# SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

## I.  GENERAL INFORMATION

| | |
|---|---|
| Device Generic Name: | Germline Gene Mutation Test |
| Device Trade Name: | BRACAnalysis CDx™ |
| Device Procode: | PJG |
| Applicant's Name and Address: | Myriad Genetic Laboratories, Inc. 320 Wakara Way Salt Lake City, UT 84108 |
| Date(s) of Panel Recommendation: | None |
| Premarket Approval Application (PMA) Number: | P140020 |
| Date of FDA Notice of Approval: | December 19, 2014 |

Priority Review: *Granted priority review status on October 15, 2014, because BRACAnalysis CDx™ addresses an unmet medical need, as there is no approved alternative and as demonstrated by significant clinically meaningful advantage.*

## II.  INDICATIONS FOR USE

BRACAnalysis CDx™ is an *in vitro* diagnostic device intended for the qualitative detection and classification of variants in the protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes using genomic DNA obtained from whole blood specimens collected in EDTA.  Single nucleotide variants and small insertions and deletions (indels) are identified by polymerase chain reaction (PCR) and Sanger sequencing.  Large deletions and duplications in *BRCA1* and *BRCA2* are detected using multiplex PCR.  Results of the test are used as an aid in identifying ovarian cancer patients with deleterious or suspected deleterious germline *BRCA* variants eligible for treatment with Lynparza™ (olaparib).  This assay is for professional use only and is to be performed only at Myriad Genetic Laboratories, a single laboratory site located at 320 Wakara Way, Salt Lake City, UT 84108.

## III.  CONTRAINDICATIONS

Patients who have undergone a previous allogeneic bone marrow transplant should not be tested with the BRACAnalysis CDx™.

## IV.  WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the BRACAnalysis CDx™ labeling.

## V.    DEVICE DESCRIPTION

BRACAnalysis CDx™ is an *in vitro* diagnostic device performed in a single laboratory, Myriad Genetic Laboratories, Inc. (Myriad), in Salt Lake City, UT.  The test includes a sample collection kit, which is sent to ordering laboratories and healthcare providers. The collection kit contains the following components:

- Monoject™ Blood Collection Tube (part no. 8881-311743);
- Test Request Form;
- Instructions for Sample Collection and Mailing;
- Technical Information Summary.

BRACAnalysis CDx™ consists of the following two assays to detect sequence variants and large rearrangements in the *BRCA1* and *BRCA2* genes:

- BRACAnalysis CDx™ Sanger Sequencing;
- BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx).

All reportable variants, including deleterious and suspected deleterious mutations, are confirmed by repeat analysis with the BRACAnalysis CDx™ Sanger Sequencing test or the BART® CDx test, or by confirmatory testing.  Approximately 98% and 99% of all variants detected by the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx tests, respectively, are confirmed by repeat testing; the remaining variants (about 2% and 1%, respectively) require confirmatory analysis by the following tests:

- Alternate Primer Sequencing (APS);
- Confirmatory PCR Analysis (CPA).

BRACAnalysis CDx™ is intended to be performed with serial number-controlled instruments, as indicated in the table below.

**Instruments for Use with BRACAnalysis CDx™**

| Instrument | Serial Number(s) |
|---|---|
| QIASymphony SP | 14957 |
| Tecan Freedom Evo 150 | 1310005128; 1402003965 |
| Tecan Infinite F200 Pro Platereader | 1309001987 |
| MasterCycler EP & MasterCycler Pro 384 & 96 well | 5344 003700; 5344 009645; 5344 010536; 5344 010537; 5344 010530; 5344 010552; 5344 010572; 5344 026026; 5344 010613; 5344 010521; 5344 012323; 5344 009646; 6324CH818083; 6324CH718080; 6324CK418379; 6324CH018081; 6324CK118382; 6324CK318376; 6324CH418082; 6324CH618076; 6324CK818381; 6324CK018387; 6324CK518383; 6324CK218385; 6324AR315529; 6324AR715518; 6324AR915513; 6324AR015528; 6324AR415521; 6324AR815510; |

| | 6321CP818805 |
|---|---|
| ABI 3730xl | 1408-038; 16112-019; 18127-015; 25193-005; 24189-002; 24180-009; 25193-003; 1412-027 |
| E-Gel iBase | 2113127 |
| E-Gel Imaging System InGenius3 | IG3/1219 |
| E-Gel Safe Imager Real-time Transilluminator | 12093074 |

Blood Collection and DNA Extraction

Peripheral whole blood (~7 mL) is collected in a blood collection tube containing EDTA, and then the sample is mailed to Myriad at ambient temperature. At Myriad, the blood sample is accessioned, aliquoted, and then processed using an automated DNA extraction process. One aliquot (1 mL) per sample is loaded onto the QIASymphony SP instrument, which is configured for silica-based isolation and purification of genomic DNA using QIAGEN Software v4.0.1. When the extraction run is complete, the DNA is suspended in ~185 µL TE buffer. The DNA is then quantified and normalized using an automated robotic platform (Tecan Freedom EVO® 150 with Tecan EVOWare v2.4.8.7) and fluorometer (Tecan Infinite® F200 PRO with Magellan v7.0 Software). DNA samples are stored at 4°C until tested for variants in the *BRCA* genes.

Detection of Sequence Variants

Sequence analysis of the *BRCA* genes is conducted with the BRACAnalysis CDx™ Sanger Sequencing test. For *BRCA1*, full sequence determination of approximately 5,400 base pairs (bp) comprising 22 coding exons and approximately 750 adjacent bp in the non-coding intervening (intron) sequences is performed. Exons 1 and 4, which are non-coding, are not analyzed. For *BRCA2*, full sequence determination of approximately 10,200 bp comprising 26 coding exons and approximately 900 adjacent bp in the non-coding intervening sequences is performed. Exon 1 is non-coding, and therefore, is not analyzed. The intronic regions of *BRCA1* and *BRCA2* that are analyzed generally do not extend more than 20 bp proximal to the 5' end and 10 bp distal to the 3' end of each exon.

The BRACAnalysis CDx™ Sanger Sequencing test uses primers that define specific base pair sequences to amplify each of the targeted regions by polymerase chain reaction (PCR). Each primer also contains an M13 tail on the 5'end to facilitate the downstream sequencing reactions. An automated robotic platform (Tecan Freedom EVO® 150 with Tecan EVOWare v2.4.8.7) is used to add the appropriate primers and DNA samples to the wells of 384-well PCR plates containing Sanger PCR MasterMix, which consists of pre-mixed reagents for the amplification reactions. Following inoculation, the PCR plates are centrifuged and then placed onto a thermocycler (Eppendorf MasterCycler EP or MasterCycler Pro 384) for PCR amplification. In total, 35 PCR reactions are carried out for *BRCA1*, and 49 PCR reactions for *BRCA2*.

The amplified products are each directly sequenced in the forward and reverse directions using fluorescent dye-labeled sequencing primers. Fluorescently-labeled Sanger

sequencing fragments are generated using the Eppendorf MasterCycler thermocyclers, and then purified prior to sequencing on the Applied Biosystems (ABI) 3730xl. To evaluate the possibility of carryover throughout the procedure, each batch run includes two negative controls for the BRACAnalysis CDx™ Sanger Sequencing test: a No Genomic DNA Control and an M13 F+R Negative Control. In each control, no DNA template is added. The No Genomic DNA Control contains all PCR components and a PCR primer pair for one targeted amplicon. The M13 F+R Negative Control includes all PCR components in addition to the M13 forward and reverse primer pair. The controls must produce the expected results in order to evaluate the data from test samples.

Chromatographic tracings of each amplicon are automatically generated with the ABI Sequence Analysis Software v5.3.1 and Gene Mapper v4.0, and subsequently analyzed using Myriad's proprietary Alignment Software v1.7.4 and Mutation Calling Software v1.9.6 to identify possible sequence variants. The variant calling software numerically compares each base of the sequencing traces to consensus wild-type sequences, and any mismatches are flagged as potential variants. The flagged variants may then be routed for review by trained data analysts. In this case, two independent reviewers visually inspect the traces to confirm the variant calls. If the analysts do not agree, the results are reviewed by a supervisor for final determination. All reportable variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and subsequent sequencing.

Detection of Large Rearrangements

BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx) is a multiplex PCR assay intended to detect large genomic rearrangements (e.g., deletions and duplications) across all coding regions, limited flanking intron regions, and the proximal promoter regions of the *BRCA1* and *BRCA2* genes. DNA is normalized to 2 ng/µL and then added to the wells of 384-well PCR plates containing pre-mixed reagents using the Tecan Freedom EVO® 15 automated robotic platform. The PCR plates are centrifuged, and then amplified products are generated using Eppendorf MasterCycler thermocyclers. During this process, fluorescently labeled primers are incorporated into the amplified DNA. In total, 11 multiplex PCR reactions are performed, and on average, there are 12 amplicons per multiplex with at least 2 amplicons per exon. The reactions are run in duplicate to obtain at least 4 data points for each region.

The amplified products are purified using the AMPure® magnetic bead system and then loaded onto the Applied Biosystems (ABI) 3730xl for fragment analysis. Chromatographic traces are generated with the ABI Gene Mapper v4.0 software and analyzed for the presence of wild-type and variant fragments. The fragment data from separation of the PCR products are sized using an internal lane standard with fragments of known sizes. Myriad's proprietary large rearrangement analysis software, MiniART Application v0.2, compares the relative peak intensities within a sample, and between samples run in the same batch, to generate statistical values and a gene dosage scatter plot. Briefly, each exon is represented by a minimum of 4 data points, so data from all amplicons of all samples in the same batch are normalized across the batch and then combined to yield one data point per sample per exon on the gene dosage scatter plot. Three housekeeping genes are used as additional copy number controls. The traces and scatter plot are reviewed by two independent trained analysts. Any sample flagged

during data review is reprocessed and reanalyzed.  Any sample with a potential large rearrangement is reviewed by a trained supervisor to verify the result.

