SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

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

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

Device Procode: PQP

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

Date(s) of Panel Recommendation: None

Premarket Approval Application (PMA) Number: P200016

Date of FDA Notice of Approval: November 6, 2020

The FoundationOne® Liquid CDx was approved on August 26, 2020 as a companion diagnostic for *BRCA1* and *BRCA2* alterations in metastatic castration-resistant prostate cancer (mCRPC) patients who may benefit from treatment with RUBRACA® (rucaparib) and *EGFR* activating mutations (Exon 19 deletions and L858R substitution mutation) in patients with advanced and metastatic non-small cell lung cancer (NSCLC) who may benefit from treatment with IRESSA® (gefitinib), TAGRISSO® (osimertinib), and TARCEVA® (erlotinib). On October 26, 2020 the FoundationOne® Liquid CDx test was approved as a companion diagnostic for *BRCA1* and *BRCA2* alterations in epithelial ovarian cancer for patients who may benefit from treatment with RUBRACA® (rucaparib), *ALK* rearrangements in non-small cell lung cancer for patients who may benefit from treatment with ALECENSA® (alectinib), and *PIK3CA* mutations patients with breast cancer who may benefit from treatment with PIQRAY® (alpelisib).

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

New Indication Being Sought in this PMA submission.

Biomarker(s) Detected	Therapy	Tumor Type
BRCA1, *BRCA2*, and *ATM* alterations	Lynparza® (olaparib)	Prostate Cancer

II. INDICATIONS FOR USE

FoundationOne® Liquid CDx is a qualitative next generation sequencing based in vitro diagnostic test that uses targeted high throughput hybridization-based capture technology

to detect and report substitutions, insertions and deletions (indels) in 311 genes, including rearrangements in four (4) genes, and copy number alterations in three (3) genes. FoundationOne® Liquid CDx utilizes circulating cell-free DNA (cfDNA) isolated from plasma derived from anti-coagulated peripheral whole blood of cancer patients collected in FoundationOne® Liquid CDx cfDNA blood collection tubes included in the FoundationOne® Liquid CDx Blood Sample Collection Kit. The test is intended to be used as a companion diagnostic to identify patients who may benefit from treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1: Companion diagnostic indications

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

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

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

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

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

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

- Alterations reported may include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic

alterations. If a reported alteration is suspected to be germline, confirmatory testing should be considered in the appropriate clinical context.

- The test is not intended to replace germline testing or to provide information about cancer predisposition.
- Patients for whom no companion diagnostic alterations are detected should be considered for confirmation with an FDA-approved tumor tissue test, if possible.

V. DEVICE DESCRIPTION

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

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

ABL1 [Exons 4-9]	*ACVR1B*	***AKT1 [Exon 3]***	*AKT2*	*AKT3*	***ALK [Exons 20-29, Introns 18,19]***	*ALOX12B*	*AMER1 (FAM123B)*	***APC***	***AR***
ARAF [Exons 4, 5, 7, 11, 13, 15, 16]	*ARFRP1*	*ARID1A*	*ASXL1*	***ATM***	***ATR***	*ATRX*	*AURKA*	*AURKB*	*AXIN1*
AXL	*BAP1*	*BARD1*	*BCL2*	*BCL2L1*	*BCL2L2*	*BCL6*	*BCOR*	*BCORL1*	*BCR* [Introns 8, 13, 14]*
***BRAF [Exons 11-18**, Introns 7-10]*	***BRCA1** [Introns 2, 7, 8, 12, 16, 19, 20]*	***BRCA2** [Intron 2]*	*BRD4*	*BRIP1*	*BTG1*	*BTG2*	***BTK [Exons 2, 15]***	*C11orf30 (EMSY)*	*C17orf39 (GID4)*
CALR	*CARD11*	*CASP8*	*CBFB*	*CBL*	***CCND1***	*CCND2*	*CCND3*	*CCNE1*	*CD22*
CD70	*CD74* [Introns 6-8]*	*CD79A*	*CD79B*	***CD274 (PD-L1)***	*CDC73*	***CDH1***	***CDK12***	***CDK4***	***CDK6***
CDK8	*CDKN1A*	*CDKN1B*	***CDKN2A***	*CDKN2B*	*CDKN2C*	*CEBPA*	*CHEK1*	***CHEK2***	*CIC*
CREBBP	***CRKL***	*CSF1R*	*CSF3R*	*CTCF*	*CTNNA1*	***CTNNB1 [Exon 3]***	*CUL3*	*CUL4A*	*CXCR4*
CYP17A1	*DAXX*	*DDR1*	***DDR2 [Exons 5, 17, 18]***	*DIS3*	*DNMT3A*	*DOT1L*	*EED*	***EGFR [Introns 7,** 15, **24-27]***	*EP300*

EPHA3	*EPHB1*	*EPHB4*	***ERBB2***	***ERBB3 [Exons 3, 6, 7, 8, 10, 12, 20, 21, 23, 24, 25]***	*ERBB4*	*ERCC4*	*ERG*	***ERRFI1***	***ESR1 [Exons 4-8]***
ETV4 [Intron 8]*	*ETV5* [Introns 6, 7]*	***ETV6* [Introns 5, 6]***	*EWSR1* [Introns 7-13]*	***EZH2 [Exons 4, 16, 17, 18]***	*EZR* [Introns 9-11]*	*FAM46C*	*FANCA*	*FANCC*	*FANCG*
FANCL	*FAS*	*FBXW7*	*FGF10*	*FGF12*	*FGF14*	*FGF19*	*FGF23*	*FGF3*	*FGF4*
FGF6	***FGFR1*** *[Introns 1, 5,* ***Intron 17]***	***FGFR2*** *[Intron 1,* ***Intron 17]***	***FGFR3 [Exons 7, 9 (alternative designation exon 10), 14, 18, Intron 17]***	*FGFR4*	*FH*	*FLCN*	*FLT1*	***FLT3 [Exons 14, 15, 20]***	***FOXL2***
FUBP1	*GABRA6*	*GATA3*	*GATA4*	*GATA6*	***GNA11 [Exons 4, 5]***	*GNA13*	***GNAQ [Exons 4, 5]***	***GNAS [Exons 1, 8]***	*GRM3*
GSK3B	*H3F3A*	*HDAC1*	*HGF*	*HNF1A*	***HRAS [Exons 2, 3]***	*HSD3B1*	*ID3*	***IDH1 [Exon 4]***	***IDH2 [Exon 4]***
IGF1R	*IKBKE*	*IKZF1*	*INPP4B*	*IRF2*	*IRF4*	*IRS2*	*JAK1*	***JAK2 [Exon 14]***	***JAK3 [Exons 5, 11, 12, 13, 15, 16]***
JUN	*KDM5A*	*KDM5C*	*KDM6A*	*KDR*	*KEAP1*	*KEL*	***KIT [Exons 8,9,11,12, 13, 17****, Intron 16]*	*KLHL6*	*KMT2A (MLL) [Introns 6, 8-11, Intron 7]*
KMT2D (MLL2)	***KRAS***	*LTK*	*LYN*	*MAF*	***MAP2K1 (MEK1) [Exons 2, 3]***	***MAP2K2 (MEK2) [Exons 2-4, 6, 7]***	*MAP2K4*	*MAP3K1*	*MAP3K13*
MAPK1	*MCL1*	***MDM2***	*MDM4*	*MED12*	*MEF2B*	*MEN1*	*MERTK*	***MET***	*MITF*
MKNK1	*MLH1*	***MPL [Exon 10]***	*MRE11A*	*MSH2 [Intron 5]*	*MSH3*	*MSH6*	*MST1R*	*MTAP*	***MTOR [Exons 19, 30, 39 40, 43-45, 47, 48, 53, 56]***
MUTYH	*MYB* [Intron 14]*	***MYC [Intron 1]***	*MYCL (MYCL1)*	***MYCN***	***MYD88 [Exon 4]***	*NBN*	***NF1***	*NF2*	*NFE2L2*
NFKBIA	*NKX2-1 (TTF-1)*	*NOTCH1*	*NOTCH2 [Intron 26]*	*NOTCH3*	***NPM1 [Exons 4-6, 8, 10]***	***NRAS [Exons 2, 3]***	*NSD3 (WHSC1L1)*	*NT5C2*	***NTRK1 [Exons 14, 15, Introns 8-11]***
NTRK2 [Intron 12]	***NTRK3 [Exons 16, 17]***	*NUTM1** ***[Intron 1]***	*P2RY8*	***PALB2***	*PARK2*	*PARP1*	*PARP2*	*PARP3*	*PAX5*
PBRM1	*PDCD1 (PD-1)*	***PDCD1LG2 (PD-L2)***	***PDGFRA [Exons 12, 18, Introns 7, 9, 11]***	***PDGFRB [Exons 12-21, 23]***	*PDK1*	*PIK3C2B*	*PIK3C2G*	***PIK3CA [Exons 2, 3, 5-8, 10, 14, 19, 21 (Coding Exons 1, 2, 4-7, 9, 13, 18, 20)]***	*PIK3CB*
PIK3R1	*PIM1*	*PMS2*	*POLD1*	*POLE*	*PPARG*	*PPP2R1A*	*PPP2R2A*	*PRDM1*	*PRKAR1A*

PRKCI	*PTCH1*	***PTEN***	***PTPN11***	*PTPRO*	*QKI*	*RAC1*	*RAD21*	*RAD51*	*RAD51B*
RAD51C	*RAD51D*	*RAD52*	*RAD54L*	***RAF1 [Exons 3, 4, 6, 7, 10, 14, 15, 17,*** *Introns 4-8]*	*RARA [Intron 2]*	***RB1***	*RBM10*	*REL*	***RET [****Introns 7, 8,* ***Exons 11, 13-16, Introns 9-11]***
RICTOR	*RNF43*	***ROS1 [Exons 31, 36-38, 40, Introns 31-35]***	*RPTOR*	*RSPO2* [Intron 1]*	*SDC4* [Intron 2]*	*SDHA*	*SDHB*	*SDHC*	*SDHD*
SETD2	*SF3B1*	*SGK1*	*SLC34A2* [Intron 4]*	*SMAD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	***SMO***	*SNCAIP*
SOCS1	*SOX2*	*SOX9*	*SPEN*	*SPOP*	*SRC*	*STAG2*	*STAT3*	***STK11 (LKB1)***	*SUFU*
SYK	*TBX3*	*TEK*	*TERC* {ncRNA}*	***TERT* {Promoter}***	*TET2*	*TGFBR2*	*TIPARP*	*TMPRSS2* [Introns 1-3]*	*TNFAIP3*
TNFRSF14	***TP53***	*TSC1*	*TSC2*	*TYRO3*	*U2AF1*	***VEGFA***	*VHL*	*WHSC1*	*WTI*
XPO1	*XRCC2*	*ZNF217*	*ZNF703*						

[1] As part of its FDA-approved intended use, the FoundationOne® Liquid CDx assay interrogates 324 genes, including 309 genes with complete exonic (coding) coverage and 15 genes with only select non-coding coverage (indicated with an *). Select genes and select exons (indicated in bold) are captured with increased sensitivity.

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

Table 3: Genes for which copy number alterations and rearrangements are reported for tumor profiling by FoundationOne® Liquid CDx

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

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

Table 4. Category Definitions

Category	FoundationOne® Liquid CDx			Comments
	Prescriptive use for a Therapeutic Product	Clinical Performance	Analytical Performance	
Category 1: Companion Diagnostic (CDx)	**Yes**	**Yes**	**Yes**	ctDNA biomarkers linked to the safe and effective use of the corresponding therapeutic product, for which FoundationOne® Liquid CDx has demonstrated clinical performance shown to support therapeutic efficacy and strong analytical performance for the biomarker.
Category 2:	No	No	**Yes**	ctDNA biomarkers with strong evidence of clinical significance

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

FoundationOne® Liquid cfDNA CDx Blood Specimen Collection Kit Contents

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

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

Instruments

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

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

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

Test Process

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

A. Specimen Collection and Preparation

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

B. DNA Extraction

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

C. Library Construction

Library Construction (LC) begins with the normalization of cfDNA. The samples are purified, using AMPure XP Beads (Agencourt). Solid-phase reversible immobilization (SPRI) purification is used subsequent to library construction with the NEBNext kits (NEB), including mixes for end repair with blunt-end and 5'-phosphorylate the cfDNA fragments using T4 Polynucleotide Kinase and T4 DNA Polymerase. This step prepares the 3'- end for dA-addition while also preparing the 5'-end of the DNA fragment for ligation. Second, dA-addition will incorporate a single dAMP to the 3'-end of the End-Repaired material. After dA-addition, a universal Y-adaptor is ligated onto each end of the DNA fragment using a DNA ligase. These steps are performed in 96-well plates (Eppendorf) on a Bravo Benchbot (Agilent) using the "with-bead" protocol to maximize reproducibility and library yield. Indexed (Foundation Medicine customized six base pair barcodes) sequencing libraries are PCR amplified with a high-fidelity DNA polymerase (HiFi™, Kapa) for ten cycles, SPRI purified and quantified by PicoGreen fluorescence assay

(Invitrogen). Process matched control (PMC) is prepared and added to the plate with other cfDNA samples at the beginning of LC.

