DRAFT FoundationFocus CDx*BRCA* LOH
Technical Information Summary

Intended 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.

Contraindication

None.

Warnings and Precautions

- *BRCA1/2* alterations reported include somatic (not inherited) or germline (inherited) alterations; however, the test does not distinguish between germline and somatic alterations. The test does not provide information about susceptibility.
- Biopsy may pose a risk to the patient when archival tissue is not available for use with the assay. The patient's physician should determine whether the patient is a candidate for biopsy.

Limitations

- For *in vitro* diagnostic use.
- For prescription use only.
- For professional use only. This test must be ordered by a qualified medical professional in accordance with clinical laboratory regulations.
- Performance of the assay has not been established for samples below 35% tumor content and with LOH scores near the cut-off of 16.
- Limited performance characteristics of the test were evaluated for insertion alterations > 4 nucleotides and deletions >10 nucleotides.
- Performance was not established for insertions > 10 nucleotides, deletions >12 nucleotides, alterations residing in polyC homopolymer runs, homozygous deletions or large rearrangements.

- Alterations in polyT homopolymer runs may not be reliably detected.
- Alterations detected at allele frequencies below the established limit of detection are not detected consistently.
- There may be potential interference of ethanol with LOH detection. The interfering effects of xylene, hemoglobin, and triglycerides on the LOH score have not been demonstrated.
- Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostic tests, and patient preferences, in accordance with the standard of care in a given community.
- The test is intended to be performed at a single site on specific serial number-controlled instruments at Foundation Medicine, Inc.

Test Principle

The FoundationFocus CDx*BRCA* LOH assay is performed using DNA extracted from ovarian cancer (OC) tumor specimen(s) and reports *BRCA1/2* alterations including somatic (not inherited) or germline (inherited) alterations. The FoundationFocus CDx*BRCA* LOH assay also reports genomic LOH. Collectively, patients with these *BRCA* alterations and/or genomic LOH are referred to as HRD (t*BRCA*+ and/or LOH high).

The NGS assay starts with extracted DNA from FFPE biopsy or surgical resection specimens. 50 ng of the sample is subjected to whole-genome shotgun library construction and hybridization-based capture of all coding exons from *BRCA1* and *BRCA2*. Using the Illumina® HiSeq™ 4000 platform, hybrid-capture–selected libraries are sequenced to high uniform depth (targeting >500X median coverage with >99% of exons at coverage >100X). The FoundationFocus CDx *BRCA* LOH assay uses a custom-developed analysis pipeline to identify *BRCA1* and *BRCA2* base substitutions and insertion/deletions (indels) up to 13bp, as well as LOH.

The genomic LOH score is assessed based on the percent of LOH in the tumor genome. To compute genomic LOH for each tumor, LOH regions will be inferred across the 22 autosomal chromosomes using the genome-wide copy number profile and minor allele frequencies of the single nucleotide polymorphisms (SNPs). Certain LOH regions will be excluded from analysis, including (1) LOH regions spanning across ≥90% of a whole chromosome or chromosome arm as these LOH events are thought to be due to non-HRD mechanisms; and, (2) regions in which LOH inference is ambiguous. A patient with genomic LOH ≥16 is regarded as "LOH high", and a patient with genomic LOH <16 is "LOH low", and a patient with indeterminable result is "LOH unknown".

Table 1 describes how genomic LOH is detected and reported as either high or low.

Table 1: LOH Classification Schema

LOH Status	Qualification Criteria	Methodology
LOH high	LOH score ≥ 16	Sequence analysis identifies LOH across the genome and summarizes it into a genomic LOH score
LOH low	LOH score < 16	Sequence analysis identifies LOH across the genome and summarizes it into a genomic LOH score

Tables 2 and 3 describe the detected variants in *BRCA1* or *BRCA2* classified according to criteria that includes presence of alterations known to be deleterious to BRCA protein function rendering the sample t*BRCA*+.

Table 2: Subgroups and Classification Schema

BRCA Status	Qualification Criteria	Sequence Classification	Methodology
tBRCA+	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 -/+ 2 bp of exon starts/ends
		Deleterious missense alterations	Curated list (see Table 3)
t*BRCA*-	Negative for t*BRCA*		

Table 3: Deleterious *BRCA1* and *BRCA2* Missense Mutations Used to Define t*BRCA*+ Status

***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)*

***BRCA1* Alterations (Protein Change)**	***BRCA2 Alterations (Protein Change)***
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 (G1788V)	

Summary and Explanation

The Foundation Medicine FoundationFocus CDx*BRCA* LOH assay is a companion diagnostic for identifying patients that may benefit from monotherapy treatment and a complementary diagnostic associated with maintenance therapy with Clovis Oncology's drug Rubraca, a poly ADP-ribose polymerase (PARP) inhibitor. See the RUBRACA product label for information about guiding therapy in specific clinical circumstances.

