Normalized LST Is an Efficient Biomarker for Homologous Recombination Deficiency and Olaparib Response in Ovarian Carcinoma

Christinat, Yann; Ho-Mohrle, Liza Kwok-Fung; Clément Leboube, Sophie; Genestie, Catherine; Sehouli, Jalid; Cinieri, Saverio; Gonzalez Martin, Antonio; Denison, Ursula; Fujiwara, Keiichi; Vergote, Ignace; Tognon, Germana; Hietanen, Sakari; Ray-Coquard, Isabelle; Pujade-Lauraine, Eric [**and 1 more**]

How to cite

CHRISTINAT, Yann et al. Normalized LST Is an Efficient Biomarker for Homologous Recombination Deficiency and Olaparib Response in Ovarian Carcinoma. In: JCO precision oncology, 2023, vol. 7, p. e2200555. doi: 10.1200/PO.22.00555

This publication URL: https://archive-ouverte.unige.ch/unige:170573

Publication DOI: 10.1200/PO.22.00555

Normalized LST Is an Efficient Biomarker for Homologous Recombination Deficiency and Olaparib Response in Ovarian Carcinoma

Yann Christinat, PhD[1]; Liza Ho, PhD[1]; Sophie Clément, PhD[2]; Catherine Genestie, MD, PhD[3]; Jalid Sehouli, MD, PhD[4]; Saverio Cinieri, MD, PhD[5]; Antonio Gonzalez Martin, MD, PhD[5]; Ursula Denison, MD[6]; Keiichi Fujiwara, MD, PhD[7]; Ignace Vergote, MD, PhD[8]; Germana Tognon, MD[9]; Sakari Hietanen, MD, PhD[10]; Isabelle Ray-Coquard, MD, PhD[11]; Eric Pujade-Lauraine, MD, PhD[12]; and Thomas A. McKee, MD, PhD[1]

DOI https://doi.org/10.1200/PO.22.00555

ABSTRACT

PURPOSE The efficiency of the Myriad Homologous Recombination Deficiency (HRD) test to guide the use of poly (ADP-ribose) polymerase (PARP) inhibitors has been demonstrated in several phase III trials. However, a need exists for alternative clinically validated tests.

METHODS A novel biomarker for HRD was developed using The Cancer Genome Atlas database and, as part of the ENGOT HRD European Initiative, applied to 469 samples from the PAOLA-1/ENGOT-ov25 trial. Results were compared with the Myriad myChoice Genomic Instability Score (GIS) with respect to the progression-free survival in the olaparib + bevacizumab and placebo + bevacizumab arms.

RESULTS Analysis of the TCGA cohort revealed that a normalization of the number of large-scale state transitions by the number of whole-genome doubling events allows a better separation and classification of HRD samples than the GIS. Analysis of the PAOLA-1 samples, using the Geneva test (OncoScan + nLST), yielded a lower failure rate (27 of 469 *v* 59 of 469) and a hazard ratio of 0.40 (95% CI, 0.28 to 0.57) compared with 0.37 for Myriad myChoice (BRCAm or GIS+) in the nLST-positive samples. In patients with BRCAwt, the Geneva test identified a novel subpopulation of patients, with a favorable 1-year PFS (85%) but a poor 2-year PFS (30%) on olaparib + bevacizumab treatment.

CONCLUSION The proposed test efficiently separates HRD-positive from HRD-negative patients, predicts response to PARP inhibition, and can be easily deployed in a clinical laboratory for routine practice. The performance is similar to the available commercial test, but its lower failure rate allows an increase in the number of patients who will receive a conclusive laboratory result.

ACCOMPANYING CONTENT

Data Sharing Statement

Data Supplement

Accepted May 25, 2023
Published June 26, 2023

JCO Precis Oncol 7:e2200555

INTRODUCTION

Ovarian cancer is the eighth most common cancer affecting women worldwide,[1] and the majority of patients present with advanced disease.[2] Numerous clinical studies have shown that patients whose tumors have a deficiency in the homologous recombination pathway (HR), with or without germline or somatic breast cancer type 1 and 2 susceptibility protein mutations (gBRCA and sBRCA), have improved survival if treated with inhibitors of poly (ADP-ribose) polymerase (PARPi), particularly as maintenance therapy (reviewed by Foo et al).[3,4] There is an unmet need for an academic assay to detect HR deficiency (HRD) in high-grade serous ovarian and perhaps in other cancers. The available approaches have shortcomings with respect to their technical characteristics and cost.

