Developing a whole exome sequencing-based homologous recombination deficiency test of epithelial ovarian cancer

Po-Han Lin  
National Taiwan University Hospital

Kuan-Ting Kuo  
National Taiwan University

Wuh-Liang Hwu  
National Taiwan University Hospital

Hsien-Neng Huang  
National Taiwan University Hospital Hsin-Chu Branch

Tzu-Ying Lin  
Takeda Pharmaceuticals Taiwan, Ltd

Chieh-Min Chen  
Takeda Pharmaceuticals Taiwan, Ltd

Wen-Fang Cheng  
National Taiwan University

Ying-Cheng Chiang ( ycchiang@ntuh.gov.tw )  
National Taiwan University

Research Article

Keywords: Epithelial ovarian cancer, Homologous recombination deficiency test, Whole-exome sequencing, scarHRD

Posted Date: May 30th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2983608/v1

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Abstract

Background:
Homologous recombination deficiency (HRD) test is an important tool to stratify epithelial ovarian cancer (EOC) patients for maintenance therapy. Using whole exome sequencing (WES)-based platform can provide information of gene mutations and HRD score, however, the clinical value of WES-based HRD test was less validated in EOC.

Methods:
We evaluated the performance of WES-based HRD test by using scarHRD software (https://github.com/sztup/scarHRD) in 44 EOC patients. Samples were concordantly examined using Myriad myChoice® and ACT Genomics. The correlation between HRD status and clinical outcomes was analyzed among the three tests.

Results:
A high correlation of HRD scores was observed between our WES-based scarHRD test and Myriad (coefficient 0.82, p < 0.001). Compared with positive HRD status in Myriad test, our WES-based scarHRD test had sensitivity, specificity, positive predictive value, and negative predictive value of 93.5%, 76.9%, 90.6%, and 83.3%, respectively. Positive HRD status by our WES-based scarHRD test and Myriad test both were highly associated with advanced FIGO stage and sensitive platinum-response. In multivariate Cox regression analysis, optimal debulking surgery (hazards ratio [HR] 0.39, 95% confidence interval [CI] 0.18–0.84, p = 0.017) and positive HRD status of our test (HR 0.42, 95% CI 0.20–0.90, p = 0.026) were independent factors for the lower risk of disease recurrence. However, the positive HRD status either by Myriad or ACT genomics was not significantly associated with an inferior trend of recurrence.

Conclusions
Our WES-based scarHRD test provides comprehensive information about gene mutations and HRD scores. It is a new feasible option to determine the HRD status in EOC patients.

Background
Epithelial ovarian cancer (EOC) is a major cause of cancer-related death in women (1, 2). Owing to the lack of specific symptoms and screening tools for identifying early-stage disease, most EOC patients are diagnosed at an advanced stage at which time the disease would have spread beyond the pelvis, with a 5-year survival of less than 50% (3). Most advanced-stage EOC patients relapse with a good response to even primary treatments including debulking surgery and adjuvant platinum-based chemotherapy (3). Patients are designated as "platinum-sensitive" when the tumor recurs beyond 6 months after completing primary treatment, and patients are designated "platinum-resistant" when the tumor recurs within 6 months after completing primary treatment; this categorization plays an important role in disease progression. The outcome of platinum-resistant EOC patients is generally worse, with a poor response to salvage chemotherapy (3).

Accumulation of DNA damage caused by replication error, oxidative stress, ultraviolet light, radiation, or cytotoxic agents leads to genomic instability, which is harmful to cells. DNA damage response (DDR) pathways repair single-strand breaks (SSBs) or double-strand breaks (DSBs) in the damaged DNA, and dysfunction of DDR is associated with carcinogenesis (4). Homologous recombination repair (HRR) is an error-proof DNA repair pathway involving several genes such as BRCA1/2, which restores the original sequence at the DSB sites (4). Homologous recombination deficiency (HRD) is the condition that when HRR is impaired, the DSBs are repaired by the error-prone pathways leading to genomic instability and subsequent apoptosis of the BRCA-mutated cancer cells (5). Maintenance PARPi therapy has proven beneficial for the management of newly diagnosed and recurrent EOC patients (6–9). The dilemma is that most expensive promising target drugs benefit only in a limited subpopulation, and it is important to select the right patients for the target therapy in clinical practice. The cost-effective analysis suggested PARPi should be reserved for EOC patients with positive HRD status (10, 11). To stratify EOC patients for PARPi is currently based on the HRD status, and Myriad myChoice CDx test was one commercial test suggested by US Food and Drug Administration (FDA) and European Medicines Agency (EMA) (6–9).