Test Kit Contents

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

- Specimen Preparation and Mailing Instructions
- Shipping Label
- Return Shipping Label
- Technical Information Summary

All other reagents, materials and equipment needed to perform the assay are used exclusively in the Foundation Medicine laboratory. The FoundationFocus CDx *BRCA* LOH assay is intended to be performed with serial number-controlled instruments.

Sample Collection and Test Ordering

To order the FoundationFocus CDx *BRCA* LOH assay, the Test Requisition Form (TRF) included in the test kit must be fully completed and signed by the ordering physician or other authorized medical professional. Please refer to Specimen Preparation Instructions and mailing instructions include in the test kit.

For more detailed information, including Performance Characteristics, please find complete Technical Information at: [website to be inserted upon sPMA approval]

Foundation Medicine, Inc.
150 Second Street, Cambridge, MA 02141
Phone: 617.418.2200

1. Intended Use

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2. Contraindication

None.

3. Warnings and Precautions

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5. Test Principle

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To order the FoundationFocus CDx*BRCA* LOH assay, the Test Requisition Form (TRF) included in the test kit must be fully completed and signed by the ordering physician or other authorized medical professional. Please refer to

Specimen Preparation Instructions and mailing instructions include in the test kit.

For more detailed information, including Performance Characteristics, please find complete Technical Information at: [website to be inserted upon sPMA approval]

9. Instruments

The FoundationFocus CDx*BRCA* LOH device is intended to be performed with the following instruments, as identified by specific serial numbers:

- Agilent Benchbot Workstation with Integrated Bravo Liquid Handler
- Beckman Biomek NxP Span-8 Liquid Handler
- Covaris Focused Ultrasonicator LE220
- Thermo Scientific Kingfisher Flex with 96 Deep-well Head
- Illumina cBot
- Illumina HiSeq 4000 System

10. Test Results and Interpretation

Patients evaluated with the FoundationFocus CDx*BRCA* LOH test who are determined to carry a deleterious or suspected deleterious *BRCA1* or *BRCA2* alteration, inclusive of both somatic and germline alterations, as well as genomic loss of heterozygosity, can be considered for treatment with Rubraca under the supervision of a physician.

Upon completion of testing at Foundation Medicine, a test report will be sent to the designated physician. The results of the identified alteration(s) including *BRCA1*, *BRCA2* and LOH status, are provided in the report. Only alterations defined as deleterious in Section 5 Test Principle classification schema are reported. A negative result indicates that no alterations consistent with the classification schema were detected.

11. Performance Characteristics

11.1 Accuracy/Concordance Study

The concordance of the *BRCA1/2* alteration detection component of the FoundationFocus CDx*BRCA* LOH assay was compared to results of an externally developed NGS assay run in a CLIA laboratory. This study included 36 t*BRCA*+ and 44 tumor *BRCA*- (tBRCA-) ovarian cancer (OC) 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). Concordance data with and without invalid results between the FoundationFocus CDx*BRCA* LOH assay and the comparator assay are shown in Table 4. Both of the discordant samples were found to be *BRCA*+ by a second validated NGS comparator method. Accuracy studies are ongoing.

Table 4: Agreement between FoundationFocus CDx*BRCA* LOH and NGS Comparator Assay

		NGS Comparator Assay			Total
		***BRCA*+**	***BRCA*-**	**Invalid**	
FoundationFocus CDx*BRCA* LOH	***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, 1 sample was detected in a region not covered by the comparator assay.

11.2 *In Silico* Assessment of LOH at BRCA

Since an accuracy study for LOH cannot be conducted due to the lack of well-validated orthogonal methods that measure the genomic LOH score, an *in-silico* assessment of LOH was performed to compare the rate of LOH at *BRCA1* and *BRCA2* genes in patient samples from study CO-338-014 (ARIEL3) as compared to published literature.[1, 2, 3, 4] The ARIEL3 patient sample counts are tabulated per LOH and t*BRCA* status and shown in Table 5. A significantly higher rate of LOH is shown 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*).

Furthermore, 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 published literatures: 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;[3] Hilton et al. (2002) found 92% (12/13) of ovarian cancer patients harboring a deleterious *BRCA2* mutation also had LOH at *BRCA2*;[1] Press et al. (2008) found 78% (7/9) of ovarian cancer patients harboring a

deleterious *BRCA1* mutation also had LOH at *BRCA1*.[4] The number of patients and their t*BRCA* and LOH status is shown below in Table 5. The results supported the published literatures that significantly higher rate of LOH was observed in the presence of t*BRCA* mutations, as compared to the rate of LOH in the absence of t*BRCA* mutations.

