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Homologous Recombination Deficiency as an Ovarian Cancer Biomarker in a Real-World Cohort

Validation of Decentralized Genomic Profiling

Carsten Denkert,* Marcel Romey,* Brad Swedlund,† Akira Hattesohl,* Julia Teply-Szymanski,* Stefan Kommoss,‡ Kristin Kaiser,† Annette Staebler,§ Andreas du Bois,¶ Albert Grass,* Christiane Knappmeyer,† Florian Heitz,¶‖ Cara Solimeno,† Thomas Ebel,** Philipp Harter,¶ Frederik Marmé,†† Paul Jank,* Timo Gaiser,‡‡ Chris Neff,† Uwe Wagner,§§ Kirsten M. Timms,† and Fiona Rodepeter*

From the Institute of Pathology and the Department of Gynecology,§§ Philipps-Universität Marburg and University Hospital Marburg, Marburg, Germany; Myriad Genetics,† Salt Lake City, Utah; the Department of Women's Health‡ and the Institute of Pathology,§ Tübingen University Hospital, Tübingen, Germany; the Department of Gynecology and Gynecologic Oncology,¶ Evangelische Kliniken Essen-Mitte, Essen, Germany; the Department of Gynecology with the Centre of Oncologic Surgery Charite Campus,‖ Virchow Klinikum, Charité Universitätsmedizin Berlin, Berlin, Germany; Zentrum für Pathologie Essen-Mitte,** Essen, Germany; the Department of Obstetrics and Gynaecology,†† University Hospital Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; and the Institute of Pathology,‡‡ University Medical Centre Mannheim, University of Heidelberg, Mannheim, Germany*

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Address correspondence to Carsten Denkert, M.D., Institute of Pathology, Philipps-Universität Marburg and University Hospital Marburg, Baldingerstr. 1, 35043 Marburg, Germany. E-mail: carsten.denkert@uni-marburg.de.

The diagnostic evaluation of homologous recombination deficiency (HRD) is central to define targeted therapy strategies for patients with ovarian carcinoma. We evaluated HRD in 514 ovarian carcinoma samples by next-generation sequencing of DNA libraries, including *BRCA1/BRCA2* and 26,523 single-nucleotide polymorphisms using the standardized Myriad HRD assay, with the predefined cut point of ≥42 for a positive genomic instability score (GIS). All samples were measured in the central Myriad laboratory and in an academic molecular pathology laboratory. A positive GIS was detected in 196 (38.1%) of tumors, whereas 318 (61.9%) were GIS negative. Combining GIS and *BRCA* mutations, a total of 200 (38.9%) of the 514 tumors were HRD positive. A positive GIS was significantly associated with high-grade serous histology ($P < 0.000001$), grade 3 tumors ($P = 0.001$), and patient age <60 years ($P = 0.0003$). The concordance between both laboratories for the GIS status was 96.9% ($P < 0.000001$), with a sensitivity of 94.6% and a specificity of 98.4%. Concordance for HRD status was 97.1% (499 of 514 tumors). The percentage of HRD-positive tumors in our real-life cohort was similar to the proportion observed in the recently published PAOLA-1 trial, with high concordance between central and local laboratories. Our results support introduction of the standardized HRD assay in academic molecular pathology laboratories, thus broadening access to personalized oncology strategies for patients with ovarian cancer worldwide. *(J Mol Diagn 2022, 24: 1254—1263; https://doi.org/10.1016/j.jmoldx.2022.09.004)*

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The concept of synthetic lethality[1] has recently entered clinical practice for patients with ovarian cancer, and poly (ADP-ribose) polymerase inhibitors have been integrated into therapy concepts in several clinical trials.[2–4] In the PAOLA-1 trial, the combination of bevacizumab and olaparib was established and registered as maintenance therapy in platinum-responsive high-grade ovarian cancer with *BRCA1/2* mutations and/or a homologous recombination deficiency (HRD).[5] The diagnostic evaluation of the genomic instability score (GIS) by molecular assays, defined by a combination of large-scale state transitions (LSTs),[6] telomeric allelic imbalance (TAI),[7] and loss of heterozygosity (LOH),[8] has been approved as a companion diagnostic approach in the United States and Europe. Currently, the Myriad myChoice assay is the only clinically validated and US Food and Drug Administration–approved molecular HRD test in ovarian cancer. In Europe, the European Medicines Agency has decided against approval of a specific test, but stated that HRD should be determined by an experienced laboratory using a validated test, including LSTs, TAI, and LOH.

From the perspective of academic research centers and molecular pathologists, it is important that the complete range of molecular assays is available in a decentralized academic setting. Clinical decisions require the integration of clinical, histopathologic, and molecular information, and many molecular pathologists and clinical oncologists are reluctant to build clinical decisions on molecular assays without the ability to control the assay parameters in their own laboratory environment. Last but not the least, in many European countries including Germany, the structure of the health system does not allow integration of diagnostic procedures performed in central laboratories in other countries.

The recent approval of olaparib in combination with bevacizumab for patients newly diagnosed with HRD-positive high-grade ovarian cancer provides a major challenge for personalized medicine. On the one hand, there is an urgent need for access to validated HRD testing for all patients with ovarian cancer; and on the other hand, there is limited access to only one clinically validated assay, which is not broadly available.