HRD is a biomarker that shows high sensitivity to PARPi or platinum-based chemotherapy (12, 13). However, currently available FDA-approved HRD tests are not economical and feasible for application in the real world, especially for patients not residing in America, because of the high cost and
time-consuming overseas transportation. A recent concept migrated the methodology from single-nucleotide polymorphism (SNP) sequencing to whole-genome sequencing (WGS) or whole-exome sequencing (WES) (14). Compared with sequencing of SNPs, WES provides more useful information about actionable genetic variants, copy number variants, microsatellite instability, and even tumor mutational burden for immune checkpoint blockade therapy. Some studies have demonstrated a very good correlation in HRD status between Myriad testing and WGS/WES method for breast cancer (14–17). However, the clinical significance of WES-based HRD analysis has not been extensively validated in EOC.

In this study, we assessed the performance of WES-based HRD detection in EOC and compared its results to those of Myriad myChoice® CDx test and another commercial HRD test (ACT Genomics Inc., Taiwan). We demonstrated the WES-based method was a feasible comprehensive HRD test, which accurately revealed both the HRD status and gene mutations of the DDR pathway. We found that the HRD status determined using the WES method had a good correlation with mutations in DDR genes. Then, we showed that the HRD status was associated with a better progression-free survival (PFS) in patients who received platinum-based chemotherapy, suggesting that the WES-based HRD method was clinically useful for stratifying patients with EOC for maintenance therapy.

Methods

Patients and specimens

The study protocol was approved by the National Taiwan University Hospital Research Ethics Committee (201807116RINA), and the study was performed in accordance with the guidelines and regulations. Specimens were retrieved from formalin-fixed, paraffin-embedded (FFPE) tissues obtained from primary debulking surgery. After pathologists’ review, FFPE specimens with a tumor purity of more than 20% were sliced at a thickness of 5–10 µm and sent for experiments.

Clinical data were obtained from patients’ medical records and included age, tumor stage, residual tumor size after debulking surgery, pathological report, adjuvant treatments, and outcomes. All patients received primary debulking surgery and adjuvant platinum-based chemotherapy. Maintenance treatment was not initiated in these patients. Optimal debulking was defined as a maximal residual tumor size of < 1 cm following surgery, and suboptimal debulking was defined as a maximal residual tumor size of ≥ 1 cm. The cancer stage was defined based on the International Federation of Gynecology and Obstetrics (FIGO) criteria, and tumor grade was defined based on the International Union Against Cancer criteria (18). All patients were followed up regularly after primary treatments. Cancer recurrence was defined as biopsy-proven disease, abnormalities reported from imaging studies (including computed tomography scan or magnetic resonance imaging), or continuous elevated levels of cancer antigen 125 (CA-125; more than twice the upper normal limit) for at least two consecutive tests with a monthly interval. Progression-free survival (PFS) was defined as the interval from the date of completing primary treatments to the date of confirmed recurrence, progression, or last follow-up. Overall survival (OS) was defined as the interval from either the date of diagnosis or the start of primary treatments to the date due to EOC or last follow-up.

DNA extraction and library preparation

Genomic DNA was isolated from the FFPE specimens using a Quick-DNA FFPE extraction kit (Zymo Research, CA, USA) according to the manufacturer’s instructions. In total, 100-ng DNA per sample was used as the input material for library preparation. DNA fragmentation and library construction were performed using KAPA HyperPlus Kits for next-generation sequencing (NGS) DNA Library Preparation. The exome library was generated with Roche KAPA HyperExome probes (Roche, Basel, Switzerland).

Sequencing and bioinformatics

The samples were sequenced using Illumina NovaSeq with paired-end reads of 300 nucleotides, and the analysis algorithm was in accordance with our previous protocol (19). Briefly, raw sequencing data were aligned with the reference human genome (December 2013, GRCh38) using Burrows-Wheeler Aligner software (version 0.5.9) (19). SAM tools (version 0.1.18) were used for data conversion, sorting, and indexing (19). We used Genome Analysis Toolkit (GATK; version 4) Mutect2 for variant calling, including nonsynonymous variants, small insertion/deletions (indels), and variants of splicing boundaries (19). After variant calling, ANNOVAR was used for annotation of the genetic variants (19, 20). ClinVar, dbSNP (version 150), Exome Sequencing Project 6500 (ESP6500), and the 1000 Genomes variant datasets ExAC and gnomAD were used for filtering common variants of sequencing results. The pathogenic/likely pathogenic variants, which were defined by guidelines for the interpretation of sequencing variants, were considered deleterious and used for further analysis (21); variants of uncertain significance were not enrolled. Because we focused on HRD, we not only calculated HRD scores but also searched for pathogenic variants of genes involved in the DDR pathways. The target gene panel was based on our previous studies (Supplementary Table S1) (19, 22). Additionally, the association between the HRD score and gene mutations was analyzed.