D. Hybrid Capture

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

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

E. Sequencing

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

A growing nucleotide chain is created on the flow cell by incorporating fluorescently labeled, 3'-blocked dNTPs. After excitation by a laser, the camera captures the emission color of the incorporated, fluorescently labeled nucleotide. The 3'-block is then removed, reverting the nucleotide to its natural form, which allows the polymerase to add another base to the growing double strand of DNA. With each

successive SBS cycle, a new fluorescently labeled 3'- blocked dNTP is added. SBS allows for millions of discrete clusters of clonal copies of DNA to be sequenced in parallel.

F. Sequence Analysis

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

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

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

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

G. Report Generation

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

H. Internal Process Controls

Positive Control

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

Sensitivity Control

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

Negative Control

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

I. CDx Classification Criteria

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

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

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

Table 7: Deleterious *BRCA* Missense Alterations in rucaparib

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

2. *ATM*, *BRCA1* and *BRCA2* alterations to identify patients eligible for olaparib in mCRPC:

Table 8: Rules Applied to the Aforementioned Genes:

Qualification Criteria	Sequence Classification	Methodology	Comments
A gene alteration that includes any of the sequence classifications	Protein truncating mutations	Sequence analysis identifies premature stop codons anywhere in the gene coding region, except: 3' of and including *BRCA2* K3326*	Does not include VUS. Includes mutations on the canonical transcript only for genes *ATM*, *BRCA1*, and *BRCA2*.
	Splice site mutations	Sequence analysis identifies variant splice sequences at intron/exon junctions -/+ 2bp of exon starts/ends	Does not include VUS. Includes indels that extend through ±2bp from the intron/exon junction. Includes mutations on the canonical transcript only for genes *ATM*, *BRCA1*, and *BRCA2*.
	Homozygous deletions	Sequence analysis identifies deletions in both gene alleles of ≥1 exon in size	Does not include VUS Only reported for *BRCA1*&2. Not reported for *ATM*.
	Large protein truncating rearrangements	Sequence analysis identifies protein truncating rearrangements	Does not include VUS
	Deleterious missense mutations	Curated list	Protein effects from list of missense mutations on the canonical transcript only for genes *ATM*, *BRCA1*, and *BRCA2*.

Alterations reported are limited to those within the alteration-calling capabilities of FMI as of March 2, 2020. ATM missense mutations were identified from the ClinVar database. Should the calling capabilities expand, additional alterations that meet the above criteria may also be reported, per FDA approval.

Table 9. List of Deleterious Missense Mutations by Protein Effect, Implemented on the Respective Canonical Transcript.

BRCA1		***BRCA2***		***ATM***	
Protein Effect (PE)	**FMI Annotated PE**	**Protein Effect (PE)**	**FMI Annotated PE**	**Protein Effect (PE)**	**FMI Annotated PE**
MIV	MIV	MIR	MIR	MIT	MIT
MlI	MlI	MlI	MlI	R2032K	R2032K
C6IG	C6IG	VI59M	VI59M	R2227C	R2227C
C64Y	C64Y	V211L	V211L	R2547 S2549del	R2547 S2549del
R7IG	R7IG	V211I	V211I	G2765S	G2765S
R7IK	R7IK	R2336P	R2336P	R2832C	R2832C
RI495M	RI495M	R2336H	R2336H	S2855 V2856delinsR1	S2855 V2856delinsRl S2855 V2856>Rl
EI559K	EI559K			R3008C	R3008C
DI692N	DI692N			R3008H	R3008H
DI692H	DI692H			[VUS from Jan 2016 HRR* List to be Excluded]	
RI699W	RI699W			V2424G	V2424G
AI708E	AI708E			[Excluded from Jan 2016 HRR List]	
G1788V	GI788V			K750K	splice site 2250G>A

HRR = Homologous Recombination Repair genes

Intronic Variants

Gene	**Chr**	**Position**	**Ref**	**Alt**	**dbSNP**	**FMI Protein Effect**
ATM	*chr11*	*108128198*	*T*	*G*	*rs730881346*	**[Variant Not Called by FMI]**
ATM	*chr11*	*108214102*	AGTGA	A	rs730881295	splice site 8418+5_8418+8delGTGA or splice site 8418+1_8418+4delGTGA

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

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

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are FDA-approved companion diagnostic (CDx) alternatives for the detection of genetic alterations using cfDNA isolated from plasma samples, as listed in Table 1 of the FoundationOne® Liquid CDx intended use statement. The approved CDx tests are listed in Table 10, below; for additional details see FDA List of Cleared or Approved Companion Diagnostic Devices at: https://www.fda.gov/media/119249/download. Each alternative has its own advantages and disadvantages. A patient should fully discuss these alternatives with his/her physician to select the method that best meets expectations and lifestyle.

Table 10: FDA-approved companion diagnostic (CDx) alternatives

Biomarker(s) Detected	Device	Company	Technology	Therapy	Indication
EGFR Exon 19 deletions and L858R Substitution Mutation	**cobas** *EGFR* Mutation Test v2	Roche Molecular Systems, Inc.	Polymerase Chain Reaction (PCR)	TARCEVA® (erlotinib), TAGRISSO® (osimertinib), and IRESSA® (gefitinib)	NSCLC
	Guardant360 CDx	Guardant Health, Inc.	NGS	TAGRISSO® (osimertinib)	
PIK3CA: C420R, E542K, E545A, E545D [1635G>T only], E545G, E545K, Q546E, Q546R, H1047L, H1047R, and H1047Y	*therascreen* *PIK3CA* RGQ PCR test	QIAGEN, Inc.	PCR	PIQRAY® (alpelisib)	Breast Cancer

There are no FDA-approved CDx alternatives using cfDNA isolated from plasma for the detection of genomic alterations of *BRCA1*, *BRCA2*, and *ATM* for the identification of mCRPC patients eligible for treatment with LYNPARZA® (olaparib).

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

assay was also approved for tumor mutation profiling for substitutions and indels to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with solid malignant neoplasms. The FoundationOne® Liquid CDx test was approved on October 26, 2020 as a companion diagnostic for *BRCA1* and *BRCA2* alterations in epithelial ovarian cancer for patients who may benefit from treatment with RUBRACA® (rucaparib), *ALK* rearrangements in NSCLC for patients who may benefit from treatment with ALECENSA® (alectinib), and *PIK3CA* mutations in patients with breast cancer who may benefit from treatment with PIQRAY® (alpelisib). This approval also included the addition of rearrangements in three (3) genes, and copy number alterations in three (3) genes for tumor profiling.

The FoundationOne® Liquid CDx assay has been marketed in the United States, the European Union, and in several other foreign countries since August 2020.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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

For the specific adverse events that occurred in the clinical study, please see the FDA approved package inserts for LYNPARZA® (olaparib) which is available at Drugs@FDA.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

Performance characteristics were established using circulating cfDNA derived from blood specimens extracted from a wide range of tumor types and performed as described in the Summary of Safety and Effectiveness Data for P190032 and P200006. Table 11 and Table 12 below provides a summary of the number of tumor types and relevant *ATM, BRCA1*, and *BRCA2* variants or variant types included in each study described below or referenced. 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 several genes.

Due to the lack of sufficient volume of clinical specimens, some of the studies used contrived samples, which consisted of enzymatically sheared cell line DNA spiked into human plasma and diluted with cfDNA isolated from healthy donor plasma. A

contrived sample functional characterization (CSFC) study (Section IX.A.1) was conducted to demonstrate comparable performance of sheared cell line DNA samples as compared to cfDNA isolated from plasma specimens obtained from cancer positive patient specimens. Clinical specimens were used to assess analytical accuracy, precision and confirmation of the estimated limit of detection (LoD), and evaluate sample stability.

The validation studies included >7,000 sample replicates, >31,000 unique variants, >30 tumor types, representing all 311 genes targeted by the assay. Please refer to the Summary of Safety and Effectiveness Data for P190032 and P200006 for the representation of tumor types and variants included in the previous device approvals.

Table 11: 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
Orthogonal Concordance	23 cancer types Contrived samples	278	0	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
FoundationOne® Liquid CDx to Validated Tumor Tissue Test Concordance: *BRCA1* and *BRCA2* Variants	Prostate cancer Ovarian cancer	279	0	2	100	87	9	2
Potentially Interfering Substances	Contrived samples	9	336	18	16	11	11	2
Hybrid Capture Bait Specificity	25 cancer types Contrived samples	3546	0	324	0	0	0	0
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	0	1	5	7	0	0
Clinical validation study for detection of deleterious alterations in *BRCA1* and *BRCA2* in prostate cancer	Prostate cancer	199	0	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
Automation Line Equivalence	Contrived samples	8	187	303	1926	337	63	4
Variant Report Curation	Breast cancer CRC Lung cancer	19	57	183	300	104	15	2

Study Title	Cancer Types Represented	# Unique Samples	# of Sample Replicates	# Unique				
				Targeted Genes	Subs	Indels	Rearrang.	Copy Number Losses
	Prostate cancer Skin cancer							
Pan-tumor performance (includes historical analysis)	20 cancer types	19868	0	0	0	0	0	0
Molecular Index Barcode Performance	25 cancer types Contrived samples	7637	0	324	0	0	0	0
FoundationOne® Liquid LDT to FoundationOne® Liquid CDx Concordance	25 cancer types	927	0	73	1815	376	109	0

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

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 post-market study (See Section XIII) 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 1 *ATM* substitutions, 2 *ATM* indels, 2 *BRCA1* (positive for 2 indels and 1 substitutions), and 3 *BRCA2* samples (positive for 5 indels). These samples were used to supplement the samples used to support the performance of the *ATM*, *BRCA1* and *BRCA2* CDx indications listed in Table 1.

1. Contrived Sample Functional Characterization (CSFC) Study:

   See Summary of Safety and Effectiveness Data for P190032 and P200006 and Section XIII.

2. Analytical Accuracy/Concordance with an Orthogonal Method:

   See Summary of Safety and Effectiveness Data for P190032 and P200006.

3. Analytical Sensitivity:

   a. Limit of Blank (LoB):

      See Summary of Safety and Effectiveness Data for P190032.

   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). For this study the initial cfDNA input was set at 45 ng for contrived samples; however, the final cfDNA input varied depending upon the starting VAF and/or tumor fraction and the targeted VAF and/or tumor fraction dilution levels. 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 12. The median LoD for targeted short variant, rearrangement, and copy number alterations were consistent with the platform LoD.

Table 12: LoD estimation for CDx alterations

Gene	Alteration Subtype	# Samples Evaluated	Median LoD[1]
ATM	Indel	1	0.51%
	ATM-EXPH5 Truncation[2]	1	1.13%
BRCA1	Substitutions	8	0.34% VAF
	Indels	1	0.38% VAF
	Rearrangement[2]	1	0.87% VAF
BRCA2	Substitutions	17	0.37% VAF
	Indels	2	0.36% VAF
	BRCA2-EDA Truncation[2]	1	0.48% VAF
	Copy Number Loss[1]	1	48.1% TF

The Estimated LoDs for one *ATM* and several *BRCA1* and *BRCA2* short variants were confirmed at values higher than the LoDs estimated for the non-CDx alterations. (see Precision: Reproducibility and Reproducibility section below, Tables 14 and 15 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.

4. Analytical Specificity:
   a. Potentially Interfering Substances:

See Summary of Safety and Effectiveness Data for P190032.

b. Hybrid Capture Bait Specificity:

See Summary of Safety and Effectiveness Data for P190032.

