



Please note that although the following Technical Information for myChoice[®] CDx is correct, this document is currently under review by the Center for Devices and Radiological Health (CDRH). As soon as CDRH has completed their review, an approved version of the myChoice CDx Technical Information will be posted.

myChoice CDx[®] Technical Information

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Intended 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 <i>BRCA1</i> and <i>BRCA2</i> 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.

Contraindication

- There are no known contraindications.

Warnings and Precautions

- There are no known warnings or precautions.

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Limitations

- For *in vitro* diagnostic use
- For professional use only
- For prescription use only
- This test identifies germline and somatic variants in the tumor but does not distinguish between the two.
- Unequal allele enrichment may result from long insertions and deletions (indels) or rearrangements under probes
 - Indels > 25 bp in length can be detected by this 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.
 - This test has been designed to detect genomic rearrangements including large rearrangements (LRs) involving the promoter and coding exons of *BRCA1* and *BRCA2*, however, 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 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 assay.

Test Principle

The myChoice CDx test determines a patient's Myriad HRD Status by detecting single nucleotide variants (SNVs), variants in homopolymer stretches, indels, and LRs in the *BRCA1* and *BRCA2* genes and determining a 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 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.

The myChoice CDx test is composed of the following major processes:

- Tumor Sample Collection and Shipping
- Tumor genomic DNA Extraction
- DNA Processing using the following assays:
 - myChoice CDx next generation sequencing is used to detect sequence variants and genomic rearrangements (ie. large deletions and duplications) in *BRCA1* or *BRCA2*,

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and genomic instability analysis

- *BRCA1* and *BRCA2* Variant Classification
- Genomic Instability Calculation (GIS)
- Results Reporting

Summary and Explanation

The myChoice CDx device is a companion diagnostic for Zejula[®] (niraparib), a poly ADP-ribose polymerase (PARP) inhibitor. Patients whose tumors have a positive GIS Status and/or pathogenic variants in *BRCA1* or *BRCA2* show improved progression free survival when treated with Zejula[®] (niraparib) compared to placebo in the treatment setting.

Test Kit Contents

A sample collection kit provided by Myriad is used by the ordering laboratories/physicians. The collection kits contain the following components:

- Slide Container
- Tumor Block Container
- Collection Instructions
- Mailing Instructions
- Ice Pack

Instruments

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

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

Sample Collection and Test Ordering

To order myChoice CDx testing, the Test Request Form (TRF) included in the test kit must be fully completed.

Please refer to the myChoice CDx Collection Instructions and Mailing Instructions for further details about collecting formalin-fixed paraffin-embedded (FFPE) tumor samples and mailing the samples to Myriad.

Test Results and Interpretation

Patients evaluated with the myChoice CDx test that have clinically relevant tumor genomic instability and/or have been determined to carry a deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation can be considered for treatment with Zejula[®] (niraparib) under the supervision

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of a physician.

Upon completion of testing at Myriad, a test report will be sent to the designated physician. The results of each test component, along with the interpretation of the *BRCA1* and *BRCA2* variant(s) identified, are provided. If multiple variants are detected, the overall test interpretation most relevant to patient management is based on the most severe variant identified. Standard interpretative information included in test reports is listed below. Note that variants determined to have a classification of favor polymorphism or polymorphism are not included on the test report.

myChoice CDx Components:

The overall results are composed of two major components, namely GIS Status, and *tBRCA1/2* Status. The combined results form the basis of an overall interpretation of the myChoice CDx Myriad HRD Status. Potential results for these two components are described below:

“Genomic Instability Status: Positive”

The test results demonstrate homologous recombination deficiency based on the GIS alone.

“Genomic Instability Status: Negative”

The test results demonstrate homologous recombination proficiency based on the GIS alone.

“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.

“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 myChoice CDx testing (see **Limitations**).

Variant Interpretations

“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 test

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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.

“Complex Large Rearrangement”: a large rearrangement involving one or more exons where there is insufficient data to definitively determine its precise structure but has a significant potential to result in abnormal protein production and/or function.

