
PTEN	RE	20.45 chimeric reads
PTEN	HD	35.25% tumor purity

3. **Precision**
   a. **Within-Laboratory (Intermediate) Precision**

   To evaluate the precision of *PIK3CA/AKT1/PTEN* alteration detection, a within-laboratory (intermediate) precision study using 14 pre-extracted DNA samples from FFPE tissue from patients with breast cancer with 18 target alterations selected to be at 1-1.5x LoD was conducted at a challenging DNA input (close to 50 ng); refer to Table 15 for the samples evaluated in the study. In total, there were 321 sample replicates, of which, 219 were valid (i.e., passed processing QC criteria) and used in the analysis.

Table 15. Samples evaluated in the Within-Laboratory (Intermediate) Precision Study.

Sample	Target Gene	Alteration	Source sample VAF (SNV)/TP (HD)	Alteration Type	Disease Ontology
1	*PTEN*	*PTEN* loss of exons 5 of 9	41.0%	HD	Breast invasive ductal carcinoma (IDC)
2	*PTEN*	*PTEN* loss of exons 9 of 9	35.5%	HD	Breast invasive ductal carcinoma (IDC)
3	*PTEN*	*PTEN* loss of exons 9 of 9	40.0%	HD	Breast carcinoma (NOS)
4	*AKT1*	E17K	6.7%	SNV	Breast invasive ductal carcinoma (IDC)
5	*AKT1*	E17K	2.1%	SNV	Breast invasive ductal carcinoma (IDC)
6	*PIK3CA*	E542K	3.6%	SNV	Breast invasive ductal carcinoma (IDC)
7	*PIK3CA*	M1043I	5.8%	SNV	Breast carcinoma (NOS)
8	*PIK3CA*	E542K	5.5%	SNV	Breast carcinoma (NOS)
9	*PIK3CA*	M1043V	5.6%	SNV	Breast carcinoma (NOS)
10	*PIK3CA*	M1043V	15.3%	SNV	Breast invasive lobular carcinoma (ILC)
11	*PIK3CA*	N345K	5.8%	SNV	Breast carcinoma (NOS)
12	*PIK3CA*	E545K	4.6%	SNV	Breast carcinoma (NOS)
13	*PIK3CA*	Q546K	1.6%	SNV	Breast invasive ductal carcinoma (IDC)
14	*PIK3CA*	E542K	2.34%	SNV	Breast invasive ductal carcinoma (IDC)
15	*PTEN*	C124R	12.4%	SNV	Breast invasive ductal carcinoma (IDC)
16	*PTEN*	C124S	37.3%	SNV	Breast invasive ductal carcinoma (IDC)
17	*PTEN*	R130Q	11.6%	SNV	Breast invasive ductal carcinoma (IDC)
18	*PTEN*	R130X	6.0%	SNV	Breast carcinoma (NOS)

For the assessment of reproducibility, sample replicates from the same source sample were processed under different conditions by varying one factor at a time. The conditions were applied on a plate-level basis and included the same operator, same day, same reagent lot, and same sequencer. The reproducibility performance was evaluated by comparing to the reference biomarker status (positive for targeted

variants) across all valid replicates. Table 16 summarizes the reproducibility statistics across samples evaluated for the 14 samples selected to have 18 targeted variants at 1-1.5x LoD. Of the 18 targeted alterations 10 target alterations that had observed levels across all valid replicates for each source sample at ≥1x LoD (samples 1-10), while 8 target alterations had observed levels at <1x LoD (samples 11-18). All 10 target alterations with observed levels ≥ 1x LoD demonstrated 100% reproducibility, while the remaining 8 alterations with observed levels <1x LoD demonstrated 50% to 100% reproducibility.

Table 16. Reproducibility for *AKT1, PIK3CA,* and *PTEN* alterations

Sample	Observed Average VAF (SNV) and TP (HD)	Fold LoD	Alteration	Variant Type	Positive Replicates	Valid Replicates	Reproducibility (95% 2-Sided Score CI) *
1	7.75%	1.27x	*AKT1* E17K	SNV	23	23	100.00% [85.69%, 100.00%]
2	6.40%	1.39x	*PTEN* R130X	SNV	22	22	100.00% [85.13%, 100.00%]
3	6.20%	1.35x	*PTEN* C124R	SNV	20	20	100.00% [83.89%, 100.00%]
4	5.72%	1.24x	*PTEN* R130Q	SNV	23	23	100.00% [85.69%, 100.00%]
5	4.96%	1.01x	*PIK3CA* M1043V	SNV	24	24	100.00% [86.20%, 100.00%]
6	6.18%	1.35x	*PTEN* C124S	SNV	23	23	100.00% [85.69%, 100.00%]
7	5.13%	1.05x	*PIK3CA* E545K	SNV	22	22	100.00% [85.13%, 100.00%]
8	35.25%	1.06x	*PTEN* loss exons 9 of 9	HD	24	24	100.00% [86.2%, 100.00%]
9	40.94%	1.23x	*PTEN* loss exons 5 of 9	HD	24	24	100.0% [86.2%, 100.00%]
10	39.65%	1.19x	*PTEN* loss exons 9 of 9	HD	23	23	100.00% [85.69 %, 100.00%]
11	1.35 %	0.22x	*AKT1* E17K	SNV	11	22	50.00%, [30.72%, 69.28%,]

12	2.09 %	0.43x	*PIK3CA* E542K	SNV	19	22	86.36%, [66.67%,95.25%]
13	1.93 %	0.39x	*PIK3CA* E542K	SNV	21	22	95.45%, [78.20%,99.19%,]
14	0.68 %	0.14x	*PIK3CA* Q546K	SNV	14	22	63.64%, [42.95%,80.27%,]
15	4.31 %	0.88x	*PIK3CA* M1043I	SNV	24	24	100.00%, [86.20%, 100.00%,]
16	4.58 %	0.93x	*PIK3CA* E542K	SNV	24	24	100%, [86.20%, 100.00%,]
17	4.88 %	1.00x	*PIK3CA* N345K	SNV	22	22	100.00%, [85.13%, 100.00%,]
18	4.68 %	0.96x	*PIK3CA* M1043V	SNV	23	23	100.00%, [85.69%, 100.00%,]

*95% 2-sided CI were calculated using the Wilson score method.

