SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

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

Device Trade Name: FoundationOne® Liquid CDx

Device Procode: PQP

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

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P190032

Date of FDA Notice of Approval: 8/26/2020

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.

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 and copy number losses only in *BRCA1* and *BRCA2*. 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. Additionally, FoundationOne® Liquid CDx is intended to provide 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.

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)
Prostate cancer	*BRCA1, BRCA2* alterations	RUBRACA® (rucaparib)

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-approve 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 *BRCA1* and *BRCA2* (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, and copy number variants and genomic rearrangements in *BRCA1* and *BRCA2*. 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 CDx[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, 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***	TGFBR2

						[Exons 3, 4, 6, 7, 10, 14, 15, 17]	
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 output of the test includes:
Category 1: Companion Diagnostic (CDx) claims noted in Table 1 of the Intended Use

Category 2: ctDNA Biomarkers with Strong Evidence of Clinical Significance in ctDNA

Category 3: Biomarkers with Evidence of Clinical Significance in tissue supported by:
3A: strong analytical validation using ctDNA
3B: analytical validation using ctDNA

Category 4: Other Biomarkers with Potential Clinical Significance

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 3, below. All instruments are qualified by Foundation Medicine, Inc. (Foundation Medicine) under Foundation Medicine's Quality System.

Table 3: 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 to 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 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. <u>*BRCA1*, *BRCA2* alterations to identify patients eligible for rucaparib in prostate cancer:</u>

   The CDx classification criteria and the list of *BRCA1/BRCA2* missense mutations for rucaparib, based on the trial prespecifications are described in Table 4 and Table 5; however, not all of the missense mutations listed below were observed in the TRITON2 clinical study.

Table 4: 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 5)

Table 5: 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				

VI. ALTERNATIVE PRACTICES AND PROCEDURES

The Roche **cobas**® *EGFR* Mutation Test v2 from Roche Molecular Systems, Inc. is an FDA-approved companion diagnostic intended for the detection of *EGFR* Exon 19 deletions (Exon 19del) and L858R substitution in plasma obtained from patients with advanced and metastatic NSCLC for treatment with TARCEVA® (erlotinib), TAGRISSO® (osimertinib), and IRESSA® (gefitinib).

There are no FDA-approved CDx alternatives for the detection of genomic alterations of *BRCA1* or *BRCA2* for the identification of prostate cancer patients eligible for treatment with RUBRACA® (rucaparib).

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 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 RUBRACA® (rucaparib) FDA approved package insert 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. Table 6 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. The validation studies included >7,000 sample replicates, >31,000 unique variants, >30 tumor types, representing all 324 genes targeted by the assay. Table 7 provides a summary of the representation of unique variants in *BRCA1* and *BRCA2*, and *EGFR*, genes associated with CDx indications for FoundationOne Liquid CDx.

Table 6. Representation of tumor types and variants* across validation studies

Study Title	**Cancer Types Represented**	**# Unique Samples**	**# of Sample Replicates**	**# Unique**				
				Targeted Genes	**Subs**	**Indels**	**Rearrang.**	**Copy Number Losses**
Contrived Sample Functional Characterization (CSFC) Study	Breast cancer Colorectal Cancer (CRC) Lung cancer Contrived samples	13	1843	228	563	81	11	1
F1 Liquid CDx to Validated Tumor Tissue Test Concordance: *BRCA1* and *BRCA2* Variants	Prostate cancer Ovarian cancer	279	N/A	2	100	87	9	2
Orthogonal Concordance	23 cancer types Contrived samples	278	N/A	64	541	12	11	0
LoD Estimation	Prostate Contrived samples	10	877	286	1490	247	32	3
LoB	Healthy Donors	28	79	322	26134	4482	911	42
Potentially Interfering Substances	Contrived samples	9	336	18	16	11	11	2
Hybrid Capture Bait Specificity	25 cancer types Contrived samples	3546	N/A	324	N/A	N/A	N/A	N/A
Reagent Stability	Contrived samples	8	142	279	1090	215	32	2
Reagent Interchangeability	Contrived samples	8	192	20	15	11	11	1
Precision study 1	Breast cancer CRC Lung cancer Ovarian cancer Prostate cancer Skin cancer Contrived samples	47	1121	280	900	229	63	5

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# Unique				
				Targeted Genes	Subs	Indels	Rearrang.	Copy Number Losses
Precision study 2	Lung cancer Prostate cancer Stomach cancer CRC Bile duct cancer Breast cancer	10	230	6	6	4	0	0
DNA Extraction	CRC Prostate cancer Breast cancer Lung cancer Skin cancer	6	72	161	265	53	2	0
Whole Blood Sample Stability	Lung cancer CRC Prostate cancer Breast cancer	11	22	66	75	15	1	0
Inverted Tube Whole Blood Sample Stability	Lung cancer CRC Breast cancer Ovarian cancer Prostate cancer	130	260	237	594	91	5	0
Cross Contamination	Contrived samples	5	376	39	9	5	4	1
Guard Banding	Contrived samples	10	375	20	17	12	12	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
Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in prostate cancer	Prostate cancer	199	N/A	2	44	55	8	1
Blood Collection Tube Equivalence	Ovarian cancer Breast cancer CRC Prostate cancer Lung cancer Skin cancer Stomach cancer	60	192	116	135	39	13	0

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# Unique				
				Targeted Genes	Subs	Indels	Rearrang.	Copy Number Losses
Automation Line Equivalence	Contrived samples	8	187	303	1926	337	63	4
Variant Report Curation	Breast cancer CRC Lung cancer Prostate cancer Skin cancer	19	57	183	300	104	15	2
Pan-tumor performance (includes historical analysis)	20 cancer types	19868	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
F1 Liquid LDT to F1 Liquid CDx Concordance	25 cancer types	927	N/A	73	1815	376	109	N/A

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

Table 7. Representation unique variants* in *BRCA1*, *BRCA2*, and *EGFR*

Study	Gene	# of Unique Substitutions	# of Unique Indels	# of Unique Rearrangements	# of Unique Copy Number Loss
CSFC Study	*BRCA1*	0	0	0	0
	BRCA2	0	2 Other DO 2 Contrived	0	0
	EGFR	1 Other DO 1 Contrived	1 NSCLC 1 Contrived	0	0
Limit of Blank (LoB)	*BRCA1*	258 HD	80 HD	18 HD	1 HD
	BRCA2	433 HD	127 HD	14 HD	1 HD
	EGFR	228 HD	33 HD	14 HD	0
Limit of Detection (LoD)	*BRCA1*	6 Other DO 10 Contrived	4 Contrived	1 Contrived	0
	BRCA2	16 Other DO 7 Contrived	5 Contrived	0	1 Prostate Cancer
	EGFR	8 Contrived	3 Contrived	0	0
Interfering Sub	*BRCA1*	1 Contrived	2 Contrived	0	0
	BRCA2	0	5 Contrived	2 Contrived	1 Contrived
	EGFR	2 Contrived	1 Contrived	0	0
Reagent Stability	*BRCA1*	10 Contrived	3 Contrived	2 Contrived	0
	BRCA2	6 Contrived	5 Contrived	1 Contrived	0
	EGFR	9 Contrived	3 Contrived	0	0
Reagent interchangeability	*BRCA1*	1 Contrived	2 Contrived	0	0
	BRCA2	0 Contrived	5 Contrived	0	0

Study	Gene	# of Unique Substitutions	# of Unique Indels	# of Unique Rearrangements	# of Unique Copy Number Loss
	EGFR	2 Contrived	1 Contrived	0	0
Precision study 1	*BRCA1*	12 Prostate Cancer 12 Other DO 12 Contrived	11 Prostate Cancer 12 Other DO 11 Contrived	4 Prostate Cancer 4 Other DO 4 Contrived	0
	BRCA2	13 Prostate Cancer 14 Other DO 13 Contrived	7 Prostate Cancer 7 Other DO 7 Contrived	1 Prostate Cancer 1 Other DO 1 Contrived	1 Prostate Cancer 1 Other DO 1 Contrived
	EGFR	5 NSCLC 5 Other DO 5 Contrived	3 NSCLC 3 Other DO 3 Contrived	1 NSCLC 1 Other DO 1 Contrived	0
Precision study 2	*BRCA1*	1 Prostate Cancer	1 Prostate Cancer	0	0
	BRCA2	1 Other DO	1 Other DO	0	0
	EGFR	1 Other DO	1 Other DO	0	0
DNA Extraction	*BRCA1*	2 Other DO	0	0	0
	BRCA2	2 Prostate Cancer 3 Other DO	1 Prostate Cancer	0	0
	EGFR	1 NSCLC	1 Other DO	0	0
Whole Blood Sample Stability	*BRCA1*	1 Other DO	1 Other DO	0	0
	BRCA2	2 Other DO	0	0	0
	EGFR	1 NSCLC	0	0	0
Inverted Tube Whole Blood Sample Stability	*BRCA1*	3 Other DO	1 Prostate Cancer 2 Other DO	0	0
	BRCA2	3 Prostate Cancer 4 Other DO	1 Other DO	0	0
	EGFR	1 Other DO	0	0	0
Cross Contamination	*BRCA1*	N/A	1 contrived	N/A	N/A
	BRCA2	N/A	N/A	N/A	N/A
	EGFR	N/A	N/A	N/A	N/A
Guard Banding	*BRCA1*	1 Contrived	2 Contrived	0	0
	BRCA2	0	4 Contrived	1 Contrived	0
	EGFR	2 Contrived	2 Contrived	0	0
F1 Liquid CDx to Validated Tumor Tissue Test Concordance: *BRCA1* and *BRCA2* Variants	*BRCA1*	6 Prostate Cancer 43 Ovarian Cancer	2 Prostate Cancer 37 Ovarian Cancer	2 Prostate Cancer 3 Ovarian Cancer	1 Prostate Cancer 1 Ovarian Cancer
	BRCA2	23 Prostate Cancer 31 Ovarian Cancer	29 Prostate Cancer 25 Ovarian Cancer	5 Prostate Cancer	1 Prostate Cancer 1 Ovarian Cancer
	EGFR	N/A	N/A	N/A	N/A
Orthogonal Concordance	*BRCA1*	1 Prostate Cancer 1 Other DO 3 Contrived	N/A	N/A	N/A
	BRCA2	4 Other DO 2 Contrived	N/A	N/A	N/A
	EGFR	7 NSCLC 4 Other DO Contrived	5 NSCLC 1 Other DO 2 Contrived	N/A	N/A
	BRCA1	16 Prostate Cancer 60 Other DO	5 Prostate Cancer 3 Other DO	3 Prostate Cancer	

<table>
<tr><th>Study</th><th>Gene</th><th># of Unique Substitutions</th><th># of Unique Indels</th><th># of Unique Rearrangements</th><th># of Unique Copy Number Loss</th></tr>
<tr><td rowspan="2">F1 Liquid LDT to F1 Liquid CDx Concordance</td><td>BRCA2</td><td>38 Prostate Cancer
92 Other DO</td><td>39 Prostate Cancer
10 Other DO</td><td>6 Prostate Cancer
1 Other DO</td><td rowspan="2">N/A
(copy loss not evaluated)</td></tr>
<tr><td>EGFR</td><td>50 NSCLC
37 Other DO</td><td>15 NSCLC
2 Other DO</td><td>3 NSCLC
2 Other DO</td></tr>
<tr><td rowspan="3">Clinical validation study for detection of deleterious alterations in BRCA1 and BRCA2 in prostate cancer</td><td>BRCA1</td><td>21 Prostate Cancer</td><td>3 Prostate Cancer</td><td>3 Prostate Cancer</td><td>0</td></tr>
<tr><td>BRCA2</td><td>53 Prostate Cancer</td><td>53 Prostate Cancer</td><td>5 Prostate Cancer</td><td>1 Prostate Cancer</td></tr>
<tr><td>EGFR</td><td>17 Prostate Cancer</td><td>1 Prostate Cancer</td><td>3 Prostate Cancer</td><td>0</td></tr>
<tr><td rowspan="3">Blood Collection Tube Equivalence</td><td>BRCA1</td><td>1 Contrived</td><td>1 Other DO
2 Contrived</td><td>0</td><td>0</td></tr>
<tr><td>BRCA2</td><td>4 Other DO</td><td>1 Other DO
5 Contrived</td><td>0</td><td>0</td></tr>
<tr><td>EGFR</td><td>2 Other DO
2 Contrived</td><td>1 Contrived</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Automation Line Equivalence</td><td>BRCA1</td><td>11 Contrived</td><td>2 Contrived</td><td>1 Contrived</td><td>0</td></tr>
<tr><td>BRCA2</td><td>13 Contrived</td><td>6 Contrived</td><td>1 Contrived</td><td>1 Contrived</td></tr>
<tr><td>EGFR</td><td>7 Contrived</td><td>3 Contrived</td><td>0</td><td>0</td></tr>
<tr><td rowspan="3">Variant Report Curation</td><td>BRCA1</td><td>2 Prostate Cancer
1 Other DO</td><td>8 Prostate Cancer
1 Other DO</td><td>1 Prostate Cancer</td><td>0</td></tr>
<tr><td>BRCA2</td><td>2 Prostate Cancer
5 Other DO</td><td>3 Other DO</td><td>0</td><td>1 Prostate Cancer</td></tr>
<tr><td>EGFR</td><td>1 NSCLC
2 Other DO</td><td>0</td><td>0</td><td>0</td></tr>
</table>

*Variant result totals may include variants classified as VUS or benign.
HD = apparently healthy donor; DO = Disease Ontology
Note: The number of unique variants in each sample type may add up to more than the total of unique variants as the same variants may be represented in multiple sample types

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. In some studies when use of clinical specimens was not feasible due to volume limitations, contrived samples were used which consisted of enzymatically sheared cell line DNA spiked into human plasma from healthy donors, extracted according to the assay's standard procedure, and the isolated cfDNA was then diluted with cfDNA. To support such use, a contrived sample functional characterization (CSFC) study was conducted to demonstrate comparable performance of sheared cell line DNA samples as compared to cfDNA isolated from plasma.