Assessment of homologous recombination deficiency (WES-based scarHRD test)

HRD was considered based on the following two conditions. First, when deleterious variants of BRCA1 or BRCA2 were detected, we considered that this tumor harbored HRD. Second, we used scarHRD software to measure the HRD score, which was a combination of loss of heterozygosity (LOH), number of chromosomal regions with allelic imbalance extending to the telomere (TAI), and large-scale state transition (LST). The LOH score is the total number of LOH regions greater than 15 Mb in length. TAI refers to the unequal contribution of parental allele sequences extended to the end of
the chromosome. LST is defined as a chromosomal break between adjacent regions, each of which being at least 10 Mb, with the distance between them not larger than 3 Mb. The scarHRD is an R package program and available for downloaded at the website (https://github.com/sztup/scarHRD).

**Myriad myChoice® CDx HRD test**

Myriad myChoice® CDx is an NGS-based diagnostic test conducted using DNA isolated from the FFPE specimens. This test performs (1) qualitative detection of single-nucleotide variants (SNVs), insertions and deletions (indels), and large genomic rearrangement variants in protein-coding regions and intron/exon boundaries of *BRCA1/2* genes and (2) determination of the genomic instability score (GIS), which is an algorithmic measurement of LOH, TAI, and LST. The positive HRD status is defined as pathogenic or likely deleterious mutations in *BRCA1/2* or a GIS ≥ 42.

**ACT HRD™ test**

ACT HRD™ test is an NGS-based diagnostic test conducted using DNA isolated from the FFPE specimens. This test performs (1) qualitative detection of SNVs, indels, copy number alterations, and large genomic rearrangement variants in *BRCA1/2* and (2) analysis of the genomic LOH status. As defined by ACT Genomics, a positive HRD status in the ACT HRD™ test refers to pathogenic or likely pathogenic mutations in *BRCA1* or *BRCA2* gene or LOH ≥ 0.4.

**Statistical analysis**

The chi-square test and Fisher's exact test were used to calculate the significant difference in variables between the groups. With Myriad myChoice® HRD as the reference, the sensitivity and specificity of our HRD and ACT HRD™ scores were assessed. The correlation of the HRD score with the clinical outcomes among the three methods was analyzed. PFS and OS were estimated using Kaplan-Meier analysis and log-rank test. The HRD status on the risks of recurrence and death was evaluated using univariate and multivariate Cox proportional hazards regression analyses with corresponding 95% confidence interval (CI). All p values were two-sided, and p values of less than 0.05 were considered statistically significant.

**Results**

**Clinical characteristics of patients**

A total of 44 serous EOC patients were enrolled (Table 1). Median patient age was 56.5 years old, and the median pretreatment CA-125 value was 889 U/ml. Overall, 42 (95.5%) patients had their diagnosis in the advanced stage (FIGO stages III and IV). All patients underwent primary debulking surgery, and optimal debulking was achieved in 16 (36.4%) patients. All patients received adjuvant platinum-based and paclitaxel chemotherapy, and 26 (59.1%) patients were platinum-sensitive. The median follow-up time was 48 months; the tumor recurred in 34 (77.3%) patients, and 23 (52.3%) patients died due to EOC.
Table 1
Characteristics of patients with epithelial ovarian cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>44</td>
</tr>
<tr>
<td>Age (years old)</td>
<td>56.5 (32–75)</td>
</tr>
<tr>
<td>Pretreatment CA-125 (U/ml)</td>
<td>889 (153–7560)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
</tr>
<tr>
<td>Serous carcinoma</td>
<td>44 (100%)</td>
</tr>
<tr>
<td>FIGO stage</td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>2 (4.5%)</td>
</tr>
<tr>
<td>Advanced</td>
<td>42 (95.5%)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1 (2.3%)</td>
</tr>
<tr>
<td>High</td>
<td>43 (97.7%)</td>
</tr>
<tr>
<td>Debulking surgery</td>
<td></td>
</tr>
<tr>
<td>Residual tumor &lt; 1 cm</td>
<td>16 (36.4%)</td>
</tr>
<tr>
<td>Residual tumor ≥ 1 cm</td>
<td>28 (63.6%)</td>
</tr>
<tr>
<td>Platinum response</td>
<td></td>
</tr>
<tr>
<td>Sensitive</td>
<td>26 (59.1%)</td>
</tr>
<tr>
<td>Resistant</td>
<td>18 (40.9%)</td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34 (77.3%)</td>
</tr>
<tr>
<td>No</td>
<td>10 (22.7%)</td>
</tr>
<tr>
<td>Death</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>23 (52.3%)</td>
</tr>
<tr>
<td>No</td>
<td>21 (47.7%)</td>
</tr>
</tbody>
</table>

