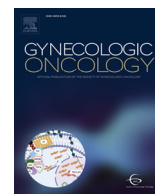




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## Concordance between an FDA-approved companion diagnostic and an alternative assay kit for assessing homologous recombination deficiency in ovarian cancer

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### HIGHLIGHTS

- HRD status was evaluated using two tests (Illumina TSO 500 HRD assay and Myriad MyChoice®CDx PLUS assay).
- Overall HRD, BRCA mutation, and HRD GIS status detection showed >93% agreement between the tests.
- Prevalence of HRD-positive and BRCA mutation status were similar between the tests.
- HRD GIS was strongly correlated between the two tests.

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### ABSTRACT

**Objective.** Authors evaluated the performance of a commercially available next-generation sequencing assay kit; this was based on genomic content from Illumina's TruSight™ Oncology 500 research assay that identifies BRCA variants and proprietary algorithms licensed from Myriad and, with additional genomic content, measures the homologous recombination deficiency (HRD) genomic instability score (GIS) in tumor tissue (TSO 500 HRD assay).

**Methods.** Data from the TSO 500 HRD assay were compared with data from the Myriad MyChoice®CDx PLUS assay (Myriad assay). Prevalence rates for overall HRD status and BRCA mutations (a deleterious or suspected deleterious BRCA1 or BRCA2 mutation or both) and assay agreement rates for HRD GIS and BRCA analysis were assessed in ovarian tumor samples. Pearson correlations of the continuous HRD GIS and analytic sensitivity and specificity were evaluated.

**Results.** The prevalence of overall HRD positivity was 51.2% (TSO 500 HRD assay) versus 49.2% (Myriad assay) and the prevalence of BRCA mutations was 27.6% (TSO 500 HRD assay) versus 25.5% (Myriad assay). After post-processing optimization, concordance of the HRD GIS was 0.980 in all samples and 0.976 in the non-BRCA mutation cohort; the area under the receiver operating characteristic curve was 0.995 and 0.992, respectively.

**Conclusions.** Comparison between the Illumina and Myriad assays showed that overall HRD status, the individual components of BRCA analysis, and HRD GIS detection results were highly concordant (>93%), suggesting the TSO 500 HRD assay will approach the analytical accuracy of the FDA-approved Myriad assay.

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## 1. Introduction

Ovarian cancer is a heterogeneous disease [1] and is the seventh most common cancer in women worldwide and the leading cause of mortality among women, mainly because of delayed diagnosis [1–3]. Platinum-based chemotherapy is recommended in the first-line and relapsed settings for patients with platinum-sensitive ovarian cancer [4]. Defects in the homologous recombination pathway may result in the homologous recombination deficiency (HRD) phenotype (a tumor with a deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation [BRCAm] or the presence of HRD-induced genomic instability), and detection of such defects can help predict response to platinum and poly (ADP-ribose) polymerase (PARP) inhibitor therapy [2,5–11]. Modulation of the DNA repair pathway using PARP inhibitors, such as olaparib, has emerged as a treatment option for patients with ovarian cancer. Olaparib is currently approved by regulatory agencies for ovarian cancer as 1) maintenance treatment for patients with platinum-sensitive relapsed disease that responded to platinum-based chemotherapy, 2) maintenance monotherapy for patients with advanced *BRCA1*- or *BRCA2*-mutated (germline and/or somatic) disease that responded after completion of first-line platinum-based chemotherapy, and 3) maintenance treatment in combination with bevacizumab for patients whose disease responded after completion of first-line platinum-based chemotherapy and whose cancer is associated with HRD-positive status [12,13]. Therefore, defining the HRD phenotype using the appropriate HRD test is key to identifying patients who may benefit from PARP inhibitor therapy.

Currently, there is no standard definition of the HRD phenotype or consensus on how HRD should be detected and quantified, although several groups are actively attempting to standardize this [14,15]. Friends of Cancer Research, a nonprofit advocacy agency, is making an ongoing effort to harmonize the definition of HRD as well as the methodology used to detect HRD across clinical laboratories, diagnostic providers, and pharmaceutical companies. In a recent publication, this group provided an overview of the biology and assessment of HRD [15]. HRD can be characterized as a phenotype caused by the inability of a cell to effectively repair double-stranded DNA breaks using the homologous recombination repair (HRR) pathway and can be determined by functional assessment (such as the RAD51 foci assay [16]). Potential causes of the HRD phenotype are alterations in the HRR pathway stemming from genetic or epigenetic events such as *BRCA1*, *BRCA2*, or other HRR gene mutations or methylation. Potential consequences of an impaired HRR pathway can be assessed by probing the genome for evidence of genomic instability, such as HRD-type chromosomal instability and other genomic signatures; examples of biomarkers that detect such alterations are genome-wide loss of heterozygosity (LOH) alone or the HRD genomic instability score (GIS; LOH plus telomeric allelic imbalance [TAI] plus large-scale state transitions [LST]) [15].