Another 3 SNVs in *PIK3CA* and 1 indel in *PTEN* with observed average VAF levels were found in the samples tested to meet the biomarker definition although they were not initially targeted for the evaluation of precision. All three variants achieved reproducibility of 100% as shown in Table 17.

Table 17. Reproducibility of additional non-targeted alterations at LoD values ≥1x

Sample	Observed Average MAF	Fold LoD	Alteration	Variant Type	Positive Replicates	Valid Replicates	Reproducibility [95% 2-Sided Score CI] *
1	15.8%	3.08x	*PIK3CA* E545K	SNV	24	24	100.00% [86.20%, 100.00%]
2	5.67%	1.23x	*PTEN* 77 78insTGAC	Indel	23	23	100.00% [85.69%, 100.00%]
3	16.34%	3.33x	*PIK3CA* C420R	SNV	22	22	100.00% [85.13%, 100.00%]
4	33.60%	6.86x	*PIK3CA* E545Q	SNV	24	24	100.00% [86.20%, 100.00%]

*95% 2-sided CI were calculated using the Wilson score method.

For the assessment of repeatability, sample replicates from the same source sample were processed under the same condition. The result was considered concordant

when the alteration statuses are the same between the two replicates run under identical measurement conditions. Table 18 presents the repeatability statistics for the 10 target alterations at levels ≥1x LoD (samples 1-10), followed by samples at levels <1 LoD (samples 11-18).

Table 18. Repeatability for *AKT1*, *PIK3CA*, and *PTEN* alterations

Sample	Observed Average VAF (SNV) and Tumor Purity (HD)	Fold LoD	Alteration	Variant Type	Positive Replicates	#Total Valid Replicates	Repeatability (95% 2-Sided Score CI)*
1	7.75%	1.27x	*AKT1* E17K	SNV	11	11	100.00% [74.12%, 100.00%]
2	6.40%	1.39x	*PTEN*R130X	SNV	11	11	100.00% [74.12%, 100.00%]
3	6.20%	1.35x	*PTEN* C124R	SNV	9	9	100.00% [N/A*]
4	5.72%	1.24x	*PTEN* R130Q	SNV	11	11	100.00% [74.12%, 100.00%]
5	4.96%	1.01x	*PIK3CA* M1043V	SNV	12	12	100.00% [75.75%, 100.00%]
6	6.18%	1.35x	*PTEN* C124S	SNV	11	11	100.00% [74.12%, 100.00%]
7	5.13%	1.05x	*PIK3CA* E545K	SNV	11	11	100.00% [74.12 %, 100%]
8	35.25%	1.06x	*PTEN* loss exons 9 of 9	HD	12	12	100.0% [75.75%, 100.00%]
9	40.94%	1.23x	*PTEN* loss exons 5 of 9	HD	12	12	100.00% [75.75%, 100.00%]
10	39.65%	1.19x	*PTEN* loss exons 9 of 9	HD	11	11	100.00% [74.12 %, 100.00%]

11	1.35	0.22x	*AKT1* E17K	SNV	2	11	18.18% [5.14%, 47.70%]
12	2.09	0.43x	*PIK3CA* E542K	SNV	8	11	72.73% [43.44%, 90.25%]
13	1.93	0.39x	*PIK3CA* E542K	SNV	10	10	100.00% [N/A**]
14	0.68	0.14x	*PIK3CA* Q546K	SNV	5	10	50.0% [N/A**]
15	4.31	0.88x	*PIK3CA* E542K	SNV	12	12	100.00% [75.75%, 100.00%]
16	4.58	0.93x	*PIK3CA* M1043I	SNV	12	12	100.00% [75.75%, 100.00%]
17	4.88	1.00x	*PIK3CA* N345K	SNV	11	11	100.00% [74.12%, 100.00%]
18	4.68	0.96x	*PIK3CA* M1043V	SNV	11	11	100.00% [74.12%, 100.00%]

*95% 2-sided CI were calculated using the Wilson score method.

**CI is not estimated for sample sizes ≤10.

Table 19 presents the repeatability statistics for the 3 SNVs in *PIK3CA* and 1 indel in *PTEN* with observed average VAF levels were found in the samples tested to meet the biomarker definition although they were not initially targeted for the evaluation of precision. All three variants achieved repeatability of 100% as shown in Table 19.

Table 19. Repeatability of additional samples at LoD values ≥1x LoD

Sample	Observed Average MAF	Fold LoD	Alteration	Variant Type	Positive Replicates	Valid Replicates	Repeatability [95% 2-Sided Score CI]*
1	15.8%	3.08x	*PIK3CA* E545K	SNV	12	12	100.00% [75.75%, 100.00%]
2	5.67%	1.23x	*PTEN* 77 78insTGAC	Indel	11	11	100.00% [74.12%, 100.00%]
3	16.34%	3.3x	*PIK3CA* C420R	SNV	10	10	100.00% [N/A**]
4	33.60%	6.86x	*PIK3CA* E545Q	SNV	12	12	100.00% [75.75%, 100.00%]

*95% 2-sided CI were calculated using the Wilson score method.