5. Carryover/Cross-Contamination:

See Summary of Safety and Effectiveness Data for P190032.

6. Precision: Repeatability and Reproducibility at LoD
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 13.

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 *ATM* alterations. 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. The cfDNA input for the library construction step was set at 45 ng for the contrived samples, and ranged from 24ng - 45ng for the clinical samples, with preference towards the lower, more challenging cfDNA input amounts. 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 13: CDx sample set assessed for precision

Targeted Alteration	**Disease Ontology of Patient from which Sample was Derived**
ATM K1773fs*3 (5318delA)	Contrived
ATM splice site 8850+1G>A	Prostate cancer

Targeted Alteration	Disease Ontology of Patient from which Sample was Derived
ATM I2012fs*4	Prostate cancer
ATM-EXPHS Truncation	Prostate cancer
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 S2988fs*12	Ovary cancer
BRCA2- EDA Truncation	Prostate cancer

The repeatability of CDx alterations is summarized in Table 14 and the reproducibility of CDx alterations is summarized in Table 15.

Table 14: Repeatability of CDx alterations targeted in precision study at ≥ 1x LoD[1]

Variant Type	Alteration	Concordant Pairs	Repeatability (%)	95% CIs (%)	VAF/TF[2] Level Tested	X LoD Tested
Short variant	*ATM* I2012fs*4	12/12	100	(73.54, 100)	0.86%	1.7
Short variant	*ATM* splice site 8850+1G>A	12/12	100	(73.54, 100)	0.56%	1.1
Short variant	*ATM* K1773fs*3	12/12	100	(73.54, 100)	0.77%	1.5
Short variant	*ATM* K1773fs*3	12/12	100	(73.54, 100)	1.04%	2.0
Rearrangement	*ATM-EXPH5 truncation*	12/12	100	(73.54, 100)	1.13%	1.0[3]
Short variant	*BRCA1* 2338C>T	12/12	100	(73.54, 100)	1.11%	3.3
Short variant	*BRCA1* 2475delC	12/12	100	(73.54, 100)	0.61%	1.6
Short variant	*BRCA1* 2475delC	12/12	100	(73.54, 100)	0.93%	3.3
Short variant	*BRCA1* 2612C>TT	11/11	100	(71.51, 100)	0.51%	1.3
Short variant	*BRCA1* 68_69delAG	12/12	100	(73.54, 100)	0.66%	1.7
Short variant	*BRCA1* P871fs*32	12/12	100	(73.54, 100)	1.08%	2.8
Rearrangement	*BRCA1-BRCA1*	12/12	100	(73.54, 100)	0.87%	1.0
Short variant	*BRCA2* 3599_3600delGT	12/12	100	(73.54, 100)	0.58%	1.6
Short variant	*BRCA2* 3599_3600delGT	12/12	100	(73.54, 100)	0.92%	2.6
Short variant	*BRCA2* 4284_4285insT	12/12	100	(73.54, 100)	0.94%	2.6
Short variant	*BRCA2* 4284_4285insT	11/11	100	(71.51, 100)	1.26%	3.5
Short variant	*BRCA2* 5351delA	12/12	100	(73.54, 100)	1.22%	3.2
Short variant	*BRCA2* 5351delA	12/12	100	(73.54, 100)	1.85%	4.9
Short variant	*BRCA2* 5351delA	11/11	100	(71.51, 100)	1.07%	2.8
Short variant	*BRCA2* 5351delA	12/12	100	(73.54, 100)	2.24%	5.9
Short variant	*BRCA2* 5465_5466insA	12/12	100	(73.54, 100)	0.92%	2.4
Short variant	*BRCA2* 5465_5466insA	11/11	100	(71.51, 100)	1.19%	3.1
Short variant	*BRCA2* 8961_8964delGAGT	12/12	100	(73.54, 100)	1.07%	2.8
Short variant	*BRCA2* 799G>T	10/12	83.33	(51.59, 97.91)	0.5%	1.5
Short variant	*BRCA2* 9097_9098insA	6/11	54.55	(23.38, 83.25)	0.71%	1.9
Short variant	*BRCA2* 9097_9098insA	10/12	83.33	(51.59, 97.91)	1.03%	2.7
Copy Number Loss	*BRCA2* loss	11/12	91.67	(61.52, 99.79)	39.43%	0.8

Variant Type	Alteration	Concordant Pairs	Repeatability (%)	95% CIs (%)	VAF/TF[2] Level Tested	X LoD Tested
Rearrangement	*BRCA2-EDA*	11/11	100	(71.51, 100)	0.48%	0.6

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

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

[3] LoD was not directly established for this variant. The %VAF tested is considered the LoD for *ATM* rearrangements.

Table 15: Reproducibility of CDx alterations targeted in precision study at ≥1x LoD[1]

Variant Type	Alteration	Concordant Replicates	Reproducibility (%)	95% CIs (%)	VAF/TF[2] Level Tested	X LoD Tested
Short variant	*ATM* I2012fs*4	24/24	100	(85.75, 100)	0.86%	1.7
Short variant	*ATM* splice site 8850+1G>A	24/24	100	(85.75, 100)	0.56%	1.1
Short variant	*ATM* K1773fs*3	24/24	100	(85.75, 100)	0.77%	1.5
Short variant	*ATM* K1773fs*3	24/24	100	(85.75, 100)	1.04%	2.0
Rearrangement	*ATM-EXPH5 truncation*	24/24	100	(85.75, 100)	1.13%	1.0[3]
Short variant	*BRCA1* 2338C>T	24/24	100	(85.75, 100)	1.11%	3.3
Short variant	*BRCA1* 2475delC	24/24	100	(85.75, 100)	0.61%	1.6
Short variant	*BRCA1* 2475delC	24/24	100	(85.75, 100)	0.93%	2.4
Short variant	*BRCA1* 2612C>TT	23/23	100	(85.18, 100)	0.51%	1.3
Short variant	*BRCA1* 68_69delAG	24/24	100	(85.75, 100)	0.66%	1.7
Short variant	*BRCA1* P871fs*32	24/24	100	(85.75, 100)	1.08%	2.8
Rearrangement	*BRCA1-BRCA1*	24/24	100	(85.75, 100)	0.87%	1.0
Short variant	*BRCA2* 3599_3600delGT	24/24	100	(85.75, 100)	0.58%	1.6
Short variant	*BRCA2* 3599_3600delGT	24/24	100	(85.75, 100)	0.92%	2.6
Short variant	*BRCA2* 4284_4285insT	24/24	100	(85.75, 100)	0.94%	2.6
Short variant	*BRCA2* 4284_4285insT	23/23	100	(85.18, 100)	1.26%	3.5
Short variant	*BRCA2* 5351delA	24/24	100	(85.75, 100)	1.22%	3.4
Short variant	*BRCA2* 5351delA	24/24	100	(85.75, 100)	1.85%	5.1
Short variant	*BRCA2* 5351delA	23/23	100	(85.18, 100)	1.07%	3.0
Short variant	*BRCA2* 5351delA	24/24	100	(85.75, 100)	2.24%	6.2
Short variant	*BRCA2* 5465_5466insA	24/24	100	(85.75, 100)	0.92%	2.6
Short variant	*BRCA2* 5465_5466insA	23/23	100	(85.18, 100)	1.19%	3.3
Short variant	*BRCA2* 799G>T	22/24	91.67	(73.0, 98.97)	0.5%	1.4
Short variant	*BRCA2* 8961_8964delGAGT	24/24	100	(85.75, 100)	1.07%	3.0
Short variant	*BRCA2* 9097_9098insA	22/24	91.67	(73.0, 98.97)	1.03%	2.9
Short variant	*BRCA2* 799G>T	22/24	91.67	(73.0, 98.97)	0.5%	1.4
Short variant	*BRCA2* 9097_9098insA	5/23	21.74	(7.46, 43.7)	0.71%	2.0
Short variant	*BRCA2* 9097_9098insA	22/24	91.67	(73.0, 98.97)	1.03%	2.9
Copy Number Loss	*BRCA2* loss	21/24	87.5	(67.64, 97.34)	39.43%	0.8
Rearrangement	*BRCA2*-EDA	23/23	100	(85.18, 100)	0.48%	1.0

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

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

[3] LoD was not directly established for this variant. The %VAF tested is considered the LoD for *ATM* rearrangements.

For repeatability, 28 samples with 19 unique targeted alterations were evaluated. Of the 19 unique alterations that were targeted in 24 of the 28

samples, 16 alterations (84.2%) demonstrated 100% repeatability. Four *BRCA2* samples demonstrated repeatability below 95% (54.6% - 91.7%). The *BRCA2* loss was tested at an 39.4%TF below the estimated LoD of 48.1%TF and used a cfDNA input below the recommended cfDNA input of 30 ng. Of the remaining 3 poorly performing samples, only one was at a %VAF (0.5% VAF) near the estimated LoD (0.37% VAF), while the remaining 2 were tested at levels higher than the estimated LoDs for each sample. Therefore, the reason for the observed performance was not determined.

Reproducibility of 100% was observed in 16 of 19 (84.2%) unique alterations in 24 of the 30 samples included in the study. Six *BRCA2* samples demonstrated reproducibility below 95% (21.7 – 91.7). The *BRCA2* loss was tested at an %TF below the estimated LoD and used a cfDNA input below the recommended cfDNA input of 30 ng. Of the remaining 5 poorly performing samples, only one was at a %VAF (0.5% VAF) near the estimated LoD (0.37% VAF), while the remaining 4 were tested at levels higher than the estimated LoDs for each sample. Therefore, the reason for the observed performance was not determined.

Samples included in the precision/reproducibility study BRCA1 and BRCA2 alterations included prostate and non-prostate specimens. Data from a post-market study will be provided using clinical samples from patients with prostate cancer to demonstrate performance in the intended specimen type. Also see the Summary of Safety and Effectiveness Data for P190032 and P200006 and Section XIII.

b. Confirmation of LoD and Precision in Clinical Specimens:
The combined confirmation of LoD and precision study was performed as described in the Summy of Safety and Effectiveness for P190032. In this study, 29 clinical cfDNA samples targeting variants at 1-1.5x LoD were evaluated to confirm LoD and precision in clinical specimens. Of the samples included in this study 9 clincial samples were associated with the olaparib indication listed in Table 1. All 9 specimens had 100% reproducibility at the levels tested. A summary of the Confirmation of LoD and precision results for a subset of highly-actionable alterations are provided in Table 16. See the Summary of Safety and Effectiveness Data for P190032 and P200006 and Section XIII.

Table 16: Confirmation of LoD* and precision in clinical specimens for CDx alterations

Target Alteration	LoD	Mean Level Tested	Reproducibility (95% CI)
ATM I2012fs*4	0.51% MAF	0.86%	100% (85.8, 100.0)
ATM splice site 8850+1G>A	0.51% MAF	0.56%	100% (85.8, 100.0)
ATM-EXPH5 truncation	Not Determined	1.13 %VAF	100% (85.8, 100.0)
BRCA1 E23fs*17	0.38% VAF	0.66% VAF	100% (85.8, 100.0)
BRCA1 Q780*	0.34% VAF	1.11%VAF	100% (85.8, 100.0)
BRCA1 Rearrangement	0.26%-.47% VAF[1]	0.87% VAF	100% (85.8, 100.0)

BRCA2 S2988fs*12	0.36% VAF	1.07% VAF	100% (85.8, 100.0)
BRCA2- EDA Truncation	0.26%-.47% VAF[1]	0.48% VAF	100% (85.2, 100.0)

In general, most of the targeted variants were tested at levels higher than estimated near LoD (~1x); therefore, the tested LoD level values (%VAF/%TF) are considered to be the confirmed LoD. The LoD for *ATM* rearrangements was not previously determined; however , as indicated previously, the variant was confirmed at 1.13% VAF which is considered to be the confirmed LoD. A post-market study is planned to demonstrate precision using samples at near the estimated LoD for those tested above or below the estimated LoD and in plasma specimens obtained from patients with prostate cancer (See Section XIII).

c. Tumor Mutation Profiling Variants:

See Summary of Safety and Effectiveness Data for P190032 and P200006 and Section XIII.

d. Reagent Lot-to-Lot Reproducibility:

See Summary of Safety and Effectiveness Data for P190032.

e. Instrument-to-Instrument Reproducibility:

See Summary of Safety and Effectiveness Data for P190032.

f. Reagent Lot Interchangeability:

See Summary of Safety and Effectiveness Data for P190032.

g. Curator Precision:

See Summary of Safety and Effectiveness Data for P190032.