Prediction of a response to PARPi is currently based on two strategies—detection of mutations in genes involved in HRD (particularly *BRCA1/2*) and identification of a HRD phenotype. HRD is a frequent alteration identified in a wide range of tumors but particularly those with a genetic predisposition associated with gBRCA mutations. The advantage of mutation testing is that it provides a simple readout (presence or absence of a pathogenic or probably pathogenic mutation in the tested genes), but other mechanisms of gene silencing are overlooked. This was highlighted in the PAOLA-1 study where 19% of patients were considered to be BRCA wild-type but HR-deficient. These patients showed a response to olaparib + bevacizumab, and more extensive mutation testing failed to reduce this percentage significantly.[5,6] The benefits of HRD phenotype testing over mutation testing were confirmed in the PRIMA[7] and VELIA[8] studies. To our knowledge, the

CONTEXT

Key Objective

Ovarian carcinoma is the deadliest gynecologic cancer, and the advance of poly (ADP-ribose) polymerase (PARP) inhibitors, such as olaparib, has greatly improved the survival of patients. However, tests that predict response to PARP inhibitors are in the commercial sector with limited accessibility. To answer the need for clinically validated academic tests, we aim to evaluate the performance of our homologous recombination deficiency (HRD) test on 469 patients from the PAOLA-1/ENGOT-ov25 phase III trial.

Knowledge Generated

The hazard ratios between the olaparib and placebo arms and the low failure rate of the Geneva HRD test demonstrate that the test can predict response to olaparib and is well adapted for clinical routine. In addition, the algorithm for HRD scoring is freely available to the community.

Relevance

The test, already in clinical routine at the Geneva University Hospitals, can be easily implemented in a pathology laboratory, which should facilitate the availability of HRD testing and thus improve patient well-being.

PAOLA-1 trial is the only trial to show no benefits of PARPi maintenance treatment in the HRD-negative population. However, the effect of the combination of olaparib and bevacizumab is yet to be assessed. Several techniques have been developed to identify HRD; the quantification of large-scale genomic alterations,[9-11] specific mutational signatures,[12] and the absence of RAD51 foci,[13,14] certain of which alone or in combination are the basis of current assays. Of note, only large-scale genomic alterations have been clinically validated in phase II/III trials.

Whole-genome doubling (WGD) is a common genomic phenotype, found in advanced neoplasms, and is particularly prevalent in high-grade serous ovarian cancers.[15] It is thought to promote tumorigenesis by facilitating genomic instability and helping to buffer against the negative effects of deleterious mutations. It is self-evident that a near doubling of genetic material in a tumor is a confounding factor for HRD analyses that depend on quantification of genomic alterations.

We report an academic laboratory–developed, clinically validated test to detect HRD. The algorithm is publicly available within an R package on GitHub[16] and R/Bioconductor to allow wide dissemination of this essential diagnostic modality.

METHODS

TCGA Cohorts

The Cancer Genome Atlas (TCGA) pan-cancer cohort (pancan12) contains 457 high-grade serous ovarian carcinomas (264 with an associated BRCA1/2 mutation status) and 112 triple-negative breast carcinomas. Copy number variation (CNV) segments and the number of WGD events computed using the ABSOLUTE software were downloaded from Synapse.[17,18] Mutational status for *BRCA1* and *BRCA2* was obtained from the TCGA article.[19] Estrogen receptor status, progesterone receptor status, and human epidermal growth factor receptor 2 status for the breast cancer samples were downloaded from cBioPortal on May 18, 2020.[20]

