SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

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

Device Trade Name: FoundationOne® Liquid CDx (F1 Liquid CDx)

Device Procode: PQP

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

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P200006

Date of FDA Notice of Approval: October 26, 2020

Breakthrough Device: Granted breakthrough device status (formerly known as the Expedited Access Pathway, or EAP) on April 25, 2018 because (1) is intended to diagnose a life threatening or irreversibly debilitating disease or condition (2) represents a breakthrough technology that provides a clinically meaningful advantage over existing legally marketed technology, and (3) the availability of the device is in the best interest of patients.

The FoundationOne® Liquid CDx was approved on August 26, 2020 as a companion diagnostic for *BRCA1* and *BRCA2* alterations in metastatic castration-resistant prostate cancer (mCRPC) patients who may benefit from treatment with RUBRACA® (rucaparib) and *EGFR* activating mutations (Exon 19 deletions and L858R substitution mutation) in patients with advanced and metastatic non-small cell lung cancer (NSCLC) who may benefit from treatment with IRESSA® (gefitinib), TAGRISSO® (osimertinib), and TARCEVA® (erlotinib).

The current PMA was submitted to include the intended use of FoundationOne® Liquid CDx as a companion diagnostic for the indications listed in the table below:

New Indications Being Sought in this PMA submission.

Biomarker(s) Detected	Therapy	Tumor Type
BRCA1 and *BRCA2* alterations	RUBRACA® (rucaparib)	Ovarian Cancer
ALK Rearrangements	ALECENSA® (alectinib)	NSCLC
PIK3CA mutations	PIQRAY® (alpelisib)	Breast Cancer

II. INDICATIONS FOR USE

FoundationOne® Liquid CDx is a qualitative next generation sequencing based in vitro diagnostic test that uses targeted high throughput hybridization-based capture technology to detect and report substitutions, insertions and deletions (indels) in 311 genes, including rearrangements in three (3) genes, and copy number alterations in three (3) genes. FoundationOne® Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne® Liquid CDx cfDNA blood collection tubes included in the FoundationOne® Liquid CDx Blood Sample Collection Kit. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1: Companion diagnostic indications

Tumor Type	Biomarker(s) Detected	Therapy
Non-small cell lung cancer (NSCLC)	*EGFR* Exon 19 deletions and *EGFR* Exon 21 L858R alteration	IRESSA® (gefitinib) TAGRISSO® (osimertinib) TARCEVA® (erlotinib)
	ALK Rearrangements	ALECENSA® (alectinib)
Prostate cancer	*BRCA1, BRCA2* alterations	RUBRACA® (rucaparib)
Ovarian Cancer	*BRCA1, BRCA2* alterations	RUBRACA® (rucaparib)
Breast Cancer	*PIK3CA* mutations C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y	PIQRAY® (alpelisib)

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

A negative result from a plasma specimen does not mean that the patient's tumor is negative for genomic findings. Patients who are negative for the mutations listed in Table 1 should be reflexed to routine biopsy and their tumor mutation status confirmed using an FDA-approved tumor tissue test, if feasible.

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

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

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.
- The test is not intended to replace germline testing or to provide information about cancer predisposition.
- Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved tumor tissue test, if possible.

V. DEVICE DESCRIPTION

The FoundationOne® Liquid CDx assay is performed exclusively as a laboratory service using circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood from patients with solid malignant neoplasms. The assay employs a single DNA extraction method to obtain cfDNA from plasma from whole blood. Extracted cfDNA undergoes whole-genome shotgun library construction and hybridization-based capture of 324 cancer-related genes. All coding exons of 309 genes are targeted; select intronic or non-coding regions are targeted in three genes (refer to Table 2 for the complete list of genes reported by FoundationOne® Liquid CDx). Hybrid-capture selected libraries are sequenced with deep coverage using the NovaSeq® 6000 platform. Sequence data are processed using a custom analysis pipeline designed to detect genomic alterations, including base substitutions and indels in 311 genes, copy number variants in three genes, and genomic rearrangements in three genes. A subset of targeted regions in 75 genes is baited for increased sensitivity.

Table 2: Genomic Regions in which Variants are Reported by FoundationOne® Liquid[1]

ABL1 [Exons 4-9]	*CALR*	*CYP17A1*	*FGFR4*	*KDM6A*	*MYCL (MYCL1)*	*POLD1*	SMAD4
ACVR1B	*CARD11*	*DAXX*	*FH*	*KDR*	***MYCN***	*POLE*	SMARCA4
AKT1 [Exon 3]	*CASP8*	*DDR1*	*FLCN*	*KEAP1*	***MYD88 [Exon 4]***	*PPARG*	SMARCB1
AKT2	*CBFB*	***DDR2 [Exons 5, 17, 18]***	*FLT1*	*KEL*	*NBN*	*PPP2R1A*	**SMO**
AKT3	*CBL*	*DIS3*	***FLT3 [Exons 14, 15, 20]***	***KIT [Exons 8, 9, 11, 12, 13, 17]***	***NF1***	*PPP2R2A*	SNCAIP
ALK [Exons 20-29]	***CCND1***	*DNMT3A*	***FOXL2***	*KLHL6*	*NF2*	*PRDM1*	SOCS1
ALOX12B	*CCND2*	*DOT1L*	*FUBP1*	*KMT2A (MLL)*	*NFE2L2*	*PRKAR1A*	SOX2
AMER1 (FAM123B)	*CCND3*	*EED*	*GABRA6*	*KMT2D (MLL2)*	*NFKBIA*	*PRKCI*	SOX9

APC	*CCNE1*	***EGFR***	*GATA3*	***KRAS***	*NKX2-1*	*PTCH1*	SPEN
AR	*CD22*	*EP300*	*GATA4*	*LTK*	*NOTCH1*	***PTEN***	SPOP
ARAF [Exons 4, 5, 7, 11, 13, 15, 16]	***CD274 (PD-L1)***	*EPHA3*	*GATA6*	*LYN*	*NOTCH2*	***PTPN11***	SRC
ARFRP1	*CD70*	*EPHB1*	***GNA11 [Exons 4, 5]***	*MAF*	*NOTCH3*	*PTPRO*	STAG2
ARID1A	*CD79A*	*EPHB4*	*GNA13*	***MAP2K1 (MEK1) [Exons 2, 3]***	***NPM1 [Exons 4-6, 8, 10]***	*QKI*	STAT3
ASXL1	*CD79B*	*ERBB2*	***GNAQ [Exons 4, 5]***	*MAP2K2 (MEK2) [Exons 2-4, 6, 7]*	***NRAS [Exons 2, 3]***	*RAC1*	**STK11**
ATM	*CDC73*	*ERBB3 [Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25]*	***GNAS [Exons 1, 8]***	*MAP2K4*	*NSD3 (WHSC1L1)*	*RAD21*	SUFU
ATR	***CDH1***	*ERBB4*	*GRM3*	*MAP3K1*	*NT5C2*	*RAD51*	SYK
ATRX	***CDK12***	*ERCC4*	*GSK3B*	*MAP3K13*	***NTRK1 [Exons 14, 15]***	*RAD51B*	TBX3
AURKA	***CDK4***	*ERG*	*H3F3A*	*MAPK1*	*NTRK2*	*RAD51C*	TEK
AURKB	***CDK6***	***ERRFI1***	*HDAC1*	*MCL1*	***NTRK3 [Exons 16, 17]***	*RAD51D*	TERC* {ncRNA}
AXIN1	*CDK8*	***ESR1 [Exons 4-8]***	*HGF*	***MDM2***	*P2RY8*	*RAD52*	**TERT* {Promoter}**
AXL	*CDKN1A*	***EZH2 [Exons 4, 16, 17, 18]***	*HNF1A*	*MDM4*	***PALB2***	*RAD54L*	TET2
BAP1	*CDKN1B*	*FAM46C*	***HRAS [Exons 2, 3]***	*MED12*	*PARK2*	***RAF1 [Exons 3, 4, 6, 7, 10, 14, 15, 17]***	TGFBR2
BARD1	***CDKN2A***	*FANCA*	*HSD3B1*	*MEF2B*	*PARP1*	*RARA*	TIPARP
BCL2	*CDKN2B*	*FANCC*	*ID3*	*MEN1*	*PARP2*	***RB1***	TNFAIP3
BCL2L1	*CDKN2C*	*FANCG*	***IDH1 [Exon 4]***	*MERTK*	*PARP3*	*RBM10*	TNFRSF14
BCL2L2	*CEBPA*	*FANCL*	***IDH2 [Exon 4]***	***MET***	*PAX5*	*REL*	**TP53**
BCL6	*CHEK1*	*FAS*	*IGF1R*	*MITF*	*PBRM1*	***RET [Exons 11, 13-16]***	TSC1
BCOR	***CHEK2***	*FBXW7*	*IKBKE*	*MKNK1*	*PDCD1 (PD-1)*	*RICTOR*	TSC2
BCORL1	*CIC*	*FGF10*	*IKZF1*	*MLH1*	***PDCD1LG2 (PD-L2)***	*RNF43*	TYRO3
BRAF [Exons 11-18]	*CREBBP*	*FGF12*	*INPP4B*	***MPL [Exon 10]***	***PDGFRA [Exons 12, 18]***	*ROS1 [Exons 31, 36-38, 40]*	U2AF1
BRCA1 {Introns 2, 7, 8, 12, 16, 19, 20}	***CRKL***	*FGF14*	*IRF2*	*MRE11A*	***PDGFRB [Exons 12-21, 23]***	*RPTOR*	**VEGFA**
BRCA2 {Intron 2}	*CSF1R*	*FGF19*	*IRF4*	*MSH2*	*PDK1*	*SDHA*	VHL

BRD4	*CSF3R*	*FGF23*	*IRS2*	*MSH3*	*PIK3C2B*	*SDHB*	WHSC1
BRIP1	*CTCF*	*FGF3*	*JAK1*	*MSH6*	*PIK3C2G*	*SDHC*	WT1
BTG1	*CTNNA1*	*FGF4*	***JAK2 [Exons 14]***	*MST1R*	***PIK3CA [Exons 2, 3, 5-8, 10, 14, 19, 21] (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)***	*SDHD*	XPO1
BTG2	***CTNNB1 [Exon 3]***	*FGF6*	***JAK3 [Exons 5, 11, 12, 13, 15, 16]***	*MTAP*	*PIK3CB*	*SETD2*	XRCC2
BTK [Exons 2, 15]	*CUL3*	***FGFR1***	*JUN*	***MTOR [Exons 19, 30, 39, 40, 43-45, 47, 48, 53, 56]***	*PIK3R1*	*SF3B1*	ZNF217
C11orf30 (EMSY)	*CUL4A*	***FGFR2***	*KDM5A*	*MUTYH*	*PIM1*	*SGK1*	ZNF703
C17orf39 (GID4)	*CXCR4*	***FGFR3 [Exons 7, 9 (alternative designation exon 10), 14, 18]***	*KDM5C*	***MYC***	*PMS2*	*SMAD2*	

[1] As part of its FDA-approved intended use, the FoundationOne® Liquid CDx assay interrogates 311 genes, including 309 genes with complete exonic (coding) coverage and 2 genes with only select non-coding coverage (indicated with an *). Select genes and select exons (indicated in bold) are captured with increased sensitivity.

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

Table 3: Genes Containing Copy Number Alterations and Rearrangements Detected and Reported by the FoundationOne® Liquid CDx

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

The test report includes variants reported in the following categories; see Table 4:

Table 4. Category Definitions

Category	FoundationOne® Liquid CDx			Comments
	Prescriptive use for a Therapeutic Product	**Clinical Performance**	**Analytical Performance**	
Category 1: Companion Diagnostic (CDx)	**Yes**	**Yes**	**Yes**	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which FoundationOne® Liquid CDx has demonstrated clinical performance shown to support therapeutic efficacy and strong analytical performance for the biomarker.
Category 2: ctDNA Biomarkers with Strong Evidence of Clinical Significance in ctDNA	No	No	**Yes**	ctDNA biomarkers with strong evidence of clinical significance presented by other FDA-approved liquid biopsy companion diagnostics for which FoundationOne® Liquid CDx has demonstrated analytical reliability but not clinical performance.
Category 3A: Biomarkers with Evidence of Clinical Significance in tissue supported by strong analytical validation using ctDNA	No	No	**Yes**	ctDNA biomarkers with evidence of clinical significance presented by tissue-based FDA-approved companion diagnostics or professional guidelines for which FoundationOne® Liquid CDx has demonstrated analytical performance including analytical accuracy, and concordance of blood-based testing to tissue-based testing for the biomarker.
Category 3B: Biomarkers with Evidence of Clinical Significance in tissue supported by analytical validation using ctDNA	No	No	**Yes**	ctDNA biomarkers with evidence of clinical significance presented by tissue-based FDA-approved companion diagnostics or professional guidelines for which FoundationOne® Liquid has demonstrated minimum analytical performance including analytical accuracy.
Category 4: Other Biomarkers with Potential Clinical Significance	No	No	**Yes**	ctDNA biomarkers with emergent evidence based on peer-reviewed publications for genes/variants in tissue, variant information from well-curated public databases, or *in-vitro* pre-clinical models, for which FoundationOne® Liquid CDx has demonstrated minimum analytical performance.

FoundationOne® Liquid cfDNA CDx Blood Specimen Collection Kit Contents

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

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

Instruments
The FoundationOne® Liquid CDx assay is intended to be performed with the serial number-controlled instruments indicated in Table 5, below. All instruments are qualified by Foundation Medicine, Inc. (Foundation Medicine or FMI) under Foundation Medicine's Quality System.

Table 5: Instruments for use with the FoundationOne® Liquid CDx assay

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

Test Process
All assay reagents included in the FoundationOne® Liquid CDx assay process are qualified by Foundation Medicine and are compliant with the medical device Quality System Regulation (QSR).

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

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

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

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

D. Hybrid Capture

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

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

E. Sequencing

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

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

F. Sequence Analysis

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

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

For the detection of short variants (e.g., substitutions and small indels) in each target region of interest, a *de novo* assembly is performed. This is done using proprietary software to generate a de Bruijn graph including all k-mers in reads mapping to a particular locus. The graph is parsed to identify paths that originate and terminate in reference nodes from the locus. Increased k-mer sizes may be used to account for ambiguities, cycles, and other problematic regions within the graph. The result of the graph traversal is a set of candidate variants. For each variant, there is a set of k-mers supporting the variant and a set of k-mers that would support the reference or another variant at the location.

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

G. Report Generation

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

H. Internal Process Controls

Positive Control

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

Sensitivity Control

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

Negative Control

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

I. CDx Classification Criteria

1. *BRCA1 and BRCA2* alterations to identify patients eligible for rucaparib in prostate and ovarian cancer:
   The CDx classification criteria and the list of *BRCA1*/*BRCA2* missense mutations for rucaparib, based on the trial prespecifications are described in Table 6 and Table 7; however, not all of the missense mutations listed below were observed in the TRITON2, ARIEL2, and Study 10 clinical studies.

Table 6: Classification Criteria for Deleterious Tumor *BRCA* Variants

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

	Large protein truncating rearrangements	Sequence analysis identifies protein truncating rearrangements
	Deleterious missense mutations	Curated list (Table 7)

Table 7: Deleterious *BRCA* Missense Alterations

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

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

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using cfDNA isolated from plasma samples, as listed in Table 1 of the FoundationOne® Liquid CDx intended use statement. The approved CDx tests are listed in Table 8, 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 8: FDA-approved companion diagnostic (CDx) alternatives

Biomarker(s) Detected	Device	Company	Technology	Therapy	Indication
EGFR: Exon 19 deletions & L858R substitution	**cobas**® *EGFR* Mutation Test v2	Roche Molecular Systems, Inc.	Polymerase Chain Reaction (PCR)	TARCEVA® (erlotinib), TAGRISSO® (osimertinib), and IRESSA® (gefitinib)	NSCLC
	FoundationOne® Liquid CDx	Foundation Medicine, Inc.	Next-Generation Sequencing (NGS)		
	Guardant360 CDx	Guardant Health, Inc.	NGS	TAGRISSO® (osimertinib)	
BRCA1/BRCA2	FoundationOne® Liquid CDx	Foundation Medicine, Inc.	NGS	RUBRACA® (rucaparib).	metastatic castration-resistant prostate cancer (mCRPC)
PIK3CA: C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y	*therascreen PIK3CA* RGQ PCR test	QIAGEN, Inc.	PCR	PIQRAY® (alpelisib)	Breast Cancer

There are no FDA-approved CDx alternatives for the detection of genomic alterations of *BRCA1* or *BRCA2* for the identification of ovarian cancer patients eligible for treatment with RUBRACA® (rucaparib) nor for the identification of *ALK* rearrangements in patients with metastatic NSCLC for treatment with Alecensa® (alectinib).

VII. MARKETING HISTORY

Foundation Medicine designed and developed FoundationOne® Liquid CDx based on previous versions of the assay, including the FoundationACT (FACT) and FoundationOne® Liquid laboratory developed test (LDT), a revised version of FACT. The first commercial sample was tested in 2016. The FACT and FoundationOne® Liquid LDTs have been used to detect the presence of genomic alterations in blood and plasma specimens. Neither the FACT nor FoundationOne® Liquid LDTs were FDA-cleared or -approved.

The FoundationOne® Liquid CDx assay was approved on August 26, 2020 for the the detection of genomic alterations of *BRCA1* or *BRCA2* for the identification of prostate cancer patients eligible for treatment with RUBRACA® (rucaparib) and the detection of *EGFR* Exon 19 deletions (Exon 19del) and L858R substitutions in plasma obtained from patients with advanced and metastatic NSCLC for treatment with TARCEVA® (erlotinib), TAGRISSO® (osimertinib), and IRESSA® (gefitinib). The FoundationOne®

Liquid CDx assay was also approved for tumor mutation profiling for substitutions and indels to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms.

