Myriad MyChoice® CDx Plus Technical Specifications

Effective Date: January 2025

Myriad Genetic Laboratories, Inc.

322 N 2200 W, Salt Lake City, UT 84116

Phone: 1-877-283-6709

www.myriad.com

CE IVD

Intended Use Statement

Myriad Genetics MyChoice® CDx Plus is a next generation sequencing based *in vitro* diagnostic device that provides sequencing and large rearrangement analyses on a panel of genes and/or detects genomic instability using DNA extracted from tumor specimens. Homologous Recombination Deficiency (HRD) is determined by assessing the results of a subset of these genes and/or the Genomic Instability Score (GIS) Status. The test may be used as a companion diagnostic to identify patients who are or may become eligible for treatment with specific therapies in accordance with the approved therapeutic product labeling. Results are to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with malignant solid tumors.

Limitations

- For *in vitro* diagnostic use
- For professional use only
- For prescription use only
- The MyChoice® CDx Plus assay identifies germline and somatic variants in the tumor but does not distinguish between the two.
- Alterations at allele frequencies below the established limit of detection may not be detected consistently.
- Reduced hybridization efficiency of DNA fragments spanning long insertions and deletions (indels), or rearrangements may result in under-representation of mutant DNA molecules in the final sequencing library. This will result in a reduction in the observed frequency of sequence reads spanning the mutation.
- Indels > 25 bp in length can be detected by the MyChoice® CDx Plus assay. However, the ability to detect any particular indel may be impacted by the location and nature of the mutation, the local sequence context, the DNA quality, and the non-tumor DNA content in the sample provided.
- The MyChoice® CDx Plus assay has been designed to detect genomic rearrangements including large rearrangements (LRs) involving the promoter and coding exons of the analyzed genes; however,
    - the promotor is not analyzed for the following genes: *CDK12*, *CHEK1*, *FANCL*, *PPP2R2A*, *RAD51B, and RAD54L*,
    - the detection of large rearrangement deletions and duplications is dependent on the quality of the submitted specimen.
- Whole gene duplications and deletions may not be detected by the MyChoice® CDx Plus assay.
- Other terminal duplications are reported as variants of uncertain significance.
- This analysis is believed to rule out the majority of abnormalities in the genes analyzed. There may be uncommon genetic abnormalities such as specific insertions, inversions, and certain regulatory mutations that will not be detected by the MyChoice® CDx Plus assay.

Myriad MyChoice® CDx Plus Technical Specifications

Effective Date: January 2025

Description of Analysis

Myriad Genetics MyChoice® CDx Plus uses next generation sequencing to assess tumor genomic instability and/or detect sequence variants and large rearrangements in up to 15 genes (*ATM, BARD1, BRCA1, BRCA2, BRIP1, CDK12, CHEK1, CHEK2, FANCL, PALB2, PPP2R2A, RAD51B, RAD51C, RAD51D, and RAD54L*). This analysis is performed on genomic DNA isolated from fixed tumor tissue.

The MyChoice® CDx Plus *in vitro* diagnostic laboratory developed test includes the following components:

Genomic Instability Score (GIS)

A comprehensive signature for Homologous Recombination Deficiency (HRD) is identified by testing genome-wide single nucleotide variants. The GIS is determined by measuring several elements including loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST). This test component may be performed alone or in combination with sequencing and LR analyses.

Sequence Analysis

Gene coding regions and portions of non-coding intronic regions are analyzed by sequence analysis and typically do not extend more than 20 base pairs (bp) proximal to the 5' end and 10 bp distal to the 3' end of each exon.

Large Rearrangement Analysis

Dosage analysis is used to determine copy number abnormalities. Coding exons and limited flanking intronic regions are examined for evidence of deletions and duplications (see Limitations section for any exceptions). Large rearrangement (LR) detection utilizes the number of reads that map to each nucleotide, normalized to the run median depth of coverage of the same nucleotide.

Description of Method

Genomic DNA from fixed tumor tissue is extracted using standard extraction reagents and methods to prepare DNA for library amplification. Following amplification, DNA is hybridized to a custom hybridization capture panel, which has biotinylated probes for over 26,000 single nucleotide polymorphism sites that are distributed across the human genome, as well as probes for exons and exon boundaries of the analyzed genes. The hybridized DNA mixture is amplified via standard PCR amplification method. The DNA libraries are then run on the next-generation sequencing instrument to generate sequences to be analyzed by Myriad's proprietary algorithm and software.

Sequence reads are mapped to targeted sequences of a pre-specified panel of genes. Allele specific copy number (ASCN) at each SNP location is determined using an algorithm to identify sequencing variants and LRs. Each variant is compared to a list of previously classified variants stored in the Myriad Variant Classification database, and the classification in the database is applied accordingly. Variants are classified in accordance with the recommendations of the American College of Medical Genetics and Genomics (ACMG) for standards in the interpretation and reporting of sequence variations. Clinically significant variants will be classified as "deleterious" or "suspected deleterious."

The GIS is calculated based upon an aggregate analysis of Loss of Heterozygosity (LOH), Telomeric Allelic Imbalance (TAI), and Large-scale State Transitions (LST). A GIS is considered to be positive if the score is at or above the current pre-specified threshold.

Interpretive Criteria:

Myriad's MyChoice® CDx Plus assay is used to detect Homologous Recombination Deficiency (HRD). Homologous Recombination Deficiency (HRD) is determined by assessing the results of a subset of these genes and/or the Genomic Instability Score (GIS) Status. The test may be used as a companion

diagnostic to identify patients who are or may become eligible for treatment with specific therapies in accordance with the approved therapeutic product labeling. Results are to be used by qualified health care professionals in accordance with professional guidelines in oncology for patients with malignant solid tumors. Follow-up germline testing may be appropriate for mutations in genes associated with hereditary cancer risk.

A GIS of 42 or greater confers a positive GIS Status in ovarian cancer.

"GIS Status: Positive"

The test results demonstrate homologous recombination deficiency based on the Genomic Instability Score.

"GIS Status: Negative"

The test results demonstrate homologous recombination proficiency based on the Genomic Instability Score.

"Tumor Mutation BRCA1/BRCA2 Status: Positive for a Clinically Significant Mutation"

The test results demonstrate the presence of a deleterious or suspected deleterious sequencing mutation or large rearrangement in *BRCA1* and/or *BRCA2*.

"Tumor Mutation BRCA1/BRCA2 Status: Negative for a Clinically Significant Mutation"

The test results do not demonstrate the presence of a deleterious or suspected deleterious sequencing mutation or large rearrangement in *BRCA1* or *BRCA2*. This category includes genetic variants for which published data demonstrate absence of substantial clinical significance and truncating mutations in *BRCA2* that occur at and distal to amino acid 3.326 (Mazoyer S et al., *Nature Genetics* 1996, 14:253-254). It also includes variants in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and base pair alterations in non-coding portions of the gene that have been demonstrated to have no pathogenic effect on the length or stability of the mRNA transcript. There may be uncommon genetic abnormalities that will not be detected by the MyChoice® CDx Plus assay (see **Limitations**).

