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$_{BRCA}$
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
Date of FDA Notice of Approval:	December 19, 2016

Priority Review: Granted priority review status on July 26, 2016, because FoundationFocus™ CDx$_{BRCA}$ addresses an unmet clinical need, as the test provides a significant clinically meaningful advantage and is in the best interest of patients.

II. INDICATIONS FOR USE

The FoundationFocus™ CDx$_{BRCA}$ is a next generation sequencing based *in vitro* diagnostic device for qualitative detection of *BRCA1* and *BRCA2* alterations in formalin-fixed paraffin-embedded (FFPE) ovarian tumor tissue. The FoundationFocus CDx$_{BRCA}$ assay detects sequence alterations in *BRCA1* and *BRCA2* (*BRCA1/2*) genes. Results of the assay are used as an aid in identifying ovarian cancer patients for whom treatment with Rubraca™ (rucaparib) is being considered. If a patient is positive for any of the deleterious alterations specified in the *BRCA1/2* classification, the patient may be eligible for treatment with Rubraca. This assay 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$_{BRCA}$ assay labeling.

V. DEVICE DESCRIPTION

The FoundationFocus CDx*BRCA* assay is an *in vitro* diagnostic device performed in a single laboratory at Foundation Medicine, Inc. in Cambridge, MA. The assay is performed using DNA from ovarian cancer tumor, and resulting *BRCA1/2* alterations reported may include somatic (not inherited) or germline (inherited) alterations. Collectively, patients with deleterious *BRCA* alterations are referred to as tumor *BRCA* positive (t*BRCA*+).

The assay uses extracted DNA from FFPE biopsy or surgical tumor resection specimens. Two hundred (200) ng of the sample is subjected to whole-genome shotgun library construction and hybridization-based capture of all coding exons and some intronic regions from *BRCA1* and *BRCA2*. Using the Illumina® HiSeq™ 4000 platform, hybrid-capture–selected libraries are sequenced to uniform depth (targeting > 500X coverage with > 99% of exons at coverage > 100X).

The FoundationFocus CDx*BRCA* assay uses a custom-developed analysis pipeline to identify *BRCA1/2* base substitutions and short insertion/deletions (indels) up to 13 bp. Certain variants including larger rearrangements and homozygous deletions have not been validated by the FoundationFocus CDx*BRCA*. 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. The sequence data then undergoes an alignment process where it is mapped to the human genome (hg19) and an analysis of sequence variant data is performed using FoundationFocus CDx*BRCA* analysis pipeline software. Variant classification is conducted according to defined classification criteria.

Tables 1 and 2 describe the criteria for classifying *BRCA1* or *BRCA2* alterations known to be deleterious to BRCA protein function rendering the sample t*BRCA*+.

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

Qualification Criteria	Sequence Classification	Methodology
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, with exception of *BRCA2* K3326* and any alteration 3' downstream of K3326
	Splice site alterations	Sequence analysis identifies alteration splice sequences at intron/exon junctions -/+ 2 bp of exon starts/ends
	Deleterious missense alterations	Curated list, Table 2*

* 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 *BRCA* Missense Alterations

***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.1A>G (M1V)	

Test Kit Contents

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

- Specimen Preparation Instructions
- Test Requisition Form

- Shipping Instructions
- Return Shipping Label
- Technical Information Summary

Instruments

The FoundationFocus CDx_{BRCA} assay is intended to be performed with serial number-controlled instruments as indicated in Table 3 below.

Table 3: Instruments for Use with the FoundationFocus CDx_{BRCA} assay

Instrument	Serial Number(s)
Illumina HiSeq 4000	K00255
Illumina HiSeq 4000	K00256
Illumina HiSeq 4000	K00257
Illumina cBot	6002629
Illumina cBot	6002630
Illumina cBot	6002631
Illumina cBot	6002632
Illumina cBot	6002633
Illumina cBot	6002634
Agilent Benchbot Workstation with Integrated Bravo Liquid Handler	USH5026802
Agilent Benchbot Workstation with Integrated Bravo Liquid Handler	USH5026803
Agilent Benchbot Workstation with Integrated Bravo Liquid Handler	USH5026801
Beckman Biomek NXP Span-8 Liquid Handler	A318400793
Beckman Biomek NXP Span-8 Liquid Handler	A318400794
Covaris Focused Ultrasonicator LE220	003181
Thermo Scientific Kingfisher Flex DW 96	711-1030
Thermo Scientific Kingfisher Flex DW 96	711-1032

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 ($\geq$ 20% tumor) 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 200 ng of genomic DNA to proceed with LC.

C. Library Construction

LC begins with the normalization of DNA to a specified amount of 200 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 diluted for quality control (QC).

QC check is performed by measuring DNA yield and by assessing fragment size. Single-stranded DNA (ssDNA) from purified libraries is quantified using the Quant-iT™ OliGreen® ssDNA Assay Kit (Life Technologies), read on a Molecular Devices Multimode SpectraMax M2 plate reader and sized on a LabChip® GX Touch (Caliper); size selection is not performed. Libraries yielding less than 1000 ng of sequencing library, or with a mean insert size < 200 bp or > 400 bp, are considered as failed.

D. Hybrid Capture

Hybrid Capture (HC) begins with normalization of each library to 2000 ng. Normalized samples then undergo solution hybridization which is performed using a > 50-fold molar excess of a pool of individually synthesized 5'-biotinylated DNA 120 bp oligonucleotides. The baits target ~1.5 Mb of the human genome including all coding exons of 310 cancer-related genes, introns or non-coding regions of 35 genes, plus > 3,500 single nucleotide polymorphisms (SNPs) located throughout the genome; however, the FoundationFocus CDx$_{BRCA}$ only reports results for *BRCA1/2*. Baits are designed by tiling overlapping 120 bp DNA sequence intervals covering target exons (60 bp overlap) and introns (20 bp overlap), with a minimum of three baits per target; SNP targets are allocated one bait each. Intronic baits are filtered for repetitive elements[2] as defined by the UCSC Genome RepeatMasker track[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.

QC for HC is performed by measuring dsDNA yield and assessing the size of the fragments. Yields of individual captured samples are measured using a Quant-iT™

PicoGreen® dsDNA Assay Kit (Life Technologies) read on a Molecular Devices Multimode SpectraMax M2 plate reader, and sized on a LabChip® GX Touch (Caliper); size selection is not performed. Captured libraries yielding less than 140 ng of sequencing library, or with a mean insert size < 200 bp or > 400 bp, are considered as failed.

E. Sequencing

Sequencing is performed using off-board clustering on the Illumina cBot with patterned flow cell technology to generate monoclonal clusters from a single DNA template followed by sequencing using sequencing by synthesis (SBS) chemistry on the Illumina HiSeq 4000. Fluorescently labeled 3'-blocked dNTPs along with a polymerase are incorporated through the flow cell to create a growing nucleotide chain that is excited by a laser. A camera captures the emission color of the incorporated base and then is cleaved off. The terminator is then removed to allow the nucleotide to revert to its natural form and to allow the polymerase to add another base to the growing chain. A new pool of fluorescently labeled 3'-blocked dNTPs 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

Sequence data is analyzed using proprietary software developed by FMI. 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]. 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 mutant allele frequency (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[8]. Key steps are:

- Collecting all read-pairs for which at least one read maps to the target region.
- Decomposing each read into constituent k-mers and constructing an enumerable graph representation (de-Bruijn) of all candidate non-reference haplotypes present.
- Evaluating the support of each alternate haplotype with respect to the raw read data to generate mutational candidates. All reads are compared to each of the candidate haplotypes via ungapped alignment, and a read 'vote' for each read is assigned to the candidate with best match. Ties between candidates are resolved by splitting the read vote, weighted by the number of reads already supporting each haplotype. This process is iterated until a 'winning' haplotype is selected.
- Aligning candidates against the reference genome to report alteration calls.