**CI is not estimated for sample sizes ≤10.

b. Site-to-site Precision

Site-to-site precision for *PIK3CA, AKT1* and *PTEN* alteration detection was evaluated via two site-to-site precision studies. A total of 7 biomarker positive samples consisting of pre-extracted DNA from FFPE tissue from breast cancer patients targeting 1-1.5x LoD alteration levels were selected for evaluation (see Table 20). These samples were selected based on harboring *AKT1* E17K mutation (2 samples), *PIK3CA* SNVs (2 samples), and *PTEN* REs (3 samples) that meet the CDx biomarker definition. Together the studies evaluated 7 biomarker-positive samples at a challenging DNA input (close to 50 ng), at two laboratory locations in Cambridge, MA (CAM) and Morrisville, NC (RTP).

The first study evaluated 2 samples with *AKT1* E17K mutation and 2 samples with *PIK3CA* SNVs. Each sample tested in duplicate at the CAM and RTP sites, with two (2) reagent lots, and three (3) unique start days (sequencer runs) per site per reagent lot. A total of 24 replicates were evaluated per sample.

The second study evaluated the site-to-site precision of the F1CDx assay in calling PTEN RE in DNA derived from FFPE tumor tissue from patients with breast cancer. Samples with PTEN REs and at least 1,200 ng remaining mass of DNA were selected for testing. Samples were titrated with biomarker-negative diluent DNA extracted from FFPE samples from breast cancer patients to achieve the desired targeted chimeric reads (1x - 1.5x LoD) and DNA input close to 50 ng. Each sample was tested in duplicate at 2 sites on 3 library construction start days with 2 reagent lots for a total of 24 replicates.

Table 20. Samples evaluated in the Site-to-Site Precision Studies

Sample	Target Gene	Alteration	Source sample VAF (SNV)/Chimeric reads (RE)	Alteration Type	Disease Ontology
1	*AKT1*	E17K	9.13%	SNV	Breast Cancer
2	*AKT1*	E17K	9.78%	SNV	Breast Cancer
3	*PIK3CA*	E545K	7.34%	SNV	Breast Cancer
4	*PIK3CA*	Q546E	7.37%	SNV	Breast Cancer
5	*PTEN*	*PTEN*_*PTEN* duplication	153*	RE	Breast carcinoma (NOS)
6	*PTEN*	*PTEN*_N/A truncation	113*	RE	Breast invasive ductal carcinoma (IDC)
7	*PTEN*	*PTEN*_N/A truncation	92*	RE	Breast invasive ductal carcinoma (IDC)

*Chimeric read data were from initial testing of the clinical samples. The targeted number of chimeric reads for each sample was 1x – 1.5x LoD.

The point estimates and 95% two-sided CIs for reproducibility of each sample are detailed in Table 21. Only five samples were ≥1x LoD and included in the reproducibility results below.

Table 21. Reproducibility for *AKT1, PIK3CA* and *PTEN* alterations

Sample	Observed Average VAF (SNV) and Chimeric Reads (RE)	Fold LoD	Alteration	Variant Type	Positive Replicates	Valid Replicates	Reproducibility (95% 2-Sided CI) *
1	9.46%	1.54x	*AKT1* E17K	SNV	24	24	100.00% [86.20%, 100.00%]
2	10.66%	1.74x	*AKT1* E17K	SNV	24	24	100.00% [86.20%, 100.00%]
3	7.41%	1.51x	*PIK3CA* E545K	SNV	24	24	100.00% [86.20%, 100.00%]
4	9.23%	1.88x	*PIK3CA* Q546E	SNV	24	24	100.00% [86.20%, 100.00%]
5	23.00	1.12x	*PTEN_PTEN* duplication	RE	24	24	100.00% [86.20%, 100.00%]
6	19.42	0.95x	*PTEN* N/A_truncation	RE	24	24	100.00% [86.20%, 100.00%]
7	20.08	0.98x	*PTEN* N/A_truncation	RE	23	24	95.83% [79.76%, 99.26%]

*95% 2-sided CI were calculated using the Wilson score method.

Repeatability was evaluated in the breast cancer samples by processing samples with two replicates from the same source sample and plate within each site, reagent lot, and start day. The result was considered in agreement if the duplicate replicates processed under identical conditions had the same detection status for the targeted alteration. The point estimates and 95% two-sided CIs for repeatability of each sample are detailed in Table 22.

Table 22. Repeatability for *AKT1, PIK3CA* and *PTEN* alterations

Sample	Observed Average VAF (SNV) and Chimeric Reads (RE)	Fold LoD	Alteration	Variant Type	Positive Replicates	Valid Replicates	Repeatability (95% 2-Sided CI)*
1	9.46%	1.54x	*AKT1* E17K	SNV	12	12	100.00% [75.75%, 100.00%]
2	10.66%	1.74x	*AKT1* E17K	SNV	12	12	100.00% [75.75%, 100.00%]
3	7.41%	1.51x	*PIK3CA* E545K	SNV	12	12	100.00% [75.75%, 100.00%]
4	9.23%	1.88x	*PIK3CA* Q546E	SNV	12	12	100.00% [75.75%, 100.00%]
5	23.00	1.12x	*PTEN_PTEN* duplication	RE	12	12	100.00% [75.75%, 100.00%]
6	19.42	0.95x	*PTEN* N/A truncation	RE	12	12	100.00% [75.75%, 100.00%]
7	20.08	0.98x	*PTEN* N/A truncation	RE	11	12	91.67% [64.61%, 98.51%]

*95% 2-sided CI were calculated using the Wilson score method.