The FoundationOne® Liquid CDx assay has not been marketed in the United States or any foreign country.

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

For the specific adverse events that occurred in the clinical study, please see the FDA approved package inserts for RUBRACA® (rucaparib); ALECENSA® (alectinib); and PIQRAY® (alpelisib) which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Performance characteristics were established using circulating cfDNA derived from blood specimens extracted from a wide range of tumor types and performed as described in the Summary of Safety and Effectiveness Data for P190032. Table 9 below provides a summary of the number of tumor types and variants included in each study. As summarized in the table below, each study included a broad range of representative alteration types (substitutions, insertion-deletions, copy number alterations, rearrangements) in various genomic contexts across a number of genes.

Due to the lack of sufficient volume of clinical specimens, some of the studies used contrived samples, which consisted of enzymatically sheared cell line DNA spiked into human plasma and diluted with cfDNA isolated from healthy donor plasma. A contrived sample functional characterization (CSFC) study (Section IX.A.1) was conducted to demonstrate comparable performance of sheared cell line DNA samples as compared to cfDNA isolated from plasma specimens obtained from cancer positive patient specimens. Clinical specimens were used to assess analytical accuracy, precision and confirmation of the estimated limit of detection (LoD), and evaluate sample stability.

The validation studies included >7,000 sample replicates, >31,000 unique variants, >30 tumor types, representing all 311 genes targeted by the assay. Please refer to the Summary of Safety and Effectiveness (SSED) for P190032 for the representation of tumor types and variants included in the original device approval.

Table 9: Representation of tumor types and variants* across validation studies

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
Contrived Sample Functional Characterization (CSFC) Study	Breast cancer Colorectal cancer Lung cancer Contrived samples	13	1843	228	563	81	11	1	1
FoundationOne Liquid CDx to Validated NGS Tumor Tissue Test Concordance: *BRCA1* and *BRCA2* Variants	Prostate cancer Ovarian cancer	279	N/A	2	100	87	9	0	2
FoundationOne Liquid CDx to Validated NGS cfDNA Assay Concordance: *PIK3CA* mutations	Breast cancer	412	N/A	1	32	5	0	0	0
Orthogonal Concordance	23 cancer types Contrived samples	278	N/A	64	541	12	11	3	0
LoD Estimation	Prostate Contrived samples	10	877	286	1490	247	32	13	3
LoB	Healthy Donors	28	79	322	26134	4482	911	222	42
Potentially Interfering Substances	Contrived samples	9	336	18	16	11	11	1	2
Hybrid Capture Bait Specificity	25 cancer types Contrived samples	3546	N/A	324	N/A	N/A	N/A	N/A	N/A
Reagent Stability	Contrived samples	8	142	279	1090	215	32	17	2
Reagent Interchangeability	Contrived samples	8	192	20	15	11	11	1	1
Precision study 1	Breast cancer Colon cancer Lung cancer Ovarian cancer Prostate cancer Skin cancer Contrived samples	47	1121	280	900	229	63	49	5

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
Precision study 2	Lung cancer Prostate cancer Stomach cancer Colorectal cancer Bile duct cancer Breast cancer	10	230	6	6	4	0	0	0
DNA Extraction	Colorectal cancer Prostate cancer Breast cancer Lung cancer Skin cancer	6	72	161	265	53	2	0	0
Whole Blood Sample Stability	Lung cancer Colorectal cancer Prostate cancer Breast cancer	11	22	66	75	15	1	0	0
Inverted Tube Whole Blood Sample Stability	Lung cancer Colorectal cancer Breast cancer Ovarian cancer Prostate cancer	130	260	237	594	91	5	5	0
Cross Contamination	Contrived samples	5	376	39	9	5	4	21	1
Guard Banding	Contrived samples	10	375	20	17	12	12	1	1
Clinical validation for detection of *EGFR* exon 19 deletions and L858R alterations: non-inferiority study	Lung cancer	177	N/A	1	5	7	N/A	N/A	N/A
Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in prostate cancer	Prostate cancer	199	N/A	2	44	55	8	0	1
Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in ovarian	Ovarian cancer	217	N/A	2	48	49	3	0	0

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# of Unique Genes	# of Unique				
					Subs	Indels	Rearrang.	Copy Number Amplif.	Copy Number Losses
cancer									
Clinical validation study for detection of PIK3CA mutations in breast cancer	Breast	359	N/A	1	28	4	0	0	0
Clinical validation study for ALK rearrangements in NSCLC	Lung cancer	249	N/A	1	13	1	11	1	0
Blood Collection Tube Equivalence	Ovarian cancer Breast cancer Colorectal cancer Prostate cancer Lung cancer Skin cancer Stomach cancer	60	192	116	135	39	13	5	0
Automation Line Equivalence	Contrived samples	8	187	303	1926	337	63	61	4
Variant Report Curation	Breast cancer Colorectal cancer Lung cancer Prostate cancer Skin cancer	19	57	183	300	104	15	11	2
Pan-tumor performance (includes historical analysis)	20 cancer types	19868	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Molecular Index Barcode Performance	25 cancer types Contrived samples	7637	N/A	324	N/A	N/A	N/A	N/A	N/A
FoundationOne Liquid LDT to FoundationOne Liquid CDx Concordance	25 cancer types	927	N/A	73	1815	376	109	46	N/A

*Variant result totals may include variants classified as variants of unknown significance (VUS) or benign.

[#] FoundationOne Liquid LDT to FoundationOne® Liquid CDx concordance.

Clinical oncology blood specimens can be constrained by factors such as limitations in blood draw volumes and cfDNA concentration. For studies where clinical samples carrying CDx biomarkers/alteration types were not evaluated due to limitations in

sample availability, a postmarket study is planned to confirm the performance of the FoundationOne® Liquid CDx test using intended use clinical specimens.

Actionable alterations were identified in the 39 contrived samples representing 17 genes and included 2 *ALK* rearrangements, 2 *BRCA1* (positive for 2 indels and 1 substitutions), and 3 *BRCA2* samples (positive for 5 indels), 5 *PIK3CA* substitutions, and 2 *ERBB2* copy number amplifications. These samples were used to supplement the samples used to support the performance of the *ALK, BRCA1, BRCA2,* and *PIK3CA* CDx indications listed in Table 1 as well as the tumor mutation profiling claims which include those genes listed in Table 3.

1. Contrived Sample Functional Characterization (CSFC) Study:
   Similar performance between clinical cfDNA samples and contrived samples was confirmed by demonstrating equivalent hit rates across comparable dilutions between the two sample types, including the LoD level. The study was conducted as described in the Summary of Safety and Effectiveness Data for P190032.

   While all matching alterations were used in the analysis, clinical specimens were selected to target some highly relevant alterations for each alteration type, including some CDx biomarkers. Comparable hit rates at targeted dilution levels between clinical and contrived samples for these targeted alterations demonstrate similar performance between contrived and clinical samples for processing with FoundationOne® Liquid CDx.

   A post-market study will be conducted to confirm the functional comparability between contrived and clinical samples positive for other specific *BRCA1* and *BRCA2* alterations, rearrangements, gene fusions, and copy number alterations (See Section XIII).

2. Analytical Accuracy/Concordance with an Orthogonal Method:

   a. Concordance data for CDx-associated alterations:
      i. Comparison with Validated NGS Plasma-Based Assay:
      Additional data was provided to that included in the Summary of Safety and Effectiveness Data for P190032 for the assessment of analytical accuracy/concordance with a validated NGS plasma-based assay for rearrangements, including gene fusions, and copy number alterations. The detection of short variants and rearrangements by the FoundationOne® Liquid CDx assay was compared to that of an externally-validated NGS assay in 74 genes common to both assays, across 278 samples that represented an array of tumor types. The study included samples selected from clinical FoundationOne® Liquid testing and contrived samples to represent rare alterations.

      For assessment of alterations that would be classified as rearrangements, FoundationOne® Liquid CDx was compared with the orthogonal method.

The samples included 3 *ALK* rearrangements of which one was discordant and not called by the orthogonal method and one was excluded/filtered for either the gene or the partner not being included in the region interrogated by comparator assay. Twelve *ERBB2* amplified clinical samples and one contrived sample were also compared and found to be concordant with the orthogonal NGS test. A summary of the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) results are shown in the table below.

Table 10. Concordance of CDx alterations called between FoundationOne® Liquid CDx and the comparator assay (n = 74)

Targeted Alteration	N	PPA (95% CI)	NPA (95% CI)
BRCA1 short variants	1	100% (2.5%-100.0%)	100% (98.7%-100.0%)
BRCA2 short variants	2	100% (15.8%-100.0%)	100% (99.3%-100.0%)
PIK3CA substitutions	49	100% (92.7%-100.0%)	100% (99.9%-100.0%)
ALK rearrangements	1	100% (2.5%-100.0%)	99.9% (99.7%-100.0%)

Since adequate samples from all CDx indications were not represented in the above study either due to sample limitations or due to inadequate coverage of panel space in the orthogonal method. Data from an additional analytical accuracy study will be provided post-market as a condition of approval study (See Section XIII).

ii. Concordance with Orthogonal cfDNA-based NGS Method #2

An additional analytical accuracy study was conducted for breast cancer patients with samples harboring *PIK3CA* mutations with residual plasma samples from the SOLAR-1 clinical study. Of the 549 residual plasma samples, 542 were previously tested with the externally-validated NGS (evNGS) method and produced valid results. Of the 459 plasma samples available for testing, only 445 samples had sufficient volume (≥ 2.5 mL) for testing with the FoundationOne® Liquid CDx test. Of those, 20 were determined to not meet the minimum assay requirement for cfDNA after extraction resulting in 425 evaluable samples.

Of the remaining 425 samples, 7 failed genomic curation due to noise resulting in 418 samples. Of those, only 415 generated valid FoundationOne® Liquid CDx results. Three (3) were excluded after it being determined they were identified as not having been processed on the evNGS method. samples resulting in a final total of 412 samples having produced valid results on both assays. One hundred ninety-two (192) positive variants were detected across 188 patients, with four patients possessing two positive variants each. The distribution of counts per positive variant is listed in Table 11.

Table 11: Distribution of Variants Detected with FoundationOne® Liquid CDx evaluable samples.

Protein Effect in *PIK3CA*	# Variant Calls (188 Positive Samples)
C420R	3
E542K	25
E545A	1
E545G	2
E545K	50
H1047L	9
H1047R	100
H1047Y	1
Q546R	1
Total	192

A total of 412 valid samples generated valid results with both assays. The primary analysis using comparator assay as the reference assay achieved a PPA (95% CI) of 97.1% (93.3%, 99.0%), and an NPA (95% CI) of 91.7% (87.5%, 94.9%) and Overall Percent Agreement (OPA) (95% CI) of 93.9% (91.2%, 96.0%) . The contingency table for this comparison is provided in Table 12 below, with counts representing number of samples (versus number of variant calls).

Table 12: Contingency Table Comparing FoundationOne® Liquid CDx with the Reference Assay, Primary Analysis with 412 Cases

		NGS Comparator #2				
		Positive	**Negative**	**Non-evaluable**	**Missing**	**Total**
FoundationOne® Liquid CDx	**Positive**	165	20	2	1	188
	Negative	5	222	1	2	230
	Invalid	0	7	0	0	7
	Total	170	249	3	3	425

The agreement calculations, relative to the orthogonal method were:

- PPA: 97.1% (93.3%, 99.0%)
- NPA: 91.7% (87.5%, 94.9%)
- OPA: 93.9% (91.2%, 96.0%)

3. Analytical Sensitivity:

   a. Limit of Blank (LoB):

   See Summary of Safety and Effectiveness Data for P190032.

A post-market LoB study will be conducted to confirm the results in accordance with the FoundationOne® Liquid CDx assay workflow (See Section XIII).

b. Limit of Detection (LoD):
The LoD study was performed as described in the Summary of Safety and Effectiveness Data for P190032. The LoD by hit rate was defined as the variant allelic fraction (VAF) value (for short variants and rearrangements) or mean tumor fraction (TF) value (for copy number alterations) at the lowest dilution level tested with at least 95% detection across replicates. The hit rate was computed as the number of replicates with positive variant calls per the total number of replicates tested at each level.

The median estimated LoD for CDx alterations are presented in Table 13. The estimated LoD for *ERBB2* copy number amplification included under the tumor profiling claim was 19.8% TF. The median LoD for targeted short variant, rearrangement, and copy number alterations were consistent with the platform LoD.

Table 13. LoD estimation for CDx alterations

Gene	Alteration Subtype	# Samples Evaluated	Median LoD[1]
BRCA1	Substitutions	8	0.34% VAF
	Indels	1	0.38% VAF
	Rearrangement[2]	1	0.87% VAF
BRCA2	Substitutions	17	0.37% VAF
	Indels	2	0.36% VAF
	BRCA2-EDA Truncation[2]	1	0.48% VAF
	Copy Number Loss[1]	1	48.1% TF
ALK	*ALK-EML4* Rearrangement[2]	1	0.24% VAF
	NPM1-ALK Rearrangement	1	0.94% VAF
PIK3CA	Substitutions	6	0.34% VAF

The Estimated LoDs for *BRCA1* and *BRCA2* subs and indels were confirmed at values higher than the LoDs estimated for the non-CDx alterations. (see Precision: Reproducibility and Reproducibility section below, Tables 15 and 16 for confirmed LoD values).

[1] The accuracy of %VAF/%TF has not been analytically validated.

[2] The LoD for these alterations were determined using clinical specimens.

The LoDs for other variants detected by the assay were determined to be similar to the median LoDs estimated for the CDx variants above. A total of 864 short variants were included in the platform LoD analysis. The enhanced sensitivity region of the bait set contains 269 of the short variants analyzed and the standard sensitivity region of the bait set contains 595 of the short variants analyzed. The median LoD for short variants was estimated at 0.40% for the enhanced sensitivity region and 0.82% of the standard sensitivity

region. The median LoD for rearrangements was estimated to be 0.37% for the enhanced sensitivity region and 0.9% for the standard sensitivity region.

4. Analytical Specificity:
   a. Potentially Interfering Substances:

      See Summary of Safety and Effectiveness Data for P190032.

   b. Hybrid Capture Bait Specificity:

      See Summary of Safety and Effectiveness Data for P190032.

5. Carryover/Cross-Contamination:

   See Summary of Safety and Effectiveness Data for P190032.

6. Precision: Repeatability and Reproducibility
   Precision was evaluated for alterations associated with CDx claims, as well as tumor mutation profiling variants. Repeatability including intra-run performance (run on the same plate under the same conditions) and reproducibility including inter-run performance (run on different plates under different conditions) were assessed and compared across three reagent lots, two sequencers, and two processing runs.

   a. Results for a subset of highly-actionable alterations
      A set of 39 unique samples were used to evaluate precision of FoundationOne® Liquid CDx for detecting a set of highly-actionable variants, including 8 contrived samples representing various targeted alterations and 31 clinical samples. Also see the Summary of Safety and Effectiveness Data for P190032. The samples representing CDx alterations that are the subject of this PMA are summarized in Table 14.

      The 31 clinical samples consisted of 7 different cancers (10 lung, 6 prostate, 3 colon, 2 melanoma, 4 ovarian, 5 breast, and 1 unknown). The samples included 30 actionable gene alterations including 8 *BRCA1* or *BRCA2* alterations, 1 ALK rearrangements, and 3 PIK3CA mutations to equal, 7 substitutions and indels, 2 rearrangements, and 2 copy number alterations (gains and losses). One lung sample included an *ALK-EML4* rearrangement and 3 of the breast cancer specimens included 3 independent *PIK3CA* mutations (E542K, E545K, and H1047R). The remaining samples included multiple other actionable genes and variant types. The samples representing the CDx alterations are summarized in the table below:

Table 14: CDx Precision Sample Set

CDx Biomarker	Targeted Alteration	Disease Ontology of Patient from which Sample was Derived
ALK rearrangements	*ALK*-EML4 Rearrangement	Contrived sample
	ALK-EML4 Rearrangement	Lung adenocarcinoma
	ALK-NPM1 Rearrangement	Contrived sample
BRCA1 and *BRCA2* alterations	*BRCA1* E23fs*17 (*BRCA1* 68_69delAG)	Ovary cancer
	BRCA1 Q780* (*BRCA1* 2338C>T)	Ovary high grade serous carcinoma
	BRCA1 Rearrangement (*BRCA1-BRCA1*)	Unknown primary malignant neoplasm
	BRCA1 2475delC	Contrived sample
	BRCA1 2612C>TT	Contrived sample
	BRCA2 3599_3600delGT	Contrived sample
	BRCA2 4284_4285insT	Contrived sample
	BRCA2 5351delA	Contrived sample
	BRCA2 G267* (*BRCA2* 799G>T)	Ovary serous carcinoma
	BRCA2 Loss (26 of 26)	Prostate acinar adenocarcinoma
	BRCA2 S2988fs*12 (*BRCA2* 8961_8964delGAGT)	Ovary cancer
	BRCA2-EDA Truncation	Prostate cancer
PIK3CA mutations	*PIK3CA* E542K	Contrived sample
	PIK3CA E542K, D549N	Contrived sample
	PIK3CA H1047R	Contrived sample
	PIK3CA E542K	Breast carcinoma
	PIK3CA E545K	Breast carcinoma
	PIK3CA H1047R	Breast cancer

Target alterations were assessed near LoD and/or 2x – 3x LoD. Each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Across 47 samples (31 clinical specimens at one dilution level and 8 contrived samples across two dilution levels), a total of 57 unique alterations were evaluated.

