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:	FoundationFocus™ CDx *BRCA* LOH
Device Procode:	PQP
Applicant's Name and Address:	Foundation Medicine, Inc. 150 Second Street, 1st Floor Cambridge, MA 02141
Date(s) of Panel Recommendation:	None
Premarket Approval Application (PMA) Number:	P160018/S001
Date of FDA Notice of Approval:	April 6, 2018

Background: The original PMA (P160018) was approved on December 19, 2016 for the detection of *BRCA1* and *BRCA2* alterations in ovarian cancer tissue to identify ovarian cancer patients for whom treatment with Rubraca (rucaparib) is being considered. The SSED to support the indication is available on the CDRH website and is incorporated by reference here. The current supplement was submitted to expand the indications for use for the FoundationFocus™ CDx*BRCA* LOH for the detection of genomic loss of heterozygosity (LOH) to determine homologous recombination deficiency (HRD) status in ovarian cancer patients.

II. INDICATIONS FOR USE

FoundationFocus™ CDx *BRCA* LOH is an assay that uses next-generation sequencing (NGS) for qualitative detection of *BRCA1* and *BRCA2* sequence alterations and genomic loss of heterozygosity (LOH) from formalin-fixed, paraffin-embedded (FFPE) ovarian tumor tissue. Results of the test are used as an aid in identifying ovarian cancer patients with deleterious tumor *BRCA* variants (t*BRCA*-positive), who may be eligible for treatment with Rubraca (rucaparib). Positive homologous recombination deficiency (HRD) status (defined as t*BRCA*-positive or LOH high) in ovarian cancer patients is associated with improved progression-free survival (PFS) from Rubraca (rucaparib) maintenance therapy. See the RUBRACA product label for information about guiding therapy in specific clinical circumstances. This test is to be performed at Foundation Medicine, Inc., a single laboratory site, located at 150 Second Street, Cambridge, MA 02141.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the FoundationFocus™ CDx *BRCA* LOH labeling.

V. DEVICE DESCRIPTION

The FoundationFocus™ CDx *BRCA* LOH is an *in vitro* diagnostic device performed in a single laboratory at Foundation Medicine, Inc. in Cambridge, MA. The NGS assay is performed using DNA extracted from FFPE biopsy or surgical tumor resection specimens, which is subjected to whole-genome shotgun library construction and hybridization-based capture of all coding exons from *BRCA1* and *BRCA2*. To compute the percent LOH for each tumor, LOH regions are inferred across the 22 autosomal chromosomes using the genome-wide copy number profile and minor allele frequencies of more than 3500 germline single nucleotide polymorphisms (SNPs). The results for *BRCA* mutation and percent LOH are used to determine homologous recombination deficiency (HRD) status.

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

- Specimen Preparation Instructions
- Test Requisition Form
- Shipping Instructions
- Return Shipping Label
- Technical Information Summary

The FoundationFocus™ CDx*BRCA* LOH assay is intended to be performed with serial number-controlled instruments, and all assay reagents included in the FoundationOne CDx™ (F1CDx) assay process are qualified by Foundation Medicine, Inc. (FMI) and are compliant with the medical device Quality System Regulation (QSR).

Test Process

A. Specimen Collection and Preparation

FFPE ovarian cancer tumor specimens may be received either as unstained slides or as an FFPE block. Prior to starting the assay, a Hematoxylin and Eosin (H&E) stained slide is prepared, and then reviewed by a board-certified pathologist to confirm disease ontology and to ensure that adequate tissue (0.6 mm^3), tumor content (≥ 20% tumor for *BRCA1/2* and ≥35% tumor for LOH score), and sufficient nucleated cells are present to proceed with the assay.

B. DNA Extraction

Specimens passing Pathology Review are queued for DNA extraction which begins with cell lysis by digestion with a Proteinase K buffer followed by automated purification using the 96-well KingFisher™ FLEX Magnetic Particle Processor. After completion of DNA extraction, double-stranded DNA (dsDNA) is quantified by the Quant-iT™ PicoGreen® Fluorescence Assay using the provided lambda DNA standards (Invitrogen) prior to library construction (LC). The specimen is quantified and must yield a minimum of 55 ng of genomic DNA to proceed with LC (minimum input requirement of 50 ng).

C. Library Construction

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

Following LC, a quality control procedure is performed by quantifying single-stranded DNA (ssDNA) from purified libraries using the Quant-iT™ OliGreen® ssDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Libraries yielding less than 545 ng of sequencing library are considered as failed.

D. Hybrid Capture

Hybrid Capture (HC) begins with normalization of each library to 500-2000 ng. Normalized samples undergo solution hybridization which is performed using a > 50-fold molar excess of a pool of individually synthesized 5'-biotinylated DNA 120 bp oligonucleotides. The baits target ~1.5 Mb of the human genome including 324 cancer-related genes, plus > 3,500 single nucleotide polymorphisms (SNPs) located throughout the genome; however, the FoundationFocus™ CDx *BRCA* LOH only reports results for HRD, inclusive of *BRCA1/2* and LOH status. Baits are designed by tiling overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; SNP targets are allocated one bait each. Intronic baits are filtered for repetitive elements2 as defined by the UCSC Genome RepeatMasker track[2].

After hybridization, the library-bait duplexes are captured on paramagnetic MyOne™ streptavidin beads (Invitrogen) and off-target material is removed by washing with 1X

SSC at 25°C and four times with 0.25X SSC at 55°C. The PCR master mix is added to directly amplify the captured library from the washed beads.[3] After amplification, the samples are 1.8X SPRI purified and then diluted for QC.

Quality Control for Hybrid Capture is performed by measuring double stranded DNA (dsDNA) yield using a Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate Reader. Captured libraries yielding less than 500 ng of sequencing library are considered as failed.

E. Sequencing

Using the Illumina® HiSeq™ 4000 platform, hybrid capture-selected libraries are sequenced to high uniform depth (targeting > 500X coverage with > 99% of exons at coverage > 100X). Briefly, sequencing is performed using off-board clustering on the Illumina cBot with patterned flow cell technology to generate monoclonal clusters from a single DNA template followed by sequencing using sequencing by synthesis (SBS) chemistry on the Illumina HiSeq 4000. Fluorescently labeled 3'-blocked dNTPs along with a polymerase are incorporated through the flow cell to create a growing nucleotide chain that is excited by a laser. A camera captures the emission color of the incorporated base and then is cleaved off. The terminator is then removed to allow the nucleotide to revert to its natural form and to allow the polymerase to add another base to the growing chain. A new pool of fluorescently labeled 3'-blocked dNTPs is added with each new sequencing cycle. This method allows for millions of discrete clusters of clonal copies of DNA to be sequenced in parallel.

F. Sequence Analysis

The FoundationFocus™ CDx *BRCA* LOH assay uses a custom-developed analysis pipeline to identify *BRCA1/2* base substitutions and short insertion/deletions (indels) up to 13 base pairs (bp). The analysis pipeline uses the raw data (output) from the targeted sequencing and aggregates the data based on the index sequence (barcode) of each read, segregates the data to a given sample and generates a FASTQ data file. Sequence data is mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA) v0.5.9[4]. PCR duplicate read removal and sequence metric collection is performed using Picard 1.47 (http://picard.sourceforge.net) and SAMtools 0.1.12a[5]. Local alignment optimization is performed using Genome Analysis Toolkit (GATK) 1.0.4705[6]. The error rate for base substitutions must be <1% and the error rate for indels must be < 0.8%. Variant calling is performed only in genomic regions targeted by the test.