Variant Interpretations

The following classifications apply to all genes analyzed.

"Deleterious"

Includes most nonsense and frameshift mutations that occur at/or before the last known deleterious amino acid position of the affected gene. In addition, specific missense mutations and non-coding intervening sequence (IVS) mutations are recognized as deleterious on the basis of data derived from linkage analysis of high-risk families, functional assays, biochemical evidence, statistical evidence, and/or demonstration of abnormal mRNA transcript processing. Deletions and duplications of an entire exon(s) identified by the MyChoice® CDx Plus test may also be interpreted to be deleterious. Deleterious large genomic rearrangements include single exon and multi-exonic deletions that are out-of-frame. Out-of-frame single or multi-exonic duplications are classified as deleterious if the orientation is determined to be in tandem and head-to-tail. In-frame deletions/duplications are interpreted on an individual basis and the specific evidence supporting the classification of these mutations is included in the individual patient report.

"Suspected Deleterious"

Includes genetic variants for which the available evidence indicates a high likelihood, but not definitive proof, that the mutation is deleterious. The specific evidence supporting an interpretation will be summarized for individual variants in the patient report.

"Special Interpretation"

Describes variants that would be typically interpreted as pathogenic according to the recommendations of the American College of Medical Genetics and Genomics (Richards S et al., Genet Med, 17(5):405-24); however, Myriad internal data suggest that these particular variants may not fully impair gene function in comparison to other pathogenic variants. Therefore, the effect of this variant on homologous recombination is currently unknown. These variants are reported under the category of "Additional Non-Clinically Significant Findings."

"Genetic variant of Uncertain Significance"

Includes missense variants and variants that occur in analyzed intronic regions whose functional significance has not yet been determined, as well as nonsense and frameshift mutations that occur distal to the last known deleterious amino acid positions of the affected genes. These variants are reported under the category of "Additional Non-Clinically Significant Findings."

"Favor Polymorphism" and "Polymorphism"

Includes genetic variants for which available evidence indicates that the variant is highly unlikely to alter protein production and/or function. Variants of this type are not reported.

Change of Interpretation

The classification and interpretation of all variants identified in the assay reflect the current state of scientific understanding at the time the report is issued. In some instances, the classification and interpretation of such variants may change as new scientific information becomes available.

Performance Characteristics

A description of the genes and tumor types evaluated in each analytical validation (AV) study is described within the respective summary. While not all genes and tumor types are represented in each of the AV studies, there have been no observed differences in the device performance based on these characteristics. The studies have been designed to evaluate representative sequence variants and large rearrangement (LR) types. Analytical results are provided for all genes and tumor types evaluated. Additionally, patient status, when reported in a study, is based on an evaluation of the *BRCA1* and *BRCA2* genes (t*BRCA1/2* Status) and a Genomic Instability Score (GIS) cutoff of ≥42. The established GIS cutoff has only been validated in clinical trials in ovarian cancer. The Myriad HRD Status is based on both the t*BRCA1/2* Status and GIS Status.

The following analytical performance characteristics are not applicable because the device is a qualitative test without any calibrators or controls: metrological traceability, linearity, limit of quantitation, measuring range. High dose hook effect is not applicable to NGS methods.

Analytical Specificity

The incidence of a false report of a genetic variant or mutation resulting from technical error or errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%).

The validation studies for *BRCA1* and *BRCA2* using breast, ovarian, and prostate fixed tumor samples demonstrated a Negative Percent Agreement (NPA) of all valid sequence non-variant base calls to be 953,408/953,409 (99.9999%).

Analytical Sensitivity

Failure to detect a genetic variant or mutation in the analyzed DNA regions may result from errors in specimen handling and tracking, hybridization, amplification and sequencing reactions, or computer-assisted analysis and data review. The rate of such errors is estimated from validation studies to be less than one percent (<1%).

The analytical sensitivity of next-generation sequencing for *BRCA1* and *BRCA2* was based on concordance of results between the test and comparator assays, using 56 individual anonymized breast, ovarian, and prostate fixed tumor samples. The comparator assay, which was performed by a second Myriad Genetic laboratory, detected 421 sequence variants observed at ≥ 10% allele frequencies. All 421 sequence variants were also detected by the MyChoice® CDx Plus assay, corresponding to a positive percent agreement (PPA) of 100%. The PPA of all valid variant calls (at allele frequencies ≥ 2%) was 425/426 (99.8%). For the *BRCA1* and *BRCA2* large rearrangement (LR) portion of the assays, 108/108 concordant valid gene LR calls were obtained corresponding to an overall percent agreement (OPA) of 100%. Comparisons were also made between the GISs generated by both assays for each of the 56 samples. The Total Analytical Error (TAE) calculated for all valid GISs generated by both assays was 3.83.

The lower Limit of Detection (LoD) for sequence variants was determined by using a total of three cell lines with multiple known sequence variants selected as "tumor" samples and one cell line with as few sequence variants as possible selected as the "normal" sample. The designated "tumor" DNA was mixed with various amount of "normal" DNA to create three sets of simulated "tumor": "normal" DNA mixtures with six levels of "tumor"-to- "normal" mix ratios. The resulting DNA mixtures were tested in multiple replicates. The results from this study revealed that the LoD for the sequencing portion of the MyChoice® CDx Plus assay is 10% allele frequency for representative sequence variants across all genes at the 200 ng DNA input level.

For large rearrangement analysis, two cell lines with large rearrangements (one single exon deletion and one multi-exon deletion) were selected as "tumor" samples and one cell line that did not carry any large rearrangements was selected as the "normal" sample. The designated "tumor" DNA was mixed with various amount of "normal" DNA to create two sets of simulated "tumor":"normal" DNA mixtures with 6 levels of "tumor"-to- "normal" dosage ratios. The resulting DNA mixtures were tested in triplicate. The results from this study demonstrate that the LoD of the LR portion of the MyChoice® CDx Plus assay is at 20% allele frequency for both the single exon deletion and multi-exon deletion at the 200 ng DNA input level.

For the GIS portion of the MyChoice® CDx Plus assay, three pairs of matched "tumor" and "normal" cell lines were evaluated in this study. Each cell line sample pair consisted of DNA from a breast cancer cell line and DNA from the corresponding, matched normal cell line that was established from the same affected individual. The resulting paired "tumor" and "normal" DNAs were mixed at eight different levels of "tumor" to "normal" dosage ratios ranging from 100% "tumor" to 100% "normal" (0% "tumor") to create three sets of simulated "tumor": "normal" DNA mixtures. These DNA mixtures were subsequently tested in multiple replicates. The Limit of Quantitation of the GIS portion of the assay was determined to be 30% tumor DNA content at the 200 ng DNA input level.