7. Comparability Across Cancer Types:

See Summary of Safety and Effectiveness Data for P190032 and P200006.

8. Stability:

a. Reagent Stability:

See Summary of Safety and Effectiveness Data for P190032.

b. Stability of cfDNA and Plasma Samples:

See Summary of Safety and Effectiveness Data for P190032.

c. Whole Blood Specimen Stability and Inverted Tube Stability:

See Summary of Safety and Effectiveness Data for P190032 and Section XIII.

9. Guard-banding and Robustness:

a. DNA Extraction:

See Summary of Safety and Effectiveness Data for P190032.

b. cfDNA Input:

See Summary of Safety and Effectiveness Data for P190032 and P200006 and Section XIII.

c. Molecular Index Barcode Performance:

See Summary of Safety and Effectiveness Data for P190032.

d. Automation Line Equivalence:

See Summary of Safety and Effectiveness Data for P190032.

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:

See Summary of Safety and Effectiveness Data for P190032 and Section XIII.

2. Concordance with a Validated Tumor-tissue NGS by Gene and Variant Type
The concordance between the FoundationOne® Liquid CDx and a validated tumor-tissue NGS orthogonal assay was performed to establish the ability of the FoundationOne® Liquid CDx test to detect *ATM*, *BRCA1*, and *BRCA2* alterations in plasma samples obtained from patients whose prostate tumors were identified as positive by a validated tumor-tissue based NGS orthogonal assay. Agreements (PPA and NPA) were calculated by gene and by variant type using the comparator method as the reference. The PPA estimate across all 3 genes was 74.29%, it was highest in the *BRCA2* gene (PPA=76.47%). PPA estimates for *ATM* and *BRCA1*

were 70.83% and 70.43% respectively. NPA point estimates in all cases were greater than 99% (Table 17).

Concordances per variant type in each gene were calculated based on the results of 325 samples from the clinical bridging study. Copy number losses in *ATM* are not reported by FoundationOne® Liquid CDx, and therefore were not assessed within this analysis. All data analysis was based on variant level data rather than at the sample level, i.e., a sample can have multiple positive (or negative) calls in the same gene of the same variant type, and as such the numbers reported in the tables below are based on targeted variant locations across all 3 genes for each sample. For short variants, the set of all observed variants in the data were considered as being either positively or negatively called in each sample. For rearrangements, all possible rearrangement types (truncation, deletion, and duplication) that could be called as a biomarker were examined. For copy number alterations, only homozygous deletions were measured, consistent with the CDx biomarker definition. Results were as follows in Table 17 and Table 18.

Table 17: Concordance by Gene and Overall Variant Type

Gene/Variant Type	F1LCDx+/ Orth+	F1LCDx–/ Orth +	F1LCDx+/ Orth–	F1LCDx–/ Orth–	PPA (%) (95% CI*%)	NPA (%) (95% CIs)
ATM	34	14	37	22990	70.83% (56.82%, 81.76%)	99.84% (99.78%, 99.88%)
BRCA1	5	2	3	2590	71.43% (35.89%, 91.78%)	99.88% (99.66%, 99.96%)
BRCA2	65	20	11	20054	76.47% (66.43%, 84.22%)	99.95% (99.90%, 99.97%)
Short Variant	90	17	44	42099	84.11% (76.02%, 89.84%)	99.90% (99.86%, 99.92%)
Rearrangement	10	8	6	2901	55.56% (33.72%, 75.44%)	99.79% (99.55%, 99.90%)
Copy Number Alteration (gain)	4	11	1	634	26.67% (10.90%, 51.95%)	99.84% (99.11%, 99.99%)
Total	104	36	51	45634	74.29% (66.47%, 80.81%)	99.89% (99.85%, 99.92%)

Abbreviations: F1LCDx = FoundationOne® Liquid CDx; Orth = orthogonal method

[1] Score method was used to estimate 95% two-sided CI

Additional analysis was performed for each of the variant types by gene.

Table 18: Concordance by Variant Type by Gene

Gene	Variant Type	F1LCDx+/ Orth+	F1LCDx–/ Orth +	F1LCDx+/ Orth–	F1LCDx–/ Orth–	PPA (%) (95% CI[1])	NPA (%) (95% CI)
ATM	SV	31	12	35	22022	72.09% (57.31%, 83.25%)	99.84% 99.78%, 99.89%)
	RE	3	2	2	318	60.00% (23.07%, 88.24%)	99.38% (97.75%, 99.83%)
BRCA1	SV	3	0	1	1296	100% (43.85%, 100%)	99.92% (99.56%, 100%[2])

Gene	Variant Type	F1LCDx+/ Orth+	F1LCDx–/ Orth +	F1LCDx+/ Orth–	F1LCDx–/ Orth–	PPA (%) (95% CI[1])	NPA (%) (95% CI)
	RE	2	2	2	969	50.00% (15.00%, 85.00%)	99.79% (98.25%, 99.94%)
	CNA	0	0	0	325	N/A[3]	100.00% (98.83%, 100%)
BRCA2	SV	56	5	8	18781	91.80% (82.21%, 96.45%)	99.96% (99.92%, 99.98%)
	RE	5	4	2	964	55.56% (26.65%, 81.12%)	99.79% (98.25%, 99.94%)
	CNA	4	11	1	309	26.67% (10.90%, 51.95%)	99.68% (98.20%, 99.98%)

Abbreviations: F1LCDx = FoundationOne® Liquid CDx; Orth = orthogonal method

[1] Score method was used to estimate 95% two-sided CI

[2] Actual upper bound is 99.996%

[3] PPA was not evaluable, as the denominator was 0.

In general, the point estimates of NPA were very high, indicating that if an alteration is not detected by the orthogonal method, it likely will not be detected in FoundationOne® Liquid CDx, providing a high level of confidence in variants reported by FoundationOne® Liquid CDx. The F1L CDx–/Orth+ discordances observed are likely due to biological differences between tumor tissue and plasma, and random chance due to small sample size. Differences in tumor fraction and %VAF values between the variants present in the tissue and liquid samples due to shedding of cfDNA into the circulation, as well as tumor heterogeneity in the tumor tissue specimens could contribute to discordant results.

Another potentially explanatory trend was sample quality. Many of the discordant samples (40 of 64 total samples with at least one discordance) had at least one in-process QC metric that was not met, resulting in qualified results. For the orthogonal method, 22 of 38 (57.89%) samples with a F1L CDx+/Orth– discordance had a QC flag in the corresponding Orthogonal method sample. Of the other samples with an FoundationOne® Liquid CDx variant detected, only 9 of 87 (10.34%) had a QC flag in the corresponding orthogonal method sample. This difference is highly significant ($p < 1x10^{-13}$). This suggests that poor quality of some of the tissue samples explains some of the discordances seen. This trend was seen in liquid samples as well, although the difference was not statistically significant. For samples with an Orth+/F1L CDx– discordance, 11 of 35 (31.43%) had a flag in the liquid samples, compared to 24 of 96 (25%) in other samples with a tumor tissue result. Moreover, the number of samples is small enough that results can be skewed due to random variance. Overall, low VAF and TF in either assay as well as poor sample quality explain most of the discordances observed.

The data provided in this study also supports the recommendation for samples yielding a negative result by the FoundationOne® Liquid CDx test should be reflexed to a FDA-approved tumor-tissue based CDx test.

D. Animal Studies

Not Applicable

X. SUMMARY OF PRIMARY CLINICAL STUDY

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

The safety and effectiveness of FoundationOne® Liquid CDx for detecting *BRCA1, BRCA2* and/or *ATM* alterations in prostate cancer patients who may benefit from treatment with olaparib versus enzalutamide or abiraterone acetate was demonstrated in a prospectively defined retrospective analysis of specimens from patients enrolled in Cohort A of the study D081DC00007 (PROfound). PROfound is a prospective, multicenter, randomized, open-label, phase III trial evaluating the efficacy and safety of olaparib versus enzalutamide or abiraterone acetate in subjects with mCRPC who have failed prior treatment with a new hormonal agent (NHA) who have qualifying mutations in one of 15 genes directly or indirectly involved in homologous recombination repair, as determined by a tumor tissue test. All patients were required to have a qualifying homologous recombination repair (HRR) mutation assessed using the FoundationOne LDT tumor tissue-based clinical trial assay (F1 LDT CTA) to be randomized. Qualifying HRR gene alterations included alterations in *BRCA1, BRCA2* and *ATM* for Cohort A, and *BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D* and *RAD54L* for Cohort B.

This indication for FoundationOne Liquid CDx was based on plasma samples available from patients enrolled into Cohort A only. Cohort A of PROfound included patients who had a qualifying tumor mutation in either *BRCA1, BRCA2*, or *ATM* genes. A bridging study was conducted to assess: 1) the concordance between *ATM, BRCA1,* and *BRCA2* status (alteration positive or negative) by the F1 LDT CTA used for enrollment and the FoundationOne® Liquid CDx and 2) the effectiveness of olaparib in patients identified to harbor *ATM, BRCA1*, or *BRCA2* alterations by the FoundationOne® Liquid CDx test. Throughout this document, the term *ATM/BRCA1/BRCA2* is used to denote patients who carry mutations in *BRCA1, BRCA2* or *ATM*, unless specified.

A. Study Design

The PROfound study is a prospective, multi-center, randomized, open-label, Phase III trial evaluating the efficacy and safety of olaparib versus enzalutamide or abiraterone acetate in patients with mCRPC who have failed prior treatment with a NHA and have a qualifying tumor mutation in one of 15 genes involved in the homologous recombination repair pathway. Patients were divided into two cohorts (Cohort A or Cohort B) based on their HRR gene mutation status. As stated above, patients randomized into Cohort A, included patients whose tumor tissue specimens were

identified as harboring alterations in *BRCA1, BRCA2* or *ATM*, while those randomized into Cohort B included those patients whose tumor tissue specimens were identified as harboring alterations in one of the remaining 11 HRR genes (i.e., *BARD1, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D* and *RAD54L).*

In the device bridging study, all available plasma samples, meeting the inclusion/exclusion criteria below, from Cohort A or screen failed patients collected at baseline prior to randomization into the AstraZeneca PROfound clinical trial were tested with FoundationOne® Liquid CDx.

1. Clinical Bridging Study Inclusion and Exclusion Criteria

    a. Sample inclusion criteria:
        - Patient provided appropriate consent for sample testing and diagnostic development
        - Specimens in frozen plasma.
        - Samples must meet minimum criteria for FoundationOne® Liquid CDx operational testing requirements
        - Samples must have a minimum plasma volume of 2.5mL.
        - For FoundationOne® Liquid CDx: Samples must have DNA content, as assessed by the TapeStation assay, >30 ng of DNA for LC input for primary analysis
        - Samples obtained in accordance with FoundationOne® Liquid CDx sample criteria.

    b. Sample exclusion criteria:
        - Tissue, other liquid samples are excluded
        - Samples that do not meet minimum FoundationOne® Liquid CDx operational testing requirements
        - Samples with plasma volume <2.5mL will be excluded.
        - Samples with <30 ng of DNA content for LC input for primary analysis
        - Samples not obtained in accordance with FoundationOne® Liquid CDx sample criteria.

3. Clinical Endpoints
    The primary efficacy endpoint of Cohort Awas radiographic progression-free survival (rPFS) as determined by Blinded independent central review (BICR) per RECIST version 1.1 and/or Prostate Cancer Clinical Trials Working Group 3 (PCWG3) (bone) criteria. Key secondary endpoints included confirmed objective response rate (ORR) (Cohort A), time to pain progression (Cohort A) and overall survival (Cohort A). The bridging study used the same primary endpoint for drug efficacy analysis.

B. Accountability of PMA Cohort

In total, 4,425 patients were screened and 387 (9.6%) were randomized into the PROfound study (Table 19). Of these 387 patients, 245 patients were randomized in Cohort A. 181 out of the 245 randomized patients in Cohort A both consented to the use of their sample for ctDNA CDx development and had a plasma sample available for testing.