B. Animal Studies

No animal studies were conducted using the F1CDx assay.

C. Additional Studies

No additional studies were conducted using the F1CDx assay.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The reasonable assurance of safety and effectiveness for F1CDx for detection of *AKT1*, *PTEN* and *PIK3CA* alterations in breast cancer patients who may benefit from treatment with TRUQAP™ (capivasertib) in combination with FASLODEX (fulvestrant), was demonstrated based on results from the CAPItello-291 trial. Patients were enrolled in the CAPItello-291 trial regardless of their biomarker status and were retrospectively tested by F1CDx to determine the *PIK3CA/AKT1/PTEN* -Altered status. Clinical efficacy was established in the overall population and the *PIK3CA/AKT1/PTEN* -altered population, as defined by the F1CDx assay.

A summary of the clinical study design is presented below.

A. Clinical Study Design

The CAPItello-291 study is a Phase III, double-blinded, placebo-controlled, parallel-group, randomized, multicenter study assessing the safety and efficacy of TRUQAP™ (capivasertib) in combination with FASLODEX (fulvestrant)versus placebo in combination with fulvestrant as treatment for locally advanced (inoperable) or metastatic hormone receptor positive, human epidermal growth factor receptor 2 negative (HR-positive/HER2-negative) breast cancer following recurrence or progression on or after treatment with an aromatase inhibitor (AI), with or without a CDK4/6 inhibitor. The major efficacy outcome measure was progression-free survival (PFS) in the overall population and the *PIK3CA/AKT1/PTEN* -altered subgroup. Patients with an *PIK3CA/AKT1/PTEN* -altered tumor were identified post randomization by F1CDx central testing of tumor tissue collected prior to randomization. The biomarker positive status was determined according to the F1CDx biomarker definition for *PIK3CA, AKT1* or *PTEN* alterations.

1. Clinical Inclusion and Exclusion Criteria

Inclusion criteria for all samples, including those processed conditionally, were based on FMI's standard process standard operation procedure (SOPs) for F1CDx.

Inclusion Criteria

Samples must be in FFPE blocks or slides

- Samples with ≥20% tumor cells as determined by FMI pathology
- Samples with ≥0.6mm^3 tissue volume
- Samples with ≥55ng of extracted DNA for quantification and library creation
- Note: A small number of samples did not meet criteria above and were processed conditionally. The specifications for conditionally processed samples are:
    - Tissue sample volume ≥ 0.2 mm^3 and < 0.6 mm^3 with tumor content ≥ 10% and < 20% to proceed with DNA extraction
    - ≥ 27 ng and < 55 ng of extracted DNA is required for DNA quantification and library creation

Exclusion Criteria

- Blood, other liquid, and fresh frozen samples
- Samples with < 10% nucleated tumor cells as determined by FMI pathology
- Samples lack of clear subject identification or label on stored patient sample
- Samples with obvious physical damage of stored patient sample
- Insufficient sample: total tissue volume < 0.2 mm^3

2. Follow-up Schedule

Study visits and safety assessments were scheduled relative to Week 1, Day 1 of each cycle. Cycles are 28 days. During Cycle 1, safety assessment visits were scheduled on W1D1, W3D1 (-1/+3), and W3D4 (-1/+3). During Cycle 2, safety assessment visits were scheduled on W1D1 (-1/+3) and W3D1 (-1/+3). Starting with Cycle 3 safety assessment visits were scheduled on W1D1 (-1+3). After discontinuation of the study drugs, the patient had a safety follow up visit 7 days later (discontinuation visit) and a safety follow up visit 30 days (+7 days) (post-treatment follow up visit).