Highly actionable alterations were identified in the 39 contrived samples representing 17 genes and included 17 substitutions, 6 indels, 6 copy number losses, and 9 rearrangements that were used across validation studies. The 39 contrived samples

included 7 *EGFR* (positive for 3 *EGFR* Ex 19 deletions, 2 *EGFR* L858R substitutions), 2 *BRCA1* (positive for 2 indels and 1 substitutions), and 3 *BRCA2* samples (positive for 5 indels). These 12 samples were used to supplement the samples used to support the performance of the *EGFR* and *BRCA1*/*BRCA2* CDx indications listed in Table 1 as well as the tumor mutation profiling claim.

1. <u>Contrived Sample Functional Characterization (CSFC) Study:</u>
   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. Similar performance between clinical and contrived samples was established by testing a dilution series of contrived and clinical specimens harboring substitutions, insertions, deletions, rearrangements, and copy number losses, totaling 924 clinical (cfDNA) sample replicates and 1069 enzymatically fragmented cell-line genomic DNA (gDNA) contrived sample replicates. All matching alterations present in both the contrived and clinical specimens at comparable levels of variant allele fraction (for substitutions, indels, rearrangements) or tumor fraction (for copy number losses) were used to determine the similar performance for a total of 654 unique variants.

   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 .

   An additional evaluation of *BRCA2* indels (*BRCA2* 5351delA and *BRCA2* 9097_9098insA) between 6 clinical replicates of each variant and 27 and 14 contrived sample replicates, respectively, for each contrived variant was performed using a regression analysis. A probit model was used to evaluate the relationship between the positive call rate and variant allele frequency. A regression analysis and formal hypothesis test confirmed no significant difference in test performance between contrived and clinical specimens as determined by a p-value > 0.05.

   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.

2. <u>Analytical Accuracy/Concordance with an Orthogonal Method:</u>

   a. Concordance with Orthogonal cfDNA-based NGS Method #1:
      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 (>50 unique disease ontologies across 23 cancer types.

The cancer types (# samples) included lung [NSCLC (75) and other (3)]; breast (54); prostate (32); colorectal [colon (27) and rectal (6)]; liver (11); ovarian (6); pancreas (9); gastrointestinal (7); bile duct (2); esophageal (5); skin (6); cervical (1); anal (1); bladder (1); gallbladder (1); salivary gland (2); thymus (1); thyroid (3); uterine (2); fallopian tube (1); head and neck (1); soft tissue (1); and unknown primary (19). The study included samples selected from clinical FoundationOne Liquid CDx testing (n=268) and contrived samples consisting of fragmented gDNA diluted in clinical cfDNA to represent rare alterations (n=10).

Using the externally validated NGS assay as the comparator, the analysis demonstrated a short variant Positive Percent Agreement (PPA) of 96.2% with a 95% two-sided CI of (94.8%, 97.4%). The short variant Negative Percent Agreement (NPA) was >99.9% with a 95% two-sided CI of (99.9%, 100.0%). The respective PPA of base substitutions and indels with a 95% two-sided CI was 96.1% (94.6%, 97.3%) and 100.0% (85.2%, 100.0%). The respective NPA and 95% two-sided CI of base substitutions and indels was >99.9% (99.9%, 100.0%) and 100.0% (99.9%, 100.0%) (Table 8).

Table 8: Concordance of short variants* called in FoundationOne Liquid CDx and the comparator assay

Variant Type	CDx(+)/ Comp(+)	CDx(-)/ Comp (+)	CDx(+)/ Comp (-)	CDx(-)/ Comp (-)	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
All Short Variants	868	34	8	152824	96.2% (94.8%, 97.4%)	>99.9% (99.9%, 100.0%)	>99.9% (99.9%, 100.0%)
Base Substitutions	845	34	8	149511	96.1% (94.6%, 97.3%)	>99.9% (99.9%, 100.0%)	>99.9% (99.9%, 100.0%)
Indels	23	0	0	3313	100.0% (85.2%, 100.0%)	100.0% (99.9%, 100.0%)	100.0% (99.9%, 100.0%)

CDx = FoundationOne Liquid CDx; Comp = Comparator
N = 902 positive variants, N= 152,832 negative variants by the comparator assay)
*Variant result totals may include variants classified as VUS or benign.

For the rearrangement detection concordance between the FoundationOne Liquid CDx and the comparator assay, the observed rearrangement PPA_{Comp} was 100.0%, with a 95% two-sided CI of (59.04%, 100.0%). The NPA_{Comp} was 99.8%, with a 95% two-sided CI (99.5%, 100.0%).

Assessment of a subset of highly-actionable alterations were compared between the two assays. The analysis resulted in a PPA of 100% across all eligible highly-actionable alterations called in the comparator assay (Table 9) and a NPA of 99.8% (99.5%, 100.0%).

Table 9. Concordance of highly actionable alterations called between FoundationOne Liquid CDx and the comparator assay

Targeted Alteration	*n*	PPA (95% CI)	NPA (95% CI)
EGFR L858R	10	100% (69.2%, 100.0%)	100% (98.7%, 100.0%)
EGFR Exon 19 deletions	11	100% (71.5%, 100.0%)	100% (99.7%, 100.0%)
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%)

These data demonstrate that the FoundationOne Liquid CDx assay and an externally validated NGS assay are highly concordant across the 74 genes common between the two panels.

Due to the rarity of some variant types in the *BRCA1* and *BRCA2* prostate cancer patient population, *BRCA1* and *BRCA2* samples from other cancers as well as other clinical samples that were shown to perform similarly were used to leverage for those samples not represented in the current study. While those samples were shown to perform similarly, an additional post-market study is planned to support the above described analytical accuracy study intended to provide additional data to confirm the performance of those *BRCA1* and *BRCA2* variants in prostate cancer specimens which were not represented in the above study either due to limitations in sample availability or due to absence of a a well-validated comparator assay for robust detection of all *BRCA1* and *BRCA2* variant types. This post-market study will also include additional samples to represent other genes and variants tested for by the FoundationOne Liquid CDx test.

b. Concordance data for CDx-associated alterations:

i. Comparison with Validated NGS Tumor Tissue Assay:

Samples from a total of 279 *BRCA1* and *BRCA2* positive prostate and ovarian cancer patients were tested and the concordance evaluated between matched FoundationOne Liquid CDx and a validated tumor tissue test for the detection of deleterious alterations in *BRCA1* or *BRCA2*. The sample set was comprised of 100 unique substitutions, 87 unique indels, 9 unique rearrangements, and 2 unique copy number loss. As summarized in Table 10 below, a PPA of 88.0% and an NPA of 95.7% were observed.

Table 10. Concordance of FoundationOne Liquid CDx and Validated NGS Tumor Tissue Test #1 in prostate and ovarian cancer patients for the detection of alterations in *BRCA1* or *BRCA2*

		Validated Tumor Tissue Method #1		
		(+)	(-)	
F1 Liquid CDx	(+)	103	7	**PPA:** 88.0% (80.9%, 92.7%)
	(-)	14	155	**NPA:** 95.7% (91.4%, 97.9%)

As summarized in Table 11 an overall PPA of 87.28% and an NPA of 99.83% were observed.

Table 11. Concordance of FoundationOne Liquid CDx and Validated NGS Tumor Tissue Test #1 in prostate and ovarian cancer patients for the detection of alterations* in *BRCA1* or *BRCA2*

	F1 Liquid CDx(+) /Tissue(+)	F1 Liquid CDx(-) /Tissue(+)	F1 Liquid CDx(+) /Tissue(-)	F1 Liquid CDx(-)/ Tissue(-)	PPA (%) CI[1]	NPA (%) CI[1]
Substitutions	77	6	29	20255	92.77 (85.1%, 96.6%)	99.86 (99.8%, 99.9%)
Indels	65	3	31	16362	95.59 (87.8%, 98.5%)	99.81 (99.7%, 99.9%)
Rearrangements	4	3	7	1939	57.14 (25.1%, 84.2%)	99.64 (99.3%, 99.8%)
Copy number loss	5	10	1	263	33.33 (15.2%, 58.3%)	99.62 (97.9%, 99.9%)
Total	151	22	68	38819	87.28 (81.5%, 91.5%)	99.83 (99.8%, 99.9%)

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

An analysis was also performed for the subset of patients with prostate cancer as summarized in Table 12. This sample set was comprised of 24 substitutions, 27 indels, 6 rearrangements, and 1 copy number loss. An overall PPA of 80.00% and an NPA of 99.69% were observed.

Table 12. Concordance of FoundationOne Liquid CDx and Validated NGS Tumor Tissue Test #1 in the subset of patients with prostate cancer for the detection of alterations* in *BRCA1/BRCA2*

	F1 Liquid CDx(+) /Tissue(+)	F1 Liquid CDx(-) /Tissue(+)	F1 Liquid CDx(+) /Tissue(-)	F1 Liquid CDx(-)/ Tissue(-)	PPA (%) CI[1]	NPA (%) CI[1]
Substitutions	23	1	5	1459	95.83 (79.8%, 99.3%)	99.66 (99.2%, 99.9%)
Indels	25	3	4	1642	89.29 (72.8%, 96.3%)	99.76 (99.4%, 99.9%)
Rearrangements	3	3	1	365	50.00 (18.8%, 81.2%)	99.73 (98.5%, 99.95%)
Copy number loss	5	7	1	49	41.67 (19.3%, 68.1%)	98.00 (89.5%, 99.7%)
Total	56	14	11	3515	80.00 (69.2%, 87.7%)	99.69 (99.4%, 99.8%)

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

Some discordance is expected based on biological differences and sampling times between tumor tissue and plasma samples. Considering the impact of biological differences between analytes, these data demonstrate

a good concordance for base substitutions but poor performance with rearrangements and copy number losses between the FoundationOne Liquid CDx and the validated tumor tissue test for the detection of deleterious alterations in *BRCA1* or *BRCA2*.

ii. Comparison with Second Orthogonal Tumor Tissue NGS Assay:
A separate exploratory analysis was also performed to demonstrate the concordance between matched plasma and FFPE samples using the FoundationOne Liquid CDx to an externally validated tissue-based orthogonal NGS method. In this study, 3 prostate specimens obtained from FMI's archives, which showed 1 *BRCA1* rearrangement, 1 *BRCA1* deletion, and 1 *BRCA2* deletion in the FFPE specimens. The results of this study showed discordant results between the plasma and tissue results for the two *BRCA* deletions. For one of the discordant samples, based on the FoundationOne Liquid CDx results there appeared to be little evidence of cfDNA, suggesting that the discordance was most likely due to biological differences between the sample analytes (i.e., low ctDNA shedding) and for the other discordant sample, the orthogonal tissue assay detected a monoallelic loss of exons 5 – 7 in the *BRCA1* gene. Hemizygous focal loss events are not expected to be called by the FoundationOne Liquid CDx assay.

3. Analytical Sensitivity:

a. Limit of Blank (LoB):
The LoB was established by sequencing plasma samples from 30 apparently healthy donors with no diagnosis of cancer using 4 replicates per sample. All donors were over the age of 60 with a median age of 68, and included 15 smokers and 15 non-smokers.

Across 30,622 short variants, 58 variants had a detection rate of greater than 5%. Of those, five variants of unknown significance (VUS) (TSC1 965T>C, IRF4 1ins87, MSH3 186_187insGCCGCAGCGCCCGCAGCG, IGF1R 568C>T, WHSC1 1582C>A) had a detection rate significantly exceeding 5%, up to 35% for one variant, on an individual variant basis. Across 264 copy number alterations and 894 rearrangements, zero variants were detected.

Table 13: Detection Rate in LoB Study

Category	Per Variant* Detection Rate
Level 1	0% (0 of 292)
Level 2	0% (0 of 10)
Level 3	0% (0 of 18)
Level 4	0.82% (47 of 5760)
VUS	0.83% (203 of 24542)
All categories	0.82% (250 of 30622)

*Variant result totals may include variants classified as VUS or benign.
Note: Per variant detection rate was calculated as the number of unique variants detected at least once across all replicates divided by the total number of unique variants included in the analysis.

All other variants were assigned an LoB of 0, as the detection rate not significantly exceeding 5%. Each cancer-related alteration detected in this study was detected in replicates from a single donor, indicating that these are likely true variants present in the sample.