**Correlation of BRCA mutation status among the three tests**

Two patients had pathogenic *BRCA1* mutation, and two other patients had *BRCA2* mutation. A strong correlation was observed between Myriad HRD test and our WES-based scarHRD test results. One *BRCA1* mutation was not detected found in the ACT HRD™ test.

**Correlation of the HRD score among the three tests**

The linear regression model was applied to analyze the correlation between the Myriad HRD score and our WES-based scarHRD score or ACT HRD score (Supplementary Figure S1A, B). Our WES-based scarHRD score showed a strong correlation with the Myriad HRD score (correlation coefficient (r): 0.82, p < 0.001), whereas the ACT HRD score showed a moderate positive correlation with the Myriad HRD score (r: 0.67, p < 0.001). Based on the regression model, we defined a positive HRD status as *BRCA* gene mutation or a score of ≥ 50 in our WES-based scarHRD test, which is equal to a score of 42 in the Myriad HRD test.

We then used the positive HRD status obtained in the Myriad test as the reference to validate the positive HRD status in the other two tests (Table 2). Compared with the positive HRD status in the Myriad test, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were 93.5% (29/31), 76.9% (10/13), 90.6% (29/32), and 83.3% (10/12), respectively, in our WES-based scarHRD test. The corresponding values in the ACT HRD™ test were 80.6% (25/31), 61.5% (8/13), 83.3% (25/30), and 57.1% (8/14).
Table 2
Validation of HRD-positive status between the WES-based scarHRD and ACT HRD™ methods.

<table>
<thead>
<tr>
<th></th>
<th>WES-based scarHRD</th>
<th>ACT HRD™</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Positive</td>
</tr>
<tr>
<td>44</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>Myriad HRD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>P value*</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

HRD scores and deleterious gene mutations

The HRD scores and deleterious gene mutations detected in the three tests are shown in Fig. 1. In our WES-based scarHRD test, the HRD score in the 44 patients ranged from 17 to 90. Overall, 32 patients had a positive HRD status (score ≥ 50). As for gene mutation, BRCA1 mutation was noted in two patients; BRCA2, two patients; ATM, one patient; BRIP1, one patient; CHEK2, one patient; FANCG, one patient; RAD51C, two patients; CDK12, one patient; MSH6, one patient; KRAS, two patients; SMARCA4, one patient; and TP53, 29 patients (Supplementary Table S2). In the Myriad HRD test, the HRD score in the 44 patients ranged from 3 to 84. Overall, 31 patients had a positive HRD status (score ≥ 42). As for gene mutation, BRCA1 mutation was noted in two patients and BRCA2 mutation in two patients. In the ACT HRD™ test, the HRD score in the 44 patients ranged from 0.1 to 0.69. Overall, 30 patients had a positive HRD status (score ≥ 0.4). As for gene mutation, BRCA1 mutation was noted in one patient; BRCA2, two patients; CDK12, one patient; RAD51C, two patients; and RAD51D, one patient.

Correlation of HRD status with clinical parameters in EOC patients

We evaluated the correlation between the HRD status and the clinicopathologic parameters in EOC patients (Table 3). In our WES-based scarHRD test, a higher percentage of advanced-stage patients had a positive HRD status than that of early-stage patients (76.2% [32/42] versus 0% [0/2], p = 0.018, chi-square test). A higher percentage of patients with a platinum-sensitive response had a positive HRD status than that of patients with a platinum-resistant response (84.6% [22/26] versus 55.6% [10/18], p = 0.033, chi-square test). No significant difference was noted in tumor grade, cancer recurrence, and death due to cancer. In the Myriad HRD test, a higher percentage of advanced-stage patients had a positive HRD status than that of early-stage patients (73.8% [31/42] versus 0% [0/2], p = 0.025, chi-square test). A higher percentage of patients with a platinum-sensitive response had a positive HRD status than that of patients with a platinum-resistant response (84.6% [22/26] versus 50% [9/18], p = 0.013, chi-square test). No significant difference was noted in tumor grade, cancer recurrence, and death due to cancer. In the ACT HRD™ test, a higher percentage of advanced-stage patients had a positive HRD status than that of early-stage patients (71.4% [30/42] versus 0% [0/2], p = 0.034, chi-square test). No significant difference was noted in tumor grade, platinum response, cancer recurrence, and death due to cancer.
Table 3
Correlation of HRD status with clinical parameters in epithelial ovarian cancer patients.