Base substitution detection is performed using a Bayesian methodology, which allows detection of novel somatic alterations at low MAF and increased sensitivity for alterations at hotspot sites through the incorporation of tissue-specific prior expectations.[7] Reads with mapping quality < 25 are discarded, as are base calls with quality ≤ 2.

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

- Collecting all read-pairs for which at least one read maps to the target region.
- Decomposing each read into constituent k-mers and constructing an enumerable graph representation (de-Bruijn) of all candidate non-reference haplotypes present.
- Evaluating the support of each alternate haplotype with respect to the raw read data to generate mutational candidates. All reads are compared to each of the candidate haplotypes via ungapped alignment, and a read 'vote' for each read is assigned to the candidate with best match. Ties between candidates are resolved by splitting the read vote, weighted by the number of reads already supporting each haplotype. This process is iterated until a 'winning' haplotype is selected. Aligning candidates against the reference genome to report alteration calls. Filtering of indel candidates is carried out similarly to base substitutions (strand bias $p < 1x10^{-10}$, MAF $\geq 3\%$ at hotspots). Briefly, it includes an empirically increased MAF threshold at repeats and adjacent sequence quality metrics as implemented in GATK: % of neighboring base mismatches < 25%, average neighboring base quality> 25, and average number of supporting read mismatches ≤ 2.

To compute the percentage of genomic LOH for each tumor, LOH segments are inferred across the 22 autosomal chromosomes using the genome-wide aneuploidy/copy number profile and minor allele frequencies of the more than 3500 SNPs sequenced in the Foundation Medicine's next-generation sequencing (NGS)-based platform. A comparative genomic hybridization (i.e., log-ratio profile of the sample) is obtained from the NGS sequencing data by normalizing the sequence coverage obtained at all exons and genome-wide SNPs against a process-matched normal control. This profile is segmented and interpreted using allele frequencies of sequenced SNPs to estimate copy number (Ci) and minor allele count (Mi) at each segment (i). A segment is determined to have LOH if $Ci \neq 0$ and $Mi = 0$. Two types of LOH segments are excluded from the calculation of percent genomic LOH: (1) LOH segments spanning $\geq 90\%$ of a whole chromosome or chromosome arm, as these LOH events usually arise through non-homologous recombination deficiency (HRD) mechanisms (e.g., mitotic nondisjunction), and (2) regions in which LOH inference is ambiguous (e.g., some small genomic regions that do not have sufficient heterozygous SNPs to support LOH calling).

After completion of the analysis pipeline, variant data is displayed in the CATi software applications with sequence QC metrics. Specimens failing any QC metrics are automatically held and cannot be released. Additionally, samples containing single nucleotide variants (SNVs) and indels with MAF < 5% will not be reported for non-hotspot bases whereas SNVs with MAF $\geq 1\%$ and indels with MAF $\geq 3\%$ will be filtered for hotspots prior to reporting. Results are released after review of associated batch records and specimen QC records and the final report sent to the Laboratory Director.

G. Interpretation of Sequencing Results

Classification of *BRCA* variants is conducted according to defined classification criteria. The criteria for classifying *BRCA1* or *BRCA2* alterations known to be deleterious to BRCA protein function, rendering the sample t*BRCA*+, are shown in the table below:

Table 1: Classification Criteria for Deleterious Tumor *BRCA* Variants

***BRCA* Status**	**Qualification Criteria**	**Sequence Classification**	**Methodology**
t*BRCA*+	A *BRCA1/2* alteration in tumor that includes any of the sequence classifications	Protein truncating alterations	Sequence analysis identifies premature stop codons anywhere in *BRCA1/2* coding regions, except 3' of *BRCA2* K3326*
		Splice site alterations	Sequence analysis identifies alteration splice sequences at intron/exon junctions $\pm$ 2 bp of exon starts/ends
		Deleterious missense alterations	Curated list* (see Table 2)
t*BRCA*-	Negative for t*BRCA*		

* The curated list of deleterious *BRCA1/2* alterations is based on the Breast Cancer Information Core (BIC) database. Each alteration included in the curated list has at least 2 records, of which ≥ 90% are classified as deleterious. The transcript IDs for the deleterious missense alterations listed in Table 2 are: *BRCA1* U14680 and *BRCA2* U43746.

Table 2: Deleterious *BRCA1* and *BRCA2* Missense Mutations

***BRCA1* Alterations (Protein Change)**	***BRCA2* Alterations (Protein Change)**
c.1A>G (M1V)	c.2T>G (M1R)
c.3G>T (M1I)	c.3G>T (M1I)
c.181T>G (C61G)	c.475G>A (V159M)
c.191G>A (C64Y)	c.631G>C (V211L)
c.211A>G (R71G)	c.631G>A (V211I)
c.212G>A (R71K)	c.7007G>C (R2336P)
c.4484G>T (R1495M)	c.7007G>A (R2336H)
c.4675G>A (E1559K)	
c.5074G>A (D1692N)	
c.5074G>C (D1692H)	
c.5095C>T (R1699W)	
c.5123C>A (A1708E)	
c.5363G>T (1788V)	

The genomic LOH score is assessed based on the percent of LOH in the tumor genome. The percentage of the genome with LOH is computed as 100 times the total length of non-excluded LOH regions divided by the total length of non-excluded regions of the genome. LOH status is defined as shown in the table below.

Table 3: LOH Classification Schema

LOH Status	Qualification Criteria
LOH high	LOH score ≥ 16
LOH low	LOH score < 16

Table 4 shows how *BRCA* and genomic LOH status contribute to an overall HRD status.

Table 4: HRD Classification Schema

HRD Status	*BRCA* Status	LOH Status
HRD+	t*BRCA*+	LOH high
		LOH low
	t*BRCA*-	LOH high
HRD-	t*BRCA*-	LOH low

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-cleared or -approved alternatives for tests intended to detect homologous recombination deficiency (HRD) status (based on t*BRCA* and LOH status) in ovarian cancer patients, which is associated with improved progression-free survival (PFS) from Rubraca (rucaparib) maintenance therapy.

VII. MARKETING HISTORY

The FoundationFocus™ CDx *BRCA* test for detection of *BRCA1* and *BRCA2* mutations has been marketed in the United States since December 19, 2016, upon approval of P160018. The FoundationFocus™ CDx *BRCA* LOH assay, which includes detection of LOH and HRD, has not been previously marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Failure of the device to perform as expected or failure to correctly interpret test results may lead to incorrect test results, and subsequently, inappropriate patient management decisions. A patient with a false positive result (erroneous results for LOH and hence HRD status) may undergo treatment with rucaparib with inappropriate expectation of improved progression-free (PFS) survival. A patient with a false negative result may have inappropriate expectation of not having improved PFS from rucaparib.