“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”.

“Uncharacterized Large Rearrangement”: a large rearrangement involving one or more exons where there is insufficient data to definitively determine its structure, breakpoints and location within the genome. It is not known if transcription and/or translation are affected by this large rearrangement. This finding should not be used for clinical management of this patient. These large rearrangements 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 and issuance of amended reports

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

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becomes available. Whenever there is a clinically significant change in the classification of a variant within a patient's test result, an amended report will be provided by Myriad Genetic Laboratories.

Whenever there is a change in the classification of a variant that affects the overall interpretation of a patient's test result, an amended report will be provided by Myriad.

PERFORMANCE CHARACTERISTICS

Summary of Non-clinical Laboratory Studies

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

1. Correlation with Orthogonal Reference Method (Accuracy)

The accuracy of the Myriad HRD Status determined by the 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 FFPE tumor specimen-derived DNA samples were tested with both the assays. Samples representing the following subgroups were tested in the study: 5 *tBRCA1/2* Status positive / GIS Status negative, 71 *tBRCA1/2* Status negative / GIS Status negative, 66 *tBRCA1/2* Status positive / GIS Status positive, 61 *tBRCA1/2* Status negative / GIS Status positive.

a. *tBRCA1/2* 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%. Including variants with $< 10\%$ allele frequency, 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 percent agreement (OPA) of 100% for

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LR calls.

The results of the accuracy study were evaluated for three patient outcomes: (i) the *tBRCA1/2* Status based on *BRCA1* and *BRCA2* sequence and LR analyses; (ii) the GIS Status based on the GIS; and (iii) the overall Myriad HRD Status based on the combined results of the *tBRCA1/2* Status and GIS Status. The agreement between the myChoice CDx device and the comparator (reference) assay is summarized below.

b. *tBRCA1/2* Status Results

Concordance analysis of all 200 valid patient results produced by both the myChoice CDx and Comparator assays resulted in an OPA of 100%.

c. GIS Status Results

Concordance analysis of all 206 valid patient results produced by both the myChoice CDx and Comparator assays revealed a PPA of 98.5%, a NPA of 97.4%, and an OPA of 98.1%.

d. Myriad HRD Status Results

Concordance analysis of all 206 valid patient results from both the myChoice CDx and Comparator assay revealed a PPA of 98.5%, a NPA of 98.6%, and an OPA of 98.5%.

2. Analytical Sensitivity

a. Limit of Blank (LoB)

Twenty-six (26) FFPE normal tissue samples were tested wherein all low frequency variants were expected to be spurious technical artifacts rather than true biological events. The distribution of allele frequencies was plotted. The frequencies of spurious variants were 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 *tBRCA1/2* 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 *tBRCA1/2* 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.

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b. Limit of Detection (LoD)

i. *tBRCA1/2* 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 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. *tBRCA1/2* 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 *tBRCA1/2* 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.

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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 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 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.

3. Analytical Specificity

a. Interference (*tBRCA1/2* Sequence Variant and LR Analytical Calls)

To evaluate the potential impact of three classes of substances (endogenous [hemoglobin, triglycerides], exogenous [tissue marking dye, paraffin wax], and method-specific interferents [ethanol, NaOH]) that can potentially interfere with the assay, this study evaluated seven FFPE specimens (5 *tBRCA1/2* Status positive / GIS Status positive and 2 *tBRCA1/2* Status negative / GIS status negative) representing single nucleotide variants, insertions or deletions of < 10 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 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.

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)

All treated samples produced valid GIS for each potential interfering substance tested. In

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addition, all samples run under each condition produced valid positive or negative patient calls showing 100% concordance when compared to samples without additional interferents.

