SUMMARY OF SAFETY AND EFFECTIVENESS DATA (SSED)

I. GENERAL INFORMATION

Device Generic Name:	Next Gen Sequencing oncology panel, somatic or germline variant detection system
Device Trade Name:	Myriad myChoice® CDx
Device Procode:	PQP
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: P190014

Date of FDA Notice of Approval: October 23, 2019

II. INDICATIONS FOR USE

Myriad myChoice® CDx is a next generation sequencing-based *in vitro* diagnostic test that assesses the qualitative detection and classification of single nucleotide variants, insertions and deletions, and large rearrangement variants in protein coding regions and intron/exon boundaries of the *BRCA1* and *BRCA2* genes and the determination of Genomic Instability Score (GIS) which is an algorithmic measurement of Loss of Heterozygosity (LOH), Telomeric Allelic Imbalance (TAI), and Large-scale State Transitions (LST) using DNA isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens. The results of the test are used as an aid in identifying ovarian cancer patients with positive homologous recombination deficiency (HRD) status for treatment with the targeted therapy listed in Table 1 in accordance with the approved therapeutic product labeling.

Table 1: Companion diagnostic indications

Tumor Type	Biomarker	Therapy
Ovarian Cancer	Myriad HRD (defined as deleterious or suspected deleterious mutations in *BRCA1* and *BRCA2* genes and/or positive Genomic Instability Score)	Zejula® (niraparib)

This assay is for professional use only and is to be performed only at Myriad Genetic Laboratories, Inc., a single laboratory site located at 320 Wakara Way, Salt Lake City, UT 84108.

III. CONTRAINDICATIONS

There are no known contraindications.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions can be found in the myChoice® CDx labeling.

V. DEVICE DESCRIPTION

The myChoice CDx device determines a patient's Myriad HRD Status by detecting single nucleotide variants (SNVs), variants in homopolymer stretches, insertions and deletions (indels), and large rearrangements (LRs) in the *BRCA1* and *BRCA2* genes, and determining a genomic instability score (GIS) using DNA obtained from FFPE ovarian tumor tissue. A positive Myriad HRD Status result is due to either the presence of a pathogenic mutation in *BRCA1* and/or *BRCA2* (sequencing and/or LR) [t*BRCA1/2* Status] and/or a GIS above a defined threshold [GIS Status].

The myChoice CDx is a single-site assay performed at Myriad Genetic Laboratories, Inc. The assay includes reagents, software, instruments and procedures for testing DNA extracted from formalin-fixed, paraffin-embedded (FFPE) tumor samples.
The assay employs a single DNA extraction method from FFPE specimens, 30-200 ng of which undergoes multiple steps including fragmentation, end repair and adenylation, adapter ligation, library construction/amplification, hybridization and capture, sequencing and data analysis.

Test Kit Contents

Slide Container
Tumor Block Container
Collection Instructions
Mailing Instructions
Ice Pack

Key reagents used for the device

Key Reagents
KAPA Hyper Prep Custom Volume Kit (96 reactions) (KAPA)
SureSelect XT2 and Index Kit-96, Custom Volume (Agilent Technologies)
HiSeq Rapid SBS Kit-v2 (200 cycle) for HiSeq 2500 (Illumina)

Key Reagents
HiSeq Rapid PE Cluster Kit-v2 Paired end cluster generation kit for HiSeq 2500 on board clustering only (Illumina)
SureSelectXT2 6-11.9Mb Custom Library-96 (up to 55k oligos): ELID 0641491 (Agilent Technologies)

Instruments

Instrument
Illumina HiSeq 2500 Next Generation Sequencer
Eppendorf MasterCycler PRO-S 96 well Thermocycler

Test Process

gDNA Extraction and Normalization

The genomic DNA (gDNA) is extracted from FFPE ovarian tumor tissue slices using automated robotic platforms. The FFPE ovarian tissue is dewaxed, digested with proteinase K and treated with RNaseA prior to the gDNA extraction process that uses silica-clad paramagnetic beads (Maxwell 16 FFPE Plus LEV DNA Purification Kit). An automated robot platform is used for the gDNA extraction process and has a preprogrammed purification protocol that uses pre-filled reagent cartridges.

gDNA normalization is an automated process that utilizes an automated robotic platform and a fluorimeter. Extracted gDNA is quantified by fluorescent methods and diluted to a specified concentration range for use in the subsequent step. The original gDNA extraction samples are stored at -20°C and the diluted gDNA samples are stored between 2-6°C until the specimen is ready for gDNA fragmentation.

Fragmentation of the gDNA

The normalized gDNA (30-200 ng) is sheared to a specific size range by ultrasonic vibrations from an automated ultrasonic instrument, using a program that is optimized to achieve the desired gDNA size range.

Purification of sheared gDNA

The sheared gDNA is purified using silica-clad paramagnetic bead technology. An automated robotic platform is used to perform this process. The sheared gDNA is mixed with a fixed volume of beads, washed, bound and eluted. The sheared and purified gDNA fragment size range is determined (200-300 basepairs) by electrophoresis on a robotic platform instrument.

gDNA End Repair/Adenylation & Index Adapter Ligation Processes

The gDNA end repair/adenylation is performed to create 5'phosphorylated blunt ends and 3'-dA tailed in a single tube on double stranded gDNA fragments. Double stranded DNA Indexed-Illumina Adapters with 3'-dTMP overhangs are then ligated to the 3'-dA tailed fragments. All reactions are set up on an automated robotic platform. All reactions are incubated using the Eppendorf PRO-S 96 Master Cycler. The index-adapter ligated double stranded gDNA fragments are then purified using silica-clad paramagnetic beads on an automated robotic platform to remove excess nucleotides, enzymes, salts, and adapter-indexes.

Library Amplification

The library is amplified on an Eppendorf PRO-S 96 thermal cycler using KAPA HiFi Ready mix and Agilent SureSelect primers. The PCR amplified library is purified using silica-clad paramagnetic beads to remove excess nucleotides, enzymes, salts, and primers. The purified gDNA Library quantity and quality is assessed by electrophoresis on an automated electrophoresis instrument.

Library Hybridization and Capture

Each sample of the genomic library is pooled at an equal molar ratio and dried down in a vacufuge. The pooled sample is then re-suspended in a hybridization buffer mix and hybridized to a custom Agilent SureSelect capture array. The capture array has biotinylated RNA probes for 54,091 single nucleotide polymorphism sites which are distributed across the human genome, and 685 biotinylated RNA probes for *BRCA1* and *BRCA2* exons and exon boundaries. The hybridization mixture is incubated on an Eppendorf PRO-S 96 thermal cycler for 24 hrs. The gDNA –capture library hybrids are captured using streptavidin-coated magnetic beads. The captured library is PCR amplified again on an Eppendorf PRO-S 96 thermal cycler. The amplified capture library is purified using silica-clad paramagnetic beads on an automated robotic platform. The purified library quantity and quality is assessed by an automated electrophoresis instrument.

Next-Generation Sequencing on HiSeq 2500

Quantified libraries are normalized to 2nM, denatured using 0.1 N NaOH, and further diluted to 6.0 pM prior to sequencing. Samples are then run on two HiSeq flow cells. The combined data from two flow cells generates forward and reverse sequences for each sample with sufficient coverage to call sequence variants, large rearrangements and GIS comprised of LOH, LST and TAI.

Data Analysis and Review

Sequence data is converted from BCL (Binary) to QSEQ (text) format using Illumina software. Sequence reads are assigned to individual samples based on index sequences (DNA barcodes). Sequence reads are trimmed to remove adapter and low-quality portions of the reads.

SNV, Indel (t*BRCA1/2* Sequence Variants) and Large Rearrangement (LR) Variant Detection

Putative sequence variants (t*BRCA1/2* sequence variants) are identified by comparing with wild type sequences of *BRCA1* and *BRCA2* genes. Normalized base and exon coverage of *BRCA1* and *BRCA2* genes are calculated to detect LRs. Putative sequence variants and base and exon coverage are reviewed using a dedicated graphical user interface (GUI). Upon completion of testing, a test report is sent to the ordering physician.

Myriad has adopted and utilizes a documented classification process for *BRCA1* and *BRCA2* variants that is consistent with American College of Medical Genetics and Genomics (ACMG) recommendations. As part of this process, the variants are classified by committee of experts within Myriad, including board-certified laboratory and medical directors, research scientists, genetic counselors, and variant support specialists. After a variant is classified it is entered into the classification database where it can be readily retrieved each time it is observed during routine testing. *BRCA1* and *BRCA2* variants are classified into one of the following five categories: Deleterious, Suspected Deleterious, Variant of Uncertain Significance, Favor Polymorphism, and Polymorphism.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

There are no other FDA cleared or approved alternatives for the testing of FFPE ovarian tumor tissue for the assessment of tumor genomic instability score (GIS) and the detection and classification of variants in the *BRCA1* and *BRCA2* genes, for the selection of patients who are eligible for treatment with niraparib.

VII. MARKETING HISTORY

The Myriad myChoice CDx test (will be refered to as myChoice CDx throughout the document) 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 appropriately, or failure to correctly interpret test results may lead to incorrect tumor *BRCA1* and *BRCA2* (t*BRCA1/2)* Status test results and GIS Status, which could impact patient treatment decisions. A false positive test result may lead to inappropriate treatment and potentially any adverse effects associated with targeted treatment with Zejula® (niraparib). A false negative test result may prevent a patient from potentially benefitting from a targeted therapy, and result in receiving other treatment options. There is also a risk of delayed results, which may lead to a delay in treatment with Zejula® (niraparib).

For the specific adverse events related to Zejula® (niraparib) that occurred in the clinical studies, please see Section X below.

IX. SUMMARY OF NONCLINICAL STUDIES

A. Laboratory Studies

The specific performance characteristics of the myChoice CDx assay were determined by studies using fixed tumor samples. Samples were selected to evaluate a range of representative tumor *BRCA1* and *BRCA2* sequence variants (i.e., single nucleotide variants, insertions or deletions, and variants in homopolymers) and LRs (i.e., deletions and duplications affecting single and multiple exons) detected by the myChoice CDx assay, as well as a representative range of Genomic Instability Scores, as reflected in the device labeling.

1. Correlation with Orthogonal Reference Method (Accuracy)

The accuracy of the Myriad HRD status determined by myChoice CDx test was demonstrated using a validated Next Generation Sequencing (NGS)-based assay with a combination of non-clinical samples and FFPE clinical specimens from cancer patients enrolled in clinical trials from whom sufficient quantity and quality of DNA was available for testing with the NGS comparator assay. A total of 209 formalin-fixed paraffin-embedded (FFPE) tumor specimen-derived DNA samples were tested with both the assays. Sample respresenting the following subgroups were tested in the study: 5 t*BRCA1/2* Status positive / GIS Status negative, 71 t*BRCA1/2* Status negative / GIS Status negative, 14 t*BRCA1/2* Status positive / GIS Status positive (42-52), 13 t*BRCA1/2* Status negative / GIS Status positive (42-52), 52 t*BRCA1/2* Status positive / GIS Status positive ($\geq$ 53), 48 t*BRCA1/2* Status negative / GIS Status positive ($\geq$ 53).

a. t*BRCA1/2* Sequence Variant and LR Analytical Calls

A total of 1,733/1,733 valid *BRCA1/2* sequence variant test calls were observed compared to the valid reference (comparator) *BRCA1/2* sequence variant calls with $\geq$ 10% allele frequencies across all samples evaluated. This corresponds to a Positive Percent Agreement (PPA) of 100%. The concordance of all valid *BRCA1/2* sequencing analytical calls across all samples, including variants at <10% allele frequencies, is shown in Table 2. A total of 1,733/1,734 valid *BRCA1/2* sequence variant calls were observed, corresponding to a PPA of 99.94% with a 95% lower confidence limit of 99.7267%. In addition, a total of 3,605,951/3,605,951 valid *BRCA1/2* sequence non-variant base calls were observed, corresponding to a negative percent agreement (NPA) of 100% with a 95% lower confidence limit of 99.9999%. A total of 402/402 concordant valid *BRCA1/2* LR calls were observed compared to the valid reference (comparator) *BRCA1/2* LR calls across all samples evaluated. This corresponds to an Overall Agreement of 100% for LR calls.