A post-market LoB study will be conducted to confirm the results in accordance with the FoundationOne Liquid CDx assay workflow.

b. Limit of Detection (LoD):
The LoD for each variant type was established by processing a total of 1,069 sample replicates across ten contrived (enzymatically fragmented cell-line gDNA) samples representing short variants, rearrangements, and copy number alterations (homozygous deletions). The LoD was determined using the conservative hit rate approach for the majority of variants. LoD by hit rate was defined as the mean VAF value (for short variants and rearrangements) or mean tumor fraction 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. Short variants with hit rates of at least 95% at all dilution levels or hit rates below 95% for all dilution levels were excluded from analysis as LoD could not be reliably estimated.

The median estimated LoD for CDx alterations are presented in Table 14. The median LoD for targeted short variant, rearrangement, and copy number alterations were consistent with the platform LoD.

Table 14. LoD estimation for CDx alterations

Gene	Alteration Subtype	# Samples Evaluated	Median LoD[1]
BRCA1	Indels	1	0.38% VAF
	Substitutions	8	0.34% 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
EGFR	Indels (Exon 19 deletions)	2	0.27% VAF
	Substitutions (L858R substitutions)	2	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 18 and 19 for confirmed LoD values).

[1] The accuracy of %VAF/%TF have not been analytically validated.
[2] The LoD for these alterations was 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 is 30.4% tumor fraction for copy number losses. Rearrangement LoD was estimated as a median of 0.37% for the enhanced sensitivity region and 0.9% for the standard sensitivity region. The LoD for copy number losses was estimated at 30.4% based on tumor fraction.

Because a major component driving the detectability of a variant is genomic context (repetitiveness of the reference genomic region), an LoD analysis for short variants was also performed within categories based on genomic context**.**

4. Analytical Specificity:
   a. Potentially Interfering Substances:
   To evaluate the robustness of the FoundationOne Liquid CDx results in the presence of potentially interfering exogenous and endogenous substances, a total of 11 potential interferents were evaluated. These potential interferents included six endogenous substances (albumin, conjugated bilirubin, unconjugated bilirubin, cholesterol, hemoglobin and triglycerides) and five exogenous substances (DNA from another source [the microorganism *Staphylococcus epidermidis*], excess anticoagulant, proteinase K, ethanol and molecular index barcodes).

   A total of 340 samples were tested to evaluate the potential interference of albumin, conjugated bilirubin, unconjugated bilirubin, cholesterol, hemoglobin, triglycerides, DNA from another source (the microorganism *Staphylococcus epidermidis*), excess anticoagulant, proteinase K, ethanol, and molecular index barcodes. An assessment of the cfDNA yield obtained during the DNA isolation, purification, and quantification steps, as well as at library construction QC (LCQC) and hybrid capture QC (HCQC) was performed. Substances were considered as non-interfering if ≥90% of samples across aggregated replicates per treatment level successfully met processing criteria. The process success rates for each step are listed in Table 15.

Table 15: Process success rates with interfering substances

Process	# Failed	# Pass	Total	Success Rate (%)	95% CI LB (%)	95% CI UB (%)
DNA Extraction	0	180	180	100.00	97.97	100.00
LC	1	339	340	99.71	98.37	99.99
HC	3	336	339	99.12	97.44	99.82
Sequencing	0	336	336	100.00	98.91	100.00

For each potential interferent, concordance of alteration calls was calculated relative to a control sample without interferent. The pre-defined variants included 27 short variants, 17 rearrangements, and 3 copy number variants. Of the 11 potential interferents tested across 16 conditions, concordance for all variant calls was 100% for 8 conditions and ≥97% for all conditions (Table 16). Potential interference from albumin (60 g/L), Bilirubin (unconjugated at 0.2 g/L), and cholesterol (at 150 mg/dL and 250 mg/dL) was observed for the single rearrangement evaluated for each of these four conditions.

Table 16: Concordance per substance for variants 1.5x LoD

Substance	Concordance	95% CI	N
Triglycerides, 37 mmol/L (or 33 g/L)	100.00%	(91.00%, 100.00%)	40
Hemoglobin, 2.0 g/L	100.00%	(91.00%, 100.00%)	39
Albumin, 60 g/L	97.56%	(87.00%, 100.00%)	41
Bilirubin (conjugated), 0.2 g/L	100.00%	(92.00%, 100.00%)	42
Bilirubin (unconjugated), 0.2 g/L	97.44%	(87.00%, 100.00%)	39
Cholesterol Level 2, 3.88 mmol (150 mg/dL)	97.56%	(87.00%, 100.00%)	41
Cholesterol Level 1, 6.47mmol (250 mg/dL)	97.37%	(86.00%, 100.00%)	38
Staphylococcus epidermidis, 1 x 106 CFU/mL	100.00%	(91.00%, 100.00%)	39
Anticoagulant, 5X nominal volume	100.00%	(91.00%, 100.00%)	41
Proteinase K, +0.6 mg/mL	98.00%	(89.00%, 100.00%)	50
Proteinase K, +0.3 mg/mL	100.00%	(92.00%, 100.00%)	46
Ethanol, +2.5%	97.96%	(89.00%, 100.00%)	49
Ethanol, +5.0%	97.92%	(89.00%, 100.00%)	48
Molecular Index barcodes, +5%	97.22%	(85.00%, 100.00%)	36
Molecular Index barcodes, +15%	100.00%	(93.00%, 100.00%)	48
Molecular Index barcodes, +30%	100.00%	(93.00%, 100.00%)	49

Taken together, these data indicate that the FoundationOne Liquid CDx assay is robust to potential specimen-related endogenous substances and exogenous contaminants or interferents.

b. Hybrid Capture Bait Specificity:
Bait specificity was addressed through an assessment of coverage of targeted regions in FoundationOne Liquid CDx using 3,546 validation study samples. Results show that targeted genomic regions have consistently high, uniform coverage. For each genomic region associated with a predefined subset of

highly-actionable alterations, between 94% to 100% of samples possessed the expected level of coverage. An in-depth, platform-wide examination of the FoundationOne Liquid CDx bait set through the analysis of HapMap process control samples revealed that, on average, 98.8% and 94.1% of platform-wide baited coding and non-coding regions, respectively, met their expected coverage levels. Samples assessed in this study consistently demonstrated high quality uniform and deep coverage across the entire genomic region targeted by the assay.

5. Carryover/Cross-Contamination:
The study demonstrated that the risk of cross contamination (intra-plate), and carry-over contamination (inter-plate) of samples during processing of the FoundationOne Liquid CDx assay is low. A total of 376 wells were examined for intra- and inter-plate contamination by processing and sequencing of contrived samples derived from cell lines at high input concentrations with known genomic backgrounds. Unique variants of each cell line were characterized by independent control sequencing runs. The samples were arrayed in a checkerboard fashion across four 96-well PCR plates to detect cross-contamination events. A cross-contamination rate of 0.53% (2/376) was observed in this study. These data demonstrate a low probability of cross contamination during the FoundationOne Liquid CDx process.

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. The samples representing CDx alterations are summarized in Table 17.

   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 7 *BRCA1* or *BRCA2* alterations and 4 *EGFR* substitutions and indels. The remaining samples included multiple other actionable genes and variant types.

   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.

Table 17: CDx sample set assessed for precision

Targeted Alteration	**Disease Ontology of Patient from which Sample was Derived**
EGFR Exon 19 deletions and *EGFR* exon 21 L858R alterations	5 contrived samples
BRCA1, BRCA2 alterations	6 contrived samples
*BRCA1 E23fs*17*	Ovary cancer
BRCA1 Q780*	Ovary high grade serous carcinoma
BRCA1 Rearrangement	Unknown primary malignant neoplasm
BRCA2 G267*	Ovary serous carcinoma
BRCA2 Loss (15 of 26)	Prostate acinar adenocarcinoma
BRCA2 Loss *(26 of 26)*	Prostate acinar adenocarcinoma
BRCA2 S2988fs*12	Ovary cancer
BRCA2- EDA Truncation	Prostate cancer
EGFR E746_A750del	Non-small cell lung carcinoma
EGFR L858R	Non-small cell lung carcinoma
EGFR L858R	Non-small cell lung carcinoma

The repeatability of CDx alterations is summarized in Table 18 and the reproducibility of CDx alterations is summarized in Table Table 19.

Table 18: Repeatability of CDx alterations targeted in precision study at >1x LoD*

Variant Type	**Alteration**	**Concordant Pairs**	**Repeatability (%)**	**95% CIs (%)**	**Level Tested****
Short variant	*BRCA1*_2338C>T	12/12	100	(73.54, 100)	1.11% VAF
Short variant	*BRCA1*_2475delC	12/12	100	(73.54, 100)	0.61% VAF
Short variant	*BRCA1*_2475delC	12/12	100	(73.54, 100)	0.93% VAF
Short variant	*BRCA1*_2612C>TT	11/11	100	(71.51, 100)	0.51% VAF
Short variant	*BRCA1*_68_69delAG	12/12	100	(73.54, 100)	0.66% VAF
Short variant	*BRCA1*_P871fs*32	12/12	100	(73.54, 100)	1.08% VAF
Rearrangement	*BRCA1-BRCA1*	12/12	100	(73.54, 100)	0.87% VAF
Short variant	*BRCA2*_3599_3600delGT	12/12	100	(73.54, 100)	0.58% VAF
Short variant	*BRCA2*_3599_3600delGT	12/12	100	(73.54, 100)	0.92% VAF
Short variant	*BRCA2*_4284_4285insT	12/12	100	(73.54, 100)	0.94% VAF
Short variant	*BRCA2*_4284_4285insT	11/11	100	(71.51, 100)	1.26% VAF
Short variant	*BRCA2*_5351delA	12/12	100	(73.54, 100)	1.22% VAF
Short variant	*BRCA2*_5351delA	12/12	100	(73.54, 100)	1.85% VAF
Short variant	*BRCA2*_5351delA	11/11	100	(71.51, 100)	1.07% VAF
Short variant	*BRCA2*_5351delA	12/12	100	(73.54, 100)	2.24% VAF
Short variant	*BRCA2*_5465_5466insA	12/12	100	(73.54, 100)	0.92% VAF
Short variant	*BRCA2*_5465_5466insA	11/11	100	(71.51, 100)	1.19% VAF
Short variant	*BRCA2*_8961_8964delGAGT	12/12	100	(73.54, 100)	1.07% VAF
Short variant	*BRCA2*_c.799G>T	10/12	83.33	(51.59, 97.91)	0.5% VAF

Variant Type	**Alteration**	**Concordant Pairs**	**Repeatability (%)**	**95% CIs (%)**	**Level Tested****
Short variant	*BRCA2*_c.9097_9098insA	6/11	54.55	(23.38, 83.25)	0.71% VAF
Short variant	*BRCA2*_c.9097_9098insA	10/12	83.33	(51.59, 97.91)	1.03% VAF
Copy Number Loss	*BRCA2*_loss	11/12	91.67	(61.52, 99.79)	39.43% TF
Rearrangement	*BRCA2*-EDA	11/11	100	(71.51, 100)	0.48% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	0.44% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	0.66% VAF
Short variant	*EGFR*_2369C>T	11/11	100	(71.51, 100)	0.36% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	0.65% VAF
Short variant	*EGFR*_2369C>T	12/12	100	(73.54, 100)	1.26% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.46% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.68% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.68% VAF
Short variant	*EGFR*_2573T>G	11/11	100	(71.51, 100)	0.95% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	0.64% VAF
Short variant	*EGFR*_2573T>G	12/12	100	(73.54, 100)	1.64% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	0.51% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	0.74% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	0.93% VAF
Short variant	*EGFR*_E746_A750del	11/11	100	(71.51, 100)	1.2% VAF
Short variant	*EGFR*_E746_A750del	11/11	100	(71.51, 100)	0.51% VAF
Short variant	*EGFR*_E746_A750del	12/12	100	(73.54, 100)	1.01% VAF
Short variant	*EGFR*_E746_A750del	11/11	100	(71.51, 100)	0.34% VAF

*Clinical samples were mostly tested at 2x – 3x LoD rather than 1x – 1.5x LoD
**The accuracy of %VAF/%TF have not been analytically validated.