PAOLA-1 Cohort Patient Samples

The PAOLA-1 study is part of the European ENGOT (European Network of Gynecological Oncology Trial) HRD initiative led by the French group GINECO (Groupe des Investigateurs Nationaux pour l'Etude des Cancers de l'Ovaire).[21] The Ethic Committee Comite de Protection des Personnes SUD-EST IV of Centre Leon Berard, Lyon, gave ethical approval for this work. To allow the ten initial participant laboratories to analyze the same samples from the PAOLA-1 trial,[5] samples with the highest content of DNA were prioritized (ARCAGY-GINECO tumor bank, Institut Curie, Paris). DNA was extracted from FFPE tumor slides, preferentially from patients untreated before surgery (76.5% [359] were taken prechemotherapy, and 21.5% [101] post-chemotherapy). 100 ng of tumor DNA samples from each patient was transferred to 96-well plates that were sent to Geneva at −80°C, after agreement from the French authority. The clinical database was locked on August 2019 (maximum follow-up of 51 months). BRCA wild-type tumors were selected for the first phase of 85 samples that was designed to test the correlation with the MYRIAD test, specifically for those BRCA wild-type tumors where the Genomic Instability Score (GIS) score has been shown to detect HRD-positive cases. The second phase involved testing 384 additional samples with high DNA content. Care was taken that this selection was representative of the global PAOLA-1 population in terms of BRCA and HRD status distribution and that the benefit of olaparib + bevacizumab

maintenance versus bevacizumab among the 469 selected cases was in the same range as that of the global PAOLA-1 population of 806 cases. These 469 samples (female patients with a high-grade ovarian adenocarcinoma; median age: 60 years) were analyzed using the OncoScan assay (see the Methods section in the Data Supplement for details).

Statistical Analysis and HRD Scores Computation

All statistical analyses and graphs were performed using R 3.5.1[22] and the packages forestplot, survival, and survminer. For the TCGA cohort, the HRD score (LOH + LST + TAI) was computed using the scarHRD v0.1.1 package.[23] The WGD-based nLST score and other OncoScan-related analyses were performed using the OncoScanR v1.0.0 package[16] and are described in the Methods section in the Data Supplement. A schema of the analyses on both cohorts is described in the Data Supplement (Figure S1).

Data Availability

All data in the present study produced from the TCGA cohort are available upon reasonable request to the authors. All data in the present study produced from the PAOLA-1 trial data are unavailable.

RESULTS

The Number of LSTs Depends of the Number of WGD Events

Using the public data sets from the TCGA, we observe that the distributions of the three components of the HRD score from the study by Telli et al (as used in the Myriad myChoice test; see the Methods section in the Data Supplement)[24] shift with the number of WGD events (Fig 1). The number of large-scale state transitions (LST) and the number of telomeric allelic imbalance (TAI) events increase with the number of WGD events, whereas the number of loss of heterozygosity events (LOH) diminishes. Interestingly, the sum of the three markers does not seem to be affected by the number of WGD events as the peaks of the bimodal distributions are aligned. However, the percentage of HRD-positive cases (defined as LST + LOH + TAI ≥42) decreases as the number of WGD events increases: 67% (170 of 255), 49% (120 of 245), and 54% (37 of 69) for no WGD, one WGD, and two WGD events, respectively. Therefore, a test based solely on the presence or absence of WGD events will have a positive predictive value of 67% (170 of 255) and a negative predictive value of 50% (157 of 314) where we would expect 57% and 43% by chance (Cohen's Kappa of 0.162; minor agreement).