As a way to resolve this problem, we evaluated the transfer of the Myriad myChoice assay to an academic molecular pathology laboratory. We present the results of the decentralized testing in comparison with the central Myriad laboratory for a large cohort of 514 samples of patients with advanced ovarian carcinomas. Furthermore, we provide data on the distribution of genomic instability in a real-world cohort of patients with ovarian carcinoma.

Materials and Methods

This study compared the assay performance of Myriad myChoice in the molecular pathology laboratory of the Philipps-Universität Marburg (UMR) with the central diagnostic laboratory of Myriad Genetics in Salt Lake City (SLC), UT. End points were concordance of HRD status and differences of GISs between the two laboratories.

Clinical Cohort

Inclusion criteria were an available formalin-fixed, paraffin-embedded (FFPE) ovarian tumor block, written informed patient consent, and available molecular results from both laboratories (UMR and SLC) for *BRCA1/2* and GIS. On the basis of these criteria, a cohort of 514 consecutive samples that were referred to the molecular pathology laboratory at UMR between November 2020 and July 2021 were included (Supplemental Figure S1). Clinical parameters were derived from the pathology report and represent the local diagnostic assessment. This study was approved by the ethics committee of the faculty of medicine, Philipps-Universität Marburg (RS-21/57).

DNA Extraction from FFPE Samples

Sections from tumor samples were split up between the UMR and SLC laboratories for DNA extraction. One hematoxylin and eosin slide for each laboratory was cut, then reviewed and marked by pathologists in Marburg and Salt Lake City, respectively, for manual dissection for enrichment of tumor-derived DNA. Generally, a minimum tumor cell content of 20%, a tumor area in the range of 1 cm^2, and at least 200 ng of extracted genomic DNA as the starting input for library preparation are recommended for optimal sample processing success rates. Additional tissue slides for microdissection were cut, and split up between both laboratories, alternatingly. All SLC slides were sent to the SLC laboratory via priority mail directly after cutting. In some cases of small tumor area, the original FFPE block was sent alongside the slides to allow preparation of additional slides.

Regions of highest tumor cell density were scraped from the slide, and DNA was extracted using the Promega Maxwell RSC FFPE Plus DNA Kit (AS1720; Promega, Madison, WI). Samples were incubated in a proteinase K lysis buffer in a shaking heat block overnight, afterwards mixed with another lysis buffer (number A826F; Promega), and loaded onto the Maxwell cartridge. The resulting genomic DNA was eluted in 50 μL.

HRD Assay Library Preparation

Myriad myChoice HRD assays were performed in batches of 15 patient samples and one cell line control sample (SeraCare, Milford, MA). Genomic sample and control DNA were quantified using a Quantus Fluorometer (Promega), normalized to 200 ng genomic DNA starting input, and sheared in an LE220-plus focused ultrasonicator (Covaris, Woburn, MA). Unfragmented DNA was spun out, whereas fragmented DNA was transferred into a fresh 96-well work plate. An epMotion pipetting robot (Eppendorf, Hamburg,

Germany) performed end repair and A-tailing protocols followed by a thermocycling step. A second epMotion program step provided adapter ligation with predefined adapters in the first two columns of 96-well adapter plates that were supplied and quality checked by Integrated DNA Technologies (Coralville, IA; catalog number 231583515).

Samples were enriched through a PCR before the post-PCR epMotion wash run was performed. Each sample was quantified and normalized for a second time using the Quantus Fluorometer. A total of 100 ng of genomic DNA from each sample was pooled together before the pooled genomic DNA was lyophilized using a VacuFuge (Eppendorf, Hamburg, Germany) at 60°C. The lyophilized DNA pool was hybridized to a custom capture panel (Probe Pool; Integrated DNA Technologies; catalog number CAPP) with biotinylated probes for >26,000 single-nucleotide polymorphisms (SNPs), distributed across the human genome. This custom hybridization panel was developed in combination by Myriad Genetics and Integrated DNA Technologies, and allows for whole genome detection of gene mutations and SNPs, with a complete sequence coverage of both *BRCA1/2*.[9,10] Large rearrangement detection for all 15 homologous recombination repair (HRR) genes is achieved by comparing the number of reads for each individual base with a normalized median read count value across all genes and SNP locations, previously set during the assay's validation process. Resulting read counts were compared with the allele-specific copy number, as well as copy number of each allele being determined, at each individual SNP location.[10]

Sequencing and Data Analysis

The DNA library pool was sequenced on an Illumina NextSeq550 Dx (Illumina, San Diego, CA), according to the manufacturer's protocol. An average run generated approximately 500 million reads, distributed across the 16 samples, with sequence data of >30 million reads generated for the average sample. To allow for post-sequence processing, each sequencing run needed to display a minimum quality score q30 value of 80%. Post-sequencing data were uploaded and analyzed by Myriad's sequencing analysis pipeline algorithm and software. Through the algorithm, all sequenced reads were aligned and mapped to a variety of targeted sequences of a specific panel of genes, by a Burrows-Wheeler transform algorithm,[11] performing a search of all exons in the Myriad's database to generate the alignment, determining the matching exon for each individual read.[10] To call variants, each read was then aligned with the expected wild-type sequence of the best-match exon from the Burrows-Wheeler transform search, using a pairwise alignment performed by JAligner (current release software version: 1.0), an open-source software that uses a Needleman-Wunsch-Gotoh algorithm (*http://jaligner.sourceforge.net/api/jaligner/NeedlemanWunschGotoh.html*, last accessed September 16, 2022).[12] Furthermore, sequence variants and all large rearrangement variants are reviewed and called by two separate reviewers, using the Myriad-developed Data Review App (version 02.06.2021).