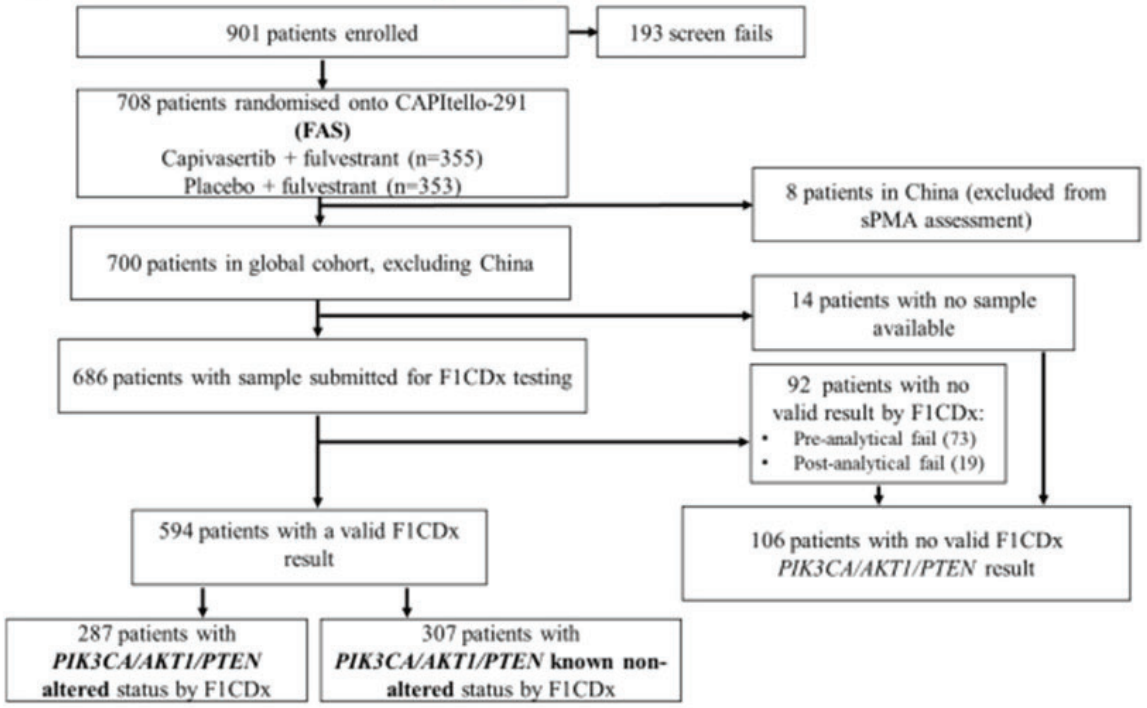
3. Clinical Endpoint

The primary objective of the clinical study was to compare the effect of capivasertib + fulvestrant relative to placebo + fulvestrant by assessment of PFS in the F1CDx *PIK3CA/AKT1/PTEN*-altered population. The dual primary endpoints were progression-free survival (PFS) in the overall population and PFS in the *PIK3CA/AKT1/PTEN*-altered subgroup. PFS was defined as the time from randomization until progression per RECIST v1.1, as assessed by the investigator at the local site, or death due to any cause.

B. Accountability of PMA Cohort

Of the 901 patients initially screened for the CAPItello-291 study, 193 were screen failures and were not randomized. The remaining 708 patients were randomized at a 1:1 ratio into the capivasertib + fulvestrant (n=355) and placebo + fulvestrant (n=353) arm of the trial. However, eight (8) patient samples originating from China and were not tested with F1CDx, while 14 patients had no sample available for testing, leaving 686 patient samples available for testing by F1CDx. Of the 686 patient samples, 92 samples either failed to meet the F1CDx input requirements (pre-analytical failure) or failed to meet the QC metrics required by the assay during processing (post-analytical failure) resulting in 594 patients with valid F1CDx results. Out of the 289 patients with *PIK3CA/AKT1/PTEN* -altered status in CAPItello-291, 287 patients determined to be *PIK3CA/AKT1/PTEN* altered by F1CDx, which constitutes the F1CDx-altered population. Two (2) patients from China were not tested with F1CDx. Refer to Figure 1 for specimen accountability.

Figure 1. Patient and Sample Disposition in CAPItello-291



C. Study Population Demographics and Baseline Parameters

The summary of selected demographic, patient and disease characteristics for the F1CDx *AKT1/PIK3CA/PTEN*-altered (n=287) population is shown in Table 23, 24, and 25.

Table 23. Demographic Characteristics for F1CDx *PIK3CA/AKT1/PTEN* Altered Population

		F1CDx *AKT1/PIK3CA/PTEN* Altered Population		
		Capivasertib + Fulvestrant (N=153)	**Placebo + Fulvestrant (N=134)**	**Total (N=287)**
Age (years)	n	153	134	287
	Mean	58.8	59.8	59.3
	SD	10.29	11.61	10.92
	Median	58.0	60.0	59.0
	Min	36	34	34
	Max	84	90	90
Age Group (years) n (%)	<50	27 (17.6)	29 (26.1)	56 (19.5)
	≥50-<65	82 (53.6)	60 (44.8)	142 (49.5)
	≥65-<75	36 (23.5)	28 (20.9)	54 (22.3)
	≥75	8 (5.2)	17 (12.7)	25 (8.7)
	Total	153	134	287
Sex n (%)	Male	2 (1.5)	0	2 (0.7)
	Female	151 (98.7)	134 (100)	285 (99.3)
	Total	153	134	287
Race n (%)	Black or African American	2 (1.3)	1 (0.7)	3 (1.0)
	Native Hawaiian or other Pacific Islander	0	0	0
	American Indian or Alaskan American	1 (0.7)	1 (0.7)	2 (0.7)
	Asian	46 (30.1)	35 (26.1)	81 (28.2)
	White	75 (49.0)	76 (56.7)	151 (52.6)
	Other	29 (19.0)	21 (15.7)	50 (17.4)
	Total	153	134	287
Ethnic Group n (%)	Hispanic or Latino	14 (9.2)	10 (7.5)	24 (8.4)
	Not Hispanic or Latino	139 (90.8)	124 (92.5)	263 (91.6)
	Total	153	134	287

Table 24. Patient Characteristics at Baseline for F1CDx *PIK3CA/AKT1/PTEN* Altered Population

		F1CDx *AKT1/PIK3CA/PTEN* Altered Population		
		Capivasertib + Fulvestrant (N=153)	Placebo + Fulvestrant (N=134)	Total (N=287)
Height (cm)	n	152	131	283
	Mean	160.4	161.4	160.8
	SD	7.05	7.00	7.03
	Median	160.0	162.0	160.0
	Min	147	146	146
	Max	185	175	185
Weight (kg)	n	152	132	284
	Mean	68.1	69.1	68.5
	SD	14.68	16.81	15.66
	Median	65.8	66.7	66.0
	Min	44	37	37
	Max	115	124	124
Weight group (kg) n (%))	n	152	132	284
	<50	13 (8.6)	12 (9.1)	25 (8.8)
	≥50-≥70	78 (51.3)	62 (47.0)	140 (49.3)
	≥70-≤90	49 (32.2)	42 (31.8)	91 (32.0)
	≥90	12 (7.9)	16 (12.1)	28 (9.9)
Body Mass Index (kg/m2)	n	152	131	283
	Mean	26.4	26.4	26.4
	SD	5.03	5.93	5.45
	Median	26.1	25.6	26.0
	Min	17.1	14.8	14.8
	Max	42.2	45.9	45.9
Body Mass Index (kg/m2)	n	152	131	283
	Underweight (<18.5)	4 (2.6)	7 (5.3)	11 (3.9)
	Normal (18.5-<25)	63 (41.4)	54 (41.2)	117 (41.3)
	Overweight (25.0-<30.0)	53 (34.9)	39 (29.8)	92 (32.5)
	Obese (>=30.0)	32 (21.1)	31 (23.7)	63 (22.3)

N = number of patients in treatment group. n= number of patients in category or analysis. SD: standard deviation. Min = minimum. Max = Maximum. The number of subjects with data is used as the denominator for calculating percentages.