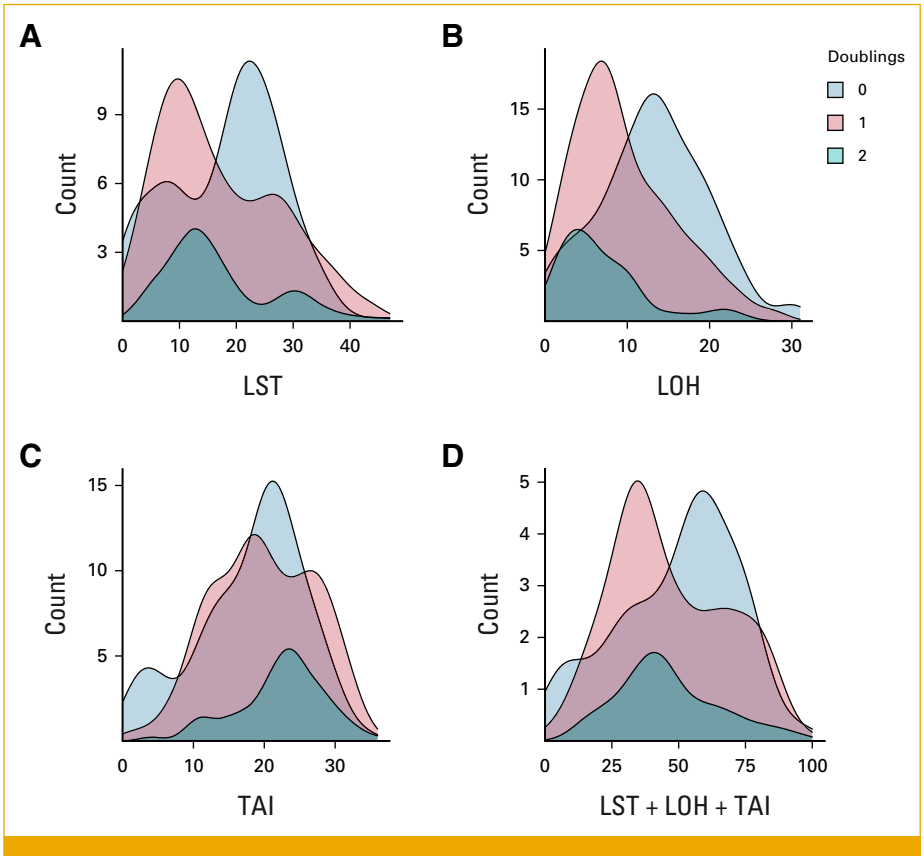

FIG 1. Distribution of (A) LST, (B) TAI, and (C) LOH scores and (D) their sum for The Cancer Genome Atlas cohort with respect to the number of WGD events as obtained using the ABSOLUTE software. LOH, loss of heterozygosity events; LST, large-scale state transitions; TAI, telomeric allelic imbalance events; WGD, whole genome doubling.

The Number of LSTs Normalized by the Number of WGD Events is an Efficient Biomarker for HR-Deficient Tumors

Since the number of LSTs is the only value to have a bimodal distribution, as expected for a biomarker identifying two distinct populations, we postulated that the numbers of LOH and TAI events in the HRD score from the study by Telli et al[24] act as surrogates to normalize the ploidy or the number of WGD events, but do not in themselves add value to a normalized LST score (nLST). To test this hypothesis, we investigated three normalization methods for the LSTs (see the Methods section in the Data Supplement):

1. A ploidy-based method, as suggested by Timms et al,[25] that shifts the LST values as a function of the ploidy of the sample.
2. A method on the basis of the average copy number that, instead of shifting the values by a discrete number, normalizes the LST directly by the amount of DNA in the cell.
3. A WGD-based method, similar to that in the study by Timms et al, but that uses the estimated number of WGD events (none, one, or two) instead of the ploidy.

The HRD-negative LST peak of the no-WGD-events samples has a value of 7 (Fig 1A) and was chosen as the baseline parameter for the ploidy- and WGD-based normalization methods (k = 7/2). Of note, the value of k=15.5 suggested by Timms et al[25] for the ploidy-based normalization method shifts all polyploid samples into the first peak and was not further considered (Data Supplement Fig S2).

These methods were compared using two performance metrics:

1. The BRCA detection rate, that is, the percentage of samples with a homozygous mutation on *BRCA1* or *BRCA2* within the positive cluster. A good test would be expected to classify all BRCA-mutated samples as positive. (Of note, this measure was only computed using the 264 ovarian cases that had been characterized for BRCA mutations.)
2. The between-clusters sum of squares (BCSS), which measures the average distance between two clusters. A good test is expected to have a clear separation and thus a large BCSS.