For the specific adverse events that occurred in the clinical studies related to Rubraca, please see Section X below.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

1. Accuracy/Concordance Study

The concordance of *BRCA1/2* alteration detection by the FoundationFocus™ CDx *BRCA* LOH assay was compared to results of an externally validated NGS assay. See Summary of Safety and Effectiveness Data for P160018.

2. *In silico* assessment of LOH

An *in silico* assessment of LOH was performed to compare the rate of LOH at the *BRCA1* and *BRCA2* genes in patient samples from the ARIEL3 clinical study. ARIEL3 was a randomized, placebo-controlled study used to support the efficacy and safety of rucaparib following response to chemotherapy in patients with platinum-sensitive ovarian cancer (refer to Section X). The results from the ARIEL3 patient samples are tabulated per LOH and t*BRCA* status in Table 5. A significantly higher rate of LOH was observed in the presence of t*BRCA* mutations (100% in *BRCA1* and 88% in *BRCA2*), as compared to the rate of LOH in the absence of t*BRCA* mutations (93% in *BRCA1* and 55% in *BRCA2*). The results met the acceptance criteria for *BRCA2* (Fisher's 2-sided p-value: pre-specified, $p<0.05$; actual, $p<0.0001$).

The results from the ARIEL3 clinical study were also compared to the LOH rates reported in published literature[9, 10, 11, 12]. The high rates of LOH at *BRCA1* (100%) and *BRCA2* (88%) in the presence of t*BRCA* mutations are comparable to the LOH rates found in the following: Osorio *et al.* (2002) found that 93% (14/15) of familial breast cancer patients harboring a deleterious *BRCA1*/2 mutation also had LOH at *BRCA1*/2[11]; Hilton *et al.* (2002) found that 92% (12/13) of ovarian cancer patients harboring a deleterious *BRCA2* mutation also had LOH at *BRCA2*[9]; and Press *et al.* (2008) found that 78% (7/9) of ovarian cancer patients harboring a deleterious *BRCA1* mutation also had LOH at *BRCA1*[12].

Table 5: Presence of LOH at *BRCA1/2* Loci in Patients from the ARIEL3 Study

t*BRCA*1 status	Patients with LOH at *BRCA1*	Patients without LOH at *BRCA1*
t*BRCA1*+	100% (86/86)	0% (0/86)
t*BRCA1*-	93% (353/379)	7% (26/379)

t*BRCA*2 status	Patients with LOH at *BRCA2*	Patients without LOH at *BRCA2*
t*BRCA2*+	88% (53/60)	12% (7/60)
t*BRCA2*-	55% (224/405)	45% (181/405)

3. Analytical Specificity

a. *In silico* Analysis of Interfering Substances

See Summary of Safety and Effectiveness Data for P160018 for interference studies for *BRCA1/2* alterations.

To evaluate the performance of the HRD component of the assay in the presence of exogenous and endogenous interfering substances, the samples included in the interfering substances study that was used to support P160018, were re-analyzed for the LOH scores. The following conditions and substances were evaluated: necrotic tissues, triglycerides, hemoglobin and xylene. For triglycerides, hemoglobin and xylene, the percent agreements for LOH classification status were computed and accepted if 100% of replicates were concordant.

Eight ovarian cancer FFPE samples with varying quantities of necrosis (5%, 10%, 15%, 25%, 40% and 50%) were assessed in duplicate. Except for one (50% necrosis), all necrotic samples were 100% concordant. The one discordant sample yielded LOH scores near the cutoff, with one replicate with LOH of 15.27 (LOH low) and the second with LOH score of 19.4 (LOH high). Additionally, three *BRCA* positive FFPE samples (one LOH high (18.3) and two LOH unknown) were assessed with three interfering substances (triglycerides, xylene and hemoglobin). Only one sample yielded evaluable results. An additional interference study will be performed in the post-approval setting to further assess the effects of triglycerides, xylene hemoglobin, as well as Proteinase K, ethanol and Molecular Index Barcodes.

b. *In silico* Analysis – Hybrid Capture Bait Specificity

The results of the hybrid capture bait specificity are described in the Summary of Safety and Effectiveness Data for P160018.

c. Carryover/Cross-contamination

DNA sample carryover and cross-contamination during the library construction (LC), hybrid capture (HC) and sequencing steps of the FoundationFocus™ CDx *BRCA* LOH assay for *BRCA1/2* alterations were assessed as part of the original PMA P160018.

Results from an *in silico* LOH precision study, in which samples with LOH low scores were placed adjacent to LOH high samples, were assessed for carryover and cross-contamination. The observed LOH scores were determined for each sample with attention to the sequencing positions in the plate. No cross-contamination or carryover was observed based on the concordance across replicates.

4. Intermediate Precision/Reproducibility from Extracted DNA

The intermediate precision study conducted for PMA P160018 (see Summary of Safety and Effectiveness Data for P160018) included intra-run, inter-run, lot-to-lot and instrument-to-instrument reproducibility from extracted DNA. A set of 25 ovarian cancer specimens were tested, in duplicate, using 3 sequencing instruments and 3 reagent lots, at and near the LoD for MAF levels, and the sample set included a range of *BRCA1* and *BRCA2* variants.

To establish intermediate precision for LOH, *in silico* analysis was performed on the same set of samples used for the intermediate precision study that supported PMA P160018. Of the 25 samples, 21 passed the LOH QC at natural (undiluted) levels and were included in the analysis. The inter-run reproducibility was evaluated by percent agreement (fraction of calls consistent with the majority call of LOH status) across 18 replicates per sample (2 duplicates × 3 sequencers × 3 reagent lots). The repeatability (intra-run reproducibility) was evaluated by assessing concordance across duplicates within the same sequencer/reagent lot. Replicates that failed the LOH QC were excluded from the results in Table 6. The results showed 100% agreement in 18 samples and 94.4% (17 out of 18 replicates) agreement in two samples (samples 6 and 9). One sample (sample 5) had < 90% agreement and the LOH score was close to the pre-specified clinical cutoff of 16. All samples were also analyzed based on HRD status, which is the final output of the device. All samples passed the acceptance criterion of concordance ≥ 90% across all replicates, except sample 5 (with a concordance of 77.8%; 95% CI: 54.3% 91.5%).

Table 6: Inter-Run Reproducibility Results of LOH Classification

Sample*	Tumor Content** (%)	LOH Avg Score (SD)	Majority Call LOH	Agreement (%)	95% CI
1	78	3.46 (0.19)	low	100%	[71.8, 100]
2	56	6.71 (1.31)	low	100%	[79.3, 100]
3	59	11.11 (0.26)	low	100%	[79.3,100]
4	71	12.42 (0.73)	low	100%	[79.3. 100]
5	58	14.55 (1.72)	low	77.8%	[54.3, 91.5]
6	72	18.69 (1.72)	high	94.4%	[72.4, 100]
7	43	21.74 (2.41)	high	100%	[79.3, 100]
8	61	18.83 (1.21)	high	100%	[79.3, 100]
9	46	17.51 (0.8)	high	94.4%	[72.4, 100]
10	73	21.48 (0.96)	high	100%	[79.3, 100]
11	85	29.21 (0.39)	high	100%	[79.3, 100]
12	58	27.16 (0.66)	high	100%	[79.3, 100]
13	96	21.88 (1.94)	high	100%	[79.3, 100]
14	76	23.88 (1.64)	high	100%	[79.3, 100]
15	60	28.73 (1.64)	high	100%	[79.3, 100]

16	54	28.25 (0.66)	high	100%	[79.3, 100]
17	46	28.6 (0.76)	high	100%	[79.3, 100]
18	53	29.5 (0.89)	high	100%	[79.3, 100]
19	42	31.09 (1.82)	high	100%	[79.3, 100]
20	66	33.61 (1.09)	high	100%	[79.3, 100]
21	88	39.58 (0.86)	high	100%	[79.3, 100]

* Sample 1 had 6 replicates, resulting a total of 12 replicates included in the study, while all other samples had 18 replicates included.