Table 2: Concordance of All Valid t*BRCA1/2* Sequence Variant and LR Analytical Calls Across All Samples

Concordant *BRCA1/2* Variant Calls	95% LCL for Seq Variant Calls	Concordant *BRCA1/2* Non-Variant Base Calls	95% LCL for Non-Variant Base Calls	Concordant *BRCA1/2* LR calls
1,733/1,734 PPA = 99.94%	99.73%	3,605,951/3,605,951 NPA = 100%	99.99%	402/402 OPA =100%

The results of the accuracy study were evaluated for three patient outcomes: (i) the t*BRCA1/2* Status based on *BRCA1* and *BRCA2* sequence and LR analyses; (ii) the GIS Status based on the Genomic Instability Score (GIS); and (iii) the overall Myriad HRD Status based on the combined results of the t*BRCA1/2* Status and GIS Status. The agreement between the myChoice CDx device and the comparator (reference) assay is summarized below.

b. t***BRCA1/2* Status Results**

All myChoice CDx and Comparator per specimen t*BRCA1/2* Status results (representing the combined interpretation of the sequencing and LR results) are shown in Table 3. In addition, the Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and Overall Percent Agreement (OPA) of the t*BRCA1/2* Status patient results, with and without invalid calls, along with 95% confidence intervals, are presented. The t*BRCA1/2* Status concordance between the Myriad myChoice CDx and Comparator assays for valid patient results was 100%.

Table 3. Summary of t*BRCA1/2* Status Per Specimen Results

		Comparator Assay			
		True Positive	True Negative	Invalid	Total
myChoice CDx t*BRCA1/2* Status[a]	Positive	71	0	1	72
	Negative	0	129	1	130
	Invalid	0	5	2	7
	Total	71	134	4	209
Agreement Including Valid Results Only (Total n=200)	PPA [95% CI]	100.0 [95.9%, 100.0%]			
	NPA [95% CI]	100.0 [97.7%, 100.0%]			

		Comparator Assay			
		True Positive	**True Negative**	**Invalid**	**Total**
	OPA [95% CI]	100.0 [98.5%, 100.0%]			
Agreement Including Invalid t*BRCA1/2* Status Results (Total n=205)	**PPA [95% CI]**	100.0 [95.9%, 100.0%]			
	NPA [95% CI]	96.3 [91.5%, 98.8%]			
	OPA [95% CI]	97.6 [94.4%, 99.2%]			

[a]The t*BRCA1/2* Status is based on tumor *BRCA1* and *BRCA2* sequence and LR results.

c. GIS Status Results

The GIS Status and Comparator patient results are shown in Table 4. Concordance analysis of all valid patient results produced by both the myChoice CDx and Comparator assays revealed a Positive Percent Agreement of 98.5%, a Negative Percent Agreement of 97.4%, and an Overall Percent Agreement of 98.1%. In addition, the Positive Percent Agreement (PPA), Negative Percent Agreement (NPA) and Overall Percent Agreement (OPA) of the patient results, with and without invalid calls and 95% confidence intervals, are presented.

Table 4. Summary of GIS Status Per Specimen Results

		Comparator Assay			
		TRUE Positive	**TRUE Negative**	**Invalid**	**Total**
myChoice CDx GIS Status	**Positive**	128	2	0	130
	Negative	2	74	2	78
	Invalid	0	1	0	1
	Total	130	77	2	209
Agreement Including Valid Results Only (Total n=206)	**PPA [95% CI]**	98.5 [94.6%, 99.8%]			
	NPA [95% CI]	97.4 [90.8%, 99.7%]			
	OPA [95% CI]	98.1 [95.1%, 99.5%]			
Agreement Including Invalid GIS Status Results (Total n=207)	**PPA [95% CI]**	98.5 [94.6%, 99.8%]			
	NPA [95% CI]	96.1 [89.0%, 99.2%]			
	OPA [95% CI]	97.6 [94.5%, 99.2%]			

d. myChoice CDx Myriad HRD Status Per Specimen Results

All myChoice CDx and Comparator per specimen Myriad HRD Status results are shown in Table 5. Concordance analysis of all valid patient results from both the myChoice CDx and Comparator assay revealed a Positive Percent Agreement of 98.5%, a Negative Percent Agreement of 98.6%, and an Overall Percent Agreement of 98.5%.

Table 5. Overall Myriad HRD Status Per Specimen Results

		Comparator Assay			
		TRUE Positive	**TRUE Negative**	**Invalid**	**Total**
myChoice CDx Myriad HRD Status	**Positive**	133	1	0	134
	Negative	2	70	2	74
	Invalid	0	1	0	1
	Total	135	72	2	209
Agreement Including Valid Results Only (Total N=206)	**PPA [95% CI]**	98.5% [94.8%, 99.8%]			
	NPA [95% CI]	98.6% [92.4%, 100.0%]			
	OPA [95% CI]	98.5% [95.8%, 99.7%]			
Agreement Including Invalid Myriad HRD Status Results (Total N=207)	**PPA [95% CI]**	98.5% [94.8%, 99.8%]			
	NPA [95% CI]	97.2% [90.3%, 99.7%]			
	OPA [95% CI]	98.1% [95.1%, 99.5%]			

2. <u>Analytical Sensitivity</u>

a. Limit of Blank (LoB)

Twenty-six (26) FFPE normal tissue samples were tested where all low frequency variants are expected to be spurious technical artifacts rather than true biological events. The distribution of allele frequencies was plotted. The frequencies of spurious variants are typically very low with the distribution decreasing very rapidly from 1% to 5%. There were no spurious variants with frequency above 5%.

One hundred thirty-six (136) FFPE t*BRCA1/2* Status negative (wildtype) tissue samples were analyzed. These samples produced 2,357,861/2,357,861 (100%) concordant non-variant base calls, resulting in a false positive rate of 0% with a 95% upper confidence limit of 0.00013%. Of the 136 samples, 70 samples were both t*BRCA1/2* Status negative / GIS Status negative. These 70 samples produced 1,213,621/1,213,621 (100%) concordant non-variant base calls, resulting in a false positive rate of 0% with a 95% upper confidence limit of 0.00025%. This retrospective analysis provides empirical data for setting a minimum allele frequency threshold of 5% to differentiate spurious background noise from real variants.

b. Analytical Sensitivity – Limit of Detection (LoD)

(i) t*BRCA1/2* Sequence Variants

DNAs from four FFPE samples with known *BRCA1/2* pathogenic sequence variants [*BRCA1* c.181T>G (p.Cys61Gly), a single nucleotide variant, *BRCA1* c.1961del, a < 10 bp deletion in an 8 bp homopolymer sequence, *BRCA1* c.5266dupC, a < 10 bp insertion and *BRCA2* c.9117_9117+11del , a ≥ 10 bp deletion] and FFPE tumor DNA samples without these *BRCA1/2* variants (WT) were used to create simulated tumor : normal DNA samples with 10%, 8%, 6.5%, 5% and 2% allele frequencies of these mutations. Twenty replicates of each of the various DNA mixes were run and *BRCA1/2* sequencing analytical calls were analyzed using CLSI's EP17-A2 probit analysis methods to determine the LoD of these mutations.

The results of this study show that the LoDs of the four pathogenic sequence variants have different ranges of allele frequencies. The LoD of a single base pair (bp) substitution was at 7.23% allele frequency. The LoD of a < 10 bp deletion in an 8 bp homopolymer sequence was 6.66%. The LoD for a < 10 bp insertion was 6.36%, and the LoD for a ≥ 10 bp deletion was 5.98%.

(ii) t*BRCA1/2* Large Rearrangements

Two FFPE tumor DNA samples, each carrying a different large rearrangement (LR): [*BRCA1* del exon 8 LR exon and *BRCA2* del exons 19–21 LR], were each mixed with a FFPE DNA with no detectable *BRCA1/2* LRs (WT) to create tumor : normal samples with 50%, 40%, 30% and 10% allele frequencies of each LR. Ten replicates of each of the various DNA mixes were run and the concordance between the *BRCA1/2* LR test calls and reference calls from each of the undiluted tumor samples was analyzed. The LoD for each LR was defined based on CLSI's EP17-A2 guidance that recommends ≥ 95% concordant, positive LR calls.

The t*BRCA1/2* LR portion of the myChoice CDx assay's LoD for the ≥ 3 exons LR is at 30% allele frequency, while the LoD for the 1-2 exons LR is at 50% allele frequency.

(iii) GIS Status

Four FFPE matched tumor-normal samples were evaluated in this LoD study. DNAs extracted from each pair of FFPE matched tumor and normal samples were mixed to create five different tumor : normal DNA mixes at 40%, 30%, 20%, 10% and 0% tumor DNA content. Ten replicates of each of the tumor : normal mixes were run along with replicates of the undiluted tumor and normal DNAs. All the tumor : normal DNA mixes were tested using the myChoice

CDx assay and final GIS Status patient results were analyzed to assess the LoD of this assay.

The results from this LoD study show that the myChoice CDx assay yields highly precise GIS results and GIS Status patient results for all samples at all tumor DNA content levels that produced valid results. Based on this study, the LoD of the GIS portion of the myChoice CDx assay is at ~ 30% tumor DNA content.

An additional LoD study will be conducted postmarket with samples at the lowest DNA input levels. Refer to section XIII.

3. Analytical Specificity

a. Interference (t*BRCA1/2* Sequence Variant and LR Analytical Calls)

To evaluate the potential impact of three classes of substances (endogenous, exogenous, and method-specific interferents) that can potentially interfere with the assay, this study evaluated seven FFPE specimens (5 t*BRCA1/2* Status positive / GIS Status positive, and 2 t*BRCA1/2* Status negative / GIS status negative) representing five variant types [Single nucleotide variants, insertions or deletions of < 10 base pairs (bp) in length, insertions or deletions of ≥ 10 bp in length, homopolymer variants (5+ bp), and large rearrangements affecting ≥ 3 exons]. The effects of potential interfering substances (Table 6) were tested at one or two replicates to determine if they would impact the myChoice CDx device, and the results were compared to the control (no additional interferents) condition.