Genomic instability related to HRD was initially reported using DNA-based genome-wide LOH [17] that was subsequently refined into the combined HRD score or HRD GIS, defined as the unweighted sum of LOH, TAI, and LST [18]. There are techniques available to assess these genomic alterations, including single-nucleotide polymorphism (SNP) arrays and next-generation sequencing (NGS) assays. Clinically, the ARIEL2 trial demonstrated that LOH alone, as determined by the Foundation Medicine T5 NGS assay, was predictive of response to rucaparib [19], and the PAOLA-1 study showed the addition of olaparib to bevacizumab provided a clinical benefit specifically in those patients with tumors identified as HRD positive, as determined by the Myriad MyChoice@CDx assay (Myriad Genetics, Inc., Salt Lake City, UT) [20]. Myriad MyChoice CDx is an NGS-based in vitro companion diagnostic (CDx) approved by the United States Food and Drug Administration (US FDA) and the Japanese Pharmaceuticals and Medical Devices Agency [21,22]. This assay determines HRD status by detecting loss-of-function variants in *BRCA1* and *BRCA2* (including deletions and large rearrangements) and assessing HRD GIS. The Myriad MyChoice CDx assay

has US regulatory approval and is used in the majority of pivotal clinical studies; this assay is currently one of only two clinically validated HRD tests (the other being FoundationOne@CDx Foundation Medicine, Cambridge, MA). Other tests are being validated on appropriate clinical sample sets and in smaller studies and are expected to lead to additional clinically validated tests.

Currently, there is interest in the field in assessing concordance between the available methods to evaluate HRD [15]. In one study, the performance of the OncoScan™ (ThermoFisher, Waltham, MA) and Infinium™ CytoSNP-850 K (Illumina, San Diego, CA) for assessing HRD genomic instability was evaluated [23]. This analysis found that the genomic metrics (as continuous variables) assessed by SNP genotyping arrays demonstrated good association with the Myriad BRCA alteration calling and genome-wide test metric (HRD GIS at a cutoff of 42). In another study, HRD scores determined using SOPHiA DDM HRD solution (SOPHiA Genetics, Rolle, Switzerland), the AmoyDx HRD Focus panel (AmoyDx, Xiamen, China), and the OncoPrint HRR Pathway predesigned panel (Thermo Fisher Scientific, Waltham, USA) had high agreement with the MyChoice CDx [24]. SOPHiA DDM HRD solution demonstrated 90.0% overall percentage agreement (OPA), 85.7% positive percentage agreement (PPA), and 92.3% negative percentage agreement (NPA) with the MyChoice CDx; the AmoyDx HRD Focus panel demonstrated 88.2% OPA, 75.0% PPA, and 100.0% NPA with the MyChoice CDx; and the OncoPrint HRR Pathway predesigned panel demonstrated 80.0% OPA, PPA, and NPA with MyChoice CDx [24].

The Illumina TruSight™ Oncology 500 HRD (San Diego, CA; hereafter referred to as the TSO 500 HRD assay) Research Use Only (RUO) assay identifies BRCA variants based on genomic content from the TruSight™ Oncology 500 (TSO 500) RUO assay and with proprietary algorithms licensed from Myriad and additional genomic content from Illumina, measures HRD GIS in tumor tissue in parallel. TSO 500 is an NGS-based assay that uses targeted high-throughput hybridization-based capture technology for detection of single nucleotide variants, insertion and deletion alterations (indels), and copy number alterations (CNAs) in 523 genes; tumor mutational burden (TMB); and microsatellite instability (MSI) using DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens [25].

To increase access to HRD testing in the European Union (EU), clinical laboratories need a distributable kit that can be performed locally and that closely replicates the analytical performance of an on-market, clinically validated HRD test in identifying patients that may respond to PARP inhibition. The current report aims to evaluate TSO 500 HRD RUO and reports the analytic concordance and quality control pass rates of the commercially available TSO 500 HRD assay vs the FDA-approved Myriad MyChoice CDx assay (hereafter referred to as the Myriad assay), both initially and after bioinformatic optimization of the TSO 500 HRD assay.