Taken together, these results demonstrate that the *tBRCA1/2* 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 $\geq 10\%$ 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 $\leq 60\%$ does not impact the myChoice CDx assay results.

c. Carryover

The purpose of this study was to demonstrate that the myChoice CDx test minimizes carryover across samples. Fourteen FFPE specimens (11 *tBRCA1/2* Status positive / GIS Status positive, 1 *tBRCA1/2* Status positive / GIS Status positive and 2 *tBRCA1/2* 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 checkerboard pattern inverted, 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 SNVs 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 patient results.

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

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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 *tBRCA1/2* Status positive / GIS Status positive, 1 *tBRCA1/2* Status negative / GIS Status negative, 1 *tBRCA1/2* Status negative / GIS Status positive, and 1 *tBRCA1/2* 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 SNVs 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 purpose of these studies was to demonstrate that the myChoice CDx assay generates highly precise/reproducible *BRCA1* and *BRCA2* sequencing and LR analytical calls and precise GIS on the tested samples from five different studies over five periods of time. In the first three studies (Studies 1–3), a total of 18 unique samples 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 (Study 5) was performed using 5 unique FFPE samples with 18 replicates per sample divided across 9 independent runs over multiple days using 3 different sequencers and 3 lots of critical reagents. Thus, a total of 23 FFPE samples were evaluated (10 *tBRCA1/2* Status positive / GIS Status positive, 2 *tBRCA1/2* Status positive / GIS Status negative, 6 *tBRCA1/2* Status negative / GIS Status positive, 3 *tBRCA1/2* Status negative / GIS Status negative, 2

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tBRCA1/2 Status unknown / GIS Status positive). The samples contained single nucleotide variants, insertions or deletions of < 10 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 a wide GIS range.

a. *tBRCA1/2* Sequence Variant and LR Analytical Calls

All 228/228 samples and replicates tested from Studies 1–3 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.

Study 4 analyzed 7 FFPE tumor samples at 50 ng DNA inputs, which had previously been evaluated at 200 ng DNA input levels in Studies 1-3. All samples and replicates run generated complete (100%) valid *BRCA1* and *BRCA2* sequence and LR calls. Altogether, all 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.

Study 5 tested five new additional ovarian tumor specimens that were *tBRCA1/2* Status negative / GIS Status positive. The specimens were tested at the lowest DNA input of 30 ng with GIS Status being low positives. The myChoice CDx device produced 700/702 concordant *BRCA1/2* sequence variant calls, at mean allele frequency (MAF) $\geq 10\%$, resulting in a 99.7% PPA. There were two false negative calls in one sample in a single nucleotide variant with a MAF of 11.2%. 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, *BRCA1/2* LR concordance was 100% (165/165).

b. *tBRCA1/2* Status Results

All samples and replicates run for all five studies produced valid positive or negative patient results, except for two samples, which were inconclusive for the *tBRCA1/2* Status result. However, both of these samples had a positive GIS Status and were therefore both Myriad HRD 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.

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c. GIS

For the GIS portion of the assay, all 23 samples and replicates at all DNA inputs (200 ng, 50 ng, and 30 ng) from the five studies produced valid, precise GIS. 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.

d. GIS Status and Myriad HRD Status 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.

These results demonstrate that *BRCAl/2* sequence variant and LR calls, GIS, and patient results of the myChoice CDx assay are highly precise/reproducible across the different process and reagent variabilities introduced in these studies.

5. Guardbanding

The myChoice CDx guardband/robustness studies challenged 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 *tBRCAl/2* Status positive / GIS Status positive, 1 *tBRCAl/2* Status positive / GIS Status negative, 4 *tBRCAl/2* Status negative / GIS Status positive, 10 *tBRCAl/2* Status negative / GIS Status negative) were run. For the hybridization temperature and library input guardband studies, seven FFPE tumor samples (3 *tBRCAl/2* Status positive / GIS Status positive, 2 *tBRCAl/2* Status positive / GIS Status negative, 2 *tBRCAl/2* Status negative / GIS Status negative) were run in triplicate at standard myChoice CDx assay conditions for the generation of reference results for each guardband condition. All analytical calls (i.e., *BRCAl/2* sequence variants, LR calls, and GIS) and patient results 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 *tBRCAl/2* portion (< 10% and ≥ 10% MAF) were reported.