Filtering of indel candidates is carried out similarly to base substitutions (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 $\leq$ 2.

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 SNVs and indels with MAF < 5% will not be reported for non-hotspot bases whereas SNVs with MAF $\geq$ 1% and indels $\geq$ 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.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-cleared or -approved alternatives for detection of deleterious sequence variants in the *BRCA1* and *BRCA2* genes using DNA extracted from FFPE ovarian cancer tumor specimens for the selection of ovarian cancer patients who are eligible for treatment with Rubraca, a poly (ADP-ribose) polymerase (PARP) inhibitor.

VII. MARKETING HISTORY

The FoundationFocus CDx$_{BRCA}$ assay has not been marketed in the United States or any foreign country.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

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

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

IX. SUMMARY OF NON-CLINICAL STUDIES

A. Laboratory Studies

a.Accuracy/Concordance Study

The concordance of *BRCA1/2* alteration detection by the FoundationFocus CDx$_{BRCA}$ assay was compared to results of an externally validated NGS assay.

This study included 36 t*BRCA*+ and 44 tumor *BRCA*-negative (t*BRCA*-) ovarian cancer samples. The sample set covered a range of *BRCA1/2* alterations including insertions ranging from 1-4 nucleotides, deletions ranging from 1-12 nucleotides, and single nucleotide variants, including variants in certain homopolymer runs. One sample failed assay QC due to low sequencing coverage and was excluded from the analysis. Additionally, six samples failed the comparator assay QC due to low coverage and/or low allele frequency and were excluded from the analysis.

Concordance excluding the invalid samples resulted in a 97.3% overall percent agreement (OPA). Two samples that were positive by the FoundationFocus CDx$_{BRCA}$ assay were negative by the comparator assay. Both samples were found to be t*BRCA*+ by a second validated NGS comparator method. Concordance data with and without invalid results between the FoundationFocus CDx$_{BRCA}$ assay and the comparator assay are shown in Table 4. The accuracy study is ongoing.

Table 4: Agreement between FoundationFocus CDx$_{BRCA}$ and an NGS Comparator

		NGS Comparator Assay			**Total**
		BRCA+	***BRCA-***	**Invalid**	
FoundationFocus CDx$_{BRCA}$	***tBRCA+***	34	2*	0	36
	tBRCA-	0	37	6	43
	Invalid	0	1	0	1
	Total	34	40	6	80
Agreement Including Valid Results Only (Total N=73)	**PPA [95% CI]**	100% [89.7%, 100%]			
	NPA [95% CI]	94.9% [82.7%, 99.4%]			
	OPA [95% CI]	97.3% [90.5%, 99.7%]			
Agreement Including Invalid Results (Total N=80)	**PPA [95% CI]**	85.0% [70.2%, 94.3%]			
	NPA [95% CI]	92.5% [79.6%, 98.4%]			
	OPA [95% CI]	88.8% [79.7%, 94.7%]			

*1 sample was below LoD of the comparator assay, and 1 sample was detected in a region not covered by the comparator assay.

b. Analytical Specificity

a. Interfering Substances

To evaluate the potential impact of interfering substances on the performance of the FoundationFocus CDx$_{BRCA}$ assay process, this study evaluated three ovarian cancer (OC) FFPE specimens in the presence of exogenous and endogenous substances. Each specimen was assessed with eight replicates, for a total of 24 samples with the addition of the following interfering substances: triglycerides (37 mmol/L of the OC FFPE volume), hemoglobin (2 mg/mL of the OC FFPE curl volume), or xylene (0.0001% of the OC FFPE volume). In addition, eight OC FFPE samples with varying quantities of necrosis, ranging from 5% to 50%, were assessed in duplicate. The percent necrosis included 5%, 10%, 15%, 25%, 40% and 50%.

Substances were considered to have no effect on assay performance when the DNA yield was sufficient to meet the standard processing requirements of DNA isolation (> 200 ng), and when the quality of DNA was sufficient to create products per the specification of library construction (> 1000 ng) for a minimum of 6 of 8 replicates. Sequence analysis was assessed as percent agreement for each sample and was calculated as the number of replicates with the correct alteration call reported, when comparing across spiked and non-spiked samples, per the total number of replicates processed.

All (100%) of the samples exhibited concordant alteration calls across the study. Necrotic tumor content, and the addition of three contaminants, did not have an impact on alteration detection as assessed by sequence concordance.

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

This study assessed whether the 120-mer baits designed for capturing DNA targets at *BRCA1/2* have homologs in other parts of the genome utilizing a Basic Local Alignment Search Tool (BLAST) search, and whether the impact of this non-specific capture leads to a significant reduction in NGS read coverage at *BRCA1/2*. The BLAST analysis for the 359 *BRCA1* oligonucleotide baits against the human genome reference sequence identified 17 baits spanning four targets with homology to non-targeted genomic regions. Coverage of regions with homology was assessed and compared to regions with no homology. This analysis demonstrated that the coverage of the targets with homology was sufficiently high (minimum read depth of 644X) not to impact assay performance in these regions. The BLAST analysis for the 271 *BRCA2* oligonucleotide baits against the human genomic database did not identify any sequences with significant homology.

c. Carryover/Cross-contamination

DNA sample carryover and cross-contamination during the LC, HC and sequencing steps of the FoundationFocus CDx$_{BRCA}$ assay were assessed. DNA from two FFPE OC samples with unique *BRCA1/2* genotypes, one including a *BRCA1* alteration and one t*BRCA*-, were set up in a checkerboard matrix pattern as alternating *BRCA*+ and *BRCA*- wells run on duplicate plates, and more than 3500 SNPs across the genome were assessed. For SNPs that are homozygous in a patient, contamination of the sample by another human leads to baseline SNP allele frequencies significantly above 0%. Therefore, the contamination level was assessed by measuring the allele frequency of homozygous SNPs.

Sample contamination was not detected in any of the analyzed samples. An assessment of *BRCA* alterations was also performed on each sample. Percent agreement (fraction of correct calls) was computed across the aggregated

replicates with 100% of samples exhibiting concordance across expected alteration calls. The lower bounds of two-sided 95% CI for concordance were 96.2% and 96.1% % for plates 1 and 2, respectively. The results are shown in Table 5 below.

Table 5: Concordance of *BRCA* Positive and *BRCA* Negative Samples

Agreement	Plate 1		Plate 2	
	Point Est.	95% CI	Point Est.	95% CI
PPA	100%	[92.5%, 100%]	100%	[92.3%, 100%]
NPA	100%	[92.5%, 100%]	100%	[92.5%, 100%]
OPA	100%	[96.2%, 100%]	100%	[96.1%, 100%]

d. Precision/Reproducibility from Extracted DNA

This study evaluated precision, including intra-run, inter-run, lot-to-lot and instrument-to-instrument reproducibility, of the FoundationFocus CDx$_{BRCA}$ assay. A set of 25 samples was tested, in duplicate, using three sequencing instruments and three reagent lots, at and near the limit of detection (LoD) for mutant allele frequency (MAF) levels. A range of *BRCA1* and *BRCA2* variants were represented in the sample set including insertions ranging from 1-10 nucleotides, deletions ranging from 1-12 nucleotides and single nucleotide variants, including variants in some homopolymer runs. Seven specimens were excluded due to technical limitations including three due to unbalanced dilutions and four samples with variants present in complex or long repeat contexts. One sample was only assessed in its natural state (i.e., not further diluted), given that the pre-screened alteration frequency was found to be near the LoD.