Table 19: Reproducibility of CDx alterations targeted in precision study at >1x LoD*

Variant Type	**Alteration**	**Concordant Replicates**	**Reproducibility (%)**	**95% CIs (%)**	**VAF/TF** Level Tested**
Short variant	*BRCA1*_2338C>T	24/24	100	(85.75, 100)	1.11%
Short variant	*BRCA1*_2475delC	24/24	100	(85.75, 100)	0.61%
Short variant	*BRCA1*_2475delC	24/24	100	(85.75, 100)	0.93%
Short variant	*BRCA1*_2612C>TT	23/23	100	(85.18, 100)	0.51%
Short variant	*BRCA1*_68_69delAG	24/24	100	(85.75, 100)	0.66%
Short variant	*BRCA1*_P871fs*32	24/24	100	(85.75, 100)	1.08%
Rearrangement	*BRCA1-BRCA1*	24/24	100	(85.75, 100)	0.87%
Short variant	*BRCA2*_3599_3600delGT	24/24	100	(85.75, 100)	0.58%
Short variant	*BRCA2*_3599_3600delGT	24/24	100	(85.75, 100)	0.92%
Short variant	*BRCA2*_4284_4285insT	24/24	100	(85.75, 100)	0.94%
Short variant	*BRCA2*_4284_4285insT	23/23	100	(85.18, 100)	1.26%
Short variant	*BRCA2*_5351delA	24/24	100	(85.75, 100)	1.22%
Short variant	*BRCA2*_5351delA	24/24	100	(85.75, 100)	1.85%
Short variant	*BRCA2*_5351delA	23/23	100	(85.18, 100)	1.07%
Short variant	*BRCA2*_5351delA	24/24	100	(85.75, 100)	2.24%
Short variant	*BRCA2*_5465_5466insA	24/24	100	(85.75, 100)	0.92%
Short variant	*BRCA2*_5465_5466insA	23/23	100	(85.18, 100)	1.19%

Variant Type	Alteration	Concordant Replicates	Reproducibility (%)	95% CIs (%)	VAF/TF** Level Tested
Short variant	*BRCA2*_799G>T	22/24	91.67	(73.0, 98.97)	0.5%
Short variant	*BRCA2*_8961_8964delGAGT	24/24	100	(85.75, 100)	1.07%
Short variant	*BRCA2*_9097_9098insA	22/24	91.67	(73.0, 98.97)	1.03%
Short variant	*BRCA2*_c.799G>T	22/24	91.67	(73.0, 98.97)	0.5%
Short variant	*BRCA2*_c.9097_9098insA	5/23	21.74	(7.46, 43.7)	0.71%
Short variant	*BRCA2*_c.9097_9098insA	22/24	91.67	(73.0, 98.97)	1.03%
Copy Number Loss	*BRCA2* loss	21/24	87.5	(67.64, 97.34)	39.43% TF
Rearrangement	*BRCA2*-EDA	23/23	100	(85.18, 100)	0.48%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	0.44%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	0.66%
Short variant	*EGFR*_2369C>T	23/23	100	(85.18, 100)	0.36%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	0.65%
Short variant	*EGFR*_2369C>T	24/24	100	(85.75, 100)	1.26%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.46%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.68%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.68%
Short variant	*EGFR*_2573T>G	23/23	100	(85.18, 100)	0.95%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	0.64%
Short variant	*EGFR*_2573T>G	24/24	100	(85.75, 100)	1.64%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	0.51%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	0.74%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	0.93%
Short variant	*EGFR*_E746_A750del	23/23	100	(85.18, 100)	1.2%
Short variant	*EGFR*_E746_A750del	23/23	100	(85.18, 100)	0.51%
Short variant	*EGFR*_E746_A750del	24/24	100	(85.75, 100)	1.01%
Short variant	*EGFR*_E746_A750del	22/22	100	(84.56, 100)	0.34%

*Clinical samples were mostly tested at 2x – 3x LoD rather than 1x – 1.5x LoD
**The accuracy of %VAF/%TF have not been analytically validated.

For repeatability, 42 samples with 53 targeted alterations were evaluated. Of the 53 alterations that were targeted, 43 alterations demonstrated 100% repeatability. Within the targeted CDx variants assessed, the overall repeatability was 96.39% (95.28%, 97.30%).

Reproducibility of 100% was observed in 42 of 55 (76.4%) alterations. These results demonstrate the robustness of FoundationOne Liquid CDx assay, as 55 targeted alterations passed the acceptance criterion for reproducibility. For the targeted CDx variants assessed, the overall reproducibility was 97.33% (96.67 %, 97.89%).

b. Confirmation of LoD and Precision in Clinical Specimens:
Twenty-nine 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 91.67% repeatability. This sample had cfDNA input below the recommended minimum. The other sample harbored a BRCA2 substitution (c.799G>T) with 91.67% reproducibility and 83.33% repeatability. The average VAF of this variant was 0.5% across replicates, which is near the LoD for this variant type (LoD of 0.4% VAF). A summary of the Confirmation of LoD and precision results for a subset of highly-actionable alterations are provided in Table 20.

Table 20: Confirmation of LoD* and precision in clinical specimens for CDx alterations

Target Alteration	LoD[1]	Mean Level Tested[2]	Reproducibility (95% CI)	95% CIs (%)
BRCA1 E23fs*17	0.38% VAF	0.66% VAF	100	(85.75, 100)
BRCA1 Q780*	0.34% VAF	1.11%VAF	100	(85.75, 100)
BRCA1 Rearrangement	0.87% VAF	0.87% VAF	100	(85.75, 100)
BRCA2 799G>T	0.40% VAF	0.50% VAF	91.6	(73.0, 98.97)
BRCA2 Loss	48.1% TF	39.43%TF	87.5	(67.64, 97.34)
BRCA2 S2988fs*12	0.36% VAF	1.07% VAF	100	(85.75, 100)
BRCA2- EDA Truncation	0.48% VAF	0.48% VAF	100	(85.18, 100)
EGFR E746_A750del	0.27% VAF	0.34% VAF	100	(84.56, 100)
EGFR L858R	0.34% VAF	1.64% VAF	100	(85.75, 100)
EGFR L858R	0.34% VAF	0.64% VAF	100	(85.75, 100)

[1] Estimated LoD levels reported in Table 14.
[2] The accuracy of %VAF/%TF have not been analytically validated

As observed in the tables above (19, 20, and 21), several *BRCA2* positive samples (c.799G>T and c.9097_9098insA, and a *BRCA2* loss) demonstrated poor performance for both repeatability and reproducibility. For the *BRCA2* specimen harboring the c.799G>T, upon investigation the average %VAF was determined to be 0.5% near the LoD of 0.4% for this variant type. The BRCA2 c.9097_9098insA variant had a 93% hit rate at the highest level tested in the LoD study, 1.16% VAF, indicating that the levels evaluated in this precision analysis were below the LoD for this variant. This variant is an insertion of an A in a highly repetitive homopolymer region of eight As, which impacts sensitivity. The replicates for the clinical sample harboring the *BRCA2* loss were processed at 24 ng cfDNA input, below the minimum cfDNA input of 30 ng.

In general most of the targeted variants were tested at levels higher than near 1x LoD; therefore the tested LoD level values (%VAF/%TF) are actually considered to be the confirmed LoD. A post-market study is planned to demonstrate precision using samples at near the estimated LoD.

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. Three had 100% reproducibility, one had 95.7% reproducibility, one had 91.7% reproducibility, and one had 91.3% 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 21.

Table 21: Confirmation of LoD and precision in clinical specimens for CDx alterations

Target Alteration	LoD	Mean Level Tested[1]	Reproducibility (95% CI)	95% CIs (%)
BRCA1 1395T>A	0.34%	0.51%	100%	[86.2%, 100%]
BRCA2 5351_5352insA	0.36%	0.34%	87.5%	[69.0%, 95.7%]
EGFR 2235_2249del	0.27%	0.45%	95.7%	[79.0%, 99.2%]

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

As summarized in Table 21 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 variant allele fraction below the LoD.

c. Tumor Mutation Profiling Variants:
Across 39 unique samples, including 8 contrived samples, and 31 clinical samples, a total of 1,126 variants were evaluated with variant types including substitutions and indels,. The number of variants in each variant bin are summarized in Table 22.

Table 22: Number of each variant type

Variant Category	N
Substitutions	898
Indels	228
Total	1126

The overall repeatability for all short variants was 99.51% with 95% 2-sided exact CIs (99.49%, 99.53%). The repeatability result for each variant type are summarized in Table 23**.**

Table 23: 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 result totals include variants classified as VUS or benign.

The overall reproducibility results were 99.62% with the 95% 2-sided exact CIs (99.61%, 99.63%). The reproducibility result for each variant type are summarized Table 24.

Table 24: 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)

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

d. Reagent Lot-to-Lot Reproducibility:
Three lots of critical reagents were assessed in a factorial design. Reagents were evaluated as internally prepared kits for each process step (LC, HC, sequencing). The variant level pairwise average positive agreement (APA) and average negative agreement (ANA) among three reagent lots and the corresponding 95% confidence interval per sample were calculated. For APA, 43 of 47 samples (91.5%) had APA results above 90%, ranging from 90.11% to 100%. For ANA, 47 of 47 samples (100%) had ANA results above 97%, ranging from 97.5% to 100%.

e. Instrument-to-Instrument Reproducibility:
Two sequencers were assessed in a factorial design. The variant level pairwise APA/ANAs among two sequencers and the corresponding 95% CI per sample were calculated. For APA, 43 of 47 samples (91.5%) had APA results above 90%, ranging from 90.74% to 100%. For ANA, 47 of 47 samples (100%) had ANA results above 97%, ranging from 97.53% to 100%.

f. Reagent Lot Interchangeability:
The interchangeability of critical reagent lots for library construction (LC), hybrid capture (HC) and sequencing within the FoundationOne Liquid CDx assay was evaluated by testing eight (8) contrived samples from either enzymatically fragmented cell line gDNA containing alterations of interest or enzymatically fragmented plasmid DNA. Each of the contrived samples was tested in triplicate using two different lots each of LC, HC, and sequencing reagents. Eight reagent pairings were assessed. A total of eight analyses for each specimen were completed. 192 tests in total were included in this study. Four Master Pool Libraries (MPLs) were evaluated on each of two flowcells on a NovaSeq 6000 sequencer, using two different Sequencing reagent lots. Of the 49 alterations assessed in the sample set, 43 had a percent agreement greater than 90% (39 alterations had percentage agreement equal to 100%, one had percent agreement equal to 95.83%, one had percent agreement equal to 95.65%, and two had percent agreement equal to 91.67%), exceeding the pre-specified acceptance criteria. For the remaining six alterations the observed detection rates for these variants were similar to the predicted detection rate based on the LoD analysis. These results demonstrate the interchangeability of critical reagent lots in the FoundationOne Liquid CDx assay.

g. Curator Precision:
This study was performed to evaluate the precision of genomic variant call curation, following analysis by the FoundationOne Liquid CDx analysis pipeline. This was established by analyzing targeted alterations, including CDx alterations, and platform-wide alterations within samples used in the FoundationOne Liquid CDx Precision and LoD and Precision Confirmation Study. The study design reflected the intermediate precision design and evaluated curator precision in reporting of targeted and platform alterations. A total of 19 samples were selected for this study. Three curators were chosen randomly amongst all qualified curators to curate variant calls in a set of randomly chosen replicates from each of the 19 samples. The variant calls were generated from each sample per curator. The overall average percent agreement for targeted alterations was 93.3% (95% CI; 83.80%, 98.15%), and for platform genomic alterations was 99.14% (95% CI; 98.47%, 99.57%).

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, each of which includes only a subset of the genes included in FoundationOne Liquid CDx. FoundationACT (FACT) included 62 genes where the FoundationOne Liquid LDT included 70 genes. The workflow is substantially similar between the two assays. The test performance of FoundationOne Liquid CDx was evaluated by comparing in-process QC metrics across tumor types using historical data from samples processed in Foundation Medicine's clinical laboratory. In order to support the use of historical data in this study, only those regions commonly baited between the respective versions of the FoundationOne Liquid LDT and the bait set used by FoundationOne Liquid CDx were included in the analysis.

The sample set for this analysis included 19,868 distinct samples from 25 tumor type categories that had previously been tested using the Foundation Medicine FoundationOne Liquid LDT and FACT assays, previous versions of FoundationOne Liquid CDx. Table 25 below provides a summary of the tissue types included in the study. Overall, 98.1% samples yielded ≥25ng cfDNA, which corresponds to a cfDNA input mass of 20 ng for library construction (LC). A total of 89.1% of samples yielded ≥36 ng of cfDNA which corresponds to a cfDNA input mass of 30 ng for LC. The proportion of samples with an LC yield greater than the minimum mass of 500 ng was 99.9%, with one sided 95% confidence interval of (99.8%, 99.9%). The proportion of samples with an HC yield greater than the minimum mass of 1000ng was 100%, with one sided 95% confidence interval of (99.99%, 100%). The proportion of samples which met coverage requirements was 96.2%, with one sided 95% confidence interval of (95.9%, 96.3%). The proportion of samples that generated a passing or qualified result

after sequencing was 95.4%, with one sided 95% confidence interval of (95.1%, 95.6%).