All clinically significant variants found were denoted as either deleterious or suspected deleterious, a notation that predates the American College of Medical Genetics and Genomics—recommended terminology of pathogenic and likely pathogenic, while the terms are analogous. For this publication, the American College of Medical Genetics and Genomics—recommended terminology was used. This variant analysis was performed for *BRCA1/2*, and 13 additional HRR genes: *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, and *RAD54L*.

Each patient's GIS was calculated using the fully automated software algorithm, based on the sum of LOH, TAI, and LST scores. These three individual parts of the GIS have been defined independently, each by separate studies that were all published in 2012.[6–8] LOH events have been described as permanent losses of one parental allele, where each event accounts for one long LOH region of ≥15 megabases, but less than a whole chromosome. The sum of these LOH events equals the LOH score, which was found to be highly positively correlated with HRD: samples that received high scores were deficient of homologous recombination and/or had a deficiency in *BRCA1* or *BRCA2* functionality.[8] The second part, TAI, adds events of unequal contribution of maternal and paternal DNA sequences, with or without changes in overall DNA copy number. This imbalance is caused by HRD, because a deficiency in double-stranded DNA repair mechanisms will lead to genomic scarring through error-prone repair mechanisms, such as nonhomologous end joining. Furthermore, tumors displaying a high number of TAI breakpoints were found to be more sensitive to treatment with cisplatin, a platinum salt chemotherapeutic that causes double-stranded DNA breaks, and enriched with copy number variants, which also suggests a defective double-stranded DNA repair mechanism. The TAI score was thus defined as the sum of events where one allele, mostly at the end of the chromosome and extending to the telomere, had been lost.[7] The third part of the GIS, LST, was defined as the sum of chromosomal breaks of adjacent regions of at least 10 megabases, after filtering of all small-scale copy number variants of ≤3 megabases, scored for each chromosome arm independently. An elevated number of these events has been found to be strongly associated with BRCA1 inactivation, and most LSTs (70%) have been linked to inter-chromosomal translocations. Because nonhomologous end joining frequently causes these translocations, HRD can be inferred for samples displaying a high number of LSTs in their genome.[6] The sum of these three scores—LOH, TAI, and LST—forms the GIS. The predefined cut points for the GIS as well as the definition of HRD-positive and HRD-negative tumors, based on a combination of GIS and *BRCA1/2* status,

are shown in Supplemental Table S1. This cut point had previously been identified by a bioinformatical algorithm, combining individual LOH, TAI, and LST scores into a combined GIS, so that at least 95% of BRCA1/2-deficient tumors are detected.[13] Furthermore, because of the combination of the three scores, the algorithm detects additional tumors without BRCA mutations, where HRD is potentially caused by different HRR genes, or other molecular alterations. When applying for US Food and Drug Administration approval of the Myriad myChoice cdx assay as a companion diagnostic in 2020, the predefined threshold for positive GIS was set at ≥42, as validated in the PRIMA (NCT02655016) and the PAOLA-1 (NCT02477644) clinical trials.[4,5]

To determine each patient's HRD status, both parts of the analysis were combined (Supplemental Table S1). If a patient's test results showed a GIS of ≥42 and/or a clinically significant *BRCA1/2* mutation, the HRD result was reported as positive. For the comparison of both laboratories, the results for SLC and UMR were used. For the distribution of GIS and *BRCA1/2* mutations, as well as the association with clinicopathologic parameters, only data from the UMR laboratory are shown.

Statistical Analysis

The analysis was based on a predefined statistical analysis plan and performed using SPSS version 27 (SPSS, IBM Corp., Armonk, NY) as well as R version 4.1.1 (*https://www.R-project.org*, last accessed September 16, 2022). The total allowable error indicates the expected deviation of 80% of the measured from the expected values. It was calculated as total allowable error $= |\mu_{\Delta GIS}| + 1.282 \times s_{\Delta GIS}$, with $|\mu_{\Delta GIS}|$ being the absolute value of $\mu_{\Delta GIS}$, with $\mu_{\Delta GIS}$ being the arithmetic mean of the ΔGIS_i values, where $\Delta GIS_i = GIS_{UMRi} - GIS_{SLCi}$ with GIS_{UMRi} and GIS_{SLCi} being the ith GIS value of Marburg and Myriad, respectively, for $i = 1...514$, and $s_{\Delta GIS}$ being the sample SD of the ΔGIS_i values. The figures were generated with R packages plotly[14] version 4.9.4.1 and shiny version 1.6.0 (*https://CRAN.R-project.org/package=shiny*, last accessed September 16, 2022).

Statistical tests were by default two sided with a significance level of 0.05.

Results

Clinical Cohort

A total of 514 FFPE samples from patients with ovarian carcinomas were evaluated. The median age of patients was 63 years (range, 22 to 86 years). Most patients (88.9%) had high-grade serous tumors. Information on International Federation of Gynecology and Obstetrics stage was available for 420 patients, and 96.2% of these patients had International Federation of Gynecology and Obstetrics stage III to IV tumors (Table 1).