In order to verify that the BART® CDx test can produce the expected results, two positive controls and one negative control are included in each run.  The positive controls are two independent cell lines with defined *BRCA* large rearrangements.  The negative control contains all of the components for the BART® CDx PCR reactions, with the exception of DNA template.  If the controls produce the expected results, then the data from the test samples are assessed.

Confirmatory Testing

All reportable variants, including deleterious and suspected deleterious mutations, are confirmed by additional testing prior to result reporting.  Approximately 98% of reportable sequence variants are confirmed by repeating the BRACAnalysis CDx™ Sanger Sequencing test for the indicated gene region(s), and about 99% of large rearrangements are confirmed by repeat BART® CDx testing.  Certain variants that cannot be confirmed with repeat testing are subject to confirmatory analysis using Alternate Primer Sequencing (APS) or Confirmatory PCR Analysis (CPA).

Approximately 2% of atypical variant results are confirmed using the APS test.  APS is based on the same PCR and Sanger sequencing methods used in the BRACAnalysis CDx™ Sanger Sequencing test; however, PCR is conducted in 96-well PCR plates.  The APS test is performed using alternative primer sequences, or primer combinations that flank the primer binding sites used in the BRACAnalysis CDx™ Sanger Sequencing assay, to allow for the identification of potential heterozygous base changes at primer binding sites that may have resulted in inefficient PCR amplification in the BRACAnalysis CDx™ Sanger Sequencing or BART® CDx tests.  For example, a heterozygous base change could yield either unequal PCR amplification in the BRACAnalysis CDx™ Sanger Sequencing test or artifacts - such as apparent single exon deletions - in the BART® CDx test.  The APS test is therefore used to confirm these types of rare results from the BRACAnalysis CDx™ Sanger Sequencing or BART® CDx tests.

The CPA test is used for follow up testing for about 1% of atypical results, such as single exon deletions, from the BART® CDx test.  Sequence-specific primers that span one or more exons are used to amplify breakpoint-specific genomic regions in a 96-well PCR plate format.  The amplified products are evaluated using gel electrophoresis and subsequent imaging (E-Gel Imaging System InGenius3, Invitrogen E-Gel® Safe Imager and E-Gel® iBASE™ Power System with GENESys Gel Documentation System v1.4.0.0 software) and may be verified by sequencing.  A size-based analysis of the PCR products is performed by comparing the PCR products from the sample of interest against wild-type PCR products.  The confirmatory assays are generally performed as needed to confirm the presence of certain variants.

Variant Classification

Upon completion of testing at Myriad, a test report is sent to the ordering physician.  The results of each test component, along with the classification of the germline variant(s) detected by the BRACAnalysis CDx™, are provided.  Variant classification is conducted

according to a defined classification process by an in-house committee consisting of Laboratory Directors, the Chief Medical Officer, representatives from Medical Services, Genetic Counselors, and other trained professionals, including PhD-level scientists, board-certified clinical molecular geneticists, or equivalent. Based on the classification criteria, each identified variant is classified into one of five categories. If multiple variants are detected with different classifications, the overall test interpretation is determined by the highest tiered variant classification, as listed below. Variants determined to have a classification of polymorphism are not included on the test report.

1. Deleterious Mutation;
2. Suspected Deleterious Mutation;
3. Variant of Uncertain Significance;
4. Favor Polymorphism;
5. Polymorphism (considered 'No Mutation Detected').

The BRACAnalysis CDx™ is intended as an aid in selecting ovarian cancer patients with deleterious or suspected deleterious germline *BRCA* variants who may be eligible for treatment with Lynparza™ (olaparib). The majority (> 90%) of deleterious or suspected deleterious variants identified by Myriad in *BRCA1* and *BRCA2* are classified using objective criteria based on the type and genomic position of the variants, as described in the table below. Deleterious or suspected deleterious mutations classified by other criteria that are based on available evidence may be subject to change.

**Objective Classification Criteria for Deleterious and Suspected Deleterious Variants**

| Classification | Variant Type | Position[*] |
|---|---|---|
| Deleterious | Nonsense | Truncates the reading frame at or before the most 3' known deleterious mutation |
| | Frameshift | Truncates the reading frame at or before the most 3' known deleterious mutation |
| | Large Rearrangement | Deletion of an exon or exons predicted to disrupt the reading frame or a critical functional domain |
| | | Duplication of a non-terminal exon or exons predicted to disrupt the reading frame or a critical functional domain |
| | | Insertion of non-*BRCA* sequence that disrupts the reading frame or a critical functional domain |
| Suspected Deleterious | Splicing | Intronic variants that disrupt the consensus splice donor sites (+1, +2 intronic position) and splice acceptor sites (-1, -2 intronic position) |

[*] There are exceptions that are classified on a case-by-case basis. For example, a silent variant in exon 9 (C197C) in *BRCA1* has been reported to affect mRNA splicing (Miki et al. 1994; Dosil et al., 2010). Also, alternative splicing of exon 11 in *BRCA1* is partially functional (Thakur et al., 1997; Kim et al., 2006; Huber et al., 2001).

Once the identified variants are classified, the completed dataset for each sample is reviewed and a report is sent to the designated physician. All mutations and genetic variants are named according to the convention of Beaudet and Tsui (1993). Nucleotide numbering starts at the first transcribed base of *BRCA1* and *BRCA2* based on GenBank entries U14680 and U43746, respectively.

## VI.  ALTERNATIVE PRACTICES AND PROCEDURES

There are no FDA-cleared or -approved alternatives for *BRCA* mutation testing of DNA isolated from whole blood specimens for the selection of ovarian cancer patients who are eligible for treatment with Lynparza™ (olaparib).

## VII.  MARKETING HISTORY

Myriad Genetic Laboratories, Inc. initially designed and developed BRACAnalysis as a laboratory developed test, and the first commercial sample was tested in 1996.  The BRACAnalysis test has been used to detect the presence of mutations within the *BRCA1* or *BRCA2* genes in the hereditary cancer predisposition setting.  BRACAnalysis is not FDA-cleared or -approved.

BRACAnalysis CDx™ 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 *BRCA* test results, and subsequently, improper patient management decisions in ovarian cancer treatment.  Patients with false positive results may undergo treatment with Lynparza™ (olaparib) without any clinical benefit, and may experience adverse reactions associated with olaparib therapy.  Patients with false negative results may not be considered for treatment with Lynparza™ (olaparib), and therefore, may receive other treatment options.  There is also a risk of delayed results, which may lead to a delay in treatment with Lynparza™ (olaparib).

For the specific adverse events related to Lynparza™ (olaparib) that occurred in the clinical studies, please see Section X below.

## IX.  SUMMARY OF PRECLINICAL STUDIES

### A. Laboratory Studies

The specific performance characteristics of the BRACAnalysis CDx™ were determined by studies using samples from ovarian cancer patients, as well as samples from breast cancer patients and unaffected individuals from families with and without a high risk for Hereditary Breast and Ovarian Cancer.  Samples were selected to represent a range of variants detected by the BRACAnalysis CDx™, as reflected in the device labeling.

1. **Accuracy**

   a. **BRACAnalysis CDx™ Sanger Sequencing Test**

      Accuracy of the BRACAnalysis CDx™ Sanger Sequencing test was verified by comparison against a validated next generation sequencing (NGS)-based

assay. The two methods sequenced overlapping regions of the *BRCA1* and *BRCA2* genes (a total of 17,337 bases), and were used to independently evaluate a panel of 100 patient-derived specimens. All specimens were tested in a blinded manner. The specimens covered a range of variants, including single nucleotide variants, deletions up to 5 bp, and insertions up to 2 bp.

After variant and non-variant calls (relative to wild-type sequences) were made for each test, the results were compared. From the set of samples tested, a total of 796 variant bases (representing 790 variants) and 1,732,907 non-variant bases were identified by the NGS-based test. For each sample tested with the BRACAnalysis CDx™ Sanger Sequencing test, successful calls were made for all amplicons that are part of the assay, and the no call rate was 0%. All variant and non-variant base calls between the two tests were concordant. The agreement analysis between the results from both tests demonstrated a positive percent agreement (PPA), negative percent agreement (NPA), and overall agreement of 100%. The lower bounds of the 95% confidence intervals (CI) for PPA and NPA were 99.62% and 99.99%, respectively, which met the pre-specified acceptance criteria for the study. The results are summarized in the table below.

**BRACAnalysis CDx™ Sanger Sequencing Test vs. NGS-Based Assay**

| Results | NGS Assay | Sanger Assay | Concordant Calls |
|---|---|---|---|
| **Variant Bases** | 796 | 796 | 796 |
| **Non-Variant Bases** | 1,732,907 | 1,732,907 | 1,732,907 |
| **Total Bases*** | 1,733,703 | 1,733,703 | 1,733,703 |
| Positive Percent Agreement (PPA) = 100% (95% CI: 99.62%, 100%) | | | |
| Negative Percent Agreement (NPA) = 100% (95% CI: 99.99%, 100%) | | | |
| Overall Agreement = 100% (95% CI: 99.99%, 100%) | | | |

*There were 3 one-base insertions in this sample set.

### b. BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)

The performance of the BART® CDx test was evaluated by comparing it to a validated microarray-based test. A set of 100 samples was processed in a blinded manner using the BART® CDx and the microarray tests. The types of large rearrangements evaluated in this study were as follows: single exon and multi-exon deletion and duplication, the Portuguese founder mutation, and multi-exon triplication.

Accuracy of the results from the BART® CDx test was demonstrated by comparison against the positive and negative calls from the validated microarray test. Based on the microarray results, 26 samples were positive for a large rearrangement in *BRCA* genes, and 74 samples were negative. For the BART® CDx test, 95 samples yielded valid results and 5 samples had invalid results, or no calls. Among the 95 samples with callable results, 94 samples had results that matched those from the microarray assay, while one did not. The miscalled, or discordant, variant was identified as a multi-exon duplication by the BART® CDx test while the microarray test detected a multi-exon triplication. Although both tests detected an increase in dosage of

the same region, the BART® CDx test is not designed to differentiate between duplications and triplications, and therefore, this is a limitation of the BART® CDx test.  Based on the results, PPA was 84.6% (95% CI: 65.1% - 95.6%) and the NPA was 97.3% (95% CI: 90.6% - 99.7%).  The overall agreement was 94% (95% CI: 87.4% - 97.8%).  The results are summarized in the table below.