Table 25: FoundationOne Liquid LDT/FACT samples per tumor type and pass rates

Tumor Type	Sample Size	Passing Rates					
		DNA Extraction (≥25 ng)	DNA Extraction (≥36 ng)	LC Yield	HC Yield	Median Coverage	Overall (≥36 ng)
Rare Tumors	1164	97.0%	86.4%	99.9%	100.0%	93.8%	94.0%
Biliary Cancer	171	99.4%	95.3%	100.0%	100.0%	98.8%	97.1%
Bladder Cancer	166	97.6%	85.5%	100.0%	100.0%	93.2%	98.8%
Breast Cancer	2775	97.6%	87.7%	99.9%	100.0%	96.4%	95.3%
Cholangio-carcinoma	377	98.9%	96.0%	99.7%	100.0%	98.7%	96.8%
CRC	1640	98.5%	92.4%	99.9%	100.0%	97.5%	96.9%
Endocrine-Neuro Cancer	75	100.0%	85.3%	100.0%	100.0%	100.0%	93.3%
Endometrial Cancer	231	98.3%	88.3%	100.0%	100.0%	96.5%	95.6%
Esophagus Cancer	291	99.7%	92.4%	100.0%	100.0%	97.6%	96.6%
Glioma Cancer	59	94.9%	72.9%	100.0%	100.0%	100.0%	76.8%
Head and Neck Cancer	154	96.1%	81.8%	100.0%	100.0%	89.2%	95.3%
Kidney Cancer	203	99.0%	87.7%	100.0%	100.0%	95.0%	95.0%
Liver Cancer	109	98.2%	95.4%	100.0%	100.0%	100.0%	95.3%
Lung, NSCLC	5919	98.2%	88.8%	99.8%	100.0%	95.5%	95.4%
Melanoma	257	96.5%	79.8%	100.0%	100.0%	92.7%	93.1%
Ovary Cancer	496	97.8%	88.5%	100.0%	100.0%	95.9%	94.2%
Pancreas Cancer	1359	98.8%	94.0%	99.9%	100.0%	97.8%	95.5%
Peripheral Nervous System (PNS)	44	100.0%	90.9%	100.0%	100.0%	100.0%	93.2%
Prostate Cancer	1778	97.3%	87.7%	99.9%	100.0%	96.9%	95.1%
Small Cell Cancer	135	98.5%	93.3%	100.0%	100.0%	99.2%	99.2%
Soft Tissue Sarcoma	130	97.7%	83.1%	100.0%	100.0%	95.3%	92.1%
Stomach Cancer	267	98.9%	89.1%	100.0%	100.0%	98.1%	93.2%
Thyroid Cancer	50	98.0%	86.0%	100.0%	100.0%	100.0%	81.6%
Unspecified	856	98.5%	89.1%	100.0%	100.0%	95.5%	96.3%
Unknown Primary Carcinoma (CUP)	1162	98.1%	89.7%	100.0%	100.0%	95.2%	95.7%

Table 26 summarizes the overall sample pass rate across tumor types as well as performance metrics from key QC points in the process. These results demonstrate comparable test performance across tumor types.

Table 26: Summary of FoundationOne Liquid LDT/FACT sample data

QC Metric	QC Pass Rate Across Tumor Types	Tumor Types with ≥ 90% QC Pass Rate
Overall report Pass/Qualified rate	76.8%~99.2%	23/25 (92%)
Library Construction	99.7%~100%	25/25 (100%)
Hybridization Capture	100%	25/25 (100%)
Median exon coverage	89.2%~100%	24/25 (96%)

A second analysis was performed to evaluate the concordance between the FoundationOne Liquid LDT, FACT, and the FoundationOne Liquid CDx assays based on the concordance of 927 unique samples processed on both the FoundationOne Liquid LDT and FoundationOne Liquid CDx assays in which a total of 3,366 unique alterations were evaluated. The concordance analysis using FoundationOne Liquid LDT or FoundationOne Liquid CDx as the reference assay is summarized by variant category in Table 27.

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 27: Concordance* between FoundationOne Liquid LDT and FoundationOne Liquid CDx

Variant**/ Mutation Type	CDx(+) / LDT(+)	CDx(-) / LDT(+)	Dx(+) / LDT(-)	CDx(-) / LDT(-)*	PPA (95% CI)	NPA (95% CI)	OPA (95% CI)
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%)
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%)

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

The overall PPA between FoundationOne Liquid LDT and FoundationOne Liquid CDx assays, with FoundationOne Liquid LDT as the reference assay, was 94.8%

with a 95% two-sided CI of (94.0%, 95.5%). The respective short variant, and rearrangement PPA values, with 95% two-sided CI, were: 95.9% (95.1%, 96.6%), 84.4% (78.7%, 89.1%), and 88.0% (82.1%, 92.5%). These results from this study supports 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:
Evaluation of the stability of critical reagents for library construction (LC), hybrid capture (HC), and sequencing within the FoundationOne Liquid CDx Assay is ongoing. The reagent stability of FoundationOne Liquid CDx is assessed by analyzing data from each of eight samples in triplicate, per each of three different lots of LC, HC, and sequencing reagents. A total of nine analyses for each specimen will be completed for each of six time points assessed. A total of 72 tests will be assessed per time period; a total of 432 samples and six time points will be included in this study overall. Each of the three sample Master Library Pools (MPLs), representing three LC and HC reagent lots will be evaluated per time point on a NovaSeq 6000 sequencer, using three different sequencing reagent lots. The analysis of baseline timepoint zero (T_0) identified the baseline variant calls for each sample. These results provide the baseline to which subsequent time points will be compared. Concordance of 12,511 variant alterations will be assessed across future time points for sample aliquots derived from eight DNA samples.

To date, timepoint T_1 (3 months) has been analyzed for reagent Lot #1, Lot #2 and Lot #3. Variants at the experimental timepoints are ≥90% concordant with the baseline variant call values as presented in Table 28. Current data demonstrates LC, HC, and sequencing reagent stability for up to 3 months. This study is ongoing and further evaluation will be performed to validate reagent stability over 12 months.

Table 28: Concordance analysis between 3 months and baseline

	Reagent Lot	**Timepoint**	**# Replicates Concordant**	**Total # Replicates**	**Concordance Percentage**	**95% CI**
Variant Calls	Lot #1	1	1921	1966	97.71%	96.95%, 98.28%
	Lot #2	1	2083	2148	96.97%	96.16%, 97.62%
	Lot #3	1	2086	2139	97.52%	96.77%, 98.10%

b. Stability of cfDNA and Plasma Samples:
The stability of plasma and cfDNA for FoundationOne Liquid CDx processing over time was assessed through evaluating concordance between FoundationOne Liquid CDx processing and historical FoundationOne Liquid processing of matched samples.

A set of 543 samples processed during multiple time periods were included in this study. As summarized in Table 29, concordance results indicate stability of frozen plasma for up to 19 months for plasma stored under appropriate laboratory conditions. Due to a small sample size in the earliest time period, a broad confidence interval is observed for rearrangement and copy number concordance.

Table 29: Concordance of variant categories over time from frozen plasma

Dates of Sample Processing		Time Between Processing	# samples	$PPA_{F1Liquid}$		
F1L LDT	F1L CDx			Short Variant	Copy Number	Rearrangement
Nov – Dec 2017	Jul – Sep 2019	19 – 22 months	54	92.5% (=86/93) [85.1%, 96.9%]	66.7% (=6/9) [29.9%, 92.5%]	50% (=3/6) [11.8%, 88.2%]
Jan – Jun 2018	Jul – Sep 2019	13 – 20 months	489	94.0% (=1061/1129) [92.4%, 95.3%]	85.4% (=41/48) [72.2%, 93.9%]	87.5% (=63/72) [77.6%, 94.1%]

$PPA_{F1\ Liquid}$ = PPA using F1 Liquid LDT as the reference.

An analysis with a set of an additional 122 samples indicated stability of frozen cfDNA for up to 33 months if stored under appropriate laboratory conditions. Sample data were analyzed across three time periods, as summarized in Table 30. Due to a small sample size in the earliest time period, a broad confidence interval is observed for rearrangement concordance. Given the number of rearrangements (n=2) in the earlier time period no conclusions can be drawn from the observed 50% concordance.

Table 30: Concordance of variant categories over time from frozen cfDNA PPA_{F1L}

Dates of Sample Processing		Time Between Processing	# Samples	$PPA_{F1Liquid}$		
F1 Liquid LDT	F1 Liquid CDx			Short Variant	Copy Number	Rearrangement
May – Dec 2016	Sep 2019	33 – 40 months	36	98.5% (=133/135) [94.8%, 99.8%]	100% (=8/8) [63.1%, 100.0%]	50% (=1/2, low *n*) [1.26%, 98.7%]
Jan – Jun 2017	Sep 2019	32 - 27 months	44	99.4% (=170/171) [96.8%, 99.9%]	88% (=15/17) [63.6%, 98.5%]	100% (=5/5) [47.8%, 100.0%]
Jul – Dec 2017	Sep 2019	22 – 26 months	42	98.7% (=236/239) [96.4%, 99.7%]	90.9% (=10/11) [58.7%, 99.8%]	100% (=8/8) [63.1%, 100.0%]

Based on the data provided, concordance analyses across specific time periods demonstrate stability of cfDNA over a minimum of 33 months and plasma over a minimum of 19 months. These data supported the use of stored sample in the clinical bridging studies as summarized in Section X, below.

c. Whole Blood Specimen Stability and Inverted Tube Stability:

Whole blood stability and the impact of tube inversion was evaluated in freshly collected whole blood samples from the following five cancer types: NSCLC, CRC, prostate, breast, and ovarian cancer.

i. A whole blood stability study is ongoing to establish sample stability parameters for whole blood stored in the collection tubes provided in the Foundation Medicine sample collection kit (FoundationOne Liquid CDx cfDNA BCT). The data presented here is from an interim analysis evaluating whole blood sample stability parameters for the period between sample receipt and processing. The recommended storage temperature is 18°C – 25°C. In this study, stress conditions were simulated through extended storage at elevated (35°C ± 2°C) and reduced (4° ± 2°C) temperatures.

In this interim analysis, 22 samples (11 sample pairs) were tested, including baseline (within 24 hours of collection) and experimental timepoints (after 10, 14, or 15 days of storage).

Overall, 100% of samples yielded a cfDNA input ≥30ng. The success rate for DNA extraction (DNAx) yield, and LC yield were 100% and the success rate of the HC yield was 96.3%. The variant analysis was conducted for variants at ≥2x LoD. For the aggregate 11 pairs of samples processed and reported, 100% agreement was observed between the baseline and experimental timepoint for short variants and rearrangements for each experimental timepoint. The percent agreement per sample also resulted in 100% agreement between the baseline and experimental timepoint for short variants and rearrangements. The data are summarized Table 31**.**

Table 31: Aggregate percent agreement per temperature and experimental timepoint

Temperature	Experimental Timepoint	N	Short Variants (95% two-sided CI)	Rearrangements
4°C	7 Days	4	100.00 (89.72, 100.00)	100.00 (39.76, 100.00)
	14 Days	3	100.00 (91.40, 100.00)	N/A
	15 Days	3	100.00 (83.89, 100.00)	N/A
35°C	14 Days	1	N/A	N/A

An additional post-market study will be performed in order to verify the study results and to incorporate lot variability.

ii. The impact of potential interferents originating from the FoundationOne Liquid CDx blood collection tube (BCT) stopper on the performance of

the FoundationOne Liquid CDx assay was assessed by comparing whole blood samples processed upon receipt at FMI (baseline samples within 24 hours of collection) to experimental samples processed after treatment in an inverted (INV) position at manufacturer's recommended storage temperature (18 – 25°C) for various durations (10, 14 and 15 days between sample receipt and processing). In addition to the standard storage temperature, the effect of tube inversion was examined in simulated stress transport conditions at reduced (4 ± 2°C) and elevated (35 ± 2°C) temperatures at the proposed time points. Additionally, storage in an upright (UPR) position was also used to further evaluate the stability of whole blood collected in the FoundationOne Liquid CDx BCT.

First, the success rate of the FoundationOne Liquid CDx assay for processing samples was assessed at the DNA extraction (DNAx), Library Construction (LC), Hybrid Capture (HC) and Sequencing step, based on product in-process quality control (QC) criteria. Samples stratified by the upright and the inverted condition exhibited comparable success rates above 94% at DNAx, LC, HC and Seq (Table 32). Thus, the stopper of the FoundationOne Liquid CDx BCT does not impact FoundationOne Liquid CDx test performance when stored between 4 and 35°C for up to 15 days after receipt of the sample at FMI.

Table 32: Process success rate by tube position

Process	Tube Position	# Passing Samples	# Total Samples	Success Rate (%)	95% CIs (%)
DNA Extraction	Upright	139	147	94.6%	(89.6%, 100%)
	Inverted	147	150	98%	(94.3%, 100%)
LC	Upright	135	136	99.3%	(96%, 100%)
	Inverted	146	146	100%	(97.4%, 100%)
HC	Upright	134	135	99.3%	(95.9%, 100%)
	Inverted	143	146	97.9%	(94.1%, 100%)
Sequencing	Upright	134	134	100%	(97.2%, 100%)
	Inverted	143	143	100%	(97.4%, 100%)

In addition to examining the effect of tube inversion on process success rates, whole blood stability and the potential impact of the blood collection tube cap were evaluated by comparing concordance between baseline and experimental samples. Positive percent agreement (PPA) and negative percent agreement (NPA) for alteration calls at ≥ 2x LoD were computed along with the corresponding two-sided 95% score confidence interval (CI) across all replicates by variant category using the baseline detection as reference. Note that NPA is under-estimated as variants not detected at any of the treatment conditions were not used in the analysis set and hence counted against the NPA calculation.

Concordance between baseline and experimental results from all samples in the upright and inverted position combined demonstrated > 99% PPA and NPA for the detection of short variants and rearrangements. Copy number alterations were only detected in samples treated in the inverted tube position and therefore, not included in this analysis. Furthermore, stratification by the treatment condition (2 tube positions × 3 temperatures × 3 durations) revealed >99.0% PPA and NPA for short variants and rearrangements across the combinations of tube positions, temperatures and durations tested. The data also demonstrate that the detection of copy number alterations is not impacted by storage of blood in the inverted position at 35°C for up to 14 days following receipt of the sample at FMI.