Sequencing Coverage

Sequenced samples in both laboratories showed similar coverage values, both across the HRR genes, where average coverage in the SLC laboratory was higher (956.3) than in the Marburg laboratory (852.7), as well as the SNP regions. Herein, a slightly higher average coverage could be found in the samples that were processed in the UMR laboratory (428.3) than those that were processed in SLC (376.7). Although also comparable, Marburg samples in general showed a broader range of coverage values (coverage range of HRR genes, 141.3 to 2366.9; coverage range of SNPs, 70.4 to 1591.8) compared with the SLC samples (coverage range of HRR genes, 385.5 to 1953.3; coverage range of SNPs, 102.6 to 778.6).

Distribution of the GISs and HRD Parameters

One-hundred and ninety-six (38.1%) of the 514 patients had tumors with a GIS of ≥42 score units and were therefore positive for genomic instability based on the predefined cut point, whereas 318 (61.9%) were GIS negative (Figure 1A). The median GIS was 30 (range, 0 to 89). Clinically significant mutations of *BRCA1* or *BRCA2* were detected by sequencing of the tumor in 64 of the 514 samples (12.5%). A list of all pathogenic and likely pathogenic BRCA1/2 variants detected in Marburg can be found in Supplemental Table S2. Most tumors with *BRCA1/2* mutations (60 of 64 patients, 93.8%) also showed a GIS of ≥42. Only four tumors (0.8%) had a clinically significant *BRCA* mutation with a GIS of <42. In addition, 21 pathogenic or likely pathogenic variants in non-BRCA HRR genes were found in GIS^+, $BRCA1/2^-$ samples (Supplemental Table S3). The HRD status combines those tumors that have a positive GIS and/or a *BRCA* mutation (Supplemental Table S1). Therefore, a total of 200 (38.9%) of the 514 tumors were HRD positive. Interestingly, there was a bimodal distribution of GISs observed with a separation of HRD-positive and HRD-negative tumors, and only a small intermediate group with GIS around the cutoff (Figure 1B). When overlaying two gaussian distribution curves to represent the bimodal distribution, they were found to intersect between GIS values of 41 and 42 (Supplemental Figure S2), further validating the cut point of 42 in a real-life cohort, and supporting the biological concept that the two peaks represent different biological phenotypes.

Typical examples of tumors with high and low GIS are shown in Figure 2, including a low-grade serous carcinoma with a GIS of 5 (HRD negative) as well as two high-grade ovarian carcinomas with GISs of 49 (HRD positive) and 35 (HRD negative). On simple visual examination, it is not possible to distinguish tumors with low or high GIS using the B-allele frequency plot.

Table 1 Clinicopathologic Parameters of Patients with Ovarian Tumors and Association with GIS Determined in the UMR Laboratory

Parameter	Overall *N*	GIS negative (<42 score units), *N* (%)	GIS positive (≥42 score units), *N* (%)	*P* value
All patients ($n = 514$)		318 (61.9)	196 (38.1)	
Age, years ($n = 514$)				0.0003*
<60	190	98 (51.6)	92 (48.4)	
≥60	324	220 (67.9)	104 (32.1)	
Grading ($n = 513$)				0.001*
G1-G2	19	18 (94.7)	1 (5.3)	
G3	494	299 (60.5)	195 (39.5)	
FIGO stage ($n = 420$)				NS
I-II	16	11 (68.8)	5 (31.3)	
III	339	206 (60.8)	133 (39.2)	
IV	65	37 (56.9)	28 (43.1)	
pT stage ($n = 466$)				NS
pT0-1	36	27 (75)	9 (25)	
pT2	47	32 (68.1)	15 (31.9)	
pT3	383	229 (59.8)	154 (40.2)	
N stage ($n = 213$)				NS
pN0	79	47 (59.5)	32 (40.5)	
pN1	134	77 (57.5)	57 (42.5)	
Histologic type ($n = 513$)				0.00002†
Low-grade serous	9	9 (100)	0 (0)	
High-grade serous	457	264 (57.8)	193 (42.2)	
Endometrioid	15	15 (100)	0 (0)	
Mucinous	6	6 (100)	0 (0)	
Clear cell	15	15 (100)	0 (0)	
Carcinosarcoma	8	6 (75)	2 (25)	
Undifferentiated	3	2 (66.7)	1 (33.3)	
HGSC vs non-HGSC ($n = 513$)				<0.000001*
HGSC	457	264 (57.8)	193 (42.2)	
Non-HGSC	56	53 (94.6)	3 (5.4)	
Tumor *BRCA1/2* status ($n = 514$)				<0.000001*
Wild type	450	314 (69.8)	136 (30.2)	
Mutated	64	4 (6.3)	60 (93.8)	

Clinical parameters (including grading) are derived from the original pathology report.

*Two-sided Fisher test.

†χ^2 Test.

FIGO, International Federation of Gynecology and Obstetrics; GIS, genomic instability score; HGSC, high-grade serous carcinoma; NS, not significant; UMR, Philipps-Universität Marburg.