The repeatability of CDx alterations is summarized in Table 15 and the reproducibility of CDx alterations is summarized in Table 16.

Table 15: Repeatability of CDx alterations targeted in precision study at ≥ 1x LoD*

Variant Type	Alteration[1]	Concordant Pairs	Repeatability (%)	95% CIs (%)	Level Tested[2]	X LoD
Short variant	*BRCA1* 2338C>T	12/12	100	(73.5, 100.0)	1.11% VAF	3.3
Short variant	*BRCA1* 2475delC	12/12	100	(73.5, 100.0)	0.61% VAF	1.6
Short variant	*BRCA1* 2475delC	11/11	100	(71.5, 100.0)	1.26% VAF	3.3
Short variant	*BRCA2* 5351delA	12/12	100	(73.5, 100.0)	1.22% VAF	3.2
Short variant	*BRCA2* 5351delA	12/12	100	(73.5, 100.0)	1.85% VAF	4.9
Short variant	*BRCA2* 5351delA	11/11	100	(71.5, 100.0)	1.07% VAF	2.8
Short variant	*BRCA2* 5351delA	12/12	100	(73.5, 100.0)	2.24% VAF	5.9
Short variant	*BRCA2* 5465_5466insA	12/12	100	(73.5, 100.0)	0.92% VAF	2.4
Short variant	*BRCA2* 5465_5466insA	11/11	100	(71.5, 100.0)	1.19% VAF	3.1
Short variant	*BRCA2* 8961_8964delGAGT	12/12	100	(73.5, 100.0)	1.07% VAF	2.8
Short variant	*BRCA2* c.799G>T	10/12	83.3	(51.6, 97.9)	0.5% VAF	1.5
Short variant	*BRCA2* c.9097_9098insA	6/11	54.6	(23.4, 83.3)	0.71% VAF	1.9

Variant Type	Alteration[1]	Concordant Pairs	Repeatability (%)	95% CIs (%)	Level Tested[2]	X LoD
Short variant	*BRCA2* c.9097_9098insA	10/12	83.3	(51.6, 97.9)	1.03% VAF	2.7
Copy Number Loss	*BRCA2* loss	11/12	91.7	(61.5, 99.8)	39.43% TF	0.8
Rearrangement	*BRCA2-EDA* Truncation	11/11	100	(71.5, 100.0)	0.48% VAF	0.6
Rearrangement	*ALK-EML4*	12/12	100	(73.5, 100.0)	0.64% VAF	2.7
Rearrangement	*ALK-EML4* (contrived)	11/11	100	(71.5, 100.0)	0.89% VAF	3.7
Rearrangement	*ALK-EML4* (contrived)	12/12	100	(73.5, 100.0)	1.39% VAF	5.8
Rearrangement	*ALK-NPM1* (contrived)	12/12	100	(73.5, 100.0)	0.64% VAF	0.7
Short variant	*PIK3CA* E542K	12/12	100	(73.5, 100.0)	0.89% VAF	2.6
Short variant	*PIK3CA* E545K	12/12	100	(73.5, 100.0)	0.45% VAF	1.3
Short variant	*PIK3CA* E545K (contrived)	12/12	100	(73.5, 100.0)	0.66% VAF	1.9
Short variant	*PIK3CA* E545K (contrived)	12/12	100	(73.5, 100.0)	0.5% VAF	1.5
Short variant	*PIK3CA* E545A (contrived)	12/12	100	(73.5, 100.0)	0.52% VAF	1.5
Short variant	*PIK3CA* E545A (contrived)	11/11	100	(71.5, 100.0)	0.7% VAF	2.1
Short variant	*PIK3CA* Q546R (contrived)	10/11	90.9	(58.7, 99.8)	0.49% VAF	1.4
Short variant	*PIK3CA* Q546R (contrived)	12/12	100	(73.5, 100.0)	0.92% VAF	2.7
Short variant	*PIK3CA* D549N (contrived)	12/12	100	(73.5, 100.0)	0.48% VAF	1.4
Short variant	*PIK3CA* D549N (contrived)	12/12	100	(73.5, 100.0)	0.73% VAF	2.1
Short variant	*PIK3CA* H1047R	11/11	100	(71.5, 100.0)	0.41% VAF	1.2
Short variant	*PIK3CA* H1047R (contrived)	12/12	100	(73.5, 100.0)	0.76% VAF	2.2
Short variant	*PIK3CA* H1047R (contrived)	12/12	100	(73.5, 100.0)	1.04% VAF	3.1

*Several clinical samples were mostly tested at 2x – 3x LoD rather than 1x – 1.5x LoD

[1] See Table 14 for *BRCA1/BRCA2* sample source type

[2] The accuracy of %VAF/%TF has not been analytically validated.

Table 16: Reproducibility of CDx alterations targeted in precision study at ≥ 1x LoD*

Variant Type	Alteration	Concordant Replicates	Reproducibility (%)	95% CIs (%)	Level Tested**	xLoD
Short variant	*BRCA1* 2338C>T	24/24	100	(85.8, 100.0)	1.11% VAF	3.3
Short variant	*BRCA1* 2475delC	24/24	100	(85.8, 100.0)	0.61% VAF	1.6
Short variant	*BRCA1* 2475delC	24/24	100	(85.8, 100.0)	0.93% VAF	2.4
Short variant	*BRCA1* 2612C>TT	23/23	100	(85.2, 100.0)	0.51% VAF	1.3
Short variant	*BRCA1* 68_69delAG	24/24	100	(85.8, 100.0)	0.66% VAF	1.7
Short variant	*BRCA1* P871fs*32	24/24	100	(85.8, 100.0)	1.08% VAF	2.8
Rearrangement	*BRCA1-BRCA1*	24/24	100	(85.8, 100.0)	0.87% VAF	1.0
Short variant	*BRCA2* 3599_3600delGT	24/24	100	(85.8, 100.0)	0.58% VAF	1.6
Short variant	*BRCA2* 3599_3600delGT	24/24	100	(85.8, 100.0)	0.92% VAF	2.6
Short variant	*BRCA2* 4284_4285insT	24/24	100	(85.8, 100.0)	0.94% VAF	2.6
Short variant	*BRCA2* 4284_4285insT	23/23	100	(85.2, 100.0)	1.26% VAF	3.5
Short variant	*BRCA2* 5351delA	24/24	100	(85.8, 100.0)	1.22% VAF	3.4
Short variant	*BRCA2* 5351delA	24/24	100	(85.8, 100.0)	1.85% VAF	5.1
Short variant	*BRCA2* 5351delA	23/23	100	(85.2, 100.0)	1.07% VAF	3.0
Short variant	*BRCA2* 5351delA	24/24	100	(85.8, 100.0)	2.24% VAF	6.2
Short variant	*BRCA2* 5465_5466insA	24/24	100	(85.8, 100.0)	0.92% VAF	2.6
Short variant	*BRCA2* 5465_5466insA	23/23	100	(85.2, 100.0)	1.19% VAF	3.3
Short variant	*BRCA2* 799G>T	22/24	91.7	(73.0, 99.0)	0.5% VAF	1.4

Variant Type	Alteration	Concordant Replicates	Reproducibility (%)	95% CIs (%)	Level Tested**	xLoD
Short variant	*BRCA2* 8961_8964delGAGT	24/24	100	(85.8, 100.0)	1.07% VAF	3.0
Short variant	*BRCA2* 9097_9098insA	22/24	91.7	(73.0, 99.0)	1.03% VAF	2.9
Short variant	*BRCA2* c.799G>T	22/24	91.7	(73.0, 99.0)	0.5% VAF	1.4
Short variant	*BRCA2*_c.9097_9098insA	5/23	21.7	(7.5, 43.7)	0.71% VAF	2.0
Short variant	*BRCA2* c.9097_9098insA	22/24	91.7	(73.0, 99.0)	1.03% VAF	2.9
Copy Number Loss	*BRCA2* loss	21/24	87.5	(67.6, 97.3)	39.43% TF	0.8
Rearrangement	*BRCA2*-EDA Truncation	23/23	100	(85.8, 100.0)	0.48% VAF	1.0
Rearrangement	*ALK-EML4*	24/24	100	(85.8, 100.0)	0.64% VAF	2.7
Rearrangement	*ALK-EML4*	23/23	100	(85.8, 100.0)	0.89% VAF	3.7
Rearrangement	*ALK-EML4*	24/24	100	(85.8, 100.0)	1.39% VAF	5.8
Rearrangement	*ALK-NPM1*	24/24	100	(85.8, 100.0)	0.64% VAF	0.7
Rearrangement	*ALK-NPM1*	18/23	78.3	(56.3, 92.5)	0.4% VAF	0.4
Short variant	*PIK3CA* E542K	24/24	100	(85.8, 100.0)	0.89% VAF	2.6
Short variant	*PIK3CA* E545K	24/24	100	(85.8, 100.0)	0.45% VAF	1.3
Short variant	*PIK3CA* E545K (contrived)	24/24	100	(85.8, 100.0)	0.66% VAF	1.9
Short variant	*PIK3CA* E545K (contrived)	24/24	100	(85.8, 100.0)	0.5% VAF	1.5
Short variant	*PIK3CA* E545A (contrived)	24/24	100	(85.8, 100.0)	0.52% VAF	1.5
Short variant	*PIK3CA* E545A (contrived)	23/23	100	(85.2, 100.0)	0.70% VAF	2.1
Short variant	*PIK3CA* Q546R (contrived)	22/23	95.7	(78.1, 99.9)	0.49% VAF	1.4
Short variant	*PIK3CA* Q546R (contrived)	24/24	100	(85.8, 100.0)	0.92% VAF	2.7
Short variant	*PIK3CA* D549N (contrived)	24/24	100	(85.8, 100.0)	0.48% VAF	1.4
Short variant	*PIK3CA* D549N (contrived)	24/24	100	(85.8, 100.0)	0.73% VAF	2.1
Short variant	*PIK3CA* H1047R	23/23	100	(85.2, 100.0)	0.41% VAF	1.2
Short variant	*PIK3CA* H1047R (contrived)	24/24	100	(85.8, 100.0)	0.76% VAF	2.2
Short variant	*PIK3CA* H1047R (contrived)	24/24	100	(85.8, 100.0)	1.04% VAF	3.1

*Clinical samples were mostly tested at 2x – 3x LoD rather than 1x – 1.5x LoD

**The accuracy of %VAF/%TF has not been analytically validated.

For repeatability, of the *ALK* fusion/rearrangements and *PIK3CA* samples assessed in this PMA, 93.8% (15/16) samples demonstrated 100% repeatability. Four *BRCA2* samples demonstrated repeatability below 95% (54.6% - 91.7%). The *BRCA2* loss was tested at an 39.4%TF below the estimated LoD of 48.1%TF and used a cfDNA input below the recommended cfDNA input of 30 ng. Of the remaining 3 poorly performing samples, only one was at a %VAF (0.5% VAF) near the estimated LoD (0.37% VAF), while the remaining 2 were tested at levels higher than the estimated LoDs for each sample. Therefore, the reason for the observed performance is not clear. One *PIK3CA* Q546R sample (tested at 1.4x LoD) demonstrated a repeatability of 90.9%. All 3 *ERBB2* amplified samples, 1 contrived (tested at 35.8%, and 39.8%TF) and 1 clinical (tested at 61.7% TF) demonstrated 100% reproducibility.

Reproducibility of 100% was observed in 16/18 (88.9%) alterations.

A single contrived *ALK-NPM1* fusion/rearrangement demonstrated poor repeatability at 54.6% and reproducibility of 78.3% due to the sample being tested below the estimated VAF of 0.94%. at 0.4% VAF which is below the estimated LoD of 0.94%. Six *BRCA2* samples demonstrated reproducibility below 95% (21.7 – 91.7). The *BRCA2* loss was tested at an %TF below the estimated LoD and used a cfDNA input below the recommended cfDNA input of 30 ng. Of the remaining 5 poorly performing samples, only one was at a %VAF (0.5% VAF) near the estimated LoD (0.37% VAF), while the remaining 4 were tested at levels higher than the estimated LoDs for each sample. Therefore, the reason for the observed performance is not clear. All 3 *ERBB2* amplified samples, 1 contrived (tested at 35.8%, and 39.8%TF) and 1 clinical (tested at 61.7% TF) demonstrated 100% reproducibility.

b. Confirmation of LoD and Precision in Clinical Specimens:
The combined confirmation of LoD and precision study was performed as described in the Summy of Safety and Effectiveness for P190032. In this study, 29 clinical cfDNA samples targeting variants at 1-1.5x LoD were evaluated to confirm LoD and precision in clinical specimens. Twenty-six had 100% reproducibility, one had 95.8% reproducibility, and two samples had reproducibility below 90%. Of these two samples, one contained a *BRCA2* loss that had 87.5% reproducibility and 91.7% repeatability. This sample had cfDNA input below the recommended minimum input. The other sample harbored a *BRCA2* substitution (c.799G>T) with 91.7% reproducibility and 83.3% repeatability. The average VAF of this variant was 0.5% across replicates, which is near the LoD for this variant type (LoD of 0.37% VAF). A summary of the Confirmation of LoD and precision results for a subset of highly-actionable alterations are provided in Table 17.

Table 17: Confirmation of LoD and precision in clinical specimens

Target Alteration	LoD	Mean Level Tested	Reproducibility (95% CI)	95% CIs (%)
ALK-EML4 Rearrangement	0.24% VAF	1.39 %VAF	100	(85.8, 100.0)
BRCA1 E23fs*17	0.38% VAF	0.66% VAF	100	(85.8, 100.0)
BRCA1 Q780*	0.34% VAF	1.11%VAF	100	(85.8, 100.0)
BRCA1 Rearrangement	0.26%-.47% VAF[1]	0.87% VAF	100	(85.8, 100.0)
BRCA2 S2988fs*12	0.36% VAF	1.07% VAF	100	(85.8, 100.0)
BRCA2- EDA Truncation	0.26%-.47% VAF[1]	0.48% VAF	100	(85.2, 100.0)
PIK3CA E542K	0.34% VAF	0.89% VAF	100	(85.8, 100.0)
PIK3CA E545K	0.34% VAF	0.5% VAF	100	(85.8, 100.0)
PIK3CA H1047R	0.34% VAF	1.04% VAF	100	(85.8, 100.0)
ERBB2 CNA	19.8% TF	61.73% TF	100	(85.8, 100.0)

[1] Estimated LoD levels reported in Table 13.
[2] The accuracy of %VAF/%TF has not been analytically validated

In general, most of the targeted variants were tested at levels higher (or lower) than estimated near LoD (1x); therefore, the tested LoD level values (%VAF/%TF) are considered to be the confirmed LoD. A post-market study

is planned to demonstrate precision using samples at near the estimated LoD for those tested above or below the estimated LoD (See Section XIII).

A second study with 10 samples targeting variants at 1-1.5x LoD was performed to confirm LoD and precision in clinical specimens. Similar to above, each sample was divided into 24 aliquots, with 12 duplicates being processed on the same plate under the same conditions. Each sample was tested across 24 replicates. Six samples were included in the primary analysis for samples with ≥30 ng DNA input. Two BRCA substitutions showed 100% repeatability and reproducibility, one *BRCA2* indel (*BRCA2* 5351_5352insA) had repeatability of 75.0% and an 87.7% reproducibility, the *PIK3CA* Q546R SNV had repeatability of 8.3.% and a 91.7% reproducibility. The other four samples had a majority of sample replicates with DNA input <30 ng. A summary of the Confirmation of LoD and Precision results for CDx alterations are provided in Table 18.

Table 18: Confirmation of LoD and precision in clinical specimens for CDx alterations

Targeted Alteration	Mean Input Mass (ng)	Concordant /Total	Repeatability (%)	Concordant/ Total	Reproducibility (%)	Average VAF (%)	Estimated LoD (VAF %)
BRCA1 1395T>A	32.8	12/12	100% (75.8%, 100%)	24/24	100% (86.2%, 100%)	0.51%	0.34%
BRCA2 5351_5352insA	36.6	9/12	75% (46.8%, 91.1%)	21/24	87.5% (69.0%, 95.7%)	0.34%	0.36%
BRCA2 8524C>T	31.5	11/11	100% (74.1%, 100%)	23/23	100% (85.7%, 100%)	0.59%	0.49%2
PIK3CA Q546R	37.5	10/12	83.3% (55.2%, 95.3%)	22/24	91.7%3 (74.2%, 97.7%)	0.44%	0.34%

[1] The accuracy of %VAF/%TF has not been analytically validated.

As summarized in Table 18 above, both CDx variants with ≥30 ng DNA input had reproducibility ≥95% with the exception of one variant (*BRCA2* 5351_5352insA) which was tested at a %VAF just below the LoD.

c. Tumor Mutation Profiling Variants:
Across 39 unique samples, including 8 contrived samples, and 31 clinical samples, a total of 1,240 variants were evaluated with variant types including 898 substitutions, 228 indels, 60 rearrangements, 49 copy number amplifications, and 5 copy number losses. The overall repeatability for all variants was 99.5% with 95% 2-sided exact CIs (99.5%, 99.5%). The repeatability result for each variant type are summarized in Table 19.

Table 19: Assessment of repeatability of tumor mutation profiling variants* per type

Variant Type	# Concordant Pairs	# Total Pairs	Repeatability (%)	95% CIs (%)
Substitution	498765	501084	99.54	(99.52, 99.56)
Indels	126475	127224	99.41	(99.37, 99.45)

Variant Type	# Concordant Pairs	# Total Pairs	Repeatability (%)	95% CIs (%)
Rearrangements	33105	33480	98.88	(98.76, 98.99)
Copy Number Alterations	29880	30132	99.16	(99.05, 99.26)

*Variant result totals include variants classified as VUS or benign.

