FOUNDATIONFOCUS™ CDx BRCA

FoundationFocus™ CDx*BRCA*
Technical Information Summary

Intended 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 test 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.

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.
- Limited performance characteristics of the test were evaluated for insertion alterations > 4 nucleotides and deletions > 10 nucleotides.
- Performance of the FoundationFocus CDx*BRCA* 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.
- Information generated by this test is an aid in the identification of patients who are most likely to benefit from the therapeutic product. 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* assay is performed using DNA from ovarian cancer (OC) 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, including splice sites, and select intronic regions of *BRCA1* and *BRCA2*. Using the Illumina® HiSeq™ 4000 platform, hybrid-capture–selected libraries are sequenced to a uniform depth (targeting >500X median 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 insertion/deletions (indels). Protein truncating alterations, splice site alterations, and missense alterations known to be deleterious are classified as t*BRCA*+.

Summary and Explanation

The Foundation Medicine FoundationFocus CDx*BRCA* assay is a companion diagnostic to Clovis Oncology's drug Rubraca, a poly (ADP-ribose) polymerase (PARP) inhibitor. Specimens that are found to have a deleterious *BRCA* alteration in their tumor tissue (t*BRCA*+) may be eligible for treatment with Rubraca therapy.

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 Instructions
- Test Requisition Form (TRF)

- Shipping Instructions
- 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$_{BRCA}$ assay is intended to be performed with serial number-controlled instruments.

Sample Collection and Test Ordering

To order the FoundationFocus CDx$_{BRCA}$ 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 included in the test kit.

For more detailed information, including Performance Characteristics, please find complete Technical Information at:

http://www.accessdata.fda.gov/cdrh_docs/pdf16/P160018B.pdf

FOUNDATIONFOCUS™ CDx BRCA

FoundationFocus CDx*BRCA*
Technical Information

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

1. Intended 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.

2. Contraindication

None.

3. 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.

4. 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.

- Limited performance characteristics of the test were evaluated for insertion alterations > 4 nucleotides and deletions > 10 nucleotides.
- Performance of the FoundationFocus CDx*BRCA* 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.
- Information generated by this test is an aid in the identification of patients who are most likely to benefit from the therapeutic product. 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.

5. Test Principle

The FoundationFocus CDx*BRCA* assay is performed using DNA from tumor, and resulting *BRCA1/2* alterations reported may include somatic (not inherited) or germline (inherited) alterations. Collectively, patients with *BRCA* alterations are referred to as tumor *BRCA* positive (t*BRCA*+).

The assay uses extracted DNA from tumor FFPE biopsy or surgical 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, including splice sites, and select intronic regions of *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 13bp. Briefly, the raw data (output) from the targeted sequencing is aggregated based on the index sequence (barcode) of each read, segregated to a given sample and a FASTQ data file is then generated. 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 and associated protein effects is based on the Breast Cancer Information Core (BIC) database. Each alteration included in the curated list has at least 2 records, of which ≥ 90% are classified as deleterious. The transcript IDs for the deleterious missense alterations listed in Table 2 are: *BRCA1* U14680 and *BRCA2* U43746.

Table 2: Deleterious *BRCA1/2* Missense Alterations 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)
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)	

6. Summary and Explanation

The Foundation Medicine FoundationFocus CDx*BRCA* assay is a companion diagnostic to Clovis Oncology's drug Rubraca, a poly (ADP-ribose) polymerase (PARP) inhibitor. Specimens that are found to have a deleterious *BRCA* alteration in their tumor tissue (t*BRCA*+) may be eligible for treatment with Rubraca therapy.

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

8. Sample Collection and Test Ordering

To order the FoundationFocus CDx*BRCA* 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:

http://www.accessdata.fda.gov/cdrh_docs/pdf16/P160018B.pdf

9. Instruments

The FoundationFocus CDx*BRCA* 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* test that are determined to carry a deleterious *BRCA1* or *BRCA2* alteration, 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 alteration(s) identified, are provided. 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* 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* assay and the comparator assay are shown in Table 3. Both of the discordant samples were found to be *BRCA*+ by a second validated NGS comparator method. The accuracy study is ongoing.

Table 3: Agreement between FoundationFocus CDx*BRCA* and NGS Comparator Assay

		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, 1 sample was detected in a region not covered by the comparator assay.

11.2 Analytical Specificity

11.2.1 Interfering Substances

To evaluate the potential impact of interfering substances on the performance of the FoundationFocus CDx$_{BRCA}$ assay process, 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.

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.

11.2.2 *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) 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.

11.3 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 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.

11.4 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* 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%.

11.5 Limit of Detection (LoD) and Limit of Blank (LoB)

LoD of the FoundationFocus CDx$_{BRCA}$ 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.

11.6 Stability

11.6.1 Reagent Stability

The stability of critical reagent lots used in the library construction (LC), hybrid capture (HC) and sequencing processes within the FoundationFocus CDx$_{BRCA}$ 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). Alteration calls were 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.

11.6.2 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.

11.6.3 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 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.

11.7 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%.

11.8 General Lab Equipment and Reagent Evaluation

11.8.1 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.

11.8.2 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.

11.9 Guard banding/Robustness

Guard banding studies were performed to evaluate the performance of the FoundationFocus CDx$_{BRCA}$ assay and the impact of process variation with regard to DNA concentration. Guard bands 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.

11.9.1 Library Construction Guard banding

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.

11.9.2 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.

11.9.3 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 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.

12. Summary of Clinical Studies

12.1 Summary of Primary Clinical Studies

The clinical benefit of the FoundationFocus CDx$_{BRCA}$ assay as a companion diagnostic (CDx) test was demonstrated in a retrospective analysis of efficacy and safety data from two Phase 2 open-label studies (Study 1 and Study 2) 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. Patients enrolled in Study 2 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 1 was based on local laboratory *BRCA* test results. In addition, *BRCA* alteration status was evaluated via the FoundationFocus CDx$_{BRCA}$ assay for Study 2 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$_{BRCA}$ assay. A clinical bridging study was performed 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.

12.2 Accountability of PMA Cohort

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$_{BRCA}$ assay.

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$_{BRCA}$ 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. 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.