Concordance of GISs and HRD Status in Both Laboratories

Figure 3A gives an overview of all 514 samples that have been analyzed in both laboratories, sorted by increased mean GIS values. Of these 514 tumors, 498 had an identical GIS status, resulting in a GIS-status concordance of 96.9% between the two laboratories. A correlation plot between the Myriad and Marburg laboratories, including a Pearson correlation coefficient and the linear regression, is shown in Supplemental Figure S3, which shows high correlation between the two laboratories, with a notable Pearson correlation coefficient of $r = 0.982$ ($P < 2.22 \times 10^{-16}$) and a strong linear regression fit ($R^2 = 0.963$). One of the 16 discordant tumors had a *BRCA* mutation and was therefore HRD positive. Therefore, the concordance for the final HRD status was 97.1% (499 of 514 tumors were concordant). The mean of the absolute values of the difference in GIS between the two measurements at UMR and SLC across all samples was 3.12 GIS units. The sensitivity for the GIS status was 94.6%, the specificity was 98.4%, and the total allowable error was calculated as 5.92 score units (Table 2).

The absolute differences between the two GIS measurements in the two laboratories are shown in Figure 3B. A total of 497 of the 514 tumors (96.7%) showed a difference of <10 GIS units; 467 (90.9%) of the 514 tumors had a difference of <7 GIS units. The 16 tumors with a discordance in GIS status are marked with a red dot in Figure 3B, indicating that only 5 (31.25%) of these

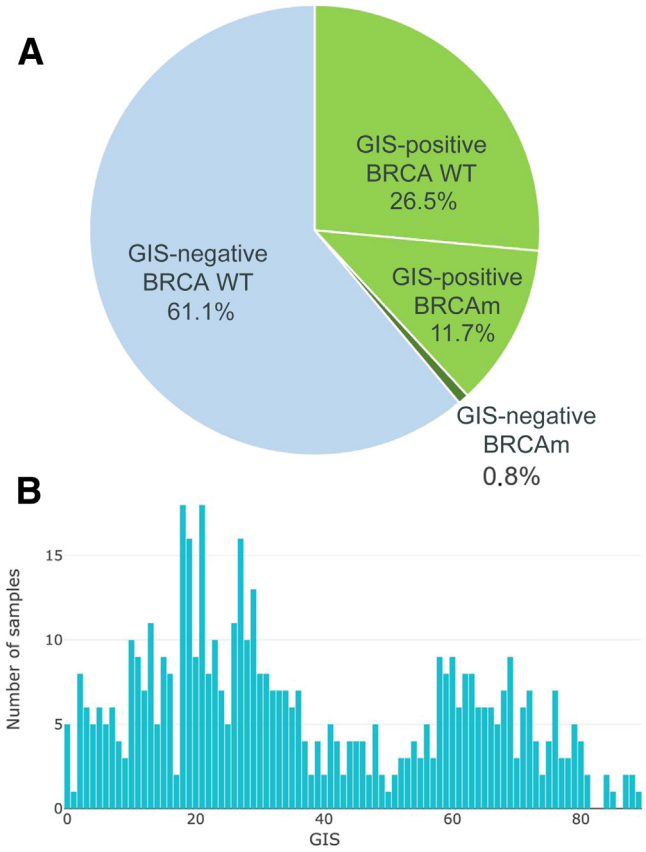

Figure 1 **A:** Distribution of genomic instability score (GIS) status and *BRCA1/2* mutations in 514 tumor samples from patients with ovarian cancer. **B:** Bimodal distribution of GIS in 514 ovarian carcinomas. Data from the Philipps-Universität Marburg laboratory are shown. BRCAm, BRCA-mutated; WT, wild type.

16 tumors had a GIS-status change and a GIS difference of >10 score units. Therefore, 11 of the 16 discordant tumors (68.75%) had GIS values close to the cut point of 42.

GIS and HRD in Different Tumor Types

To evaluate a possible enrichment of HRD in different tumor subtypes, the HRD evaluation in different subgroups of tumor defined by clinicopathologic parameters was compared. Table 1 shows the differences in GIS status in different groups of tumors defined by staging, grading, and histologic parameters.

A positive GIS status was significantly associated with high-grade serous carcinoma (HGSC) histology, 42.3% of HGSCs, but only 5.4% of nonserous carcinomas were GIS positive ($P < 0.000001$). Similarly, a positive GIS status was significantly associated with grade 3 tumors ($P = 0.001$) and patient age <60 years ($P = 0.0003$). In the subgroup of tumors with available staging information, there were no significant differences in different tumor stage groups defined by pT, pN, and International Federation of Gynecology and Obstetrics stage.

The distribution of continuous GISs is different in tumors with different patient age, grading, and histologic type (Figure 4). The distribution of GIS in tumors with different histologic types is shown in Supplemental Figure S4. Low GIS levels were observed in low-grade serous carcinoma and mucinous carcinomas. In contrast, in carcinosarcomas, clear cell carcinomas, and endometrioid carcinoma, the GIS was closer to the cut point of 42.