Precision – Repeatability and Reproducibility

Analytical validation studies included a precision study for NGS. In a separate study, seven fixed tumor samples (comprised of ovarian, breast, and prostate samples) were analyzed by the MyChoice® CDx Plus assay. Each sample was run in duplicate on four independent runs performed over different days, using two sets of different instruments, reagents lots, and operators. This study demonstrated a 99.74% PPA (1,157/1,160) for all valid sequence variant calls, 99.9998% NPA (3,256,283/3,256,291) for all valid sequence non-variant base calls, and 100% OPA (834/834) for all valid large rearrangement calls across all samples and replicates evaluated. GISs were also assessed across the same samples and demonstrated an overall standard deviation (SD) of 2.20 for valid scores across all replicates evaluated.

Additional Tumor Type Validation

A total of 35 fixed tumor tissue samples from 3 lung, 3 pancreatic, 9 colon, 3 endometrial, 3 esophageal, 3 head and neck, 3 gastric, 3 bladder, and 5 sarcoma (including 5 MSI+ tumors) were evaluated against all 15 genes at four DNA input levels: 200 ng (highest input), 100 ng, 25 ng, and 7.5 ng (lowest input) to demonstrate there is no performance difference in the assay based on tumor type. Samples were run in triplicate at 200 ng to generate the reference results. All other inputs were run in singlet. The reference results for all genes contained representative variants, including 4,589 single base substitutions, 331 insertions or deletions < 10 bp in length, 7 insertions or deletions ≥ 10 bp in length, and 271 variants in 5+ bp homopolymer stretches. Three samples failed the whole assay in this study, one sample at 25 ng and two samples at 7.5 ng.

Table 1. Summary of Concordance and TAE of Analytical Results

Analytical Component	Analysis	DNA Input	All 15 Genes
Sequence Variants (≥ 10% Reference AF)	PPA	200 ng	2,471/2,472 (99.96%)
		100 ng	824/824 (100%)
		25 ng	806/806 (100%)
		7.5 ng	776/781 (99.4%)
Sequence Variants (All AFs)	PPA	200 ng	2,485/2,487 (99.92%)
		100 ng	828/829 (99.9%)
		25 ng	810/811 (99.9%)
		7.5 ng	777/785 (99.0%)
Sequence Non-Variant Base Calls	NPA	200 ng	6,105,995/6,105,997 (99.99997%)
		100 ng	2,035,249/2,035,251 (99.99990%)
		25 ng	1,976,711/1,976,715 (99.99980%)
		7.5 ng	1,798,416/1,798,435 (99.99894%)
Large Rearrangements	NPA	200 ng	1,492/1,492 (100%)
		100 ng	508/508 (100%)
		25 ng	497/497 (100%)
		7.5 ng	417/417 (100%)
GIS	TAE	200 ng	NA
		100 ng	3.34
		25 ng	2.55
		7.5 ng	4.36

*There were no LRs in this study and therefore PPA is not applicable and NPA and OPA are the same.

Cross-reactivity

Cross-reactivity studies for sequence-based assays are intended to differentiate between target analyte sequences and sequences generated from other sources. Three types of spurious sequences that could potentially be mistaken for target sequences, e.g., pseudogenes or other genomic regions that are

highly homologous to targeted genes and regions, off-target regions that hybridize to hybridization baits or DNA sequences that carry-through the process, and process artifacts and low-quality sequences. The purpose of this study was to demonstrate that captured sequences not originating from target regions do not materially affect the assay. Sequence data from 6 fixed breast and ovarian tumor tissue samples run in quadruplicate, yielding 24 total tests results, were mapped to the human genome sequence to reveal off-target sequences due to pseudogene and cross-reactivity of hybridization baits (capture probes). The assay has two methods for mitigating the impact of pseudogenes, 1) the capture baits were designed to minimize the capture of pseudogene regions, and 2) the analysis algorithm identifies and excludes pseudogene-derived sequences as part of variant detection and LR calling. No off-target sequences were found to affect the *BRCA1* and *BRCA2* sequencing component of the assay. Only 0.32% of single base substitutions (SBSs) used to calculate the GIS were affected by off-target sequences with a minimal effect on the reported score. This analysis suggests that cross-reactivity poses a minimal risk to the assay.

Interfering substances

Interference studies were performed to evaluate potential interference of selected substances such as tissue marking dye, paraffin wax, hemoglobin, triglycerides, ethanol, sodium hydroxide and proteinase K. High levels of each substance were tested and only if an acceptance criterion was failed then the lower level of substance was tested. All substances except for sodium hydroxide and proteinase K passed all acceptance criteria at the high level tested. Sodium hydroxide and proteinase K passed all acceptance criteria at the lower level tested. Factoring in the likelihood of occurrence of the levels of substances evaluated in this study, the overall results demonstrate that the assay exhibits minimal interference from these substances.

Specimen Stability

This study evaluated the stability of fixed tumor blocks and fixed tumor sections on slides using ovarian and breast fixed tumor tissue, stored at laboratory temperature (a.k.a. room temperature). This stability study mimics the testing of aged, patient fixed tumor blocks and slides sent to and/or stored at Myriad. This study evaluated *BRCA1* and *BRCA2* genes, as well as GIS. For a fixed tumor block, the initial time 0 (t0) is defined based on the date of surgery. For fixed tumor sections on slides, t0 was defined based on the date of sectioning. Results from the earliest time point tested in at least triplicate were used as the reference result. Since the date of surgery, time of first testing, and fixed tumor block remaining all vary per sample, not all fixed tumor blocks were tested at each time-point. TAE analysis was only performed if there were 5 valid GISs. In total, 25 unique ovarian and breast tumor samples were tested for fixed tumor block stability and 10 unique ovarian and breast samples were tested for fixed sections on slides stability. All time-points tested so far have passed all acceptance criteria. The final stability of fixed tumor blocks and sections on slides will be defined at a time point that is shorter by at least 3 months than the longest time point to successfully pass the acceptance criteria. This study is ongoing. The latest validated timepoints for specimen stability are:

- Fixed tumor tissue blocks: 8.75 years
- Fixed tumor tissue sections on slides: 5 years

The next time points to be assessed will be ≥12.25 years for fixed tumor tissue blocks and ≥8.75 years for fixed tumor tissue sections on slides.