Among the 18 samples that produced successful calls, all variants were detected with 100% concordance at their natural levels. The pair-wise agreements in the 18 samples at diluted MAF (range, 5% - 30% MAF) were 100% concordant (two-sided 95% CI) based on Clopper-Pearson method: 15 samples with CI of (95%, 100%) and 1 sample with CI of (93%, 100%) for sequencer-to-sequencer agreements and CI of (90%, 100%) for lot-to-lot agreements for nearly all replicates with the exception of two samples with 80% to 91% APA in certain sequencer-to-sequencer and lot-to-lot comparisons. Two-sided 95% CIs were calculated with bootstrap method when agreements (APA, NPA) were not 100%. The precision was calculated in terms of mean, %CV and SD based on MAF. The MAF data was analyzed for each sample at natural level and diluted level using a two-way Analysis of Variance (ANOVA) model with main effects and interaction of sequencer and reagent lot. The precision estimates are reported in following two tables.

Table 6: Precision MAF Results by Samples Evaluated at the Natural Level

Sample	Mean	Repeatability		Lot		Sequencer		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.614	0.012	1.995	0.000	0.000	0.004	0.692	0.013	2.112
2	0.889	0.015	1.718	0.007	0.795	0.000	0.000	0.017	1.894
3	0.792	0.011	1.364	0.010	1.315	0.000	0.000	0.015	1.895
4	0.704	0.013	1.893	0.004	0.626	0.000	0.000	0.014	1.994
5	0.559	0.026	4.658	0.011	1.955	0.006	1.033	0.029	5.157
6	0.408	0.031	7.504	0.002	0.577	0.002	0.577	0.031	7.548
7	0.299	0.018	6.006	0.008	2.703	0.004	1.308	0.020	6.715
8	0.557	0.026	4.691	0.002	0.423	0.000	0.000	0.026	4.710
9	0.463	0.015	3.217	0.000	0.000	0.000	0.000	0.015	3.217
10	0.591	0.016	2.763	0.020	3.300	0.008	1.352	0.027	4.511
11	0.581	0.020	3.372	0.007	1.235	0.000	0.000	0.021	3.591
12	0.337	0.019	5.548	0.027	7.946	0.013	3.907	0.035	10.449
13	0.278	0.013	4.648	0.010	3.447	0.000	0.000	0.016	5.768
14	0.181	0.015	8.424	0.008	4.531	0.005	2.483	0.018	9.882
15	0.791	0.028	3.525	0.010	1.264	0.002	0.298	0.030	3.757
16	0.721	0.015	2.145	0.010	1.359	0.000	0.000	0.018	2.539
17	0.251	0.024	9.454	0.008	3.293	0.000	0.000	0.025	10.011
18	0.402	0.016	4.023	0.000	0.000	0.008	1.924	0.018	4.459
19	0.475	0.023	4.785	0.000	0.000	0.006	1.289	0.024	4.956
20	0.796	0.009	1.109	0.006	0.726	0.000	0.000	0.012	1.539
21	0.464	0.031	6.694	0.026	5.668	0.010	2.255	0.042	9.057
22	0.572	0.015	2.669	0.000	0.000	0.000	0.000	0.017	2.977
23	0.787	0.013	1.641	0.009	1.080	0.000	0.000	0.015	1.965
24	0.564	0.017	2.953	0.009	1.576	0.005	0.861	0.020	3.456
25	0.386	0.031	8.162	0.000	0.000	0.000	0.000	0.031	8.162

Table 7: Precision Results by Samples Evaluated at Diluted Level*

Sample	Mean	Repeatability		Lot		Sequencer		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.189	0.022	11.605	0.000	0.000	0.000	0.000	0.022	11.605
2	0.208	0.015	6.993	0.013	6.417	0.006	3.107	0.021	9.987
3	0.213	0.028	12.966	0.002	0.783	0.003	1.357	0.028	13.060
4	0.224	0.025	10.991	0.013	5.838	0.000	0.000	0.028	12.445
5	0.274	0.016	5.773	0.002	0.609	0.009	3.277	0.018	6.666
6	0.088	0.016	18.212	0.000	0.000	0.000	0.000	0.016	18.360
7	0.271	0.014	5.216	0.011	3.984	0.004	1.506	0.018	6.734
8	0.056	0.009	16.459	0.007	13.180	0.002	3.030	0.012	21.302
9	0.122	0.012	9.448	0.000	0.000	0.000	0.000	0.012	9.448
10	0.217	0.024	10.905	0.000	0.000	0.000	0.000	0.024	10.905
11	0.236	0.021	8.760	0.011	4.520	0.000	0.000	0.023	9.857
12	0.189	0.015	7.692	0.000	0.000	0.000	0.000	0.023	12.369
13	0.101	0.013	12.768	0.002	1.648	0.005	4.662	0.014	13.692
14	0.276	0.017	5.976	0.002	0.739	0.001	0.427	0.017	6.036
15	0.214	0.011	5.155	0.021	9.707	0.003	1.346	0.024	11.236
16	0.031	0.004	11.340	0.000	0.741	0.002	5.257	0.004	12.522
17	0.192	0.023	11.839	0.008	4.118	0.002	0.824	0.024	12.561

*7 samples were excluded due to technical reasons and one sample was only assessed in its natural state as the pre-screened alteration frequency was found to be near the LoD.

To examine operator-to-operator reproducibility, ten operators contributed to the execution of the lab processes. Nine of the operators were assigned to various steps in the processes across many several days to encourage high variability in operator contribution. No difference in performance was observed.

To examine reagent lot-to-lot reproducibility, three lots of critical reagents were assessed for each of the three portions of the assay including LC, HC, and sequencing. Reagents were evaluated as internally prepared kits for each process step (LC, HC, and sequencing). The use of three different lots of reagents did not significantly impact performance. Results are summarized in Table 8 below.

Table 8: Lot-to-Lot Reproducibility Results

Reagent Kit Lot	% Concordance [95% CI]
1	99.6% [97.9%, 100.0%]
2	100.0% [98.6%, 100.0%]
3	99.2% [97.1, 99.9%]

To examine instrument-to-instrument reproducibility, each of the sample plates processed was sequenced on each of three Illumina HiSeq4000 sequencers. Results are summarized in Table 9 below.

Table 9: Instrument-to-Instrument Reproducibility Results

HiSeq4000	% Concordance [95% CI]
sequencer 1	100.0% [98.6%, 100.0%]
sequencer 2	99.6% [97.9%, 100.0%]
sequencer 3	99.2% [97.2%, 99.9%]

e. Limit of Detection (LoD) and Limit of Blank (LoB)

LoD of the FoundationFocus CDx$_{BRCA}$ assay for *BRCA1/2* alterations was assessed. Seven OC samples possessing different categories of *BRCA1/2* alterations were evaluated; including samples with insertions ranging from 1-4 nucleotides, deletions ranging from 1-11 nucleotides and single nucleotide variants, including variants in some homopolymer runs. Using logistic regression, the LoD for alterations in non-repetitive regions or homopolymer repeats < 4 nucleotides, is 6% MAF. The LoD for representative alterations in a homopolymer region > 4 nucleotides, is 15.3% MAF. Alterations detected at allele frequencies below the established limit of detection are not detected consistently.