Table 25. Disease Characteristics for F1CDx *PIK3CA/AKT1/PTEN* Altered Population

		F1CDx *PIK3CA/AKT1/PTEN* Altered Population		
		Capivasertib + Fulvestrant (N=153)	**Placebo + Fulvestrant (N=134)**	**Total (N=287)**
ECOG Status	Normal Activity	93 (60.8)	97 (72.4)	190 (66.2)
	Restricted Activity	60 (39.2)	36 (26.9)	96 (33.4)
	In bed ≤50% time	0	1 (0.7)	1 (0.3)
Primary Tumor location	Breast	153 (100)	134 (100)	287 (100)
	Other	0	0	0
Histology Type	Ductal carcinoma in situ	9 (5.9)	7 (5.2)	16 (5.6)
	Lobular carcinoma in situ	1 (0.7)	4 (3.0)	5 (1.7)
	Invasive carcinoma (NOS)	24 (15.1)	24 (17.9)	48 (16.7)
	Invasive ductal	72 (47.1)	63 (47.0)	135 (47.0)
	Invasive ductal carcinoma with extensive intraductal component	3 (2.0)	2 (1.5)	5 (1.7)
	Invasive lobular	20 (13.1)	18 (13.4)	38 (13.2)
	Tubular	0	1 (0.7)	1 (0.3)
	Cribriform	0	1 (0.7)	1 (0.3)
	Carcinoma w/metaplasia: Squamous	0	0	0
	Inflammatory	0	1 (0.3)	1 (0.3)
	Non-Invasive Carcinoma (NOS)	4 (2.6)	3 (2.2)	7 (2.4)
	Mucinous	0	0	0
	Medullary	0	0	0
	Papillary	0	0	0
	Other	20 (13.1)	10 (7.5)	30 (10.5)
	Missing	0	0	0
Tumor Grade	Well differentiated (G1)	21 (13.7)	11 (8.2)	32 (11.1)
	Mod. Differentiated (G2)	64 (41.8)	71 (53.0)	135 (47.0)
	Poorly differentiated (G3)	42 (27.5)	29 (21.6)	71 (24.7)
	Unassessable (GX)	21 (13.7)	16 (11.9)	37 (12.9)
	High grade	3 (2.0)	3 (2.2)	6 (2.1)
	Low grade	1 (0.7)	0	1 (0.3)
	Missing	1 (0.7)	4 (3.0)	5 (1.7)
AJCC staging [a]	Stage 0	1 (0.7)	1 (0.7)	2 (0.7)
	Stage IA	17 (11.1)	14 (10.4)	31 (10.8)
	Stage IB	1 (0.7)	1 (0.7)	2(0.7)
	Stage IIA	28 (18.3)	17 (12.7)	45 (15.7)

	Stage IIB	15 (9.8)	20 (14.9)	35 (12.2)
	Stage IIIA	25 (16.3)	12 (9.0)	37 (12.9)
	Stage IIIB	5 (3.3)	5 (3.7)	10 (3.5)
	Stage IIIC	5 (3.3)	12 (9.0)	17 (5.9)
	Stage IV	50 (32.7)	44 (32.8)	94 (32.8)
	Missing	6 (3.9)	8 (6.0)	14 (4.9)
Overall disease classification	Metastatic [b]	153 (100)	132 (98.5)	285 (99.3)
	Locally advanced [c]	0	2 (1.5)	2 (0.7)
	Missing [d]	0	0	0
Historical BRCA status	*BRCA1*	0	0	0
	BRCA2	4 (2.6)	1 (0.7)	5 (1.7)
	BRCA1 and BRCA2	1 (0.7)	0	1 (0.3)
	Unknown	148 (96.7)	133 (99.3)	281 (97.9)
Menopausal status (female only)	Pre/peri-menopausal	23 (15.0)	29 (21.6)	52 (18.1)
	Post-menopausal	128 (83.7)	105 (78.4)	233 (81.2)
Type of endocrine resistance	Primary	60 (39.2)	55 (41.0)	115 (40.1)
	Secondary	93 (60.8)	79 (59.0)	172 (59.9)
Diabetic status	Diabetic	17 (11.1)	8 (6.0)	25 (8.7)
	No diabetes	136 (88.9)	126 (94.0)	262 (91.3)
Estrogen receptor status	Positive	153 (100)	134 (100)	287 (100)
	Negative	0	0	0
Progesterone receptor status	Positive	115 (75.2)	101 (75.4)	216 (75.3)
	Negative	34 (22.2)	31 (23.1)	65 (22.6)
	Unknown	4 (2.6)	2 (1.5)	6 (2.1)
Receptor status	ER+/PR+	115 (75.2)	101 (75.4)	216 (75.3)
	ER+/PR-	34 (22.2)	31 (23.1)	65 (22.6)
	ER+/PR unknown	4 (2.6)	2 (1.5)	6 (2.1)
	ER- [e]	0	0	0
HER2 receptor status	Negative	153 (100)	134 (100)	287 (100)
	Unknown	0	0	0
Prior CDK4/6 inhibitors	Yes	113 (73.9)	93 (69.4)	206 (71.8)
	Yes: (neo) adjuvant treatment only	0	2 (1.5)	2 (0.7)
	Yes: Locally advanced (inoperable)/metastatic treatment	113 (73.9)	91 (67.4)	204 (71.1)
	No	40 (26.1)	41 (30.6)	81 (28.2)
Reason prior CDK4/6 treatment not taken	Treatment not approved	10 (25)	11 (26.8)	21 (25.9)
	Treatment not affordable or not reimbursed	8 (20.0)	5 (12.2)	13 (16.0)
	Tolerability concerns - hematologic	1 (2.5)	1 (2.4)	2 (2.5)
	Tolerability concerns – non-hematologic	0	0	0
	Patient’s preference	7 (17.5)	7 (17.1)	14 (17.3)