Table 6: Potential Interfering Substances Assessed

Class	Substance	Treated Material	Low Test Level	High Test Level
Exogenous	Tissue Marking Dye	FFPE Tumor Resections	17%	29%
	Paraffin wax	FFPE Tumor Resections	23%	35%
Endogenous	Hemoglobin	FFPE Tumor Resections	1 mg/mL	2 mg/mL
	Triglycerides	FFPE Tumor Resections	19 mM	37 mM
Method-Specific	Ethanol	Post-ligation product	5%	10%
	Sodium Hydroxide	Diluted library	0.4 N	1.0 N

Class	Substance	Treated Material	Low Test Level	High Test Level
Controls	Untreated Control	FFPE Tumor Resections	N.A.	
	Water Diluent Control	FFPE Tumor Resections	4 µl	
	Chloroform DiluentControl	FFPE Tumor Resections	3.6 µl	

All treated samples across all six substances at the high test levels passed the acceptance criteria, with the exception of method-specific NaOH at the high test level (1.0 N), which failed as 10/14 (71.4%) tests successfully generated valid *BRCA1/2* sequencing and LR results. However, 14/14 (100%) of the tests at the low 0.4 N NaOH level passed. All samples run under each condition produced valid positive or negative patient calls. The positive and negative patient calls were 100% concordant when compared to samples without additional interferents.

b. Interference (GIS)

The purpose of this study was to reveal if the performance of the GIS is affected by the presence of potential interfering substances. The experimental procedure and classes of potential interfering substances are the same as described above. Seven samples that were treated with each of these substances at the high test levels were processed with the myChoice CDx assay through generation of final GIS. All valid GIS were generated for each potential interfering substance tested. In addition, all samples run under each condition produced valid positive or negative patient calls showing 100% concordance when compared samples without additional interferents.

Taken together, these results demonstrate that the t*BRCA1/2* sequencing and GIS portions of the assay are minimally impacted or not impacted by the presence of any of the substances tested in this study.

Necrosis of ≥ 10% of the tumor area was observed in 11% (n = 66) of tumor samples from the QUADRA study. Only 3% of samples had necrosis in > 10% of the tumor area, and no samples were identified with necrosis involving > 60% of the tumor area. The standard procedure in Myriad's Anatomic Pathology laboratory is to macro-dissect the fixed tumor tissues on slides to maximize tumor content, i.e. minimize the inclusion of non-tumor content including necrotic tissue. Thus, based on

the above analysis, it was concluded that necrosis of ≤ 60% does not impact the myChoice CDx assay results.

An additional interference study will be performed postmarket to evaluate the effects of any potential interference due to use of hybrid capture probes and proteinase K. Refer to section XIII.

c. Carryover

The purpose of this study was to demonstrate that the myChoice CDx test minimizes carryover across samples. Fourteen FFPE specimens (11 t*BRCA1/2* Status positive / GIS Status positive, 1 t*BRCA1/2* Status positive / GIS Status positive and 2 t*BRCA1/2* Status negative / GIS Status negative) were processed consecutively through DNA extraction. The DNA from these 14 FFPE samples were then set up in a checkerboard pattern, alternating between low (50 ng) and high (200 ng) inputs. Two consecutive batches were set up in this pattern, with one checkboard pattern inversed, to assess intra-run (1st batch) and inter-run (2nd batch) carryover. An additional reference batch was run with all samples at 200 ng to compare for concordance. For both the intra-run and inter-run batches, all 14/14 samples produced complete, valid analytical calls that were 100% concordant for *BRCA1* and *BRCA2* sequence and LR calls, and all GIS were precise. In addition, the patient results of all samples run in both batches were 100% concordant.

Additionally, using the SNPs analyzed in the assay, carryover was quantified within and between batches. The highest intra-run carryover observed was 0.2% and the highest inter-run carryover observed was 0.1%. The average intra-run carryover observed was 0.14% and average inter-run carryover was 0.10%**.** The overall analytical results show that the myChoice CDx device has very low intra-run and inter-run sample carryover and poses minimal risk to the myChoice CDx device.

d. 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 is to demonstrate that captured sequences not originating from target regions do not materially affect the myChoice CDx test.

Sequencing data for 7 FFPE tissue samples (4 t*BRCA1/2* Status positive /GIS Status positive, 1 t*BRCA1/2* Status negative / GIS Status negative, 1 t*BRCA1/2* Status negative / GIS Status positive, and 1 t*BRCA1/2* Status unknown / GIS Status positive) were processed through myChoice CDx in quadruplicate, yielding 28 total tests results that were mapped to the human genome sequence to reveal off-target sequences due to pseudogene and cross-reactivity of hybridization baits (capture probes).

The myChoice CDx test has two methods for mitigating the impact of pseudogenes. The capture baits were designed to minimize the capture of pseudogene regions, and the analysis algorithm identifies and excludes pseudogene-derived sequences as part of *BRCA1/2* variant detection and LR calling.

No off-target sequences were found to affect the tumor *BRCA1* and *BRCA2* component of the device. Only 1.2% of SNPs used to calculate the GIS were affected by off-target sequences with a minimal effect on the reported score. This retrospective analysis suggests that cross-reactivity poses a minimal risk to the myChoice CDx device.

4. Repeatability and Reproducibility

The repeatability and reproducibility of the myChoice CDx was investigated by testing DNA extracted from FFPE clinical specimens. The samples cover single nucleotide variants, insertions or deletions of < 10 base pairs (bp) in length, insertions or deletions of ≥ 10 bp in length, homopolymer variants (5+ bp), large rearrangements affecting 1-2 exons and large rearrangements affecting ≥ 3 exons. The tested samples had GIS ranging from 7 to 81.

a. t*BRCA1/2* Sequence and LR Analytical Calls

The purpose of this study was to demonstrate that the tumor *BRCA1* and *BRCA2* portion of the myChoice CDx assay *(*t*BRCA1/2*) generates highly reproducible *BRCA1* and *BRCA2* sequencing and large rearrangement (LR) analytical calls on the tested samples from four different studies over four periods of time. In the first three studies (Study 1 to 3), a total of 18 unique formalin-fixed, paraffin-embedded (FFPE) tumor specimens and/or extracted FFPE tumor specimen DNAs were tested. All but two of these samples were tested at the DNA input amount of 200 ng per assay. The fourth study (Study 4) evaluated 7 FFPE tumor specimens from the first three studies at 50 ng DNA input. The samples were run in duplicate per run, over 6 runs, using 3 lots of reagents, 3 different sets of instruments, 6 different operators and 3 different data reviewers. An additional study (fifth study) was performed using 5 unique FFPE samples (below). Thus, a total of 23 FFPE samples (10 t*BRCA1/2* Status positive / GIS Status positive, 2 t*BRCA1/2* Status positive / GIS Status negative, 6 t*BRCA1/2* Status negative / GIS Status positive, 3 t*BRCA1/2* Status negative /

GIS Status negative, 2 t*BRCA1/2* Status unknown / GIS Status positive) were tested in the study.

All 228/228 samples and replicates tested from the first three studies produced complete, valid *BRCA1* and *BRCA2* sequencing and LR analytical calls. Overall, 2,220/2,220 *BRCA1* and *BRCA2* sequence variant calls, 3,951,603/3,951,603 non-variant bases and 456/456 LR calls across all samples/replicates tested were 100% concordant (as shown in Table 7) .

Table 7. Overall Analytical Concordance of the t*BRCA1/2* Portion of the myChoice CDx Assay Across Three Precision Studies (Studies 1 to 3).

Overall Concordant *BRCA1/2* Sequence Variant Calls	Overall Concordant *BRCA1/2* Sequence Non-Variant Base Calls	95% LCL For Sequence Non-Variant Base Calls	Overall Concordant *BRCA1/2* LR Calls
2,220/2,220 (100%)	3,951,603/3,951,603 (100%)	99.99%	456/456 (100%)

A fourth study analyzed 7 fixed tumor samples at 50 ng DNA inputs, which had previously been evaluated at 200 ng DNA input levels in the earlier studies. All samples and replicates run generated complete (100%) valid *BRCA1* and *BRCA2* sequence and LR calls. Altogether, 708/708 *BRCA1* and *BRCA2* sequence variant calls, 1,450,393/1,450,393 non-variant bases and 168/168 LR calls across all samples/replicates tested at the 50 ng DNA input level were 100% concordant. All *BRCA1* and *BRCA2* sequence variant and LR calls from the 7 samples run at 50 ng DNA inputs were 100% concordant with analytical calls from the same samples run at 200 ng DNA inputs as shown in Table 8.

Table 8: Overall Analytical Concordance of t*BRCA1/2* Portion of the myChoice CDx Assay at the 50 ng DNA Input Level (Study 4)

Concordant *BRCA1/2* Sequence Variant Calls	Overall Concordant *BRCA1/2* Sequence Non-Variant Base Calls	95% LCL for Sequence Non-Variant Base Calls	Overall Concordant *BRCA1/2* LR Calls
708/708 (100%)	1,450,393/1,450,393 (100%)	99.99%	168/168 (100%)

Per sample *BRCA1* and *BRCA2* sequence variant decomposition of variance at 200 ng and 50 ng DNA input was calculated by random effects models using restricted maximum likelihood estimation and is presented below (Table 9).

Table 9: *BRCA1* and *BRCA2* Sequence Variant Decomposition of Variance at 200 ng and 50 ng DNA Input

Sample	Variant Type	MAF (%)	Between Run	Between Reagents Lot	Between Instrument	Within Run	Total Variability
			SD	SD	SD	SD	SD
200 ng DNA Input							
1	Ins/del < 10 bp	48.8	1.10	0.81	0.00	2.16	2.56
2	SNV	58.9	1.22	0.00	1.46	3.22	3.74
3	Ins/del < 10 bp	78.9	0.00	2.21	0.31	1.96	2.97
4	SNV	22.0	0.49	0.37	0.00	2.50	2.58
5	SNV	57.1	1.78	0.00	1.66	1.79	3.03
6	SNV	27.8	0.93	0.00	0.00	1.23	1.54
	SNV	27.4	0.00	1.09	0.06	2.16	2.42
7	Ins/del ≥ 10 bp	80.4	0.00	2.98	0.00	3.28	4.43
8	SNV	15.8	0.00	1.11	0.76	1.62	2.10
	SNV	14.3	0.00	0.00	0.00	1.76	1.76
	Ins/del < 10 bp	74.5	2.66	0.00	0.00	1.65	3.13
9	Ins/del ≥ 10 bp	30.4	1.52	1.30	0.00	2.41	3.13
10	SNV	44.9	0.00	0.68	1.03	2.08	2.42
11	SNV	15.7	0.00	0.00	0.00	2.90	2.90
	Ins/del < 10 bp	80.0	0.00	0.00	0.00	2.98	2.98
	Hp +5 bp						
	SNV	16.2	0.00	0.00	0.00	3.43	3.43
12	SNV	24.5	1.05	0.00	0.00	0.91	1.39
	SNV	22.6	0.00	0.00	0.00	2.67	2.67
	SNV	21.9	0.00	0.00	0.88	1.86	2.06
	SNV	18.1	0.70	0.00	0.00	1.86	1.99