Figure 2A shows that a normalization by the number of WGD events provides the highest cluster separation (BCSS) and the highest BRCA detection rate for any given BCSS. The number of normalized LSTs, irrespective of the normalization method, also allows a better classification of BRCA-mutated samples and a better cluster separation than the HRD score from the study by Telli et al as computed using scarHRD.

For the number of LSTs normalized by the WGD events, a cutoff point of 15 yields the highest BCSS and was chosen for the test. Figure 2B shows that the nLST method using the cutoff of 15 results in a lower BRCA detection rate but a better cluster separation than the tripartite score from the study by Telli et al at the recommended cutoff of 42.

The OncoScan + nLST Test Yields a Lower Failure Rate Than Myriad on PAOLA-1 Samples

The OncoScan assay yielded a technical failure rate of 2% (10 of 469), and in 17 samples, the ChAS software (Thermo

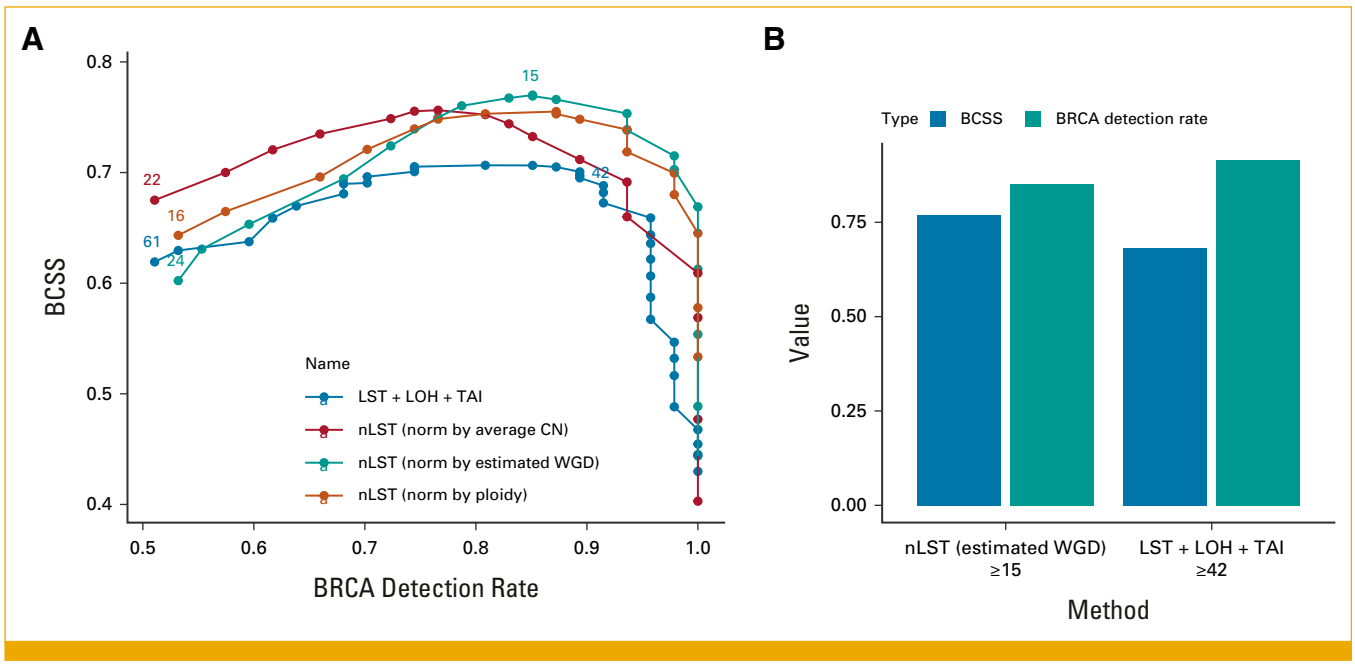

FIG 2. Evaluation of different methods on The Cancer Genome Atlas cohort: (A) results at different cutoffs and (B) results at the recommended cutoffs. BCSS, between-clusters sum of squares; CN, copy number; LOH, loss of heterozygosity events; LST, large-scale state transitions; TAI, telomeric allelic imbalance events; nLST, normalized LST; WGD, whole-genome doubling.