	Healthcare provider's preference	13 (32.5)	17 (41.5)	30 (37.0)
	Other	1 (2.5)	0	1 (1.2)
Prior chemotherapy	(Neo)adjuvant treatment only	62 (40.5)	61 (45.5)	123 (42.9)
	Locally advanced (inoperable)/metastatic treatment	28 (18.3)	23 (17.2)	51 (17.8)
Prior (neo)adjuvant chemotherapy	Yes	77 (50.3)	67 (50.0)	144 (50.2)
	No	76 (49.7)	67 (50.0)	143 (49.8)
Prior lines of endocrine based therapy for locally advanced (inoperable) or metastatic disease	0	13 (8.5)	20 (14.9)	33 (11.5)
	1	129 (84.3)	96 (71.6)	225 (78.4)
	2	11 (7.2)	18 (13.4)	29 (10.1)
Prior lines of endocrine based therapy for locally advanced (inoperable) or metastatic disease - AI therapies only	0	15 (9.8)	21 (15.7)	38 (12.5)
	1	134 (87.6)	103 (76.9)	237 (82.6)
	2	4 (2.6)	10 (7.5)	14 (4.9)
Prior lines of therapy for locally advanced (inoperable) or metastatic disease (includes endocrine or chemotherapy)	0	12 (7.8)	20 (14.9)	32 (11.1)
	1	107 (69.9)	79 (59.0)	186 (64.8)
	2	29 (19.0)	29 (21.6)	58 (20.2)
	3	5 (3.3)	6 (4.5)	11 (3.8)

AI= Aromatase inhibitor. [a] Stages according to 7th edition of the American Joint Committee on Cancer (AJCC) staging manual. [b] Metastatic disease – patient has any metastatic site of disease. [c] Locally advanced – patient has only locally advanced sites of disease. [d] [d] Subject E7016001 didn't have any site of disease as during treatment phase it turned out the lung lesion with which patient was randomized was not metastasis but primary lung cancer. [e]Due to very limited number of patients expected under this category, patients with different PR status are reported together. Diabetes is reported based on med history records. Patients with no diabetes records or no medical history records are reported as having no diabetes. WHO/ECOG performance status, diabetic status, prior use of CDK4/6, prior chemotherapy collected at screening, receptor status if metastatic biopsy available collected at screening or at primary diagnosis if not, and all other parameters are based at time of initial diagnosis. WHO = World Health Organization. ECOG = Eastern Cooperative Oncology Group. BRCA= Breast cancer gene. NOS = not otherwise specified. Mod = moderately.

D. Safety and Effectiveness Results

1. Safety Results

The safety with respect to treatment with capivasertib in combination with fulvestrant was addressed during the review of the New Drug Application (NDA) and is not addressed in detail in this SSED. 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 tissue specimens only

Please refer to Drugs@FDA for complete safety information on capivasertib and fulvestrant.

2. Effectiveness Results

The CAPItello-291 study demonstrated that treatment with capivasertib + fulvestrant resulted in a clinically meaningful and statistically significant improvement in Investigator-assessed PFS by RECIST v1.1 compared with placebo + fulvestrant in the *PIK3CA/AKT1/PTEN* -altered Population.

As presented in Table 26, the median PFS in the F1CDx *PIK3CA/AKT1/PTEN* -Altered Population treated with capivasertib + fulvestrant was 7.3 months [95% CI: 5.5-9.1] versus 3.1 months [95% CI: 2.0-3.7] in the placebo + fulvestrant cohort. In the F1CDx *PIK3CA/AKT1/PTEN* -Altered Population, a 51% reduction in the risk of progression in favor of capivasertib + fulvestrant was observed (HR: 0.49; 95% CI: 0.38-0.64; $p<0.001$). These results are comparable to those observed in the overall *PIK3CA/AKT1/PTEN* Altered population which consisted of 289 patients, 287 which had F1CDx results as shown in Table 26.