Table 5: Presence of LOH at *BRCA1/2* Loci in Patients with and without BRCA Mutations

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

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

11.3 Analytical Specificity

11.3.1 Interfering Substances

To evaluate the potential impact of interfering substances on the performance of the FoundationFocus CDx$_{BRCA\ LOH}$ assay, this study evaluated formalin-fixed paraffin-embedded (FFPE) tumor samples in the presence of exogenous and endogenous substances. Three OC FFPE samples were assessed with eight replicates each, 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.

For *BRCA* classification, all of the samples exhibited 100% 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.

Additional analysis to study the impact of interfering substances (triglycerides, xylene, hemoglobin, Proteinase K, ethanol and Molecular Bar Indexes) on LOH will be performed as a post-marketing commitment.

11.3.2 *In silico* Analysis – Hybrid Capture Bait Specificity

The specificity of the hybrid capture oligonucleotide 120-mer baits at exons of *BRCA*1/2 genes was evaluated for FoundationFocus CDx *BRCA* LOH. The analysis was performed *in silico* and retrospectively on process control samples. It was limited to only the genes pertinent to FoundationFocus CDx*BRCA* LOH assay: *BRCA1* and *BRCA2,* for a total of 359 and 271 baits for the genes, respectively. This study assessed whether the 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 as having 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 to not 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.

11.4 Carryover/Cross-contamination

DNA sample carryover and cross-contamination during the library construction, hybrid capture 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 single nucleotide polymorphisms (SNPs) that are homozygous in a patient, contamination of the sample by another human sample leads to baseline SNP allele frequencies significantly above 0%. Therefore, the contamination level was detected 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.

Cross-contamination of LOH was examined, as part of the precision study, where samples with low scores in apposition to samples with high scores in several plates demonstrate no carryover or cross-contamination.

11.5 Precision/Reproducibility from Extracted DNA

Precision, including intra-run, inter-run, lot-to-lot and sequencing instrument-to-instrument reproducibility, of the FoundationFocus CDx$_{BRCA\ LOH}$ assay from extracted DNA was evaluated. Precision was assessed by testing a set of 25 OC samples representing different variant types and genomic contexts, in duplicate, using three instruments and three reagent lots, at and near the limit of detection (LoD) for mutant allele frequency (MAF) levels. 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%.

To establish intermediate precision for LOH, *in silico* reanalysis was performed on the same set of samples used for the original intermediate precision study. Of the 25 samples, 21 passed the LOH QC status in natural (undiluted) level 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 the 18 replicates (2 duplicates × 3 sequencers × 3 reagent lots) and the repeatability (intra-run reproducibility) was evaluated by concordance across duplicates within the same sequencer/reagent lot. Replicates that failed the LOH QC were excluded from the primary analysis. The results showed 100% reproducibility in 18 samples and 94.4% (17 out of 18 replicates) reproducibility in 2 samples. The only sample that showed <90% reproducibility has an LOH score close to the pre-specified clinical cutoff of 16, which is expected to be more sensitive to variability across technical replicates (Table 6). For repeatability, majority of the samples showed high repeatability; the 3 samples that had discordant LOH classification have LOH scores close to the cutoff of 16.

Table 6: Reproducibility Results of LOH classification

Sample	Tumor Content*	LOH Score Avg (Stdev)	Majority Call	Reproducibility	95% CI
1[#]	78%	3.46 (0.19)	LOH low	100%	[71.8% 100%]
2	56%	6.71 (1.31)	LOH low	100%	[79.3% 100%]
3	59%	11.11 (0.26)	LOH low	100%	[79.3% 100%]
4	71%	12.42 (0.73)	LOH low	100%	[79.3% 100%]
5	58%	14.55 (1.72)	LOH low	77.8%	[54.3% 91.5%]
6	72%	18.69 (1.72)	LOH high	94.4%	[72.4% 100%]
7	43%	21.74 (2.41)	LOH high	100%	[79.3% 100%]
8	61%	18.83 (1.21)	LOH high	100%	[79.3% 100%]
9	46%	17.51 (0.8)	LOH high	94.4%	[72.4% 100%]
10	73%	21.48 (0.96)	LOH high	100%	[79.3% 100%]
11	85%	29.21 (0.39)	LOH high	100%	[79.3% 100%]
12	58%	27.16 (0.66)	LOH high	100%	[79.3% 100%]
13	96%	21.88 (1.94)	LOH high	100%	[79.3% 100%]
14	76%	23.88 (1.64)	LOH high	100%	[79.3% 100%]
15	60%	28.73 (1.64)	LOH high	100%	[79.3% 100%]
16	54%	28.25 (0.66)	LOH high	100%	[79.3% 100%]
17	46%	28.6 (0.76)	LOH high	100%	[79.3% 100%]
18	53%	29.5 (0.89)	LOH high	100%	[79.3% 100%]
19	42%	31.09 (1.82)	LOH high	100%	[79.3% 100%]
20	66%	33.61 (1.09)	LOH high	100%	[79.3% 100%]
21	88%	39.58 (0.86)	LOH high	100%	[79.3% 100%]