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For the *tBRCA1/2* portion of the myChoice CDx assay, all valid *BRCA1* and *BRCA2* sequence variant calls from 300 ng to 10 ng DNA input levels were 99.8% concordant and all valid *BRCA1* and *BRCA2* non-variant base calls were 99.99% 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 *tBRCA1/2* 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 $\pm 1^{\circ}\text{C}$ and $\pm 2^{\circ}\text{C}$ from the 65°C 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 precise GIS analytical results and all patient results were 100% 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 were precise and 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

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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.

i. 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 were precise 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.

ii. 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 were precise and all patient results were 100% concordant across all specimens tested. The stability study has thus far been confirmed up to 1-year time point.

b. FFPE Tumor Extracted DNA Stability

The stability of DNAs extracted from 9 FFPE tumor specimens and stored at -20 °C 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 were precise 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

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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 at each aged reagents time point. 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 generated were precise, and all the patient results were 100% concordant across all lots of aged reagents.

Summary of Clinical Studies

The niraparib clinical study PR-30-5020-C (QUADRA) was an open-label, single-arm clinical trial designed to evaluate the efficacy and safety of niraparib in patients with advanced, relapsed, high-grade serous epithelial ovarian, fallopian tube or primary peritoneal cancer who had received three or more previous chemotherapy regimens.

1. Accountability of PMA

To determine patient eligibility, a tumor sample was sent to a centralized laboratory for immediate myChoice CDx (HRD) testing. Archival or fresh tumor tissue was required. *(Note: for patients enrolled after implementation of protocol version 3 [amendment 2; 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 HRD testing prior to enrollment unless they had a previously detected germline breast cancer gene (BRCA) mutation (gBRCAmut). Blood samples were also collected for all patients during screening for determination of gBRCAmut status. If gBRCA status was previously confirmed positive, then it was not necessary to wait for HRD testing results for enrollment into the study; however, confirmatory HRD testing still needed to be performed.

2. Effectiveness Results

The primary efficacy endpoint was Investigator-assessed confirmed ORR as defined by RECIST (version 1.1). The primary analysis population for this endpoint included all patients with HRDpos tumors who received 3 or 4 prior LOT and whose disease was sensitive to the last platinum based therapy. Patients with prior PARPi treatment were not included in the primary analysis population. The patient's best overall response (BOR) was determined based on the overall responses at all timepoints between the date of the first dose and the date of first documented radiological disease progression, the date of subsequent anticancer therapy, or the date of study discontinuation, whichever occurred first. Patients with a BOR of either confirmed complete response (CR) or confirmed

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partial response (PR) were considered to have responded to treatment (“responders”). All other patients were considered not to have responded to treatment (“non-responders”).

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

- The study met the primary endpoint, with an ORR as defined by RECIST (version 1.1) of 27.7% (95% CI: 15.6%, 42.6%; 1-sided p-value=0.0005) in the primary analysis population
- (n=47 patients with 3 or 4 prior LOT, HRDpos tumors, platinum-sensitive disease, and PARPi-naïve).

Post hoc analyses were also conducted in subgroups defined by platinum sensitivity and biomarkers.

Investigator-assessed confirmed ORR as defined by RECIST (version 1.1) was analyzed for the biomarker-defined population overall and in the following biomarker subsets:

- BRCAmut (regardless of platinum sensitivity)
- Non-BRCAmut/HRDpos platinum-sensitive

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

- In the HRDpos cohort (n=98), the ORR was 24% and the median DOR was 8.3 months.
- In patients with BRCAmut tumors (n=63), the ORR was 27% 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 39%; patients with BRCAmut platinum-resistant disease (n=21) had an ORR of 29%; and patients with BRCAmut platinum-refractory disease (n=16) had an ORR of 19%.
- In patients with non-BRCAmut HRDpos platinum-sensitive disease (n=35), the ORR was 20.0% and median DOR was 6.6 months.