<table>
<thead>
<tr>
<th>HRD positive status</th>
<th>FIGO stage</th>
<th>Tumor grade</th>
<th>Platinum response</th>
<th>Recurrence</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Advanced</td>
<td>Low</td>
<td>High</td>
<td>Sensitive</td>
</tr>
<tr>
<td>WES-based scarHRD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2(100%)</td>
<td>10(23.8%)</td>
<td>1(100%)</td>
<td>11(25.6%)</td>
<td>4(15.4%)</td>
</tr>
<tr>
<td>Positive</td>
<td>32(76.2%)</td>
<td>0(0%)</td>
<td>32(74.4%)</td>
<td>22(84.6%)</td>
<td>10(55.6%)</td>
</tr>
<tr>
<td>p value*</td>
<td>0.018</td>
<td>0.099</td>
<td>0.033</td>
<td>0.163</td>
<td>0.622</td>
</tr>
<tr>
<td>Myriad HRD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>12(100%)</td>
<td>11(26.2%)</td>
<td>1(100%)</td>
<td>12(27.9%)</td>
<td>4(15.4%)</td>
</tr>
<tr>
<td>Positive</td>
<td>31(73.8%)</td>
<td>0(0%)</td>
<td>31(72.1%)</td>
<td>22(84.6%)</td>
<td>9(50%)</td>
</tr>
<tr>
<td>p value*</td>
<td>0.025</td>
<td>0.118</td>
<td>0.013</td>
<td>0.123</td>
<td>0.892</td>
</tr>
<tr>
<td>ACT HRD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14(100%)</td>
<td>12(28.6%)</td>
<td>1(100%)</td>
<td>13(30.2%)</td>
<td>7(26.9%)</td>
</tr>
<tr>
<td>Positive</td>
<td>30(71.4%)</td>
<td>0(0%)</td>
<td>30(69.8%)</td>
<td>19(73.1%)</td>
<td>11(61.1%)</td>
</tr>
<tr>
<td>p value*</td>
<td>0.034</td>
<td>0.139</td>
<td>0.402</td>
<td>0.361</td>
<td>0.837</td>
</tr>
</tbody>
</table>

Correlation of HRD status with prognosis of EOC patients

EOC patients who underwent optimal debulking surgery had longer PFS (p = 0.037, log-rank test; Fig. 2A) and OS (p = 0.008, log-rank test; Fig. 2E) than those who underwent suboptimal debulking surgery. In our WES-based scarHRD test, patients with a positive HRD status had longer PFS (Fig. 2B). In the Myriad HRD test, patients with a positive HRD status had longer PFS (p = 0.012, log-rank test; Fig. 2C). In the ACT HRD™ test, patients with a positive HRD status did not have significantly longer PFS (p = 0.194, log-rank test; Fig. 2D). The predictive value of OS in the three HRD tests was not satisfactory (Fig. 2F, G, H).

The Cox regression model was used to evaluate the risk of recurrence (Table 4). Optimal debulking surgery (hazard ratio [HR] 0.39, 95% CI 0.18–0.84, p = 0.017) and positive status in our WES-based scarHRD test (HR 0.42, 95% CI 0.20–0.90, p = 0.026) were independent factors for disease recurrence in multivariate analysis. When the model incorporated the other two HRD tests, only optimal debulking surgery was an independent factor for disease recurrence. Tumor HRD assessed using the Myriad HRD test (HR 0.99, 95% CI 0.97–1.00, p = 0.083) or ACT HRD™ test (HR 0.59, 95% CI 0.24–1.05, p = 0.068) was not statistically significant. The Cox regression model that evaluated the risk of death revealed only optimal debulking surgery as an independent factor in multivariate analysis.

Table 4
Cox regression model for evaluating the risk factors for recurrence and death in all patients (n = 44).