Table 10: Limit of Detection Results

Sample	Gene	Alteration	LoD
1	BRCA1	5263_5264insC	2.6%
2	BRCA1	66_67delAG	2.5%
3	BRCA1	1954_1954delA	15.3%
4	BRCA2	3978_3979insTGCT	4.5%
5	BRCA1	5170_5173delAAAG	4.5%
6	BRCA1	3479_3489delAGGAAGATACT	4.7%
7	BRCA2	8878C>T	5.9%

Eleven t*BRCA*- samples were assessed for LoB with each sample processed in nine replicates. A total of 93 sample replicates proceeded to sequencing with 6 samples failing QC after the HC step due to insufficient DNA. All samples that proceeded to sequencing (100%) were in agreement as *BRCA*-negative variant calls and there were no false positive *BRCA* calls; thus, confirming the LoB of zero.

f. Stability

a. Reagent Stability

The stability of critical reagent lots used in the LC, HC and sequencing processes within the FoundationFocus CDx$_{BRCA}$ assay were evaluated in this study. Three lots of each set of reagents were stored under the manufacturer's specified temperature conditions (ranging from 4 to -20°C) and then tested at defined time points. Under all of the test conditions, results from each time point were compared against samples tested at day 0 (time point T_0). Alteration calls are concordant for critical

reagents assessed for time points up to 90 days. Stability testing for these reagents is ongoing and will span a time of 120 days.

b. DNA Stability

To define the storage conditions and evaluate the stability of DNA extracted from FFPE OC samples, stability at defined temperatures and durations was assessed. Three DNA samples containing alterations in the *BRCA1/2* genes were assessed in triplicate at day zero (T_0), 6 weeks at 4 ºC, and 3 months at -20 ºC. Results from each time point were compared to those from T_0 to determine if the same results were obtained. Alteration calls were concordant at all tested time points. DNA stability testing is ongoing; additional data will be collected and evaluated over a period of 3 years at fourteen different time points.

c. FFPE Sample Stability

The stability of the FFPE specimens used for FoundationFocus CDx$_{BRCA}$ assay was evaluated retrospectively by examining DNA extraction yields from FFPE OC tissue samples. A total of 3,195 OC samples were binned into seven groups according to block age. The oldest block examined was collected 9.83 years before DNA extraction. All samples categorized by the block ages in years (< 0.5, 0.5-1, 1-2, 2-3, 3-4, 4-5, and > 5 years) demonstrated greater than 95%, yielding sufficient DNA for the FoundationFocus CDx$_{BRCA}$ assay with no significant difference. There was no significant deterioration of yield with increasing block age and no significant downward trend with block age up to 5 years post specimen-collection. A prospective study to assess the stability of cut FFPE on slides over five time points up to 15 months is ongoing.

g.Reagent Lot Interchangeability

Reagent lot interchangeability was assessed by testing four FFPE OC samples containing alterations in the *BRCA1/2* genes in duplicate using two different lots each of LC, HC, and sequencing reagents in eight different lot combinations resulting in a total of 64 samples processed. One of the four OC samples had relatively low HC yields resulting in 6 failed replicates. The failure is indicative of a specimen quality issue and not reflective of a reagent failure since all failed replicates came from the same sample and had low HC yields across all plates. For the 58 sample replicates that proceeded to sequencing, all passed all sequencing metrics. Of the 58 sequenced samples, 58 (100%) of the samples had concordant sequence calls. The lower bound of the 95% two-sided 95% CI for this result is 93.84%.

h. General Lab Equipment and Reagent Evaluation

a. DNA Amplification

Thermal cycler interchangeability during the post-LC and post-HC process steps was evaluated for the FoundationFocus CDx$_{BRCA}$ assay. Eight replicate aliquots for each of three OC FFPE samples were

processed in parallel, with two replicates amplified on each of four different thermal cycler pairings. A total of 24 aliquots were evaluated, with 100% of the samples concordant among the replicates across all thermal cycler pairings.

b. DNA Extraction

The performance of the DNA extraction from FFPE OC tumor specimens was evaluated. The study included 46 FFPE specimens, tested in triplicate using two different KingFisher Flex Magnetic Particle Processors and three extraction reagent lots. A total of 405 of 414 samples exhibited DNA yields ≥ 200 ng after the DNA extraction step, for a 97.8% success rate.

i. Guardbanding/Robustness

Guardbanding studies were performed to evaluate the performance of the FoundationFocus CDx$_{BRCA}$ assay and the impact of process variation with regard to DNA concentration. Guardbands were evaluated relative to observed and measured process variability for LC, HC, and sequencing. For each of the experiments, the sample set included five samples containing variants in the *BRCA1/2* genes.

a. Library Construction Guardbanding

Five samples were run in duplicate over five different DNA input levels representing ±25% and ±50% of the required amount needed for LC (100-300 ng). All replicates resulted in libraries with sufficient DNA yield for 100% of samples. No significant difference was observed in the resulting concentration of the library, regardless of the input DNA quantity.

b. Hybrid Capture Guardbanding

Five samples were run in duplicate over each of five DNA input levels representing ±25% and ±50% of the required input amount needed for HC (0.5-2.5 µg) were tested. For each of the two lower DNA input levels (0.5 µg and 1.0 µg), nine of the ten replicates met the required specification for HC yield. At the higher input levels (1.5 µg, 2.0 µg, and 2.5 µg), 100% success rates were observed. These results support a DNA input amount of 1.5 µg to 2.0 µg DNA for HC.

c. Sequencing Guardbanding

The third component of the guard banding study evaluated the captured DNA input into the sequencing reaction. Five samples were run in duplicate over five different DNA input levels representing ±25% and ±50% of the required amount needed for sequencing (1.4-2.1 nM). For each of the five input DNA levels evaluated, 100% of the samples met all required sequencing metrics (e.g., median read depth, maximum error rate, number of total reads per sample). An analysis of median depth of

coverage did not indicate a significant difference in the resulting sequencing content, supporting assay performance for all tested input levels.

B. Animal Studies

No animal studies were conducted using the FoundationFocus CDx$_{BRCA}$.

C. Additional Studies

No additional studies were conducted using the FoundationFocus CDx$_{BRCA}$.

X. SUMMARY OF PRIMARY CLINICAL STUDIES

The clinical performance of the FoundationFocus CDx$_{BRCA}$ was established based on two clinical studies to support Rubraca (rucaparib) treatment of ovarian cancer patients with deleterious *BRCA* mutations who have been treated with two or more chemotherapies. The studies included US and ex-US sites, and the results are described in NDA 209115. Data from these two clinical studies, Study 1 and Study 2, were used to support PMA approval. A clinical bridging study was performed to determine concordance of the Foundation Medicine Clinical Trial Assay (CTA) test results and local laboratory test results with the FoundationFocus CDx$_{BRCA}$ assay for the detection of a deleterious *BRCA* alteration, and to compare outcome data for patients identified by the FoundationFocus CDx$_{BRCA}$ assay with data from those patients identified by another method to ensure the results are comparable.

A. Study Design

a. Study 1 Design

Study 1 was a 3-part, single-arm, open-label Phase 1/2 study evaluating rucaparib as oral monotherapy in patients with advanced solid tumors who had progressed on prior treatment. The primary objectives of Parts 1 and 3 were to characterize the pharmacokinetics and safety of rucaparib tablets, while the primary objective of Part 2A was to evaluate the efficacy of rucaparib in ovarian cancer patients with a germline *BRCA* (g*BRCA*) alteration by assessment of the overall response rate (ORR) according to Response Evaluation Criteria in Solid Tumors Version 1.1 (RECIST v1.1). Key secondary efficacy endpoints included duration of response (DOR), ORR by RECIST and Gynecologic Intergroup (GCIG) cancer antigen 125 (CA-125) criteria, and progression-free survival (PFS). Only efficacy data from Part 2A patients were used to support the performance of the FoundationFocus CDx$_{BRCA}$.