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

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

Variance component analysis based on LOH scores (quantitative measures) was also performed. The percent coefficient of variation (%CV) for repeatability and reproducibility are both ≤30% for samples with mean LOH score ≥10. This evaluation passed the pre-specified acceptance criteria. The results are summarized in Table 7 below:

Table 7: Results of variance component analysis of LOH score

Sample	Mean LOH Score	SD (Repeatability)	CV (Repeatability)	SD (Lot)	CV (Lot)	SD (Seq)	CV (Seq)	SD (Reproducibility)	CV (Reproducibility)
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%

Analysis was performed to evaluate different sequencers and lots for each sample. Concordance was calculated as the total number of same reagent and lot runs with consistent LOH status between the two within-run replicates divided by total number across lots and sequencers. The results are shown in Table 8 (95% exact CI included). As expected, the three samples that have less than 100% reproducibility (Samples 5, 6, and 9) for LOH status also show less than 100% overall repeatability, primarily due to the LOH scores being close to the cutoff of 16 (a low level of variability in LOH score would result in changes in LOH classification based on the cutoff).

Table 8. Repeatability assessment as overall percent agreement of duplicates within the same run across lots and sequencers, for LOH status

Sample	Number Concordant	Total Number	Concordance	95% CI
1	6	6	100%	[54.1% 100%]
2	9	9	100%	[66.4% 100%]
3	9	9	100%	[66.4% 100%]
4	9	9	100%	[66.4% 100%]
5	5	9	55.6%	[21.2% 86.3%]
6	8	9	88.9%	[51.8% 99.7%]
7	9	9	100%	[66.4% 100%]
8	9	9	100%	[66.4% 100%]
9	8	9	88.9%	[51.8% 99.7%]
10	9	9	100%	[66.4% 100%]
11	9	9	100%	[66.4% 100%]
12	9	9	100%	[66.4% 100%]
13	9	9	100%	[66.4% 100%]
14	9	9	100%	[66.4% 100%]
15	9	9	100%	[66.4% 100%]
16	9	9	100%	[66.4% 100%]
17	9	9	100%	[66.4% 100%]
18	9	9	100%	[66.4% 100%]
19	9	9	100%	[66.4% 100%]
20	9	9	100%	[66.4% 100%]
21	9	9	100%	[66.4% 100%]

The repeatability was also assessed for HRD status and the results are shown in Table 9 (95% exact CI included). All samples are included except Sample 5 (with an overall agreement of 55.56%).

Table 9. Repeatability assessment as overall percent agreement of duplicates within the same run across lots and sequencers, for HRD status

Sample	Number Concordant	Total Number	Concordance	95% CI
1	6	6	100%	[54.1% 100%]

Sample	Number Concordant	Total Number	Concordance	95% CI
2	9	9	100%	[66.4% 100%]
3	9	9	100%	[66.4% 100%]
4	9	9	100%	[66.4% 100%]
5	5	9	55.6%	[21.2% 86.3%]
6	9	9	100%	[66.4% 100%]
7	9	9	100%	[66.4% 100%]
8	9	9	100%	[66.4% 100%]
9	9	9	100%	[66.4% 100%]
10	9	9	100%	[66.4% 100%]
11	9	9	100%	[66.4% 100%]
12	9	9	100%	[66.4% 100%]
13	9	9	100%	[66.4% 100%]
14	9	9	100%	[66.4% 100%]
15	9	9	100%	[66.4% 100%]
16	9	9	100%	[66.4% 100%]
17	9	9	100%	[66.4% 100%]
18	9	9	100%	[66.4% 100%]
19	9	9	100%	[66.4% 100%]
20	9	9	100%	[66.4% 100%]
21	9	9	100%	[66.4% 100%]

The majority of samples show 100% overall repeatability within the same run across lots and sequencers, except three samples for LOH status and one sample for HRD status with less than 100% agreement, primarily due to the LOH scores of those samples being close to the cutoff of 16.

11.6 Limit of Detection (LoD), Limit of Blank (LoB) and Analytical Sensitivity

LoD of the FoundationFocus CDx$_{BRCA\ LOH}$ assay for *BRCA*1/2 alterations was assessed. Seven OC samples possessing different categories of *BRCA*1/2 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.

Eleven t*BRCA*- samples were assessed for LoB with each sample processed in nine replicates. A total of 93 samples 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*-variant calls and there were no false positive *BRCA* calls; thus, confirming the LoB of zero.