<table>
<thead>
<tr>
<th></th>
<th>Univariate</th>
<th>Multivariate</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>H.R. (95% C.I.)</td>
<td>p</td>
<td>H.R. (95% C.I.)</td>
</tr>
<tr>
<td>Debulking surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual tumor ≥ 1 cm</td>
<td>28</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>Residual tumor &lt; 1 cm</td>
<td>16</td>
<td>0.422(0.194–0.914)</td>
<td>0.029</td>
<td>0.386(0.177–0.844)</td>
</tr>
<tr>
<td>WES-based scarHRD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>Positive</td>
<td>32</td>
<td>0.474(0.224–1.001)</td>
<td>0.050</td>
<td>0.419(0.195–0.900)</td>
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</tbody>
</table>
Genomic scars represent a record that reflects the repair of DNA damage in response to harmful exposure through multiple pathways in cells (28). Currently available methods for detecting “genomic scars” use SNP-based microarray or NGS to measure copy number variation in tumor specimens, including LOH, TAI, and LST (29). LOH is defined as a permanent loss of an allele copy in DNA of more than 15 Mb, which makes the tumor cell homozygous at the locus (12). TAI is defined as different allele copy numbers of more than 11 Mb in the sub-telomere region of DNA that does not cross the centromere (30). LST is defined as chromosomal breaks between adjacent genomic regions of more than 10 Mb resulting from translocations, copy gains, or losses (31). GIS combines information derived from LOH, TAI, and LST to represent the degree of genomic instability.

Our WES-based scarHRD test provides comprehensive information of HR genes, other oncogenes, and tumor suppressor genes. BRCA mutations that should be included in the testing list remains undetermined. BRCA1/2 genes that should be included in the testing list includes ATM, BRCAl/2, BRIP1, MLH1, MSH2, MSH6, PALB2, RAD51C, and RAD51D (23), and it has an increased detection rate for gene mutations related to the DDR pathway when compared with the BRCA test alone. The percentage of BRCA1/2 somatic mutation in serous EOC in this study was 9.1% (4/44), consistent with the findings of previous literature (24–26). Approximately 11%-18% of high-grade serous EOC patients had a germline BRCA mutation and another 6%-7% of patients with somatic BRCA mutations could be identified from tumor specimens (24–26). In addition to the BRCA mutated EOC patients who get the greatest benefit from PARPi therapy, the patients with non-BRCA HR gene mutations, include ATM, BRIP1, NBN, PALB2, RAD51B, RAD51C, RAD51D...etc, also derive a survival benefit (25, 27). Furthermore, the number of non-BRCA HR genes that should be included in the testing list remains undetermined. Our WES-based scarHRD test provided comprehensive information of HR genes, other oncogenes, and tumor suppressor genes.

Discussion

Our WES-based scarHRD test provides information about DDR gene mutations and HRD scores in EOC patients. Our previous study showed varied proportions of DDR gene mutations in the histological subtypes including BRCA1/2 and homologous recombination (HR) genes (22). The DDR genes are involved in DSB repair, SSB repair, and cell cycle regulation. Our WES-based scarHRD test assesses genes recommended by the National Comprehensive Cancer Network guidelines, including ATM, BRCAl/2, BRIP1, MLH1, MSH2, MSH6, PALB2, RAD51C, and RAD51D (23), and it has an increased detection rate for gene mutations related to the DDR pathway when compared with the BRCA test alone. The percentage of BRCA1/2 somatic mutation in serous EOC in this study was 9.1% (4/44), consistent with the findings of previous literature (24–26). Approximately 11%-18% of high-grade serous EOC patients had a germline BRCA mutation and another 6%-7% of patients with somatic BRCA mutations could be identified from tumor specimens (24–26). In addition to the BRCA mutated EOC patients who get the greatest benefit from PARPi therapy, the patients with non-BRCA HR gene mutations, include ATM, BRIP1, NBN, PALB2, RAD51B, RAD51C, RAD51D...etc, also derive a survival benefit (25, 27). Furthermore, the number of non-BRCA HR genes that should be included in the testing list remains undetermined. Our WES-based scarHRD test provided comprehensive information of HR genes, other oncogenes, and tumor suppressor genes.