Sample	Variant Type	MAF (%)	Between Run	Between Reagents Lot	Between Instrument	Within Run	Total Variability
			SD	SD	SD	SD	SD
13	Ins/del < 10 bp	60.9	0.00	0.76	0.00	1.49	1.67
	Hp +5 bp						
14	Ins/del < 10 bp	80.9	0.06	0.95	0.00	0.87	1.29
	Ins/del ≥ 10 bp	26.1	0.49	0.00	0.84	1.31	1.63
15	Ins/del < 10 bp	76.3	0.00	0.48	0.00	1.85	1.91
	Hp +5 bp						
	SNV	20.6	0.00	0.00	1.11	1.77	2.09
	SNV	19.9	0.00	0.00	0.00	2.45	2.45
16	SNV	17.8	0.87	0.00	0.78	0.80	1.41
	SNV	18.6	0.00	1.49	0.00	1.70	2.26
	SNV	19.7	0.00	0.00	0.00	1.21	1.21
17	SNV	49.4	0.00	0.00	0.00	1.52	1.52
18	Ins/del ≥ 10 bp	59.7	0.00	0.00	4.06	2.72	4.89
50 ng DNA Input							
1	Ins/del < 10 bp	47.9	0.00	0.00	0.01	2.46	2.46
7	Ins/del ≥ 10 bp	80.8	0.65	0.00	0.00	3.04	3.11
9	Ins/del ≥ 10 bp	29.7	0.00	0.00	0.00	2.46	2.46
11	SNV	17.1	1.55	2.17	0.00	1.60	3.11
	Ins/del < 10 bp	76.2	0.00	1.25	1.56	2.04	2.86
	Hp +5 bp						
	SNV	16.5	0.00	0.00	0.00	3.40	3.40
12	SNV	9.0	0.00	0.00	0.51	1.41	1.50

Sample	Variant Type	MAF (%)	Between Run	Between Reagents Lot	Between Instrument	Within Run	Total Variability
			SD	SD	SD	SD	SD
	SNV	8.8	0.00	0.00	0.29	1.41	1.43
	SNV	9.2	0.00	0.00	0.00	1.75	1.75
	SNV	9.2	0.62	0.00	1.03	1.01	1.57
13	Ins/del < 10 bp	68.9	0.00	0.00	0.00	3.36	3.36
	Hp +5 bp						
14	Ins/del < 10 bp	80.6	0.00	0.00	0.78	2.65	2.77
	Ins/del ≥ 10 bp	26.6	0.00	0.00	0.00	3.09	3.09

Hp = Homopolymer

An additional study (fifth study) evaluated the inter- and intra-run precision/reproducibility of the myChoice CDx test by testing five new additional ovarian tumor specimens that were t*BRCA1/2* Status negative / GIS Status positive. The specimens were tested at the lowest DNA input of 30 ng with GIS ranging between 42-52. Eighteen replicates per sample divided across nine independent runs over multiple days using three different sequencers and three lots of critical reagents were evaluated. The myChoice CDx device produced 700/702 concordant *BRCA1/2* sequence variant calls, at a mean allele frequency (MAF) ≥ 10%, resulting in a 99.7% PPA. Two false negative calls in one sample in a single nucleotide variant with a MAF of 11.2%, were the only discordances in this portion of the study. When all valid *BRCA1/2* sequence variants were analyzed, including those below 10% MAF, the myChoice CDx device produced 788/792 concordant sequence variant calls resulting in a 99.5% PPA and 99.99% (1,553,331/1,553,332) concordance for non-variant base calls. For these five samples, *BRCA 1/2* LR concordance was 100% (165/165).

Variance decomposition for the valid *BRCA1/2* sequence variants at 30 ng DNA input was calculated by random effects models using restricted maximum likelihood estimation and is presented below (Table 10).

Table 10: *BRCA1* and *BRCA2* Sequence Variant Decomposition of Variance at 30 ng DNA Input

Sample	Variant Type	MAF (%)	Between Run	Between Day	Between Operator	Between Reagents Lot	Between Instrument	Within Run	Total Variability
			SD	SD	SD	SD	SD	SD	SD
30 ng DNA Input									
1	SNV	49.81	0.00	0.00	0.83	0.87	0.00	3.89	4.07
	SNV	50.95	0.00	0.55	2.54	2.94	0.00	2.81	4.83
2	SNV	9.86	0.00	0.00	1.72	1.73	2.03	2.09	3.80
	SNV	95.51	0.00	0.00	0.29	0.00	0.08	0.86	0.91
	SNV	95.69	0.00	0.00	0.29	0.00	0.00	1.04	1.08
3	SNV	9.26	0.00	0.00	1.02	0.00	0.87	1.39	1.93
	SNV	9.32	0.00	0.67	0.21	0.00	0.33	0.99	1.25
	SNV	9.47	0.40	0.00	0.00	0.56	0.00	1.37	1.53
	SNV	9.77	0.00	0.00	0.00	0.16	0.88	1.14	1.45
	SNV	10.13	0.00	0.00	0.00	0.00	0.00	1.60	1.60
	SNV	10.15	0.00	0.00	0.40	0.00	0.66	2.03	2.17
	SNV	10.49	0.00	0.00	1.07	0.00	0.00	2.03	2.30
	SNV	35.05	0.00	0.00	0.00	0.62	1.47	3.28	3.65
	SNV	36.15	0.00	0.00	0.77	0.59	0.00	1.34	1.65
4	SNV	83.67	0.00	0.00	0.48	0.00	0.00	1.85	1.91
	SNV	84.89	0.00	0.00	0.49	0.91	0.00	2.53	2.74
5	SNV	11.33	0.00	0.00	0.50	1.22	1.36	2.06	2.80
	SNV	88.75	0.00	0.00	0.00	0.41	0.93	2.96	3.14
	SNV	89.32	0.51	0.00	0.00	0.00	1.68	3.37	3.80

LR call precision per sample across all studies, presented in the table below (Table 11), demonstrates the myChoice CDx device is able to reproducibly detect LRs in single and multiple exons at both the high and low DNA inputs within runs and across runs. 95% CIs were calculated using a binomial distribution with Clopper-Pearson intervals.

Table 11. Precision of *BRCA1* and *BRCA2* LR Calls

Sample	200 ng DNA Input	50 ng DNA Input				
			Gene	LR call	Overall Percent Agreement (OPA)	95% CI
6	12	0	*BRCA1*	**≥ 3 exons LR**	24/24 (100%)	85.8%, 100%
12[a]	12	0	*BRCA2*	**≥ 3 exons LR**	24/24 (100%)	85.8%, 100%
12[b]	0	12	*BRCA2*	**≥ 3 exons LR**	24/24 (100%)	85.8%, 100%
16	12	0	*BRCA2*	**1-2 exon LR**	24/24 (100%)	85.8%, 100%
17	12	0	*BRCA1*	**1-2 exon LR**	24/24 (100%)	85.8%, 100%

Samples 12[a] and 12[b] were two separate slices from different regions of the same ovarian tumor specimen.

b. **t*BRCA1/2* Status Patient Results**

All samples produced valid positive or negative patient results, except for Sample 13 from Studies 1 – 3 and Sample 4 from the fifth study, which were inconclusive for the t*BRCA1/2* Status patient result. However, both samples had positive GIS statues and therefore, were both determined to be Myriad HRD Status positive (patient is either t*BRCA1/2* Status positive and/or GIS Status positive). All valid positive and negative patient calls were 100% concordant, resulting in 100% PPA and NPA. In addition, valid patient results for all samples run at 50 ng DNA input level were 100% concordant with their corresponding sample run at the 200 ng DNA input level.

c. **GIS**

The sample sets (18 unique FFPE samples) and the study design used for GIS precision were the same as those used for evaluating the precision of t*BRCA1/2* portion of the myChoice CDx assay. For the GIS, four different studies (at 200 ng DNA input for Studies 1 to 3 and at 50 ng DNA input in the Study 4) were performed. The standard deviation for the GIS calculated from all samples/replicates across the first three studies was 1.43, and for the fourth study was 1.19, meeting the pre-specified acceptance criteria. In the fifth study

at the 30 ng DNA input, the GI score standard deviation (SD) across all samples and replicates was 1.635 which also met pre-specified acceptance criteria. In addition to the mean and variance of GIS, the %CV for each sample in all five studies was calculated. The overall 95% confidence interval for the true proportion of majority calls across samples correspond to 98.8% - 100% for the first four studies and 81.5% - 100% for the fifth study. Per sample decomposition of GIS variance at the 200 ng, 50 ng, and 30 ng DNA inputs were calculated by random effects models using restricted maximum likelihood estimation and are presented in the tables below (Tables 12, 13 and 14).

Table 12. GIS Variance Decomposition at 200 ng DNA Input

Sample	Mean GIS	Between Run	Between Reagent Lot	Between Instrument	Within Run	Total Variability
		SD	SD	SD	SD	SD
1	14	0.00	0.15	0.00	0.64	0.66
2	81	0.00	0.00	0.00	1.98	1.98
3	65	0.00	0.00	0.08	1.03	1.03
4	56	0.46	0.12	0.00	0.50	0.69
5	31	0.65	0.80	0.00	1.44	1.77
6	54	1.12	0.00	1.99	1.29	2.63
7	52	0.00	0.00	0.53	1.11	1.22
8	78	0.00	0.00	1.86	2.10	2.81
9	13	0.00	0.00	0.24	0.33	0.41
10	7	0.00	0.00	0.00	0.00	0.00
11	37	0.00	0.00	0.64	1.17	1.33
12[a]	78	0.00	0.18	0.18	0.72	0.76
13	65	0.02	0.72	0.00	0.71	1.01
14	54	0.00	0.12	0.00	1.24	1.24
15	65	0.00	0.00	0.24	0.88	0.91
16	69	1.60	0.00	0.00	1.63	2.29
17	50	0.04	0.66	0.00	1.22	1.39
18	70	0.76	0.29	0.00	1.61	1.80

Table 13. GIS Variance Decomposition at 50 ng DNA Input

Sample	Mean GIS	Between Run	Between Reagent Lot	Between Instrument	Within Run	Total Variability
		SD	SD	SD	SD	SD
1	14	0.00	0.17	0.00	0.47	0.50
7	52	0.00	0.00	0.00	1.61	1.61
9	13	0.37	0.00	0.00	0.29	0.47
11	37	0.00	0.00	0.00	1.06	1.06
12[b]	71	0.58	0.00	1.47	0.87	1.80
13	64	0.50	0.52	0.00	1.29	1.48
14	54	0.65	0.20	0.00	0.76	1.02

Table 14: GIS Variance Decomposition at 30 ng DNA Input

Sample	Mean GIS	Between Run	Between Day	Between Operatotr	Between Reagent Lot	Between Instrument	Within Run	Total Variability
		SD	SD	SD	SD	SD	SD	SD
1	47	0.00	0.00	0.39	0.36	0.00	0.89	1.04
2	51	0.00	0.00	0.00	0.89	0.00	2.45	2.60
3	49	0.00	0.00	0.00	0.68	0.00	1.19	1.37
4	47	0.00	0.00	0.95	0.00	1.00	0.92	1.66
5	47	0.67	0.00	0.17	0.00	0.44	1.15	1.41

Samples 12a and 12b were two separate slices from different regions of the same ovarian tumor specimen. Due to tumor heterogeneity, samples 12a and 12b contained the same *BRCA1* and *BRCA2* variants at different allele frequencies. Consequently, their data was analyzed separately.

d. GIS Status and Myriad HRD Status Patient Results

All samples and replicates run for all five studies produced complete, valid, positive or negative patient calls in both sets of patient results. All positive and negative patient calls were 100% concordant, resulting in 100% PPA and NPA. In addition, all 7 samples run at the 50 ng and 200 ng DNA input levels were 100% concordant.

An additional repeatability and reproducibility study will be conducted postmarket using a wild type sample (t*BRCA1/2* Status negative / GIS Status negative) and samples with the lowest DNA input, low MAF, and variant representation that were not included in the study described here. Refer to section XIII.