## 2. Materials and methods

### 2.1. Study setup

This evaluation of the TSO 500 HRD assay was divided into two sections: Part 1 describes the evaluation of the initial analytical accuracy of the TSO 500 HRD assay and HRD status calling algorithm in comparison to the Myriad assay; and Part 2 describes the performance of the TSO 500 HRD assay after bioinformatic optimization of the HRD calling algorithm.

### 2.2. Samples

Commercial ovarian cancer samples were obtained from BioOptions (Brea, CA), Indivumed (Hamburg, Germany), and ProteoGenex (Inglewood, CA) and were collected under informed consent. Samples from clinical trials were obtained from KEYNOTE-100 ([clinicaltrials.gov](http://clinicaltrials.gov), NCT02674061;  $N = 376$ ), which comprised patients with epithelial

ovarian cancer, fallopian tube cancer, or primary peritoneal cancer who demonstrated recurrent disease following primary or interval cytoreductive/debulking surgery and standard front-line, platinum-based combination therapy. All patients provided written informed consent before enrollment in the clinical trial. The study protocol and all amendments were approved by the institutional review board or ethics committee at each institution. The study was conducted in accordance with the protocol, its amendments, the ethical principles originating from the Declaration of Helsinki, and Good Clinical Practice guidelines.

### 2.3. Sample analysis

Ovarian cancer tissue samples were collected, and DNA was extracted using the Qiagen QIAamp DNA (Qiagen, Hilden, Germany) FFPE tissue kit at a central laboratory (Almac, Craigavon, UK). Extracted DNA was tested in the Myriad Genetics laboratory (Salt Lake City, UT) and the Illumina laboratory (San Diego, CA); the DNA input for the assay was 200 ng for the Myriad assay and 40 ng for the TSO 500 HRD assay. The subset of samples that failed post-sequencing quality control during Part 1 of the TSO 500 HRD assay development were retested with increased DNA input as available (range: 52–193 ng), as many of the commercially procured samples that were incorporated into the study were noted to have poor quality DNA upon extraction. The analysis for Part 2 was conducted on 40 ng of DNA input, per recommendations from Illumina.

### 2.4. Probe build for the HRD component of the TSO 500 HRD kit

To develop the HRD GIS component of TSO 500 HRD in collaboration with Myriad, probes were designed by selecting known polymorphic sites from the 1000 Genomes Project [26,27] and applying the following selection criteria: (1) targeted interprobe distance of ~100 kilobases, optimized to meet coverage requirements for the HRD sub-pool to meet the required LOH, TAI, and LST resolution while simultaneously meeting the coverage requirements for the TSO 500 component running in parallel. (2) compatibility with the TSO 500 probe set, (3) population allele frequency with a range of  $0.5 \pm 0.15$  averaged across sub-ethnicities and  $0.5 \pm 0.3$  for each ethnicity, (4) applied GC content and filter ability to be aligned, and (5) applied criteria for the uniqueness of capture region.

### 2.5. TSO 500 HRD assay workflow

The TSO 500 HRD assay workflow is depicted in Fig. 1. The Illumina HRD test was an add-on to the TSO 500 workflow. The TSO 500 DNA library was split into two hybridization reactions, one with TSO 500 DNA probes and the other with HRD probes. Both DNA libraries were then pooled with the enriched TSO 500 RNA library at a 65%:15%:20% ratio (TSO 500 DNA library: HRD library: TSO 500 RNA library) prior to sequencing on the NextSeq™ 550 sequencer (Illumina, San Diego, CA). The combined product created a complete end-to-end solution with all reagents and software components necessary to obtain a combined comprehensive genomic profile and HRD variant output from nucleic acids.

### 2.6. HRD GIS algorithm implementation on TSO 500 HRD

HRD GIS was calculated using a combination of LOH, TAI, and LST and based on previous work [17,18]. The overall score was calculated as the sum of the single events for LOH, TAI, and LST. HRD status was considered positive if the tumor sample carried a pathologic BRCAm variant and/or had an HRD GIS  $\geq 42$ ; otherwise, the HRD sample was considered negative.

The proprietary HRD GIS algorithm used on the Illumina sequencing data was licensed from Myriad, re-implemented as part of the

DRAGEN™ Bio-IT software suite (Illumina), and integrated into the TSO 500 analysis workflow. After initial concordance calculations (Part 1), in which Illumina was blinded to the Myriad result, data processing steps upstream to the unmodified algorithm licensed from Myriad were revised to minimize noise and biases in the data (Part 2).