Discussion

The combination of olaparib and bevacizumab as a maintenance therapy in high-grade ovarian cancer opens new therapeutic options for patients with ovarian cancer. This combination has been shown to improve progression-free survival in the PAOLA-1 trial from 17.7 to 37.2 months in the subcohort of patients with a positive HRD status.[5]

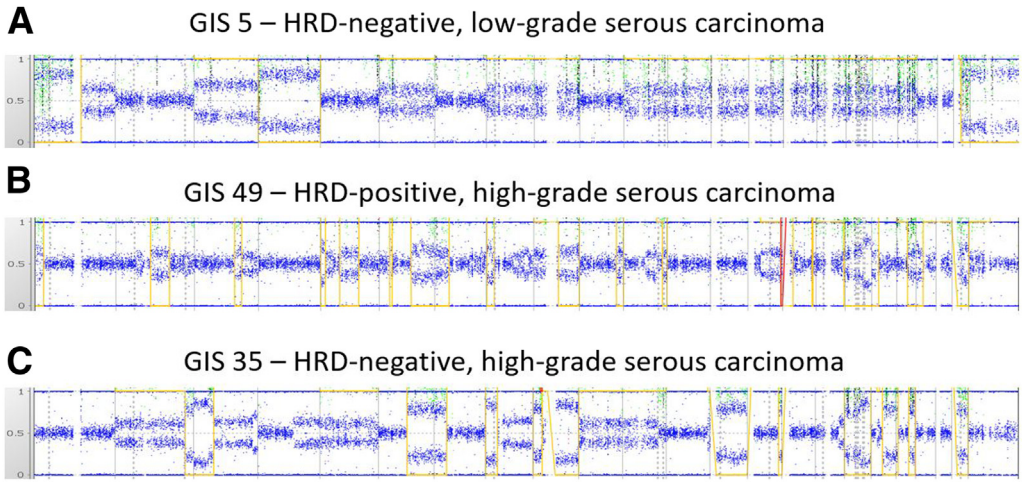

Figure 2 B-allele frequency plots of tumors with different genomic instability scores (GISs) measured at the Philipps-Universität Marburg laboratory. **A:** GIS 5, homologous recombination deficiency (HRD)—negative, low-grade serous carcinoma. **B:** GIS 49, HRD-positive, high-grade serous carcinoma. **C:** GIS 35, HRD-negative, high-grade serous carcinoma (**red lines**: estimated total copy number; **yellow lines**: estimated minor allele copy number).

Table 2 Comparison of Genomic Instability Status between the Two Laboratories (UMR and SLC)

Parameter	Overall *N*	GIS negative (UMR), *N* (%)	GIS positive (UMR), *N* (%)	*P* value
All patients ($N = 514$)	514	318 (61.9)	196 (38.1)	<0.000001*
GIS negative (SLC)	312	307 (98.4)	5 (1.6)	
GIS positive (SLC)	202	11 (5.4)	191 (94.6)	
Sensitivity, %			94.6	
Specificity, %			98.4	
Positive predictive value, %			97.5	
Negative predictive value, %			96.5	
Total allowable error			5.92	
Overall concordance (GIS status), %			96.9	
Overall concordance (HRD status), %			97.1†	

*Two-sided Fisher test.
†One tumor had a *BRCA* mutation, and therefore had a positive HRD status.
GIS, genomic instability score; HRD, homologous recombination deficiency; SLC, Salt Lake City; UMR, Philipps-Universität Marburg.

These positive results and the consecutive approval of olaparib plus bevacizumab introduced an immediate challenge for diagnostic pathology to provide validated HRD testing to all patients who might be eligible for this therapy. This obstacle could only be approached by building a network of local academic laboratories being able to provide a validated test. This study represents the first step in this program, where the decentralized