**BART® CDx Test vs. Microarray Assay**

| LR* Status | Variant Type | Array Results | BART® CDx Results | | |
|---|---|---|---|---|---|
| | | | Concordant | Miscall | No Call |
| Positive | Single exon deletion | 7 | 5 | 0 | 2 |
| | Single exon duplication | 3 | 2 | 0 | 1 |
| | Multi-exon deletion | 11 | 11 | 0 | 0 |
| | Multi-exon duplication | 3 | 3 | 0 | 0 |
| | Multi-exon triplication | 1 | 0 | 1 | 0 |
| | Portuguese founder mutation | 1 | 1 | 0 | 0 |
| | TOTAL | 26 | 22 | 1 | 3 |
| Negative | NMD** | 74 | 72 | 0 | 2 |
| | TOTAL | 100 | 94 | 1 | 5 |

* Large Rearrangement
** No Mutation Detected

2. **Analytical Sensitivity – DNA Input**

a. **BRACAnalysis CDx™ Sanger Sequencing Test**

PCR Amplification is the critical step in the BRACAnalysis CDx™ Sanger Sequencing test for generating high levels of specific amplicons for the sequencing reactions.  To assess the acceptable range of genomic DNA input to achieve the PCR performance requirements of the test, DNA extracted from five clinical specimens were each diluted to evaluate six DNA input concentrations (0.2 ng, 1 ng, 4 ng, 20 ng, 40 ng, and 100 ng) per PCR reaction.  The specimens carried variant types, such as single nucleotide variant and small deletion up to 5 bp.  Each sample was tested for eight representative amplicons in duplicate.  The amplicons were selected to represent the range of amplicon sizes, GC-content, and coverage applicable to the BRACAnalysis CDx™ Sanger Sequencing test, as indicated in the table below.  The rate of successful calls at each DNA input level was assessed, in addition to the concordance between the replicates per amplicon.

**Amplicon Characteristics**

| Gene | Amplicon | Amplicon Description | | |
|---|---|---|---|---|
| | | Length (bp) | % GC | Coverage |
| BRCA1 | 1 | 496 | 41 | Bi-directional |
| | 2 | 506[a] | 42 | Bi-directional |
| | 3 | 292 | 42 | Bi-directional |
| | 4 | 279 | 55[c] | Bi-directional |
| | 5 | 82[b] | 29 | Uni-directional[e] |
| | 6 | 257 | 36 | Bi-directional |

| | | | | |
|---|---|---|---|---|
| *BRCA2* | 7 | 445 | 36 | Bi-directional |
| | 8 | 290 | 27[d] | Bi-directional |

[a] Longest amplicon in the assay.
[b] Shortest amplicon in the assay.
[c] Most GC-rich amplicon in the assay.
[d] Least GC-rich amplicon in the assay.
[e] The presence of a homopolymer track renders one sequencing direction unreadable.

The optimal DNA input concentration for PCR amplification is 20 ng, as specified in the protocol for the BRACAnalysis CDx™. At this level, all of the results for each sample met the quality criteria and generated callable results. The duplicate results for each amplicon were fully concordant for all of the variant and non-variant calls. For input levels at 100 ng and 40 ng, the no call rate was 0%, and all results were fully concordant. At 4 ng, the no call rate was 1%, and all callable results were concordant. At 1 ng, the no call rate was 4% and no miscalls were observed. At 0.2 ng, although the callable results were concordant, the no call rate was 44%, and therefore the acceptance criteria were not met for this input level. The DNA input level of 20 ng, which is specified in the standard protocol, is within the tolerated range of tested DNA input concentrations from 1 ng to 100 ng.

## b. BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)

The BART® CDx test is a multiplex PCR assay that amplifies specific regions in the *BRCA1* and *BRCA2* genes. To evaluate the DNA input range for the PCR step, DNA concentrations higher and lower than the optimal DNA input amount of 8 ng, which is specified in the assay protocol, were evaluated. DNA samples from five patient specimens were each diluted and tested at seven different DNA input levels: 2 ng, 4, ng, 6 ng, 8 ng, 10 ng, 12 ng, and 14 ng. Samples with and without large rearrangements (e.g., multi-exon deletions) were included.

The rate of successful calls for each sample per DNA input level was assessed, as well as the concordance across DNA concentrations. DNA input levels ranging from 2 ng to 12 ng produced callable results for all samples tested, and the results were fully concordant. At 14 ng, one sample did not yield callable results and, therefore, failed to meet the acceptance criteria of the study. The results demonstrate that DNA input levels from 2 ng to 12 ng generate similar results to those at 8ng, which is the DNA concentration specified for the BART® CDx test.

## 3. Analytical Specificity – Cross Reactivity

## a. BRACAnalysis CDx™ Sanger Sequencing Test

The ability of the BRACAnalysis CDx™ Sanger Sequencing test to detect sequence variants is highly dependent upon the specificity of the primers for PCR amplification. To assess the potential for amplification of non-specific products from human genomic DNA, *in silico* analysis of the PCR primers

used in the assay was performed.  Non-standard primer combinations were not evaluated since the assay consists of only singleplex PCR reactions.  No non-specific products were predicted for any of primer pair combinations.

**b.  BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)**

A specificity analysis was conducted to determine if the PCR primers used in the BART® CDx test have the potential to amplify non-target sequences in the human genome.  A bioinformatics program was used to align primer pairs against genomic sequence to predict if there may be any non-specific amplicons.  Every possible primer pair combination per multiplex reaction was evaluated.  In total, 3,016 combinations were assessed.  Non-specific products were not predicted for any of the potentially cross-reactive primer pairs in any of the BART® CDx multiplex PCR reactions.

4. **Interference**

To evaluate how potential interfering substances may impact the performance of the BRACAnalysis CDx™, the effects of three classes of substances were assessed: 1) endogenous substances normally present in human whole blood (i.e., hemoglobin, Albumin, IgG, and bilirubin); 2) an exogenous substance (i.e., $K_3$EDTA); and 3) substances used in the standard process of the device (i.e., ethanol and bleach).  Three whole blood samples from healthy subjects carrying a total of 30 different *BRCA* variants were evaluated.  The types of variants included single nucleotide variant and small deletion up to 2 bp.  The endogenous and exogenous substances were spiked into each blood sample, and then the samples were processed, along with a corresponding set of unspiked blood samples.  The concentrations tested for some of the endogenous substances were based on the CLSI guideline document EP7-A2.  In accordance with standard protocol for the assay, the method-specific substances - ethanol and bleach - were added to genomic DNA samples after the DNA quantification step.  All of the samples were tested with the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx test.

The variant and non-variant calls were compared across the spiked and unspiked samples to determine if the potential interferents may lead to alterations in the test results.  All non-spiked blood specimens yielded 100% callable and concordant results that passed the acceptance criteria for both the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx test.  Treatment with each potentially interfering substance at the maximum concentration tested, with the exception of IgG at 60 g/L, did not affect the performance of either test (i.e., hemoglobin at 20 g/dL, albumin at 50 g/L, conjugated bilirubin at 5 mg/dL, $K_3$EDTA at 5%, ethanol at 12.75%, and 10% bleach at 0.5%).  Samples with IgG at 60 g/L yielded a no call rate of 33%, which failed to meet the acceptance criteria for the BART® CDx test and demonstrated that IgG concentrations at 60 g/L interfere with the assay performance.  At an IgG concentration of 9.5 g/L, which is near the average level typically detected in blood, all samples generated callable results that matched those of the corresponding unspiked samples.  Thus, at 9.5 g/L of IgG, the acceptance criteria were met.

5. **Reproducibility and Repeatability**

   a. **Combined Reproducibility**

   Reproducibility of the BRACAnalysis CDx™ was assessed by testing a set of 20 patient-derived samples over three independent runs using two or three instruments of each type, three reagent lots, and three operators for each manual processing step.  The three runs were conducted over non-consecutive days using the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx test.  The confirmatory assays were also performed, in accordance with the standard protocols.

   Across all tested samples, sequencing variants (single nucleotide variants and deletions up to 5 bp) and large rearrangements (single exon deletion, single exon duplication, and multi-exon deletion) were represented.  For both tests, the no call rate was 0%, since all runs produced successful calls for all samples.  For each sample, all calls were consistent across all runs.  The positive percent agreement (PPA), negative percent agreement (NPA), and overall agreement were all 100%, which met the acceptance criteria for the study.

   b. **Intra-Run Repeatability**

   i. **BRACAnalysis CDx™ Sanger Sequencing Test**

   To determine if the BRACAnalysis CDx™ Sanger Sequencing test is reproducible across replicates of the same sample within a single batch run, three blood samples were tested in triplicate using ten amplicons of the test.  The amplicons were selected to represent the range of genomic regions that are evaluated in the BRACAnalysis CDx™ Sanger Sequencing test.  These include the longest amplicon, the shortest amplicon, the most GC-rich amplicon, and the least GC-rich amplicon.  Regions for which the presence of a homopolymer track renders only one sequencing direction readable were also included.  The amplicons were tested in two batches, such that 9 amplicons were run in one batch and 1 amplicon in another.  For the batch with 9 amplicons, all of the replicates for each sample produced 100% callable and concordant results.  For the batch with only one amplicon, 8 of 9 reactions were callable and the callable results were concordant within each sample.

   ii. **BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)**

   To demonstrate that the BART® CDx test can generate repeatable results across replicates of the same samples within a single batch run, ten specimens were processed in triplicate within one run.  Variants of the following types were represented in the study: single exon duplication and multi-exon deletion.  All replicates for each of the samples passed the

quality criteria and therefore produced callable results, all of which were fully concordant.