### 2.7. BRCA mutation classification

The TSO 500 HRD assay defines BRCAm status based on a set algorithm for single-nucleotide variants, large rearrangement variants, and insertions/deletion alterations (indels). Deleterious BRCAm variants are defined by the TSO 500 HRD kit based on the annotated gene consequences including mutations that result in protein truncation (stop gains, transcript truncations, frame shift alterations excluding mutations at/after *BRCA2* K3326\*), splice variants, and a list of deleterious missense mutations confirmed by ClinVar. This static list differs from the Myriad method, which has a dynamic list of mutations that is frequently reviewed by a professional variant classification team.

### 2.8. Quality control

The NGSCheckMate algorithm was used to rule out sample misalignment [28]. Genomic SNP fingerprints at loci expected to have robust germline variation were calculated for each sample using the TSO HRD 500 and Myriad assay raw FASTQ sequencing files, and the pairwise correlation between the resulting fingerprints was assessed for each pair of samples analyzed using the two assays, suggesting very good concordance among samples from the same patient and no cross-contamination among samples from different patients (**Supplemental Fig. 1**). The prevalence of overall HRD positivity and BRCAm status was estimated using both tests.

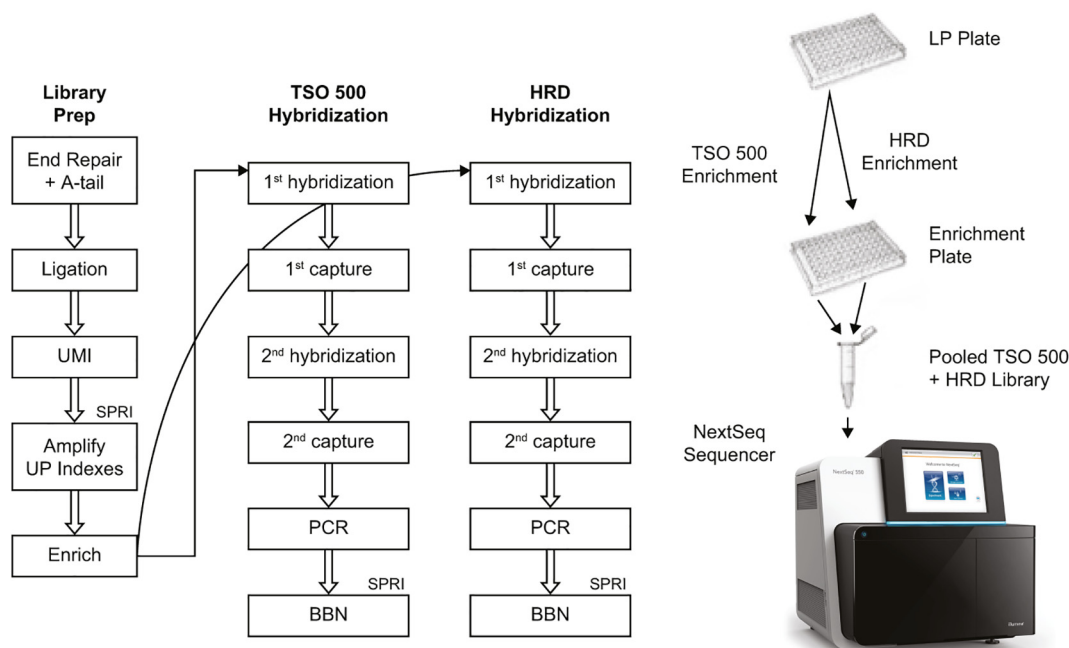
### 2.9. Assessment and analyses

The agreement rates for the TSO 500 HRD assay vs the reference (Myriad assay) were summarized descriptively for BRCAm status, HRD GIS, and overall HRD status (includes both BRCAm status and HRD GIS) in terms of OPA (the number of samples with status in agreement by both Illumina and Myriad assays divided by the total number of samples), PPA (the number of positives identified by both the Illumina and Myriad assays divided by the total number of positives by the Myriad assay), and NPA (the number of negatives identified by both the Illumina and Myriad assays divided by the total number of negatives by the Myriad assay). For both the overall and non-BRCAm cohorts, correlation between the continuous HRD GIS of the TSO 500 HRD assay and the reference test (Myriad assay) was estimated. The analytic sensitivity and specificity of the Illumina-derived HRD GIS to classify HRD status was evaluated. *BRCA1* and *BRCA2* variant classification was also evaluated between the two tests. Pearson correlation was used to test the association between the continuous HRD GIS of the Illumina and Myriad assays. The area under the receiver operating characteristic (AUROC) curve was used to evaluate the analytic sensitivity and specificity of the Illumina-derived HRD GIS to classify HRD status using the Myriad assay result as true finding.