** Tumor content was calculated as the average computational tumor purity across all replicates per sample.

For repeatability, 18 of 21 samples had 100% concordance. The three (5, 6 and 9) samples that had discordant LOH classification results had LOH scores close to the cut-off of 16. The results were also assessed for HRD status, and all but one sample (sample 5) demonstrated 100% concordance. Thus, the results based on HRD status are similar to the results for LOH status.

For inter-run and intra-run precision, variance component analysis based on LOH scores was also performed. The percent of coefficient of variation (%CV) for intra-run repeatability ranged from 1.30% to 17.6%, while the %CV for inter-run reproducibility ranged from 1.70% to 22.30%. Variance component analysis was also performed for inter-lot and inter-instrument variability (Table 7).

Table 7: Results of Variance Component Analysis of LOH Score

Sample	Mean LOH	Intra-Run		Inter-Lot		Inter-Instrument		Inter-Run	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	3.46	0.19	5.60	0	0.00	0.05	1.40	0.2	5.70
2	6.71	1.18	17.60	0	0.00	0	0.00	1.5	22.30
3	11.11	0.15	1.30	0.18	1.60	0.13	1.20	0.29	2.60
4	12.42	0.79	6.40	0.24	2.00	0	0.00	0.83	6.70
5	14.56	1.41	9.70	0.98	6.70	0.56	3.80	1.82	12.50
6	18.69	1.38	7.40	0	0.00	0	0.00	2.03	10.90
7	21.74	2.63	12.10	1.05	4.80	1.04	4.80	3.02	13.90
8	18.83	1.24	6.60	0	0.00	0	0.00	1.32	7.00
9	17.51	0.88	5.00	0.26	1.50	0	0.00	0.92	5.20
10	21.48	1.16	5.40	0.26	1.20	0	0.00	1.19	5.50
11	29.21	0.44	1.50	0.18	0.60	0.12	0.40	0.49	1.70
12	27.16	0.68	2.50	0	0.00	0.36	1.30	0.77	2.80
13	21.88	1.9	8.70	0	0.00	0	0.00	2.06	9.40
14	23.88	1.62	6.80	0.47	2.00	0	0.00	1.69	7.10
15	28.73	1.66	5.80	1.11	3.90	0	0.00	2	7.00
16	28.25	0.59	2.10	0.29	1.00	0.3	1.10	0.72	2.50

17	28.6	0.9	3.20	0	0.00	0.22	0.80	0.93	3.20
18	29.5	0.82	2.80	0.25	0.80	0	0.00	0.96	3.20
19	31.09	1.98	6.40	0.98	3.10	0	0.00	2.21	7.10
20	33.61	0.86	2.60	0.76	2.30	0.15	0.40	1.17	3.50
21	39.58	0.75	1.90	0.3	0.70	0	0.00	0.95	2.40

5. Limit of Detection (LoD) / Analytical Sensitivity

See the Summary of Safety and Effectiveness Data for P160018 the details about the LoD study for the FoundationFocus™ CDx *BRCA* LOH assay for *BRCA1/2* alterations.

Analytical sensitivity for tumor purity for the LOH classification was assessed using ovarian cancer samples that represent a range of LOH scores. Two (2) LOH low samples (with pre-screen LOH scores of 2.38 and 14.03) and 2 LOH high samples (with pre-screen LOH scores of 17.91 and 29.12) were used. DNA from each sample was mixed with matched normal DNA to attain five tumor purity levels (10%, 20%, 30%, 40%, and 50%). The hit rate was calculated at each level for the LOH low and LOH high samples (Table 8). The minimum tumor fraction at which at least 95% of the replicates yielded positive calls was 35%.

Table 8: Tumor Purity Results

Targeted Tumor Purity	Average Computational Tumor Purity	LOH High Call	LOH Low Call	LOH No Call	Hit Rate
LOH High Sample 1					
10%	11.7%	0	0	20	0
20%	22.5%	10	10	0	0.5
30%	34.9%	20	0	0	1
40%	46.6%	20	0	0	1
50%	55.0%	10	0	0	1
LOH High Sample 2					
10%	12.6%	0	0	20	0
20%	19.9%	0	0	20	0
30%	30%	19	1	0	0.95
40%	41.6%	20	0	0	1
50%	52.2%	10	0	0	1
LOH Low Sample 1					
10%	11.3%	0	4	16	0.2
20%	22.5%	1	19	0	1
30%	33%	0	19	0	1
40%	43.%	0	20	0	1
50%	55%	0	10	0	1
LOH Low Sample 2					
10%	15.9%	0	16	4	0.8

20%	27.4%	0	20	0	1
30%	35.8%	0	20	0	1
40%	42.7%	0	20	0	1
50%	48.1%	0	10	0	1

6. Stability

a. Reagent Stability

Stability of critical reagents for library construction (LC), hybrid capture (HC), and sequencing was evaluated for the FoundationFocus™ CDx *BRCA* LOH Assay with respect to LOH classification. Three (3) lots of each set of reagents (stored under the manufacturer's recommended conditions ranging from 4°C to -20°C) were evaluated in the stability study. Three (3) ovarian cancer samples containing alterations in the *BRCA1/2* genes that were used in the stability study for P160018 were evaluated for this study. Based on the pre-specified cut-off for LOH score of 16, two samples were classified as LOH high and one sample was LOH low. Each sample was tested with five replicates using each reagent lot for the 6 time points (with 30-day intervals: T_0, T_{30}, T_{60}, T_{90}, T_{120}, and T_{150}). Results from each time point were compared against those from samples tested at time point T_0. Out of 270 replicates, 10 replicates were excluded because of process failure. All remaining 260 replicates were concordant, with respect to *BRCA* mutation and LOH classification. In addition to the concordance of *BRCA1/2* and LOH classification, concordance based on the HRD status was 100%. The results suggest that the LC and HC reagents are stable up to 150 days and the sequencing kit is stable for 120 days. The claimed reagent stability is 120 days for the LC and HC kits, and 90 days for the sequencing kits, when stored between 4°C and -20°C.

b. DNA Stability

The DNA stability study for 3 FFPE ovarian cancer samples with defined *BRCA* alterations and LOH scores is ongoing with data available at time zero (T_0), 45 days (T_1), 3 months (T_2), 6 months (T_3), 9 months (T_4), 12 months (T_5) and 15 months (T_6) at -20°C. To date, 100% of sample aliquots up to T_6 were successfully processed. Agreement across *BRCA 1/2* variant calls and LOH status is 100% concordant with baseline calls at T_0 for each of the three samples assessed. The concordance based on HRD is also 100%. Stability of DNA testing is ongoing and will span a time of 3 years.

c. FFPE Sample Stability

The objective of this study is to evaluate the stability of FFPE tumor tissue prepared as slides prior to extraction. This is an ongoing study and data for the first two time points [T_0 and T_1 (30 days)] is available. To date, 20 of 20 samples

were successfully processed at all tested time points. All sample replicates were concordant for their respective *BRCA1/2* variants and LOH status, as well as for HRD status. Stability of FFPE sample testing is ongoing and will span a time of 15 months.