Study 1 Part 2A enrolled ovarian cancer patients who had received 2 to 4 prior treatment regimens and were known to harbor a deleterious gBRCA alteration, determined by a local laboratory test. Archival formalin-fixed paraffin-embedded (FFPE) tumor tissue samples were retrospectively requested to confirm the presence of a *BRCA* alteration utilizing the FoundationFocus CDx$_{BRCA}$.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in Study 1 was limited to patients who met the following key inclusion criteria:

- Age ≥ 18 years
- Understood and voluntarily signed an Institution Review Board/Independent Ethics Committee-approved informed consent form
- Had a histologically confirmed diagnosis of high-grade serous or endometrioid epithelial ovarian, fallopian tube, or primary peritoneal cancer.
- Had received at least two, but no more than four, prior chemotherapy regimens and had relapsed disease as confirmed by radiologic assessment
- Had evidence of measurable disease as defined by RECIST v1.1
- Had a known deleterious germline *BRCA* mutation as determined by a local laboratory that has received an international or country-specific quality standards certification
- Had adequate organ function and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1

Patients were not permitted to enroll in Study 1 if they met any of the following key exclusion criteria:

- Received prior treatment with a PARP inhibitor
- Untreated or symptomatic central nervous system (CNS) metastases. Patients with asymptomatic CNS metastases were eligible provided they had been clinically stable for at least 4 weeks
- Received treatment with chemotherapy, radiation, antibody therapy or other immunotherapy, gene therapy, vaccine therapy, angiogenesis inhibitors, or experimental drugs ≤ 14 days prior to first dose of rucaparib and/or ongoing adverse effects from such treatment > National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Grade 1. Ongoing Grade 2 non-hematologic toxicity related to the most recent treatment regimen was permitted with prior advanced approval.
- Pre-existing duodenal stent and/or any gastrointestinal disorder of defect that would, in the opinion of the investigator, interfere with adsorption of rucaparib
- Presence of any other condition that may increase the risk associated with study participation or may interfere with the interpretation of study results, and in the opinion of the investigator, would make the patient inappropriate for entry into the study

2. Follow-up Schedule

Tumor assessments were performed at baseline then every 8 weeks up to 6 months after starting study treatment. If the tumor had not progressed after 6 months, the tumor assessments were performed every 12 weeks. If the tumor had not progressed after 18 months from the start of treatment, scans were performed every 16 weeks until progression.

3. Clinical Endpoints

The primary efficacy endpoint was objective response rate (ORR), with responses assessed by the investigator. The confirmed response rate by RECIST v1.1 is defined as the proportion of patients with a confirmed complete response (CR) or partial response (PR) on subsequent a tumor assessment at least 28 days after first response documentation. Duration of response (DOR) was also analyzed using Kaplan-Meier methodology where any patient with an ongoing response was censored at the date of the last post-baseline scan. The efficacy evaluation in Study 1 Part 2A was performed for all treated patients, as all had received ≥ 2 prior chemotherapy regimens and had a deleterious *BRCA* alteration.

For the clinical bridging study, the objectives were agreement between the FoundationFocus CDx$_{BRCA}$ and the local test results, and assessment of the ORR by RECIST v1.1 in the subset of patients with a *BRCA1/2* alteration identified by the FoundationFocus CDx$_{BRCA}$ as compared to ORR in the overall primary efficacy population.

b. Study 2 Design

Study 2 was a 2-part, open-label Phase 2 study of oral monotherapy rucaparib designed to identify patients with relapsed ovarian cancer who were likely to respond to rucaparib. Part 1 enrolled patients who had received at least 1 prior platinum-based regimen and had platinum-sensitive disease. Part 2 enrolled patients with relapsed ovarian cancer who received at least 3, but no more than 4, prior chemotherapy regimens.

As a condition of enrollment in Study 2, all patients were required to undergo a biopsy procedure prior to enrollment, with the exception of patients in Part 2 who were known to harbor a deleterious *BRCA* alteration based on results of a local test recorded in their medical record. Submission of archival FFPE tumor tissue was also required for all patients. Patients were classified based on analysis of the most recent tumor specimen available (i.e., biopsy collected during screening period if available, or archival tumor). The primary objective of Part 1 was to assess PFS in the 3 molecularly-defined subgroups using RECIST v1.1. Key secondary efficacy endpoints included ORR and DOR by RECIST v1.1 and ORR by RECIST and GCIG CA-125 criteria. The primary objective of Part 2 was to assess ORR by RECIST v1.1 in the 3 molecularly-defined subgroups. Key secondary efficacy endpoints included DOR and PFS by RECIST v1.1 and ORR by RECIST and GCIG CA-125 criteria. Only efficacy data from patients who had received ≥ 2 prior chemotherapy regimens and were identified as having a deleterious *BRCA* alteration were used to support approval of FoundationFocus CDx$_{BRCA}$.

1. Clinical Inclusion and Exclusion Criteria

Enrollment in Study 2 was limited to patients who met the following key inclusion criteria:

- Age ≥ 18 years
- Understood and voluntarily signed an Institution Review Board/Independent Ethics Committee-approved informed consent form
- Had a histologically confirmed diagnosis of high-grade serous or Grade 2 or Grade 3 endometrioid epithelial ovarian, fallopian tube, or primary peritoneal cancer
- Had relapsed/progressive disease as confirmed by radiologic assessment
- Had evidence of measurable disease as defined by RECIST v1.1
- Had adequate organ function and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 1
- Had received ≥1 prior platinum-based treatment regimen **(Part 1)** or
- Had received at least 3, but not more than 4, prior chemotherapy regimens **(Part 2)**
- **Part 1 only:** if <55 years of age at diagnosis, or has prior history of breast cancer, or has a close relative (first or second degree) with ovarian cancer or early onset (<age 50) breast cancer, must have been previously tested for a gBRCA mutation. (Enrollment of patients known to harbor a germline *BRCA* mutation was capped at 15).
- Have undergone a biopsy of tumor tissue prior to the first dose of study drug and had tumor tissue confirmed by central laboratory as being of adequate quality. (Biopsy was optional for patients in Part 2 known to harbor a deleterious gBRCA mutation)
- Had sufficient archival formalin fixed paraffin embedded (FFPE) tumor tissue available for planned analyses

Patients were <u>not</u> permitted to enroll in Study 2 if they met any of the following key exclusion criteria:

- Diagnosis of low-grade serous or Grade 1 endometrioid cancer
- Received prior treatment with a PARP inhibitor
- Untreated or symptomatic central nervous system (CNS) metastases. Patients with asymptomatic CNS metastases were eligible provided they had been clinically stable for at least 4 weeks.
- Received treatment with chemotherapy, radiation, antibody therapy or other immunotherapy, gene therapy, vaccine therapy, angiogenesis inhibitors, or experimental drugs ≤ 14 days prior to first dose of rucaparib and/or ongoing adverse effects from such treatment > National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Grade 1. Ongoing Grade 2 non-hematologic toxicity related to the most recent treatment regimen was permitted with prior advanced approval.
- Pre-existing duodenal stent and/or any gastrointestinal disorder of defect that would, in the opinion of the investigator, interfere with adsorption of rucaparib
- Presence of any other condition that may increase the risk associated with study participation or may interfere with the interpretation of study results,

and in the opinion of the investigator, would make the patient inappropriate for entry into the study

- **Part 2 only:** Hospitalization for bowel obstruction within 3 months prior to enrollment

2. Follow-up Schedule
   Tumor assessments were performed at baseline and every 8 weeks until disease progression or initiation of subsequent anticancer treatment. Following disease progression, patients discontinued rucaparib treatment, and were continued to be contacted to assess survival status until death or the data cutoff for the primary analysis.