These results demonstrate that blood is stable in the FoundationOne Liquid CDx BCT when stored between 4°C and 35°C for up to 15 days following receipt of the sample at FMI, in an upright or inverted position. Additional data will be generated to further evaluate whole blood stability, sample stability in the FoundationOne Liquid CDx cfDNA BCTs between time of collection and receipt at FMI, and potential interference of the blood collection tube cap.

9. <u>Guard-banding and Robustness:</u>

   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. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) 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 33). 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 34).

Table 33: 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 34: 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:
The purpose of this validation study was to evaluate the impact on FoundationOne Liquid CDx test performance due to potential process variation with regard to uncertainty in the measurement of cfDNA concentration. This guard banding evaluation assessed the cfDNA input into

each of the main process steps of the FoundationOne Liquid CDx assay (LC, HC, and sequencing).

Guard bands were evaluated relative to calculated process variability for LC, HC, and sequencing. The assessment of multiple cfDNA input levels into LC demonstrated robust performance and tolerance of various cfDNA input levels. The observed results of HC guard banding showed that the HC process is robust within the predefined specifications 1000ng to 2000ng of cfDNA input into HC. For sequencing, the observed distribution of coverage indicated robust performance within the predefined specifications of 1.0nM of cfDNA input concentration into sequencing.

The percentages of process pass rates are summarized Table 35, below.

Table 35: Summary of process pass and failure rate at each guard banding cfDNA input level

Process	Input Level		# of Pass	Pass Rate (%)
LC	-33%	20ng	20/20	100
	-20%	24ng	20/20	100
	Recommended lower limit	30ng	20/20	100
	Low input	45ng	20/20	100
	Mid-point	55ng	20/20	100
	Upper limit	80ng	20/20	100
	+20%	96ng	19/20 [a]	95
	+33%	106ng	20/20	100
HC	-50%	500ng	18/20	90
	-20%	800ng	20/20	100
	Lower limit	1000ng	20/20	100
	Upper limit	2000ng	20/20	100
	+20%	2400ng	20/20	100
	+50%	3000ng	18/20	90
Sequencing	-50%	0.5nM	20/20	100
	-20%	0.8nM	20/20	100
	Normal input	1.0nM	20/20	100
	+20%	1.2nM	20/20	100
	+50%	1.5nM	20/20	100

Note: [a] This one (1) failure was due to failure of HC PICO DNA yield rather than LC PICO DNA yield.

Because the guard-bands for the test were only established using contrived specimens, a post-market study to further assess cfDNA input is planned to confirm performance using clinical specimens.

c. Molecular Index Barcode Performance:
   To evaluate the molecular index barcode performance, a total of 7,641 sequenced samples from FoundationOne Liquid CDx validation studies were analyzed with the FoundationOne Liquid CDx assay.

   The overall coefficient of variation (% CV) of sequencing coverage across all barcodes was 8.95% for the enhanced sensitivity regions and 7.64% for the standard sensitivity regions. This observed small % CV includes both sample variability and barcode variability as these two components were confounded and inseparable. Results demonstrated that all 480 barcodes analyzed are detectable with low differences in sample coverage variance between barcodes, indicating comparable performance of the barcodes.

d. Automation Line Equivalence:
   An intermediate precision study was performed to establish equivalence between the Hamilton instrumentation and the Biomek/Bravo instrumentation. The study consisted of eight contrived samples run in triplicate across four runs and both instrumentation platforms resulting in a total of 192 sample replicates included in the study overall. The analysis evaluated the negative call rate (NCR) and positive call rate (PCR) for 1,309 variants from eight contrived samples. The PCR and NCR were also evaluated by the seven variant categories.

   The Mann-Whitney test was used for the comparison of PCR and NCR across liquid handling platforms for each sample, all samples in aggregate, and for each variant type. The NCR across platforms for each analysis set (per sample, all samples in aggregate, per variant type) were not statistically significant ($p > 0.05$). The PCR across platforms were not statistically significant ($p > 0.05$) with the exception of contrived sample #3, the aggregate of all samples, and substitutions in a non-repetitive region or a repetitive region of ≤7 base pairs. The PCRs for the Hamilton liquid handling platform were slightly higher than the PCRs for the Biomek/Bravo platform (92.08% versus 90.15% for sample #3, 90.75% versus 89.67% for all samples, and 91.14 versus 90.10% for substitutions in a non-repetitive region or repetitive region of ≤7 base pairs). The statistical significance observed was due to large sample sizes allowing for the detection of slight differences that were likely not meaningful in practice; therefore, the Hamilton and Biomek/Bravo liquid handling platforms are considered to be interchangeable in the FoundationOne Liquid CDx assay.

B. Animal Studies

Not Applicable.

C. Additional Studies

The following studies in this section were performed in support of the clinical validation studies.

1. Blood Collection Tube Equivalence:

   The purpose of this study was to establish FoundationOne Liquid CDx blood collection tube (BCT) equivalence to enable the use of samples collected in two other collection tubes (BCT1 and BCT2) for retrospective clinical bridging studies and other validation studies requiring plasma.

   In Phase I of this study, whole blood from 28 patients of 7 tumor types were collected in either the FoundationOne Liquid CDx BCT vs. BCT1 or the FoundationOne Liquid CDx BCT vs. BCT2. A total of 56 samples were processed as part of the Phase I study. In Phase II, whole blood from 24 healthy donors were collected in FoundationOne Liquid CDx tube, BCT1, and BCT2. Eight contrived cfDNA samples were tested, with each of three plasma samples from healthy donors spiked with fragmented gDNA, collected in three tube types. A total of 144 samples were processed as part of Phase II of the study. In total, 200 replicates were evaluated in this study.

   The process success rates for each step in the FoundationOne Liquid CDx assay were determined:

   - DNAx: 100% success rate with a 95% two-sided CI of (98.17%, 100.00%)
   - LCQC: 100% success rate with a 95% two-sided CI of (98.17%, 100.00%)
   - HCQC: 99.50% success rate with a 95% two-sided CI of (97.25%, 99.99%)
   - Sequencing: 96.48% success rate with a 95% two-sided CI of (92.89%, 98.57%)

   In Phase I of the study, concordance of expected variants was calculated for the FoundationOne Liquid CDx tube vs. BCT1, and the FoundationOne Liquid CDx tube vs. BCT2. The PPA between BCT1 and the FoundationOne Liquid CDx tube was 100% with a 95% two-sided CI of (96.31%, 100.00%). The PPA between BCT2 and the FoundationOne Liquid CDx tube was 100% with a 95% two-sided CI of (95.32%, 100.00%).

   In Phase II of the study, concordance of expected variants was calculated between BCT1 and the FoundationOne Liquid CDx tube and between BCT2 and the FoundationOne Liquid CDx tube. For the variants at ≥ 2xLoD, positive percent agreement was 93.81% with a 95% two-sided CI of (87.50%,100.00%) for BCT1 compared to the FoundationOne Liquid CDx tube and 90.57% with a 95% two-sided CI of (84.38%, 93.75%) for BCT2 compared to the FoundationOne Liquid CDx BCT.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The applicant performed two clinical studies, in the form of a clinical bridging study for

BRCA1 and *BRCA2* for the metastatic castration-resistant prostate cancer (mCRPC) indication and a follow-on non-inferiority study for *EGFR* Exon 19 deletion and *EGFR* Exon 21 *L858R* alteration for the NSCLC indication, intended to establish a reasonable assurance of safety and effectiveness of the FoundationOne Liquid CDx for indications listed in Table 1 of the Intended Use/Indications for Use. Data from these clinical studies were the basis for the PMA approval decision. A summary of the clinical studies are presented below.

The TRITON2 clinical study (NCT02952534) is a multicenter, open-label Phase 2 study of rucaparib in patients with mCRPC associated with homologous recombination deficiency. Patients with deleterious mutations in *BRCA1* and *BRCA2* genes that are associated with sensitivity to inhibitors of the enzyme poly ADP ribose polymerase (PARPi). In this study patients must also have progressed after 1 prior line of taxane-based chemotherapy for mCRPC.

A. Clinical Bridging Study for *BRCA1/BRCA2* positive metastatic castration-resistant prostate cancer (mCRPC)

1. <u>Study Design - TRITON2</u>
   The clinical performance of FoundationOne Liquid CDx as a companion diagnostic to identify patients with metastatic castration-resistant prostate cancer (mCRPC) harboring breast cancer gene 1 or 2 (*BRCA1* or *BRCA2*) alterations for treatment with rucaparib was demonstrated using pre-rucaparib treatment blood samples from TRITON2. The clinical data supporting the use of rucaparib in the proposed indication was submitted as New Drug Application (NDA) 209115/S-004.

2. <u>Clinical Bridging Study:</u>
   A bridging study was conducted to evaluate: 1) the concordance between *BRCA1* and *BRCA2* alteration status by the clinical trial assays (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.

   The sample inclusion and exclusion criteria for the the retrospective testing of the clinical bridging study were:
   a. Sample inclusion criteria:
      - Frozen plasma specimens with a minimum plasma volume of 2.5mL collected in BCT2 described above
      - Samples were required to meet minimum criteria for FoundationOne Liquid CDx operational testing requirements

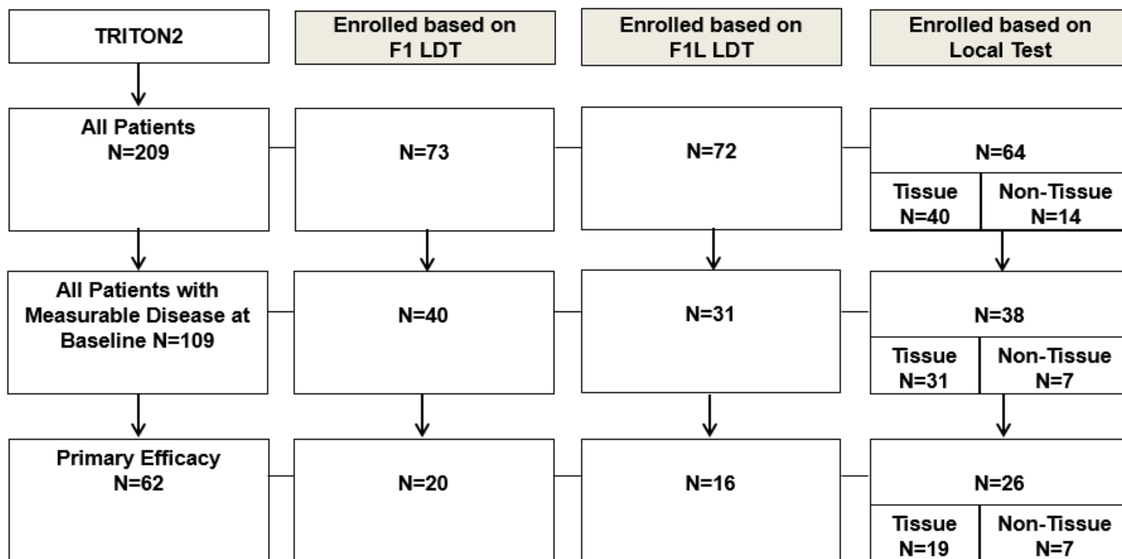
   b. Sample exclusion criteria:
      - Tissue and other liquid samples
      - Samples with plasma volume <2.5mL

Specimens included in the clinical bridging study were tested according to the standard testing protocol for the FoundationOne Liquid CDx test with a minimum recommended cfDNA input of ≥ 30 ng for the library construction step. Nineteen patient specimens were also tested at lower cfDNA inputs of between 20 ng and 29 ng cfDNA input based on pre-specified assay procedures and processed only if the samples passed pre-specified in-process quality criteria.

3. <u>Clinical Endpoints</u>
The primary efficacy endpoint for TRITON2 patients included in this study was confirmed objective response rate (ORR) per mRECIST v1.1/PCWG3 criteria by Independent Radiologic Review (IRR) in patients with a *BRCA1* or *BRCA2* alteration and measurable disease at baseline per IRR.

4. <u>Accountability of PMA Cohort</u>
A total of 209 patients (All Patients) from TRITON2 were included in NDA 209115/S-004. Genomic status was determined using either of the central tissue test [FoundationOne Laboratory Developed Test (F1 LDT)] and the central

Figure 1: TRITON2 Patient Enrollment by Enrolling Assay.



plasma test (FoundationOne Liquid LDT), or local tests which included tissue and non-tissue based (i.e., plasma, whole blood, or saliva) tests, as summarized in Figure 1.

Pre-rucaparib treatment plasma samples were available for 92% (192/209) of the patients for retesting by FoundationOne Liquid CDx. Of those 192 available plasma samples, sufficient cfDNA was available for 183 samples with cfDNA input ≥ 20ng. Of those 183 samples, only 178 of all the patients in TRITON2 had evaluable FoundationOne Liquid CDx results.