7. Reagent Lot Interchangeability

For reagent lot interchangeability performance data, see the Summary of Safety and Effectiveness Data for P160018.

8. General Lab Equipment and Reagent Evaluation

General laboratory equipment and reagent evaluation, including DNA amplification and DNA extraction, are described in the Summary of Safety and Effectiveness Data for P160018.

9. Guardbanding/Robustness

For guard band studies for *BRCA1/2* variants, see the Summary of Safety and Effectiveness Data for P160018. Data from the DNA input guard banding study that was described in P160018 were reanalyzed for LOH and HRD status. Five (5) DNA levels (100 ng, 160 ng, 200 ng, 240 ng and 300 ng) were evaluated relative to measured process variability for LC, HC, and sequencing. The results showed that LOH status is 100% concordant for all DNA input levels. Similarly, all samples showed 100 concordance for HRD status.

To evaluate the impact of additional DNA input levels for detection of *BRCA1/2* variants and LOH classification, a guard banding study was conducted using 6 ovarian cancer samples run in triplicate (N=18) over 5 DNA input levels (10, 20, 50, 100, and 200 ng). Out of 90 samples, 83 samples passed library construction quality control (LCQC) and 7 samples failed at LCQC. A total of 7 samples failed LCQC (5 samples did not yield 545 ng DNA and 2 failed due to higher %CV). Six (6) failures were observed at hybrid capture quality control (HCQC) for insufficient yield. All of the HCQC failures originated from a single FFPE specimen. Samples and replicates that failed to meet the processing specifications for LC, HC and sequencing were predominantly in the lowest DNA input levels and were excluded from the analysis. All replicates at 10 ng failed and four replicates at 20 ng failed. For 50 ng input, there were three failed samples (two HCQC and one sample failed due to low sequencing coverage). For the samples that successfully met the sequencing metrics, including those with 50 ng LC DNA input, 100% concordance in *BRCA1/2* variant calling was observed (Table 9). Similarly, the LOH status was highly concordant (≥ 93.8%). There was one discordant sample, which had a LOH score close to the cutoff of 16. These results support a minimum DNA input of 50 ng for LC for detection of *BRCA1/2* variants and LOH classification.

Table 9: Percent Concordance Relative to Standard DNA Input (200 ng)

Level (ng)	Replicates	LOH Concordance	*BRCA1/2* Concordance
20	14	100.0% (14/14)	100%
50	15	93.8% (14/15)	100%
100	18	94.4% (17/18)	100%

B. Additional Studies

No animal studies were conducted using the FoundationFocus™ CDx *BRCA* LOH.

C. Additional Studies

No additional studies were conducted using the FoundationFocus™ CDx *BRCA* LOH.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The clinical performance of the FoundationFocus™ CDx *BRCA* LOH was established based on results from ARIEL3, a Phase 3, global, randomized, double-blind clinical study. The results support use of the device to aid in identifying ovarian cancer patients with deleterious tumor *BRCA* variants (t*BRCA*-positive) and patients with positive HRD status for Rubraca maintenance treatment of platinum sensitive ovarian cancer patients who have been treated with two or more lines of chemotherapy. The studies included US and ex-US sites, and the results are described in NDA 209115/S003. Data from the clinical study ARIEL3 were used to support PMA supplement approval.

A. Study Design

ARIEL3 was a Phase 3, randomized, double-blind study of oral rucaparib monotherapy versus placebo as switch maintenance treatment in patients with platinum-sensitive, relapsed, high-grade ovarian cancer, fallopian tube cancer, or primary peritoneal cancer who achieved a response to platinum-based chemotherapy. A total of 564 patients were enrolled and randomized with a ratio of 2:1 to receive either rucaparib or placebo. The primary objective of the study was to evaluate progression-free survival (PFS) by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1, as assessed by the investigator (invPFS). The intent-to-treat (ITT) population consisted of all randomized patients, and patients were stratified for randomization at study entry into biomarker-defined subgroups, t*BRCA* and HRD, based on results from a clinical trial assay (CTA), as defined as:

- t*BRCA*: "tumor *BRCA*" defined as deleterious alteration in *BRCA1* or *BRCA2*, derived from tumor tissue;
- HRD (t*BRCA* or non-t*BRCA*, LOH high): "homologous recombination deficient" defined as having a t*BRCA* mutation and/or having genomic LOH ≥ 16% in the tumor tissue.

The population analyzed for drug efficacy comprised all 564 patients randomized [i.e., ITT population] to either rucaparib (n=375) or placebo (n=189). The primary endpoint was tested among the t*BRCA*, HRD (t*BRCA*-positive or LOH high), and ITT populations using an ordered step-down multiple comparisons procedure. Statistically significant differences between rucaparib and placebo groups were tested at a one-sided significance level starting with investigator-assessed PFS (invPFS) in the t*BRCA* subgroup.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in the study was limited to patients who met the following key inclusion criteria:

- Patients with age 18 years or older.
- Confirmed diagnosis of high-grade serous or endometrioid epithelial ovarian, primary peritoneal, or fallopian tube cancer.
- Received ≥ 2 prior platinum-based treatment regimens including platinum based regimen that must have been administered immediately prior to maintenance therapy in this trial.
- Received no more than 1 non-platinum chemotherapy regimen. Prior hormonal therapy will not be counted as a non-platinum regimen.
- Must have had at least a 6-month disease-free period following prior treatment with the penultimate platinum-based chemotherapy and achieved a response.
- For the last chemotherapy course prior to study entry, patients must have received a platinum-based doublet chemotherapy regimen and have achieved a CR or PR (as defined by RECIST) and/or a GCIG CA-125 response.
- Have sufficient archival tumor tissue for analysis.

Patients were not permitted to enroll in the study if they met any of the following key exclusion criteria:

- History of prior cancer except for non-melanoma skin cancer, breast cancer curatively > 3 years ago, curatively treated solid tumor (> 5 years ago without evidence of recurrence), and synchronous endometrial cancer (Stage 1A) with ovarian cancer.
- Prior treatment with any PARP inhibitor, including rucaparib. Patients who received prior iniparib are eligible.
- Untreated or symptomatic central nervous system metastases.
- Pre-existing duodental stent and/or any gastrointestinal disorder or defect that would, in the opinion of the Investigator, interfere with absorption of study drug.
- Required drainage of ascites during the final 2 cycles of their last platinum-based regimen and/or during the period between the last dose of chemotherapy of that regimen and randomization to maintenance treatment in this study.