Analytical sensitivity for tumor purity for the LOH classification was assessed in OC samples that represent a wide range of LOH scores: close to 0, just below or above the LOH threshold of 16, and appreciably above the threshold. Based on the criteria, 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 selected and the extracted DNA from each sample was mixed with matched normal control to attain five levels of tumor purity ranging from 10% to 50% (10%, 20%, 30%, 40%, and 50%). The Hit Rate method was used to determine the minimum tumor fraction required to support the robustness of the test for assessing LOH low and LOH high samples. The data validated that the minimum tumor purity is of 35%, meeting the required acceptance criteria.

11.7 Stability

11.7.1 Reagent Stability

The stability of critical reagent lots used in the library construction (LC), hybrid capture (HC) and sequencing processes within the FoundationFocus CDx*BRCA* LOH assay were evaluated in this study. Three lots of each set of reagents were stored under the manufacturer's specified temperature conditions (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 those from samples immediately tested (time point T_0). Six (6) stability time points (T_0, T_{30}, T_{60}, T_{90}, T_{120}, and T_{150}), spanning zero to 150 days, were evaluated herein. The results suggest that 100% of the *BRCA1/2* variant calls and LOH status for each of the three reagent lots, across all time points, were concordant to those variants identified during the baseline evaluation (T_0). Reagent lot performance and stability were validated for 5 months (150 days) for the LC and HC kits, and 4 months (120 days) for the sequencing kits. The claimed reagent kit stability is: 4 months (120 days) for the LC and HC kits, and 3 months (90 days) for the sequencing kits.

11.7.2 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 *BRCA*1/2 genes were assessed in triplicate at day zero, T_0, 6 weeks at 4ºC, and 3 months at -20ºC. Results from each time point were compared to those from T_0 to determine if the same results were obtained from stored samples. 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. To date, 100% of sample aliquots were successfully processed (T_0 through T_6). Agreement across *BRCA1/2* variant calls and LOH status is 100% concordant with baseline calls for each of the FFPE DNA assessed.

11.7.3 FFPE Sample Stability

The stability of the FFPE specimens used for FoundationFocus CDx*BRCA* LOH assay was evaluated retrospectively by examining DNA extraction yields from FFPE OC tissue samples. A total of 3195 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. To date, all twenty samples of T_0 and T_1 were successfully processed. All samples replicates are concordant for both their respective *BRCA1/2* variants and LOH status.

11.8 Reagent Lot Interchangeability

Reagent lot interchangeability was assessed by testing four OC samples containing alterations in the *BRCA1/2* genes in duplicate using two different lots each of Library Construction, Hybrid Capture, 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%.

11.9 General Lab Equipment and Reagent Evaluation

11.9.1 DNA Amplification

Thermal cycler interchangeability during the post-library construction (LC) and post-HC process steps was evaluated for the FoundationFocus CDx*BRCA* LOH assay. Eight replicate aliquots for each of three OC FFPE samples were processed in parallel, with two replicates being 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 four thermal cycler pairings

11.9.2 DNA Extraction

The performance of the DNA extraction from FFPE ovarian cancer tumor specimens was evaluated. The DNA extraction procedure for the FoundationFocus CDx*BRCA* LOH assay was assessed by testing 46 FFPE specimens, in triplicate (employing two different KingFisher Flex Magnetic Particle Processors) and comparing across three extraction reagent lots. Overall success was 405 of 414 samples exhibiting DNA yields ≥200 ng after the DNA extraction step, for a 97.8% success rate.

11.9.3 Guard Banding/Robustness

Guard banding studies were completed to evaluate the performance of the FoundationFocus CDx*BRCA* LOH assay and the impact of process variation with regard to uncertainty in the measurement of DNA concentration at various stages of the process. Guard bands were evaluated relative to observed and measured process variability for Library Construction (LC), Hybrid Capture (HC), and Sequencing.

11.9.4 Library Construction Guard Banding

To evaluate the impact of DNA input into LC on detection of *BRCA1/2* variants and LOH classification, a LC guard banding study was conducted. Six samples were run in triplicate (N=18) over five different DNA input levels (10, 20, 50, 100, and 200 ng) representing 5%-100% of the standard DNA input (200 ng) as defined by the LC guard banding study for the original PMA p160018. Samples and replicates that failed to meet the processing specifications for LC, HC and sequencing were predominantly in the lowest DNA input levels (10 and 20 ng) and excluded from the concordance analysis for *BRCA1/2* variants and LOH status. 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. Similarly, the LOH status was highly concordant (≥93.8%) and exceeded the acceptance criterion (>90%). The only one discordant sample has LOH scores close to the cutoff of 16, which rendered disagreement in LOH status within its replicates. These results support a DNA input of 50 ng into LC for detection of *BRCA1/2* variants and LOH classification.

11.9.5 Hybrid Capture Guard Banding

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.