"Genomic scars" represent a record that reflects the repair of DNA damage in response to harmful exposure through multiple pathways in cells (28). Currently available methods for detecting “genomic scars” use SNP-based microarray or NGS to measure copy number variation in tumor specimens, including LOH, TAI, and LST (29). LOH is defined as a permanent loss of an allele copy in DNA of more than 15 Mb, which makes the tumor cell homozygous at the locus (12). TAI is defined as different allele copy numbers of more than 11 Mb in the sub-telomere region of DNA that does not cross the centromere (30). LST is defined as chromosomal breaks between adjacent genomic regions of more than 10 Mb resulting from translocations, copy gains, or losses (31). GIS combines information derived from LOH, TAI, and LST to represent the degree of genomic instability.

myChoice® CDx (Myriad Genetics) and Foundation Focus CDx BRCA LOH (Foundation Medicine) are currently the FDA-approved diagnostic HRD tests. The positive HRD status in the Myriad test was determined by BRCA mutation or GIS ≥ 42 in PAOLA-1 (32) and PRIMA (6) but by the BRCA mutation or GIS ≥ 33 in VELIA (33). The cut-off value of GIS was determined retrospectively from exploratory analyses, and these PARPi trials were
not prospectively designed to stratify the patients by the HRD tests. The Foundation HRD test includes BRCA mutation and genomic LOH, calculated as the fraction of genome regions with LOH by sequencing SNPs in tumor specimens. Moreover, 14% genomic LOH was considered a positive HRD status in the ARIEL2 trial (34), but 16% genomic LOH was considered the threshold in the ARIEL3 trial (35). The compatibility between HRD defined by GIS and percent genomic LOH also needs to be determined.

The recent concept of HRD testing migrated from SNP-based array to the NGS-based method. The use of WGS or WES methods to assess the HRD status of a tumor is in development. For example, HRDetect, a WGS-based assay, could predict BRCA deficiency with sensitivity of 98.7% and nearly 100% in 560 breast cancer cases and 73 ovarian cancer cases, respectively, in the validation cohort (15). However, the ability of HRDetect to predict the PARPi response in EOC has not been confirmed (16, 17). WGS analyzes the whole genome, whereas WES analyzes all coding regions in the tumor DNA, which comprise 1%-2% of the genome (36, 37). The original data provided by WGS are quite larger than those provided by WES; therefore, WGS is more time-consuming and expensive than WES (38, 39). Thus, WES is more affordable in clinical practice if WES-based HRD is accurate. scarHRD is an open-source R program that can be freely downloaded, and WGS or WES data can be used to calculate GIS. The result of our WES-based scarHRD test correlated well with that of the Myriad HRD test, and the WES-based test provided a significantly predictive value for clinical outcomes. This finding suggested that the WES-based HRD test is suitable for guiding precision oncology.

The cutoff value for HRD in our WES-based scarHRD test was 50, which was different from that of 42 for the Myriad test definition. The use of different methods and a baseline reference to measure the HRD score may generate different thresholds to define a positive HRD status (40). The linear regression model revealed a very high positive correlation between our WES-based scarHRD score and the Myriad HRD score. Thus, we defined the threshold of our WES-based scarHRD score according to the results of linear regression analysis when the Myriad HRD result was equal to a score of 42. In general, the WES-based scarHRD score was higher than the Myriad HRD score, suggesting more genomic lesions detected by the WES-based method. The reason may be that the WES-based scarHRD method provided a more comprehensive coverage in the human genome than the SNPs used in the Myriad test. We then evaluated the clinical value of HRD status defined by WES-based methodology. A higher percentage of EOC patients with a positive HRD status had pathogenic variants of HR genes (28.1% [9/32]) than that of patients with a negative HRD status (16.7% [2/12]). The median HRD score in EOC patients with HR gene mutation (64; range: 45–84) was higher than that in EOC patients without the mutation (56; range: 17–90). Previous studies revealed that EOC patients with a positive HRD status had a better response to platinum-paclitaxel chemotherapy. Thus, the clinical utility of our WES-based scarHRD test, Myriad HRD test, and ACT HRD™ test was evaluated by the survival analysis to evaluate whether these tests can distinguish patients with a favorable response to platinum-paclitaxel chemotherapy. The positive HRD status defined by our test had favorable sensitivity, specificity, and PPV/NPV when compared with the HRD status defined by the Myriad HRD test. A higher percentage of advanced-stage patients and platinum-sensitive response patients had a positive HRD status as defined by our test or Myriad test. EOC patients with a positive HRD status defined by our test or Myriad test had longer PFS.

The present study had limitations. First, the number of cases was not large. In the present study, the preliminary results of our WES-based scarHRD test were encouraging, and in future studies, we will recruit more participants for the validation of our results. Second, there was no information about clinical response to PARPi in the cohort. We used platinum sensitivity as the clinical surrogate marker for developing our WES-based scarHRD test because platinum sensitivity was used as the indicator of response in obtaining GIS (12, 29–31). Our WES-based scarHRD test showed good predictability of clinical outcomes, including platinum response. In the future, a validation cohort comprising EOC patients who are prescribed PARPi should be assessed.