## 3. Results

### 3.1. Part 1: Initial analytical performance

The investigators sought to evaluate the success rate of the Illumina and Myriad assays, defined as the ability to pass post-sequencing quality control metrics and generate an evaluable BRCAm status, HRD GIS score, and overall HRD status. For the current analysis, there were 227 samples available for the TSO 500 HRD assay; 196 were commercial ovarian samples and 31 were clinical trial samples. There were 254 samples available for the Myriad assay; 223 were commercial ovarian samples



**Fig. 1.** TSO 500 HRD assay workflow. A single library was split into two parallel enrichments and the final enriched library was pooled for sequencing. BBN, bead-based normalization; HRD, homologous recombination deficiency; LP, library preparation; SPRI, solid-phase reversible immobilization; UMI, unique molecular identifier; UP, unique index primer.

and 31 were clinical trial samples. Of these, 227 samples overlapped and were assessed by both the Illumina and the Myriad assays. Initial success rates for the TSO 500 HRD assay vs. the Myriad assay (all tested samples) were 86.8% vs 94.1% for overall HRD status, 88.1% vs 97.6% for BRCAm status, and 91.2% vs 93.7% for HRD GIS (Table 1). Success rates using the overlapping 227 samples that were assessed by both the Illumina and Myriad assays were 86.8% vs 95.2%, 88.1% vs 97.8%, and 91.2% vs 94.7%, respectively (Table 1). The majority of samples used in Part 1 of the study were commercially procured samples that were subsequently shown to have lower-quality extracted DNA. Forty-three samples that failed quality control after the sequencing step were retested with increased extracted DNA input. The success rates for the TSO 500 HRD assay, including these retested samples, were 90.3%, 92.5%, and 93.4%, respectively (Table 1). The prevalence of overall HRD positivity was 51.0% with the TSO 500 HRD assay and 49.2% with the Myriad assay; the prevalence of BRCAm was 27.6% and 25.5%, respectively. For overall HRD status, BRCAm status, and HRD GIS, agreement rates ranged from 91.3% to 92.9% for PPA, 96.7% to 98.6% for NPA, and 94.3% to 96.9% for OPA (Table 2). For HRD GIS, the Pearson correlation between the two tests was 0.980 for all samples and 0.975 for the non-BRCAm cohort (Fig. 2A); the AUROC was 0.992 and 0.988, respectively. When evaluating HRD status classification as determined by HRD GIS, most discordant cases in Part 1 were near the cutoff of 42 (Supplemental Table 1). In 9 of 11 HRD GIS discordant cases (81.8%), the HRD GIS was positive using the Myriad assay and negative using the Illumina assay (Supplemental Table 1). BRCAm status agreement analysis found that of the 197 samples with data available from both the Illumina and Myriad assays, 139 were identified as non-BRCAm and 52 as BRCAm by both tests (Table 3). Disagreement was observed for four samples classified as non-BRCAm with the TSO 500 HRD assay vs BRCAm with the Myriad assay, and two samples were classified as BRCAm with the TSO 500 HRD assay vs non-BRCAm with the Myriad assay. Examination of NGS raw sequencing files from both tests in these six discordant samples revealed in five cases that the identical variant was detected by both tests, but the variant was differentially classified by the Illumina and Myriad evaluation algorithms (Supplemental Table 2). In the other discordant sample, no BRCAm variants were

detected using the Myriad assay, whereas a frame shift *BRCA2* mutation was detected with the TSO 500 HRD assay with only two supporting reads in the NGS raw data.

### 3.2. Part 2: Optimized analytical performance

After the initial assessment (Part 1), further optimization of the HRD GIS concordance was pursued by reducing allele dosage noise/bias in the copy number estimates. Specifically, 49 normal samples were run on the HRD probe set and an estimate of probe-specific biases in the b-allele copy number estimate was obtained. After post-processing optimization, success rates for the TSO 500 HRD assay vs the Myriad assay were 89.0% vs. 94.1% for overall HRD status, 88.1% vs 97.6% for BRCAm status, and 93.4% vs 93.7% for HRD GIS (Table 1). The prevalence of overall HRD positivity was 51.2% with the TSO 500 HRD assay and 49.2% with the Myriad assay; the prevalence of BRCAm remained 27.6% and 25.5%, respectively. For overall HRD status, BRCAm status, and HRD GIS, agreement rates ranged