11.9.6 Sequencing Guard Banding

The third component of the guard banding study evaluated the captured DNA input into the sequencing reaction. Five samples were run in replicate over five

different DNA input levels - 100 ng, 160 ng, 200 ng, 240 ng and 300 ng - representing ±25% and ±50% of the required amount needed for sequencing (n=50). For each of the five input DNA levels evaluated, 100% of the samples met all required sequencing metrics. An analysis of median depth of coverage did not indicate a significant difference in the resulting sequencing content, supporting assay performance for input levels mentioned above.

11.10 Bridging

The purpose of this study was to evaluate agreement between the Foundation Medicine clinical trial assay (CTA) 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 Objective Response Rate (ORR) by RECIST v1.1, was evaluated in the subset of patients with a *BRCA1/2* alteration identified by the CDx and in the primary efficacy population. A total of 64 patients from primary efficacy (PE) population identified as *BRCA*+ by the CTA had sufficient remaining DNA samples and passed QC for bridging to the CDx test. A high positive percent agreement (100%, 64 of 64, two-sided exact 95% CI [94.4%, 100%]) was found between the CTA and CDx results. Note that three samples included certain genetic alterations (e.g. indels>13bp, homozygous deletion) not included in other analytic validation studies. In addition, a perfect negative percent agreement (100.0%, 79 of 79, two-sided exact 95% CI [95.4% 100.0%]) was found between the CTA and CDx results for the randomly selected BRCA- cases in the bridging study. Combined BRCA+ and BRCA- results show a high overall percent agreement (100%, two-sided exact 95% CI [97.5% 100.0%]) between the CTA and CDx results.

No bridging study was required for the supplemental PMA submission, because the clinical outcome for ARIEL3 (Table 10) was measured directly with the FoundationFocus CDx*BRCA* LOH test.

11.11 Clinical Validation

An analysis was performed to evaluate positive percent agreement between CTA and Complementary Dx. Of the 564 patients randomized in this study, 518 (92%) were successfully tested and available for concordance analysis. The overall efficacy result based on Complementary Dx is very robust like the results from the CTA HRD groups. The overall concordance was high between the CTA and Complementary Dx with a PPA greater than 90% for all patients. The exploration of the effect of missing data on the efficacy results showed the hazard ratios for the rucaparib-treated group vs the placebo-treated group to be robust and similar for patients classified by either the CTA or the Complementary Dx. The results for all patients (irrespective of treatment) is presented and the agreement is also explored by each randomized treatment group which has similar distributions. The primary endpoint was achieved with a highly statistically significant difference in investigator-assessed PFS (invPFS) between rucaparib

treatment compared to placebo for each of the three primary efficacy analysis populations (ITT, HRD, and t*BRCA*). See Table 10 for a summary of the data.

Table 10. Clinical Efficacy Results

Cohort	Hazard Ratio Rucaparib vs Placebo	invPFS (months)		95% CI
ITT	0.377 P value: <0.0001 95% CI: 0.302, 0.469	10.4	Rucaparib	8.3, 11.1
		5.4	Placebo	5.3, 5.5
HRD	0.292 P value: <0.0001 95% CI: 0.217, 0.393	13.7	Rucaparib	11.0, 17.1
		5.4	Placebo	5.1, 5.6
tBRCA	0.228 P value: <0.0001 95% CI: 0.150, 0.346	17.1	Rucaparib	13.6, 23.2
		5.4	Placebo	4.9, 7.1

The benefit observed for the HRD and ITT populations was not driven solely by the t*BRCA* or HRD subpopulations, respectively, as shown by the prolonged median PFS for the rucaparib group in the non-t*BRCA* LOH high and non-t*BRCA* LOH low subgroups.

12. Summary of Clinical Studies

12.1 Summary of Primary Clinical Studies

The clinical benefit of the FoundationFocus CDx$_{BRCA\ LOH}$ test was demonstrated in a retrospective analysis of efficacy and safety data from two Phase 2 open-label studies (Study 10 and ARIEL2) that evaluated rucaparib for treatment of patients with advanced ovarian cancer. The Integrated Summary of Efficacy (ISE) population includes 123 patients who were positive for a deleterious *BRCA* alteration as determined by either a local laboratory test or by the Foundation Medicine clinical trial assay (CTA). Included within the ISE population are 106 patients who received 2 or more prior chemotherapy regimens and 17 patients who received only 1 prior chemotherapy regimen. The 106 patients with 2 or more prior chemotherapy regimens are considered to be the primary efficacy analysis population based on the indication accepted for consideration in the New Drug Application (NDA) 209,115 for Rubraca (rucaparib). A subset (64 of 106) of these patients was confirmed by the FoundationFocus CDx$_{BRCA}$ assay to have a deleterious *BRCA* alteration in tumor tissue in a clinical bridging study.