5. Guardbanding

The objective of the guardbanding study was to establish the robustness of the myChoice CDx assay. This guardband/robustness study challenges the performance of the assay across three key parameters: (i) amount of FFPE tumor tissue-derived DNA input into the myChoice CDx assay, (ii) hybridization temperature for probe capture, and (iii) library input onto the HiSeq instrument.

For the DNA input guardband study, 28 FFPE tumor samples (13 t*BRCA1/2* Status positive / GIS Status positive, 1 t*BRCA1/2* Status positive / GIS Status negative, 4, t*BRCA1/2* Status negative / GIS Status positive, 10 t*BRCA1/2* Status negative / GIS Status negative) were run. For the hybridization temprature and library input all guardband studies, seven FFPE tumor samples (3 t*BRCA1/2* Status positive / GIS Status positive, 2 t*BRCA1/2* Status positive / GIS Status negative, 2 t*BRCA1/2* Status negative / GIS Status negative) were run in triplicate with the myChoice CDx assay at standard conditions for the generation of reference results for each guardband condition. All analytical calls, i.e., *BRCA1/2* sequence variants, LR calls and GIS, for the reference and test samples and replicates were reported.

a. DNA input guardband/robustness

Two studies were performed to evaluate the range of FFPE tumor extracted DNA input into the assay. In the first study, 14 samples were run in triplicate at 200 ng (used as the reference), 100 ng and 50 ng, and in singlet at 300 ng, 40 ng, 30 ng, 20 ng, and 10 ng. The second study ran a different set of 14 samples in triplicate at 200 ng (used as the reference) and 30 ng. The results from samples with different DNA input and samples near LoD of the t*BRCA1/2* portion (< 10% and ≥ 10% MAF) were reported.

For the tumor *BRCA1* and *BRCA2* mutation assay, all valid *BRCA1/2* sequence variant calls from 300 ng to 10 ng DNA input levels were 99.8% concordant.

All valid *BRCA1* and *BRCA2* LR calls produced from 300 ng to 20 ng DNA input levels were 100% concordant (no valid LR calls were produced at the 10 ng input level). For the GIS portion of the myChoice CDx assay, the total allowable error (TAE) analysis of valid GIS displayed acceptable amounts of bias and variation by passing pre-defined acceptance criteria from 300 ng to 30 ng input levels. In addition, the valid patient calls of all tests run were 100% concordant, except for a single false negative GIS Status patient call in one out of three replicates of one sample tested at 30 ng. All replicates of this sample had t*BRCA1/2* Status positive patient results, and as such, all patient results were Myriad HRD Status positive.

b. Hybridization Temperature for Probe Capture

The hybridization temperature for probe capture was evaluated by varying the temperature by ± 1ºC and ± 2ºC from the 65ºC standard condition. Seven FFPE samples were run at standard hybridization temperature to generate reference results. The results of the 7 samples from each guardband test condition were compared to the results obtained at the standard hybridization temperature. All samples across all guardband test conditions generated complete, valid *BRCA1* and *BRCA2* sequencing and LR results, as well as valid GIS. All *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant. Additionally, all samples tested at all guardband conditions produced 100% valid GIS analytical and patient results that were concordant.

c. Library input onto the HiSeq instrument

The library input onto the HiSeq instrument was evaluated by varying the library concentration by ± 2 pM and ± 4 pM from the 6 pM standard condition. Seven FFPE samples were run at the standard library input amounts to generate reference results. The results of the 7 samples from each guardband test condition were compared to the results obtained at the standard library input amounts. Samples across all guardband test conditions generated complete, valid *BRCA1* and *BRCA2* sequencing and LR results, as well as GIS. All *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant. All GIS generated at the different test conditions passed the pre-defined acceptance criteria. In addition, all patient results were 100% concordant. These results show that the myChoice CDx test is robust and is not affected by these process variations.

6. Stability Studies

a. Stability of FFPE Clinical Specimens

This study evaluated the real-time stability of FFPE tumor blocks and FFPE tumor sections on slides, stored at laboratory temperature. Reference results were defined from replicates of each specimen run at the initial (earliest) time point. The analytical and patient results of aged specimens, run in singlet, were compared to the reference calls for concordance.

FFPE Tumor Blocks.

Stability data was analyzed for 16 unique FFPE tumor blocks for up to the 5.5-year time point. Analytical calls from aged blocks at each time-point were compared to those from the initial (T0), reference time point. Analytical results have been obtained for 12, 7 and 6 samples at the 3.5, 5.0, and 5.5 year time points, respectively. For *BRCA1* and *BRCA2* sequencing, a single false positive *BRCA2* sequence variant was called at an allele frequency of 8.3% at the 3.5 year time point. All other *BRCA1* and *BRCA2* sequence variant calls were 100% concordant across all aged FFPE tumor blocks tested at each of the time points. All *BRCA1* and *BRCA2* LR calls were 100% concordant. In addition, the GIS and patient results were 100% concordant across all aged blocks tested at the different time points. The stability study has been confirmed up to 5.5 years. Stability testing of these FFPE tumor blocks is ongoing. The results of the ongoing tests will be reported in annual reports.

FFPE Tumor Sections.

The stability of unique FFPE tumor sections on slides is being evaluated at the following time points: 0, 1, 3, 5 and 5.5 years. Analytical calls from aged tumor sections at each testing time-point were compared to those from the initial (T0), reference time point. At the 1 year time point, all 10/10 samples generated complete, valid analytical calls and patient results. All *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant. In addition, all GIS and all patient results were 100% concordant across all specimens tested. So far, the stability study has been confirmed up to 1-year time point. Stability testing of these FFPE tumor sections is ongoing and will be reported in annual reports.

b. FFPE Tumor Extracted DNA Stability

The stability of DNAs extracted from 9 FFPE tumor specimens and stored at -20 ºC was evaluated at 0 (T0), 30 days, 60 days, 90 days and 6 months. Analytical calls from aged DNAs at each time-point were compared to those from the initial (T0), reference time point. All tests performed from

30 days to 6 months produced complete, valid analytical calls and patient results. For each of the stability testing time points, all *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant. In addition, the GIS and patient results were 100% concordant across all aged FFPE tumor extracted DNAs. The claimed stability for the extracted DNA is 5 months at -20°C.

c. Reagents Stability

The real-time stability of critical reagents used in the device was evaluated. Three lots of each of these reagents were stored at specified conditions and run at 0, 1 month and 4 months. Each reagent lot was tested with 6 FFPE tumor DNAs in duplicate. The lots of each reagent was stored at specified storage conditions and tested at the timepoints defined in Table 15. Analytical calls from aged reagents at each time-point were compared to those from the initial (T0), reference time point. All tests performed at 1 month and 4 months produced complete, valid analytical calls and patient results. For each of the stability testing time points, all *BRCA1* and *BRCA2* sequence and LR calls were 100% concordant, the GIS and all the patient results were 100% concordant across all lots of aged reagents.

Table 15: Key assay reagents evaluated in the stability study.

Reagent	Storage Condition	Shelf-Life	StabilityTesting Time-Points (months)
SureSelect XT2 and Index Kit	-20 °C	1 year	t = 0, 4
KAPA Custom Volume Hyper Prep Kit	-20 °C	1 year	t = 0, 4
HiSeq Rapid SBS Kit-v2 (200 cycle)	Ambient	1 year	t = 0, 1, 4
200 cycle SBS kit for HiSeq 2500	-20 °C	1 year	t = 0, 1, 4
HiSeq Rapid PE Cluster Kit v2	-20 °C	1 year	t = 0, 1, 4
HiSeq Rapid Flow Cell v2	4 °C	6 mth	t = 0, 1, 4
SureSelectXT2 Custom 6 -11.9Mb library	-80 °C	1 year	t = 0, 4

The stability of critical reagents has been assessed up to four (4) months. Stability testing for reagents is ongoing and will span up to 15 to 30 months depending on the reagent. The results of the ongoing tests will be reported in annual reports.

B. Animal Studies

None.

C. Additional Studies

None.

X. SUMMARY OF PRIMARY CLINICAL STUDY

The Myriad Genetics Laboratories, Inc. performed a clinical performance study to establish a reasonable assurance of safety and effectiveness of the myChoice CDx test as an aid to clinicians in identifying ovarian cancer patients who may be eligible for treatment with Zejula (niraparib), based on HRD positive status defined by the t*BRCA1/2* mutation(s) or positive GIS Status that has progressed more than six months after response to last platinum based chemotherapy. The Phase II study for niraparib (QUADRA), and the bridging study between the Clinical Trial Assay (CTA) and the myChoice CDx were the clinical basis of the PMA approval decision.

QUADRA study protocol was approved in December 17, 2014, with first patient enrolled on April 1, 2015, aiming to evaluate the activity in all patients with recurrent high-grade serous ovarian cancer with at least three prior lines of treatment. The patients with HRD positive (t*BRCA*m and/or GIS Status positive) were selected and enrolled in QUADRA trial using myChoice Clinical Trial Assay (CTA). This clinical study report (CSR) presents data as of the database lock date of April 11, 2018. At that time, enrollment was complete, but patients remained on treatment.

Compared to the CTA, the current myChoice CDx assay includes improvements to downstream data analysis and data review procedures. No substantive changes were made to the upfront sample processing portions of the assay in the laboratory. The myChoice CDx assay was used to retrospectively reanalyze all tissue samples from patients enrolled in QUARDA trial based on the CTA to support a clinical bridging study.

QUADRA, a Phase 2, open-label, single-arm study to evaluate the safety and efficacy of niraparib in patients with advanced, relapsed, high-grade serous epithelial ovarian, fallopian tube, or primary peritoneal cancer who have received three or four previous chemotherapy regimens.

Four analysis populations were predefined for testing the endpoints, and one additional analysis population was defined post hoc (the biomarker-defined population). The biomarker-defined population (n=98) was defined as all patients who had received at least 3 prior lines of therapy (LOT), had t*BRCA1/2* mutation (regardless of platinum sensitivity) or GIS status positive platinum-sensitive tumors, and were PARPi-naïve.

A summary of the clinical study is presented below.

A. Study Design

The study was initiated at 60 sites in 2 countries (53 sites in the US and 7 sites in Canada), and patients were enrolled at 50 sites in 2 countries, the United States (US; 43 sites) and Canada (7 sites). The original protocol (dated December 17, 2014) was amended 5 times across all study sites to modify study procedures or patient eligibility criteria.

To determine patient eligibility, a tumor sample was sent to a centralized laboratory for immediate GIS testing by CTA. Archival or fresh tumor tissue was required. For patients enrolled after implementation of protocol version 3 amendment on 24 May 2016, the sample was required prior to enrollment and could be sent in advance of the protocol-defined screening period in order to facilitate the screening and enrollment process. Patients were to wait for the results from the on-study centralized testing (CTA) prior to enrollment unless they had a previously detected *gBRCA*m. Blood samples were also collected for all patients during screening for determination of *gBRCA*m status. If *gBRCA* status was previously confirmed positive, then it was not necessary to wait for GIS testing results for enrollment into the study; however, confirmatory GIS testing still needed to be performed. In order to evaluate tumor markers, an optional fresh biopsy could be done at screening and at the end of treatment (EOT) visit.

A total of 463 enrolled patients, based on CTA, represented the safety population, of whom 461 patients were included in the ITT population. A biomarker-defined population was identified post hoc, defined as patients who received 3 or more prior LOT; had either tumor *BRCA1/2* mutation positive (t*BRCA*m) (regardless of platinum sensitivity) or GIS status positive platinum-sensitive tumors, and were PARPi-naïve; this population included 98 patients.