**Table 1**  
Reportable data from the Illumina and the Myriad assays.

	Overall HRD status		BRCAm status		HRD GIS	
	n/N	%	n/N	%	n/N	%
Initial results						
Illumina (40 ng)	197/227	86.8	200/227	88.1	207/227	91.2
Illumina including re-run of failed samples	205/227	90.3	210/227	92.5	212/227	93.4
Myriad (200 ng)	239/254	94.1	248/254	97.6	238/254	93.7
Myriad sample set restricted to overlap with Illumina	216/227	95.2	222/227	97.8	215/227	94.7
Results after post-processing optimization						
Illumina (40 ng)	202/227	89.0	200/227	88.1	212/227	93.4
Myriad (200 ng)	239/254	94.1	248/254	97.6	238/254	93.7

Reportable data from the Illumina and the Myriad assays. BRCAm, deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation or both; GIS, genomic instability score; HRD, homologous recombination deficiency.

**Table 2**  
Agreement rates for the TSO 500 HRD assay vs the Myriad assay.

	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)
Initial results			
Overall HRD status (N = 194)	92.3 (85.6–96.1)	96.7 (90.7–98.9)	94.3 (90.1–96.8)
BRCAM status (N = 197)	92.9 (83.0–97.2)	98.6 (95.0–99.6)	96.9 (93.5–98.6)
HRD GIS (N = 204)	91.3 (84.2–95.3)	98.0 (93.1–99.5)	94.6 (90.6–97.0)
Updated results			
Overall HRD status (N = 198)	95.2 (89.2–97.9)	96.8 (91.0–98.9)	96.0 (92.2–97.9)
BRCAM status (N = 197)	92.9 (83.0–97.2)	98.6 (95.0–99.6)	96.9 (93.5–98.6)
HRD GIS (N = 207)	95.1 (89.1–97.9)	97.1 (91.9–99.0)	96.1 (92.6–98.0)

Agreement rates for the TSO 500 HRD assay vs the Myriad assay. BRCAM, deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation or both; GIS, genomic instability score; HRD, homologous recombination deficiency; NPA, negative percentage agreement; OPA, overall percentage agreement; PPA, positive percentage agreement.

from 92.9% to 95.2% for PPA, from 96.8% to 98.6% for NPA, and from 96.0% to 96.9% for OPA (Table 2). For HRD GIS, the Pearson correlation between the two tests was 0.980 for all samples and 0.976 for the non-BRCAM cohort; the correlation was the same between the initial and post-processing optimization results (Fig. 2B). The AUROC was 0.995 for all samples and 0.992 for the non-BRCAM cohort. When evaluating HRD status classification as determined by HRD GIS, most discordant cases in Part 2 were near the cutoff of 42 (Table 4).

#### 4. Discussion

##### 4.1. Correlation of the TSO 500 HRD assay to the Myriad assay

The overall HRD status, BRCAM status, and HRD GIS detection results showed >93% agreement between the Illumina and Myriad assays. Prevalence estimates of overall HRD-positive status and BRCAM were comparable between the two tests and consistent with previously published reports (HRD-positive status, ~50%; BRCAM, ~6%–23%) [29]. HRD GIS was strongly correlated between the two tests (0.98).

**Table 3**  
BRCAM, HRD GIS, and overall HRD status agreement between Illumina and Myriad assays.

BRCAM status		
	TSO 500 HRD assay positive	TSO 500 HRD assay negative
Myriad assay positive	52	4
Myriad assay negative	2	139
HRD GIS		
	TSO 500 HRD assay ≥42	TSO 500 HRD assay <42
Myriad assay ≥42	94	9
Myriad assay <42	2	99
Overall HRD status		
	TSO 500 HRD assay positive	TSO 500 HRD assay negative
Myriad assay positive	96	8
Myriad assay negative	3	87