Table 2. Summary of Concordance and TAE of Analytical Results

Analytical Component	Analysis	Study Arm	Time Point (Years)	*BRCA1* and *BRCA2* Genes
Sequence Variants	PPA	Fixed Tumor Blocks	t_0	1,535/1,535 (100%)
			1.5	4/4 (100%)
			2.0	27/27 (100%)

Analytical Component	Analysis	Study Arm	Time Point (Years)	*BRCA1* and *BRCA2* Genes
(≥ 10% Reference AF)			2.5	11/11 (100%)
			3.0	25/25 (100%)
			3.5	107/107 (100%)
			5.0	93/93 (100%)
			5.5	67/67 (100%)
			7.5	11/11 (100%)
			8.0	11/11 (100%)
			8.75	95/95 (100%)
			12.25	42/42 (100%)
		Fixed Sections on Slides	t_0	312/312 (100%)
			1.0	104/104 (100%)
			3.75	183/183 (100%)
			5.0	104/104 (100%)
Sequence Variants (All AFs)	PPA	Fixed Tumor Blocks	t_0	1,552/1,552 (100%)
			1.5	4/4 (100%)
			2.0	27/27 (100%)
			2.5	11/11 (100%)
			3.0	25/25 (100%)
			3.5	108/108 (100%)
			5.0	94/94 (100%)
			5.5	68/68 (100%)
			7.5	11/11 (100%)
			8.0	11/11 (100%)
			8.75	98/98 (100%)
			12.25	42/42 (100%)
		Fixed Sections on Slides	t_0	318/318 (100%)
			1.0	106/106 (100%)
			3.75	187/187 (100%)
			5.0	105/106 (99.06%)
Sequence Non-Variant Base Calls	NPA [95% LCL]	Fixed Tumor Blocks	t_0	2,700,875/2,700,875 (100%) [99.9999%]
			1.5	17,344/17,344 (100%) [99.9827%]
			2.0	52,016/52,016 (100%) [99.9942%]
			2.5	17,336/17,336 (100%) [99.9827%]
			3.0	51,979/51,979 (100%) [99.9942%]
			3.5	207,273/207,274 (99.9995%) [99.9977%]
			5.0	172,590/172,590 (100%) [99.9983%]
			5.5	118,877/118,877 (100%) [99.9975%]
			7.5	17,336/17,336 (100%) [99.9827%]

Analytical Component	Analysis	Study Arm	Time Point (Years)	*BRCA1* and *BRCA2* Genes
			8.0	17,336/17,336 (100%) [99.9827%]
			8.75	171,272/171,272 (100%) [99.9983%]
			12.25	69,346/69,346 (100%) [99.9957%]
		Fixed Sections on Slides	t_0	517,263/517,264 (99.9998%) [99.9989%]
			1.0	172,508/172,508 (100%) [99.9983%]
			3.75	305,006/305,006 (100%) [99.9990%]
			5.0	173,224/173,224 (100%) [99.9983%]
Large Rearrangements	PPA, NPA, OPA	Fixed Tumor Blocks	t_0	15/15 (100%), 249/249 (100%), 264/264 (100%)
			1.5	0/0 (N/A), 2/2 (100%), 2/2 (100%)
			2.0	0/0 (NA), 6/6 (100%), 6/6 (100%)
			2.5	0/0 (N/A), 2/2 (100%), 2/2 (100%)
			3.0	1/1 (100%), 5/5 (100%), 6/6 (100%)
			3.5	2/2 (100%), 22/22 (100%), 24/24 (100%)
			5.0	2/2 (100%), 18/18 (100%), 20/20 (100%)
			5.5	2/2 (100%), 11/11 (100%), 13/13 (100%)
			7.5	0/0 (N/A), 2/2 (100%), 2/2 (100%)
			8.0	0/0 (N/A), 2/2 (100%), 2/2 (100%)
			8.75	1/1 (100%), 17/17 (100%), 18/18 (100%)
			12.25	0/0 (N/A), 8/8 (100%), 8/8 (100%)
		Fixed Sections on Slides	t_0	3/3 (100%), 57/57 (100%), 60/60 (100%)
			1.0	1/1 (100%), 19/19 (100%), 20/20 (100%)
			3.75	2/2 (100%), 33/33 (100%), 35/35 (100%)
			5.0	1/1 (100%), 19/19 (100%), 20/20 (100%)
GIS	TAE	Fixed Tumor Blocks	t_0	NA
			1.5	NA
			2.0	NA
			2.5	NA

Analytical Component	Analysis	Study Arm	Time Point (Years)	*BRCA1* and *BRCA2* Genes
			3.0	NA
			3.5	3.25
			5.0	6.94
			5.5	7.76
			7.5	NA
			8.0	NA
			8.75	4.33
			12.25	NA
		Fixed Sections on Slides	t_0	NA
			1.0	1.94
			3.75	4.02
			5.0	2.98

Table 3. Summary of Concordance of Valid Patient Status Results

	Study Arm	Time Point (Years)	t*BRCA1/2* Status	GIS Status	Myriad HRD Status
PPA	Fixed Tumor Blocks	t_0	73/73 (100%)	70/70 (100%)	85/85 (100%)
		1.5	1/1 (100%)	NA	1/1 (100%)
		2.0	1/1 (100%)	1/1 (100%)	2/2 (100%)
		2.5	NA	NA	NA
		3.0	3/3 (100%)	3/3 (100%)	3/3 (100%)
		3.5	6/6 (100%)	6/6 (100%)	7/7 (100%)
		5.0	4/4 (100%)	5/5 (100%)	5/5 (100%)
		5.5	4/4 (100%)	5/5 (100%)	5/5 (100%)
		7.5	NA	NA	NA
		8.0	NA	NA	NA
		8.75	3/3 (100%)	5/5 (100%)	5/5 (100%)
		12.25	NA	NA	NA
	Fixed Sections on Slides	t_0	12/12 (100%)	15/15 (100%)	15/15 (100%)
		1.0	4/4 (100%)	5/5 (100%)	5/5 (100%)
		3.75	7/7 (100%)	9/9 (100%)	9/9 (100%)
		5.0	4/4 (100%)	5/5 (100%)	5/5 (100%)
NPA	Fixed Tumor Blocks	t_0	83/83 (100%)	86/86 (100%)	71/71 (100%)
		1.5	NA	1/1 (100%)	NA
		2.0	2/2 (100%)	2/2 (100%)	1/1 (100%)
		2.5	1/1 (100%)	1/1 (100%)	1/1 (100%)
		3.0	NA	NA	NA
		3.5	6/6 (100%)	6/6 (100%)	5/5 (100%)
		5.0	6/6 (100%)	5/5 (100%)	5/5 (100%)
		5.5	3/3 (100%)	2/2 (100%)	2/2 (100%)
		7.5	1/1 (100%)	1/1 (100%)	1/1 (100%)
		8.0	1/1 (100%)	1/1 (100%)	1/1 (100%)
		8.75	7/7 (100%)	5/5 (100%)	5/5 (100%)
		12.25	4/4 (100%)	4/4 (100%)	4/4 (100%)
	Fixed Sections on Slides	t_0	18/18 (100%)	15/15 (100%)	15/15 (100%)
		1.0	6/6 (100%)	5/5 (100%)	5/5 (100%)
		3.75	11/11 (100%)	9/9 (100%)	9/9 (100%)