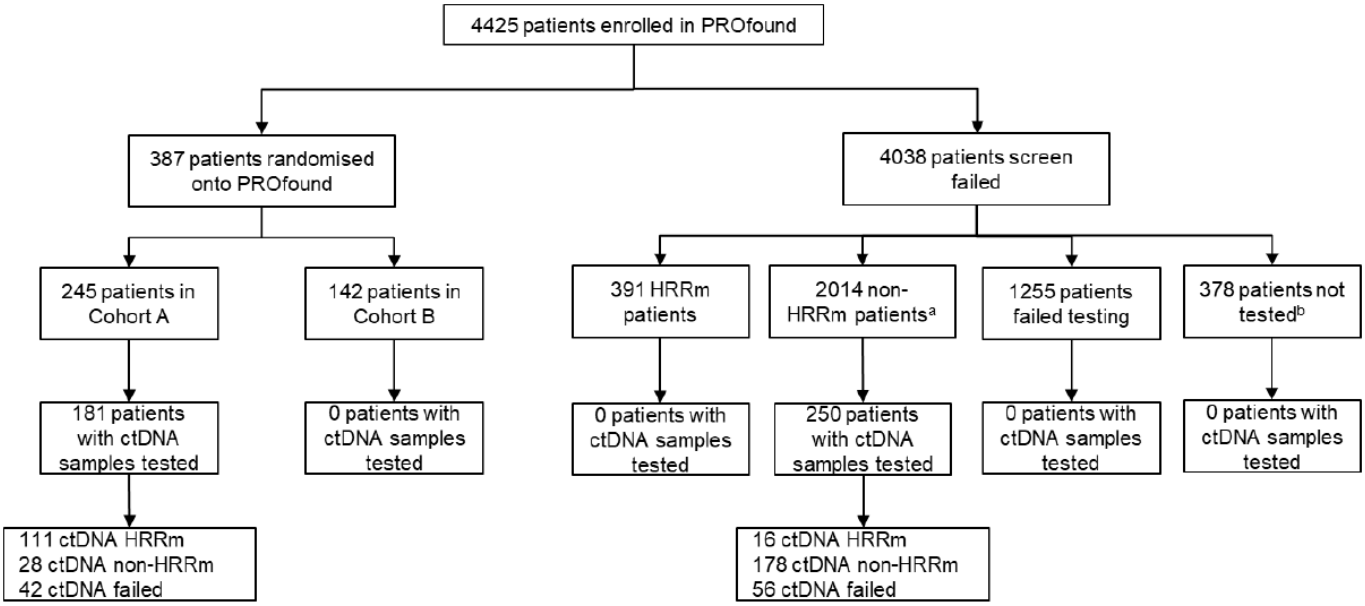
In total, 181/245 (73.9%) of the Cohort A patients were tested using the FoundationOne® Liquid CDx assay. Of these, 139 (76.8%) Cohort A patients had a successful FoundationOne® Liquid CDx test result and 42 Cohort A patients had a failed FoundationOne® Liquid CDx test result. This represents 56.7% (139/245) of total Cohort A patients. In addition, for the bridging study, 250 non-*ATM/BRCA1/BRCA2* patient samples determined by F1 LDT CTA were randomly selected from the screen failure population and their matched plasma samples were tested by FoundationOne® Liquid CDx to determine the NPA between the FoundationOne® Liquid CDx test and the F1 LDT CTA. One hundred ninety-four (194) of 250 (77.6%) screen failed non-*ATM/BRCA1/BRCA2* patients were successfully tested using the FoundationOne® Liquid CDx test. In total, 98/431 (22.7%) samples failed testing with the FoundationOne® Liquid CDx test. Eight (8) samples failed tests were due to insufficient DNA yield and 90 samples failed post DNA-extraction.

Of the 139 successfully tested Cohort A patients by the FoundationOne® Liquid CDx, 111 patients were reported as *BRCA1, BRCA2,* and/or *ATM* mutation positive and 28 randomized patients were reported as non-*ATM/BRCA1/BRCA2*. Sample accountability for this clinical bridging study is summarized in Table 19 and Figure 1.

Table 19: Sample accountability for olaparib clinical bridging study

Description	# patients
Patients randomized into PROfound	387
BRCA1m, *BRCA2m* or *ATMm* (Cohort A) patients	245
Cohort A patient samples tested with FoundationOne® Liquid CDx	181
FoundationOne® Liquid CDx results available	139
Cohort A patients *ATM/BRCA1/BRCA2* positive by FoundationOne® Liquid CDx	111

Figure 1 Overview of patients screened for the PROfound study



[a] Includes 2013 patients who were non-ATM/BRCA1/BRCA2 using the F1 LDT CTA and 1 patient non-*ATM/BRCA1/BRCA2* by a prior FoundationOne® test. In the figure above HRRm and non-HRRm refers to patients with or without *ATM/BRCA1/BRCA2* alterations, respecitively.

[b] Includes patients who did not supply a sample for testing, patients who supplied an ineligible sample type and/or patient who supplied a sample after the testing window closed.

C. Study Population Demographics and Baseline Parameters

Screened patients who were tissue positive for the pre-defined HRR genes were the primary population of interest. The demographics and baseline clinical characteristics of the Full Analysis Set (FAS)-confirmed F1 LDT CTA and the FoundationOne® Liquid CDx subgroups are summarized in Table 20 and Table 21. The demographics and baseline characteristics of the patients in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive subgroup were generally balanced between treatment arms and consistent with those of the patients in the FAS. However, interpretation of the demographics and baseline characteristics of patients in the FoundationOne® Liquid CDx non-*ATM/BRCA1/BRCA2* subgroup should be undertaken with caution due to the small numbers in this group.

Table 20: Demographic Characteristics for Cohort A Patients (FAS, Confirmed FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* and FoundationOne® Liquid CDx non-*ATM/BRCA1/BRCA2* patients)

		FAS		Confirmed FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2*		FoundationOne® Liquid CDx non-*ATM/BRCA1/BRCA2*	
		Olaparib 300 mg bd (N=162)	Investigators choice of NHA (N=83)	Olaparib 300 mg bd (N=73)	Investigators choice of NHA (N=38)	Olaparib 300 mg bd (N=20)	Investigators choice of NHA (N=8)
Age (years)	n	162	83	73	38	20	8
	Mean	68.0	68.1	65.9	68.4	71.8	65.6
	SD	8.23	7.36	8.32	7.7	6.01	7.93
	Median	68.0	67.0	65.0	68.5	72.5	65.5
	Min	47	49	47	49	62	55
	Max	86	86	81	86	86	75
Age group (years), n (%)	<65	54 (33.3)	23 (27.7)	32 (43.8)	11 (28.9)	3 (15.0)	4 (50.0)
	≥65	108 (66.7)	60 (72.3)	41 (56.2)	27 (71.1)	17 (85,0)	4 (50.0)
Race, n (%)	White	109 (67.3)	55 (66.3)	56 (76.7)	30 (78.0)	15 (75.0)	5 (62.5)
	Black or African American	2 (1.2)	1 (1.2)	1 (1.4)	0	0	0
	Asian	43 (26.5)	19 (22.9)	10 (13.7)	4 (10.5)	5 (25.0)	2 (25.0)
	Other	1 (0.6)	1 (1.2)	0	0	0	0
	Missing	7 (4.3)	7 (8.4)	6 (8.2)	4 (10.5)	0	1 (12.5)
Ethnic group, n (%)	Hispanic or Latino	12 (7.4)	9 (10.8)	4 (5.5)	2 (5.3)	1 (5.0)	2 (25.0)
	Not Hispanic or Latino	145 (89.5)	69 (83.1)	65 (89.0)	33 (86.8)	19 (95.0)	5 (62.5)
	Missing	5 (3.1)	5 (6.0)	4 (5.5)	3 (7.9)	0	1 (12.5)

SD = standard deviation

Table 21: Disease characteristics at baseline for Cohort A patients (FAS, confirmed F1 Liquid CDx *ATM/BRCA1/BRCA2* and F1 Liquid CDx non-*ATM/BRCA1/BRCA2* patients)

		FAS		Confirmed F1 Liquid CDx *ATM/BRCA1/BRCA2*		F1 Liquid CDx non-*ATM/BRCA1/BRCA2*	
		Olaparib 300 mg bd (N=162)	Investigators choice of NHA (N=83)	Olaparib 300 mg bd (N=73)	Investigators choice of NHA (N=38)	Olaparib 300 mg bd (N=20)	Investigators choice of NHA (N=8)
Time from CRPC to randomization (months)	n	160	82	73	37	19	8
	Median	24.2	23.7	23.3	30.2	24.9	22.3
	Min, max	-6[a], 189	1, 175	-6,189	1,175	6,69	4,87
Time from mCRPC to randomization (months)	n	160	82	73	37	19	8
	Median	23.3	22.5	22.9	27.8	22.6	22.3
	Min, max	-6[a], 121	1, 105	-6,113	1,105	6,56	3,87
Histology type at diagnosis	Adenocarcinoma	160 (98.8)	80 (96.4)	72 (98.6)	36 (94.7)	20 (100)	8 (100)
	Small cell carcinoma	0	0	0	0	0	0
	Other	0	2 (2.4)	0	1 (2.6)	0	0
	Missing	2 (1.2)	1 (1.2)	1 (1.4)	1 (2.6)	0	0
Total Gleason	1	0	0	0	0	0	0

		FAS		Confirmed F1 Liquid CDx *ATM/BRCA1/BRCA2*		F1 Liquid CDx non-*ATM/BRCA1/BRCA2*	
		Olaparib 300 mg bd (N=162)	**Investigators choice of NHA (N=83)**	**Olaparib 300 mg bd (N=73)**	**Investigators choice of NHA (N=38)**	**Olaparib 300 mg bd (N=20)**	**Investigators choice of NHA (N=8)**
Score at diagnosis	2	1 (0.6)	0	0	0	1 (5.0)	0
	3	0	0	0	0	0	0
	4	2 (1.2)	0	1 (1.4)	0	0	0
	5	2 (1.2)	1 (1.2)	2 (2.7)	0	0	1 (12.5)
	6	6 (3.7)	3 (3.6)	3 (4.1)	2 (5.3)	1 (5.0)	0
	7	41 (25.3)	22 (26.5)	20 (27.4)	11 (28.9)	7 (35.0)	2 (25.0)
	8	36 (22.2)	12 (14.5)	13 (17.8)	6 (15.8)	5 (25.0)	2 (25.0)
	9	59 (36.4)	35 (42.2)	26 (35.6)	14 (36.8)	6 (30.0)	2 (25.0)
	10	10 (6.2)	7 (8.4)	5 (6.8)	3 (7.9)	0	1 (12.5)
	Missing	5 (3.1)	3 (3.6)	3 (4.1)	2 (5.3)	0	0
Sites of disease at baseline[b]	Total	162 (100)	83 (100)	73 (100)	38 (100)	20 (100)	8 (100)
	Prostate	27 (16.7)	12 (14.5)	11 (15.1)	3 (7.9)	4 (20.0)	2 (25.0)
	Loco-regional LNs	35 (21.6)	17 (20.5)	15 (20.5)	10 (26.3)	10 (50.0)	1 (12.5)
	Distant LNs	59 (36.4)	35 (42.2)	31 (42.5)	16 (42.1)	8 (40.0)	5 (62.5)
	Bone	140 (86.4)	73 (88.0)	64 (87.7)	34 (89.5)	17 (85.0)	7 (87.5)
	Respiratory	30 (18.5)	11 (13.3)	18 (24.7)	6 (15.8)	2 (10.0)	0
	Liver	18 (11.1)	13 (15.7)	8 (11.0)	8 (21.1)	1 (5.0)	2 (25.0)
	Other distant sites	34 (21.0)	15 (18.1)	17 (23.3)	9 (23.7)	3 (15.0)	1 (12.5)
	Bone only	42 (25.9)	25 (30.1)	16 (21.9)	11 (28.9)	3 (15.0)	1 (12.5)
	LN only	13 (8.0)	5 (6.0)	7 (9.6)	3 (7.9)	2 (10.0)	0
	Bone and LN only	26 (16.0)	14 (16.9)	14 (19.2)	5 (13.2)	5 (25.0)	2 (25.0)
ECOG performance status at baseline	(0) Fully active	84 (51.9)	34 (41.0)	35 (47.9)	13 (34.2)	11 (55.0)	2 (25.0)
	(1) Restricted in physically strenuous activity	67 (41.4)	46 (55.4)	32 (43.8)	23 (60.5)	9 (45.0)	6 (75.0)
	(2) Ambulatory and capable of self-care	11 (6.8)	3 (3.6)	6 (8.2)	2 (5.3)	0	0
	Missing	0	0	0	0	0	0
Baseline pain score	0 to <2	83 (51.2)	37 (44.6)	30 (41.1)	16 (42.1)	15 (75.0)	4 (50.0)
	2 to 3	17 (10.5)	9 (10.8)	8 (11.0)	5 (13.2)	2 (10.0)	0
	>3	56 (34.6)	34 (41.0)	32 (43.8)	14 (36.8)	2 (10.0)	4 (50.0)
	Missing	6 (3.7)	3 (3.6)	3 (4.1)	3 (7.9)	1 (5.0)	0
Baseline PSA (µg/L)	n	160	81	72	38	20	8
	Median	62.180	112.920	51.650	193.800	50.725	83.800
	Min, max	0.20, 7240.74	1.85, 7115.00	0.22, 2980.75	1.85, 7115.00	0.92, 1768.50	24.69, 695.90
Baseline hemoglobin (g/L)	n	162	83	73	38	20	8
	Mean (SD)	122.6 (12.87)	122.5 (13.95)	121 (12.06)	121.8 (14.96)	127.0 (9.65)	121.0 (12.42)
Baseline ALP (U/L)	n	162	83	73	38	20	8
	Mean (SD)	172.2 (201.75)	182.7 (203.14)	196.2 (238.64)	224.8 (248.13)	155.0 (208.15)	123.4 (79.49)
Baseline LDH (U/L)	n	160	80	73	38	20	8
	Mean (SD)	268.0 (254.07)	267.3 (185.02)	306.5 (345.68)	298.6 (245.5)	205.3 (51.36)	237.3 (78.25)
Patient positive by F1CDx test	Yes	157 (96.9)	83 (100)	71 (97.3)	38 (100)	19 (95.0)	8 (100)
	No	5 (3.1)	0	2 (2.7)	0	1 (5.0)	0
Received prior taxane therapy[c]	Yes	106 (65.4)	52 (62.7)	54 (74.0)	25 (65.8)	10 (50.0)	5 (62.5)
	No	56 (34.6)	31 (37.3)	19 (26.0)	13 (34.2)	10 (50.0)	3 (37.5)