The overall reproducibility results were 99.59% with the 95% 2-sided exact CIs (99.58%, 99.60%). The reproducibility result for each variant type are summarized Table 20.

Table 20: Assessment of reproducibility of tumor mutation profiling variants* per type

Variant Type	# of Concordant Replicates	# of Total Replicates	Reproducibility (%)	95% CIs (%)
Substitution	1002981	1006658	99.63	(99.62, 99.65)
Indels	254509	255588	99.58	(99.55, 99.60)
Rearrangements	66723	67260	99.20	(99.13, 99.27)
Copy Number Alterations	60115	60534	99.31	(99.24, 99.37)

*Variant result totals include variants classified as VUS or benign.

d. Reagent Lot-to-Lot Reproducibility:

See Summary of Safety and Effectiveness Data for P190032.

e. Instrument-to-Instrument Reproducibility:

See Summary of Safety and Effectiveness Data for P190032.

f. Reagent Lot Interchangeability:

See Summary of Safety and Effectiveness Data for P190032.

g. Curator Precision:

See Summary of Safety and Effectiveness Data for P190032.

7. Comparability Across Cancer Types:
A large-scale retrospective analysis was performed to demonstrate consistent test performance of FoundationOne® Liquid CDx across samples derived from patients with different tumor types based on the performance of two prior versions of the FoundationOne® Liquid CDx assay. The FoundationOne® Liquid CDx assay was developed based on two versions of the FoundationOne® Liquid LDT assay and the FoundationACT (FACT), each of which includes only a subset of the genes included in FoundationOne® Liquid CDx. A summary of this study is found in the Summary of Safety and Effectiveness Data for P190032.

Additional data was provided for the second analysis that was performed to evaluate the concordance between the FoundationOne® Liquid LDT, FACT, and the FoundationOne® Liquid CDx assays based on the concordance of unique samples processed on both the FoundationOne® Liquid LDT and FoundationOne® Liquid CDx assays positive for additional gene rearrangements. The concordance analysis using FoundationOne® Liquid LDT or FoundationOne® Liquid CDx as the reference assay is summarized by variant category in Table 21.

Samples, sequence, and variant data were drawn from different clinical studies being used to support the approval of the FoundationOne® Liquid CDx assay. Only those regions commonly baited between the assays were included in the analysis. All comparisons were performed using FoundationOne® Liquid LDT results, which have been analyzed using the latest version of the that test's analysis pipeline. As with the study above, for samples processed using the FoundationOne® Liquid LDT and FACT assays, only those regions commonly baited between the respective version of the FoundationOne® Liquid LDT and the bait set used by FoundationOne® Liquid CDx were included in the analysis (and thus the variants contained therein). Copy number losses are not called by the FoundationOne® Liquid LDT and therefore were not consisered in the analysis.

Table 21: Concordance* between FoundationOne® Liquid LDT and FoundationOne® Liquid CDx

Variant*/ Mutation Type	CDx(+) / LDT(+)	CDx(-) / LDT(+)	CDx(+) / LDT(-)	CDx(-) / LDT(-)	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
Including VUS Results							
All Short Variants	2871	123	32	1171180	95.9% (95.1%, 96.6%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Base Substitutions	2415	104	31	999032	95.9% (95.0%, 96.6%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Indels	456	19	1	172148	96.0% (93.8%, 97.6%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Copy Number Alterations (gains)	173	32	110	59463	84.4% (78.7%, 89.1%)	99.8% (99.8%, 99.8%)	99.8% (99.7%-99.8%)
Rearrangements	147	20	24	59587	88.0% (82.1%, 92.5%)	>99.9% (>99.9%, 100.0%)	99.9% (99.9%, 99.9%)
Total	3191	175	166	1290230	94.8% (94.0%, 95.5%)	>99.9% (>99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Excluding VUS Results:							
All Short Variants	1635	66	28	534382	96.1% (95.1%, 97.0%)	>99.9% >99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Base Substitutions	1264	49	27	400278	96.3% (95.1%, 97.2%)	>99.9% >99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Indels	371	17	1	134104	95.6% (93.1%, 97.4%)	>99.9% >99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)
Copy Number Alterations (gains)	155	18	69	59536	89.6% (84.1%, 93.7%)	99.9% (99.9%, 99.9%)	99.9% (99.8%, 99.9%)
Rearrangements	100	13	16	59649	88.5%	>99.9%	>99.9%

Variant*/ Mutation Type	CDx(+) / LDT(+)	CDx(-) / LDT(+)	CDx(+) / LDT(-)	CDx(-) / LDT(-)	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
					(81.1%, 93.7%)	>99.9%, 100.0%)	(99.9%, 100.0%)
Totals	1890	97	113	653567	95.1% (94.1%, 96.0%)	>99.9% >99.9%, 100.0%)	>99.9% (>99.9%, 100.0%)

* Concordance was assessed between two version of the F1 Liquid LDT and F1 Liquid CDx. Only those regions that are commonly baited between the 3 tests were included in the analyses.

The overall PPA between FoundationOne® Liquid LDT and FoundationOne® Liquid CDx assays, with FoundationOne® Liquid LDT as the reference assay, was 95.1% with a 95% two-sided CI of (94.1%, 96.0%). The respective short variant, CNAs, and rearrangement PPA values (excluding VUS results), with 95% two-sided CI, were: 96.1% (95.1%, 97.0%), 89.6% (84.1%, 93.7%), and 88.5% (81.1%, 93.7%). The PPA values when VUS results were included were relatively similar. Despite the study only including regions that were commonly baited between the tests discordances were noted, including those that were identified as being uniquely identified by either test. For short variants (substitutions and small indels), discordant results were due primarily to lower VAF values when tested with the FoundationOne® Liquid CDx. For copy number alterations, the 32 calls reported on FoundationOne® Liquid LDT but not on FoundationOne® Liquid CDx test was primarily due to copy number events that were observed by the analysis pipeline and were near to, but did not meet the ploidy-based copy number threshold. For rearrangements, discordant calls unique to the FoundationOne® Liquid LDT test tended to have lower VAF values and those variant calls with lower VAFs that were closer to the LoD for each assay tended to demonstrate lower concordance. The results from this study support the agreement between FoundationOne® Liquid LDT and FoundationOne® Liquid CDx and the applicability of the tumor comparability analysis performed using historical FoundationOne® Liquid data.

8. Stability:

   a. Reagent Stability:

      See Summary of Safety and Effectiveness Data for P190032.

   b. Stability of cfDNA and Plasma Samples:

      See Summary of Safety and Effectiveness Data for P190032.

   c. Whole Blood Specimen Stability and Inverted Tube Stability:

      See Summary of Safety and Effectiveness Data for P190032.

9. Guard-banding and Robustness:

a. DNA Extraction:
DNA extraction evaluated 72 samples across five cancer types: lung cancer (including NSCLC), CRC, prostate cancer, breast cancer, and skin cancer (melanoma, sarcoma), using three reagent lots and two KingFisher Magnetic Particle processors.

Reproducibility of the FoundationOne® Liquid CDx DNA extraction process across King Fisher instruments and extraction reagent lots were analyzed utilizing a factorial design (3 reagent lots × 2 KingFisher instruments × 2 replicates). The success rate of the DNA extraction (DNAx) yield for three reagent lots range from 95.8% to 100.0% and two KingFisher instruments ranged from 97.2% to 100.0%.

Variant calls included in the concordance analysis were identified based on the majority call across all 12 replicates for a given disease ontology. Agreements were computed across the replicates for each somatic alteration for each sample, and aggregated by variant type (deletion, insertion, rearrangement, and substitution) for variants at ≥1x LoD. The percent agreements by disease ontologies were from 90.3% to 99.8 % for PPA, and 99.1% to 100.0% for NPA (Table 22). The percent agreement results across all variant types (deletion, insertion, rearrangement and substitution) evaluated at ≥1x LoD were from 90.6% to 96.8% for PPA and 98.9% to 100.0% for NPA (Table 23).

Table 22: Concordance summary by disease ontology at ≥1x LoD for cfDNA extraction study

Disease Ontology	Positive Detected/ Positive Total*	PPA (95% CI)	Negative Detected/ Negative Total*	NPA (95% CI)	Overall Detected/ Total*	OPA (95% CI)
Breast Cancer	347/348	99.7% (98.4%,100.0%)	3144/3144	100.0% (99.9%,100.0%)	3491/3492	100.0% (99.8%,100.0%)
CRC	1122/1188	94.4% (93.0%,95.7%)	2284/2304	99.1% (98.7%,99.5%)	3406/3492	97.5% (97.0%,98.0%)
Lung Cancer	431/432	99.8% (98.7%,100.0%)	3053/3060	99.8% (99.5%,99.9%)	3484/3492	99.8% (99.5%,99.9%)
NSCLC	600/612	98.0% (96.6%,99.0%)	2878/2880	99.9% (99.7%,100.0%)	3478/3492	99.6% (99.3%,99.8%)
Prostate Cancer	486/492	98.8% (97.4%,99.6%)	2987/3000	99.6% (99.3%,99.8%)	3473/3492	99.5% (99.2%,99.7%)
Skin Cancer	455/504	90.3% (87.4%,92.7%)	2987/2988	100.0% (99.8%,100.0%)	3442/3492	98.6% (98.1%,98.9%)

*Variant result totals may include variants classified as VUS or benign.

Table 23: Concordance summary by variant type at ≥1x LoD for cfDNA extraction study

Variant Type	Positive Detected/ Positive Total*	PPA (95% CI)	Negative Detected/ Negative Total*	NPA (95% CI)	Overall Detected/ Total*	OPA (95% CI)
Deletions	386/408	94.6% (91.9%, 96.6%)	2036/2040	99.8% (99.5%, 99.9%)	2422/2448	98.9% (98.4%, 99.3%)
Insertions	163/180	90.6% (85.3%, 94.4%)	819/828	98.9% (97.9%, 99.5%)	982/1008	97.4% (96.2%,98.3%)
Rearrangements	23/24	95.8% (78.9%, 99.9%)	120/120	100.0% (97.0%, 100.0%)	143/144	99.3% (96.2%, 100.0%)
Substitutions	2869/2964	96.8% (96.1%, 97.4%)	14358/14388	99.8% (99.7%, 99.9%)	17227/17352	99.3% (99.1%, 99.4%)

*Variant result totals may include variants classified as VUS or benign.

These results demonstrate robustness of the FoundationOne® Liquid CDx DNA extraction process across KingFisher instruments, extraction reagent lots, and cancer types.

b. cfDNA Input:

See the Summary of Safety and Effectiveness Data for P190032.

c. Molecular Index Barcode Performance:

See the Summary of Safety and Effectiveness Data for P190032.

d. Automation Line Equivalence:

See the Summary of Safety and Effectiveness Data for P190032.

B. Animal Studies

Not Applicable.

C. Additional Studies

Foundation Medicine performed additional studies, including Blood Collection Tube Equivalence, Whole Blood Stability, and Stability of cfDNA and Plasma Samples to support of the clinical validation studies. These studies are described in the Summary of Safety and Effectiveness Data for P190032.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

Foundation Medicine performed three separate clinical bridging studies to establish a reasonable assurance of safety and effectiveness of the FoundationOne® Liquid CDx for the three new CDx indications being sought. Data from this clinical study were the basis for the PMA approval decision. A summary of the clinical studies are presented below.

A. Clinical Bridging Study: Detection of *PIK3CA* mutations to Determine Eligibility for Treatment with Alpelisib

Clinical validity of using FoundationOne® Liquid CDx to identify breast cancer patients harboring *PIK3CA* mutations eligible for treatment with alpelisib was assessed through retrospective testing of plasma samples collected prior to study treatment from advanced or metastatic breast cancer patients enrolled in clinical trial CBYL719C2301 (SOLAR-1). Alpelisib was approved under NDA 212526 on May 24, 2019.

1. Study Design

SOLAR-1 was a randomized, double-blind, placebo-controlled phase III clinical trial to evaluate the safety and efficacy of alpelisib in combination with fulvestrant for men and postmenopausal women with hormone receptor positive, *HER2*-negative advanced breast cancer which progressed on or after aromatase inhibitor treatment.

In the device bridging study, all available plasma samples from patients collected at baseline prior to randomization into the Novartis SOLAR-1 clinical trial were tested with FoundationOne® Liquid CDx.

a. Bridging Study Inclusion and Exclusion Criteria

A bridging study was conducted to evaluate: 1) the concordance between *PIK3CA* mutation status by the clinical trial assays (CTA) and FoundationOne® Liquid CDx, and 2) the clinical efficacy of alpelisib treatment in patients that would be eligible for therapy based on *PIK3CA* mutation status as determined by FoundationOne® Liquid CDx.

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

Sample inclusion criteria:

- Samples from all randomized patients in the SOLAR-1 trial collected prior to start of SOLAR-1 study treatment
- Availability of adequate sample to generate a CDx test result including ≥ 2.5 mL plasma volume

Sample exclusion criteria:

- Lack of clear subject identification or label on stored patient sample
- Obvious physical damage of stored patient sample
- Insufficient sample (< 2.5 mL)

b. Clinical Endpoints

The primary endpoint for the study was progression-free survival (PFS) using Response Evaluation Criteria in Solid Tumors (RECIST v1.1), based on investigator assessment in advanced or metastatic breast cancer patients

enrolled with a *PIK3CA* alteration. Safety and tolerability were evaluated by assessment of type, frequency, and severity of adverse events and laboratory toxicities per Common Terminology Criteria for Adverse Events (CTCAE) v4.03.

2. Accountability of PMA Cohort

Of the 572 SOLAR-1 randomized patients [341 *PIK3CA*-positive and 231 *PIK3CA*-negative, as determined by the enrolling clinical trial assays (CTA1 or CTA2)], all had either been prospectively enrolled by CTA1 (n = 395) or were enrolled by CTA2 (n = 177). All 395 CTA1 enrolled samples were retrospectively tested with CTA2. Baseline samples from 432 of the 572 patients enrolled in SOLAR-1 were tested using FoundationOne® Liquid CDx, among which 375 (including 12 FoundationOne® Liquid CDx invalids and 4 CTA2 invalids) were tested withDNA input ≥ 30ng. An additional 57 samples were tested with DNA input ≥ 20 ng and < 30 ng (52 valid results and 5 invalids). Sample accountability for this clinical bridging study is summarized in Table 24.

Table 24: Sample accountability for alpelisib clinical bridging study

Description	**# Samples**
Patients enrolled in SOLAR-1 study	572
Available baseline plasma samples	432
Samples tested with DNA input ≥ 30 ng	375
Samples with invalid FoundationOne® Liquid CDx or CTA2 results and DNA input ≥30 ng	16
Total samples with valid results and DNA input ≥30 ng (primary analysis)	359 (375-16)
Samples tested with DNA input ≥ 20 ng and < 30 ng DNA input	57
Samples with invalid FoundationOne® Liquid CDx or CTA2 results and DNA input ≥ 20 ng and < 30 ng	6
Total samples with valid results and DNA input ≥20 ng (secondary analysis)	410 (359+57-6)

3. Study Population Demographics and Baseline Parameters

A comparison of the clinical outcomes and baseline characteristics between the CDx-evaluable population and the CDx non-evaluable population in SOLAR-1 demonstrated that the CDx-evaluable population was representative of the SOLAR-1 patient population. The comparison of demographics and baseline clinical characteristics between the FoundationOne® Liquid CDx evaluable and FoundationOne® Liquid CDx non-evaluable populations are provided in Table 25 and Table 26.

Table 25: Comparison of demographic and baseline clinical characteristics between the CDx-evaluable patients and the CDx non-evaluable patients in the *PIK3CA*(+) patients based on the enrolled results (primary analysis set)

Baseline characteristics	FoundationOne® Liquid CDx		
	Evaluable N=230	Non-evaluable N=111	All N=341
Age (years)			
n	230	111	341
Mean (SD)	63.5 (10.36)	63.0 (9.62)	63.3 (10.11)
Median	63.5	63.0	63.0
Q1-Q3	57.0 – 71.0	58.0 – 70.0	57.0 – 70.0
Min-Max	25 – 92	37 – 85	25 – 92
Sex-n (%)			
Female	229 (99.6)	111 (100.0)	340 (99.7)
Male	1 (0.4)	0	1 (0.3)
Race-n (%)			
White	144 (62.6)	82 (73.9)	226 (66.3)
Black or African American	3 (1.3)	1 (0.9)	4 (1.2)
Asian	55 (23.9)	19 (17.1)	74 (21.7)
American Indian or Alaska Native	3 (1.3)	0	3 (0.9)
Other	11 (4.8)	7 (6.3)	18 (5.3)
Unknown	14 (6.1)	2 (1.8)	16 (4.7)
Region-n (%)			
Europe	112 (48.7)	61 (55.0)	173 (50.7)
North America	31 (13.5)	12 (10.8)	43 (12.6)
Asia	52 (22.6)	18 (16.2)	70 (20.5)
Latin America	20 (8.7)	11 (9.9)	31 (9.1)
Other	15 (6.5)	9 (8.1)	24 (7.0)
ECOG performance status-n (%)			
0	144 (62.6)	81 (73.0)	225 (66.0)
1	86 (37.4)	28 (25.2)	114 (33.4)
Missing	0	2 (1.8)	2 (0.6)
Visceral disease-n (%)			
Yes	123 (53.5)	70 (63.1)	193 (56.6)
No	107 (46.5)	41 (36.9)	148 (43.4)
Lung and/or Liver Metastases-n (%)			
Present	110 (47.8)	60 (54.1)	170 (49.9)
Absent	120 (52.2)	51 (45.9)	171 (50.1)
Prior CDK4/6 Inhibitor Usage-n (%)			
Prior use	15 (6.5)	5 (4.5)	20 (5.9)
No prior use	215 (93.5)	106 (95.5)	321 (94.1)
Prior chemotherapy use-n (%)			
Adjuvant	101 (43.9)	60 (54.1)	161 (47.2)
Neoadjuvant	34 (14.8)	12 (10.8)	46 (13.5)
No prior use	94 (40.9)	39 (35.1)	133 (39.0)
Missing	1 (0.4)	0	1 (0.3)
Prior tamoxifen use-n (%)			
Yes	86 (37.4)	35 (31.5)	121 (35.5)
No	144 (62.6)	76 (68.5)	220 (64.5)
Estrogen receptor status-n (%)			
Positive	229 (99.6)	110 (99.1)	339 (99.4)

Baseline characteristics	FoundationOne® Liquid CDx		
	Evaluable N=230	Non-evaluable N=111	All N=341
Negative	1 (0.4)	1 (0.9)	2 (0.6)
Progesterone receptor status-n (%)			
Positive	165 (71.7)	87 (78.4)	252 (73.9)
Negative	61 (26.5)	23 (20.7)	84 (24.6)
Missing	4 (1.7)	1 (0.9)	5 (1.5)
Patient population based on endocrine status and line of therapy-n (%)			
First line endocrine sensitive (1)	26 (11.3)	13 (11.7)	39 (11.4)
First line endocrine resistant (2)	91 (39.6)	47 (42.3)	138 (40.5)
Second line (progression following (neo)adjuvant/ metastatic treatment) (3)	35 (15.2)	11 (9.9)	46 (13.5)
Second line (progression following metastatic treatment only) (4)	55 (23.9)	32 (28.8)	87 (25.5)
Other	23 (10.0)	8 (7.2)	31 (9.1)
Number of metastatic sites-n (%)			
<3	161 (70.0)	73 (65.8)	234 (68.6)
≥3	69 (30.0)	38 (34.2)	107 (31.4)

(1) Patients relapsed with documented evidence of progression more than 12 months from completion of (neo)adjuvant endocrine therapy with no treatment for metastatic disease.