3. Clinical Endpoints
   The efficacy evaluation in Study 2 was performed for the 64 patients in Part 1 and Part 2 who had received ≥ 2 prior chemotherapy regimens and had a deleterious *BRCA* alteration. The efficacy variables used to assess clinical benefit were ORR and DOR by RECIST v1.1.

   For the clinical bridging study, the objectives were agreement between the FoundationFocus™ CDx$_{BRCA}$ and the CTA / local laboratory test (Local Test) result and assessment of the ORR by RECIST v1.1 in the subset of patients with a *BRCA1/2* alteration identified by the FoundationFocus™ CDx$_{BRCA}$ as compared to ORR in the overall primary efficacy population.

B. Accountability of PMA Cohort

A total of 42 patients was enrolled in Study 1 Part 2A between February 2014 and April 2015 at 11 investigational sites in North America, Europe, and Israel. The data to support this PMA reflected efficacy data collected through 30 November 2015. Confirmation of *BRCA* alteration status for a subset of Study 1 patients was subsequently performed using the FoundationFocus CDx$_{BRCA}$.

A total of 204 patients were enrolled in Study 2 Part 1 between October 2013 and December 2014 at 14 investigational sites in North America, Europe and Australia. A total of 41 patients were identified as having a deleterious *BRCA* alteration by either a local laboratory test or by analysis of a screening biopsy or archival tumor sample by the Foundation Medicine CTA. Of these, 24 patients had received ≥ 2 prior chemotherapy regimens. The data to support this PMA reflected efficacy data collected through 29 February 2016 for these 24 patients. Confirmation of *BRCA* alteration status for a subset of Study 2 Part 1 patients was subsequently performed using the FoundationFocus CDx$_{BRCA}$.

A total of 111 patients were enrolled in Study 2 Part 2 between March 2015 and October 2015 at 16 investigational sites in North America, Europe, and Australia. The data to support this PMA reflected efficacy data collected through 29 February 2016 for the 40 patients identified as having a deleterious *BRCA* alteration by either a local laboratory test or analysis of a screening biopsy or archival tumor sample by the

Foundation Medicine CTA. Confirmation of the *BRCA* alteration status for a subset of Part 2 patients was subsequently performed using the FoundationFocus CDx$_{BRCA}$.

At the time of database lock, 106 patients who had been treated with 2 or more prior chemotherapy regimens (N = 42 from Study 1 and N = 64 from Study 2), and were identified as *BRCA*+ (as detected by any method) were included in the clinical bridging study. These 106 patients compose the primary efficacy analysis population for rucaparib clinical efficacy studies and represent the intended use population to support the clinical performance of the device. Out of the 106 patients, specimens from 67 patients were available for retrospective testing with the FoundationFocus CDx$_{BRCA}$ in the clinical bridging study. A total of 64 out of 106 patients from the primary efficacy population identified as *BRCA*+ by the CTA had sufficient remaining DNA samples and passed QC for bridging to the FoundationFocus CDx$_{BRCA}$ test.

C. Study Population Demographics and Baseline Parameters

Overall, enrollment of the 106 patients included in the primary efficacy population occurred at 24 clinical sites in 6 countries (Australia, Canada, France, Spain, United Kingdom, and United States). Twenty-six (26) patients were enrolled in the US.

Demographic and other baseline characteristics for the primary efficacy analysis population are summarized in Table 11. The overall population included 106 female patients with ovarian cancer who received ≥ 2 prior chemotherapy regimens, had a deleterious *BRCA* alteration, and received at least 1 dose of 600 mg rucaparib. Also shown are the characteristics among patients stratified by availability of FoundationFocus CDx$_{BRCA}$ test results; 64 patients with confirmed tumor *BRCA* alteration results with the FoundationFocus CDx$_{BRCA}$ and 42 patients who were not tested with the FoundationFocus CDx$_{BRCA}$.

In general, the demographics and baseline characteristics for patients with and without a FoundationFocus CDx$_{BRCA}$ result are similar, with the exception of geographic region of enrollment. The difference in testing across regions is due to incomplete retrospective collection of tumor tissue samples from patients enrolled in Study 1; thus more patients in this study had only a local test result. The imbalance of demographic and baseline disease characteristics of patients was assessed between patients with and without a FoundationFocus CDx$_{BRCA}$ result. There was very little or no association between the demographic and baseline characteristics versus the presence of a FoundationFocus CDx$_{BRCA}$ result.

Table 11: Demographic Characteristics

Characteristic	Patients with a *BRCA* Alteration Confirmed by FoundationFocus CDx_{BRCA} (N=64)	Patients with a *BRCA* Alteration Identified by Local Test [a] (N=42)	All Patients (N=106)
Age (years)			
Mean (SD)	59.4 (10.6)	58.9 (11.2)	59.2 (10.8)
Median	59.0	58.5	59.0
Min, Max	33.0, 82.0	42.0, 84.0	33.0, 84.0
Age Group (n [%])			
< 65 years	(62.5)	28 (66.7)	68 (64.2)
≥ 65 years	24 (37.5)	14 (33.3)	38 (35.8)
Gender (n [%])			
Male	0	0	0
Female	49 (100.0)	57 (100.0)	106 (100.0)
Race (n [%])			
American Indian or Alaska Native	0	0	0
Asian	4 (6.3)	34 (7.1)	7 (6.6)
Black or African American	2 (3.1)	2 (4.8)	4 (3.8)
Native Hawaiian or Other Pacific Islander	0	0	0
White	51 (79.7)	32 (76.2)	83 (78.3)
Other	0	1 (2.4)	1 (0.9)
Multiple	1 (1.6)	0	1 (0.9)
Missing	6 (9.4)	4 (9.5)	10 (9.4)
Ethnicity (n [%])			
Hispanic or Latino	2 (3.1)	1 (2.4)	3 (2.8)
Not Hispanic or Latino	53 (82.8)	35 (83.3)	88 (83.0)
Not Reported	7 (10.9)	6 (14.3)	13 (12.3)
Missing	2 (3.1)	0	2 (1.9)
Geographic Region (n [%])			
North America	37 (75.5)	20 (5.1)	57 (53.8)
Europe	11 (22.4)	20 (35.1)	31 (29.2)
Other	1 (2.0)	17 (29.8)	18 (17.0)
Geographic Region (n [%])			

Characteristic	Patients with a *BRCA* Alteration Confirmed by FoundationFocus CDx_{BRCA} (N=64)	Patients with a *BRCA* Alteration Identified by Local Test [a] (N=42)	All Patients (N=106)
US Region	19 (29.7)	7 (16.7)	26 (24.5)
Non-US Region	45 (70.3)	35 (83.3)	80 (75.5)
ECOG at Baseline (n [%])			
0	40 (62.5)	25 (59.5)	65 (61.3)
1	24 (37.5)	17 (40.5)	41 (38.7)
≥ 2	0	0	0
Type of Ovarian Cancer (n [%])			
Epithelial Ovarian Cancer	57 (89.1)	34 (81.0)	91 (85.8)
Fallopian Tube Cancer	5 (7.8)	4 (9.5)	9 (8.5)
Primary Peritoneal Cancer	2 (3.1)	4 (9.5)	6 (5.7)
Histological Classification (n [%])			
Serous	58 (90.6)	39 (92.9)	97 (91.5)
Mixed	4 (6.3)	1 (2.4)	5 (4.7)
Endometrioid	2 (3.1)	1 (2.4)	3 (2.8)
Other	0	1 (2.4)	1 (0.9)

[a] Includes 4 patients classified as negative (i.e., not confirmed) for a *BRCA* alteration by the FoundationFocus CDx_{BRCA}.