	Study Arm	Time Point (Years)	t*BRCA1/2* Status	GIS Status	Myriad HRD Status
		5.0	6/6 (100%)	5/5 (100%)	5/5 (100%)
OPA	Fixed Tumor Blocks	t_0	132/132 (100%)	132/132 (100%)	132/132 (100%)
		1.5	1/1 (100%)	1/1 (100%)	1/1 (100%)
		2.0	3/3 (100%)	3/3 (100%)	3/3 (100%)
		2.5	1/1 (100%)	1/1 (100%)	1/1 (100%)
		3.0	3/3 (100%)	3/3 (100%)	3/3 (100%)
		3.5	12/12 (100%)	12/12 (100%)	12/12 (100%)
		5.0	10/10 (100%)	10/10 (100%)	10/10 (100%)
		5.5	7/7 (100%)	7/7 (100%)	7/7 (100%)
		7.5	1/1 (100%)	1/1 (100%)	1/1 (100%)
		8.0	1/1 (100%)	1/1 (100%)	1/1 (100%)
		8.75	10/10 (100%)	10/10 (100%)	10/10 (100%)
		12.25	4/4 (100%)	4/4 (100%)	4/4 (100%)
	Fixed Sections on Slides	t_0	30/30 (100%)	30/30 (100%)	30/30 (100%)
		1.0	10/10 (100%)	10/10 (100%)	10/10 (100%)
		3.75	18/18 (100%)	18/18 (100%)	18/18 (100%)
		5.0	10/10 (100%)	10/10 (100%)	10/10 (100%)

Assay Migration

The following assay migration studies were performed to support the migration of MyChoice® CDx Plus from the HiSeq 2500 NGS to the NovaSeq 6000 NGS: concordance between the HiSeq 2500 and NovaSeq 6000 NGS, precision, LoB, LoD and reagent stability. No statistically significant differences ($\alpha = 0.05$, $p \leq 0.05$) in the rates of test validity or analytical and patient status agreements were observed in any study, indicating equivalent performance using the HiSeq 2500 and NovaSeq 6000 NGS.

Correlation between the HiSeq 2500 NGS and NovaSeq 6000 NGS

The concordance was based on results between MyChoice® CDx Plus using the HiSeq2500 NGS and NovaSeq 6000 NGS using 175 ovarian, 71 breast, 7 colon, 5 prostate, 5 sarcoma, 3 bladder, 3 endometrial, 3 gastric/stomach, 3 head and neck, 3 lung, and 3 pancreatic fixed tumor samples run at four DNA inputs: 200 ng (220 samples), 100 ng (11 samples), 30 ng (25 samples), and 7.5 ng (25 samples). The reference results for all 15 genes contained representative variants, including 5,775 single base substitutions, 287 insertions or deletions < 10 bp in length, 33 insertions or deletions ≥ 10 bp in length, 169 variants in 5+ bp homopolymer stretches, 16 LR affecting 1-2 exons, and 22 LR affecting ≥ 3 exons. The reference GISs ranged from 0-90. Patient status results were evaluated based on the 175 ovarian and 71 breast samples and contained all possible combinations of patient statuses (123 t*BRCA1/2* Status negative / GIS Status negative, 44 t*BRCA1/2* Status negative / GIS Status positive, 5 t*BRCA1/2* Status positive /GIS Status negative, and 62 t*BRCA1/2* Status positive / GIS Status positive). Four samples failed the assay in this study, 2 breast, 1 ovarian and 1 prostate sample.

Table 4. Summary of Concordance and TAE of Analytical Results

Analytical Component	Analysis	All 15 Genes
Sequence Variants (≥ 10% Comparator AFs)	PPA [95% LCL]	5,905/5,914 (99.85%) [99.71%]
Sequence Variants (All AFs)	PPA [95% LCL]	6,026/6,046 (99.67%) [99.49%]

Analytical Component	Analysis	All 15 Genes
Sequence Non-Variant Base Calls	NPA [95% LCL]	16,108,176/16,108,210 (99.999789%) [99.999705%]
Large Rearrangements	PPA NPA OPA	27/28 (96%) 3,785/3,791 (99.84%) 3,812/3,819 (99.82%)
GIS	TAE	3.880

Table 5. Concordance Summary of Valid Patient Status Results

	t*BRCA1/2* Status	GIS Status	Myriad HRD Status
PPA	68/69 (98.6%)	102/105 (97.1%)	108/112 (96.4%)
NPA	173/174 (99.4%)	123/125 (98.4%)	119/120 (99.2%)
OPA	241/243 (99.2%)	225/230 (97.8%)	227/232 (97.8%)

Limit of Blank (LoB)

Fifty-three (53) breast and ovarian fixed normal tissue samples were each tested across two lots of NGS reagents wherein all low frequency variants were expected to be spurious technical artifacts rather than true biological events. For all 15 genes a false positive call rate of 0.0001136% with a 95% upper confidence limit (UCL) of 0.0002340% for sequence variants was produced.

Limit of Detection (LoD)

While the lowest input into the device is 7.5 ng, Myriad performed all LoD studies using fixed tumor samples at both the highest (200 ng) and lowest (7.5 ng) input, as greater than 90% of commercial samples are run at 200 ng.

Sequence Variants LoD

The sequence variant LoD claims were established at the lowest and highest DNA input levels (7.5 ng and 200 ng) using fixed tumor samples with representative pathogenic (deleterious or suspected deleterious) sequence variants (SBS, insertions and/or deletions (indels), and variants in homopolymer stretches 5+bp). Contrived tumor:normal mixes were titrated to 4-5 different allele frequencies (AFs) and tested across either 10 or 20 replicates. LoD was based on a 95% hit rate approach. Based on these results Myriad claims an approximate 10% AF LoD for representative sequence variants at the highest (200 ng) input and 20% AF LoD for representative sequence variants at the lowest (7.5 ng) input.

Table 6. Summary of Representative Sequence Variant LoD

Variant	Variant Type	LoD AF 200 ng	LoD AF 7.5 ng
***BRCA1* c.5503C>T(p.Arg1835*)**	**Single Base Substitution**	8%	20%
***BRCA1* c.3648del (p.Leu1216Phefs*19)**	**Indel < 10 bp**	5%	20%
***BRCA1* c.4964_4982del (p.Ser1655Tyrfs*16)**	**Indel ≥ 10 bp**	5%	15%
***BRCA2* c.5350_5351del (p.Asn1784Hisfs*2)**	**Homopolymer +5 bp and Indel < 10 bp**	5%	10%

Large Rearrangements (LR) LoD

The LR LoD claims were established at the lowest and highest DNA input levels (7.5 ng and 200 ng) using fixed tumor samples with representative LRs. Contrived tumor:normal mixes were titrated to 5 different AFs and tested across 10 replicates. LoD was based on a 95% hit rate approach.

Table 7. Summary of Representative LR LoD

LR	LR Type	LoD AF 200 ng	LoD AF 7.5 ng
***BRCA1* del exons 9-10**	**1-2 exon LR**	30%	50%
***BRCA2* ULR**	**≥ 3 exon LR**	30%	60%

Abbreviation: ULR = uncharacterized large rearrangement

Genomic Instability Status (GIS) Status LoD

The GIS Status LoD claims were established at the lowest and highest DNA input levels (7.5 ng and 200 ng) using matched fixed tumor and normal ovarian samples. Tumor:normal mixes were titrated to 5 different tumor content (TC) percentage levels and tested across either 10 or 20 replicates per level. LoD was based on a 95% hit rate approach.