(2) Patients relapsed with documented evidence of progression while on (neo) adjuvant endocrine therapy or within 12 months from completion of (neo)adjuvant endocrine therapy with no treatment for metastatic disease.

(3) Patients relapsed with documented evidence of progression more than 12 months from completion of (neo)adjuvant endocrine therapy and then subsequently progressed with documented evidence of progression while on or after only one line of endocrine therapy for metastatic disease.

(4) Patients with newly diagnosed advanced breast cancer, then relapsed with documented evidence of progression while on or after only one line of endocrine therapy.

All percentages calculated using N as the denominator

Table 26. Comparison of demographic and baseline clinical characteristics between the CDx-evaluable patients and the CDx non-evaluable patients in the *PIK3CA*(-) patients based on the enrolled results (primary analysis set)

Baseline characteristics	FoundationOne® Liquid CDx		
	Evaluable N=129	Non-evaluable N=96	All N=225
Age (years)			
n	129	96	225
Mean (SD)	63.8 (9.01)	60.6 (10.39)	62.4 (9.73)
Median	64.0	60.0	63.0
Q1-Q3	58.0 – 70.0	55.0 – 67.0	57.0 – 69.0
Min-Max	40 – 88	32 – 82	32 – 88
Sex-n (%)			
Female	129 (100.0)	96 (100.0)	225 (100.0)
Race-n (%)			
White	79 (61.2)	68 (70.8)	147 (65.3)
Black or African American	3 (2.3)	1 (1.0)	4 (1.8)
Asian	31 (24.0)	19 (19.8)	50 (22.2)
American Indian or Alaska Native	2 (1.6)	0	2 (0.9)
Other	4 (3.1)	4 (4.2)	8 (3.6)

Baseline characteristics	FoundationOne® Liquid CDx		
	Evaluable N=129	Non-evaluable N=96	All N=225
Unknown	10 (7.8)	4 (4.2)	14 (6.2)
Region-n (%)			
Europe	64 (49.6)	57 (59.4)	121 (53.8)
North America	20 (15.5)	3 (3.1)	23 (10.2)
Asia	30 (23.3)	19 (19.8)	49 (21.8)
Latin America	6 (4.7)	6 (6.3)	12 (5.3)
Other	9 (7.0)	11 (11.5)	20 (8.9)
ECOG performance status-n (%)			
0	83 (64.3)	77 (80.2)	160 (71.1)
1	46 (35.7)	18 (18.8)	64 (28.4)
Missing	0	1 (1.0)	1 (0.4)
Visceral disease-n (%)			
Yes	84 (65.1)	51 (53.1)	135 (60.0)
No	45 (34.9)	45 (46.9)	90 (40.0)
Lung and/or Liver Metastases-n (%)			
Present	66 (51.2)	43 (44.8)	109 (48.4)
Absent	63 (48.8)	53 (55.2)	116 (51.6)
Prior CDK4/6 Inhibitor Usage-n (%)			
Prior use	11 (8.5)	4 (4.2)	15 (6.7)
No prior use	118 (91.5)	92 (95.8)	210 (93.3)
Prior chemotherapy use-n (%)			
Adjuvant	68 (52.7)	49 (51.0)	117 (52.0)
Neoadjuvant	13 (10.1)	16 (16.7)	29 (12.9)
No prior use	48 (37.2)	31 (32.3)	79 (35.1)
Prior tamoxifen use-n (%)			
Yes	46 (35.7)	36 (37.5)	82 (36.4)
No	83 (64.3)	60 (62.5)	143 (63.6)
Estrogen receptor status-n (%)			
Positive	128 (99.2)	95 (99.0)	223 (99.1)
Negative	1 (0.8)	1 (1.0)	2 (0.9)
Progesterone receptor status-n (%)			
Positive	97 (75.2)	70 (72.9)	167 (74.2)
Negative	28 (21.7)	25 (26.0)	53 (23.6)
Missing	4 (3.1)	1 (1.0)	5 (2.2)
Patient population based on endocrine status and line of therapy- n (%)			
First line endocrine sensitive (1)	16 (12.4)	15 (15.6)	31 (13.8)
First line endocrine resistant (2)	52 (40.3)	46 (47.9)	98 (43.6)
Second line (progression following (neo)adjuvant/ metastatic treatment) (3)	27 (20.9)	7 (7.3)	34 (15.1)
Second line (progression following metastatic treatment only) (4)	26 (20.2)	19 (19.8)	45 (20.0)
Other	8 (6.2)	9 (9.4)	17 (7.6)
Number of metastatic sites-n (%)			
<3	76 (58.9)	71 (74.0)	147 (65.3)
≥3	53 (41.1)	25 (26.0)	78 (34.7)

(1) Patients relapsed with documented evidence of progression more than 12 months from completion of (neo)adjuvant endocrine therapy with no treatment for metastatic disease.

(2) Patients relapsed with documented evidence of progression while on (neo) adjuvant endocrine therapy or within 12 months from completion of (neo)adjuvant endocrine therapy with no treatment for

metastatic disease.
(3) Patients relapsed with documented evidence of progression more than 12 months from completion of (neo)adjuvant endocrine therapy and then subsequently progressed with documented evidence of progression while on or after only one line of endocrine therapy for metastatic disease.
(4) Patients with newly diagnosed advanced breast cancer, then relapsed with documented evidence of progression while on or after only one line of endocrine therapy.
All percentages calculated using N as the denominator

A multivariate Cox regression analysis was used to identify covariates that were associated with PFS in the SOLAR-1 mutant and non-mutant cohorts. Cox regression models were fit with the SOLAR-1 full analysis dataset (FAS) and stratified by the presence of lung and/or liver metastases and previous treatment with any CDK4/6 inhibitor. Using a 20% significance level, an analysis of all potentially relevant covariates was assessed between the *PIK3CA*-positive and *PIK3CA*-negative cohorts. For the *PIK3CA*-positive cohort, 9 clinically relevant covariates were identified (i.e., geographical region, number of metastatic sites, race, endocrine status and lines of therapy, prior chemotherapy, ECOG status, age, presence of bone lesions, and visceral disease) whereas only the first 4 were were found to be relevant in the *PIK3CA*-negative cohort.

The imbalance on the identified clinically relevant covariates were checked between the CDx-evaluable patients and the CDx non-evaluable patients in the mutant cohort and non-mutant cohort. The covariate imbalance between the CDx-evaluable and CDx non-evaluable sets were assessed individually for each covariate. Fisher's exact test was used for categorical covariates. The comparison of identified clinically relevant covariates between the CDx-evaluable patients and CDx non-evaluable patients in the mutant cohort were not found to be significantly imbalanced for most identified clinical covariates, except that ECOG performance status was imbalanced between the CDx-evaluable patients and CDx non-evaluable patients. In the non-mutant cohort, age, geographical region, ECOG performance status, and number or metastatic sites were found to be imbalanced at a significant level (<0.05).

The propensity score, defined as the probability of missing CDx results conditional on the PFS, censoring information, and the identified clinically relevant covariates were also calculated within the *PIK3CA*-positive cohort and the *PIK3CA*-negative cohort. A comparison of the distribution of propensity scores between the CDx-evaluable patients and the CDx non-evaluable patients are shown for the mutation positive cohort, and the mutation negative cohort. The propensity scores showed good overlap between the two groups of patients, which lended support to assume missing at random when handling missing CDx values in the subsequent analyses via multiple imputation.

4. Safety and Effectiveness Results

a. Safety Results

The safety with respect to treatment with alpelisib was addressed in the original drug approval and is summarized in the alpelisib NDA 212526. Refer to Drugs@FDA for safety information on alpelisib.

b. Effectiveness Results

i. Clinical Bridging Study Results (primary analysis for all samples with DNA input ≥ 30 ng)

There were 375 subjects with ≥ 30 ng DNA tested by FoundationOne® Liquid CDx. Excluding those with invalid results for either CTA2 or CDx (4, 12, respectively), the primary efficacy analyses were conducted using data from the 359 subjects who were CTA2-evaluable and CDx-evaluable. A concordance analysis was conducted with the CTA2-evaluable and FoundationOne® Liquid CDx-evaluable samples as summarized in Table 27.

Table 27: Concordance between FoundationOne® Liquid CDx and CTA2 (samples with ≥ 30 ng DNA)

		CTA2			
		Pos	**Neg**	**Invalid**	**Total**
FoundationOne® Liquid CDx	**Pos**	165	0	1	166
	Neg	65	129	3	197
	Invalid	7	5	0	12
	Total	237	134	4	375

Samples not tested are excluded from the analysis.
Samples tested with DNA input < 30 ng are excluded from the analysis.

The point estimates of PPA and NPA between FoundationOne® Liquid CDx and the CTA2 assay and the corresponding 95% confidence intervals were:

- PPA (95% CI): 71.7% (65.4%, 77.5%)
- NPA (95% CI): 100% (97.2%, 100%)

The primary efficacy analysis in the *PIK3CA*-positive population identified by FoundationOne® Liquid CDx was based on PFS by local investigator assessment per RECIST 1.1 criteria. Clinical efficacy of alpelisib in combination with fulvestrant for the FoundationOne® Liquid CDx-positive population with DNA input ≥30 ng (N=165) was demonstrated with an estimated 54% risk reduction in disease progression or death in the alpelisib plus fulvestrant arm compared to the placebo plus fulvestrant arm [Hazard Ratio (HR) = 0.46, 95% CI: 0.30, 0.70].

A sensitivity analysis was performed to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne® Liquid

CDx results was performed using the multivariate imputation by chained equations (MICE) method using the CTA2 results and all clinically relevant covariates described in the Section X.A.3., above. After imputing the missing FoundationOne® Liquid CDx results, the hazard ratio was estimated to be 0.63 (95% CI: 0.45, 0.87) which is similar to that [HR = 0.65 (95% CI: 0.50, 0.85) observed in the SOLAR-1 *PIK3CA*-positive population.

ii. Concordance Study Results (secondary analysis for all samples with DNA input ≥ 20 ng)

There were 432 subjects with ≥ 20 ng DNA tested by FoundationOne® Liquid CDx. Excluding those with invalid results for either CTA2 or CDx (5, 17, respectively), the secondary concordance analyses were conducted using data from the 410 subjects who were CTA2-evaluable and CDx-evaluable as summarized in Table 28.

Table 28: Concordance between FoundationOne® Liquid CDx and CTA2 (samples with ≥ 20 ng DNA)

		CTA2			
		Pos	**Neg**	**Invalid**	**Total**
FoundationOne® Liquid CDx	**Pos**	185	0	1	186
	Neg	77	148	4	229
	Invalid	9	8	0	17
	Total	271	156	5	432

Samples not tested are excluded from the analysis.

The point estimates of PPA and NPA between FoundationOne® Liquid CDx and the CTA2 assay (excluding invalid results) and the corresponding 95% confidence intervals were:

- PPA (95% CI): 70.6% (64.7%, 76.1%)
- NPA (95% CI): 100% (97.5%, 100%)

The concordance between FoundationOne® Liquid CDx and CTA2 from the secondary analysis (samples with ≥ 20 ng DNA) is comparable to the results from the primary analysis (samples with ≥ 30 ng DNA).

c. Effectiveness Conclusions

The clinical effectiveness of FoundationOne® Liquid CDx as a companion diagnostic to identify breast cancer patients with *PIK3CA*-mutation positive for treatment with alpelisib was demonstrated using plasma samples from the SOLAR-1 study.

Concordance of the FoundationOne® Liquid CDx with the CTA2 assay was demonstrated with the CDx-evaluable population. Clinical utility of the FoundationOne® Liquid CDx was demonstrated by estimation of clinical

efficacy in the CDx *PIK3CA* mutation positive population based on PFS as assessed by the local investigator assessment per RECIST 1.1 criteria. Secondary analysis for estimation of clinical efficacy in the CDx *PIK3CA* mutation negative population demonstrated similar results to the SOLAR-1 efficacy analyses in the *PIK3CA* non-mutant cohort. Sensitivity analysis against the missing CDx results demonstrated the robustness of the concordance analysis and efficacy analysis.

Clinical efficacy of alpelisib in combination with fulvestrant for the CDx plasma *PIK3CA*-positive population was demonstrated with an estimated 54% risk reduction in disease progression or death compared to placebo plus fulvestrant (HR=0.46, 95% CI: 0.30, 0.70). This compares favorably with PFS results in the CTA2-positive population (HR = 0.64, 95% CI: 0.48, 0.85) and in the SOLAR-1 *PIK3CA*-positive cohort as determined by the enrolling tissue assays (HR = 0.65; 95% CI: 0.50, 0.85). Sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne® Liquid CDx results was performed using the multiple imputation method. PPA was calculated assuming the proportion of CTA2-positive, CDx-missing patients (N = 117), that are CDx-positive (C) ranged from 0 to 100%. The PPA ranged from 47.6% (assuming all 117 CDx-missing results were discordant) to 81.3% (assuming all 117 CDx-missing result were concordant. NPA was calculated assuming the proportion of CDx-negative, CDx-missing patients (N = 90) that are CDx-negative (C) ranged from 0 to 100%. The NPA ranged from 58.9% (assuming all 90 CDx-missing results were discordant) to 100% (assuming all 90 CDx-missing results were concordant). After imputing the missing FoundationOne® Liquid CDx results, the HR was estimated to be 0.63 (0.45, 0.87).

The data provided showed that FoundationOne® Liquid CDx identified *PIK3CA*-positive breast cancer, treated with alpelisib, had a HR similar to that observed in the clinical study. These results support the clinical effectiveness of FoundationOne® Liquid CDx for the identification of *PIK3CA*-positive breast cancer treated with alpelisib.

Agreement between the tissue-based CTA (CTA2) and FoundationOne® Liquid CDx [PPA 70.6% (64.7%, 76.1%)]. Since FoundationOne® Liquid CDx failed to detect a significant proportion of the patients, a reflex testing using tissue specimens to an FDA approved tissue test will be required, if feasible, if the plasma test is negative.

d. Pediatric Extrapolation

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

B. Clinical Bridging Study: Detection of *ALK* Rearrangements to Determine Eligibility for Treatment with Alectinib

The clinical validity of using FoundationOne® Liquid CDx as a companion diagnostic to identify patients with non-small cell lung cancer (NSCLC) harboring *ALK* rearrangements for treatment with alectinib was assessed through a clinical bridging study using screening (i.e., pre-alectinib treatment) plasma samples from Cohort A of the Blood First Assay Screening Trial (BFAST, BO29554). Alectinib was approved under NDA 208434 on December 11, 2015.

The BFAST trial is a Phase II/III multicenter study, evaluating the safety and efficacy of alectinib as a treatment for patients with advanced or metastatic NSCLC who tested positive for an *ALK* rearrangement as determined by a blood-based NGS assay (FoundationACT, FACT) under IDE # G170102.

1. Study Design

BFAST was a Phase II/III, global, multicenter, open-label, multi-cohort study designed to evaluate the safety and efficacy of targeted therapies or immunotherapy as single agents or in combination in patients with unresectable, advanced or metastatic NSCLC determined to harbor oncogenic somatic mutations (e.g., *ALK*, *RET*) or positive by tumor mutational burden (TMB) assay as identified by a blood-based NGS circulating tumor DNA (ctDNA) assay.

In the device bridging study, plasma samples from the BFAST clinical trial collected at baseline were tested with FoundationOne® Liquid CDx.

a. Clinical Inclusion and Exclusion Criteria

A bridging study was conducted to evaluate: 1) the concordance between *ALK* rearrangement status by the CTA and FoundationOne® Liquid CDx, and 2) the clinical efficacy of alectinib treatment in patients that would be eligible for therapy based on *ALK* rearrangement status as determined by FoundationOne® Liquid CDx.