Table 26. Progression-free Survival Based on Investigator Assessments, per RECIST v1.1 for F1CDx *PIK3CA/AKT1/PTEN* -Altered Population in CAPITello-291

	F1CDx *PIK3CA/AKT1/PTEN* Altered		*PIK3CA/AKT1/PTEN* Altered Overall[a]	
	Capivasertib + fulvestrant (N=153)	**Placebo + fulvestrant (N=134)**	**Capivasertib + fulvestrant (N=155)**	**Placebo + fulvestrant (N=134)**
Investigator-assessed Progression Free Survival (PFS)				
Total number of patients with events, n (%)	119 (77.8)	115 (85.8)	121 (78.1)	115 (85.8)
Median PFS (95% CI) * [months]	7.3 (5.5-9.1)	3.1 (2.0-3.7)	7.3 (5.5-9.0)	3.1 (2.0-3.7)
Hazard ratio (95% CI) *	0.49 (0.38-0.64)		0.50 (0.38-0.65)	
2-sided p-value	<0.001		<0.001	
Investigator-assessed Objective Response Rate (ORR)				
Patients with measurable disease	Capivasertib= 130 Placebo= 124		Capivasertib= 132 Placebo= 124	
ORR (95% CI)	Capivasertib= 28.5 (20.9, 37.0) Placebo= 9.7 (5.1, 16.3)		Capivasertib= 28.8 (21.2, 37.3) Placebo= 9.7 (5.1, 16.3)	
Complete response rate	Capivasertib= 3 (3.2%) Placebo= 0		Capivasertib= 3 (3.2%) Placebo= 0	

* 95% CI were calculated using the Exact Method
Unadjusted ORR (95% CI) is an exact binomial confidence interval
[a] The Altered Overall population contains 2 additional patient samples that were tested using an alternative testing method. The results were used in the drug efficacy results but not were tested by F1CDx.

These data establish that there is statistically meaningful, improved PFS in F1CDx *PIK3CA/AKT1/PTEN* -Altered patients treated with capivasertib + fulvestrant (n=153) versus those treated with placebo + fulvestrant (n=134), with a PFS HR of 0.49 (95% CI: 0.38-0.64; p<0.001). Patients without *PIK3CA/AKT1/PTEN*-Altered status did not derive a statistically meaningful benefit from capivasertib + fulvestrant when compared to placebo + fulvestrant.

E. Pediatric Extrapolation

In this pre-market application, existing clinical data were not leveraged to support approval of a pediatric patient population

F. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information

concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator who was a full-time employee of the sponsor and had disclosable financial interests/arrangements as defined in 21 CFR 54.2(a), (b), (c) and (f) and described below:

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

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

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

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The effectiveness of F1CDx to identify breast cancer patients with *AKT1*, *PIK3CA,* and *PTEN* alterations who may experience increased benefit from TRUQAP (capivasertib) in combination with FASLODEX (fulvestrant) treatment is supported by the results from the clinical validation study. This study was performed using specimens from patients enrolled in the CAPItello-291 clinical trial. The data from the analytical validation and clinical validation 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 CAPItello-291 trial show that patients who had a qualifying *PIK3CA, AKT1* and *PTEN* alteration received increased benefit from treatment with capivasertib + fulvestrant support the addition of the CDx indication to F1CDx. The efficacy data in the F1CDx-Altered population also demonstrated the ability of F1CDx to detect *PIK3CA, AKT1* and *PTEN*-altered patients that may experience increased benefit from TRUQAP (capivasertib) therapy in combination with FASLODEX (fulvestrant).

B. Safety Conclusions

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

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

C. Benefit-Risk Determination

The probable benefits of F1CDx in identification for *PIK3CA, AKT1* and *PTEN* alterations for treatment with TRUQAP in combination with FASLODEX are based on data collected in the CAPItello-291 clinical trial and the clinical and analytical validation performed for this device. The clinical benefit of the F1CDx assay for the selection of breast cancer patients with *PIK3CA, AKT1* and *PTEN* alterations was demonstrated through the evaluation of clinical efficacy of TRUQAP in combination with FASLODEX in breast cancer patients identified by F1CDx. The analyses in the F1CDx *PIK3CA/AKT1/PTEN*-altered population supported the conclusion that *PIK3CA, AKT1* and *PTEN*-altered patients demonstrated a 51% reduction in the risk of death in favor of capivasertib + fulvestrant when compared with placebo + fulvestrant. Additionally, the median PFS in the *PIK3CA, AKT1* and *PTEN* -altered population treated with capivasertib + fulvestrant was 7.3 months [95% CI 5.5-9.1] versus 3.1 months [95% CI 2.0-3.7] in the placebo + fulvestrant cohort indicating a meaningful clinical benefit in this population. This supports the probable benefit of F1CDx in selecting *PIK3CA, AKT1* and *PTEN*-altered patients for treatment with TRUQAP in combination with FASLODEX.

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

In addition, the analytical concordance study of F1CDx for the detection of *PIK3CA, AKT1* and *PTEN* alterations with the externally validated NGS (evNGS) comparator

method further supports this conclusion. The analytical concordance study with an evNGS comparator method demonstrated supportive PPV and NPV values of 94.12% (95% CI [89.20%, 96.87%]) and 99.96% (95% CI [99.90%, 99.98%]), respectively, for *AKT1/PIK3CA/PTEN* alterations, which partially mitigates the risks of this test.

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

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

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

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the analytical and clinical studies support the performance of F1CDx as an aid for the identification of *PIK3CA, AKT1* and *PTEN* alterations in breast cancer patients for whom TRUQAP in combination with FASLODEX may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on |November 16, 2023. The final non-clinical conditions of approval cited in the approval order are described below.

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

1. FMI must 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 v3.27.0 (AP v3.27), and the AP versions used in the clinical validation studies in this supplement.
2. FMI must 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 must provide evidence, acceptable to FDA, that performance expectations with the currently deployed genomics platform, including AP v3.27.0, are representative of the performance in the clinical validation study in this supplement and analytical validation studies that are leveraged. Such evidence may include regression testing using the clinical and analytical datasets to perform in silico reanalysis of the results obtained in the validation studies and confirmation that there is little or no deviation in the quality metrics for each of the samples to support that the performance of the assay remains the same.

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XV. REFERENCES

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