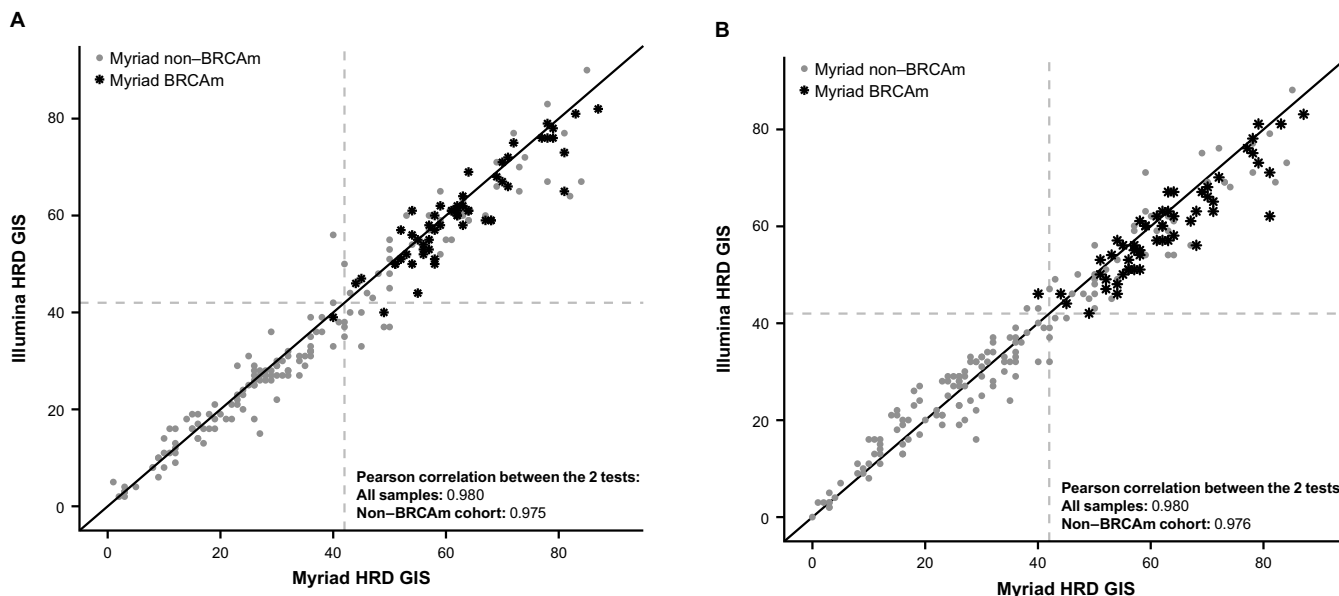
BRCAM, HRD GIS, and overall HRD status agreement between Illumina and Myriad assays. BRCAM, deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation or both; GIS, genomic instability score; HRD, homologous recombination deficiency.

##### 4.2. Discordance investigation

The current analysis demonstrated that post-processing optimization of the raw data to minimize the allele dosage noise/bias can further improve HRD GIS concordance and overall assay analytical performance. These improvements have been incorporated into the distributed Illumina TSO 500 HRD kit. Additionally, five of six observed discordant cases of BRCAM detection were a result of different variant classification at Illumina and Myriad, suggesting it may be possible to further improve overall HRD status concordance by harmonizing BRCAM variant status determination.

##### 4.3. HRD harmonization

The use of this TSO 500 HRD assay will contribute to the broader effort of HRD harmonization and may also benefit clinical laboratories inside and outside of the EU that need access to a distributable HRD kit for their patients. In addition, comparing each available test to the Myriad assay is difficult for individual laboratories because of the cost and difficulty of shipping to the US for Myriad testing. The availability of the TSO 500 HRD assay will enable broad access to a test with high concordance



**Fig. 2.** Correlation of TSO 500 HRD GIS vs Myriad HRD GIS in the overall and BRCAM cohorts during (A) Part 1 and (B) Part 2. BRCAM, a tumor with a deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation; GIS, genomic instability score; HRD, homologous recombination repair.

**Table 4**  
Discordant HRD GIS status (Part 2).

Myriad HRD GIS	Illumina HRD GIS	Myriad BRCAm status	Illumina BRCAm status
42	37	No	No
40	46	Yes	Yes
45	41	No	No
40	43	No	No
42	39	No	No
43	41	No	No
38	43	No	No
42	32	No	No
42	37	No	No
40	46	Yes	Yes
45	41	No	No

Discordant HRD GIS status (Part 2). BRCAm, deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation or both; GIS, genomic instability score; HRD, homologous recombination deficiency.

HRD GIS was considered to be positive using a cutoff of 42.

to the Myriad assay, which may improve the ability to compare results among tests more easily.

#### 4.4. Potential future work

Clinical validation of the Myriad assay has demonstrated substantial clinical benefit for patients whose tumors are HRD positive and who are treated with a PARP inhibitor [30]. Noting that the availability of the Myriad assay varies globally, the commercial availability of the Illumina TSO 500 HRD kit may provide research and development opportunities for HRD applications in underserved markets, especially since there is a need for additional health authority-approved in vitro diagnostics in the European market. One limitation of the study is the use of extracted DNA as input which removes one important variable from the clinical process while potentially boosting concordance.