Sample inclusion criteria:

- Samples from patients in the BFAST clinical trial collected prior to start of study treatment
- Availability of adequate sample to generate a CDx test result including ≥ 2.5 mL plasma volume

Sample exclusion criteria:

- Lack of clear subject identification or label on stored patient sample
- Obvious physical damage of stored patient sample
- Insufficient sample (< 2.5 mL)

b. Clinical Endpoints

The primary endpoint for the study was investigator-assessed ORR based on confirmed objective response (indicated by two objective response assessments based on RECIST v1.1. Safety were evaluated by assessment of incidence, type, and severity of adverse events (based on the NCI CTCAE v4.0), including SAEs and AEs of special interest.

2. Accountability of PMA Cohort

The bridging study included 287 samples from the BFAST trial: 87 *ALK*-positive samples determined with clinical trial assay (CTA) (FACT) from all patients enrolled in Cohort A and 200 CTA *ALK*-negative samples from patients not enrolled into Cohort A. Five samples were not available for testing and 12 samples did not meet the FoundationOne Liquid CDx test in process QC metrics. Valid CTA and FoundationOne® Liquid CDx results were available for 270 samples. An additional 21 samples with valid FoundationOne® Liquid CDx results had ≥ 20 ng and < 30 ng of DNA input. The final number of samples available for the primary analysis was 249.

The primary analyses included samples with DNA input mass ≥ 30 ng for FoundationOne® Liquid CDx. A secondary analysis was performed for all samples with DNA input mass ≥ 20 ng. The sample accountability for this clinical bridging study is summarized in Table 29.

Table 29: Sample accountability for alectinib clinical bridging study

Description	**# Samples**
Patients enrolled in Cohort A	87
CTA *ALK*-negative patients available (screen-failed or enrolled in other BFAST study cohorts based on other biomarkers)	200
Total samples available	287 (87 + 200)
Samples not available for testing	5
Samples that did not meet FoundationOne® Liquid CDx in-process QC metrics	12
Samples missing that were CTA positive	4
Total samples without a valid FoundationOne® Liquid CDx result	17 (5 + 12)
Total samples with valid FoundationOne® Liquid CDx result	270 (282 –17)
Samples with valid results and DNA input ≥ 20 ng and < 30 ng	21
Total samples included in the primary analysis (samples with DNA input mass ≥ 30 ng)	249 (270 – 21)

3. Safety and Effectiveness Results

a. Safety Results

The safety with respect to treatment with alectinib was addressed in the original drug approval and is summarized in the alectinib NDA 208434. Refer to Drugs@FDA for safety information on alectinib.

b. Effectiveness Results

i. Clinical Bridging Study Results (primary analysis for all samples with DNA input ≥ 30 ng)

The concordance between FoundationOne® Liquid CDx and the CTA was evaluated as summarized in Table 30.

Table 30: Concordance between FoundationOne® Liquid CDx and the CTA for the detection of *ALK* rearrangements (samples with ≥ 30 ng of DNA)

		CTA		
		Pos	**Neg**	**Total**
FoundationOne® Liquid CDx	**Pos**	63	0	63
	Neg	12	174	186
	Missing	4	9	13
	Total	79	183	262

The PPA and NPA between FoundationOne® Liquid CDx and the CTA using the CTA as the reference for the primary analysis set and the corresponding 95% confidence intervals were:

- PPA (95% CI): 84.0% (73.7%, 91.4%)
- NPA (95% CI) : 100% (97.9%, 100.0%)

After adjusting for a 5% prevalence of *ALK* rearrangements in the intended use population, the Positive Predictive Value (PPV), and Negative Predictive Value (NPV) calculated using the CTA as the reference and the corresponding 95% confidence intervals were:

- PPV [63/(63+0)] (95% CI): 100.0% (94.3%, 100.0%)
- NPV [174/(174+12)] (95% CI): 93.5% (89%, 96.6%)

The median Overall Response Rate (ORR) and the corresponding 95% confidence intervals was 88.9% (78.4%, 95.4%) for the FoundationOne® Liquid CDx *ALK*-positive population which is comparable with the observed ORR and the corresponding 95% confidence intervals of 87.4% (78.5%, 93.5%) for the CTA *ALK*-positive population (BFAST Cohort A).

To account for enrolled patients with missing FoundationOne® Liquid CDx results (13.8%, 12 of 87), a sensitivity analysis was performed using a univariate logistic-model where the CTA result, clinical

outcome, and any baseline characteristic covariates (race, histology, stage, baseline CNS metastases, tobacco use, and tissue availablity),. The sensitivity analysis for the concordance between FoundationOne® Liquid CDx and the CTA demonstrated:

- PPA (95% CI): 83.9% (74.5%, 90.9%)
- NPA (95% CI): 100.0% (97.9 %,100.0%)
- Adjusted PPV (95% CI): 100.0% (95.1 v%, 100.0%)
- Adjusted NPV (95% CI): 92.6% % (87.8%, 95.9 %)

A sensitivity analysis was performed to estimate the clinical efficacy of treating patients with alectinib. The median of ORR for the CTA-positive/ FoundationOne® Liquid CDx-positive (CTA+/F1L CDx+) population across the 100 imputed datasets is 90.5%, which is comparable to ORR 88.9% in observed data of 63 CTA+ and F1L CDx+ patients.

ii. Clinical Bridging Study Results (secondary analysis for all samples with DNA input ≥ 20 ng)

A second analysis was performed based on the inclusion of all samples with cfDNA input of input ≥ 20 ng which is summarized in Table 31, below.

Table 31: Concordance between FoundationOne® Liquid CDx and the CTA for the detection of *ALK* rearrangements (samples with ≥ 20 ng of DNA)

		CTA		
		Pos	**Neg**	**Total**
FoundationOne® Liquid CDx	**Pos**	69	0	69
	Neg	14	187	201
	Missing	4	13	17
	Total	87	200	287

PPA and NPA between FoundationOne® Liquid CDx and the CTA using the CTA as the reference for the primary analysis set were:

- PPA (95% CI): 83.1% (73.3%, 90.5%)
- NPA (95% CI): 100.0% (98.0%, 100.0%)

The median ORR was 89.9% (80.2%, 95.8%) for the FoundationOne® Liquid CDx *ALK*-positive population (samples with ≥ 20 ng of DNA) which is comparable with the observed ORR of 87.4% (78.5%, 93.5%) for the CTA *ALK*-positive population (BFAST Cohort A).

To account for enrolled patients with missing FoundationOne® Liquid CDx results (4.6%, 4 of 87), a sensitivity analysis was performed. The sensitivity analysis for the concordance between FoundationOne® Liquid CDx and the CTA resulted in:

- PPA (95% CI): 83.9% (74.5%, 90.9%)
- NPA (95% CI): 100.0% (97.9%, 100%)

A sensitivity analysis was performed to estimate the clinical efficacy of treating patients with alectinib using the same covariates as the primary analysis. The estimated ORR and the corresponding 95% confidence intervals were 90.4% (90.2%, 90.4%) for the patient population that are both CTA *ALK*+ and FoundationOne® Liquid CDx *ALK*+, which is comparable to the primary analyses results.

This clinical bridging study demonstrated an ORR of 89.9% (95% CI: 80.2%, 95.8%) for the FoundationOne® Liquid CDx *ALK*-positive population, demonstrating the clinical validity of using FoundationOne® Liquid CDx as a CDx for alectinib. The ORR estimated from this bridging study is comparable to the ORR of 87.4% observed in the CTA *ALK*-positive population.

The CTA used to enroll patients into the BFAST study was the Foundation Medicine FACT (FACT) assay, a precursor to the FoundationOne® Liquid CDx test. Therefore the expectation was that the bridging study PPA should have been higher. The reasons underlying the discordant calls for the 14 samples positive by the FACT assay but negative by the FoundationOne® Liquid CDx (FACT+/F1L CDx-) are primarily due to rearrangements not being detected by the pipeline (N=4), insufficient reads (N=5), detected but filtered out for not passing the in-process QC metrics (N=3) and being removed during curation as an artifact (N=1) or due to contamination (N=1). Twelve (12) of the 14 samples included in the primary analysis set (≥30ng DNA) in which 2 samples had a library construction (LC) input < 30 ng. Four of the 14 calls positive by the FACT assay had no evidence of an *ALK*-rearrangement detected by the FoundationOne® Liquid CDx pipeline, while the remainder were detected but filtered out for not passing the in-process QC metrics. Of the four samples with no evidence of an ALK rearrangement observed, three had an F1L CDx tumor fraction of 0, while one did not meet the expected coverage-based QC threshold.

iii. Analysis to support the reflexing of patients who are *ALK*(-) by the FoundationOne® Liquid CDx test to an FDA approved *ALK* tumor tissue test:
To support the recommendation to reflex NSCLC patients identified as *ALK*(-) by the FoundationOne® Liquid CDx to an FDA approved *ALK*

tumor tissue test, data from a concordance study between a validated NGS plasma based assay and an FDA approved tumor tissue test was provided using samples from a Phase III clinical trial. The ALEX trial was a randomized, active-controlled, multicenter Phase III open-label study in patients (≥ 18 years old) with treatment-naive anaplastic lymphoma kinase (*ALK*)-positive advanced/recurrent or metastatic NSCLC with histologically or cytologically confirmed *ALK* rearrangements in pretreatment tumor tissue by immunohistochemistry (IHC) using the VENTANA *ALK* (D5F3) IHC test. In this study patients were randomized 1:1 into one of two treatment arms (alectinib or crizotinib). Patients were treated until disease progression, unacceptable toxicity, withdrawal of consent, or death.

A total of 303 patients were randomized to the ALEX study, 151 patients to the crizotinib arm, and 152 patients to the alectinib arm. These patients were included in the Intent-to-Treat (ITT) population. All randomized patients received at least one dose of alectinib or crizotinib based on their randomized cohort. All patients in the ITT had *ALK*-positive NSCLC according to central analysis by the FDA approved VENTANA *ALK* (D5F3) IHC test. All patients in the ITT had measurable disease at baseline according to the investigator per inclusion criteria and were therefore included in the Investigator Response Evaluable Population.

The results of an exploratory retrospective analysis were provided to demonstrate the PPA between the VENTANA *ALK* (D5F3) IHC test and *ALK* status in cfDNA from plasma assessed by a targeted NGS test, the Foundation Assay for Circulating Tumor DNA™ (FACT), a precursor to the FoundationOne® Liquid CDx test. A total of 149 patients were included in the bridging study (73 in the crizotinib and 76 in the alectinib treatment arm). All patients in the bridging study had *ALK*-positive NSCLC according to central analysis by VENTANA *ALK* (D5F3) IHC test on tissue and were either *ALK*-positive or *ALK*-negative according to plasma FACT test.

Plasma *ALK*-positive population included 105 patients (52 in the crizotinib and 53 in the alectinib treatment arm) in the bridging study who were *ALK*-positive according to the plasma FACT test. Plasma *ALK*-negative population included 44 patients (21 in the crizotinib and 23 in the alectinib treatment arm) in the bridging study who were *ALK*-negative according to the plasma FACT test.

Since all patients enrolled in the study (ITT population) had *ALK*-positive NSCLC according to central analysis by VENTANA *ALK* (D5F3) IHC test as per protocol inclusion criteria, only PPA could be evaluated. Exploratory concordance PPA between *ALK*-positive by IHC

and *ALK*-positive by plasma was 70.5% (62.5, 77.7), and was comparable between the 2 treatment arms, 71.2% (59.6, 81.2) in the crizotinib and 69.7% (58.1, 79.8) in the alectinib arm, respectively.

The poor agreement between the FDA approved VENTANA (D5F3) *ALK* IHC assay and the Foundation Medicine FACT assay supposts the reflex recommendations for plasma negative samples to an FDA-approved tissue test.

c. Effectiveness Conclusions

The clinical effectiveness of FoundationOne®Liquid CDx as a companion diagnostic to identify patients with NSCLC harboring *ALK* rearrangements for treatment with alectinib was demonstrated using plasma samples from the BFAST study.

Samples from all 87 patients in Cohort A were included in this study with 12 (4 samples with missing FoundationOne® Liquid CDx test results and 8 additional samples with DNA input < 30ng) missing FoundationOne® Liquid CDx results which were imputed in the sensitivity analysis; 200 *ALK*-negative samples from the BFAST trial were also included with 26 (13 samples with missing FoundationOne® Liquid CDx results and 13 additional samples with DNA input <30ng) samples missing FoundationOne® Liquid CDx results which were also imputed in the sensitivity analysis. A total of 249 samples were included in the primary analyses for this bridging study. The observed concordance between FoundationOne® Liquid CDx and CTA results based on samples with a cfDNA input of ≥30 ng only (n=249) with a PPA of 84.0% [63 of 75] (73.7%, 91.4%) and an NPA of 100.0% [174 of 174] (97.9%, 100.0%). After adjusting for the prevalence of *ALK* rearrangements at 5%, PPV was determined to be 100.0% (95.1%, 100.0%) and NPV was determined to be 93.5 % (89%, 96.6%). The 95% Cl for PPV was computed based on the lower bounds of PPA and NPA; the 95% Cl for NPV is based on 20,000 bootstrap samples.

This clinical bridging study demonstrated an ORR of 88.9%, 95% two-sided Cl (78.4%,95.4%) for the FoundationOne® Liquid CDx *ALK*-positive population, demonstrating the clinical validity of using FoundationOne® Liquid CDx as a CDx for alectinib. The ORR estimated from this bridging study is comparable to the ORR of 87.4% observed in the CTA *ALK*-positive population. The sensitivity analyses demonstrated that the PPA was 83.9% (74.5%, 90.9%), the NPA was 100% (97.7%, 100.0%), the adjusted PPV was 100.0% (95.1%, 100.0%), and the adjusted NPV was 92.6 % (87.8%, 95.9%). The missing data sensitivity analysis of the clinical efficacy for the CTA *ALK*-positive and FoundationOne® Liquid CDx *ALK*-positive group resulted in a median ORR of 90.5% (90.1 %, 90.7%). The sensitivity analysis for the CTA *ALK*-positive and FoundationOne® Liquid CDx *ALK*-negative group resulted in an ORR of 71.4% (66.7%, 75%). The missing data sensitivity analysis results were comparable to the observed results, demonstrating the robustness of the analysis results.

While all patients enrolled into Cohort A of the BFAST study were enrolled based on plasma specimens, the concordance between patients identified between *ALK*-rearrangment positive tumor tissue specimens (by an FDA-approved *ALK* tumor tissue test using IHC) and plasma was not known. Therefore concordance between the two sample types was evaluated using a similar patient population of patients enrolled into a separate clinical study (ALEX) that were enrolled based on tumor tissue results and plasma samples collected from those patients were retrospectively tested with the Foundation Medicine FACT test, a predessor of the FoundationOne® Liquid CDx test. The observed PPA between *ALK*-positive by IHC and *ALK*-positive by plasma was 70.5% (62.5, 77.7), and was comparable between the studies 2 treatment arms, 71.2% (59.6, 81.2). The observed results demonstrated that NSCLC patients with tumors harboring *ALK* rearrangements would be missed if only plasma results were used, did not support a standalone CDx claim and that NSCLC patients with *ALK*-negative results should be reflexed to an FDA-approved *ALK* tumor tissue based companion diagnostic test.

The data provided showed that FoundationOne® Liquid CDx identified NSCLC *ALK*-rearrangement positive NSCLC, treated with alectinib, had a ORR similar to that observed in the clinical study. These results support the clinical effectiveness of FoundationOne® Liquid CDx for the identification of *ALK*-positive NSCLC treated with alectinib.

d. Pediatric Extrapolation

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

C. Clinical Bridging Study: Detection of *BRCA1* and *BRCA2* Alterations to Determine Eligibility of Ovarian Cancer Patients for Treatment with Rucaparib

The clinical performance of FoundationOne® Liquid CDx as a companion diagnostic to identify patients with ovarian cancer harboring *BRCA1* or *BRCA2* alterations for treatment with rucaparib was demonstrated using pre-rucaparib treatment blood samples from the ARIEL2 study.

Rucaparib is approved in the United States (US) for the treatment of adult patients with deleterious breast cancer gene (*BRCA*) alteration (germline and/or somatic)-associated epithelial ovarian (EOC), fallopian tube (FTC) or primary peritoneal (PPC) cancer who have been treated with 2 or more prior chemotherapies. Clinical data supporting this indication (NDA 209115) was pooled from CO-338-010 (Study 10) and CO-338- 017 (ARIEL2); however, no samples from Study 10 were included in this bridging study

because no blood samples were collected. Rucaparib was approved under NDA 208434 on December 19, 2016.

The bridging study was conducted to evaluate: 1) the concordance between *BRCA1* and *BRCA2* alteration status by the CTA and FoundationOne® Liquid CDx, and 2) the clinical efficacy of rucaparib treatment in patients that would be eligible for therapy based on *BRCA1* and *BRCA2* alteration status as determined by FoundationOne® Liquid CDx.

1. Study Design

The primary basis for the safe and efficacious use of rucaparib as monotherapy for the treatment of ovarian cancer are results from the 491 patients (All Patients) enrolled in the ARIEL2 study.

ARIEL2 was a two-part, single-arm, open-label Phase 2 efficacy study of oral rucaparib in patients with relapsed high-grade serous or endometrioid epithelial ovarian, fallopian tube, or primary peritoneal cancer.

- The primary efficacy population is comprised of 106 patients with *BRCA1* and/or *BRCA2* alterations ("*BRCA* positive" by CTA).
- All patients includes a total of 491 patients with both *BRCA* positive patients (n=124) and *BRCA* negative patients (n=367). The *BRCA* negative patients are used for part of the bridging study.

a. Clinical Inclusion and Exclusion Criteria

In both parts of the ARIEL2 study, patients were aged ≥ 18 years, had Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1, and had adequate organ function. All patients were required to provide tumor tissue for central genomic testing by Foundation Medicine (CTA refers to these test results).