6. **Guardbanding**

   a. **BRACAnalysis CDx™ Sanger Sequencing Test**

   Guardbanding studies were performed to evaluate if the performance of the BRACAnalysis CDx™ Sanger Sequencing test is robust to withstand process variations around two key parameters: PCR annealing temperature, and sequencing annealing temperature. Five samples were tested in duplicate per tested condition, and variant types included single nucleotide variant and small deletion (up to 5 bp).

   i. **PCR Annealing Temperature**

   The thermal cycling profile was guardbanded by varying the PCR annealing temperature by ±1°C, ±2°C and ±3°C. The expected call for each sample was defined by the results obtained under the PCR annealing temperature specified in the standard protocol. The results from each test condition were compared to the expected calls. For three test conditions (+1°C, -2°C and -3°C), all replicates for each amplicon tested per sample yielded callable results that matched the expected call. Similar results were observed for the other test conditions (-1°C, +2°C and +3°C), with the exception that only one replicate of one of the tested amplicons for one sample generated no call. Overall, the acceptance criteria were met, and all test conditions were tolerated.

   ii. **Sequencing Reaction Annealing Temperature**

   The annealing temperature for the sequencing reaction was challenged by varying the temperature by ±1°C, ±2°C and ±3°C. The expected call for each sample was defined by the results obtained under the PCR annealing temperature specified in the standard protocol. The results from each test condition were compared to the expected calls. For three test conditions (-1°C, +2°C and -3°C), all replicates for each amplicon tested per sample yielded callable results that were in agreement with the expected call. For the other test conditions (+1°C, -2°C and +3°C), one replicate for one of the tested amplicons for one sample generated no call, while all other replicates generated results that matched the expected call. Overall, the acceptance criteria were met, and all test conditions were tolerated.

   b. **BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)**

   The robustness of two critical parameters of the BART® CDx test was assessed: PCR annealing temperature and injection time of the PCR product input for capillary electrophoresis. In both cases, the same set of 28 unique samples was assessed and analyzed, of which two were run in duplicate. Two samples were positive for multi-exon deletions.

### i. PCR Annealing Temperature

The PCR annealing temperature was varied by ±1°C, ±2°C, and ±3°C. The expected call for each sample was defined by the results obtained under the PCR annealing temperature specified in the standard protocol. The results from each test condition were compared to the expected calls. Four test conditions (±1°C, -2°C, and -3°C) yielded reportable and concordant calls for all samples. At the two other conditions (+2°C and +3°C), one sample yielded a miscalled deletion result. Upon confirmatory testing, a variant in one of the BART® CDx PCR primer annealing sites was identified to be the cause for the decreased primer binding efficiency at the two elevated temperatures, leading to the miscall result.

### ii. Injection Time

Different levels of PCR product injected onto the ABI 3730xl platform were assessed by altering the injection time of the PCR product. The injection time was set at 2, 4, 5, 6, 7, 10, or 20 seconds, while the voltage was held constant (2 kV), resulting in 4, 8, 10, 12, 14, 20 or 40 kV·s, respectively. Results obtained under optimal conditions (i.e., 12 kV·s) were used to compare results obtained under the test conditions. All conditions, except 40 kV·s, resulted in callable, concordant results for all samples. At 40 kV·s, calls of acceptable quality were not obtained for any sample, indicating that this setting falls outside of the linear detection range of the capillary electrophoresis instrument. Thus, the optimal condition of 12 kV·s is within the acceptable PCR input injection conditions from 4 kV·s to 20 kV·s.

## 7. Cross Contamination

The potential for crossover contamination within a run and between runs was evaluated for three processes of the BRACAnalysis CDx™: 1) DNA extraction from whole blood specimens, 2) the BRACAnalysis CDx™ Sanger Sequencing test, and 3) the BART® CDx test. Specimens with different *BRCA* genotypes (for sequence variants and large rearrangements) were processed adjacent to each other in microtiter plate formats to maximize the potential for carryover between wells within a plate and between plates in separate batch runs. Two sequential batches were evaluated for inter-run carryover, and each run was evaluated separately for intra-run carryover.

### a. DNA Extraction

DNA extraction from whole blood samples is an automated process using the QIASymphony robotic platform. Four blood samples were processed in triplicate from DNA extraction to data review. After isolation of genomic DNA, two samples were processed through the BRACAnalysis CDx™ Sanger Sequencing test and all four samples went through the BART® CDx test. For all samples in all batches, callable results were generated. All replicates were

fully concordant within each run and between runs.  Thus, sample crossover events were not detected.

b.  **BRACAnalysis CDx™ Sanger Sequencing test**

Two samples with unique BRCA sequence variants were set up within one PCR plate in a checkerboard pattern at alternating high (20ng for the first sample) and low (4 ng for the second sample) DNA input levels.  In the first run, there were 84/90 (93%) reportable calls and 6/90 no calls for the two samples tested.  All callable results were concordant.  In the second run, there were 89/90 (99%) reportable calls, all of which were concordant.  Although the no call rate was higher than observed in some other analytical studies, no miscall results were generated.

c.  **BRACAnalysis CDx™ Large Rearrangement Test (BART® CDx)**

For the BART® CDx test, a total of ten samples were evaluated.  In each batch, eight unique samples without *BRCA* large rearrangements were included with two samples with a large rearrangement.  The two samples that were positive for a large rearrangement were tested in triplicate per run, while the samples that did not carry a large rearrangement were tested in six replicates per run.  In the first run, two pairs of samples were set up in triplicate at high (12 ng for the large rearrangement negative samples) and low (4 ng for the samples with large rearrangements) DNA input levels.  For all replicates of all samples in each batch, callable results were generated and were fully concordant with the expected results.  Carryover events leading to miscall results were not observed.

8.  **Stability**

a.  **Specimen Stability**

To define the storage conditions and evaluate the stability of whole blood specimens for use with the BRACAnalysis CDx™, blood samples stored at defined temperatures and durations were assessed.  Blood samples (25-30 mL) from five individuals were collected in EDTA tubes, aliquoted, and immediately tested (time point $T_0$) or stored at two temperatures, 4°C (storage temperature specified in the standard protocol) and 30°C, for some specified amounts of time.  Blood samples were stored at 4°C for 14 days, 30 days, and 37 days.  At 30°C, samples were stored for 3 days, 5 days, and 7 days.  At each time point, duplicate aliquots per sample were processed and analyzed with the BRACAnalysis CDx™ Sanger Sequencing test and the BART® CDx test.  No large rearrangements were detected in the samples, and sequence variants included single nucleotide variants.

Results from each time point were compared to those from time point $T_0$ to determine if the same results may be obtained from stored samples.  For all indicated time points at 4°C and 30°C, all replicates per sample yielded callable results that matched the results from $T_0$.  These data support that whole blood

specimens are stable for up to 30 days when stored at 4°C and up to 5 days at 30°C.

## b. Reagent Stability

The storage and stability conditions for a defined set of reagents used in the BRACAnalysis CDx™ were evaluated. Three lots of each set of reagents were stored under specified temperature conditions and then tested at defined time points. Critical reagents used in three processes were evaluated: the BRACAnalysis CDx™ Sanger Sequencing test, the BART® CDx test, and DNA quantification and normalization. Under all of the test conditions, results from each time point were compared against those from time point $T_0$. The parameters tested for each reagent are listed in the table below.

**Reagent Stability Storage Conditions**

| Assay / Step | Reagent | Temp | Expiration | Time Points Tested |
|---|---|---|---|---|
| Sanger sequencing | PCR plate | -20 °C | TBD* | 0 mo** |
| | | 30 °C | 24 hr | 0, 12, 24, 36 hr |
| | Oligo plate | -80 °C | TBD* | 0 mo** |
| | | 4 °C | < 30 d | 0, 30 d** |
| | | 30 °C | 7 d | 0, 4, 7, 11 d |
| | CAPSeq plate | -20 °C | TBD | 0 mo** |
| BART® | BART® PCR | -80 °C | TBD | 0 mo** |
| Quantification | Standards | 30 °C | 48 hr | 0, 24, 48, 72 hr |
| | | 4 °C | 30 d | 0, 15, 30, 45 d |

\* To Be Determined,
\*\* Real-time stability studies are underway.

For the BRACAnalysis CDx™ Sanger Sequencing test, three reagent sets were assessed: PCR plates with pre-mixed reagents (excluding DNA template and PCR primers), Oligo plates which contain the PCR primers, and CAPSeq plates which contain the components of the PCR reaction for sequencing. A set of five specimens with single nucleotide variants or deletions up to 2 bp were tested. For all time points and storage conditions tested for each reagent, variant and non-variant calls were successfully generated and concordant with the corresponding results from $T_0$. Expiration dating for each reagent is shown in the table above. Real-time stability testing for some reagents is ongoing.

For the BART® CDx test, PCR plates with pre-mixed reagents (except for DNA template) were evaluated using a different set of five samples. A multi-exon deletion was detected in one sample, while the others did not contain a large rearrangement. Stability testing for the BART® CDx PCR plate at -80°C is ongoing.

For the DNA quantification and normalization process, the stability of the standards used for quantification was tested. Duplicate calibration curves were generated for the three lots at each time point, and the concentrations of two control DNA samples were then calculated. Across all tested time points,

minimal variation was observed for the calculated concentrations of the control DNA samples, supporting the expiration dating of 30 days at 4°C for the quantification standards.

### c. Control Stability

The stability of eight assay controls used in the BRACAnalysis CDx™ was evaluated. Three lots of each control used in the BRACAnalysis CDx™ Sanger Sequencing test and BART® CDx test were produced and assessed. For the CPA assay, two lots of each control were evaluated. If three lots were tested, five replicates of each control lot were tested at each time point, for a total of 15 replicates. If two lots were available, the 15 replicates of each control were also tested, where eight replicates were tested with one lot and seven replicates with the second lot. For the positive controls, testing to the observable positive control endpoint was evaluated. For the negative controls, two testing endpoints were defined; the first test observed the expected negative outcome of each control, and the second test was designed to yield a positive outcome by spiking the samples with amplifiable DNA template to demonstrate that the expected negative outcome in the first test was not due to defective PCR reagents or a processing error. The controls and storage conditions for this study are listed in the table below.