In conclusion, the totality of data presented here suggest that the TSO 500 HRD assay, a distributable RUO kit solution for HRD testing, has analytical performance characteristics that approach the performance of the FDA-approved Myriad MyChoice CDx PLUS. These findings are an important step to conduct research to identify patient populations who may benefit from PARP inhibitor treatment.

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#### CRedit authorship contribution statement

**Amy K. Wehn:** Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – original draft, Writing – review & editing. **Ping Qiu:** Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. **Jared Lunceford:** Formal analysis, Investigation, Validation, Writing – review & editing. **Alexander Yarunin:** Conceptualization, Writing – review & editing. **Razvan Cristescu:** Conceptualization, Data curation, Formal analysis, Investigation, Validation, Writing – review & editing. **Li Liu:** Conceptualization, Data curation, Investigation, Validation, Writing – review & editing. **Kyria Roessler:** Conceptualization, Data curation, Writing – review & editing. **Sven Bilke:** Formal analysis, Investigation, Validation, Writing – review & editing. **John R. Day:** Data curation, Investigation, Validation, Writing – review & editing. **Kirsten M. Timms:** Data curation, Writing – review & editing. **Wilko Weichert:** Investigation, Validation, Writing – original draft, Writing – review & editing.

**Matthew J. Marton:** Conceptualization, Investigation, Validation, Writing – original draft, Writing – review & editing.

#### Data Availability

Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA (MSD), is committed to providing qualified scientific researchers access to anonymized data and clinical study reports from the company's clinical trials for the purpose of conducting legitimate scientific research. MSD is also obligated to protect the rights and privacy of trial participants and, as such, has a procedure in place for evaluating and fulfilling requests for sharing company clinical trial data with qualified external scientific researchers. The MSD data sharing website (available at: [http://engagezone.msd.com/ds\\_documentation.php](http://engagezone.msd.com/ds_documentation.php)) outlines the process and requirements for submitting a data request. Applications will be promptly assessed for completeness and policy compliance. Feasible requests will be reviewed by a committee of MSD subject matter experts to assess the scientific validity of the request and the qualifications of the requestors. In line with data privacy legislation, submitters of approved requests must enter into a standard data-sharing agreement with MSD before data access is granted. Data will be made available for request after product approval in the US and EU or after product development is discontinued. There are circumstances that may prevent MSD from sharing requested data, including country or region-specific regulations. If the request is declined, it will be communicated to the investigator. Access to genetic or exploratory biomarker data requires a detailed, hypothesis-driven statistical analysis plan that is collaboratively developed by the requestor and MSD subject matter experts; after approval of the statistical analysis plan and execution of a data-sharing agreement, MSD will either perform the proposed analyses and share the results with the requestor or will construct biomarker covariates and add them to a file with clinical data that is uploaded to an analysis portal so that the requestor can perform the proposed analyses.

#### Declaration of competing interest

A.K.W., P.Q., and M.J.M. are employees of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA, and stockholders of Merck & Co., Inc., Rahway, NJ, USA. J.L. is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA, and stockholder of Merck & Co., Inc., Rahway, NJ, USA, and reports two pending patents (ANGIOGENESIS AND mMDSC GENE EXPRESSION BASED BIOMARKER OF TUMOR RESPONSE TO PD-1 ANTAGONISTS; Patent WO/2020/167619). A.Y. is an employee and stockholder of AstraZeneca. R.C. is an employee of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA, and stockholder of Merck & Co., Inc., Rahway, NJ, USA, and reports two pending patents (ANGIOGENESIS AND mMDSC GENE EXPRESSION BASED BIOMARKER OF TUMOR RESPONSE TO PD-1 ANTAGONISTS; Patent WO 2020/167619). L.L., K.R., S.B., and J.R.D. are employees of and hold stock or stock options with Illumina. K.M.T. reports patents planned, issued, or pending at Myriad Genetics, Inc. (self and institution); stock or stock options in Myriad Genetics, Inc. (self) and Illumina (spouse); and employment at Myriad Genetics, Inc. (self) and Illumina (spouse). W.W. has attended and given talks at advisory boards; advised and served as speaker on national and international conferences for Roche, MSD, BMS, AstraZeneca, Pfizer, Merck, Lilly, Boehringer, Novartis, Takeda, Bayer, Amgen, Astellas, Eisai, Johnson & Johnson, Janssen, Illumina, Siemens, Agilent, ADC, GSK, and Molecular Health; and receives research funding from Roche, MSD, BMS, and AstraZeneca.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ygyno.2024.01.016>.

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