Fisher) failed to correctly detect the copy number alterations. Combining these categories resulted in an inconclusive rate of 6% for the Geneva test. Of note, a further 14 samples displayed no copy number alterations nor any sign of CNV calling errors and were classified as HRD-negative. In the Myriad myChoice assay, the percentage of inconclusive tests for the GIS score stands at 13% (59 of 469), which is reduced to 9% when the BRCA mutational status is included. Among the 59 Myriad GIS–inconclusive samples, 12 samples were found to be HRD-positive by the Geneva test, 16 were HRD-negative, 20 were inconclusive, and 11 showed no CNV alterations.

The Myriad myChoice assay requires samples with at least 30% tumor, whereas the OncoScan can detect CNVs down to a 15% tumor content, according to Thermo Fisher. An analysis of the nLST value distribution with respect to the tumor content (inferred from the OncoScan data) showed no statistically significant bias in samples with a 15%-30% tumor content (t-test P value of .0861 for 15%-30% v ≥30%; Data Supplement Fig S3A).

The nLST Score is Highly Correlated With the Myriad GIS

On the 403 patients from the PAOLA-1 trial with a conclusive result from both tests, we observe a tight correlation between the nLST score and the Myriad instability score (Pearson's correlation coefficient of 0.88, P value $<2.2 \times 10^{-16}$; Data Supplement Fig S4). Furthermore, in the 19 samples with 15%-30% tumor, the distribution of the difference between the GIS and nLST scores was similar to the other samples (Data Supplement Fig S3B), indicating a good concordance between the assays even at low tumoral DNA content.

With the default nLST threshold of 15, the Geneva test yielded a Cohen's Kappa of 0.80 and positive and negative agreement values of 98% (204 of 209) and 81% (158 of 194), respectively, with the GIS (Table 1). The Geneva test with the nLST threshold of 15 thus identifies almost all GIS-positive samples but adds an extra set of 36 patients who are Myriad GIS–negative. However, the selected thresholds for each test are not equivalent. Through a linear regression, we could deduce that a GIS threshold of 42 is equivalent to a nLST threshold of 18 and, reciprocally, a nLST threshold of 15 is equivalent to a GIS threshold of 38.

TABLE 1. Homologous Recombination Deficiency Calls Comparison (nLST v Myriad GIS) of 469 Patients From the PAOLA-1 Trial

		nLST ≥15		
		Positive	Negative	Inconclusive
GIS ≥42	Positive	204	5	4
	Negative	36	158	3
	Inconclusive	12	27	20

Abbreviation: GIS, Genomic Instability Score; nLST, normalized LST.

With a nLST threshold of 18, Cohen's Kappa reaches 0.83, which indicates an almost perfect agreement (Data Supplement Table S1).