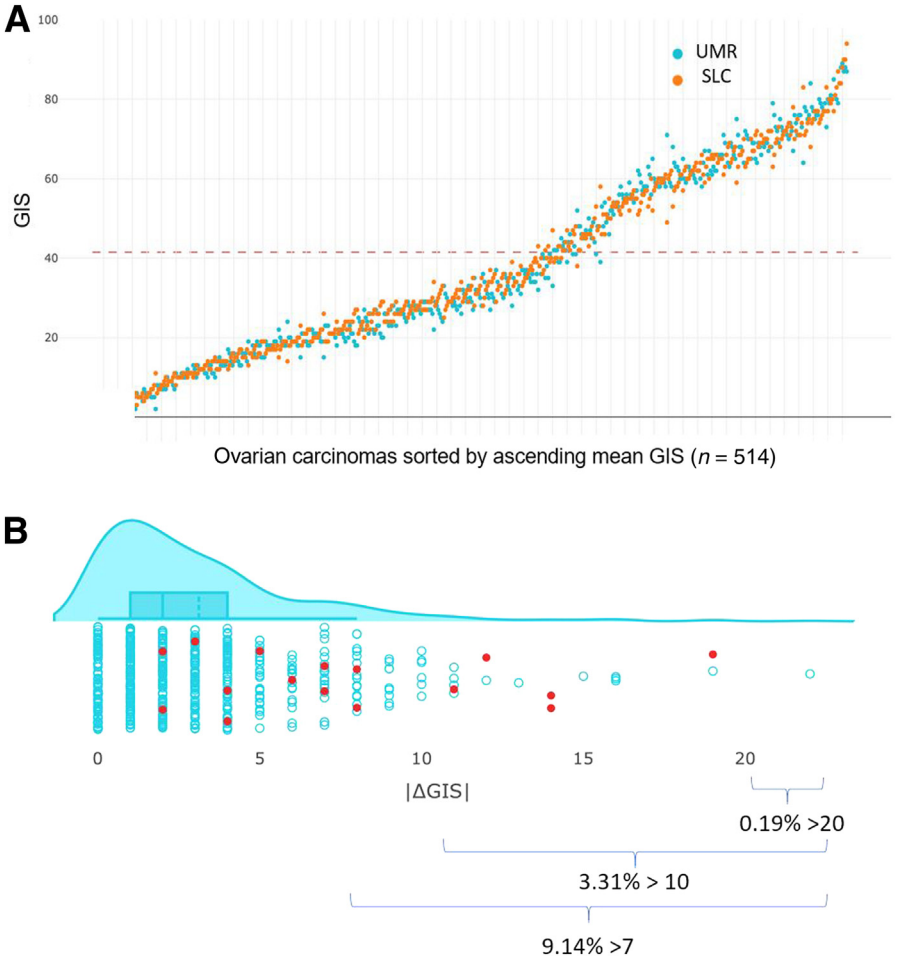

Figure 3 **A:** Comparison of genomic instability score (GIS) from two laboratories [Salt Lake City (SLC) and Philipps-Universität Marburg (UMR)] in 514 ovarian tumors. Samples are sorted by ascending mean GIS of both laboratories; **dashed red line** shows the cut point at GIS ≥42. **B:** Overview of the distribution of the absolute difference between the GIS in both laboratories. Samples with a change in GIS status are highlighted by **red dots**.

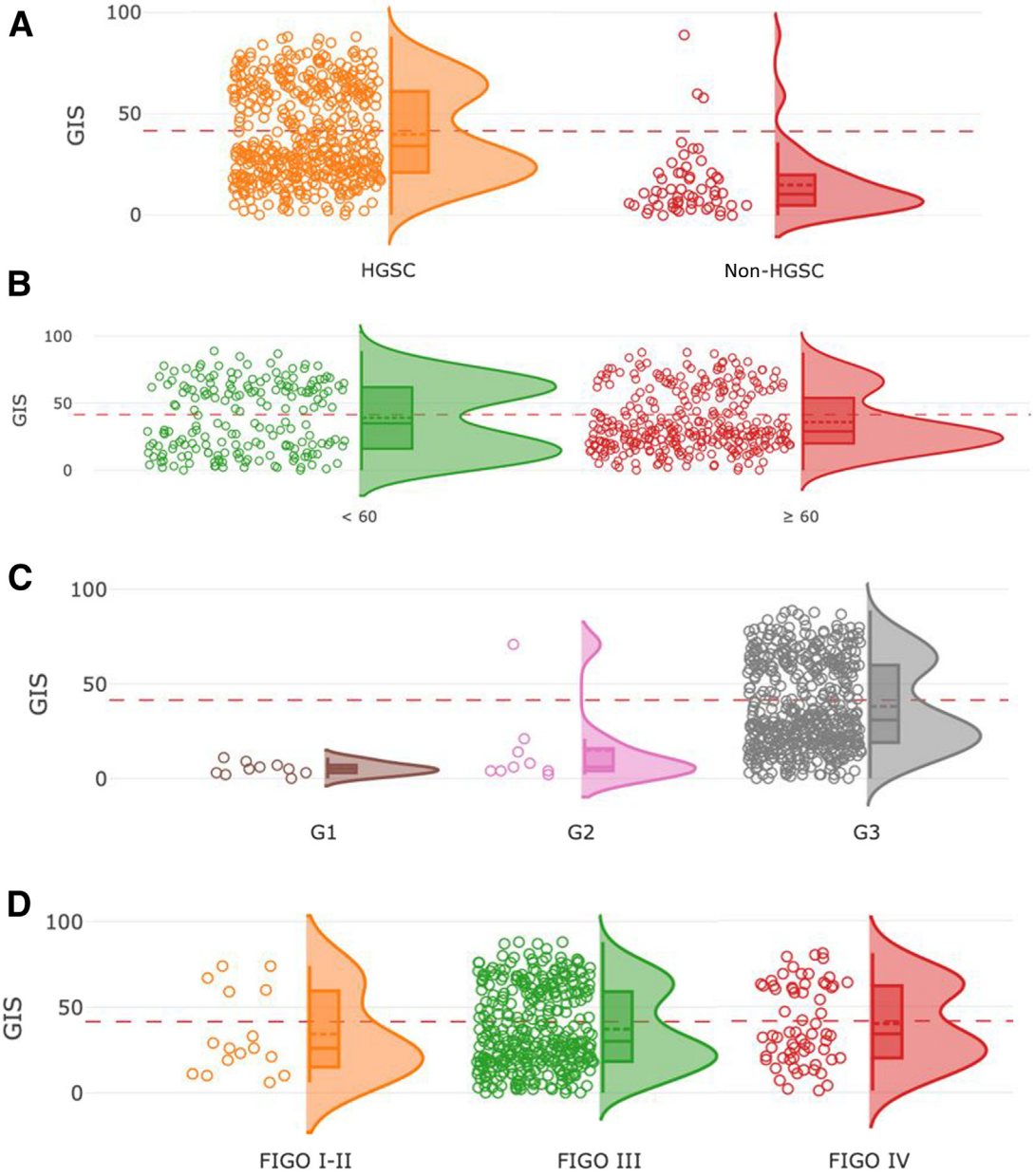

Figure 4 Genomic instability in ovarian tumors: distribution of genomic instability score (GIS) in different subgroups of ovarian tumors. **A:** Histologic type. **B:** Patient age. **C:** Grading. **D:** International Federation of Gynecology and Obstetrics (FIGO) stage. Data from the Philipps-Universität Marburg laboratory are shown; **dashed red lines** show the cut point at GIS ≥42. HGSC, high-grade serous carcinoma.

evaluation of the GIS using the molecular assay developed by Myriad was validated, showing that it is feasible to perform this sequencing assay in an academic molecular pathology laboratory with high concordance rates. The two laboratories showed similar values in average sequencing coverage, both for HRR gene and SNP regions. In comparison of 514 tumors, the concordance for the GIS was 96.9% and the mean difference between the results at UMR and SLC was 3.11 score units. Among the 16 tumors with a change of GIS status between the two laboratories, only 31.2% had a difference between the two measurements of >10 score units, whereas most had differences of ≤10 score units. This suggests that these were tumors with GIS values close to the cut point, and the changes in GIS status are therefore not due to the inaccuracy of the assay.