Table 8. Summary of Representative GIS Status LoD

GIS Status	Mean Reference GIS	LoD TC 200 ng	LoD TC 7.5 ng
Negative	**37.0**	30%	40%
Positive	**54.7**	30%	49%

Precision: Repeatability and Reproducibility

The precision study demonstrated the repeatability and reproducibility of the assay by using 14 breast and ovarian fixed tumor samples tested in duplicate per batch across three reagents lots, three sequencers, three operators, and at least 3 days, at both the highest and lowest DNA input levels (200 ng and 7.5 ng), resulting in 18 total replicates per sample, per DNA input level. The reference results for all 15 genes contained representative variants, including 333 single base substitutions, 17 insertions or deletions < 10 bp in length, 5 insertions or deletions ≥ 10 bp in length, 11 variants in 5+ bp homopolymer stretches, 3 LRs affecting 1-2 exons, and 6 LRs affecting ≥ 3 exons. The reference GISs ranged from 17.72 – 77.28. All possible combinations of patient statuses were also included, (4 t*BRCA1/2* Status negative / GIS Status negative, 3 t*BRCA1/2* Status negative / GIS Status positive, 2 t*BRCA1/2* Status positive /GIS Status negative, and 5 t*BRCA1/2* Status positive / GIS Status positive). Two samples each had one of the 18 replicates fail the whole assay at the 7.5 ng DNA input level.

Table 9. Summary of Concordance, SD and TAE of Analytical Results

Analytical Component	Analysis	DNA Input	All 15 Genes
Sequence Variants (≥ 10% Reference AFs)	PPA	200 ng	6,138/6,138 (100%)
		7.5 ng	6,004/6,091 (98.57%)
Sequence Variants (All AFs)	PPA	200 ng	6,370/6,390 (99.69%)
		7.5 ng	6,188/6,343 (97.56%)
Sequence Non-Variant Base Calls	NPA	200 ng	14,652,576/14,652,584 (99.999945%)

Analytical Component	Analysis	DNA Input	All 15 Genes
		7.5 ng	14,536,211/14,536,380 (99.998837%)
Large Rearrangements	PPA NPA OPA	200 ng	148/148 (100%) 3,551/3,551 (100%) 3,699/3,699 (100%)
		7.5 ng	127/130 (97.7%) 3,491/3,492 (99.97%) 3,618/3,622 (99.89%)
GIS	SD	200 ng	2.17
	TAE	7.5 ng	6.38

Table 10. Concordance Summary of Valid Patient Status Results

	DNA Input	t*BRCA1/2* Status	GIS Status	Myriad HRD Status
PPA	200 ng	126/126 (100%)	144/144 (100%)	180/180 (100%)
	7.5 ng	125/125 (100%)	139/139 (100%)	178/178 (100%)
NPA	200 ng	126/126 (100%)	108/108 (100%)	72/72 (100%)
	7.5 ng	124/125 (99.2%)	90/90 (100%)	62/63 (98.4%)
OPA	200 ng	252/252 (100%)	252/252 (100%)	252/252 (100%)
	7.5 ng	249/250 (99.6%)	229/229 (100%)	240/241 (99.6%)

Reagents Stability

The reagents stability study verified the storage and stability of critical assay reagents, including the IDT capture probes and NovaSeq NGS reagents. IDT capture probes were also subjected to freeze-thaw test treatments at each time point. The total (cumulative) number of freeze-thaw cycles is shown across the different stability testing time points. Time zero (t0) was defined as the first testing timepoint and reagents were then aged to 2, 9 and 15 months. Three breast and four ovarian fixed tumor samples were tested in duplicate across three different reagent lots for each time point. The reference results for all 15 genes contained representative variants, including 166 single base substitutions, 8 insertions or deletions < 10 bp in length, 2 insertions or deletions ≥ 10 bp in length, 4 variants in 5+ bp homopolymer stretches, and 1 LR affecting ≥ 3 exons. The reference GISs ranged from 5.8 – 72.2. The reference patient statuses included (2 t*BRCA1/2* Status negative / GIS Status negative, 1 t*BRCA1/2* Status negative / GIS Status positive, and 4 t*BRCA1/2* Status positive / GIS Status positive.) Results were assessed across all lots of reagents at each time point. The final stability of reagents is defined as the time point that is shorter by at least 15% than the longest time point to successfully pass the acceptance criteria. At t0 and all stability time points tested, 100% of tests produced valid sequencing, LR, and GIS analytical results and passed all acceptance criteria. Therefore, the final reagents stability claim is 12.75 months.

Table 11. Summary of Concordance and TAE of Analytical Results

Analytical Component	Analysis	Time Point/ Freeze Thaw	All 15 Genes
Sequence Variants (≥ 10% Reference AF)	PPA	t_0 / 0	1,032/1,032 (100%)
		2 months / 3	1,032/1,032 (100%)
		9 months / 6	1,028/1,032 (99.61%)

Analytical Component	Analysis	Time Point/ Freeze Thaw	All 15 Genes
		15 months / 9	1,032/1,032 (100%)
Sequence Variants (All AFs)	PPA	t_0 / 0	1,038/1,038 (100%)
		2 months / 3	1,037/1,038 (99.90%)
		9 months / 6	1,031/1,038 (99.33%)
		15 months / 9	1,038/1,038 (100%)
Sequence Non-Variant Base Calls	NPA	t_0 / 0	2,442,162/2,442,162 (100%)
		2 months / 3	2,442,159/2,442,162 (99.99988%)
		9 months / 6	2,442,162/2,442,164 (99.99992%)
		15 months / 9	2,442,162/2,442,162 (100%)
Large Rearrangements	PPA, NPA, OPA	t_0 / 0	3/6 (50%), 618/618 (100%), 621/624 (99.5%)
		2 months / 3	5/5 (100%), 622/622 (100%), 627/627 (100%)
		9 months / 6	6/6 (100%), 614/614 (100%), 620/620 (100%)
		15 months / 9	6/6 (100%), 615/615 (100%), 621/621 (100%)
GIS	TAE	t_0 / 0	NA
		2 months / 3	2.826
		9 months / 6	2.667
		15 months / 9	3.914

Table 12. Summary of Concordance of Valid Patient Status Results

	Time Point	t*BRCA1/2* Status	GIS Status	Myriad HRD Status
PPA	t_0	24/24 (100%)	30/30 (100%)	30/30 (100%)
	2 months	24/24 (100%)	30/30 (100%)	30/30 (100%)
	9 months	24/24 (100%)	30/30 (100%)	30/30 (100%)
	15 months	24/24 (100%)	30/30 (100%)	30/30 (100%)
NPA	t_0	18/18 (100%)	12/12 (100%)	12/12 (100%)
	2 months	18/18 (100%)	12/12 (100%)	12/12 (100%)
	9 months	18/18 (100%)	12/12 (100%)	12/12 (100%)
	15 months	18/18 (100%)	12/12 (100%)	12/12 (100%)
OPA	t_0	42/42 (100%)	42/42 (100%)	42/42 (100%)
	2 months	42/42 (100%)	42/42 (100%)	42/42 (100%)
	9 months	42/42 (100%)	42/42 (100%)	42/42 (100%)
	15 months	42/42 (100%)	42/42 (100%)	42/42 (100%)

Clinical Significance

The clinical utility of the Myriad MyChoice® CDx Plus for use as a companion diagnostic has been evaluated in multiple clinical studies.