Part 1

The study initially enrolled patients with relapsed ovarian cancer who had received at least 1 prior platinum-based regimen and had platinum-sensitive disease following their most recent platinum regimen. All patients in this portion of the study were required to have disease that could be biopsied prior to treatment as well as measurable disease that could be assessed by RECIST Version 1.1. Enrollment of patients known to harbor a deleterious germline *BRCA1* or *BRCA2* alteration was limited to 15.

Part 2

Upon completion of enrollment into Part 1, the protocol was amended to evaluate rucaparib in ovarian cancer patients who had received at least 3, but no more than 4, prior chemotherapy regimens. Part 2 of the study enrolled ovarian cancer patients who were resistant or refractory, as well as patients

who were sensitive, to their last platinum-based regimen. Patients who were resistant or refractory to their last platinum may have received nonplatinum based chemotherapy before initiating treatment with rucaparib. All patients were required to provide an archival tumor tissue sample and have measurable disease that could be assessed by RECIST Version 1.1. Like Part 1, patients were required to have disease that could be biopsied prior to treatment; however, an exception was made for patients who were known to harbor a known deleterious *BRCA1* or *BRCA2* alteration.

b. Clinical Endpoints

The primary efficacy endpoint evaluated for ARIEL2 in this PMA was:

- Confirmed ORR per RECIST v1.1 by Investigator

Secondary endpoints include:

- Duration of confirmed response (DOR)

2. Accountability of PMA Cohort

The ARIEL2 study is complete and enrolled 491 patients (All Patients). Pre-rucaparib treatment plasma samples were available for 55% (271/491) of patients dosed in ARIEL2. FoundationOne® Liquid CDx data were available for 80% (217/271) of the patients with samples tested; 49 failures were due to insufficient remaining plasma volume or insufficient DNA extraction yield. In total, FoundationOne® Liquid CDx results were available for 44% (217/491) of All Patients.

Of the 106 patients from the ARIEL2 drug primary efficacy population, only 42% (27/64) of the 64 BRCA positive samples, as defined by the original clinical trial's tissue-based CTA, were available for testing with the FoundationOne® Liquid CDx test and included in the bridging study. The sample accountability for this clinical validation study is summarized in Table 32.

Table 32: Sample accountability for rucaparib ovarian clinical bridging study

Description	Number
All Patients	491
Total samples available	271
Patients with FoundationOne® Liquid CDx data (All Patients)	217
Patients with FoundationOne® Liquid CDx data (device Primary Efficacy Population)	27

Study Population Demographics and Baseline Parameters

The key demographics and baseline characteristics for *BRCA* Positive, *BRCA* Negative, and *BRCA* Unknown patients based on FoundationOne® Liquid CDx results in the Primary Efficacy Population and All Patients are shown in Table 33. In general, there does not appear to be any clinically significant differences in demographics variables across the *BRCA* status subgroups (including patients with Known and Unknown *BRCA* status). However, there are a few exceptions,

such as the number of prior chemotherapy regimens, which is lower for the *BRCA* Unknown compared to those patients with *BRCA* Known subgroup. The progression-free interval after most recent platinum regimen was longer, and the percentage of patients with a platinum status of sensitive is higher in the *BRCA* Unknown subgroup compared to those with *BRCA* Known subgroup. These variables are linked to the enrollment criteria for each study part, and a higher proportion of samples were missing from the earlier study part (ARIEL2 Part 1) compared to the later study part (ARIEL2 Part 2); therefore, these results are expected.

Table 33: Baseline Demographics and Clinical Characteristics from ARIEL2 Primary Efficacy Population vs. All ARIEL2 Patients Tested by the FoundationOne® Liquid CDx

	FoundationOne® Liquid CDx						
	Device Primary Efficacy Population				**All Patients**		
	BRCA Pos. (N=26)	**BRCA Neg. (N=1)**	**BRCA Unkn. (N=37)**	**Total (N=64)**	***BRCA* Pos. N = 64**	***BRCA* Neg. N = 153**	***BRCA* Unkn. N = 274**
Age (years), n (%)							
Median, n (range)	60.5 (41.0%, 80.0%)	62.0 (62.0%, 62.0%)	57.0 (33.0%, 79.0%)	59.5 (33.0%, 80.0%)	62 (41.0%, 82.0%)	63 (41.0%, 86.0%)	63 (31.0%, 91.0%)
≤ 50	5 (19.2%)	0	12 (32.4%)	17 (26.6%)	8 (12.5%)	17 (11.1%)	33 (12.0%)
51-60	8 (30.8%)	0	9 (24.3%)	17 (26.6%)	23 (35.9%)	36 (23.5%)	78 (28.5%)
61-70	10 (38.5%)	1 (100.0%)	11 (29.7%)	22 (34.4%)	25 (39.1%)	59 (38.6%)	96 (35.0%)
71-80	3 (11.5%)	0	5 (13.5%)	8 (12.5%)	6 (9.4%)	34 (22.2%)	63 (23.0%)
81-90	0	0	0	0	2 (3.1%)	7 (4.6%)	3 (1.1%)
>90	0	0	0	0	0	0	1 (0.4%)
Race							
Asian	4 (15.4%)	0	1 (2.7%)	5 (7.8%)	4 (6.3%)	12 (7.8%)	11 (4.0%)
Black or African American	0	0	1 (2.7%)	1 (1.6%)	0	1 (0.7%)	5 (1.8%)
White	19 (73.1%)	1 (100.0%)	28 (75.7%)	48 (75.0%)	47 (73.4%)	113 (73.9%)	211 (77.0%)
Other/unknown	0	0	0	0	13 (20.3%)	27 (17.6%)	47 (17.2%)
Missing	3 (11.5%)	0	7 (18.9%)	10 (15.6%)	0	0	0
ECOG at Baseline							
0	13 (50.0%)	1 (100.0%)	25 (67.6%)	39 (60.9%)	28 (43.8%)	70 (45.8%)	170 (62.0%)
1	13 (50.0%)	0	12 (32.4%)	25 (39.1%)	36 (56.3%)	81 (52.9%)	104 (38.0%)
≥ 2	0	0	0	0	0	2 (1.3%)	0
Type of Cancer							
Epithelial Ovarian	20 (76.9%)	1 (100.0%)	34 (91.9%)	55 (85.9%)	50 (78.1%)	122 (79.7%)	226 (82.5%)
Fallopian Tube	4 (15.4%)	0	1 (2.7%)	5 (7.8%)	10 (15.6%)	14 (9.2%)	17 (6.2%)
Primary Peritoneal	2 (7.7%)	0	2 (5.4%)	4 (6.3%)	4 (6.3%)	17 (11.1%)	31 (11.3%)
Other	0	0	0	0			
Histological Classification							
Endometrioid	1 (3.8%)	0	1 (2.7%)	2 (3.1%)	1 (1.6%)	8 (5.2%)	7 (2.6%)
Mixed	2 (7.7%)	0	0	2 (3.1%)	2 (3.1%)	5 (3.3%)	2 (0.7%)
Serous	23 (88.5%)	1 (100.0%)	36 (97.3%)	60 (93.8%)	61 (95.3%)	140 (91.5%)	265 (96.7%)
Number of Prior Chemotherapy Regimens							
Median, n (range)	3.0 (3.0%, 5.0%)	6.0 (6.0%, 6.0%)	3.0 (2.0%, 4.0%)	3.0 (2.0%, 6.0%)	3 (1%, 5%)	3 (1%, 6%)	2 (1%, 5%)
1	0	0	0	0	4 (6.3%)	20 (13.1%)	95 (34.7%)
2	0	0	14 (37.8%)	14 (21.9%)	0	7 (4.6%)	47 (17.2%)
3	19 (73.1%)	0	14 (37.8%)	33 (51.6%)	37 (57.8%)	83 (54.2%)	90 (32.8%)
>3	7 (26.9%)	1 (100.0%)	9 (24.3%)	17 (26.6%)	23 (35.9%)	43 (28.1%)	42 (15.3%)

	FoundationOne® Liquid CDx						
	Device Primary Efficacy Population				All Patients		
	BRCA Pos. (N=26)	**BRCA Neg. (N=1)**	**BRCA Unkn. (N=37)**	**Total (N=64)**	***BRCA* Pos. N = 64**	***BRCA* Neg. N = 153**	***BRCA* Unkn. N = 274**
Number of Prior Platinum-Based Chemotherapy Regimens							
Median, n (range)	3.0 (2.0%, 5.0%)	5.0 (5.0%, 5.0%)	2.0 (2.0%, 4.0%)	3.0 (2.0%, 5.0%)	2.5 (1%, 5%)	2.0 (1%, 5%)	2.0 (1%, 4%)
1	0	0	0	0	6 (9.4%)	26 (17.0%)	103 (37.6%)
2	0	0	0	0	26 (40.6%)	63 (41.2%)	95 (34.7%)
3	12 (46.2%)	0	19 (51.4%)	31 (48.4%)	27 (42.2%)	58 (37.9%)	70 (25.5%)
>3	13 (50.0%)	0	15 (40.5%)	28 (43.8%)	5 (7.8%)	6 (3.9%)	6 (2.2%)
Progression-free interval to last platinum (months)							
Median, n (range)	4.9 (0.0, 26.5%)	8.1 (8.1, 8.1%)	8.3 (-0.7, 26.0%)	7.8 (-0.7, 26.5%)	4.9 (-1.0, 33.8%)	5.3 (-2.3, 71.5%)	8.6 (-0.8, 74.4%)
<6	16 (61.5%)	0	10 (27.0%)	26 (40.6%)	38 (59.4%)	85 (55.6%)	85 (31.0%)
≥ 6-12	6 (23.1%)	1 (100.0%)	17 (45.9%)	24 (37.5%)	17 (26.6%)	42 (27.5%)	90 (32.8%)
>12-24	3 (11.5%)	0	8 (21.6%)	11 (17.2%)	6 (9.4%)	19 (12.4%)	69 (25.2%)
>24	1 (3.8%)	0	2 (5.4%)	3 (4.7%)	3 (4.7%)	7 (4.6%)	30 (10.9%)
Platinum Status, n (%)							
Refractory	2 (7.7%)	0	5 (13.5%)	7 (10.9%)	4 (6.3%)	19 (12.4%)	25 (9.1%)
Resistant	14 (53.8%)	0	5 (13.5%)	19 (29.7%)	34 (53.1%)	66 (43.1%)	60 (21.9%)
Sensitive	10 (38.5%)	1 (100.0%)	27 (73.0%)	38 (59.4%)	26 (40.6%)	68 (44.4%)	189 (69.0%)

Abbreviations: *BRCA* = breast cancer gene, includes *BRCA1* and *BRCA2*, ECOG = Eastern Cooperative Oncology Group

3. Safety and Effectiveness Results

A bridging study was conducted to compare the performance of the FoundationOne® Liquid CDx assay to the clinical trial tissue assay that was used to enroll patients into the ARIEL2 clincial study. In addition to the concordance between these two tests, an analysis was performed to demonstrate the effectiveness of the FoundationOne® Liquid CDx test, to select patients for treatment with rucaparib.

a. Safety Results

The safety with respect to treatment with rucaparib was addressed in the original drug approval and is summarized in the rucaparib NDA 208434. Refer to Drugs@FDA for safety information on rucaparib.

b. Effectiveness Results

i. Concordance to tumor tissue CTA:

The concordance between FoundationOne® Liquid CDx and CTA tumor tissue test results was evaluated from the device Primary Efficacy Population and in All Patients enrolled into the ARIEL2 study are summarized in Table 34 and Table 35, respectively.

Table 34: Concordance between FoundationOne® Liquid CDx and the CTA for the detection of *BRCA1* or *BRCA2* alterations in the device primary efficacy population

		CTA		
		Pos	**Neg**	**Total**
FoundationOne® Liquid CDx	**Pos**	26	0	26
	Neg	0	1	1
	Missing	35	2	37
	Total	61	3	64

The PPA and NPA between FoundationOne® Liquid CDx and the CTA were determined using the CTA as the reference for the device Primary Efficacy Population:

- PPA (95% CI): 100% (86.8%, 100.0%)
- NPA (95% CI): 100% (2.5%, 100.0%)

Due to the low number of *BRCA1/BRCA2* negatives identified in the Primary Efficacy Population bridging study and because 80.8% of the patients included in the device Primary Efficacy Population (21/26) were identified as carrying germline *BRCA1/BRCA2* alterations, the PPA and NPA were also evaluated in the entire ARIEL2 patient population.

Table 35: Concordance between FoundationOne® Liquid CDx and the CTA for the detection of *BRCA1* or *BRCA2* alterations in All Patients

		CTA		
		Pos	**Neg**	**Total**
FoundationOne® Liquid CDx	**Pos**	60	4	64
	Neg	4	149	153
	Missing	60	214	274
	Total	124	367	491

The PPA and NPA between FoundationOne® Liquid CDx and the CTA were determined using the CTA as the reference for All Patients:

- PPA (95% CI): 93.8% (84.8%, 98.3%)
- NPA (95% CI): 97.4% (93.4%, 99.3%)

BRCA1 and *BRCA2* alteration status was verified retrospectively by FoundationOne® Liquid CDx in 41% (26/64) of the patients in the device Primary Efficacy Population.

The clinical effectiveness of FoundationOne® Liquid CDx to identify ovarian cancer patients with *BRCA1/BRCA2* alterations who may benefit from rucaparib treatment is based on 42% of the drug primary efficacy

population. Additionally, as stated above, of the patients that were retested, 21/26 (80.8%) were patients carrying germline (marginally higher than the prevalence of germline and somatic alterations in this patient population of 70% to 30%) alterations. To address the uncertainties due to the large proportion of missing data, a post-market study to provide real-world evidence with additional ovarian cancer patients will be conducted to confirm the clinical effectiveness of FoundationOne® Liquid CDx for rucaparib (See Section XIII).

ii. Demonstration of effectiveness:
The ORR in the primary efficacy population was 53.8% (33.4%, 73.4%) in *BRCA* Positive patients as determined by FoundationOne® Liquid CDx, which is comparable to the ORR of 54.1% (40.8%, 66.9%) in patients identified by the CTA (Table 36).

Table 36: ORR in the primary efficacy population by CTA and FoundationOne® Liquid CDx test results

	FoundationOne® Liquid CDx *BRCA* Positive N = 26	**CTA *BRCA* Positive N = 61**
Confirmed ORR (CR + PR), % (n)	53.8% (14)	54.1% (33)
95% CI	33.4%, 73.4%	40.8%, 66.9%

CR = complete response; PR = partial response

Additional analyses included in the PMA which support the clinical utility of the FoundationOne® Liquid CDx assay:

The ORR (95% CI) in All Patients was evaluated for *BRCA* Positive and *BRCA* Negative patients. The ORR in *BRCA* Positive patients identified from FoundationOne® Liquid CDx was 40.6% (28.5%, 53.6%) compared to the ORR of 46.8% (37.8%, 55.9%) in *BRCA*-positive patients based on the CTA. The ORR in *BRCA*-negative patients by FoundationOne® Liquid CDx and the CTA was 5.9% (2.7%, 10.9%) and 13.1% (9.8%, 17.0%), respectively.

An evaluation of key demographics and baseline characteristics variables based on cancer history and prior anticancer treatment showed, in general, to be similar and balanced between those patients with a known classification by FoundationOne® Liquid CDx and those with missing FoundationOne® Liquid CDx test result. A sensitivity analysis to evaluate the robustness of the clinical efficacy estimate against the unknown FoundationOne® Liquid CDx results was performed using the multiple imputation method in All Patients. After imputing the missing FoundationOne® Liquid CDx results, the weighted ORR (95% CI) across the imputed datasets was 45.2% (36.3%, 54.1%) was similar to ORR rates

for the known BRCA Positive group by FoundationOne® Liquid CDx [40.6% (95% CI, 28.5-53.6)].

Due to the low ascertainment of samples from the clinical study, additional clinical data will be provided post-market to supplement the existing effectiveness data provided.

c. Effectiveness Conclusions

The clinical effectiveness of FoundationOne®Liquid CDx as a companion diagnostic to identify patients with ovarian cancer harboring *BRCA1* and/or *BRCA2* alterations for treatment with rucaparib was demonstrated using plasma samples from the ARIEL2 study.

The ARIEL2 study enrolled 491 patients. Plasma samples were available for 55% (271/491) of patients dosed in ARIEL2, and FoundationOne® Liquid CDx data was obtained for 80% (217/271) of the patients with samples tested. In total, FoundationOne® Liquid CDx results were available for 44% (217/491) of All Patients, and for 42% (27/64) of the patients in the device Primary Efficacy Population.

The ORR (95% CI) in the device Primary Efficacy Population was 53.8% (33.4%, 73.4%) in *BRCA* Positive patients as determined by FoundationOne® Liquid CDx, which is comparable to the ORR of 54.1% (40.8%, 66.9%) in patients identified by CTA. The median DOR (95% CI) in the device Primary Efficacy Population was 225 days (115, 403) and 288 days (170, 403) in *BRCA* Positive patients by FoundationOne® Liquid CDx and CTA, respectively.