Patients were enrolled from multiple centers in North America, Europe, Israel, and Australia in ARIEL2. Patients enrolled in ARIEL2 provided tumor tissue prospectively for evaluation of *BRCA* alteration status by the CTA

and the FoundationFocus CDx*BRCA* assay. Patients may have also had *BRCA* test results from a local laboratory test. Eligibility for patients in Study 10 was based on local laboratory *BRCA* test results. In addition, *BRCA* alteration status was evaluated via the FoundationFocus CDx*BRCA* assay for Study 10 patients who provided tumor tissue retrospectively. Foundation Medicine, Inc. (Cambridge, Massachusetts, US) served as the central laboratory for both the clinical trial assay (CTA) and the FoundationFocus CDx*BRCA* LOH assay. A clinical bridging study was performed to determine concordance of the CTA test results with the FoundationFocus CDx*BRCA* LOH assay for the detection of a deleterious *BRCA* alteration, and to compare outcome data for patients identified by the FoundationFocus CDx*BRCA* LOH assay with those patients identified by another method to ensure the results are comparable.

The clinical benefit of the FoundationFocus CDx*BRCA* LOH assay as a complementary diagnostic test was based on ARIEL3 study. In the randomized, double-blind, placebo-controlled, phase 3 trial – ARIEL3, patients were recruited from 87 hospitals and cancer centers across 11 countries. Eligible patients were aged 18 years or older, had a platinum-sensitive, high grade serous or endometrioid ovarian, primary peritoneal, or fallopian tube carcinoma, had received at least two previous platinum-based chemotherapy regimens, had achieved complete or partial response to their last platinum based regimen, had a cancer antigen 125 concentration of less than the upper limit of normal, had a performance status of 0–1, and had adequate organ function. Patients were ineligible if they had symptomatic or untreated central nervous system metastases, had received anticancer therapy 14 days or fewer before starting the study, or had received previous treatment with a poly(ADP-ribose) polymerase inhibitor. Patients were randomly allocated 2:1 to receive oral rucaparib 600 mg twice daily or placebo in 28-day cycles using a computer-generated sequence (block size of six, stratified by homologous recombination repair gene mutation status, progression-free interval after the penultimate platinum-based regimen, and best response to the most recent platinum-based regimen). Patients, investigators, site staff, assessors, and the funder were masked to assignments and to the HRD status or the proposed LOH cut-off of 16.

12.2 Accountability of PMA Cohort

For ARIEL2, at the time of database lock, 106 patients who had been treated with 2 or more prior chemotherapy regimens and were identified as *BRCA*+ (as detected by local tests or CTA) were included in the clinical bridging study. These 106 patients compose the primary efficacy analysis population for rucaparib clinical efficacy studies and were used to support the clinical performance of the FoundationFocus CDx*BRCA* assay. Out of the 106 patients, specimens from 67 patients were available for retrospective

testing with the FoundationFocus CDx*BRCA* in the clinical bridging study. Among the 67 patients, 64 were identified as positive for a *BRCA* alteration using the FoundationFocus CDx*BRCA* LOH assay.

A total of 564 patients from ARIEL3 were included in the clinical outcome analysis, of which 518 had a passing FoundationFocus CDx*BRCA* LOH result available. Of the 518 patients with the FoundationFocus CDx*BRCA* LOH test available, 27 had a status of non-t*BRCA* LOH Unknown. Among the remaining 491 patients, 184 had an observed FoundationFocus CDx*BRCA* LOH status of t*BRCA*, 142 were non-t*BRCA* LOH high, 165 were Non-t*BRCA* LOH low. Table 10 above shows the clinical outcome for patients identified with the FoundationFocus CDx*BRCA* LOH result with the associated invPFS and 95% CI respectively.

12.3 Effectiveness Results

The analysis of effectiveness was based on efficacy data from 106 patients included in the primary efficacy population for Rubraca (rucaparib). Analysis of efficacy was based on confirmed Overall Response Rate (ORR) and Duration of Response (DOR) according to RECIST v1.1 as assessed by the investigator. In the 106 patients, confirmed Overall Response Rate (ORR) was 53.8% (95% CI: 43.8% to 63.5%) and the median Duration of Response (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.

A clinical bridging study was performed to evaluate agreement between the Foundation Medicine clinical trial assay (CTA) and local laboratory tests used to identify patients during the clinical trials and the FoundationFocus CDx*BRCA* assay (CDx) 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. A total of 64 patients from primary efficacy (PE) population identified as *BRCA*-positive by the clinical trial assay (CTA) had sufficient remaining DNA samples and passed QC for bridging to the CDx test. Of note, two samples included patients with large rearrangements that were detected by a local test, but not by the FoundationFocus CDx*BRCA* test. 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 (N= 29) 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* LOH results.