The Geneva Test is Predictive of the Response to Olaparib + Bevacizumab

The Myriad myChoice test includes a genomic instability score and the BRCA mutation status. The Geneva test (OncoScan + nLST), in contrast, is not designed to identify BRCA mutations, but, in practice, the BRCA mutational status is already known or is assessed via sequencing. The PFS hazard ratio with respect to the treatment arm (olaparib + bevacizumab or placebo + bevacizumab) and the test status (HRD-positive, negative, or unknown) was compared in relation to the BRCA mutation status provided by the Myriad test (Fig 3). In the 151 BRCA-mutated samples, the hazard ratio was 0.36 (95% CI, 0.22 to 0.57), which is similar but slightly higher than the hazard ratio reported in the full PAOLA-1 cohort (0.31; 95% CI, 0.20 to 0.47). In the 311 BRCA wild-type samples (Fig 3B), the nLST test classified 119 samples as HRD-positive, which yielded a hazard ratio of 0.56 (95% CI, 0.35 to 0.89). In the same subpopulation, the Myriad GIS with a threshold of 42 produced a hazard ratio of 0.41 (95% CI, 0.24 to 0.70) but classified only 91 samples as HRD-positive. With a threshold of 18 in the nLST test, the equivalent to a GIS of 42, the Geneva test resulted in a hazard ratio of 0.47 (95% CI, 0.28 to 0.81; 94 patients) in the HRD-positive population. When considering the tests as a whole, irrespective of the BRCA status, the two tests yielded very similar hazard ratios on the PFS when olaparib is added to the bevacizumab maintenance treatment (Fig 3A). The addition of the BRCA status to the nLST score adds 22 patients to the HRD-positive population, but does not improve the hazard ratio (from 0.40 [95% CI, 0.28 to 0.56] to 0.43 [95% CI, 0.31 to 0.60]). A similar effect is seen for the Myriad GIS. Interestingly, three BRCA-mutated but nLST-negative patients received olaparib and all had a low Myriad GIS and a low PFS (7.9, 13.9 and 18.7 months). In particular, the patient with the lowest PFS had a heterozygous mutation, which concurs with the negative HRD evaluations, indicating that there may be no deficiency in the HR pathway.

BRCA Wild-Type Patients With an Intermediate nLST Score Derive a Short-Term Benefit From Olaparib + Bevacizumab

The nLST test yielded more HRD-positive samples in the BRCA wild-type population than the Myriad test, which is mostly due to the use of different thresholds. These nLST-positive but Myriad GIS–negative patients display a poorer PFS with olaparib + bevacizumab treatment than nLST-positive and Myriad GIS–positive patients (hazard ratio, 0.71; 95% CI, 0.26 to 1.90; 28 samples; Data Supplement Fig S5). As most nLST-positive but GIS-negative samples have an nLST score below 20 (third quartile at 19), we investigated the characteristics of these patients using a three-way classification: nLST <15 (negative), 15 ≤nLST <20 (positive-mid),