The successful decentralized validation of the Myriad HRD assay opens up new opportunities for HRD testing, because it allows academic centers to use the validated clinical trial assay with full control of diagnostic procedures and to perform in-house validations and quality checks. This validation shows that the Myriad HRD assay is a method that can be implemented in other academic research laboratories as well, and technical details can be provided by Myriad for laboratories interested in implementing the assay into their workflow. It also strengthens the interaction of

molecular pathology workflows and classic histopathology, which is important for the positioning of molecular pathology as an academic discipline.

With this data set including clinical and molecular data of 514 patients, it was also possible to generate important results on the distribution and prevalence of HRD in ovarian cancer in the real-world setting outside a clinical trial. The latter is especially of interest because the two frontline studies that established the Myriad score of $\geq$42 were performed in markedly selected patient populations, representing only a minority of real-world patients (eg, in PRIMA, only patients with incompletely resected tumors and a strong response to chemotherapy were included).[4] In this context, it is even more important to demonstrate that a positive HRD status is a common feature in ovarian cancer in a real-world cohort with almost 40% positive results. A mutation in *BRCA1/2* was observed in 12.5% of samples in this cohort, which was lower than the rate of *BRCA1/2* mutations in the PAOLA-1 cohort (29%). This difference can be explained by the fact that some centers performed germline or somatic *BRCA* testing as a first step, and only those tumors that were *BRCA* wild type were sent for additional HRD testing. The Myriad HRD test combines the evaluation of the GIS with the analysis of mutations of *BRCA1/2*. To accelerate the diagnostic workup, it is therefore possible to get the complete information needed for clinical decisions in ovarian cancer from a single assay. In addition to *BRCA1/2*, the assay includes also 13 additional HRR genes (*ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C*, *RAD51D*, and *RAD54L*). These genes are currently not relevant for therapeutic decisions in ovarian cancer but could give information that these patients and their families might have a hereditary risk and should be offered germline testing and consultation.

Interestingly, the GIS showed a bimodal distribution, which is similar to the one observed in the first studies establishing the GIS cut point. This suggests that there are two biologically different groups of ovarian carcinomas, and that the HRD assay is able to distinguish between both groups. A total of 93.8% of the tumors with pathogenic *BRCA* mutations were found to also display a positive GIS status, whereas only 6.3% of the tumors with *BRCA* mutations had a GIS below the cut point of 42. These results also show great similarities to the original study describing the cut point,[15] which presented a 95% sensitivity to detect *BRCA1/2*-deficient tumors. Still, additional cut points have been under investigation that are reported to have a highly significant association with patient prognosis.[16] Likewise, other HRD assays using sequencing-based molecular tests with a predefined cutoff have been published and implemented in the academic setting (eg, AmoyDx HRD Focus Panel) and as a companion diagnostic (FoundationOneCDx).[17,18]

In this study, all types of tumors that were sent to the laboratory were included, without restricting the analysis to certain subtypes. Most tumors were HGSCs, but 10.9% of the tumors had a non-HGSC histology. Intriguingly, only 5.4% of the non-HGSCs had a positive GIS status, suggesting that genomic instability is mainly a feature of high-grade serous tumors, as described before.

Furthermore, differences in the level of the GIS were observed in different non-HGSC subgroups, with low GIS levels in low-grade serous carcinoma and mucinous carcinomas. In carcinosarcomas, clear cell carcinomas, and endometrioid carcinoma, the GIS was closer to the cut point of 42. This suggests that further studies investigating specific histologic subtypes are needed, which could also establish subtype-specific clinically relevant cut points.

In summary, the results of this study have important implications for standardized diagnostic concepts in ovarian cancer, because the concordance analysis results show that a standardized central laboratory test can be transferred to an academic molecular pathology laboratory with highly consistent results. Validated HRD testing should be offered to all patients with high-grade ovarian cancer.

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Author Contributions

C.D., M.R., A.H., K.M.T., and F.R. designed the study; C.D., M.R., B.S., A.G., P.J., K.M.T., and F.R. acquired data; C.D., A.H., K.M.T., and F.R. analyzed data; M.R., B.S., J.T.-S., S.K., K.K., A.S., A.d.B., A.G., C.K., F.H., C.S., T.E., P.H., F.M., P.J., T.G., C.N., U.W., K.M.T., and F.R. recruited patients and collected samples and data; all authors interpreted the data; C.D. wrote the first draft of the report; C.D., M.R., A.H., K.M.T., and F.R verified underlying data. The decision to submit the report for publication was made by all the authors. All authors reviewed the article.

Supplemental Data

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