2. Follow-up Schedule

Patients were assessed for disease status per RECIST v1.1 every 12 calendar weeks (up to 1 week prior was permitted) following initiation of study treatment on Day 1 of Cycle 1. Patients experiencing disease progression by RECIST v1.1, as assessed by the investigator, were discontinued from treatment and entered follow-up.

3. Clinical Endpoints

In ARIEL3, the primary endpoint was PFS, as assessed by invPFS, in the molecularly-defined subgroups (t*BRCA* and HRD) using the CTA, and the ITT population. PFS by an independent radiology review was also conducted. Tumor response was interpreted using RECIST v1.1. Disease progression was only determined by RECIST v1.1.

To support the clinical performance of the FoundationFocus™ CDx *BRCA* LOH, those trial samples with sufficient remaining extracted DNA were submitted for retrospective testing using the FoundationFocus™ CDx *BRCA* LOH assay (see Section X.D. below). Concordance was determined based on positive percent agreement (PPA) of the CTA test results with the FoundationFocus™ CDx *BRCA* LOH assay results.

B. Accountability of PMA Cohort

A total of 564 patients was enrolled in the ARIEL3 trial between January 2014 and April 2017 from 87 global sites in 11 countries. The population analyzed for drug efficacy comprised all 564 patients randomized 2:1, (i.e., ITT population) to either rucaparib (n=375) or placebo (n=189). Of these 564 patients, 130 t*BRCA* patients and 245 t*BRCA* wild-type, LOH high patients were treated with rucaparib; and 66 t*BRCA* patients and 123 t*BRCA* wild-type, LOH high patients were treated with placebo. Of the 130 t*BRCA* patients in the rucaparib arm, 82 were identified as germline *BRCA* mutants, 40 were *BRCA* somatic and 8 unidentified. Out of the 66 t*BRCA* patients in the placebo arm, 48 were germline *BRCA* mutant, 16 were *BRCA* somatic, and 2 unidentified.

For clinical validation of the FoundationFocus™ CDx *BRCA* LOH test, a total of 518 (out of 564 patients) (92%) patients had a passing FoundationFocus™ CDx *BRCA* LOH test result. Of these patients, 27 had a status of non-t*BRCA*, LOH unknown. Among the remaining 491 patients, 184 had t*BRCA* status, 142 were non-t*BRCA*, LOH high and 165 were non-t*BRCA*, LOH low.

Of the samples evaluated with both the CTA and the FoundationFocus™ CDx *BRCA* LOH test, HRD-positive status was confirmed by the FoundationFocus™ CDx *BRCA* LOH test for 94.3% (313/332) of HRD-positive patients determined by the CTA. Among these, t*BRCA* status was confirmed by the FoundationFocus™ CDx *BRCA* LOH test for

99.4% (177/178) of t*BRCA*-positive patients determined by the CTA. Blood samples for 94% (186/196) of the t*BRCA*-positive patients were evaluated using a central blood germline *BRCA* test. Based on these results, 70% (130/186) of the t*BRCA* patients had a germline *BRCA* mutation and 30% (56/186) of the patients had a somatic *BRCA* mutation.

C. Study Population Demographics and Baseline Parameters

Demographic and other baseline characteristics for the primary efficacy analysis population are summarized in Table 10. The demographic characteristics of the two treatment groups were well balanced. All patients were female, with an overall median age of 61.0 years (range: 36-85 years) and median body mass index (BMI) of 26.4 kg/m2. The majority of patients had epithelial ovarian cancer, and most patients had serous histology and had been initially diagnosed with extensive disease with International Federation of Gynecology and Obstetrics (FIGO) Stage IIIC and FIGO Stage IV. The median time since diagnosis was 37.5 months in the overall population, which was similar for both treatment groups.

Table 10: Study Population Demographics and Baseline Parameters

Characteristics	Patients with FoundationFocus™ CDx *BRCA* LOH test	Patients without the FoundationFocus™ CDx *BRCA* LOH test	All Patients tested with the CTA
Age (yr)			
n	518	46	564
Mean (SD)	60.6 (9.27)	60.5 (11.04)	60.6 (9.42)
Median	61	57.5	61
Min, Max	36.0, 85.0	41.0, 81.0	36.0, 85.0
Age Group			
< 65	326 (62.9%)	28 (60.9%)	354 (62.8%)
65-74	166 (32.0%)	11 (23.9%)	177 (31.4%)
75-85	26 (5.0%)	7 (15.2%)	33 (5 .9%)
Gender			
Female	518 (100.0%)	46 (100.0%)	564 (100.0%)
Race			
American Indian or	4 (0.8%)	0 (0 %)	4 (0 .7%)
Asian	21 (4.1%)	0 (0 %)	21 (3 .7%)
Black or African	6 (1.2%)	2 (4 .3%)	8 (1 .4%)
Native Hawaiian or	0 (0%)	0 (0 %)	0 (0 %)
White	415 (80.1%)	36 (78.3%)	451 (80.0%)
Other	6 (1.2%)	0 (0 %)	6 (1 .1%)
Missing	66 (12.7%)	8 (17.4%)	74 (13.1%)
Geographic Region			
US	122 (23.6%)	6 (13.0%)	128 (22.7%)
Non-US	396 (76.4%)	40 (87.0%)	436 (77.3%)
ECOG at Baseline			

0	390 (75.3%)	26 (56.5%)	416 (73.8%)
1	128 (24.7%)	20 (43.5%)	148 (26.2%)
≥ 2	0 (0%)	0 (0 %)	0 (0 %)
Smoking Status			
Current Smoker	35 (6.8%)	2 (4 .3%)	37 (6 .6%)
Former Smoker	141 (27.2%)	10 (21.7%)	151 (26.8%)
Never Smoked	338 (65.3%)	34 (73.9%)	372 (66.0%)
Missing	4 (0.8%)	0 (0 %)	4 (0 .7%)

D. Safety and Effectiveness Results

1. Safety Results

Safety with respect to treatment with Rubraca™ (rucaparib) will not be addressed here in detail for the FoundationFocus™ CDx *BRCA* LOH. Briefly, the safety population consisted of 561 patients: 372 patients in the rucaparib group and 189 patients in the placebo group. The most common treatment emergent adverse events (TEAEs) that occurred in the rucaparib group were nausea (75.3%), combined asthenia/fatigue (69.4%), dysgeusia (39.2%), and combined anemia/low or decreased hemoglobin (37.4%). Refer to the drug label for more information.

2. Effectiveness Results

The primary endpoint of invPFS had a statistically significant improvement comparing rucaparib to placebo for all patients irrespective of whether the CTA or FoundationFocus™ CDx *BRCA* LOH test was used (Table 11). The median invPFS in the ITT population was 10.4 months (95% CI: 8.3, 11.1) for the rucaparib group and 5.4 months (95% CI: 5.3, 5.5) for the placebo group; the median invPFS in the HRD population was 13.7 months (95% CI: 11.0, 17.1) for the rucaparib group and 5.4 months (95% CI: 5.1, 5.6) for the placebo group; the median invPFS in the t*BRCA* population was 17.1 months (95% CI: 13.6, 23.2) for the rucaparib group and 5.4 months (95% CI: 4.9, 7.1) for the placebo group; and the median invPFS in the non-t*BRCA*, LOH high population was 8.5 months (95% CI: 8.0, 13.6) for the rucaparib group and 5.4 months (95% CI: 2.9, 5.6) for the placebo group.