Conclusions

In summary, our WES-based scarHRD test provided comprehensive information about gene mutations and HRD scores. Based on these findings, our WES-based scarHRD test is a new feasible option for HRD test in EOC patients and has the potential for clinical application in the future.

Abbreviations

DDR
DNA damage response
DSBs
Double-strand breaks
EMA
European Medicines Agency
EOC
Epithelial ovarian cancer
FDA
Food and Drug Administration
FFPE
Formalin-fixed, paraffin-embedded
FIGO
Declarations

Ethics approval and consent to participate

The study protocol was approved by the National Taiwan University Hospital Research Ethics Committee, and written informed consents were provided by all participants.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from
the corresponding author on reasonable request.

**Competing interests**

T.-Y.L. and C.-M.C. are employees of Takeda Pharmaceuticals Taiwan, Ltd. All the other authors declare that they have no competing interests.

**Funding**

This study was funded by Takeda Pharmaceuticals Taiwan, Ltd that was not involved in study design, the collection, analysis and interpretation of data, and the preparation of the manuscript.

**Authors’ contributions**


**Acknowledgements**

The authors thank the Department of Medical Genetics of the National Taiwan University Hospital for the NGS platform and the National Applied Research Laboratories for providing access to a high-performance computer to analyze NGS data for supporting the work.

**Authors’ information (optional)**

1 Department of Medical Genetics, National Taiwan University Hospital, Taipei City, Taiwan

2 Graduate Institute of Medical Genomics and Proteomics, College of Medicine, National Taiwan University, Taipei City, Taiwan

3 Department of Pathology, College of Medicine, National Taiwan University, Taipei City, Taiwan

4 Department of Pediatrics, National Taiwan University Hospital, Taipei City, Taiwan

5 Department of Pathology, National Taiwan University Hospital Hsin-Chu Branch, Hsinchu, Taiwan

6 Takeda Pharmaceuticals Taiwan, Ltd., Taipei City, Taiwan

7 Department of Obstetrics and Gynecology, College of Medicine, National Taiwan University, Taipei City, Taiwan

8 Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei City, Taiwan

9 Graduate Institute of Clinical Medicine, College of Medicine, National Taiwan University, Taipei City, Taiwan

10 Graduate Institute of Oncology, College of Medicine, National Taiwan University, Taipei City, Taiwan

**References**


Figures

**Figure 1**

**Positive HRD status (by Myriad)**

**Negative HRD status (by Myriad)**

**WES-based scarHRD**

**Myriad HRD**

**ACT HRD**

**HRD scores and deleterious gene mutations obtained using the three HRD tests in 44 epithelial ovarian carcinoma patients.** Note: The numbers indicate the HRD score from the three tests. The yellow color indicates a positive HRD status obtained using our WES-based scarHRD test. The orange color indicates a positive HRD status obtained using the Myriad HRD test. The light blue color indicates a positive HRD status obtained using the ACT HRD test. The red color indicates BRCA1/2 gene mutations. The green color indicates other gene mutations.
Figure 2

Kaplan-Meier analysis of PFS and OS in 44 EOC patients. (A) PFS of 44 EOC patients who did or did not undergo optimal debulking surgery. Note: EOC patients who underwent optimal debulking surgery had better PFS than those who underwent suboptimal debulking surgery (p=0.037, log-rank test). (B) PFS of patients with a positive or negative HRD status obtained using our WES-based scarHRD test. Note: EOC patients with a positive HRD status had better PFS than those with a negative HRD status (p=0.037, log-rank test). (C) PFS of patients with a positive or negative HRD status obtained using the Myriad HRD test. Note: EOC patients with a positive HRD status had better PFS than those with a negative HRD status (p=0.012, log-rank test). (D) PFS of patients with a positive or negative HRD status obtained using the ACT HRD™ test. Note: EOC patients with a positive HRD status had better PFS than those with a negative HRD status, although no significant difference was noted. (E) OS of patients who did or did not undergo optimal debulking surgery. Note: EOC patients who underwent optimal debulking surgery had better OS than those who underwent suboptimal debulking surgery (p=0.008, log-rank test). (F) OS of patients with a positive or negative HRD status obtained using our WES-based scarHRD test. Note: No significant difference was noted in EOC patients with a positive or negative HRD status. (G) OS of patients with a positive or negative HRD status obtained using the Myriad HRD test. Note: No significant difference was noted in EOC patients with a positive or negative HRD status. (H) OS of patients with a positive or negative HRD status obtained using the ACT HRD™ test. Note: No significant difference was noted in EOC patients with a positive or negative HRD status.

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