		FAS		Confirmed F1 Liquid CDx *ATM/BRCA1/BRCA2*		F1 Liquid CDx non-*ATM/BRCA1/BRCA2*	
		Olaparib 300 mg bd (N=162)	**Investigators choice of NHA (N=83)**	**Olaparib 300 mg bd (N=73)**	**Investigators choice of NHA (N=38)**	**Olaparib 300 mg bd (N=20)**	**Investigators choice of NHA (N=8)**
Personal history of 2nd malignancy apart from prostate cancer	Yes	14 (8.6)	10 (12.0)	7 (9.6)	6 (15.8)	2 (10.0)	0
	No	148 (91.4)	73 (88.0)	66 (90.4)	32 (84.2)	18 (90.0)	8 (100.0)
Family history of prostate cancer	Yes	33 (20.4)	16 (19.3)	15 (20.5)	7 (18.4)	5 (25.0)	1 (12.5)
	No	129 (79.6)	67 (80.7)	58 (79.5)	31 (81.6)	15 (75.0)	7 (87.5)
Family history of other cancers	Yes	88 (54.3)	40 (48.2)	44 (60.3)	19 (50.0)	7 (35.0)	3 (37.5)
	No	74 (45.7)	43 (51.8)	29 (39.7)	19 (50.0)	13 (65.0)	5 (62.5)

[a] CRPC for Patient E5004001 occurred prior to randomization but is misreported in this table due to a data entry error.
[b] As per investigator assessment, patients with multiple sites of disease within the same category of extent of disease are counted only once in that category.
[c] Derived from eCRF data.
ALP = alkaline phosphatase; bd = twice daily; BPI-SF = Brief Pain Inventory – Short Form; ECOG = Eastern Cooperative Oncology Group; eCRF = electronic case report form; LDH = lactate dehydrogenase; LN = lymph node; mCRPC = metastatic castration-resistant prostate cancer; min = minimum; NHA = new hormonal agent; PSA = prostate-specific antigen; SD = standard deviation..

To ensure no biases were introduced during the random selection of the 250 non-*ATM/BRCA1/BRCA2* screen failed patients tested using the FoundationOne Liquid CDx test, a comparison of demographics and baseline characteristics was performed between the overall screen failed population and the 250 patients selected for ctDNA testing. The demographic and disease characteristics for screen failed patients successfully tested with the FoundationOne Liquid CDx assay were broadly consistent with those of the overall screen failed population. A slightly higher proportion of samples from Asian patients were tested compared with the overall screen failure population (34% tested compared with 19% overall). However, the majority of screen failed and tested patients were white. From this it can be interpreted that the non-*ATM/BRCA1/BRCA2* patients selected for ctDNA testing were broadly representative of the overall screen failed population. Additionally, demographic characteristics were also broadly comparable between screen failed patients and patients who were randomised into the FAS or patients in the *ATM/BRCA1/BRCA2* and non-*ATM/BRCA1/BRCA2* FoundationOne® Liquid CDx subgroups. Differences were seen between screen failed and randomized patients in the proportions of patients with a personal history of second malignancy or a family history of prostate or other cancers. Given patients who are randomized onto PROfound carried mutations in *ATM*, *BRCA1*, and *BRCA2* genes, some of which were germline in origin was not unexpected.

A comparison was also performed between the FoundationOne® Liquid CDx evaluable and non-evaluable patient populations to determine if the missing samples introduced any potential bias. For Cohort A, as shown in Table 22, the key baseline

and patient characteristics were balanced between FoundationOne® Liquid CDx evaluable and non-evaluable subgroups.

Table 22: Demographics and baseline characteristics for patients with FoundationOne® Liquid CDx evaluable and non-evaluable test results in Cohort A

Covariates	Evaluable by FoundationOne® Liquid CDx (N=139)	Non-Evaluable by FoundationOne® Liquid CDx (N=106)	p-value[a]
ECOG Performance Status			
0	61 (43.9)	57 (53.8)	0.3055
1	70 (50.4)	43 (40.6)	
2	8 (5.8)	6 (5.7)	
Age Group			
<65 years	50 (36.0)	27 (25.5)	0.0957
≥65 years	89 (64.0)	79 (74.5)	
Location of metastases at Baseline			
Bone only	38 (27.3)	42 (39.6)	0.1568
Visceral	46 (33.1)	32 (30.2)	
Other	47 (33.8)	25 (23.6)	
Missing	8 (5.8)	7 (6.6)	
Prior taxane			
Prior taxane use	94 (67.6)	63 (59.4)	0.2264
No prior taxane use	45 (32.4)	43 (40.6)	
Measurable disease at baseline			
Measurable disease at baseline	91 (65.5)	59 (55.7)	0.1454
No measurable disease at baseline	48 (34.5)	47 (44.3)	

[a] p-values were calculated by Fisher-Freeman-Halton tests

D. Safety and Effectiveness Results

A bridging study was conducted to compare the performance of the FoundationOne® Liquid CDx assay using a cfDNA input of ≥ 30 ng to the clinical trial tissue assay that was used to enroll patients into the PROfound clinical study. In addition to the concordance between these two tests, an analysis was performed to demonstrate the effectiveness of the FoundationOne® Liquid CDx test, to select patients for treatment with olaparib.

1. Safety Results

   The safety with respect to treatment with olaparib was addressed in the original drug approval and is summarized in the olaparib NDA 208558. Refer to Drugs@FDA for safety information on olaparib.

2. Effectiveness Results

A clinical bridging study was conducted with all available plasma samples with cfDNA input of ≥ 30 ng. Of the 431 (181 randomized F1 LDT CTA *ATM/BRCA1/BRCA2* patients and 250 screen-failed F1 LDT CTA non-*ATM/BRCA1/BRCA2*) patients who were sent for FoundationOne® Liquid CDx testing, one hundred thirty-nine (139) of the 245 F1 LDT CTA *ATM/BRCA1/BRCA2* patients in Cohort A and 194 of 250 F1 LDT CTA non-*ATM/BRCA1/BRCA2* screen failure patients had evaluable FoundationOne® Liquid CDx results.

For the primary analyses, clinical efficacy of olaparib versus investigator choice of NHA in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2*-positive population was evaluated based on the endpoint rPFS as assessed by BICR per RECIST 1.1 criteria and/or PCWG-3. The rPFS estimates for FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2*-positive and F1 LDT CTA *ATM/BRCA1/BRCA2*-positive enrolled patients (rPFS HR=0.33, two-sided 95% CI: 0.21, 0.53) were comparable to the Cohort A FAS (rPFS HR=0.34, 95% Cl: 0.25, 0.47). Sensitivity analyses to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne® Liquid CDx results were performed using the multiple imputation method using a logistic regression model.

a. <u>Concordance between tumor tissue F1 LDT CTA and matched plasma FoundationOne® Liquid CDx tested specimens</u>
The agreement between the tissue-based F1 LDT CTA (as reference) and the FoundationOne® Liquid CDx assay using matched tumor tissue and plasma specimens was evaluated by calculation of the positive percent agreement (PPA) and negative percent agreement (NPA). The point estimates of PPA and NPA between FoundationOne® Liquid CDx and the F1 LDT CTA assay and the corresponding 95% confidence intervals (calculated using Clopper-Pearson method) were: PPA = 79.9% (72.2, 86.2); NPA = 91.8% (87.0, 95.2).

Table 23 below shows the agreement analysis between F1 LDT CTA (tissue test) and the FoundationOne® Liquid CDx results for PROfound patients, including Invalid and Not Tested results.

Table 23: Summary of agreement analyses for FoundationOne® Liquid CDx compared against F1 LDT CTA tissue test, including Invalid and Not Tested results

			F1 LDT CTA Results (n=495)		
			ATM/BRCA1/BRCA2		Total
			Positive	Negative	
FoundationOne® Liquid CDx assay	*ATM/BRCA1 / BRCA2*	Positive	111	16	127
		Negative	28	178	206
		Invalid	42	56	98
		Not Tested	64	0	64
		Total Valid Results	139	194	233
		Total	245	200	495

PPA (95% CI) = 79.9% (72.2%, 86.2%)
NPA (95% CI) = 91.8% (87.0%, 95.2%)

Overall, 28 patients were reported as *ATM/BRCA1/BRCA2* by the F1 LDT CTA tumor test but non-*ATM/BRCA1/BRCA2* by the FoundationOne® Liquid CDx test. This includes four (4) patients with homozygous deletions or large rearrangements in *ATM* reported by the F1 LDT CTA tissue test which are not reported by the FoundationOne® Liquid CDx test. In patients with a non-*ATM/BRCA1/BRCA2* status determined by the F1 LDT CTA, the NPA was 91.8% (178/194) in patients with valid results by both tests. Overall, 16 patients reported an *ATM*, *BRCA1* or *BRCA2* gene alteration by the FoundationOne® Liquid CDx test which was not reported by the F1 LDT CTA tissue test. In these samples the estimated %VAF ranged from 0.17% to 27.24%; however, because the screen negative patients were not enrolled into the PROfound study, efficacy data for these patients were not available.

The resulting agreements between the FoundationOne® Liquid CDx and the tumor tissue F1 LDT CTA results, based on only valid results were: PPA (111/139) = 79.9% (72.2, 86.2) and NPA (178/194) = 91.8% (87.0, 95.2).

b. Efficacy analysis for the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive patients in the primary efficacy population

The primary efficacy analysis in the *ATM/BRCA1/BRCA2* alteration positive population identified by FoundationOne® Liquid CDx was based on PFS by BICR assessment per RECIST 1.1 criteria. As shown in Table 24, the estimated radiological progression-free survival (rPFS) HR and the corresponding 95% confidence intervals were 0.33 [0.21, 0.53] for the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive and F1 LDT CTA *ATM/BRCA1/BRCA2* positive population, which were comparable with the observed rPFS HR and the corresponding 95% confidence intervals of 0.34 [0.25, 0.47] for the F1 LDT CTA *ATM/BRCA1/BRCA2* positive population (PROfound Cohort A). Kaplan-Meier analysis of rPFS in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive and F1 LDT CTA

ATM/BRCA1/BRCA2 positive patients in the primary efficacy population is presented in Figure 2.