As shown in Table 12, the stratified Cox proportional hazard model for all populations based on FoundationFocus™ CDx *BRCA* LOH testing showed a statistically significant improvement in invPFS with rucaparib treatment compared to placebo [HR 0.377 (95% CI: 0.30, 0.47); $p < 0.0001$ for the ITT population; HR 0.292 [95% CI: 0.217, 0.393]; $p < 0.0001$ for the HRD population; HR 0.228 [95% CI: 0.150, 0.346]; $p < 0.0001$ for t*BRCA* population and HR 0.361 [95% CI: 0.234, 0.559]; $p < 0.0001$ for the non-t*BRCA* LOH high population).

Table 11: lnvPFS by CTA and FoundationFocus™ CDx *BRCA* LOH (FF CDx)

Cohort	InvPFS (Months)		95% CI
ITT	**Rucaparib (FF CDx)**	**10.4**	[8.3, 11.1]
	Rucaparib (CTA)	10.8	[8.3, 11.4]
	Placebo	5.4	[5.3, 5.5
HRD	**Rucaparib (FF CDx)**	**13.7**	[11.0, 17.1]
	Rucaparib (CTA)	13.6	[10.9, 16.2]
	Placebo	5.4	[5.1, 5.6]
t*BRCA*	**Rucaparib (FF CDx)**	**17.1**	[13.6, 23.2]
	Rucaparib (CTA)	16.6	[13.4, 22.9]
	Placebo	5.4	[4.9, 7.1]
Non-t*BRCA* LOH high	**Rucaparib (FF CDx)**	**8.5**	[8.0, 13.6]
	Rucaparib (CTA)	9.7	[7.9, 13.1]
	Placebo	5.4	[3.9, 5.6]
Non-t*BRCA* LOH low	**Rucaparib (FF CDx)**	**6.3**	[5.4, 8.3]
	Rucaparib (CTA)	6.7	[5.4, 9.1]
	Placebo	5.4	[3.9, 5.6]
Non-t*BRCA* LOH Unknown	**Rucaparib (FF CDx)**	**7.2**	[4.9, 10.5]
	Rucaparib (CTA)	8.3	[5.6, 16.5]
	Placebo	4.2	[2.3, 8.2]

Table 12: Cox Proportional Hazard Model of Primary Endpoint: invPFS – All Populations Based on FoundationFocus™ CDx *BRCA* LOH Testing

Analysis for Rucaparib vs. Placebo	Hazard Ratio	95% CI	P-value
ITT	0.377	[0.30, 0.47]	<.0001
t*BRCA*	0.228	[0.15, 0.34]	<.0001
HRD	0.292	[0.22, 0.39]	<.0001
Non-t*BRCA* LOH high	0.361	[0.23, 0.56]	<.0001
Non-t*BRCA* LOH low	0.606	[0.41, 0.88]	0.0084
Non-t*BRCA* LOH Unknown	0.277	[0.09, 0.83]	0.0217
Patients without FoundationFocus™ CDx *BRCA* LOH Testing	0.239	[0.09, 0.65]	0.0053

Concordance Study

This study was designed to determine concordance based on positive percent agreement (PPA) of the CTA test results with the results from the FoundationFocus™ CDx *BRCA* LOH assay. A summary of the patients in each molecular subgroup based on the CTA and FoundationFocus™ CDx *BRCA* LOH assay is presented in Table 13. Table 14 summarizes the PPA between the CTA and FoundationFocus™ CDx *BRCA* LOH for all patients and each of the subgroups by pooled treatment. The PPA ranged from 81.3% to 99.4%, suggesting high overall concordance between the CTA and FoundationFocus™ CDx *BRCA* LOH.

Table 13: Agreement between CTA and FoundationFocus™ CDx *BRCA* LOH (FF CDx) Classification

		CTA subgroup			
		t*BRCA*	**Non-t*BRCA* LOH high**	**Non-t*BRCA* LOH low**	**Non-t*BRCA* LOH Unknown**
FF CDx subgroup	**t*BRCA***	177	7	0	0
	Non-t*BRCA* LOH high	0	129	11	2
	Non-t*BRCA* LOH low	1	17	143	4
	Non-t*BRCA* LOH Unknown	0	1	0	26
	No Result	18	4	7	17
	Overall	196	158	161	49

Table 14: PPA across Subgroups

	PPA[a]	
Population	**Estimate (%)**	**95% CI**
All patients[b]	91.7	[89.0, 93.9]
HRD	94.3	[91.2, 96.5]
t*BRCA*	99.4	[96.9, 100.0]
Non-t*BRCA* LOH high	83.8	[77.0, 89.2]
Non-t*BRCA* LOH low	92.9	[87.6, 96.4]
Non-t*BRCA* LOH Unknown	81.3	[63.6, 92.8]

[a] Positive Percent Agreement (PPA) is calculated based on samples tested with the CTA and FoundationFocus™ CDx *BRCA* LOH.
[b] All patients subgroup is defined as all patients in the ITT population with available FoundationFocus™ CDx *BRCA* LOH results (n=518).

3. Subgroup Analyses

To support that the FoundationFocus™ CDx *BRCA* LOH assay is clinically meaningful and provides information that will aid in benefit-risk assessments for individual patients, the clinical trial data were analyzed using Cox Proportional Hazard statistical models to demonstrate that there is an interaction between the test results (HRD status) and the corresponding drug in the ITT population. The results of the Cox Proportional Hazard models of invPFS are presented in Table 15 and Table 16. Table 15 includes all patients with HRD status of t*BRCA*, Non-t*BRCA*, LOH high or Non-t*BRCA*, LOH low. The p-value for the interaction

term is p=0.0072. Table 16 excludes the t*BRCA* patients and therefore only includes patients with Non-t*BRCA*, LOH high or Non-t*BRCA*, LOH low in order to evaluate the treatment interaction with LOH status. The p-value for the interaction between treatment and LOH status is p=0.0917. Data were also analyzed using the Cox Proportional Hazard models of invPFS to adjust for clinically relevant covariates. The inclusion of the clinically relevant covariates had a small effect on the estimates of the treatment by HRD status interaction terms.