D. Safety and Effectiveness Results

1. Safety Results

   Safety with respect to treatment with Rubraca™ (rucaparib) will not be addressed in detail in this SSED for the FoundationFocus $CDx_{BRCA.}$ The most common adverse reactions occurring in ≥ 20% of ovarian cancer patients treated with Rubraca™ (rucaparib) were nausea, fatigue (including asthenia), vomiting, anemia, abdominal pain, dysgeusia, constipation, decreased appetite, diarrhea, thrombocytopenia, and dyspnea. Refer to the drug label for more information.

2. Effectiveness Results

   The analysis of effectiveness was based on efficacy data from 106 patients included in the primary efficacy population for Rubraca (rucaparib). Key effectiveness outcomes are presented in Table 12. Analysis of efficacy was based on confirmed ORR and DOR according to RECIST v1.1 as assessed by the investigator. In the 106 patients, confirmed ORR was 53.8% (95% CI: 43.8% to 63.5%) and the median DOR was 9.2 months (95% CI: 6.6 to 11.7 months). The observed ORR is likely to predict clinical benefit in the indicated population.

Response rates were comparable between patients with a tumor *BRCA* alteration detected by the FoundationFocus CDx$_{BRCA}$ test (confirmed ORR of 53.1% [95% CI: 40.2% to 65.7%, N=64]) and those with a *BRCA* alteration identified by another method (confirmed ORR of 54.8% [95% CI: 38.7% to 70.2%, N=42]).

Table 12: Tumor Response by Investigator Assessment

Parameter	*BRCA* Alteration Confirmed by FoundationFocus CDx$_{BRCA}$ (N=64)	*BRCA* Alteration Identified by Local Test[a] (N=42)	All Patients (N=106)
Confirmed Response Rate, n (%)	34 (53.1)	23 (54.8)	57 (53.8)
95% CI	40.2 - 65.7	38.7 - 70.2	43.8 - 63.5

[a] Includes 4 patients classified as negative (i.e., not confirmed) for a *BRCA* alteration by the FoundationFocus CDx$_{BRCA}$.

a.Assay Bridging Study

The purpose of this study was to evaluate agreement between the Foundation Medicine CTA and local laboratory tests used to identify patients during the clinical trials and the FoundationFocus CDx$_{BRCA}$ assay for the detection of *BRCA1/2* alterations. In addition, the primary efficacy endpoint of ORR by RECIST v1.1, was evaluated in the subset of patients with a *BRCA1/2* alteration identified by the FoundationFocus CDx$_{BRCA}$ and in the primary efficacy population. In these analyses, only CTA negative test samples are included as samples identified as negative by local laboratory tests were not available for retesting with the FoundationFocus CDx$_{BRCA}$ assay. A high positive percent agreement (97%, 64 of 66, two-sided exact 95% CI [89.5%, 99.6%]) was found between the CTA and FoundationFocus CDx$_{BRCA}$ results. In addition, a perfect negative percent agreement (100.0%, 29 of 29, two-sided exact 95% CI [88.1%, 100.0%]) was found between the CTA and FoundationFocus CDx$_{BRCA}$ results for the randomly selected *BRCA* cases in the bridging study. Combined *BRCA*+ and *BRCA*- results show a high overall percent agreement (97.9%, two-sided exact 95% CI [92.6%, 99.7%]) between the CTA and FoundationFocus CDx$_{BRCA}$ results (Table 13).

Table 13: Bridging Study Agreement Results

	Positive by CTA/Local Tests	Negative by CTA Test	Total
Positive by FoundationFocus CDx_{BRCA}	64	0	64
Negative by FoundationFocus CDx_{BRCA}	2*	29	31
Total	66	29	95
Agreement	**PPA [95% CI]**	97.0% [89.5%, 99.6%]	
	NPA [95% CI]	100% [88.1%, 100.0%]	
	OPA [95% CI]	97.9% [92.6%, 99.7%]	

*Two patients had a large rearrangement detected by a local test, but not by the FoundationFocus CDx_{BRCA}.

3. Subgroup Analyses

To further evaluate if there was bias in the subset of patients who were not tested with the FoundationFocus CDx_{BRCA} test, an imputation analysis was performed to address the subset of patients who were not tested with the FoundationFocus CDx_{BRCA} assay and to evaluate how the rate of missing data might affect the drug efficacy analysis in the intended population for the device. Two efficacy sets were analyzed separately. The first efficacy set included a total of 236 patients who had received at least 2 prior chemotherapies in both clinical studies. The second efficacy set included 194 patients who had received at least 2 prior chemotherapies in Study 2 only.

Two different logistic regression models were used to identify clinically relevant covariates. The first model identified covariates associated with a FoundationFocus CDx_{BRCA} result where the dependent variable was FoundationFocus CDx_{BRCA}-positive vs. FoundationFocus CDx_{BRCA}-negative. The second model identified covariates associated with clinical outcome where the dependent variable was ORR responder (CR or PR) vs. non-responder as assessed by the investigator. Covariates with a p-value < 0.20 from a univariate model with either dependent variable are considered to be clinically relevant. The following is a list of covariates that were investigated in both models:

- CTA/Local Tests result (positive if either the CTA or Local Tests result is positive);
- Age;
- Race (White vs non-White);
- Region (North America vs Rest of World);
- ECOG performance status;
- Height;

- Weight;
- Ovarian cancer sub-type;
- Time since diagnosis;
- Number of prior chemotherapies;
- Progression-free interval following last platinum.

For the model with the FoundationFocus CDx$_{BRCA}$ result as the dependent variable in efficacy set 1, the covariates of region (North America vs. Rest of World), baseline height, time since diagnosis, and platinum-free interval had a p-value < 0.20. No additional covariates had a p-value < 0.20 for this model using efficacy set 2.

The number of prior chemotherapies was the only additional covariate with a p-value < 0.20 in the model with ORR as the dependent variable in either efficacy set 1 or 2. Therefore, the logistic regressions along with the CTA/Local Tests results and ORR comprise the following list of 7 clinically relevant covariates:

- CTA/Local Tests result (positive if either the CTA or Local Tests result is positive);
- ORR;
- Region;
- Height;
- Time since diagnosis;
- Number of prior chemotherapies;
- Platinum-free interval.

The CTA/Local Test results and the platinum-free interval are known to have an impact on the outcome and FoundationFocus CDx$_{BRCA}$ response.

Confirmation of *BRCA* status by the FoundationFocus CDx$_{BRCA}$ test was available for 95 of 236 patients in efficacy set 1. For the 141 patients with an unknown FoundationFocus CDx$_{BRCA}$ result, the *BRCA* status was imputed based on a probability distribution based on the CTA/Local Tests and clinically relevant covariates identified above. The 'FIRTH' option in the SAS logistic regression procedure was used in order to account for the high association between the CTA/Local Test results and the FoundationFocus CDx$_{BRCA}$ results. The results of the multiple imputation using patient-specific probabilities are summarized in Tables 14 and Table 15 for efficacy set 1 and efficacy set 2, respectively.