Table 24: Summary of analysis of rPFS based on BICR (FAS and FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* and F1 LDT CTA *ATM/BRCA1/BRCA2*; Cohort A)

	Full Analysis Set		FoundationOne® Liquid *ATM/BRCA1/BRCA2* positive and F1 LDT CTA *ATM/BRCA1/BRCA2* positive	
	Olaparib 300 mg bd (N=162)	**Investigators choice of NHA (N=83)**	**Olaparib 300 mg bd (N=73)**	**Investigators choice of NHA (N=38)**
n (%) of events[a]	106 (65.4)	68 (81.9)	49 (67.1)	34 (89.5)
Median rPFS (95% CI) [months]	7.39 (6.24, 9.33)	3.55 (1.91, 3.71)	7.39 (5.65, 10.38)	3.53 (1.77, 3.71)
HR (95% CI)[b]	0.34 (0.25, 0.47)		0.331 (0.21, 0.53)	
2-sided p-value[c]	<0.0001		<0.0001[d]	

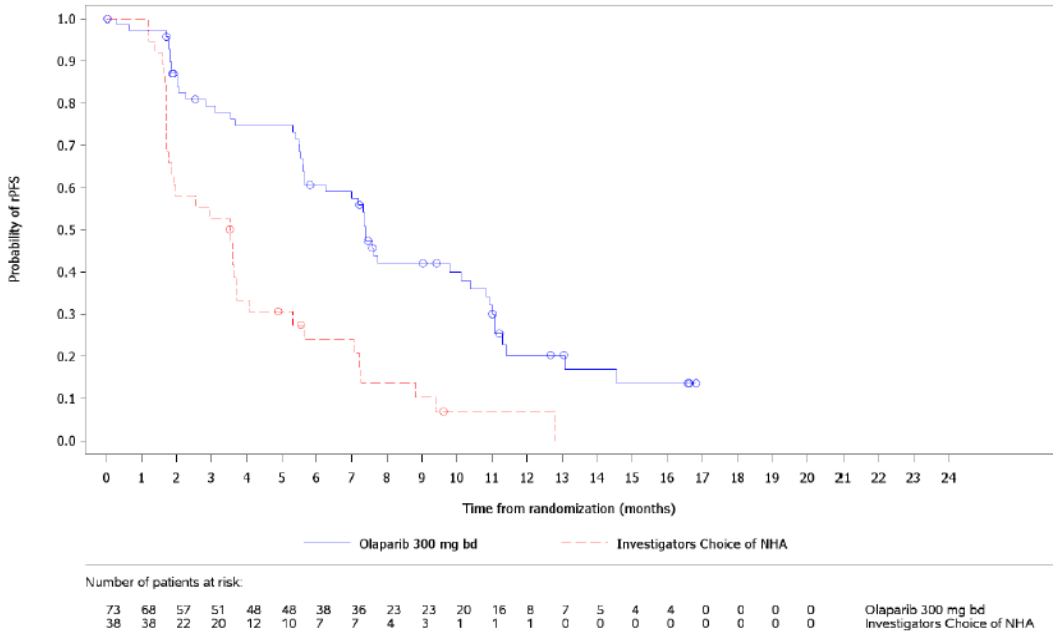
[a] Progression, as assessed by BICR, was defined by RECIST 1.1 and/or PCWG-3 or death (by any cause in the absence of progression) regardless of whether the patient withdrew from randomized therapy or received another anticancer therapy prior to progression.

[b] The HR and CI were calculated using a Cox proportional hazards model adjusted for the variables selected in the primary pooling strategy (prior taxane use and measurable disease in Cohort A). The Efron approach was used for handling ties. An HR <1 favors 300 mg bd olaparib.

[c] The analysis was performed using the l/og-rank test stratified by the variables selected in the primary pooling strategy (prior taxane use and measurable disease in Cohort A) using the Breslow method for handling ties.

[d] P-values for the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive subgroup analysis were nominal

Figure 2: Kaplan-Meier plot of rPFS (by BICR) in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive and CTA *ATM/BRCA1/BRCA2* positive patients



3. Sensitivity analysis for the rPFS in the FoundationOne® Liquid CDx positive population

Enrollment into PROfound was based on tissue testing performed using the F1 LDT CTA or a prior FoundationOne® result (denoted in the drug efficacy expressions below as CTA). As a result, patients who were determined as non-*ATM/BRCA1*/*BRCA2* in tissue were not randomized or treated. In the FoundationOne® Liquid CDx *ATM/BRCA1*/*BRCA2* positive patients in intended use population, a proportion of patients with a tissue non- *ATM/BRCA1*/*BRCA2* status were determined as *ATM/BRCA1*/*BRCA2* positive by the FoundationOne® Liquid CDx test whose drug efficacy was unknown because the drug efficacy in tissue non-*ATM/BRCA1/BRCA2* patients was not evaluated in the Profound drug trial. Also, for F1 LDT CTA *ATM/BRCA1/BRCA2* positive enrolled trial patients, notno all of them had evaluable FoundationOne® Liquid CDx test results. To investigate the robustness of the drug efficacy in FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* poisitive patients on the missing FoundationOne® Liquid CDx test results, additional analyses to evaluate the impact of missing Foundation Liquid CDx test results on the HR were conducted. Based on the imputation analysis, missing FoundationOne® Liquid CDx data did not appear to have any meaningful impact on the PPA or the FoundationOne® Liquid CDx rPFS results.

The drug efficacy (log(HR)) in FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* mutation positive patients (denoted as F1 Liquid CDx+) was estimated as a weighted efficacy of patients with (CTA+, F1 Liquid CDx+) and patients with (CTA-, F1 Liquid CDx+), and the weight was Pr(CTA+| F1 Liquid CDx+). The HR for the (F1 Liquid CDx+, CTA+) was calculated from the Profound trial. The HR for (F1 Liquid CDx+, CTA-) was assumed to be c-value times of that observed HRs of (FoundationOne® Liquid CDx+, CTA+) with c ranging from 0 (no efficacy) to 1.0 (having the same efficacy as F1 Liquid CDx+/ CTA+ patients). Thus the calculations of the estimated PFS HR for the olaparib efficacy in FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive population were based on:

- The HR obtained in the subset of patients in PROfound who were *ATM/BRCA1/BRCA2* positive reported by both the FoundationOne® Liquid CDx test and the tissue test (HR 0.33, 95% CI: 0.21, 0.53);
- The proportion of ctDNA *ATM/BRCA1/BRCA2* patients who were 'ctDNA *ATM/BRCA1/BRCA2* positive/tissue *ATM/BRCA1/BRCA2* negative', that is Pr(CTA-| FoundationOne® Liquid CDx+) which equals to 1- Pr(CTA+|F1 Liquid CDx+). The prevalence rate of BRCA1/ *BRCA2* and ATM mutation positive patients in PROfound trial was about 17.3%. Based on this prevalence rate, and the concordance between F1 Liquid CDx and F1 LDT CTA (PPA= 79.9%; NPA = 91.8%), pr(CTA+|F1 Liquid CDx+) was estimated as 66.6% with 95% CI (56.0%, 77.2%).

Table 25 shows the sensitivity analysis of HRs in FoundationOne® Liquid CDx+ population. c-values (relative efficacy as denoted in the following table) vary

from 0% to 100%. The point estimate 66.6%, lower bound 56% and upper bound 77.2% of Pr(CTA+|F1 Liquid CDx+) were used for sensitivity analysis. Thus Pr(CTA-|F1 Liquid CDx+) was assumed to be 0.334, 0.228 and 0.440 in the following sensitivity analysis.

Table 25: Sensitivity analysis on Hazard Ratio (95% CI) in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2*-positive population based on observed data

ctDNA *ATM/BRCA1/BRCA2* patients who are tissue *ATM/BRCA1/BRCA2* negative (assumed proportion)	**Assumed relative efficacy in ctDNA *ATM/BRCA1/BRCA2* positive/tissue *ATM/BRCA1/BRCA2* negative patients**					
	0%	**10%**	**20%**	**30%**	**40%**	**50%**
0.228 (1-0.772)	0.426 (0.293, 0.619)	0.415 (0.286, 0.603)	0.405 (0.279, 0.588)	0.395 (0.272, 0.573)	0.385 (0.265, 0.559)	0.375 (0.258, 0.545)
0.334 (1-0.666)	0.479 (0.338, 0.677)	0.461 (0.326, 0.652)	0.444 (0.315, 0.628)	0.428 (0.303, 0.605)	0.413 (0.292, 0.583)	0.398 (0.282, 0.562)
0.440 (1-0.560)	0.538 (0.386, 0.749)	0.513 (0.368, 0.714)	0.488 (0.351, 0.680)	0.465 (0.334, 0.647)	0.443 (0.318, 0.616)	0.422 (0.303, 0.587)

ctDNA *ATM/BRCA1/BRCA2* patients who are tissue *ATM/BRCA1/BRCA2* negative (assumed proportion)	**Assumed relative efficacy in ctDNA *ATM/BRCA1/BRCA2* positive/tissue *ATM/BRCA1/BRCA2* negative patients**				
	60%	**70%**	**80%**	**90%**	**100%**
0.228 (1-0.772)	0.366 (0.252, 0.531)	0.357 (0.246, 0.518)	0.348 (0.240, 0.505)	0.339 (0.234, 0.492)	0.331 (0.228, 0.480)
0.334 (1-0.666)	0.383 (0.272, 0.541)	0.369 (0.262, 0.522)	0.356 (0.252, 0.503)	0.343 (0.243, 0.484)	0.331 (0.234, 0.467)
0.440 (1-0.560)	0.402 (0.289, 0.559)	0.383 (0.275, 0.532)	0.365 (0.262, 0.507)	0.347 (0.250, 0.483)	0.331 (0.238, 0.460)

A sensitivity analyses was conducted to evaluate the robustness of the clinical efficacy estimate against the missing FoundationOne® Liquid CDx results using the multiple imputation method with logistic regression model. Relevant covariates that were associated with FoundationOne® Liquid CDx test results or clinical outcome or unbalanced between FoundationOne® Liquid CDx evaluable and unevaluable were identified using logistic regression models. The distribution of the propensity scores among the group of patients with FoundationOne Liquid CDx results and the group with missing FoundationOne® Liquid CDx results were assessed. Missing FoundationOne® Liquid CDx results were imputed by the imputation model which included clinical outcome, the identified covariates (prior taxane use, baseline ECOG, age group, location of metastases at baseline). Measurable disease at baseline was also included in the model since it was a known co-variate associated with clinical outcome. The sensitivity analysis result was shown in the table below. The sensitivity demonstrated that the estimated drug efficacy in FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* patients is robust to missing FoundationOne® Liquid CDx test results.

Table 26: Sensitivity analysis on Hazard Ratio (95% CI) in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive population based on combined observed and imputed data

	Mean imputed HR in F1 Liquid CDx *ATM/BRCA1/BRCA2* positive patients	**95% CI**
c=0%	0.516	0.386, 0.691
c=10%	0.499	0.372, 0.668
c=20%	0.482	0.359, 0.646
c=30%	0.466	0.347, 0.625
c=40%	0.450	0.334, 0.605
c=50%	0.435	0.322, 0.586
c=60%	0.420	0.311, 0.567
c=70%	0.406	0.300, 0.549
c=80%	0.392	0.289, 0.531
c=90%	0.379	0.279, 0.514
c=100%	0.366	0.269, 0.498

Imputation model includes the rPFS event indicator, randomized treatment, prior taxane use, measurable disease at baseline, age group, baseline ECOG score and location of metastases at baseline.
The HRs were calculated adjusting for prior taxane use and measurable disease at baseline, as in the Cohort A primary analysis. 95% CI was calculated to account for within-imputation and between-imputation variations according to Rubin's rules.