Summary of Clinical Study - Olaparib D0817C00003 (PAOLA-1)

Overview

A phase III, randomized, double-blind, placebo-controlled, multicenter study was conducted in 806 patients (537 patients in the olaparib/bevacizumab group, 269 patients with placebo/bevacizumab) with newly diagnosed, advanced (FIGO stage IIIB-IV), high grade epithelial ovarian, fallopian tube, or primary peritoneal cancer treated with standard first-line treatment, combining platinum-taxane chemotherapy and bevacizumab concurrent with chemotherapy and in maintenance to evaluate the efficacy and safety of olaparib (tablet) 300 mg given twice daily in combination with bevacizumab) in comparison with placebo in combination with bevacizumab.

Additionally, progression free survival (PFS) was investigated in exploratory biomarkers subgroups using the Myriad MyChoice® HRD Plus test, a research use assay, to further understand the consistency of treatment effect of olaparib across potential predictive and prognostic factors.

Myriad HRD status is based on the Genomic Instability Score (GIS) and/or Tumor Mutation *BRCA1/BRCA2* Status (*tBRCAm)*. A positive Myriad HRD status is determined either by presence of a *tBRCA1/2* mutation or by a GIS at or above cut-off of 42 in the absence of a *tBRCA1/2* mutation.

Of the 806 randomized patients in PAOLA-1, 755 (93.7%) had a Myriad *tBRCA1/2* mutation status available and 664 (82.4%) had an available Myriad HRD status with the myChoice® HRD Plus test. With the MyChoice® CDx test, a total of 755/806 (93.7%) patients were considered for testing. A total of 700/806 (86.8%) patients had a valid *tBRCA1/2* mutation status result and 643/806 (79.8%) had a valid HRD status result.

Concordance between the Myriad MyChoice® HRD Plus RUO test and the Myriad MyChoice® CDx in PAOLA-1:

The concordance between the MyChoice® HRD Plus test and the MyChoice® CDx test for HRD status at a cut-off 42 for patients with a valid result for both tests, was as follows:

- OPA: 97.8% (95% CI 96.3%, 98.8%)
- PPA: 98.6% (95% CI 96.8%, 99.6%)
- NPA: 96.6% (95% CI 93.7%, 98.4%)

Comparison of efficacy between PAOLA-1 full analysis set and the subgroups determined by Myriad MyChoice® CDx:

PAOLA-1 met its primary objective, demonstrating a statistically significant and clinically meaningful improvement in PFS in the Full Analysis Set (FAS) for olaparib vs placebo when added to bevacizumab (HR 0.59; 95% CI 0.49 to 0.72; p<0.0001). Olaparib prolonged PFS by a median of 5.5 months over placebo. The clinical outcome data for the FAS and the efficacy data from post-database lock exploratory analyses performed in the subset of patients for whom Myriad HRD status for cut-off ≥42 defined by the Myriad MyChoice® CDx test are shown in the following table.

Clinical outcome (PFS by investigator assessment) of PAOLA-1

Table 13

FAS		MyChoice® CDx Myriad HRD status positive		MyChoice® CDx Myriad HRD status negative		MyChoice® CDx Myriad HRD status unknown	
Olaparib/ bevacizumab (n=537)	**Placebo/ bevacizumab (n=269)**	**Olaparib/ bevacizumab (n=251)**	**Placebo/ bevacizumab (n=125)**	**Olaparib/ bevacizumab (n=180)**	**Placebo/ bevacizumab (n=87)**	**Olaparib/ bevacizumab (n=106)**	**Placebo/ bevacizumab (n=57)**
Number of events/total number of patients (%)							
280/537 (52.1)	194/269 (72.1)	87/251 (34.7)	86/125 (68.8)	135/180 (75.0)	68/87 (78.2)	58/106 (54.7)	40/57 (70.2)
Median PFS (months)							
22.1	16.6	39.3	17.7	16.6	16.2	22.1	15.3
HR (95% CI)							
0.59 (0.49, 0.72)		0.35 (0.26-0.48)		1.00 (0.75-1.34)		0.68 (0.45-1.02)	

Summary of Clinical Study – Olaparib D0818C00001 (SOLO1)

Overview

The olaparib clinical study D0818C00001 (SOLO1) was a Phase III, randomized, double blind, placebo controlled, multicenter study to assess the efficacy of olaparib maintenance monotherapy in advanced ovarian cancer patients (including patients with primary peritoneal and/or fallopian tube cancer) who had responded following first-line platinum based chemotherapy and carrying *BRCA* mutations (documented mutation in *BRCA1* or *BRCA2*) that were predicted to be deleterious or suspected deleterious (known or predicted to be detrimental/lead to loss of function). A total of 391 patients were enrolled from 118 centers in 15 countries, including the United States. Patients were required to have documented evidence of a deleterious or suspected deleterious mutation in either *BRCA1* or *BRCA2* to be enrolled into the study. Evidence of a qualifying *BRCA* mutation could be from either an existing *BRCA* mutation result from local testing, or from prospective testing performed by Myriad (Salt Lake City, UT) using the Myriad Integrated BRAC*Analysis*® or for Chinese patients by BGI Clinical Laboratories (Shenzhen, China) using a local validated test.

A post-randomization analysis of a subset of the SOLO1 tumor samples was performed to identify the population that would be defined as tumor *BRCA1/2 (tBRCA1/2)* by the Myriad MyChoice® CDx test. The clinical performance of the MyChoice® CDx test for the SOLO1 study was based on the Tumor Mutation *BRCA1*/*BRCA2* (t*BRCAm*) Status and was established based on available residual extracted DNA generated from tumor FFPE samples collected from patients enrolled on the SOLO1 study.

Accountability of PMA Cohort

Out of the 391 patients randomized in the SOLO1 study, extracted FFPE DNA samples from 333 patients were available for retrospective MyChoice® CDx testing. Samples from 298 patients were tested using MyChoice® CDx test, and valid *tBRCA1/2* mutation status results were reported for 292 patients. Among those, 284 patients were confirmed to carry a deleterious or suspected deleterious *tBRCA1/2* mutation and 8 patients were not confirmed to carry a deleterious or suspected deleterious *tBRCA1/2* mutation by the MyChoice® CDx test. The PMA cohort represented 74.7% (292/391) of the full analysis set in SOLO1.