Table 14: Efficacy Set 1 Results from 30 Total Imputations

	Positive by FoundationFocus CDx_{BRCA}	Negative by FoundationFocus CDx_{BRCA}	Total (N=236)
Observed status	61	34	95
Mean among patients with unknown result	40	101	141
Mean total number	101	135	236
Combined ORR	52.5	11.8	29.2
95% CI	42.4, 62.60	6.0, 17.6	23.5, 35.5

Table 15: Efficacy Set 2 Results from 30 Total Imputations

	Positive by FoundationFocus CDx_{BRCA}	Negative by FoundationFocus CDx_{BRCA}	Total (N=194)
Observed status	47	33	80
Mean among patients with unknown result	16	98	114
Mean total number	63	131	194
Combined ORR	49.2	9.9	22.7
95% CI	36.4, 61.9	4.6, 15.1	17.0, 29.2

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 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

The clinical benefit of the FoundationFocus CDx$_{BRCA}$ was demonstrated through analysis of efficacy data obtained from two open-label, single-arm studies, in which rucaparib demonstrated a clinically meaningful confirmed ORR and DOR in 106 ovarian cancer patients with a deleterious *BRCA* alteration who had received treatment with 2 or more prior chemotherapy regimens. Among the 106 patients, tumor specimens from 67 patients were available for retrospective testing by the FoundationFocus CDx$_{BRCA}$. Upon testing, 64 of these 67 patients (95%) were verified to have a deleterious *BRCA* alteration in tumor tissue per the FoundationFocus CDx$_{BRCA}$.

Analysis of the subset of 64 patients identified as harboring a *BRCA* alteration in tumor tissue by FoundationFocus CDx$_{BRCA}$ demonstrated a confirmed ORR of 53.1% (95% CI: 40.2% to 65.7%, N=64). These results were similar to those observed in overall population of 106, for which the ORR was 53.8% (95% CI: 43.8% to 63.5%). In a sensitivity analysis of patients with only a local laboratory test result, the confirmed ORR was 54.8% (95% CI: 38.7% to 70.2%, N=42). These results demonstrate that the ORR and DOR for the patients with a FoundationFocus CDx$_{BRCA}$ result are comparable to the ORR and DOR for the patients identified by another method. Additional robustness and worst case scenario analyses supported these results.

Analytical validation studies demonstrated that the analytical sensitivity of the FoundationFocus CDx$_{BRCA}$ is 6% MAF in non-repetitive genomic regions and 15.3% MAF in certain homopolymer regions when using 200 ng of DNA for LC. Reproducibility, repeatability and guard band studies support consistent and robust performance of the assay. Further, there was 97.0% positive agreement and 100% negative agreement between the FoundationFocus CDx$_{BRCA}$ versus a validated comparator method.

B. Safety Conclusions

The risks of the device are based on data collected in the validation studies conducted to support PMA approval as described above. The FoundationFocus CDx$_{BRCA}$ assay is an in vitro diagnostic test, which involves testing of DNA extracted from OC FFPE tumor tissue.

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 ovarian cancer treatment. Patients with false positive results may undergo treatment with Rubraca without clinical benefit, and may experience adverse reactions associated with Rubraca therapy. Patients with false negative results

may not be considered for treatment with Rubraca. There is also a risk of delayed results, which may lead to delay of treatment with Rubraca.

C. Benefit-Risk Determination

The probable benefits of the device are also based on data collected in two clinical studies conducted to support PMA approval as described above. The clinical benefit of the FoundationFocus CDx*BRCA* assay was demonstrated in a retrospective analysis of efficacy and safety data obtained from these two open-label, non-randomized studies in which rucaparib demonstrated a clinically meaningful ORR and DOR in patients with BRCA mutated advanced ovarian cancer treated with 2 or more chemotherapy regimens. Tumor tissue samples from a subset of enrolled patients were subsequently tested with the FoundationFocus CDx*BRCA* assay to verify *BRCA* alteration status. The observed clinical benefit in the subset of patients with *BRCA* alteration confirmed by the FoundationFocus CDx*BRCA* assay was comparable to that observed for all patients in the primary efficacy analysis population and to that observed in the subset of patients with a *BRCA* alteration identified by another method. In the group of 106 patients included in the primary efficacy population, the confirmed ORR was 53.8% and the median DOR was 9.2 months.

Additional factors to be considered in determining probable risks and benefits for the FoundationFocus CDx*BRCA* included: analytical performance of the device, representation of variants, additional and ongoing analytical testing, and the lack of availability of alternative tests for the safe and efficacious use of rucaparib. Notably, certain variants including larger rearrangements and homozygous deletions have not been validated by the FoundationFocus CDx*BRCA* and were therefore excluded from the efficacy analyses. Additional analytical testing will be performed in the post-approval setting. Further, test results from confirmatory clinical studies will be assessed to examine a more comprehensive set of variants. Risks associated with the FoundationFocus CDx*BRCA* assay include the possibility of inaccurate results that may lead to mismanagement of patient treatment. The available information supports the use of FoundationFocus CDx*BRCA* assay as an aid in the identification of OC patients for whom Rubraca treatment may be indicated.

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* assay the probable benefits outweigh the probable risks.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical studies, in which Rubraca demonstrated a clinically meaningful response rate and DOR in ovarian cancer patients with a deleterious BRCA alteration,

support the indicated use of this device as an aid for identification of ovarian cancer patients for whom Rubraca therapy may be indicated.

XIII. CDRH DECISION

CDRH issued an approval order on December 19, 2016. The final conditions of approval cited in the approval order are described below.

1. As reflected in the labeling for FoundationFocus CDx$_{BRCA}$, a limited range of variant types were included in some of the analytical validation studies. Additional testing of samples is required to establish the analytical performance characteristics of your device for all variant types that may be detected. Please ensure that samples adequately cover the range of insertions, deletions, and large rearrangements detected by your device, with consideration to variant lengths and genomic contexts. The results from these studies should be included in the labeling.

2. A limited amount of clinical samples were able to be bridged to FoundationFocus CDx$_{BRCA}$ (64/106 = 60%) in the clinical validation study, and these samples included a limited range of variant types. Please plan to provide FoundationFocus CDx$_{BRCA}$ results from the ongoing ARIEL 2 study upon completion of the trial. If patients were initially screened for *BRCA1/2* using a local test or clinical trial assay (CTA), a bridging study between the local test/CTA and FoundationFocus CDx$_{BRCA}$ will be required. Please be advised that results from this study could lead to labeling changes.

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

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

1. Fisher S., Barry A., Abreu J., et al. A scalable, fully automated process for construction of sequence-ready human exome targeted capture libraries. *Genome Biol* **12**, R1 (2011).
2. Karolchik, D., Hinrichs AS, Kent WJ., et al. The UCSC Table Browser data retrieval tool. *Nucleic Acids Res* **32**, D493-496 (2004).
3. Gnirke A., Melnikov A., Maguire J., et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. *Nat Biotechnol* **27**, 182-189 (2009).

4. Li H. & Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* **26**, 589-595 (2010).
5. Li H., Handsaker B., Wysoker A., et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078-2079 (2009).
6. DePristo MA., Banks E., Poplin R., et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* **43**, 491-498 (2011).
7. Forbes SA, Bindal N., Bamford S., et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. *Nucleic Acids Res* **39**, D945-950 (2011).
8. Compeau PE., Pevzner PA., Tesler, G. How to apply de Bruijn graphs to genome assembly. *Nat Biotechnol* **29**, 987-991 (2011).