Overall, central *BRCA* testing using the FoundationFocus CDx$_{BRCA}$ assay indicated a 97% positive agreement with the CTA and a 95% positive agreement with local *BRCA* tests. Rucaparib demonstrated a response rate of 53.8% (95% CI, 43.8% to 63.5%, N = 106) and median DOR of 9.2 months (95% CI: 6.6 to 11.7 months) in the primary efficacy analysis population for the original PMA. Response rates were comparable between patients with a *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]. The clinical outcome results were also supported by additional sensitivity analyses evaluating the impact missing data. Taken together, the data support the use of the FoundationFocus CDx$_{BRCA}$ assay as a CDx test for patients with advanced ovarian cancer who may be eligible for rucaparib treatment.

In this supplemental PMA, via ARIEL3 study, the invPFS shows a statistically significant improvement comparing rucaparib to placebo for all patients irrespective of whether the CTA or CDx test was used. 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, 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, and the median invPFS in the non-t*BRCA* LOH+ 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. The stratified Cox proportional hazard model, showed a statistically significant improvement in invPFS with rucaparib treatment compared to placebo (HR 0.228 [95% CI: 0.150, 0.346]; $p < 0.0001$ for t*BRCA* population by CDx; HR 0.292 [95% CI: 0.217, 0.393]; $p < 0.0001$ for HRD population by CDx; HR 0.361 [95% CI: 0.234, 0.559]; $p < 0.0001$ for non-t*BRCA* LOH high population by CDx).

12.4 Clinical Outcome Data

In the original PMA, via ARIEL2 study, the clinical benefit of FoundationFocus CDx$_{BRCA}$ assay as a companion diagnostic test was demonstrated in a retrospective analysis of efficacy and safety data from two Phase 2 open-label studies (Study 10 and ARIEL2) that evaluated rucaparib for treatment of patients with advanced ovarian cancer. The Integrated Summary of Efficacy (ISE) population includes 123 patients who were positive for a deleterious *BRCA* alteration as determined by either a local laboratory test or by the Foundation Medicine clinical trial assay (CTA). A subset of these patients was confirmed by the FoundationFocus CDx$_{BRCA}$ assay to have a deleterious *BRCA* alteration in tumor tissue in a clinical bridging study. Included within the ISE population are 106 patients who received two or more prior chemotherapy regimens and 17 patients who received only one prior chemotherapy regimen.

The 106 patients with 2 or more prior chemotherapy regimens are considered to be the primary efficacy analysis population based on the indication accepted for consideration in the New Drug Application (NDA) 209,115 for Rubraca (rucaparib).

Patients were enrolled from multiple centers in North America, Europe, Israel, and Australia. Patients enrolled in ARIEL2 study provided tumor tissue prospectively for evaluation of *BRCA* alteration status by the CTA and the FoundationFocus CDx$_{BRCA}$ assay. Patients may have also had BRCA test results from a local laboratory test. Eligibility for patients in Study 10 Part 2A was based on local laboratory BRCA test results. In addition, *BRCA* alteration status was also evaluated via the FoundationFocus CDx $_{BRCA\ \mathrm{LOH}}$ assay for Study 10 Part 2A patients who provided tumor tissue retrospectively. Foundation Medicine, Inc (Cambridge, Massachusetts, US) served as the central laboratory for both the CTA and the FoundationFocus CDx$_{BRCA\ \mathrm{LOH}}$ assay.

The objectives of the bridging study were to determine concordance of the CTA 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 those patients identified by another method to ensure the results are comparable.

Overall, central BRCA testing using the FoundationFocus CDx$_{BRCA}$ assay indicated a 100% positive agreement with the CTA and a 95% positive agreement with local BRCA tests. Rucaparib demonstrated a response rate of 53.8% (95% confidence interval [CI], 43.8% to 63.5%, N = 106) in the primary efficacy analysis population. The magnitude of responses in the subset of patients tested with the FoundationFocus CDx$_{BRCA}$ assay was comparable to that in the population with a *BRCA* alteration detected by another method. The outcome results were also supported by additional robustness analyses. Data support the use of the FoundationFocus CDx$_{BRCA\ \mathrm{LOH}}$ assay as a CDx test for patients with advanced ovarian cancer potentially eligible for rucaparib treatment.

In the ARIEL3 trial that supported the PMA supplemental approval (P160018 S001) and designation of the test as a complementary diagnostic, rucaparib maintenance treatment was found to be efficacious not only in patients with relapsed, platinum-sensitive, high-grade ovarian carcinoma with a BRCA mutation, but also in those with *BRCA* wild-type carcinomas with high genomic loss of heterozygosity (LOH), a potential marker of homologous recombination deficiency (HRD) and thus PARP inhibitor activity.[5,6,7] The FoundationFocus CDx$_{BRCA\ \mathrm{LOH}}$ assay used in ARIEL3 now combines mutation analysis of *BRCA1* and *BRCA2* genes with measurement of the percentage of genome-wide LOH in the cancer tissue as a biomarker for sensitivity to rucaparib treatment.[8]

13. References

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