Effectiveness Results

The primary objective of this study was to determine the efficacy by PFS (using investigator assessment of scans according to modified Response Evaluation Criteria in Solid Tumors (RECIST (version 1.1)) of olaparib maintenance monotherapy compared with placebo in *BRCA* mutated high risk advanced ovarian cancer patients who are in clinical complete response (CR) or partial response (PR) following first line platinum-based chemotherapy. The study population consisted of 391 ovarian cancer patients with a deleterious or suspected deleterious germline *BRCA* mutation as detected by the Myriad Integrated BRAC*Analysis*® test, BRACAnalysis CDx® test, BGI test or local test results. A statistically

significant and clinically relevant improvement in investigator assessed PFS, as evidenced by the magnitude of effect: a 70% reduction in the risk of disease progression or death at any point in time for olaparib vs placebo treated patients (HR 0.30; 95% CI 0.23-0.41; p<0.0001), with a median PFS not reached in the olaparib arm vs 13.8 months for placebo. Median follow-up for PFS defined as time from randomisation to date of censoring was approximately 41 months on both arms.

The effectiveness of the *tBRCA1/2* mutation status of the Myriad MyChoice® CDx test was based on a subset of 284 confirmed ovarian cancer patients with deleterious or suspected deleterious *tBRCA1/2* mutations.

The clinical outcome data for the 284 patients with a confirmed *tBRCA1*/2 mutation was as follows: a 71% reduction in the risk of disease progression or death at any point in time for olaparib vs placebo treated patients (HR 0.29; 95% CI 0.21-0.41; p<0.0001), with a median PFS not reached in the olaparib arm vs 13.8 months for placebo. Median follow-up for PFS defined as time from randomisation to date of censoring was approximately 41 months on both arms. Taken together, these results are very similar to those observed in the 391 patients in the SOLO1 study, which supports the effectiveness of the test.

The clinical outcome results for cases classified as having a deleterious or suspected deleterious tumor *BRCA* mutation by the Myriad MyChoice® CDx test are shown in the table below.

Table 14

	SOLO1		**Myriad MyChoice® CDx**	
	Olaparib 300 mg bd[a]	**placebo**	**Olaparib 300 mg bd[a]**	**placebo**
PFS				
Number of events: total number of patients (%)	102/206 (39)	96/131 (73)	75/191 (39.3)	69/93 (74.2)
Median PFS (months)	not reached	13.8	not reached	13.8
HR (95% CI)	0.30 (0.23-0.41)		0.29 (0.21-0.41)	
P-value (2-sided)	<0.0001		<0.0001	

a- tablet formulation

Summary of Clinical Study - Niraparib PR-30-5017-C (PRIMA) for maintenance treatment

Overview

The niraparib clinical study PR-30-5017-C (PRIMA) was a randomized, double-blind, placebo-controlled, multicenter Phase 3 study design in subjects with ovarian, fallopian tube, and primary peritoneal cancer, collectively referred to as ovarian cancer. The objective of PRIMA was to evaluate the therapeutic effect of maintenance niraparib treatment following response to first-line platinum-based chemotherapy in patients with advanced ovarian cancer.

Accountability of PMA Cohort

A total of 733 patients were screened into the PRIMA study for homologous recombination deficiency (HRD) testing. Test results were required prior to randomization. Subjects with a documented deleterious germline (gBRCA) or somatic (sBRCA) BRCA mutation by local results were considered to have homologous recombination deficient tumors for stratification and randomization purposes; the tumor test was performed concurrently to confirm local results. Of these 733 randomized patients, 487 niraparib-treated and 246 placebo-treated patients were included in the PMA cohort. The retrospective analysis included the testing of 713 ovarian FFPE tumor specimens (7 samples were retests from existing patients and 4 samples from 4 patients who were identified as screen failures after re-matching ID with clinical database) from PRIMA, where biomarker calls from the CTA were compared with those from the CDx. Therefore, the final PMA cohort by CTA/CDx includes a total of 733 patients:

373 HRD positive patients, 249 HRD negative patients, 80 patients who had inconclusive results from HRD testing, and 31 patients without sufficient sample for HRD testing.

Effectiveness Results

The primary endpoint was PFS, defined as the time from treatment randomization to the earlier date of assessment of progression (by BICR) or death by any cause in the absence of progression. PFS was based on radiology assessment using RECIST v1.1 criteria. Efficacy analyses were primarily conducted on the intent to treat (ITT) population, in the overall and homologous recombination deficient populations.

Conclusions

The main efficacy conclusions for the prespecified primary analysis population were as follows:

- The study met its primary efficacy objective; treatment with niraparib prolonged median PFS by 11.5 months compared to placebo in subjects with homologous recombination deficient advanced ovarian cancer following response to front-line platinum-based chemotherapy. Median PFS as determined by BICR based on RECIST (version 1.1) was 21.9 months in the niraparib arm and 10.4 months in the placebo arm (HR 0.43 [95% CI: 0.310,0.588]; p<0.0001).
- In the overall population, treatment with niraparib prolonged median PFS by 5.6 months compared to placebo. Median PFS as determined by BICR based on RECIST (version 1.1) was 13.8 months in the niraparib arm and 8.2 months in the placebo arm (HR 0.62 [95% CI: 0.502, 0.755]; p<0.0001).

Table 15 Efficacy Results[a] in PRIMA (Biomarker-Defined Population)

	HRD Positive Population		**Overall Population[b]**	
	ZEJULA (N=247)	Placebo (N=126)	ZEJULA (N=487)	Placebo (N=246)
PFS events, n (%)	81 (33)	73 (58)	232 (48)	155 (63)
PFS Median (95% CI), in months	21.9 (19.3, NE)	10.4 (8.1, 12.1)	13.8 (11.5, 14.9)	8.2 (7.3, 8.5)
Hazard Ratio (HR)[c] (95% CI)	0.43 (0.31, 0.59)		0.62 (0.50, 0.76)	
p-value[d]	<0.0001		<0.0001	

[a] efficacy analysis was based on blinded independent central review (BICR).
[b] In the HR proficient (HRD negative) population (N=249), a hazard ratio of 0.68 (95% CI [0.49, 0.94]) was observed.
In the HR not determined (HRnd) population (N=111), a hazard ratio of 0.85 (95% CI [0.51,1.43]) was observed.
[c] based on a stratified Cox proportional hazards model
[d] based on a stratified log-rank test
NE=Not Evaluable

Myriad Genetic Laboratories, Inc.
322 N 2200 W
Salt Lake City, Utah 84116
PH: +1 (877) 283-6709
FX: +1 (801) 883-8998
www.myriad.com
Outside U.S.A
Email: CustomerSupport@myriadgenetics.eu

QbD RepS BV
Groenenborgerlaan 16
2610 Wilrijk
Belgium

Qarad Suisse S.A.
World Trade Center
Avenue Gratta-Paille 2
1018 Lausanne
Switzerland

The Myriad MyChoice® CDx Plus test was developed and performance characteristics were determined by Myriad Genetic Laboratories, Inc. and in compliance to In-Vitro Diagnostic Device Directive (98/79/EC) and is CE marked. Myriad is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) as qualified to perform high complexity clinical laboratory testing. Myriad is compliant with multiple international standards including, ISO 13485:2016 and ISO 15189: 2012 as applicable.