Table 15: Interaction between FoundationFocus™ CDx *BRCA* LOH Results (HRD Status including t*BRCA*) and Treatment Effect

Analysis	Hazard Ratio	95% CI	P-value
Treatment			<.0001
t*BRCA*	0.24	[0.16, 0.35]	
Non-t*BRCA* LOH high	0.35	[0.23, 0.54]	
Non-t*BRCA* LOH low	0.56	[0.39, 0.81]	
HRD			0.3382
Treatment HRD			0.0072

Table 16: Interaction between FoundationFocus™ CDx *BRCA* LOH Results (HRD Status without t*BRCA*) and Treatment Effect

Analysis	Hazard Ratio	95% CI	P-value
Treatment			<.0001
Non-t*BRCA* LOH high	0.35	[0.23, 0.54]	
Non-t*BRCA* LOH low	0.57	[0.39, 0.82]	
HRD			0.7213
Treatment HRD			0.0917

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. None of the clinical investigators had any 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. SUMMARY OF SUPPLEMENTAL CLINICAL INFORMATION

Not applicable

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

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

XIII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

The clinical benefit of the FoundationFocus™ CDx *BRCA* LOH was demonstrated through analysis of efficacy data obtained from a randomized, double-blinded study (ARIEL3), in which Rubraca demonstrated a clinically meaningful PFS. Although the hazard ratio shows a statistically significant improvement comparing rucaparib to placebo for all patients irrespective of biomarker status, the stratified Cox Proportional Hazard model showed a statistically significant improvement in invPFS with rucaparib treatment compared to placebo in the biomarker subgroups, as detected by the FoundationFocus™ CDx *BRCA* LOH: HR 0.228 [95% CI: 0.150, 0.346], $p < 0.0001$ for t*BRCA* population; HR 0.292 [95% CI: 0.217, 0.393], $p < 0.0001$ for HRD population; and HR 0.361 [95% CI: 0.234, 0.559], $p < 0.0001$ for non-t*BRCA* LOH high population.

Further, the median invPFS in the ITT population was 10.4 months (95% CI: 8.3, 11.1) for the rucaparib group and 5.4 months (95% CI: 5.3, 5.5) for the placebo group. Improved PFS was observed for patients with HRD-positive status, whereby the median invPFS was 13.7 months (95% CI: 11.0, 17.1) compared to the median invPFS in the HRD-negative (Non-t*BRCA*, LOH low) subgroup, which was 6.3 months (95% CI: 5.4-8.3) for the rucaparib. Overall, there was a statistically significant difference in PFS between rucaparib treatment compared to placebo for each of the three populations (t*BRCA*, HRD, and ITT). In addition, the results support an improvement in PFS in ovarian cancer patients who are HRD-positive, as detected by the FoundationFocus™ CDx *BRCA* LOH.

The performance of the FoundationFocus™ CDx *BRCA* LOH was also supported by the analytical validation studies.

B. Safety Conclusions

The FoundationFocus™ CDx*BRCA* LOH assay is an *in vitro* diagnostic test, which involves testing of DNA extracted from ovarian cancer FFPE tumor tissue. The risks of the device are based on data collected in the validation studies conducted to support

approval. Risks of the test are associated with failure of the device to perform as expected or to correctly interpret test results, which may lead to incorrect test results, and subsequently, inappropriate patient management decisions in ovarian cancer treatment. Patients with false positive results may undergo treatment with Rubraca without significant clinical benefit, and may experience adverse reactions associated with Rubraca therapy.

In the maintenance setting for Rubraca therapy, the device results provide information to the physician about the HRD status to potentially inform treatment decisions. Although misclassification of HRD status can lead to inaccurate benefit-risk assessments for individual patients, this risk is mitigated because clinical benefit of Rubraca maintenance treatment was observed in the ITT population in the ARIEL3 study.

C. Benefit-Risk Determination

The use of the FoundationFocus™ CDx*BRCA* LOH assay has significant potential benefit in identification of ovarian cancer patients for treatment with Rubraca (rucaparib) based on t*BRCA* mutations, which is supported by the prior PMA approval (P160018), and on additional information in this application regarding HRD-positive status, which is associated with improved PFS in the maintenance setting.

The data to support the improved PFS in HRD-positive patients was based on the results of ARIEL3, a Phase 3, randomized, double-blind study, that included 564 patients. The results from this trial support the use of Rubraca (rucaparib) as a maintenance treatment option in the management of patients with recurrent, platinum-sensitive advanced ovarian cancer, who have received at least two prior lines of platinum-containing chemotherapy. The primary endpoint was achieved with a clinically meaningful, statistically significant difference in invPFS between rucaparib treatment compared to placebo for each of the three primary efficacy analysis populations (t*BRCA*, HRD, and ITT). In the ITT population, the reduction in risk of disease progression or death (HR 0.377 with $p < 0.0001$; invPFS rucaparib 10.4 months vs. 5.4 months placebo) demonstrates that patients with platinum-sensitive ovarian carcinoma can derive robust clinical benefit from maintenance treatment. In the HRD-positive population (defined as t*BRCA*-positive or LOH high), as identified by this test, improved PFS from Rubraca (rucaparib) maintenance therapy was observed (rucaparib 13.7 months vs. placebo 5.4 months). In contrast, in HRD-negative patients (Non-t*BRCA*, LOH low), there was no improved PFS for rucaparib (6.3 months) versus placebo (5.4 months). Thus, the FoundationFocus™ CDx*BRCA* LOH can potentially provide meaningful information to aid benefit-risk assessments for individual patients.

Additional factors to be considered in determining probable risks and benefits for the FoundationFocus™ CDx*BRCA* LOH include the risks of false results, the analytical performance of the device, and the availability of alternative tests. Erroneous results for the t*BRCA* mutation and LOH status may pose a risk to patients. While in the maintenance setting this device plays a complementary role for determining HRD

status, inaccurate or false results can lead to erroneous physician interpretation of results, potentially impacting patient treatment. However, the risk of misclassification of HRD dependent on LOH is attenuated by the fact that in the maintenance setting, Rubraca (rucaparib) is indicated for the all-comers, intent-to-treat population. While the indication for use partially mitigates the risk associated with erroneous physician interpretation, the analytical studies for LOH indicated variability for precision around the cut-off of 16, necessitating a flag in the patient report, informing users that LOH values near the cut-off may be variable. In addition, in terms of the analytical validity, there is the risk for interference data that was limiting in the application, but interference testing will be performed in the post-approval setting. Thus, the risks associated with the FoundationFocus™ CDx*BRCA* LOH are mitigated by the clinical indication for use, the device labeling, and ongoing analytical testing.

1. Patient Perspectives

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

In conclusion, given the available information above, the data support that for the FoundationFocus™ CDx *BRCA* LOH assay the probable benefits outweigh the probable risks.

D. Overall Conclusions

The analytical and clinical validation data support the reasonable assurance of safety and effectiveness of the FoundationFocus™ CDx *BRCA* LOH assay when used in accordance with the indications for use and product labeling. The provided studies, as well as those for PMA P160018, support use of the test to detect *BRCA* variants and genomic LOH from FFPE ovarian tumor tissue, to identify HRD-positive (defined as t*BRCA*-positive or LOH high) ovarian cancer patients who may be associated with improved PFS from Rubraca (rucaparib) maintenance therapy.

XIV. CDRH DECISION

CDRH issued an approval order on April 6, 2018. The final conditions of approval cited in the approval order are described below.

Limited interference study results were included in the PMA supplement. Additional testing and data are required to determine the impact of potential interfering substances (i.e., xylene, hemoglobin and triglycerides, Proteinase K, ethanol and Molecular Index Barcodes) on the performance of the FoundationFocus CDx *BRCA* LOH assay. This will ensure reliable performance of the device with the intended use specimens.

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

XV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

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

Post-approval Requirements and Restrictions: See approval order.

XVI. REFERENCES

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