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

E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical bridging studies described above included a single investigator. The clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

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

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

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

Matched plasma samples were collected at the screening part 1 visit from all patients, where possible. Within the group of 387 patients randomized into PROfound, 293 patients (75.7%) consented to ctDNA CDx development and had a plasma sample available for testing. As FoundationOne® Liquid CDx development was being undertaken for *BRCA1*, *BRCA2* and *ATM* only, testing was restricted to patients in Cohort A only. In total 181/245 (73.9%) of Cohort A patients were sent for testing with the FoundationOne® Liquid CDx assay. There were 2014 patients who were screen failed as they were reported as non-*ATM/BRCA1/BRCA2* by the F1 LDT CTA. Samples from 250 such patients were randomly selected (from those with sufficient plasma and consent in place) for testing using the FoundationOne® Liquid CDx assay to assess the negative percent agreement (NPA) with the F1 LDT CTA tissue test. Data from all tested samples were used for agreement calculations, using the confirmed F1 LDT CTA subgroup data as the reference. Only data from randomised patients were used for drug efficacy analysis.

In total, 139/181 (76.8%) Cohort A patients had a successful FoundationOne Liquid CDx test result and 42 Cohort A patients had a failed FoundationOne Liquid CDx test result. This represents 56.7% (139/245) of total Cohort A patients. In addition, 194/250 (77.6%) screen failed non-*ATM/BRCA1/BRCA2* patients were successfully tested using the FoundationOne Liquid CDx test. In total, 98/431 (22.7%) samples failed testing with the FoundationOne Liquid CDx test. Of the 139 successfully tested Cohort A patients, 111 patients were reported as *ATM/BRCA1/BRCA2* mutation positive and 28 randomised patients were reported as non-*ATM/BRCA1/BRCA2*. Therefore, the FoundationOne® Liquid CDx ctDNA *ATM/BRCA1/BRCA2* positive subgroup comprises 111 patients with *ATM/BRCA1/BRCA2* mutations. The agreement analysis presented in Table 23 demonstrated a PPA between the F1 LDT CTA tissue test result and FoundationOne Liquid CDx test result for *ATM/BRCA1/BRCA2* positive patients (PPA=79.9% [111/139]). Overall, 28 patients were reported as *ATM/BRCA1/BRCA2* by the F1 LDT CTA tumor test but non-*ATM/BRCA1/BRCA2* by the FoundationOne Liquid CDx test. This includes 4 patents with homozygous deletions or large rearrangements in *ATM* reported by the F1CDx tissue test but are not reported by the FoundationOne Liquid CDx test. In patients with a non-*ATM/BRCA1/BRCA2* status determined by the F1 LDT CTA, the NPA was 91.8% (178/194) in patients with valid results by both tests. Overall, 16 patients reported an *ATM/BRCA1/BRCA2* gene mutation by the FoundationOne® Liquid CDx test which was not reported by the F1 LDT CTA tissue test.

The rPFS analyses in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2*-positive and F1 LDT CTA *ATM/BRCA1/BRCA2*-positive patients (rPFS HR 0.33, 95% CI: 0.21, 0.53) are comparable to the Cohort A FAS (rPFS HR 0.34, 95% CI: 0.25, 0.47).

It is important to note that all patients in the FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* subgroup were determined as *ATM/BRCA1/BRCA2* in tissue by the Fl LDT CTA test used to enroll patients into the study. The sensitivity analysis on the drug efficacy of FoundationOne® Liquid CDx *ATM/BRCA1/BRCA2* positive patients based on combined observed and imputed data demonstrated the drug efficacy is robust to missing F1 Liquid CDx test results.

Based on the data provided, the clinical benefit of the FoundationOne® Liquid CDx assay in the detection of alterations listed in Table 1 of the intended use statement was demonstrated in clinical bridging and clinical concordance studies summarized in above sections. The clinical efficacy and concordance observed support the effectiveness of the FoundationOne® Liquid CDx assay to identify patients whose tumors are positive for the alterations listed in Table 1 of the intended use and are eligible for the associated therapeutics listed in the same table. However, as shown in Table 14 and Table 15, and the concordance between matched tumor tissue and cfDNA isolated from plasma using a validated orthogonal tumor tissue based NGS test and the FoundationOne® Liquid CDx, respectively, along with the concordance results from the clinical bridging study, the FoundationOne® Liquid CDx test does not identify all *ATM*, *BRCA1*, or *BRCA2* alterations identified in tumor tissue specimens. These observed discordances support the reflex recommendation that patients whose plasma specimens yield a negative result using the FoundationOne® Liquid CDx should have their mutation status verified by reflexing to a FDA-approved, tumor tissue based test.

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. There is also a risk of delayed results, which may lead to delay of treatment with indicated therapy. Based on the clinical bridging study, 16 potential false positive results were identified in the *ATM/BRCA1/BRCA2* screen negative cohort and 28 potential false negative results were observed. Since the screen negative patient population was not enrolled into the PROfound study, it cannot be determined whether these patients might have responded to olaparib therapy.

According to the FDA-approved drug label, olaparib has been associated with a variety of adverse reactions, and there are also several warnings and precautions. These warnings, precautions, and reported adverse events are included in sections 5 and 6 of the FDA-approved drug label. Please refer to Drugs@FDA for complete safety information on LYNPARZA (olaparib).

Data from the clinical bridging study supports the effectiveness of FoundationOne® Liquid CDx as an aid in identifying mCRPC patients with alterations in *ATM*, *BRCA1*, and/or *BRCA2* who may benefit from treatment with olaparib.

C. Benefit-Risk Determination

Treatment with olaparib provides meaning clinical benefit to metastatic castration resistant prostate cancer (mCRPC) patients with alterations in *BRCA1*, *BRCA2* or *ATM*. The probable benefit of FoundationOne Liquid CDx to identify mCRPC alterations in *BRCA1*, *BRCA2* or *ATM* who may benefit from treatment with olaparib, was demonstrated through a clinical bridging study using residual plasma specimens collected from patients enrolled into the PROfound study. PROfound is a prospective, multicenter, randomized, open-label, phase III trial evaluating the efficacy and safety of olaparib versus enzalutamide or abiraterone acetate in subjects with mCRPC who have failed prior treatment with a new hormonal agent (NHA) who have qualifying mutations in genes directly or indirectly involved in homologous recombination repair, as determined by a tumor tissue test. Cohort A of PROfound included patients who have a qualifying tumor mutation in either *BRCA1*, *BRCA2*, or *ATM* genes. A bridging study was conducted and demonstrated 1) concordance between *BRCA1*, *BRCA2* and *ATM* status between FoundationOne® Liquid CDx and the tumor tissue clinical trial assay (CTA) and 2) the effectiveness of olaparib in patients identified to harbor *ATM*, *BRCA1*, or *BRCA2* alteration identified by the FoundationOne® Liquid CDx test. For the primary analyses, clinical efficacy of olaparib versus investigator choice of NHA in the FoundationOne® Liquid CDx *BRCA1*, *BRCA2*, *ATM*-positive population was evaluated based on the endpoint rPFS, which was comparable between the FoundationOne® Liquid CDx+, CTA+ enrolled patients with alterations in *BRCA1*, *BRCA2* or *ATM* (rPFS HR = 0.33, two-sided 95% CI: 0.21, 0.53) and the Cohort A full -analysis set (FAS) (rPFS HR = 0.34, 95% Cl: 0.25, 0.47). The hazard ratio (HR) in FoundationOne® Liquid CDx positive enrolled patients, accounting for proportion and the range of efficacies of the CTA negative, FoundationOne® Liquid CDx positive population, was estimated to be between 0.33 – 0.48 (rPFS HR). The totality of the data provides evidence that there is probable benefit of FoundationOne Liquid CDx to identify mCRPC patients with *BRCA1*, *BRCA2* or *ATM* alterations for treatment with olaparib.

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 key risks of the FoundationOne® Liquid CDx for the selection of mCRPC patients with *BRCA1*, *BRCA2* or *ATM* alterations for treatment with olaparib are associated with the potential mismanagement of patient's treatment resulting from false results of the test. The agreement analysis between a tissue-based CTA and FoundationOne® Liquid CDx demonstrated concordance, with a PPA of 79.9% for identification of *BRCA1*, *BRCA2* and *ATM* and an NPA of 91 .8% in patients with valid results by both tests. Patients who are determined to be false positive by the test may be exposed to a drug that is not beneficial and may lead to adverse events or may have delayed access to other treatments that could be more

beneficial. With an NPA of 91.8%, there is a potential risk of false positivity with this test; however, given that the comparison here was between a tissue-based test and FoundationOne® Liquid, there is a degree of uncertainty about the false positivity rate of this device, due to the potential contribution of intra-tumoral heterogeneity to this observed discordance. A false negative result may prevent a patient from accessing a potentially beneficial therapeutic regimen. The likelihood of false results was assessed by an analytical and clinical validation studies and partially mitigate the risk of the FoundationOne® Liquid CDx device.

The clinical and analytical performance of the device included in this submission demonstrate that the assay is expected to perform with acceptable performance, in light of the understanding that the risks of a false negative result are partially mitigated by a recommendation that those patients whose plasma generate a negative result for those biomarkers included in Table 1 should have their tumor mutation status verified by using a FDA approved tumor test.

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

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

1. Patient Perspectives

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

In conclusion, given the available clinical and analytical information above, the data support that the probable benefit exceeds the probable risks for the use of FoundationOne® Liquid CDx for the selection of mCRPC patients with alterations in *BRCA1*, *BRCA2*, or *ATM* for treatment with olaparib.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical bridging study support the effectiveness of the FoundationOne® Liquid CDx test as an aid for the identification of mCRPC patients

with specific *ATM, BRCA1, and BRCA2* alteration who may benefit from treatment with olaparib.

XIII. CDRH DECISION

CDRH issued an approval order on November 6, 2020. The final clinical conditions of approval cited in the approval order are described below.

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

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

3. For the *ATM* alterations and *BRCA1/BRCA2* companion diagnostic (CDx) claim (olaparib), 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 limit of detection (LoD) concentration for all 4 *BRCA1* and 4 *BRCA2* CDx variant types [i.e., base substitutions, insertion/deletion (indel), rearrangement (RE), and homozygous deletions (HD)]. The level of precision at the LoD must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

    b. FMI will provide a robust and high confidence data set to confirm the analytical accuracy/concordance to a validated orthogonal next-generation sequencing (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 each *ATM* alteration type and *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 demonstrate that clinically significant inaccurate results are minimized 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 each of the *ATM* alterations, and *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 demonstrate that clinically significant inaccurate results are minimized 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 the FoundationOne® Liquid CDx your assay to confirm the specifications for cfDNA input. This study must be designed to assess cfDNA concentrations minimally including 2X below the minimum recommended cfDNA input level to confirm the cfDNA input guard-bands for *ATM*, *BRCA1* and *BRCA2* CDx variant types. The study must assess *ATM* CDx variant types along with *BRCA1* and *BRCA2* indels, HD, and RE. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

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 workflow for each replicate tested to confirm that the LoB of this assay is as claimed. The LoB data from this study must also be provided to FDA with and without germline alteration, and white blood cells must also be sequenced to confirm germline variants. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens from the intended use population.

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 demonstrate that clinically significant inaccurate results are minimized 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 substitutions and 10 pathogenic indels that are identified by the FoundationOne® Liquid CDx assay. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne® Liquid 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 cfDNA Blood Collection tubes when used in conjunction with the FoundationOne® Liquid CDx assay. FMI must provide evidence that when samples from the same patient collected in newly manufactured tubes, as well as in tubes that are at the end of their shelf life, are used in the FoundationOne® Liquid CDx assay, the FoundationOne® Liquid CDx assay performance meets the clinical and analytical performance claim in the FoundationOne® Liquid CDx assay authorized labeling.

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

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

collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.

e. FMI must provide robust and high confidence data from a stability study which demonstrates acceptable stability of whole blood collected from the CDx intended use patients and stored in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes. The study must confirm the claimed cfDNA storage stability and must confirm the suppression of white blood cells lysis across multiple lots. This study must also use the amount of cfDNA isolated and electropherogram data as a comparator method, in addition to sequencing results and quality metrics. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when used on specimens collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.
f. FMI must demonstrate clinically insignificant variability on the performance of the FoundationOne® Liquid CDx assay when specimens collected in FoundationOne® Liquid CDx cfDNA Blood Collection tubes are handled at different centrifugation conditions. The study must assess conditions that are below and above recommended relative centrifugal force and centrifugation time to account for potential performance issues that could occur due to centrifuge malfunction or operator errors. The data from this study must be adequate to demonstrate that clinically significant inaccurate results are minimized when expected handling conditions are used on specimens collected in the FoundationOne® Liquid CDx cfDNA Blood Collection tubes in the intended use population.

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 the 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 November 6, 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.

XV. REFERENCES