**Control Stability Storage Conditions**

| Assay | Control | Temp | Expiration | Time Points Tested |
|-------|---------|------|------------|--------------------|
| Sanger | M13 F+R Negative Control | $\leq$ -65 °C | TBD* | 0 mo** |
| BART® | Cell Line Positive Control | Quantified, normalized gDNA at 4 °C | 2 mo | 0, 1, 2, 3 mo |
| | Alternate Positive Control | Quantified, normalized gDNA at 4 °C | < 1 mo | 0, 1, 2, 3 mo |
| | Amplicon Negative Control | -20 °C | TBD* | 0 mo** |
| CPA | PCR Amplification Control 1 | Quantified, normalized gDNA at 4 °C | 2 mo | 0, 1, 2, 3 mo |
| | PCR Amplification Control 2 | Quantified, normalized gDNA at 4 °C | 2 mo | 0, 1, 2, 3 mo |
| | No Genomic DNA Control 1 | -20 °C | TBD* | 0 mo** |
| | No Genomic DNA Control 2 | -20 °C | TBD* | 0 mo** |

\* To Be Determined
\*\* Real-time stability studies are underway.

Results from each time point for the stored controls were compared to the corresponding $T_0$ results. At $T_0$, all replicates for each control, except one,

yielded callable, concordant results. The one exception was the M13 F+R Negative Control that was spiked with an M13-tailed PCR amplicon. In this case, one replicate resulted in no call for one lot. As a result, there was a total of 14/15 (93%) successful calls for the spiked M13 Negative Control, and all calls were concordant.

Time points for the following controls are complete: the BART® Cell Line Positive and BART® Alternative Positive Controls (1, 2, and 3 mo), and the CPA PCR Amplification Controls 1 and 2 (1 and 2 mo). For the BART® Cell Line Positive Control, all tested time points met the acceptance criteria. For example, all replicates at 1 month and 3 months produced callable results that were fully concordant, and at the 2 month time point, there was one no call for one lot, while all other replicates had reportable and concordant results. Thus, the 2 month expiration for the BART® Cell Line Positive Control was supported.

For the BART® Alternative Positive Control, all replicates at 1 month and 3 months produced callable results that were fully concordant; however, the 2 month time point failed to meet the acceptance criteria, as there were three no call results. For the CPA PCR Amplification Controls 1 and 2, all replicates at the 1 and 2 month time points generated reportable results that completely corresponded to the $T_0$ results. Taken together, the results support a current expiration of less than 1 month for the BART® Alternative Positive Control, and less than 2 months for the CPA PCR Amplification Controls 1 and 2. Additional real-time testing is ongoing for the CPA PCR Amplification Controls 1 and 2, as well as the M13 Negative Control, the BART® Amplicon Negative Control, and the CPA No Genomic DNA Controls 1 and 2.

d. **Intermediate Product Stability**

The stability of intermediate products of the BRACAnalysis CDx™ was evaluated under specific storage conditions. Intermediate products generated from each of five samples were tested in duplicate. Three samples contained sequence variants including single nucleotide variants and a deletion up to 19 bp; one sample had a large rearrangement. In all of the stability evaluations, the results of the tested time points were compared to the initial $T_0$ results for successful calls and concordance. The intermediate products and storage conditions that were tested are listed in the table below.

**Intermediate Product Storage Conditions**

| Assay | Int. Product | Temp | Stability | Time Points Tested |
|---|---|---|---|---|
| Sanger | Pre-PCRs in 384-well plates | 4 °C | 6 hr | 0, 6, 9 hr |
| | PCR in 96-well plates | 4 °C | 4 d | 0, 4, 7 d |
| | Post-DNA inoculated Pre-PCR plates | 4 °C | 4 hr | 0, 4, 8 hr |
| | | 18-32 °C* | 2 hr | 0, 2, 4 hr |

| BART® | Post-Elution | 4 °C | 7 d | 0, 4, 7 10 d |
| | | 18-32 °C* | 6 hr | 0, 9, 15 hr |
| CPA | Post-PCR | 4 °C | 14 d | 0, 7, 14, 21 d |

\* Ambient temperature in the laboratory

For the BRACAnalysis CDx™ Sanger Sequencing test, all results at $T_0$ produced callable results that were fully concordant. The results for all test conditions, except the PCR intermediate product in 96-well plates stored at 4 °C for 4 days, produced successful sequencing calls. Although the acceptance criteria were also met for the PCR intermediate product in 96-well plates stored at 4 °C for 4 days, one replicate for one amplicon in one sample failed to yield a callable result. All of the variant and non-variant calls for all time points were concordant with the results from $T_0$.

For the BART® CDx test, all results at $T_0$ generated calls, which were concordant for each of the 5 samples. For all time points tested for the two intermediate products, all samples produced successful BART® CDx positive and negative calls that matched the $T_0$ results. No miscalls were detected. For the confirmatory CPA assay, the post-PCR intermediate product from one sample with a large rearrangement (as detected by BART® CDx) was evaluated, in accordance with the standard protocol. All tested conditions generated results that were fully concordant with the $T_0$ result. The results support the stability results shown in the table above.

## B. Animal Studies

None

## C. Additional Studies

Variant Classification Study

To evaluate the robustness and reliability of the variant classification process, a set of 262 unique *BRCA* variants was subjected to classification as if they were new variant observations. The variants were classified in a blinded manner according to defined classification criteria. The resulting classifications for each variant were compared to the existing classifications in Myriad's database, and the concordance rate was determined. One variant that was not previously observed at Myriad - and therefore was not previously classified - was excluded from the study. The majority of variants (185/262) were identified from clinical studies for Lynparza™ (olaparib), and the remaining variants (77/262) were selected for inclusion into the study in order to adequately cover the spectrum of variant types for classification. The results are summarized in the tables below.

**Classification Results**

| Classification | Previous Classification | New Classification |
|---|---|---|
| Deleterious (DM) | 130 | 126 |
| Suspected Deleterious (SD) | 7 | 11 |

| | | |
|---|---|---|
| Uncertain Significance (VUS) | 34 | 32 |
| Favor Polymorphism (FP) | 9 | 17 |
| Polymorphism (PM) | 81 | 75 |
| Excluded from study | 1 | 1 |
| TOTAL | 262 | 262 |

**Discordant Results by Classification**

| Previous Classification | New Classification | Discordant Results | |
|---|---|---|---|
| | | Classification | Treatment |
| DM | SD | 5 | 0 |
| SD | DM | 1 | 0 |
| SD | VUS | 1 | 1 |
| VUS | SD | 1 | 1 |
| VUS | FP | 2 | 0 |
| PM | FP | 6 | 0 |
| TOTAL | | 16 | 2 |

Comparison of the new classifications to the previous classifications resulted in agreement for 245 variants (93.9%, 95% CI: 90.2% - 96.5%). There were 16 variants that had inconsistent results; treatment eligibility for Lynparza™ (olaparib) would not be affected by 14 of the inconsistent classifications, but the different classifications for two variants would affect patient selection. Of the two, one is a missense variant classified as suspected deleterious (SD), while the previous classification was a variant of uncertain significance (VUS). The change in classification resulted from structural and functional evidence, which recently became available, to support the SD classification. The other inconsistent result was an intronic splicing variant classified as VUS in this study, but previously was SD. Again, the change in classification was due to recently available evidence. Upon comparison of the two separate and independent variant classifications leading to the same eligibility status for treatment with Lynparza™ (olaparib), the concordance rate was 99.2% (259/261, 95% CI: 97.2% - 99.9%).

## X.    SUMMARY OF PRIMARY CLINICAL STUDY

The clinical benefit of the BRACAnalysis CDx™ was demonstrated in a retrospective analysis of efficacy and safety data obtained from the open-label, non-randomized study to assess the safety and efficacy of olaparib treatment in patients with ovarian cancer who have a deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*) and who have been previously treated with at least three lines of prior chemotherapy. Patients were enrolled from 13 centers in six countries, including the United States. Local test results for *BRCA* status were used to assess patient eligibility for the trial. Samples from a subset of enrolled patients from the intended population were retrospectively evaluated at one laboratory, Myriad (Salt Lake City, UT), using the BRACAnalysis CDx™, in a clinical bridging study. In the bridging study, there were two objectives: 1) agreement between the BRACAnalysis CDx™ and the local test results for *gBRCAm* detection, and 2) the clinical outcomes (i.e., ORR and DoR) for the patients with ovarian cancer who had received three or more prior lines of chemotherapy, who had measurable disease, and who were positive for deleterious or suspected deleterious germline *BRCA* mutations, as identified by

the BRACAnalysis CDx™. Lynparza™ (olaparib) demonstrated a robust overall response rate with a clinically meaningful duration of response in *gBRCAm* patients with ovarian cancer who had received three or more prior lines of chemotherapy. The magnitude of response in the subset tested with the BRACAnalysis CDx™ was comparable to that in the locally tested *gBRCAm* study population. These results were also supported by additional robustness analyses. Data from this bridging study were used to support PMA approval.

## A. Study Design

The major effectiveness study was a single-arm, open-label, multi-center study to assess the safety and efficacy of olaparib treatment in patients with advanced cancers who have a deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*). Patients were treated with 400 mg olaparib twice daily until disease progression or intolerance to study treatment. After starting study treatment, patients attended periodic clinic visits for assessment of safety and efficacy until confirmed objective disease progression. The primary objective of the study was to assess the efficacy of oral olaparib in patients with advanced cancers who have a confirmed genetic *BRCA1* and/or *BRCA2* mutation by assessment of tumor response. Other objectives were to assess the efficacy of oral olaparib in patients with advanced cancers who have a confirmed genetic *BRCA1* and/or *BRCA2* mutation, by assessment of objective response rate (ORR), progression-free survival (PFS), overall survival (OS), duration of response (DoR), and disease control rate (DCR). Safety information was collected throughout the study. To support approval of Lynparza™ (olaparib), the efficacy evaluation was performed in patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer who had received three or more prior lines of chemotherapy and who had measurable disease based on ORR and DoR. The results of this clinical study are described in NDA 206162.