The primary endpoint for the study was ORR as defined by RECIST v1.1, in ovarian cancer patients who received 3 or 4 prior lines of anticancer therapy and whose disease was platinum-sensitive to the last platinum-based therapy. The key secondary endpoints were ORR in all patients and Median DOR (duration of response) in all patients.

1. Patient Information and Consent

In the QUADRA study, written informed consent was obtained from each patient before enrollment according to the regulatory and legal requirements of the participating countries. As part of this procedure, the Investigator explained orally and in writing the nature, duration, and purpose of the study, and the action of the study drug in such a manner that the patient was aware of the potential risks, inconveniences, or adverse events (AEs) that could occur. The patient was informed that he/she was free to withdraw from the study at any time. The patient received all information that was required by regulatory

authorities and International Conference on Harmonisation guidelines. The Investigator (or designee) provided the Sponsor with a copy of the IRB/IEC-approved Informed Consent Form (ICF) prior to the start of the study.

The ICF was signed and dated; 1 copy was given to the patient, and the Investigator retained a copy as part of the clinical study records. The Investigator did not undertake any investigation specifically required for the clinical study until written consent had been obtained.

2. Tumor Specimens and Testing

Patient samples consisted of FFPE blocks 40 μm thick with 25 mm^2 of tumor or 10 μm thick slides, plus one positively charges glass slide for H&E staining were sent for pathologist review at Myriad Genetic Laboratories (clinical testing site). Samples were kept at room temperature and shipped as soon as available to Myriad Genetic Laboratories with Test Request Form and Surgical Pathology Report and subject ID. Myriad performed gDNA extraction and the original gDNA extraction samples were stored at - 20° C and the diluted gDNA samples were stored between 2-6° C until the specimen was ready for gDNA fragmentation.

3. Key Clinical Inclusion and Exclusion Criteria

Enrollment in the QUADRA study was limited to patients who met the following inclusion criteria:

- Patients were to be female and at least 18 years of age.
- Patients must have provided written informed consent.
- Patients must have agreed to undergo tumor HRD testing and blood *gBRCA*mut status testing.

    a. This test result must have shown that patients had an HRDpos tumor, defined by the presence of a deleterious or suspected deleterious *BRCA*mut or be positive for genomic instability. *Note: The study HRD test result must have been received prior to enrollment. The tumor sample could have been submitted for HRD testing prior to the screening period if it appeared the patient was likely to meet other eligibility requirements. To facilitate early testing, a separate ICF specific for HRD testing was required to be signed prior to testing.*

    b. If historic blood *gBRCA*mut was detected by a central gBRCAmut testing, then tumor HRD sample test results were not required prior to enrollment; however, HRD testing needed to be performed. *Note: If gBRCAmut status was known by a local test, then a fresh sample was to be submitted for centralized gBRCAmut testing. Local gBRCAmut results were not acceptable*

for enrollment. Only patients with centralized gBRCAmut and/or HRDpos samples could be enrolled in this study.

- Patients must have had histologically diagnosed high-grade (Grade 2 or 3) serous epithelial ovarian, fallopian tube, or primary peritoneal cancer with recurrent disease and must have been previously treated with chemotherapy and experienced a response lasting at least 6 months to first-line platinum-based therapy. [*Note: The requirement that patients had a response to first-line platinum based therapy lasting at least 6 months was added in protocol version 2 (amendment 1; 30 October 2015*)].

- Patients must have completed 3 or 4 previous chemotherapy regimens (e.g., gemcitabine, doxorubicin, topotecan, carboplatin, oxaliplatin, cisplatin, bevacizumab, or PARPi as single agents or in combination per standard of care). (*Note: The restriction of prior lines of anticancer therapy to 3 or 4 was added in protocol version 2 [amendment 1; 30 October 2015]; in the original version of the protocol, patients with 3 or more previous chemotherapy regimens were eligible.*)

    c. Patients must have completed their last chemotherapy regimen > 4 weeks prior to treatment initiation.

- Patients must have had measurable disease according to RECIST (version 1.1).

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

- Patients were not to have any known, persistent (> 4 weeks), Grade ≥ 3 hematologic toxicity during the last cancer therapy.
- Patients were not to have any known, persistent (> 4 weeks), Grade ≥ 3 fatigue during the last cancer therapy.
- Patients must not have received pelvic radiotherapy as treatment for primary or recurrent disease within 1 year of the first dose of study drug.
- Patients were not to have symptomatic uncontrolled brain or leptomeningeal metastases.
- Patients were not to be considered a poor medical risk due to a serious, uncontrolled medical disorder, nonmalignant systemic disease, or active, uncontrolled infection. Examples included, but were not limited to, uncontrolled ventricular arrhythmia, recent (within 90 days) myocardial infarction, uncontrolled major seizure disorder, unstable spinal cord compression, superior vena cava syndrome, small bowel obstruction or other serious gastrointestinal disorder, or any psychiatric disorder that prohibited the obtaining of informed consent.

- Patients must not have received a transfusion (platelets or red blood cells) within 4 weeks of the first dose of study drug.
- Patients were not to have a known history or current diagnosis of MDS, AML, or second primary malignancy other than MDS/AML

4. Follow up schedule

Safety follow-up

Patients who discontinued from study drug continued to receive post-treatment assessments as part of the study, unless they were discontinued from the study.

Subjects who discontinued study treatment were followed for safety, until 30 days after last study treatment administration, except in the case of death, loss to follow-up, or withdrawal of consent.

Efficacy follow-up

Patients could be discontinued from the study for any of the following reasons: withdrawal of consent by the patient, who was at any time free to discontinue her participation in the study, without prejudice to further treatment or death from any cause, or lost to follow-up or the end of the study. If a patient discontinued treatment for a reason other than disease progression as defined in the protocol or death, withdrawal of consent, or loss to follow-up, then scans and CA-125 testing were to continue at the specified intervals (until the start of subsequent anticancer treatment).

Following disease progression, all patients were to be followed every 90 days; post-treatment assessments included RECIST tumor assessment, CA-125, assessment of survival status, and administration of subsequent anticancer therapy.

Survival follow-up

The post-treatment follow-up assessments conducted every 90 days were to include information on any new malignancies.

5. Clinical Bridging study

The bridging study between Myriad CTA and myChoice CDx, wherein the former was used to enroll patients in the QUADRA study prior to the use of the myChoice CDx was performed. Retropective analysis was conducted to establish the concordance between the CTA and the final myChoice CDx. There were no changes in the biomarker status of the patient sample in the biomarker defined population when comparing CTA to CDx. The concordance between the CTA and CDx was almost 100% (see Bridging of CTA to the myChoice CDx below).

The bridging study was performed to establish the clinical validity of the myChoiceCDx in identifying the Myriad HRD Status of the patients for treatment with niraparb by assessing efficacy of the myChoice CDx. The results of the efficacy based on the myChoice CDx test demonstrated that the clinical outcome associated with the CTA test results is maintained.

The disposition of samples from the clinical trial through to bridging study results is illustrated in "Accountability of PMA Cohort" section below.

B. Accountability of PMA Cohort

A total of 728 patients were screened for entry into QUADRA study which resulted in 265 screen-failure and 463 treated patients. Of these 463 patients, 435 niraparib treated patients were included in the PMA cohort. The retrospective analysis included the testing of 590 ovarian FFPE tumor specimens (20 samples were retests from existing patients) from QUADRA 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 570 patients: 435 niraparib treated patients, 131 patients who were enrolled and later determined as screen-failures, and 4 patients whose tumor samples were submitted for HRD pre-screening but who were not enrolled into the study.

Figure 1 below outlines the specimen accountability. The efficacy of niraparib was studied in 98 patients with t*BRCAm* or GIS status positive advanced ovarian cancer in the single-arm trial. Out of 98 patients, 63 patients were t*BRCAm* positive and 35 were non-t*BRCA*/GIS status positive patients. A break-down of QUADRA study population by PMA cohort is shown in Table 16. A break-down of PMA cohort (CTA/CDx) by Study population and Biomarker status is shown in Table 17.

Figure 1. Specimen accountability (Biomarker defined population)

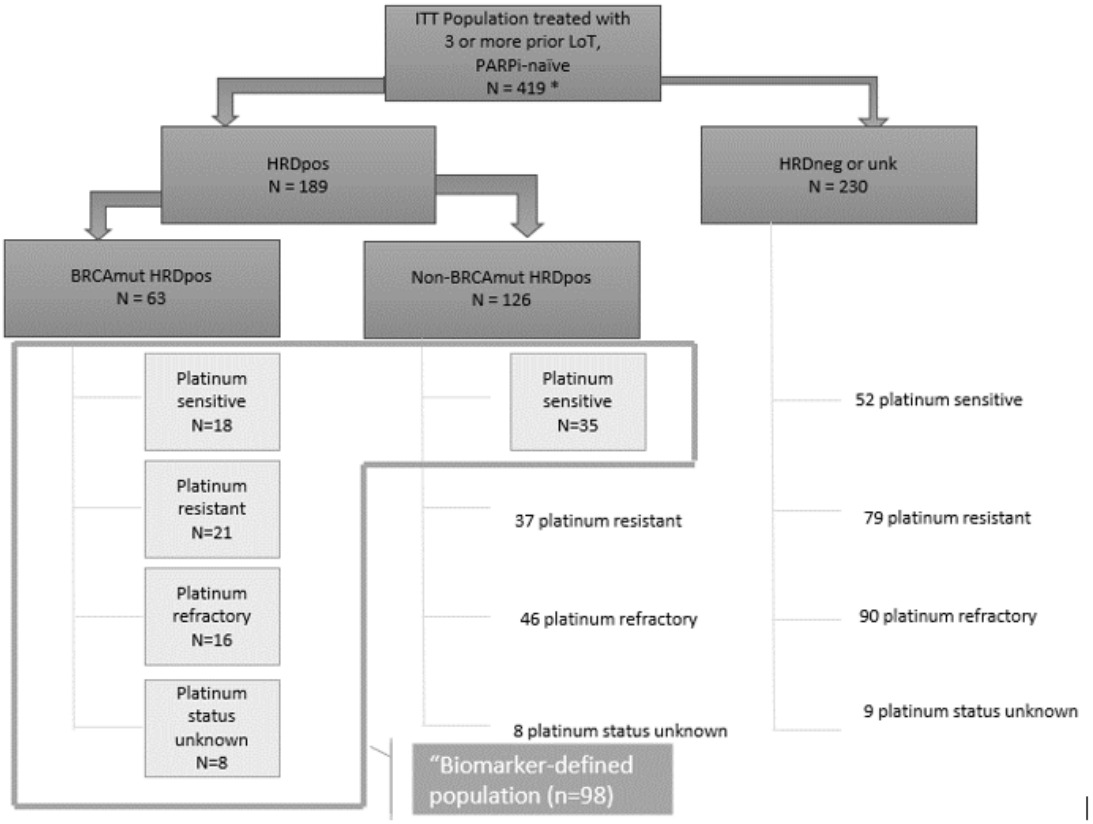

Table 16. Break-down of QUADRA Study Population by PMA Cohort

	Overall (N=732)	PMA cohort (N=570)	Not In PMA cohort (N=162)
HRD pre-screen/didnot enroll (1)	4	4	0
Screened Patients	728	566	162
Screen Failure Patients	265	131	134
Safety Population (2)	463	435	28
ITT Population (3)	461	433	28
Biomarker-defined Population (4)	98	95	3

(1): Patient submitted sample for HRD pre-screening consent but did not sign main informed consent for screening.