The 209 patients enrolled into TRITON2 (All Patients) included patients with deleterious alterations in *BRCA1*, *BRCA2*, *ATM* with measurable or non-measurable disease, and an exploratory cohort of patients with an alteration in one of 12 other homologous recombination repair (HRR) genes with or without measurable disease. Among 115 patients with a *BRCA1* or *BRCA2* alteration, 62 had measurable disease and 53 had non-measurable disease. In addition, TRITON2 enrolled 94 patients with a non-*BRCA* HRR gene alteration, 47 with and 47 without measurable disease. The accelerated approval of rucaparib for treatment of patients with mCRPC and a *BRCA1* or *BRCA2* alteration was based on the ORR by IRR in 62 patients with a *BRCA1* or *BRCA2* alteration and measurable disease by IRR at baseline (Primary Efficacy Population).

All 62 patients in the Primary Efficacy Population in TRITON2 had a deleterious somatic or germline *BRCA* alteration detected from either central plasma (26%), central tissue (32%), or local (42%) testing. For FoundationOne Liquid CDx, the minimum recommended cfDNA input for library construction is 30 ng; however, samples were also run at lower cfDNA inputs between 20 ng – 29 ng, Of the 62 patients in the Primary Efficacy Population in TRITON2, 50 samples had cfDNA input ≥ 30 ng by FoundationOne Liquid CDx while four (4) samples had cfDNA input of 20 ng – 39 ng. Forty-eight samples out of the 50 samples with cfDNA input ≥ 30ng had evaluable FoundationOne Liquid CDx results (38 *BRCA* positive and 10 *BRCA* negative). For the 54 samples with cfDNA input ≥ 20 ng, 52 samples had evaluable FoundationOne Liquid CDx results (41 *BRCA* positive and 11 *BRCA* negative). So, evaluable FoundationOne Liquid CDx test results were obtained for 84% (52/62) in the primary efficacy ppoulation. The sample accountability for this clinical bridging study is summarized in Table 36 below.

Table 36: Sample accountability for rucaparib prostate clinical bridging study

Description	Number
All Patients in TRITON2	209
Total samples available for retesting by FoundationOne Liquid CDx	192
Samples with evaluable FoundationOne Liquid CDx data and cfDNA input ≥ 30ng (All Patients)	161
Samples with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (All Patients)	178
Primary efficacy population (PEP) in TRITON2	62
Samples with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 30ng (PEP)	48
Samples with evaluable FoundationOne Liquid CDx test results and cfDNA input ≥ 20ng (PEP)	52

5. Study Population Demographics and Baseline Parameters:
   Key baseline characteristics associated with disease status for the Primary Efficacy Population based on FoundationOne Liquid CDx results in each of the *BRCA* status subgroups are summarized in Table 37. There does not appear to be

any clinically significant differences in demographics or other baseline clinical characteristics characteristics for patients with FoundationOne Liquid CDx-evaluable and FoundationOne Liquid CDx-unevaluable test results. Twenty nanograms (20 ng) was used as minimum cfDNA input threshold for Table 37 summary. Samples identified as unknown were those with cfDNA < 20 ng an input ≥ 20 ng ,which failed QC or sencencing.

Table 37: Key demographics and baseline characteristics in the Primary Efficacy Population by FoundationOne Liquid CDx BRCA status

Primary Efficacy Population	*BRCA* Positive N = 41	*BRCA* Negative N = 11	*BRCA* Known N = 52	*BRCA* Unknown N = 10
Sites of Metastatic Disease per IRR, n (%)[a,b]				
Bone	32 (78.0%)	5 (45.5%)	37 (71.2%)	7 (70.0%)
Nodal	34 (82.9%)	9 (81.8%)	43 (82.7%)	10 (100.0%)
Visceral	23 (56.1%)	4 (36.4%)	27 (51.9%)	9 (80.0%)
Hepatic	12 (29.3%)	1 (9.1%)	13 (25.0%)	0 (0%)
Number of Bone Lesions per IRR, n (%)				
< 10	21 (51.2%)	7 (63.6%)	28 (53.8%)	9 (90.0%)
≥ 10	20 (48.8%)	4 (36.4%)	24 (46.2%)	1 (10.0%)
Baseline PSA, ng/mL				
Mean	349.59 (804.116)	592.24 (1376.664)	400.92 (942.741)	76.68
Median	123.90	52.00	95.00	33.22
Min, Max	4.3, 4782.0	1.8, 4669.0	1.8, 4782.0	3.5, 249.0
Number of Prior CRPC Therapies Group, n (%)				
2	18 (43.9%)	7 (63.6%)	25 (48.1%)	7 (70.0%)
3	16 (39.0%)	3 (27.3%)	19 (36.5%)	1 (10%)
4	6 (14.6%)	0 (0%)	6 (11.5%)	1 (10%)
5	0 (0%)	1 (9.1%)	1 (1.9%)	1 (10.0%)
>5	1 (2.4%)	0 (0%)	1 (1.9%)	0 (0%)

CRPC = castrate-resistant prostate cancer; CT = computed tomography; HRD = homologous recombination deficiency; IRR = independent radiology review; Max = maximum; Min = minimum; MRI = magnetic resonance imaging; PSA=prostate-specific antigen; StD = standard deviation.

[a] Categories are not mutually exclusive; thus, patients can be counted in more than 1 category.

[b] Bone = patients with ≥ 1 bone lesion reported on the baseline bone scan per IRR; nodal = patients with ≥ 1 target or non-target lymph node lesion identified by CT/MRI per IRR; visceral = patients with ≥ 1 target or non-target non-lymph node lesion identified by CT/MRI per IRR; hepatic = patients with ≥ 1 target or non-target hepatic lesion identified by CT/MRI per IRR.

6. Safety and Effectiveness Results

a. Safety Results:

Safety with respect to treatment with RUBRACA® (rucaparib) will not be addressed in detail in this SSED for the FoundationOne Liquid CDx. The most common adverse reactions (≥ 20%) among patients with BRCA-mutated mCRPC were fatigue (including asthenia), nausea, anemia, ALT/AST

increased, decreased appetite, rash, constipation, thrombocytopenia, vomiting, diarrhea. For additional information please see the RUBRACA® (rucaparib) package insert available at Drugs@FDA.

b. Effectiveness Results (Concordance):
The concordance of BRCA status between FoundationOne Liquid CDx and CTA test results were evaluated for all patients enrolled into TRITON2 study, as summarized in Tables 38 and 39, based on cfDNA input amounts. The CTA included central tissue (FoundationOne LDT), central plasma (FoundationOne Liquid LDT) and local tests.

Table 38: Concordance between FoundationOne Liquid CDx BRCA status and CTA BRCA status for All patients with cfDNA input ≥ 30ng

All Patients		**CTA**		
		***BRCA* Positive**	***BRCA* Negative**	**Total**
F1 Liquid CDx	***BRCA* Positive**	75	1	76
	***BRCA* Negative**	16	69	85
	***BRCA* Unknown**	2	1	3
	Total	93	71	164

The PPA and NPA between FoundationOne Liquid CDx and the CTA BRCA statuses using CTA as reference for all patients that had cfDNA input ≥ 30ng were:

PPA (95% CI): 82.4% (73.0%, 89.6%)
NPA (95% CI): 98.6% (92.3%, 100.0%)

Table 39: Concordance between FoundationOne Liquid CDx BRCA status and CTA BRCA status for All patients with FoundationOne Liquid CDx cfDNA input ≥20ng

All Patients		**CTA**		
		***BRCA* Positive**	***BRCA* Negative**	**Total**
F1 Liquid CDx	***BRCA* Positive**	82	1	83
	***BRCA* Negative**	18	77	95
	***BRCA* Unknown**	3	2	5
	Total	103	80	183

The PPA and NPA between FoundationOne Liquid CDx and the CTA BRCA statuses using CTA as reference for all patients that had cfDNA input ≥ 20ng were:

PPA (95% CI): 82.0% (73.1%, 89.0%)
NPA (95% CI): 98.7% (93.1%, 100%)

As indicated above, mCRPC patients with deleterious *BRCA1* or *BRCA2* alterations were enrolled in the TRITON2 clinical study based on central

tissue and plasma tests as well as local laboratory tests. Based on the agreement calculations above, a post-market commitment was included for the development of a tumor tissue based CDx assay under the accelerated approval of rucaparib (see 209115/S-004 approval letter at Drugs@FDA).

c. Efficacy Results:

i. Clinical efficacy results in the TRITON2 primary efficacy population: The ORR (95% CI) in the Primary Efficacy Population was 46.3% (30.7%, 62.6%) in *BRCA* positive patients determined by FoundationOne Liquid CDx , which is comparable to the ORR of 43.5% (31.0% – 56.7%) in patients identified by CTA (Table 40).

Table 40: ORR in the primary efficacy population by CTA and FoundationOne Liquid CDx test results for BRCA alterations

Primary Efficacy Population	FoundationOne Liquid CDx		CTA
	***BRCA* Positive N=38 (≥ 30 ng cfDNA input)**	***BRCA* Positive N = 41 (≥ 20 ng cfDNA input)**	***BRCA* Positive N = 62**
Confirmed ORR (CR + PR), n (%)	18 (47.4)	19 (46.3)	27 (43.5)
95% CI(%)	31.0, 64.2	30.7, 62.6	31.0, 56.7

ORR = objective response rate; CR = complete response; PR = partial response.

ii. Clinical Efficacy Results in the FoundationOne Liquid CDx-positive Population:
Efficacy analyses were performed for BRCA positive patients in the primary efficacy population (patients with evaluable disease) as determined by FoundationOne Liquid CDx assay and compared to the efficacy results of the BRCA mutation positive patients as determined by CTA of TRITON2 (i.e., ORR 43.5%).

The drug efficacy (ORRs) of FoundationOne Liquid CDx+ was calculated as a weighted efficacy of patients with (CTA+, FoundationOne Liquid CDx+) and patients with (CTA-, FoundationOne Liquid CDx+), and the weight was Pr(CTA+|FoundationOne Liquid CDx+). The ORRs for the (FoundationOne Liquid CDx+, CTA+) were calculated from the TRITON2 trial. The ORRs for (FoundationOne Liquid CDx+, CTA-) was assumed to be c-value times of that observed ORRs of (FoundationOne Liquid CDx+, CTA+) with c ranging from 0 (no efficacy) to 1.0 (having the same efficacy). These results are summarized in Table 41. Prevalence of 12% for BRCA1/2 positive as determined by CTA was used to calculate the Pr(CTA+|F1 Liquid CDx+).

Table 41: Estimated Rucaparib Efficacy for Patients Selected with FoundationOne Liquid CDx BRCA+ Patients by cfDNA input

≥ 30ng	ORRs	95% CI of ORRs
c=100%	47.4%	32.8, 62.3%
c=70%	45.8%	31.2, 60.7%
c=50%	44.7%	30.2, 59.6%
c=30%	43.6%	29.1, 58.5%
c=0%	42.0%	27.5, 56.9%
≥ 20ng	**ORRs**	**95% CI of ORRs**
c=100%	46.3%	32.3, 60.9%
c=70%	44.9%	30.8, 59.5%
c=50%	44.0%	29.9, 58.5%
c=30%	43.0%	28.9, 57.6%
c=0%	41.6%	27.5, 56.1%

iii. Sensitivity analysis:
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.

Multivariate logistic regression analyses were performed to identify the clinically relevant covariates that are associated with the device outputs and clinical outcomes, respectively. Given the limited sample size, a significance level of 0.2 was used as the criteria to select covariates in the logistic regression models. Any relevant covariates not identified in the analysis and known to be clinically important to the clinical outcome or FoundationOne Liquid CDx test results were also be included in the imputation model. Covariate imbalance was assessed for imbalance of distributions between CDx-evaluable and CDx-unevaluable sets within all enrolled CTA-positive patients. The distribution of the propensity scores among the group of patients with CDx results and the group without CDx results were assessed. Missing FoundationOne Liquid CDx results in the primary efficacy population were imputed. The sensitivity analysis results demonstrated that the drug efficacy in FoundationOne Liquid CDx positive population is robust to missing FoundationOne Liquid CDx results.

B. FoundationOne Liquid CDx Concordance Study for *EGFR* Exon 19 deletion and *EGFR* Exon 21 L858R Alteration

1. Non-Inferiority Study Design:
Clinical validity of FoundationOne Liquid CDx assay was established as a companion diagnostic to identifying patients with advanced NSCLC who may be eligible for treatment with TARCEVA® (erlotinib), IRESSA® (gefitinib), or TAGRISSO® (osimertinib). Two hundred and eighty (280) retrospective samples

from NSCLC patients were included in this study, which were tested for *EGFR* Exon 19 deletion and exon 21 L858R alterations (*EGFR* alterations) by the FoundationOne Liquid CDx assay and the previously approved **cobas**® EGFR Mutation Test v2 (Roche Molecular Systems, referred to cobas assay). Both *EGFR* mutation-positive and *EGFR* mutation-negative samples (based on the cobas assay results) were selected from the screen failed population of an unrelated clinical trial in NSCLC. To avoid selection bias, the samples were selected starting with a specific testing date until the predefined number of 150 *EGFR* alteration-positive and 100 *EGFR* alteration-negative samples were fulfilled. Samples were tested across two replicates by the cobas assay (denoted as CCD1 and CCD2) and one replicate by FoundationOne Liquid CDx. The tested samples, from NSCLC patients, were compared against the intended use (IU) population with respect to gender to ensure the screening population was representative of the IU population.