To demonstrate clinical utility of the BRACAnalysis CDx™, a bridging study was conducted. Archived samples were available from a subset of patients with ovarian cancer enrolled in the open-label, non-randomized trial for retrospective analysis of *gBRCAm* status with the BRACAnalysis CDx™. The samples were tested in a blinded manner at Myriad (Salt Lake City, UT). The clinical utility of the BRACAnalysis CDx™ was established by comparing the mutation results and the associated clinical outcomes for the locally tested *gBRCAm* population to those for the subset of patients with confirmed *gBRCA* status upon retrospective testing with the BRACAnalysis CDx™. Additional robustness analyses were also performed.

1. Clinical Inclusion and Exclusion Criteria
   Enrollment in the clinical study was limited to patients who met the following inclusion criteria:

   1. Provision of fully informed consent prior to any study-specific procedures.

   2. Patients must be > 18 years of age.

   3. Confirmed documented deleterious or suspected deleterious *BRCA* mutation. (The presence of a loss-of-function germline mutation in the *BRCA1* and/or *BRCA2* gene must be confirmed prior to consent according to local practice).

4. Histologically or, where appropriate, cytologically confirmed malignant solid tumour refractory to standard therapy and for which no suitable effective standard therapy exists. Haematological malignancies will be considered if there is a component of disease that can be assessed and followed for response by RECIST v1.1.

5. For the breast cancer setting, patients must have failed at least three previous lines of chemotherapy (not including tyrosine kinase inhibitors and hormonal treatments) in the metastatic/advanced setting. Patients who are hormone receptor positive must have also failed prior hormonal therapy. Patients who are HER2 receptor-positive must have failed prior trastuzumab.

6. For the ovarian cancer setting patients must have documented progressive or recurrent disease according to either RECIST v1.1 or Gynecologic Cancer Intergroup (GCIG) criteria either during or within 6 months of completion of their most recent platinum-based chemotherapy regimen OR greater than 6 months from completion of most recent platinum-based chemotherapy, but not suitable for further platinum therapy. This should be discussed with the AstraZeneca Study Physician prior to obtaining consent.

   Note that in the ovarian cancer setting, eligibility also includes patients who have developed recurrent ovarian cancer with macroscopic peritoneal metastases outside the pelvis or distant metastases. In addition, patients with primary peritoneal carcinoma or Fallopian tube carcinoma may be considered for the study.

7. For pancreatic cancer setting, patients must have failed systematic chemotherapy in the advanced or metastatic setting.

8. For the prostate cancer setting, patients must have:

   - hormone-refractory disease, defined as a testosterone value in the castration range

   - at least 2 consecutive rising PSA values above their nadir and measured at least two weeks apart

   - at least 6 weeks from discontinuation of anti-androgen therapy.

   - must have failed at least one systemic therapy for metastatic hormone-refractory disease.

9. Patients must have normal organ and bone marrow function measured within 28 days prior to administration of study treatment as defined below:

   - Hemoglobin ≥9.0 g/dL

   - Absolute neutrophil count (ANC) ≥1.5 x $10^9$/L

   - White blood cells (WBC) >3 x $10^9$/L

   - Platelet count ≥100 x $10^9$/L

   - Total bilirubin ≤1.5 x institutional upper limit of normal (ULN).

- Aspartate transaminase (AST) (SGOT)/ALT (SGPT) ≤2.5 x institutional upper limit of normal unless liver metastases are present in which case it must be ≤ 5x ULN

- Serum creatinine ≤1.5 x institutional upper limit of normal (ULN)

10. ECOG performance status ≤2

11. Patients must have a life expectancy ≥12 weeks

12. Evidence of non-childbearing status for women of childbearing potential, or postmenopausal status: negative urine or serum pregnancy test within 28 days of study treatment, confirmed prior to treatment on day 1.

    Postmenopausal is defined as:

    - Amenorrheic for 1 year or more following cessation of exogenous hormonal treatments

    - Luteinizing hormone and follicle-stimulating hormone levels in the postmenopausal range for women under 50

    - radiation-induced oophorectomy with last menses >1 year ago

    - chemotherapy-induced menopause with >1 year interval since last menses

    - or surgical sterilization (bilateral oophorectomy or hysterectomy).

13. Patient is willing and able to comply with the protocol for the duration of the study including undergoing treatment and scheduled visits and examinations.

14. At least one lesion (measurable and/or non-measurable) at baseline that can be accurately assessed by CT/MRI and is suitable for repeated assessment at follow-up visits.

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

1. Involvement in the planning and/or conduct of the study (applies to both AstraZeneca staff and/or staff at the study site)

2. Any previous treatment with a PARP inhibitor, including olaparib.

3. Patient with any other malignancy which has been active or treated within the previous 5 years, with the exception of a second suspected BRCA-related malignancy, adequately treated cone-biopsied in situ carcinoma of the cervix uteri, endometrial carcinoma stage 1A or 1, or non-melanoma skin lesions.

4. Patients receiving any systemic chemotherapy, radiotherapy (except for palliative reasons), within 2 weeks from the last dose prior to study treatment (or a longer period depending on the defined characteristics of the agents used). The patient can receive a stable dose of bisphosphonates for bone metastases, before and during the study as long as these were started at least 4 weeks prior to treatment. Prostate cancer patients may also continue to receive Luteinizing Hormone-Releasing Hormone (LHRH).

5. Patients receiving the following classes of inhibitors of CYP3A4

- Azole antifungals

- Macrolide antibiotics

- Protease inhibitors

6. Persistent toxicities (>CTCAE grade 2), excluding alopecia, caused by previous cancer therapy.

7. Patients with symptomatic uncontrolled brain metastases. A scan to confirm the absence of brain metastases is not required. The patient can receive a stable dose of corticosteroids before and during the study as long as these were started at least 4 weeks prior to treatment.

8. Patients with spinal cord compression, unless they have received definitive treatment for this and have evidence of clinically stable disease for at least 28 days prior to study entry

9. Major surgery within 2 weeks of starting study treatment and patients must have recovered from any effects of any major surgery.

10. Patients considered a poor medical risk due to a serious, uncontrolled medical disorder, non-malignant systemic disease or active, uncontrolled infection. Examples include, but are not limited to, uncontrolled ventricular arrhythmia, recent (within 3 months) myocardial infarction, uncontrolled major seizure disorder, unstable spinal cord compression, superior vena cava syndrome, or any psychiatric disorder that prohibits obtaining informed consent.

11. Patients unable to swallow orally administered medication and patients with gastrointestinal disorders likely to interfere with absorption of the study medication.

12. Breast-feeding women.

13. Immunocompromised patients, e.g., patients who are known to be serologically positive for human immunodeficiency virus.

14. Patients with known active hepatic disease (i.e., Hepatitis B or C).

15. Patients with a known hypersensitivity to olaparib or any of the excipients of the product.

16. Patients with uncontrolled seizures.

17. Previous enrollment in the present study.

18. Treatment with any investigational product during the last 14 days (or a longer period depending on the defined characteristics of the agents used).

2. Follow-up Schedule

Tumor assessments were performed at baseline and at regular intervals thereafter (every 8 weeks $\pm$ 1 week) until objective disease progression or up to 6 months after starting study treatment. If a patient had not progressed after 6 months, the tumor assessments were performed at extended intervals (12 weeks) until disease progression. Following confirmed disease progression, patients discontinued olaparib treatment, and were continued to be contacted to assess survival status

until death or the data cutoff for the primary analysis.

3. Clinical Endpoints

With regard to safety, information about adverse events was collected from time of signed informed consent throughout the treatment period and up to and including 30 days after the patient discontinues olaparib treatment.

With regard to effectiveness, the efficacy evaluation was performed in patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer who had received three or more prior lines of chemotherapy and who had measurable disease. The efficacy variables used to assess clinical benefit were objective response rate (ORR) and duration of response (DoR). The analyses were based on the assessment of response based on RECIST 1.1.

For the clinical bridging study, there were two objectives: 1) agreement between the BRACAnalysis CDx™ and the local test results for *gBRCAm* detection, and 2) the clinical outcomes (i.e., ORR and DoR) for the patients with ovarian cancer who had received three or more prior lines of chemotherapy, who had measurable disease, and who were positive for deleterious or suspected deleterious germline *BRCA* mutations, as identified by the BRACAnalysis CDx™.

## B. Accountability of PMA Cohort

Based on local test results, a total of 317 patients with advanced cancers were enrolled in the study. There were 193 patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer, among whom 137 had measurable disease and had received three or more lines of prior chemotherapy. Out of the 137 patients, specimens from 61 patients were available for retrospective testing with the BRACAnalysis CDx™ in the clinical bridging study.

## C. Study Population Demographics and Baseline Parameters

Study enrollment occurred at 13 centers in six countries (Australia, Germany, Spain, Israel, Sweden, and United States). Among the 137 *gBRCAm* patients with ovarian cancer who had received three or more lines of chemotherapy, 40 patients (29%) were enrolled in the US.

Baseline demographic characteristics and tumor information for the 137 *gBRCAm* patients with ovarian cancer who had measurable disease and who had received three or more lines of chemotherapy are provided in the table below. Also shown are the characteristics for the subset of 59 patients with confirmed *gBRCAm* results with the BRACAnalysis CDx™ and for 78 patients who were not tested with the BRACAnalysis CDx™ test (due to unavailability of archived specimens). In general, the demographics for patients with and without a BRACAnalysis CDx™ test result are similar, apart from the ECOG Performance status for which there was a higher proportion of patients with a performance status of 0 in the subgroup with a BRACAnalysis CDx™ test result.