(2): Safety Population includes all treated patients. 28 patients in the safety population were not in the PMA cohort because they did not submit a sample or the sample submitted was insufficient for testing.

(3): ITT Population includes all treated patient who had measurable disease at baseline per RECIST v1.1.

(4): Biomarker-defined Population is the Device Intended Use Population. 3 patients in the Biomarker-defined Population was not in the PMA cohort because 1 patient did not submit sample for testing and 2 patients had canceled test for insufficient sample. All 3 patient have *gBRCA* mutation by blood test therefore were considered HRD positive.

Table 17. Break-down of PMA cohort by Study Population and Biomarker status

PMA cohort	PMA cohort (N=570)	HRD prescreen (N=4)	Screen Failure (N=131)	Safety Population (N=435)	ITT population (N=433)	Biomarker-defined Population [a] (N=95)
HRD positive	247	3	27	217	216	95
t*BRCA*m	92	1	13	78	78	56
t*BRCA*wt	131	2	14	115	114	28
t*BRCA* not-determined	24	0	0	24	24	11
HRD negative	292	1	96	195	195	0
t*BRCA*m	0	0	0	0	0	0
t*BRCA*wt	292	1	92	184	184	0
t*BRCA* not-determined	0	0	4	11	11	0
HRD	31	0	8	23	22	0
t*BRCA*m	0	0	0	0	0	0
t*BRCA*wt	31	0	8	20	19	0
t*BRCA* not-determined	0	0	0	3	3	0

[a]Biomarker-defined Population is the Device Intended Use Population.

Bridging of CTA to the myChoice CDx

This study was performed to demonstrate analytic concordance between the CTA version of the test and the final myChoice CDx test. Concordance of the myChoice CDx to the CTA at the variant level is described below (Table 18). A total of 590 ovarian FFPE tumor specimens from the QUADRA clinical study where biomarker calls from the Myriad CTA and myChoice CDx were compared. A total of 5,043/5,043 (100% positive percent agreement, PPA) valid, concordant *BRCA1/2* sequence variant calls (including the < 10% and ≥ 10% allele frequency variants) were observed, corresponding to a 95% lower confidence level (LCL) of 99.9406%.

The Table also shows the concordance of all valid *BRCA1/2* sequence non-variant base calls (at < 10% and ≥ 10% allele frequency variants and including the ten expected *BRCA1/2* sequencing call due to improved data analysis and data review procedures) from the myChoice CDx test with previous valid Myriad CTA sequence non-variant base calls. A total of 10,225,816/10,225,859 (i.e., 99.9997% negative percent agreement, NPA) concordant *BRCA1/2* sequence non-variant base calls were observed, corresponding to a 95% LCL of 99.9996%.

In addition, a total of 1,128/1,128 (100% overall percent agreement, OPA) concordant *BRCA1/2* large rearrangement (LR) calls were observed, corresponding to a 95% LCL of 99.7348%. However, there was one expected *BRCA1/2* LR call change due to improved data analysis and data review procedures. Including the one expected *BRCA1/2* LR call change, the OPA was 99.9114% (1128/1129) with 95% CI of 99.50%.

GIS were in total (100%) agreement between the Myriad CTA and the myChoice CDx tests. The Total Allowable Error analysis across all samples that produced valid GIS with both assays was 0.00.

Table 18. Concordance of All Valid t*BRCA1/2* Sequencing Analytical Calls

Concordance of All Valid *BRCA1/2* Seq Variant Calls	**95% LCL of *BRCA1/2* Seq Variant Calls**	**Concordance of All Valid *BRCA1/2* Seq Non-Variant Base Calls**	**95% LCL of *BRCA1/2* Seq Non-Variant Base Calls**
5,043/5,043 (PPA = 100%)	99.9406%	10,225,816/10,225,859 (NPA = 99.9996%)	99.9994%

In addition to variant level concordance, the concordance of patient (sample level) results were calculated for each of the three test outputs: t*BRCA* patient status, GIS status, and myChoice HRD CDx patient status. Sample level CTA and myChoice CDx agreements and 95% confidence intervals are provided for t*BRCA1/2* Status patient results in Table 19, GIS Status in Table 20, and Myriad HRD Status in Table 21.

The point estimates of PPA, NPA and OPA were 100.0% for all t*BRCA1/2* Status, GIS Status and Myriad HRD Status. When invalid calls were included in the agreement analyses, the PPA, NPA and OPA were 99.7% to 100% for all three statuses.

Table 19. CTA Versus myChoice CDx t*BRCA1/2* Status per Specimen Status Agreement

		CTA Comparator Results			Total
		t*BRCA* +	t*BRCA* -	Invalid	
myChoice CDx *tBRCA1/2* Status Result	t*BRCA* +	93[a]	0	2	95
	t*BRCA* -	0	462	17	479
	Invalid	0	1	15	16
	Total	93	463	34	590
Agreement Including Valid Results Only (Total N=555)	PPA [95% CI]	100.0 [96.1%, 100.0%]			
	NPA [95% CI]	100.0 [99.2%, 100.0%]			
	OPA [95% CI]	100.0 [99.3%, 100.0%]			
Agreement Including Invalid Results (Total N=556)	PPA [95% CI]	100.0 [96.1%, 100.0%]			
	NPA [95% CI]	99.8 [98.8%, 100.0%]			
	OPA [95% CI]	99.8 [99.0%, 100.0%]			

[a] One patient sample contained a *BRCA1* c.5152+5G>T variant that was reclassified from a variant of uncertain significance to a suspected deleterious mutation on 1/25/2019. Following standard procedures, this classification update changed the t*BRCA1/2* Status patient result to positive in both the CTA and CDx databases.

Table 20 CTA Versus myChoice CDx GIS Status Per Specimen Status Agreement

		CTA Comparator Results			Total
		GIS +	GIS -	Invalid	
myChoice CDx GIS Status Result	GIS Status +	227	0	0	227
	GIS Status -	0	299	0	299
	Invalid	0	0	64	64
	Total	227	299	64	590
Agreement Including Valid Results Only (Total N=526)	PPA [95% CI]	100.0 [98.4%, 100.0%]			
	NPA [95% CI]	100.0 [98.8%, 100.0%]			
	OPA [95% CI]	100.0 [99.3%, 100.0%]			
Agreement Including Invalid Results (Total N=526)	PPA [95% CI]	100.0 [98.4%, 100.0%]			
	NPA [95% CI]	100.0 [98.8%, 100.0%]			
	OPA [95% CI]	100.0 [99.3%, 100.0%]			

Table 21. CTA Versus myChoice CDx Overall Myriad HRD Status Per Specimen Status Agreement

		CTA Comparator Results			Total
		Positive	Negative	Invalid	
myChoice CDx Overall Myriad HRD Status Results	Positive	247	0	0	247
	Negative	0	291	0	291
	Invalid	0	1	51	52
	Total	247	292	51	590
Agreement Including Valid Results Only (Total N=538)	PPA [95% CI]	100.0 [98.5%, 100.0%]			
	NPA [95% CI]	100.0 [98.7%, 100.0%]			
	OPA [95% CI]	100.0 [99.3%, 100.0%]			
Agreement Including Invalid Results (Total N=539)	PPA [95% CI]	100.0 [98.5%, 100.0%]			
	NPA [95% CI]	99.7 [98.1%, 100.0%]			
	OPA [95% CI]	99.8 [99.0%, 100.0%]			

The results from the analytical concordance of CTA and myChoice CDx showed the concordance (PPA and NPA) of HRD+ vs. HRD- between CTA and CDx are sufficiently high (100% for both PPA and NPA), therefore, the clinical bridging study is not required to estimate the efficacy in the CDx defined population enrolled in the QUADRA clinical trial.

C. Study Population Demographics and Baseline Parameters

In the safety population, the median age was 65 years (range: 29 to 91 years), and 13.4% of patients were ≥75 years of age. Most patients were White (85.1%); 57.7% had an ECOG performance status of 0 at study entry, and 42.3% had an ECOG performance status of 1 at study entry. In the biomarker-defined population, the median age was 63 years (range: 39 to 91 years), and 10.2% of patients were ≥75 years of age. Most patients were White (81.6%); 59.2% had an ECOG performance status of 0 at study entry, and 40.8% had an ECOG performance status of 1 at study entry. Demographics for the overall safety population and for the biomarker-defined population are summarized in Table 22. Overall, 421 (90.9%) patients were treated at US sites and 42 (9.1%) patients were treated at Canadian sites.

Table 22. Demographics (Safety Population and Biomarker-Defined Population)

Demographic/Baseline Characteristic	Safety Population (N=463)	Biomarker-Defined Population (N=98)
Age (years)		
Mean (StD)	64.3 (9.28)	63.0 (9.51)

Demographic/Baseline Characteristic	Safety Population (N=463)	Biomarker-Defined Population (N=98)
Median	65.0	63.0
Min, max	29, 91	39, 91
Age (years), n (%)		
18 to <65	231 (49.9)	52 (53.1)
65 to <75	170 (36.7)	36 (36.7)
≥65	232 (50.1)	46 (46.9)
≥75	62 (13.4)	10 (10.2)
Race, n (%)		
White	394 (85.1)	80 (81.6)
Black	20 (4.3)	5 (5.1)
Asian	16 (3.5)	5 (5.1)
American Indian or	1 (0.2)	0
Other	14 (3.0)	4 (4.1)
Unknown	18 (3.9)	4 (4.1)
Weight (kg)		
n	463	98
Mean (StD)	72.2 (18.13)	73.5 (16.33)
Median	70.1	72.9
Min, max	36, 147	36, 124
ECOG PS at baseline, n (%)		
0	267 (57.7)	58 (59.2)
1	196 (42.3)	40 (40.8)
2+	0	0
Geographic Region, n (%)		
US	421 (90.9)	88 (89.8)
Canada	42 (9.1)	10 (10.2)

Baseline disease characteristics, including site of the primary tumor, histologic subtype, and duration, are provided for the safety population and the biomarker-defined population in Table 23.