The data provided showed that FoundationOne® Liquid CDx identified *BRCA*-positive ovarian cancer patients treated with rucaparib with an ORR and clinically meaningful DOR similar to that observed in the clinical study. These results support the effectiveness of FoundationOne® Liquid CDx for the identification of *BRCA*-positive ovarian cancer patients for rucaparib treatment. However, while the observed PPA from the device Primary Efficacy Population was high, as noted above, 80.8% of the patients included in the bridging study were determined to carry germline *BRCA1/BRCA2* alterations. Because the prevalence of ovarian to somatic *BRCA* alterations is approximately 70:30, respectively, and the PPA observed in the All Patients group was 93.8%, this supports the recommendation to reflex plasma negative results to a FDA-approved tumor tissue based test.

d. Subgroup Analysis

Platinum sensitivity status (i.e., sensitivity, resistant, and refractory) is a well-established predictor of response to poly ADP ribose polymerase (PARP) inhibitors in ovarian cancer patients. Thus, the ORR of *BRCA* positive patients as identified by the FoundationOne® Liquid CDx was further explored in subgroups by platinum status. As expected, the ORR (95% CI) was higher in

platinum-sensitive [57.7% (36.9%, 76.6%)] compared to platinum-resistant [32.4% (17.4%, 50.5%)] and platinum-refractory [0% (0%, 60.2%)] subgroups.

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

D. 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 bridging studies described above included a single investigator. The clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). 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

To support the Intended Use and Indications for Use of the FoundationOne® Liquid CDx to identify NSCLC patients with *ALK* rearrangements who may benefit from treatment with alectinib, safety and effectiveness was demonstrated through a clinical bridging study using residual plasma specimens collected from patients enrolled into the BFAST study. The safety and effectiveness of the FoundationOne® Liquid CDx to identify breast cancer patients with specific *PIK3CA* mutations who may benefit from treatement with alpelisib was demonstrated using residual plasma specimens collected from patients enrolled into the SOLAR-1 study. The safety and effectiveness of the FoundationOne® Liquid CDx to identify ovarian cancer patients with somatic or germline *BRCA1/BRCA2* alterations who may benefit from treatement with rucaparib was demonstrated using plasma specimens collected from patients enrolled into the ARIEL2 clincial study. The data from the analytical and clinical bridging studies support the reasonable assurance of safety and effectiveness of the FoundationOne® Liquid CDx assay when used in accordance with the indications for use.

For the tumor mutation profiling indication, analytical performance studies were conducted with the FoundationOne® Liquid CDx assay using cfDNA extracted from plasma from patients with a variety of cancer types. When the test is used in accordance with the directions provided, the sensitivity for detecting the tested variants is shown in sections above. Additionally, the analytical performance studies support the use of FoundationOne® Liquid CDx 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.

B. Safety Conclusions

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, and accordingly may forgo therapy that would have been of benefit. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy.

According to the FDA-approved labeling, all three agents have been associated with a variety of adverse reactions, and there are also several warnings and precautions. Warnings and Precautions: For alectinib: cases of hepatotoxicity, interstitial lung disease (ild)/pneumonitis, renal impairment, bradycardia, severe myalgia and creatine phosphokinase (cpk) elevation, and embryo-fetal toxicity. For alpelisib: cases of severe cutaneous reactions, including Stevens-Johnson syndrome (SJS) and erythema multiforme (EM), hyperglycemia, ketoacidosis, pneumonitis, and diarrhea have been reported. For rucaparib, cases of Myelodysplastic syndromes/Acute Myeloid Leukemia, some fatal have been reported; for osimertinib: pulmonary and cardiac toxicities as well as keratitis, SJS, and EM have been reported.

Adverse reactions (most commonly reported): For alectinib: were pneumonia, renal impairment, sudden death, and cardiac arrest. For alpelisib: Most common adverse reactions including laboratory abnormalities included hyperglycemia, increased creatinine, diarrhea, rash, lymphocyte count decreased, gamma-glutamyl transferase (GGT) increased, nausea, alanine aminotransferase (ALT) increased, fatigue, hemoglobin decreased, lipase increased, decreased appetite, stomatitis, vomiting, weight decreased, calcium decreased, hypoglycemia, partial thromboplastin time (aPTT) prolonged, and alopecia. For rucaparib: nausea, fatigue (including asthenia), vomiting, anemia, dysgeusia, aspartate aminotransferase (AST)/ALT elevation, constipation, decreased appetite, diarrhea, thrombocytopenia, neutropenia, stomatitis, nasopharyngitis/upper respiratory infection, rash, abdominal pain/distention, and dyspnea.

C. Benefit-Risk Determination

Treatment with alectinib provides meaningful clinical benefit to NSCLC patients with *ALK* rearrangements. The probable benefit of FoundationOne® Liquid CDx to identify NSCLC patients with *ALK* rearrangements who may benefit from treatment with alectinib, was demonstrated through a clinical bridging study using residual plasma specimens collected from patients enrolled into the BFAST study. In comparison to the clinical trial assay the PPA was 84% and NPA was 100%. The ORR for patients positive by FoundationOne® Liquid CDx for the *ALK* rearrangement was 88.9 (95% CI: 78.4-95.4%), providing evidence that there is probable benefit of FoundationOne® Liquid CDx to identify NSCLC patients with *ALK* rearrangements for treatment with alectinib.

In addition, treatment with alpelisib provides meaningful clinical benefit to breast cancer patients with specific *PIK3CA* mutations. The probable benefit of FoundationOne® Liquid CDx to identify breast cancer patients with specific *PIK3CA* mutations who may benefit from treatment with alpelisib was demonstrated using residual plasma specimens collected from patients enrolled into the SOLAR-1 study. In comparison to the clinical trial assays, which included tissue-based assays, the PPA for this assay was 71.7% and NPA was 100%. In addition, the hazard ratio for patients positive for specific *PIK3CA* alterations by FoundationOne® Liquid CDx was 0.46, providing evidence that there is a probable benefit of FoundationOne® Liquid CDx in identifying breast cancer patients with specific *PIK3CA* mutations for treatment with alpelisib.

Also, treatment with rucaparib provides meaningful clinical benefit for ovarian cancer patients with somatic or germline *BRCA1/BRCA2* alterations. The probable benefit of FoundationOne® Liquid CDx to identify ovarian cancer patients with somatic or germline *BRCA1*/*BRCA2* alterations, who may benefit from treatment with rucaparib was demonstrated using plasma specimens collected from patients enrolled into the ARIEL2 clinical study. In comparison to the clinical trial assay, the PPA for this assay was 93.8% and NPA was 97.4%. In addition, the ORR for patients positive for *BRCA1/BRCA2* alterations by FoundationOne® Liquid CDx was 53.8%, maintaining the efficacy observed in the intent to treat population and providing evidence that there is probable benefit of FoundationOne® Liquid CDx in identifying ovarian cancer patients with *BRCA1/BRCA2* alterations, for treatment with rucaparib.

There is potential risk associated with the use of this device, mainly due to 1) false positive, false negatives, or failure to provide a result and 2) incorrect interpretation of test results by the user.

The risks of the FoundationOne® Liquid CDx for the selection of NSCLC patients with *ALK* rearrangements for treatment with alectinib, breast cancer patients with specific *PIK3CA* mutations for treatment with alpelisib and ovarian cancer patients with *BRCA1/BRCA2* mutations for treatment with rucaparib, are associated with the potential mismanagement of patient's treatment resulting from false results of the test. Patients who are determined to be false positive by the test may be exposed to a drug combination that is not beneficial and may lead to adverse events or may have

delayed access to other treatments that could be more beneficial. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen. The risks of a false results are partially mitigated by the validation results. The analytical and clinical validation studies provided above partially mitigate the risks of false positives and false negatives.

The likelihood of false results was assessed by an analytical and clinical validation studies, which partially mitigate the risk of the FoundationOne® Liquid CDx device. The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with acceptable performance, in light of the understanding that the risks of a false negative result are partially mitigated by a recommendation that those patients whose plasma generate a negative result for those included in Table 1 should have their tumor mutation status verified by using a FDA approved tumor test.

Additional factors to be considered in determining probable risks and benefits for FoundationOne® Liquid CDx, for the indications noted here included: analytical performance of the device, representation of variants in the major effectiveness studies, and the availability of alternative tests. The FoundationOne Liquid CDx assay has been analytically validated as summarized above; however, multiple post-market studies are also planned to confirm the data provided for. To supplement the premarket data, some post-market studies are planned as summarized in Section XIII, below. The data support that for the FoundationOne® Liquid CDx assay, and the indications noted in the intended use statement, the probable benefits outweigh the probable risks.

To supplement the premarket data, some post-market studies are planned as summarized in Section XIII, below.

1. Patient Perspectives

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

In conclusion, given the available clinical and analytical information above, the data support that the probable benefit exceeds the probable risks for the use of FoundationOne® Liquid CDx for the selection of NSCLC patients with *ALK* rearrangements for treatment with alectinib, breast cancer patients with specific *PIK3CA* mutations for treatment with alpelisib and ovarian cancer patients with *BRCA1/BRCA2* mutations for treatment with rucaparib.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical studies support the clinical utility of the FoundationOne®

Liquid CDx assay as an aid for the identification of cancer patients for whom the therapies listed in Table 1 of the Intended Use/Indications for Use statement may be indicated.

Data from the clinical bridging study supports the utility of FoundationOne® Liquid CDx as an aid in identifying NSCLC patients with *ALK* rearrangements who may benefit from treatment with alectinib, breast cancer patients with specific *PIK3CA* mutations who may benefit from treatement with alpelisib, and ovarian cancer patients with somatic or germline *BRCA1*/*BRCA2* alterations who may be eligible for treatment with rucaparib.

XIII. CDRH DECISION

CDRH issued an approval order on October 26, 2020. The final clinical conditions of approval cited in the approval order are described below.

1. FMI must provide robust and detailed protocols, including acceptance criteria where appropriate, for the studies that are conditions of approval required by this order. These studies must be adequate to confirm the safety and effectiveness of the FoundationOne® Liquid CDx device and must include a detailed description of the numbers of sample to be tested, the type of samples to be tested, the tumor types for each sample, the complete testing protocol, and a robust statistical analysis plan. These protocols must be submitted to FDA no later than 60 days after approval.

2. All requested data must be generated, and a complete set of the requested data required by this order must be submitted within 1 year, unless otherwise specified.

3. FMI will provide robust and high confidence data from well-designed and well-controlled study using cell free-DNA (cfDNA) input (at a target concentration of 30 ng) from intended use specimens across other cancer types, for ALK fusions/rearrangements (NSCLC), BRCA1/BRCA2 (ovarian cancer) alterations, PIK3CA mutations, and ERBB2 copy number amplifications to confirm an acceptable level of precision at or near the LoD concentration. FMI should provide data for each of the 4 different CDx BRCA1 and BRCA2 variant types [i.e., base substitutions (SNV), insertion/deletion (indel), rearrangement (RE), and homozygous deletions (HD)], ALK fusions/rearrangements, and a representative number of PIK3CA mutations. FMI should also include additional samples with ERBB2 copy number amplifications across other cancer types to support a tumor profiling claim. The level of precision and reproducibility at the LoD must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

4. For the BRCA1/BRCA2 companion diagnostic (CDx) claim (rucaparib) for the ovarian cancer indication, you must provide the following:

a. FMI will provide a robust and high confidence data set to confirm the analytical accuracy/concordance to a validated orthogonal NGS method that has been accepted by the FDA (as part of the protocol review) as suitable for this purpose. These studies must be performed to collect data for BRCA1 and BRCA2 indels, HD, and RE using the accepted comparator assay, using intended use ovarian cancer specimens. The level of analytical accuracy/concordance must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

b. FMI will provide a robust and high confidence data set from a well-designed and well-controlled contrived sample functional characterization study to demonstrate similar performance between ovarian cancer clinical cfDNA samples and contrived samples. The study should utilize clinical samples harboring *BRCA1* and *BRCA2* SNV, HD, and RE alterations and contrived samples with the same alterations, and demonstrate equivalent hit rates across comparable dilutions close to and below LoD levels between the two sample types. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

c. FMI will provide robust and high confidence data from a guard-band study to test the limits of the FoundationOne® Liquid CDx assay to confirm the specifications for cfDNA input. This study must be designed to assess cfDNA concentrations minimally including 2X below the minimum recommended cfDNA input level to confirm the cfDNA input guard-bands for *BRCA1* and *BRCA2* CDx variant types. The study must assess *BRCA1* and *BRCA2* indels, HD, and RE. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

5. FMI must provide robust and high confidence data from an appropriately designed limit of blank (LoB) study. The study should be performed using all steps in the FoundationOne® Liquid CDx assay's workflow for each replicate tested to confirm that the LoB of this assay is as claimed. The LoB data from this study must also be provided to FDA with and without germline alteration, and white blood cells must also be sequenced to confirm germline variants. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

6. FMI must provide data from a well-designed and well-controlled accuracy/concordance study using a comparator assay that has been accepted by the FDA (as part of the protocol review) as suitable for this purpose to confirm accuracy of the FoundationOne® Liquid CDx test results to a validated orthogonal method. The samples tested in this study must include SNVs and indels of genes (i.e., 78% of the total panel genes) that have not been tested in the existing premarket accuracy/concordance study as well as additional ALK fusions/rearrangements in lung cancer, other cancers, and ERBB2 copy number amplifications in additional cancers other than breast. The level of analytical accuracy/concordance must be

adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

7. Blood Collection Tubes:

   a. FMI must demonstrate clinically insignificant variability when different lots of the FoundationOne® Liquid CDx Blood Collection tube are used with the FoundationOne® Liquid CDx assay. FMI must provide data from a robust and high confidence precision study. This study must confirm the FoundationOne® Liquid CDx assay's precision when the FoundationOne® Liquid CDx cfDNA Blood Collection tubes are used and must use replicate samples from each of multiple different patients. Each patient who donates specimens for this study must have plasma collected in a total of four tubes, each from two tube lots; three lots are required to be represented in the study. This is important to assess variability between tube lots and across patient specimens. Each replicate must be run at or near the minimum standardized cfDNA input (i.e., at a target concentration of 30 ng). The samples must be collected from patients with at least 10 different tumor types and the study must include at least 10 pathogenic SNVs and 10 pathogenic indels that are identified by the FoundationOne® Liquid CDx assay. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.

   b. FMI must provide robust and high confidence data from a well-designed and well-controlled study which is intended to confirm the shelf-life claims for the FoundationOne® Liquid CDx Blood Collection tubes when used in conjunction with the FoundationOne® Liquid CDx assay. FMI must provide evidence that when samples from the same patient collected in newly manufactured tubes, as well as in tubes that are at the end of their shelf life, are used in the FoundationOne® Liquid CDx assay, the FoundationOne® Liquid CDx assay performance meets the clinical and analytical performance claim in the FoundationOne® Liquid CDx assay authorized labeling.

   c. FMI must provide robust and high confidence data that the impact of preanalytical variables associated with the use of the FoundationOne® Liquid CDx cfDNA Blood Collection tubes, such as hemolysis, has been validated for the FoundationOne® Liquid CDx test system and that any impact of these factors on the FoundationOne® Liquid CDx assay has been appropriately mitigated. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.

   d. To support use of results submitted in FMI's clinical study generated from samples collected within 24 hours from cancer patients, you must provide robust

and high confidence data from an appropriately designed study to confirm the claimed stability of cfDNA in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes. This study must compare FoundationOne® Liquid CDx results generated from freshly drawn blood specimens to FoundationOne® Liquid CDx assay results generated from matched specimens (i.e., collected at the same time from the same patient) stored in the FoundationOne® Liquid CDx cfDNA Blood Collection tube for a minimum of 24 hours. This study must be performed in replicate samples, when feasible, at each time point, and the samples tested must adequately represent all variant types across several tumor types at each tested time point. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.

e. FMI must provide robust and high confidence data from a stability study which demonstrates acceptable stability of whole blood collected from the CDx intended use patients and stored in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes. The study must confirm the claimed cfDNA storage stability and must confirm the suppression of white blood cells lysis across multiple lots. This study must also use the amount of cfDNA isolated and electropherogram data as a comparator method, in addition to sequencing results and quality metrics. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.

f. FMI must demonstrate clinically insignificant variability on the performance of the FoundationOne® Liquid CDx assay when specimens collected in FoundationOne® Liquid CDx cfDNA Blood Collection tubes are handled at different centrifugation conditions. The study must assess conditions that are below and above recommended relative centrifugal force and centrifugation time to account for potential performance issues that could occur due to centrifuge malfunction or operator errors. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when expected handling conditions are used on specimens collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.

8. Software:

a. FMI must appropriately validate modifications to the curating and reporting of variant results, to align with the approved clinical CDx indications and the genes and variants authorized under the tumor profiling claim. FMI must provide software validation documentation, including example reports, adequate to demonstrate that these modifications do not adversely affect the safety and

effectiveness of the device. The software modifications and validation documentation must be provided within 30 days of approval.

b. FMI must appropriately validate modifications to the curating and reporting of variant results, including reporting levels for mutation profiling, and modifications to the report formatting that were made to the software following review. FMI must provide software validation documentation adequate to demonstrate that these modifications do not adversely affect he safety and effectiveness of the device. ‘

c. FMI must appropriately validate software infrastructure changes and migration of the analysis pipeline and associated software to cloud services, including any impact of these software modifications on the cybersecurity of FoundationOne® Liquid CDx assay test system. FMI must provide software validation documentation adequate to demonstrate that these modifications do not adversely affect he safety and effectiveness of the device.

9. FMI must provide robust and high confidence data in the form of real-world evidence from BRCA1/BRCA2 positive ovarian cancer patients consistent with the patient population enrolled into ARIEL2 clinical study to support the safety and effectiveness of the FoundationOne® Liquid CDx to identify patients who may benefit from treatment with rucaparib. This post-approval study should provide additional clinical outcome data in terms of confirmed objective response rate to confirm the clinical effectiveness of FoundationOne® Liquid CDx as a CDx device for identification of ovarian cancer patients with BRCA1/BRCA2 alterations who may benefit from treatment with rucaparib

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.