An additional comparison was provided to compare the non-inferiority study population to the FLAURA clinical study population used for the approval of the cobas assay for the detection of *EGFR* Exon 19del and L858R for the selection of NSCLC patients for treatment with TAGRISSO (osimertinib). As seen in Table 42, the greatest differences appears to be race, smoking status, and gender. FLAURA was conducted primarily in Asia due to the higher prevalence of *EGFR* Exon 19del and L858R in the Asian lung cancer population.

Table 42: Comparison of FoundationOne Liquid CDx *EGFR* Non-Inferiority Study vs. FLAURA Clinical Study

Baseline Characteristics	FLAURA (Global Population) (N = 556)	FoundationOne Liquid CDx *EGFR* NI Study (N = 275)
Age		
Mean (Standard Deviation)	63.0 (10.79)	65.2 (11.2)
Sex		
Female	350	138
	62.9%	50.2%
Male	206	137
	37.1%	49.8%
Race		
American Indian or Alaska Native	2	2
	0.4%	0.7%
Asian	347	84
	62.4%	30.5%
Native Hawaiian or Other Pacific Islander	0	0
	0.0%	0.0%
Black or African American	4	2
	0.7%	0.7%
White	201	175
	36.2%	63.6%
More than one race	0	0

Baseline Characteristics	FLAURA (Global Population) (N = 556)	FoundationOne Liquid CDx *EGFR* NI Study (N = 275)
	0.0%	0.0%
Unknown or Not Reported	2	12
	0.4%	4.4%
Smoking status		
Never smoked-Global Cohort	357	102
	64.2%	37.1%
Current smokers-Global Cohort	17	41
	3.1%	14.9%
Former smokers-Global Cohort	182	123
	32.7%	44.7%
Unknown	0	9
	0.00%	3.3%

The variant calls were evaluated based on the agreement between both the FoundationOne Liquid CDx and the cobas assay results and between the two cobas assay replicates. For any samples in which there was insufficient plasma to process both CCD1 and CCD2, processing was not performed. In total there were 177 samples with complete test results available for analysis.

2. Study Results
   The concordance of *EGFR* mutations as detected by FoundationOne Liquid CDx and the **cobas** assay were assessed and the data are summarized in Tables 43 – Table 48 for Exon 19 deletions and L858R separately and in aggregate.

Table 43: Concordance table for *EGFR* Exon 19 deletion and L858R with CCD1, CCD2, and FoundationOne Liquid CDx results with eligible samples (complete data)

		CCD1+			CCD1-		
		CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
Exon 19del (N = 135)	**F1 Liquid CDx(+)**	41	1	42	1	3	4
	F1 Liquid CDx(-)	2	0	2	0	87	87
	Total	43	1	44	1	90	91
L858R (N = 133)	**F1 Liquid CDx(+)**	39	3	42	1	3	4
	F1 Liquid CDx(-)	0	0	0	0	87	87
	Total	39	3	42	1	90	91

CCD1 = cobas Replicate 1; CCD2 = cobas Replicate 2

Table 44: Agreement analysis results for *EGFR* Exon 19 deletion and L858R separately

Exon 19del	PPA_{C1F}	95.5%	NPA_{C1F}	95.6%
	PPA_{C1C2}	97.7%	NPA_{C1C2}	98.9%
	PPA_{C2F}	95.5%	NPA_{C2F}	96.0%
	PPA_{C2C1}	96.2%	NPA_{C2C1}	99.4%

L858R	PPA_{C1F}	100.0%	NPA_{C1F}	95.6%
	PPA_{C1C2}	92.9%	NPA_{C1C2}	98.9%
	PPA_{C2F}	100.0%	NPA_{C2F}	94.7%
	PPA_{C2C1}	96.0%	NPA_{C2C1}	98.0%

C1 = cobas Replicate 1; C2 = cobas Replicate 2
F = FoundationOne Liquid CDx

Table 45: Point estimate of ζPPA1, ζNPA1, ζPPA2, and ζNPA2 for *EGFR* Exon 19 deletion and L858R separately

	***EGFR* Exon 19 deletion**	***EGFR* L858R**
	Point Estimate	**Point Estimate**
ζPPA1	2.3%	-7.1%
ζNPA1	3.3%	3.3%
ζPPA2	0.7%	-4.0%
ζNPA2	3.3%	3.3%

ζPPA1 = (PPA_{C1C2} = PPA_{C1F}); ζNPA1 = (NPA_{C1C2} = NPA_{C1F})
ζPPA2 = (PPA_{C2C1} = PPA_{C2F}); ζNPA2 = (NPA_{C2C1} = NPA_{C2F})

Table 46: Concordance among CCD1, CCD2 and FoundationOne Liquid CDx results with eligible samples (n=177)

		CCD1+			**CCD1-**		
		CCD2+	**CCD2-**	**Total**	**CCD2+**	**CCD2-**	**Total**
F1 Liquid CDx	+	80	4	84	1	3	4
	–	2	0	2	0	87	87
Total		82	4	86	1	90	91

The agreement analysis results between FoundationOne Liquid CDx and the cobas assay are presented in Table 47 below.

Table 47: Agreement analysis results

	PPA	**NPA**
CCD2 | CCD1*	95.3%	98.9%
CCD1 | CCD2**	96.1%	98.7%
F1 Liquid CDx | CCD1*	97.7%	95.6%
F1 Liquid CDx | CCD2**	97.7%	95.4%

* CCD1: the 1st replicate of cobas assay as the reference
** CCD2: the 2nd replicate of cobas assay as the reference

The estimates of ζPPA1, ζPPA2, ζNPA1 and ζNPA2 and the corresponding one-sided 95% upper bounds confidence limit computed using the bootstrap method are presented in Table 48 below.

48: Point estimate and one-sided 95% upper CI of ζPPA1, ζNPA1, ζPPA2, and ζNPA

	Point Estimate	Mean one-sided 95% upper confidence limit
ζ_{PPA1}	-2.3%	2.3%
ζ_{NPA1}	3.3%	6.6%
ζ_{PPA2}	-1.6%	4.7%
ζ_{NPA2}	3.3%	6.6%

$\zeta PPA1 = (PPA_{C1C2} = PPA_{C1F})$; $\zeta NPA1 = (NPA_{C1C}2 = NPA_{C1F})$
$\zeta PPA2 = (PPA_{C2C1} = PPA_{C2F})$; $\zeta NPA2 = (NPA_{C2C1} = NPA_{C2F})$

Based on these results, FoundationOne Liquid CDx has been demonstrated to be non-inferior to the cobas assay for the detection of *EGFR* Exon 19 deletions and *EGFR* exon 21 L858R mutations. This study establishes the clinical validity of the FoundationOne Liquid CDx assay to identifying patients eligible for treatment with erlotinib, gefitinib, and osimertinib.

3. Pediatric Extrapolation (for both clinical studies)

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

C. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study included one investigator. None of 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 mCRPC patients with a *BRCA1* and/or *BRCA2* alteration for

treatment with rucaparib, safety and effectiveness was demonstrated through a clinical bridging study using plasma specimens collected from patients enrolled into the TRITON2 study. In addition, to identify NSCLC patients with *EGFR* Exon 19 deletions and L858R mutations for treatment with erlotinib, gefitinib or osimertinib, a follow-on non-inferiority study to the Roche cobas *EGFR* Mutation Test v2 was performed. 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.

Data from the TRITON2 study shows that patients identified with *BRCA1* or *BRCA2* deleterious alterations in plasma specimens benefit from treatment with rucaparib and support the CDx indication to FoundationOne Liquid CDx. Data from the non-inferiority study comparing results for the detecton of *EGFR* Exon 19 deletions and L858R mutations in plasma shows that the FoundationOne Liquid CDx assay is non-inferior to the Roche cobas *EGFR* Mutation Test v2 that is FDA-approved for the same indication.

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-approve labeling, all four agents have been associated with a variety of adverse reactions, and there are also several warnings and precautions.

Warnings and Precautions: For rucaparib, cases of MDS/AML, some fatal; for osimertinib: pulmonary and cardiac toxicities as well as keratitis, Stevens-Johnson Syndrome, erythema multiforme; for erlotinib: interstitial lung disease, renal failure, hepatotoxicity, gastrointestinal perforation, bullous and exfoliative skin disorders, cerebrovascular accident, microangiopathic hemolytic anemia, ocular disorders, hemorrhage; for gefitinib: interstitial lung disease, hepatotoxicity, gastrointestinal

perforation, diarrhea, ocular disorders, bullous and exfoliative skin disorders. All four agents can cause embryo-fetal toxicity.

Adverse reactions (most commonly reported): For rucaparib: nausea, fatigue (including asthenia), vomiting, anemia, dysgeusia, AST/ALT elevation, constipation, decreased appetite, diarrhea, thrombocytopenia, neutropenia, stomatitis, nasopharyngitis/URI, rash, abdominal pain/distention, and dyspnea; for osimertinib: diarrhea, rash, dry skin, nail toxicity, stomatitis, fatigue and decreased appetite; for erlotinib: rash, diarrhea, anorexia, fatigue, dyspnea, cough, nausea, and vomiting; for gefitinib: skin reactions and diarrhea.

C. Benefit-Risk Determination

Treatment with rucaparib provides meaningful clinical benefit to mCRPC patients with germline or somatic alterations in *BRCA1* or *BRCA2* as demonstrated in the TRITON2 clinical study. The probable benefits of the FoundationOne Liquid CDx device are based on data collected in the clinical study, showing improved ORR in mCRPC patients with measurable visceral and/or nodal disease at baseline and a deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation disease population of patients with mCRPC, with a clinically meaningful overall response rate. Given the available information, the data supports the conclusion that FoundationOne Liquid CDx has probable benefit in selecting patients with alterations in *BRCA1* and/or *BRCA2* genes, for treatment with rucaparib.

Treatment with erlotinib, gefitinib, or osimertinib provides meaningful clinical benefit to patients with NSCLC harboring *EGFR* Exon 19 deletions or *EGFR* L858R substitution mutations. Based on the data provided in the non-inferiority study which compared the ability of the FoundationOne Liquid CDx assay to detect these *EGFR* activating mutations to that of the Roche cobas *EGFR* Mutation Test v2 in NSCLC, FoundationOne Liquid CDx is non-inferior to the cobas test and has probable benefit in selecting patients with *EGFR* Exon 19 deletions or L858R mutations for treatment with erlotinib, gefitinib, or osimertinib.

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 prostate cancer patients with alterations in *BRCA1* or *BRCA2* genes, for treatment with rucaparib or NSCLC patients with *EGFR* Exon 19 deletions or L858R mutations for treatment with elaparib, gefitinib, or osimertinib 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

summarized above. In addition, the risks of false negative results 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 F1CDx 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 information above, the data support that for the selection of prostate cancer patients with alterations in *BRCA1* or *BRCA2* genes, for treatment with rucaparib and for the selection of NSCLC patients with *EGFR* Exon 19 deletions or L858R mutations for treatment with erlotinib, gefitinib, or osimertinib the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical 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 selecting patients with previously treated mCRPC who may be eligible for treatment with rucaparib. In addition, data from the non-inferiority study supports the conclusion that the FoundationOne Liquid CDx assay is non-inferior to the FDA approved **cobas** EGFR Mutation Test v2 for the selection of NSCLC patients with *EGFR* Exon 19 deletions and L858R mutatons for treatment with erlotinib, gefitinib, or osimertinib.

XIII. CDRH DECISION

CDRH issued an approval order on August 26, 2020 The final 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 30 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.

3. For the *BRCA1/BRCA2* companion diagnostic (CDx) claim (rucaparib) for the prostate indication, you must provide the following:
   a. 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 (prostate cancer) specimens to confirm an acceptable level of precision at or near the LoD concentration for all 4 BRCA1 and 4 BRCA2 CDx variant types [i.e., base substitutions (SNV), insertion/deletion (indel), rearrangement (RE), and homozygous deletions (HD)]. The level of precision at the LoD must be adequate to minimize clinically significant inaccurate results when used on specimens from the intended use population.

   b. 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 prostate cancer specimens. The level of analytical accuracy/concordance must be adequate to minimize clinically significant inaccurate results when used on specimens from the intended use population.

   c. 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 prostate 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 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 minimize clinically significant inaccurate results when used on specimens from the intended use population.

d. FMI will provide robust and high confidence data from a guard-band study to test the limits of FoundationOne Liquid CDx assay's 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 minimize clinically significant inaccurate results when used on specimens from the intended use population.

4. 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 minimize clinically significant inaccurate results when used on specimens from the intended use population.

5. 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. The level of analytical accuracy/concordance must be adequate to minimize clinically significant inaccurate results when used on specimens from the intended use population.

6. 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 minimize clinically significant inaccurate results 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 minimize clinically significant inaccurate results 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 minimize clinically significant inaccurate results 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 minimum clinically significant inaccurate results 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 minimize clinically significant inaccurate results when expected handling conditions are used on specimens collected in the FoundationOne Liquid CDx cfDNA Blood Collection tubes in the intended use population.

7. Software:
   a. 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.

   b. FMI must appropriately validate software infrastructure changes and migration to 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.

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.