Table 23. Baseline Disease Characteristics (Safety Population and Biomarker-Defined Population)

Baseline Disease Characteristic	Safety Population (N=463)	Biomarker-Defined Population (N=98)
Time from first diagnosis to first dose (years)		
Mean (StD)	4.6 (2.65)	5.6 (2.80)

Baseline Disease Characteristic	Safety Population (N=463)	Biomarker-Defined Population (N=98)
Median	4.0	4.9
Min, max	1, 15	1, 14
Primary tumor site, n (%)		
Ovarian	367 (79.3)	89 (90.8)
Primary peritoneal	47 (10.2)	7 (7.1)
Fallopian Tube	49 (10.6)	2 (2.0)
Histologic subtype, n (%)		
Serous	445 (96.1)	94 (95.9)
Endometrioid	0	0
Mucinous	0	0
Other	7 (1.5)	0
HRD status, n (%)		
HRDpos	222 (47.9)	98 (100)
*BRCA*m	87 (18.8)	63 (64.3)
*BRCA*wt/*BRCA*unk and HRDpos	135 (29.2)	35 (35.7)
HRDneg	195 (42.1)	0
HRDunk	46 (9.9)	0
***BRCA* status, n (%)**		
*BRCA*m	87 (18.8)	63 (64.3)
g*BRCA*m	58 (12.5)	36 (36.7)
s*BRCA*m	29 (6.3)	27 (27.6)
*BRCA*wt	319 (68.9)	28 (28.6)
Baseline Disease Characteristic	**Safety Population (N=463)**	**Biomarker-Defined Population (N=98)**
*BRCA*unk	57 (12.3)	7 (7.1)[d]
g*BRCA*m variant, n (%)	58 (12.5)	36 (36.7)
*BRCA*1	35 (7.6)	20 (20.4)
*BRCA*2	18 (3.9)	12 (12.2)
*BRCA*1/2 large rearrangement	11 (2.4)	8 (8.2)
t*BRCA*m variant, n (%)	78 (16.8)	56 (57.1)
t*BRCA*1	53 (11.4)	38 (38.8)
t*BRCA*2	25 (5.4)	18 (18.4)

D. Safety and Effectiveness Results

1. Safety Results

The safety results are presented overall for the 463 patients who received at least 1 dose of niraparib (safety population) and for the 98 patients in the biomarker-defined population to provide a comprehensive evaluation of the safety profile of niraparib in these patient populations. However, the safety with respect to treatment with niraparib is not comprehensively addressed in the SSED for the myChoice CDx. The evaluation of safety was based on the analysis of adverse events (AEs), clinical laboratory evaluations, physical examinations, and vital signs.

The safety profile observed in the QUADRA study was consistent with the known safety profile of niraparib and other PARPi, including gastrointestinal and hematologic events. The most common treatment-emergent adverse events (TEAEs) experienced in at least 20% of patients were nausea (67.4%), fatigue (51.2%), anemia (49.5%), vomiting (44.3%), constipation and thrombocytopenia (34.3% each), decreased appetite (26.3%), decreased platelet count (21.8%), insomnia (21.2%), and abdominal pain (21.0%). Hematologic toxicities could be effectively managed by appropriate dose modifications, and in general, the AE profile consisted of AEs that are commonly managed in the patient population of advanced ovarian cancer.

Patients with advanced cancer and significant history of chemotherapy exposure, such as the QUADRA population, have an increased risk of developing therapy-related MDS/AML. Other Grade 3 and higher AEs and adverse events of special interest (AESIs) were generally manageable with dose reductions. Refer to Zejula (niraparib) drug label for more information.

2. Effectiveness Results

a. Overall Efficacy

Efficacy analyses were performed to characterize efficacy in a biomarker-defined population, defined as patients who received 3 or more prior LOT, had either GIS positive platinum-sensitive tumors or had t*BRCAm* tumors regardless of platinum sensitivity, and were PARPi-naïve (n=98). As shown in the Table 24, the observed ORR in the biomarker-defined population (n=98) was 24.4% (95% CI: 0.16, 0.34), and median DOR was 8.3 months (95% CI 6.6, not estimable). The favorable results were also observed in the subgroups of patients with t*BRCAm* tumors and those with non-BRCAm/GIS positive platinum-sensitive tumors:

- In patients with t*BRCAm* tumors (n=63), the ORR was 28.6% overall and was 38.9% in patients with platinum-sensitive disease (n=18), 33.3% in patients with platinum-resistant disease (n=21), and 18.8% in patients with platinum-refractory disease (n=16).

- In patients with non-tBRCA/GIS positive and platinum-sensitive disease (n=35), the ORR was 20.0%.

Table 24. Efficacy Results in QUADRA (Biomarker-Defined Population)

Population	ORR % (95% CI)	DOR months (95% CI)
Biomarker-defined (n=98) regardless of platinum sensitivity	24.4% (24/98) (0.16, 0.34)	8.3 (6.6, not estimable, NE)
t*BRCAm* (n=63) tumor regardless of platinum sensitivity	28.6 % (18/63) (0.18, 0.41)	9.2 (7.4, NE)
Platinum sensitive (n=18)	38.9% (7/18) (0.17, 0.64)	NE (6.5, NE)
Platinum-resistant (n=21)	28.5% (6/21) (0.11, 0.52)	7.4 (4.7, NE)
Platinum-refractory (n=16)	18.8% (3/16) (0.04, 0.46	Not estimable (3.8, NE)
Non-t*BRCA*/GIS positive (n=35) platinum sensitive	20.0% (7/35) (0.08, 0.37)	6.6 (3.5,15.2)

3. Pediatric Extrapolation

In this premarket application, existing clinical data was not leveraged to support approval of a pediatric patient population since it not applicable for the ovarian cancer indication.

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 included 322 investigators. None of the clinical investigators had disclosable financial interests/arrangements as defined in sections 54.2(a), (b), (c), and (f). The information provided does not raise any questions about the reliability of the data.

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

In accordance with the provisions of section 515(c)(3) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Molecular and Clinical Genetics Panel of Medical Devices, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XII. CONCLUSIONS DRAWN FROM PRECLINICAL AND CLINICAL STUDIES

A. Effectiveness Conclusions

In the biomarker-defined population of patients who received 3 or more prior LOT, had either GIS status positive platinum-sensitive tumors or t*BRCAm* tumors regardless of platinum sensitivity, and were PARPi-naïve (n=98), the observed ORR was 25.5% (95% CI: 0.172, 0.353) and median DOR was 8.3 months (95% CI: 6.6, NE).

In the biomarker-defined population, meaningful ORR and DOR were observed among all subgroups, including the following:

- In patients with t*BRCAm* tumors (n=63), the ORR was 28.6% and median DOR was 9.2 months. When this group was analyzed by platinum sensitivity status, patients with BRCAmut platinum-sensitive disease (n=18) had an ORR of 38.9% and a median DOR of 9.2 months; patients with BRCAmut platinum-resistant disease (n=21) had an ORR of 33.3% and a median DOR of 7.4 months; and patients with t*BRCAm* platinum-refractory disease (n=16) had an ORR of 18.8% and median DOR was not estimable.
- In patients with non-t*BRCA*/GIS status positive platinum-sensitive disease (n=35), the ORR was 20.0% and median DOR was 6.6 months.

In summary, the efficacy results in the QUADRA study demonstrated the clinical benefit defined by clinical and molecular biomarkers, with the highest response rates observed among patients with tumors that were HRD positive and platinum-sensitive or t*BRCAm* regardless of platinum sensitivity status. The observed ORR in this population was meaningful and higher than would be expected with approved chemotherapy in this late-line disease setting, including for patients with t*BRCAm* disease regardless of platinum sensitivity (ORR: 28.6%; median DOR: 9.2 months) and patients with non-t*BRCA*/GIS status positive platinum-sensitive disease (ORR: 20.0%; median DOR: 6.6 months).

The performance of the next generation sequencing-based myChoice CDx was also supported by the analytical validation studies. As demonstrated in the analytical specificity study, the assay is highly specific to detect variants in the *BRCA1* and *BRCA2* genes and large rearrangements in both *BRCA1* and *BRCA2* genes. The device also demonstrated consistent performance to assess tumor genomic instability score (GIS) which is a linear combination of three components genomic status (LOH, LST and TAI). Further, sequencing, large rearrangement results and GIS from the myChoice CDx correlated with results obtained from validated comparator method.

B. Safety Conclusions

The myChoice CDx is an *in vitro* diagnostic device, which involves testing formalin fixed paraffin embedded tumor tissues 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 myChoice 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 Zejula® (niraparib) without any clinical benefit and may experience adverse reactions associated with niraparib therapy. Patients with false negative results may not be considered for treatment with Zejula® (niraparib), and therefore, may forgo potentially beneficial treatment with niraparib with a demonstrated ORR benefit of about 8-9 months or may receive other treatment options. There is also a risk of delayed results, which may lead to a delay in treatment with Zejula® (niraparib).

C. Benefit-Risk Determination

The probable benefits of the myChoice CDx device are based on data collected in the clinical study, showing improved ORR in a defined population of patients with ovarian cancer, which were used to support PMA approval as described above. The clinical benefit of myChoice CDx was demonstrated following an analysis of efficacy and safety data obtained from an open-label single-arm study in which niraparib demonstrated a statistically significant overall response rate with a clinically meaningful duration of response in patients with deleterious or suspected deleterious *BRCA* mutation or positive GIS status and has had disease progression of more than six months after last platinum-based chemotherapy associated with ovarian cancer in patients who had received three or more prior lines of chemotherapy. Overall, the response rate in patients with t*BRCAm*-or GIS status pos platinum sensitivity-associated ovarian cancer is better than what would be expected of available therapy and represents an improvement in a surrogate endpoint that is reasonably likely to predict clinical benefit.

Additional factors to be considered in determining probable risks and benefits for myChoice CDx included: analytical performance of the device, representation of variants in the major effectiveness study, and the availability of alternative tests. Analytical accuracy of the device showed a high degree of agreement with the comparator.

The risks of this device are associated with the potential mismanagement of patients resulting from false results of the test or a failure to receive results. Patients who are determined to be false positive by the test may be exposed to a drug that may not be beneficial and has potential adverse events. A false negative result may prevent a patient access to a potentially beneficial drug. The likelihood of false results was assessed in the analytical evaluation and showed acceptable performance with overall agreement for the myChoice CDx to the comparator was 98.1% including invalids. The reported PPA and NPA was 98.5% and 97.2%, respectively including test invalids (worst concordance case). While there is potential risk with the use of

this device, there is a moderate degree of uncertainty around risk due to the nature of certain analytical studies provided for the device.

Treatment with niraparib provides meaningful clinical benefit measured by overall magnitude of the response rate, with a moderate degree of uncertainty due to the small number of subjects in some of the subgroups.

In conclusion, given the available information above, the data support the use of the myChoice CDx test as an aid in identifying ovarian cancer patients with deleterious or suspected deleterious t*BRCA* mutations or positive genomic instability score for the treatment with niraparib, and the probable benefits outweigh the probable risks.

Patient Perspectives

This submission did not include specific information on patient perspectives for this device.

D. Overall Conclusions

The data in this application support the reasonable assurance of safety and effectiveness of this device when used in accordance with the indications for use. Data from the clinical study support the utility of myChoice CDx as an aid in selecting patients with previously treated ovarian cancer who may be eligible for treatment with niraparib. Niraparib 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 *BRCA1* and *BRCA2* mutations or positive GIS status, as identified with myChoice CDx.

In summary, considering all factors including conditions of approval (postmarket actions), the benefits of the use of myChoice CDx in patients with HRD status (defined by t*BRCA*m or positive GIS) are judged to outweigh the risks.

XIII. CDRH DECISION

CDRH issued an approval order with conditions for approval on October 23 , 2019. The final conditions of approval cited in the approval order are described below.

1. Obtain additional precision/reproducibility data using samples at the lowest DNA input, low MAF, and variant representation according to the agreed upon study proposal and update the labeling.

2. Obtain additional LoD data: (a) LoD of the BRCA1/2 sequence variant analytic calls using samples at the lowest DNA input level and (b) LoD of the GIS status using samples at low DNA input and low tumor content levels according to the agreed upon study proposal and update the labeling.

3. Obtain additional potential interference data: due to (a) Agilent SureSelect XT2 capture probes library and (b) proteinase K on the outcome of the myChoice CDx test and update the labeling.

The final study data, study conclusions, and labeling revisions should be submitted within 1 years of the PMA approval date.

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

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.