**Demographic characteristics for patients with ovarian cancer, 3 or more prior lines of chemotherapy and measurable disease at baseline**

| Characteristic | All patients (n=137) | Patients with BRACAnalysis CDx™ result (n=59) | Patients without BRACAnalysis CDx™ result (n=78) |
|---|---|---|---|
| | n (%) | n (%) | n (%) |
| Age (years) | | | |
|    Mean (SD) | 57.5 (9.02) | 56.4 (7.89) | 58.3 (9.75) |
|    Median (range) | 58.0 (35-79) | 56.0 (36-75) | 59 (35-79) |
| Age group (years) | | | |
|    <50 | 26 (19.0) | 11 (18.6) | 15 (19.2) |
|    ≥50 to <65 | 83 (60.6) | 39 (66.1) | 44 (56.4) |
|    ≥65 | 28 (20.4) | 9 (15.3) | 19 (24.4) |
| Sex | | | |
|    Female | 137 (100) | 59 (100) | 78 (100) |
| Race | | | |
|    White | 129 (94.2) | 56 (94.9) | 73 (93.6) |
|    Black/African-American | 1 (0.7) | 1 (1.7) | 0 (0) |
|    Asian | 6 (4.4) | 2 (3.4) | 4 (5.1) |
|    Other | 1 (0.7) | 0 (0) | 1 (1.3) |
| ECOG performance status | | | |
|    0 | 76 (55.5) | 41 (69.5) | 35 (44.9) |
|    1 | 52 (38.0) | 17 (28.8) | 35 (44.9) |
|    2 | 8 (5.8) | 1 (1.7) | 7 (9.0) |
|    Missing | 1 (0.7) | 0 (0) | 1 (1.3) |
| Site of tumour | | | |
|    Ovary | 125 (91.2) | 55 (93.2) | 70 (89.7) |
|    Fallopian tube | 3 (2.2) | 1 (1.7) | 2 (2.6) |
|    Peritoneum | 7 (5.1) | 3 (5.1) | 4 (5.1) |
|    Primary peritoneal | 2 (1.5) | 0 (0) | 2 (2.6) |

Due to rounding of % values, some groups may sum to greater than 100.0%.

An assessment of the distribution of *BRCA* mutations between the patients with and without a BRACAnalysis CDx™ test result who had measurable disease and who received at least three lines of prior chemotherapy are summarized in the table below. The distributions of the mutations are generally similar between the groups.

**_BRCA_ mutation characteristics for patients with ovarian cancer, 3 or more prior lines of chemotherapy and measurable disease at baseline**

| Characteristic | All patients (n=137) | Patients with BRACAnalysis CDx™ result (n=59) | Patients without BRACAnalysis CDx™ result (n=78) |
|---|---|---|---|
| | n (%) | n (%) | n (%) |
| _BRCA1_ variants | 106 (77.4) | 41 (69.5) | 65 (83.3) |
| _BRCA2_ variants | 30 (21.9) | 18 (30.5) | 12 (15.4) |
| _BRCA1 & BRCA2_ variants | 1 (0.7) | 0 (0) | 1 (1.3) |
| Frameshift | 102 (74.5) | 39 (66.1) | 63 (80.8) |
| Nonsense | 18 (13.1) | 11 (18.6) | 7 (9.0) |
| Missense | 7 (5.1) | 4 (6.8) | 3 (3.8) |
| Splice site | 4 (2.9) | 2 (3.4) | 2 (2.6) |
| Large rearrangement | 2 (1.5) | 1 (1.7) | 1 (1.3) |
| Synonymous variant | 1 (0.7) | 1 (1.7) | 0 (0.0) |
| In-frame deletion | 2 (1.5) | 1 (1.7) | 1 (1.3) |
| Intronic variant | 1 (0.7) | 0 (0) | 1 (1.3) |

Due to rounding of % values, some groups may sum to greater than 100.0%.

## D. Safety and Effectiveness Results

### 1. Safety Results

The safety, with respect to treatment with Lynparza™ (olaparib), will not be addressed in detail in the SSED for the BRACAnalysis CDx™. Adverse event information was obtained from multiple clinical studies. Major safety considerations related to the drug include the potential for an increased risk for the development of myelodysplastic syndrome/Acute Myeloid Leukemia (MDS/AML) and the risk of non-infectious pneumonitis. The most common adverse reactions ($\geq$20%) in clinical trials were anemia, nausea, fatigue (including asthenia), vomiting, diarrhea, dyspepsia, headache, decreased appetite, nasopharyngitis/URI, arthralgia/musculoskeletal pain, myalgia, back pain, dermatitis/rash and upper abdominal pain. Refer to the drug label for more information.

### 2. Effectiveness Results

The analysis of efficacy analysis was based on objective response rate (ORR) and duration of response (DoR) observed in 137 patients with deleterious or suspected deleterious germline _BRCA_ mutation (_gBRCAm_)-associated ovarian cancer who had received three or more prior lines of chemotherapy and who had measurable disease. In this cohort, the ORR was 34% (95% CI: 26% - 42%) with a median

DoR of 7.9 months. The observed ORR represents an improvement over existing therapies and is reasonably likely to predict clinical benefit in the indicated population. The results are listed in the table below.

The effectiveness analysis for the BRACAnalysis CDx™ was based on a subset of 61 *gBRCAm* patients with ovarian cancer who had received three or more prior lines of chemotherapy, who had measurable disease, and for whom specimens were available for retesting with the BRACAnalysis CDx™. The level of concordance between the local test results, as reported in the Case Report Form, and the results from the BRACAnalysis CDx™ was determined to be 96.7% (59/61, 95% CI: 88.7% - 99.6%). Among the discordant results, one sample did not yield a callable result with the BRACAnalysis CDx™, and another sample had different classification results between the local test and the BRACAnalysis CDx™ (deleterious vs. variant of unknown significance, respectively), although the specific variant that was detected by both tests matched. The clinical outcome data for the 59 patients with confirmed *gBRCAm* status was as follows: ORR was 41% (95% CI: 28% - 54%), and median DoR was 8.0 months. Taken together, the results in the subset of *gBRCAm* patients tested with the BRACAnalysis CDx™ were comparable to those observed in the cohort of 137 patients, which supports effectiveness of the device. The results are summarized in the table below.

**Clinical Study Results**

| Subset* | Total Subjects n | Subjects with Response n (%) | ORR | 95% CI | Progressed n (%) | Median DoR (months) | 95% CI |
|---------|------------------|------------------------------|-----|--------|------------------|---------------------|--------|
| All | 137 | 46 (33.6) | 0.34 | (0.26, 0.42) | 30 (65.2) | 7.9 | (5.6, 9.6) |
| With BRACAnalysis CDx™ result | 59 | 24 (40.7) | 0.41 | (0.28, 0.54) | 14 (58.3) | 8.0 | (3.8, NC) |
| No BRACAnalysis CDx™ result | 78 | 22 (28.2) | 0.28 | (0.19, 0.40) | 16 (72.7) | 7.9 | (6.0, 9.6) |

*Ovarian cancer patients with measurable disease who received at least three lines of prior chemotherapy

3. Subgroup Analyses

Additional robustness analyses were conducted to consider the potential impact of missing data arising from patients with a positive BRACAnalysis CDx™ test result, but who may have been negative by the local test. Patients with such test results are part of the intended use population of the BRACAnalysis CDx™; however, they were excluded from the clinical trial due to negative results upon local test screening. To account for this missing data, the efficacy of olaparib treatment in patients with positive results from the BRACAnalysis CDx™ was estimated assuming different combinations for the following parameters:

- The objective response rate (ORR) among patients with positive results with both the BRACAnalysis CDx™ and local tests was fixed at 41%, which was observed from the trial.

- The missing ORR among patients with positive BRACAnalysis CDx™ and negative local test results was assumed to be 5%, 10%, 15%, 20%, 25%, 30%, 35%, or 40% to exhaust all possibilities that do not exceed the ORR estimated from patients with positive results with both the BRACAnalysis CDx™ and local tests.

- The proportion of cases with negative local test results are assumed to be 5%, 10%, 15%, 20%, 25%, or 30%. Based on published literature, the germline *BRCA* mutation rate in unselected ovarian cancers is from 11% to 15% (Hennessy, et al. 2010; Pal, et al. 2005).

- The negative percent agreement (NPA) of the two tests (i.e., negative results by both tests) was fixed at 0.988 (159/161), as was observed from multiple clinical studies as well as literature (Kurian et al. 2014).

Combining all of the above assumed parameter values, the ORR modeled for the BRACAnalysis CDx™ test-positive population, including those who may have tested negative by local tests, was calculated. The confidence intervals are calculated based on the imputed ORR from the subset of 137 patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer who had received three or more prior lines of chemotherapy and who had measurable disease in the study. The smallest ORR value estimated for the BRACAnalysis CDx™ test-positive population, including those who may have tested negative by local tests, is 34% (95% CI: 26% - 43%), which is not significantly different from that observed for the overall subpopulation of 137 patients who had measurable disease and who had received 3 or more lines of prior chemotherapy (34%, 95% CI: 26% - 42%). The results are listed in the table below.

**Estimated ORR for the BRACAnalysis CDx™-Positive Population**

| | | Assumed ORR for BRACAnalysis CDx™-positive and Local Test-Negative | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 5% % (95%CI) | 10% % (95%CI) | 15% % (95%CI) | 20% % (95%CI) | 25% % (95%CI) | 30% % (95%CI) | 35% % (95%CI) | 40% % (95%CI) |
| **Assumed Prevalence Local Test-Negative** | **5%** | 34% (26, 43) | 35% (27, 44) | 36% (28, 44) | 37% (29, 46) | 38% (30, 47) | 39% (30, 47) | 40% (32, 49) | 41% (33, 50) |
| | **10%** | 37% (29, 46) | 38% (30, 47) | 38% (30, 47) | 39% (30, 47) | 39% (30, 47) | 40% (32, 49) | 40% (32, 49) | 41% (33, 50) |
| | **15%** | 38% (30, 47) | 39% (30, 47) | 39% (30, 47) | 39% (30, 47) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 41% (33, 50) |
| | **20%** | 39% (30, 47) | 39% (30, 47) | 39% (30, 47) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 41% (33, 50) |
| | **25%** | 39% (30, 47) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 41% (33, 50) |
| | **30%** | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 40% (32, 49) | 41% (33, 50) | 41% (33, 50) |

## E. Financial Disclosure

The Financial Disclosure by Clinical Investigators regulation (21 CFR 54) requires applicants who submit a marketing application to include certain information concerning the compensation to, and financial interests and arrangement of, any

clinical investigator conducting clinical studies covered by the regulation. The pivotal clinical study was supplemented by retrospective testing at one site. 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)(2) 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.

## XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

### A. Effectiveness Conclusions

The clinical benefit of the BRACAnalysis CDx™ was demonstrated in a retrospective analysis of efficacy and safety data obtained from the open-label, non-randomized study in which Lynparza™ (olaparib) demonstrated a robust overall response rate with a clinically meaningful duration of response in patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer who had received three or more prior lines of chemotherapy. Results from local testing for *gBRCAm* were used to determine patient eligibility for the clinical study. The study enrolled 193 *gBRCAm* ovarian cancer patients, among whom 137 had received at least three lines of prior chemotherapy with measurable disease at baseline. Of the 137 patients, specimens from 61 patients were available for retrospective confirmation of g*BRCAm* status using the BRACAnalysis CDx™. After testing, 59 cases (96.7%) were verified to have deleterious or suspected deleterious germline *BRCA* mutations. Analysis of the subset of 59 patients revealed that the response rate was 41% (95% CI: 28% - 54%) and the median duration of response was 8.0 months. The results are similar to those observed in the overall population of 137 patients, for which the response rate was 34% (95% CI: 26% - 42%) and the median duration of response was 7.9 months. Additional robustness and worst case scenario analyses to include missing results supported an improvement in response rate in *gBRCAm* ovarian cancer patients.

The performance of the BRACAnalysis CDx™ was also supported by the analytical validation studies. As demonstrated in the analytical specificity study, the assay is highly specific for all targeted regions in the *BRCA1* and *BRCA2* genes. The device also demonstrated consistent performance to detect specific sequence variants and large rearrangements in the *BRCA* genes. Further, sequencing and large rearrangement results from the BRACAnalysis CDx™ correlated with results obtained from validated comparator methods.

### B. Safety Conclusions

The BRACAnalysis CDx™ is an *in vitro* diagnostic device, which involves testing whole blood specimens collected from patients with ovarian cancer. The risks of the device are based on data collected in the clinical study conducted to support PMA

approval as described above. Risks of the BRACAnalysis CDx™ are associated with failure of the device to perform as expected or failure to correctly interpret test results. If incorrect, or false, results are reported, then ovarian cancer patients may not receive the proper treatment. Patients with false positive results may undergo treatment with Lynparza™ (olaparib) without any clinical benefit, and may experience adverse reactions associated with olaparib therapy. Patients with false negative results may not be considered for treatment with Lynparza™ (olaparib), and therefore, may receive other treatment options. There is also a risk of delayed results, which may lead to a delay in treatment with Lynparza™ (olaparib).

## C. Benefit-Risk Conclusions

The probable benefits of the device are based on data collected in the clinical study, which were used to support PMA approval as described above. The clinical benefit of the BRACAnalysis CDx™ was demonstrated in a retrospective analysis of efficacy and safety data obtained from the open-label, non-randomized study in which Lynparza™ (olaparib) demonstrated a robust overall response rate with a clinically meaningful duration of response in patients with deleterious or suspected deleterious germline *BRCA* mutation (*gBRCAm*)-associated ovarian cancer who had received three or more prior lines of chemotherapy. Patients were enrolled into the clinical study based on local testing results for *gBRCAm*. Samples from a subset of enrolled patients with measurable disease who had received at least three lines of prior chemotherapy were subsequently tested with the BRACAnalysis CDx™ to verify *gBRCAm* status. The overall concordance rate between the local test results and those from BRACAnalysis CDx™ was 96.7% (95% CI: 88.7% - 99.6%). The observed clinical benefit in the subset of confirmed *gBRCAm* patients was comparable to that observed in the overall population. In the overall population of *gBRCAm* patients with previously treated ovarian cancer, the response rate was 34% (95% CI: 26% - 42%) and the median duration of response was 7.9 months. These results were also supported by additional robustness analyses. Overall, the response rate in patients with *gBRCAm*-associated ovarian cancer is better than what would be expected of available therapy and represents an improvement on a surrogate endpoint that is reasonably likely to predict clinical benefit.

Additional factors to be considered in determining probable risks and benefits for the BRACAnalysis CDx™ included: analytical performance of the device, representation of variants in the major effectiveness study, and the availability of alternative tests. First, the primary risks associated with the BRACAnalysis CDx™ are the possibility of inaccurate, or false, results that may lead to mismanagement of patient treatment. The performance of the device is supported by analytical validation studies, and additional analytical testing will be conducted in the post-approval setting. Second, a limited range of variant types were included in the clinical bridging study. Confirmatory clinical studies are being conducted to verify the results of the major effectiveness study, and additional variant types will likely be represented. Third, there is currently no FDA-approved or -cleared device for the selection of *gBRCAm* patients with previously treated ovarian cancer for treatment with Lynparza™ (olaparib).

In conclusion, given the available information above, the data support the use of the BRACAnalysis CDx™ as an aid in identifying ovarian cancer patients with deleterious or suspected deleterious germline *BRCA* mutations for fourth-line treatment with Lynparza™ (olaparib), and 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 open-label, non-randomized clinical study and the associated bridging study support the utility of the BRACAnalysis CDx™ as an aid in selecting patients with previously treated ovarian cancer who may be eligible for treatment with Lynparza™ (olaparib). Lynparza™ (olaparib) demonstrated improvement in objective response rate and duration of response in ovarian cancer patients who have been previously treated with at least three lines of prior chemotherapy and who have deleterious or suspected deleterious germline *BRCA* mutations, as identified with the BRACAnalysis CDx™.

## XIII. CDRH DECISION

CDRH issued an approval order on December 19, 2014. The final conditions of approval cited in the approval order are described below.

1. As reflected in the labeling for BRACAnalysis CDx™, a limited range of variant types was included in some of the analytical validation studies. Additional testing of samples is required to establish the analytical performance characteristics of the BRACAnalysis CDx™ for all variant types that may be detected. Samples that adequately cover the range of small deletions, small insertions, and large rearrangements detected by the device, should be included, with consideration to variant lengths and genomic contexts. The results from these studies should be included in the labeling, and the results should be submitted within 7 months from the date of the approval order.

2. Since a limited range of variant types were included in the clinical validation study, results from the ongoing clinical trials (Study D0816C00002 and Study D0816C00010) using the BRACAnalysis CDx™ should be provided upon completion of the trials. If patients were enrolled based on results from a clinical trial assay (CTA), a bridging study between the CTA and BRACAnalysis CDx™ will be required. The results from these studies should be reflected in the labeling.

3. Defined criteria are used to classify variants detected by BRACAnalysis CDx™. Variant classifications may be subject to change over time based on newly available evidence that is evaluated in your classification process. To monitor the robustness of the variant classification process, continued evaluation of the process will be needed. When samples are received to be tested with BRACAnalysis CDx™, all variants that are detected should be treated as new variants and classified according to the current classification criteria. The current classification results should then be compared to the penultimate classifications (if variants were previously identified), with tabulation of agreement between the two classification results. A summary of the results should

be provided annually.  The following should be included: the variants detected, the agreement between the previous and current classifications per category, the numbers of variants that changed per classification category, description(s) of the classification changes, and the criteria used for each classification (e.g., the criteria used for the previous classification, the criteria used for the current classification, and the rationale for any differences or changes to the classification criteria).  The results from these studies could lead to labeling changes.

4.  There was limited representation of deleterious and suspected deleterious germline *BRCA1* and *BRCA2* variants in the registrational clinical study. As treatment outcome data (e.g., literature) becomes available with broader representation of variants, the sponsor should assess and report on whether or not the variant classification criteria are in line with the drug efficacy results.

5.  The BRACAnalysis CDx™ is intended to be used with EDTA blood collection tubes. The sponsor should track and report on the results from samples provided in $K_2$EDTA and $K_3$EDTA collection tubes.  If the results from these studies lead to labeling changes, then a future PMA supplement would be required.

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.  Refer to drug label for Lynparza™ (olaparib) for additional information related to use of the drug.

Post-approval Requirements and Restrictions:  See approval order.

## XV.  REFERENCES

Beaudet AL and Tsui LC. (1993). A suggested nomenclature for designating mutations. *Hum Mutat* 2(4): 245-248.

Dosil V, et al. (2010). Alternative splicing and molecular characterization of splice site variant: BRCA1 c.519C>T as a case study. *Clin Chem* 56(1):53-61.

Hennessey BTJ, et al. (2010). Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP Ribose) polymerase inhibitors in ovarian cancer.  *J Clin Oncol* 28(22):3570-3576.

Huber LJ, et al. (2001). Impaired DNA damage response in cells expressing an exon 11-deleted murine Brca1 variant that localizes to nuclear foci. *Mol Cell Biol* 21(12):4005-4015.

Kim SS, et al. (2006). Hyperplasia and spontaneous tumor development in the gynecologic system in mice lacking the BRCA1-Δ11 isoform. *Mol Cell Biol* 26(18):6983-6992.

Kurian AW, et al. (2014). Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. *J Clin Oncol* 32(19): 2001-2009.

Miki Y, et al. (1994). A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science* 266(5182):66-71.

Pal T, et al. (2010). BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases.  *Cancer* 104(12):2807-2816.

Thakur S, et al. (1997). Localization of BRCA1 and a splice variant identified the nuclear localization signal. *Mol Cell Biol* 17(1): 444-452.