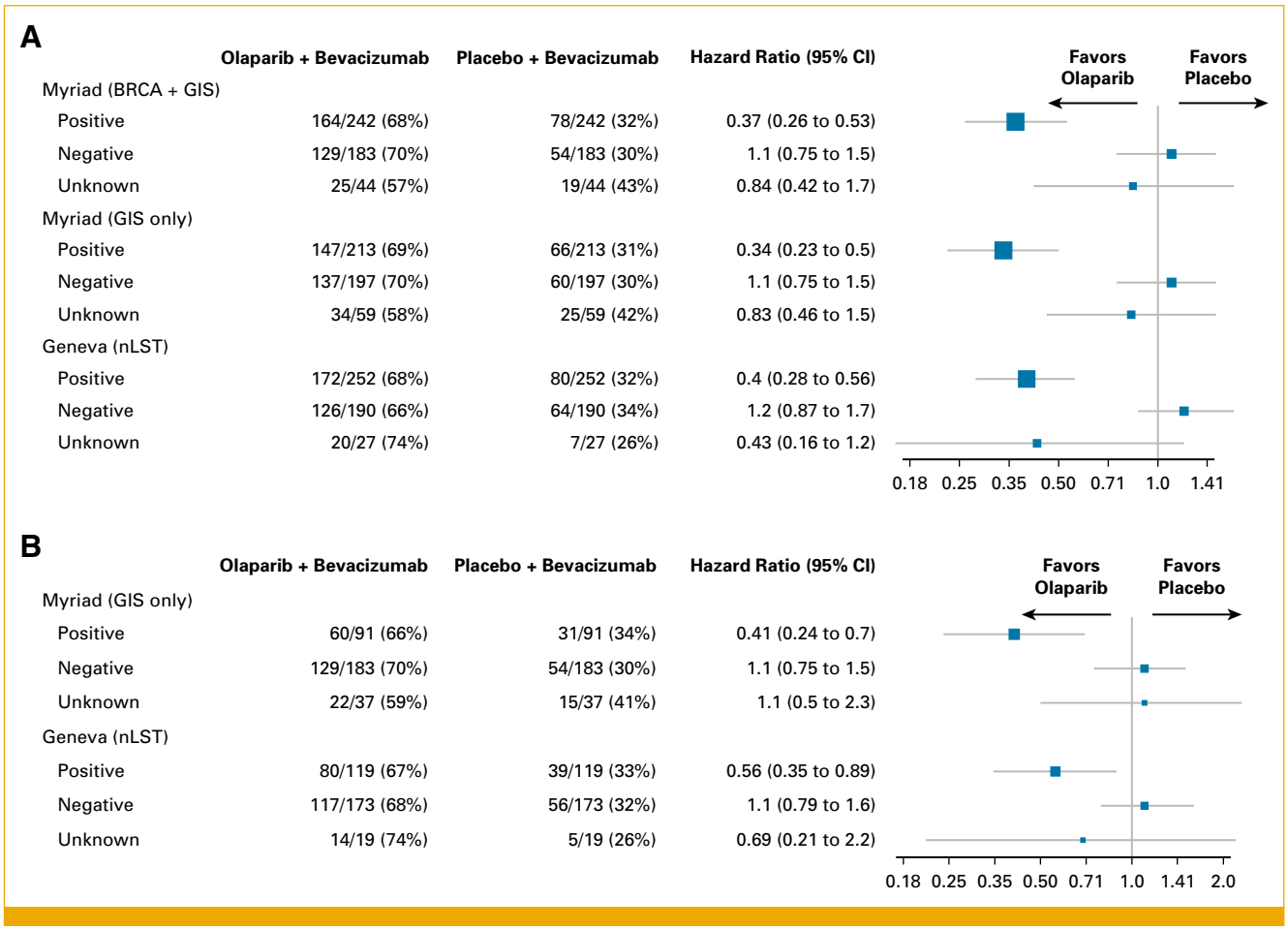

FIG 3. Hazard ratio for the Myriad test (GIS + BRCA mutation status) and the Geneva HRD test: (A) full EHEI cohort (469 patients) and (B) BRCA wild-type patients (according to the Myriad myChoice test; 311 patients). GIS, Genomic Instability Score; HRD, homologous recombination deficiency.

and nLST ≥20 (positive-high). Of note, two thirds of the positive-mid patients were GIS-negative (23 of 33). We observe that the nLST positive-mid population is enriched in patients with tumor recurrence during their second year of treatment (Fig 4A). No such difference was observed in the BRCA-mutated population (Fig 4B) nor in the placebo arm (Data Supplement Fig S6). Patients with positive-mid and positive-high values show a similar 1-year PFS (85% and 89%, respectively), but these two groups show a different 2-year PFS (30% and 66%), significant with a Fisher's exact test (*P* value of .00457, and Table 2). Patients with negative nLST values showed a lower 1-year PFS (66%) compared with those with positive-mid values (Fisher's exact test *P* value of .0246) but a 2-year PFS close to the positive-mid samples (23% *v* 30%; Fisher's exact test *P* value .3).

DISCUSSION

Many studies have shown that inhibition of PARP is an effective treatment that prolongs PFS for high-grade serous carcinoma of the ovary.[3] The efficacy of this treatment is in great part dependent on the loss of the homologous recombination pathway for DNA repair.[3,4] However, the adverse effects of PARPi can be serious, and thus, careful selection of patients is important.[26] We report here the development and technical and clinical validation of a novel assay for HRD, which is available to the academic community. This test has been integrated into our routine laboratory workflow and represents a low-cost alternative to commercial assays. The algorithm on which it is based could be applied to other data sources as long as confident calling of copy number can be assured. The OncoScan technology is particularly well adapted to calculating the HRD value using FFPE tissues and circumvents one of the main barriers to genomic testing, DNA quality, as no enzymatic reactions is performed on the tumor DNA,[27] in contrast to the capture-based next-generation sequencing assays. Low tumor cell percentage remains a barrier to accurate genomic analysis, and the OncoScan CNV assay is theoretically more sensitive than the Myriad myChoice. Further investigations with a ground truth for tumoral content (eg, the *TP53* mutation allele frequency) are required to truly assess the limit of detection of the Geneva HRD test.

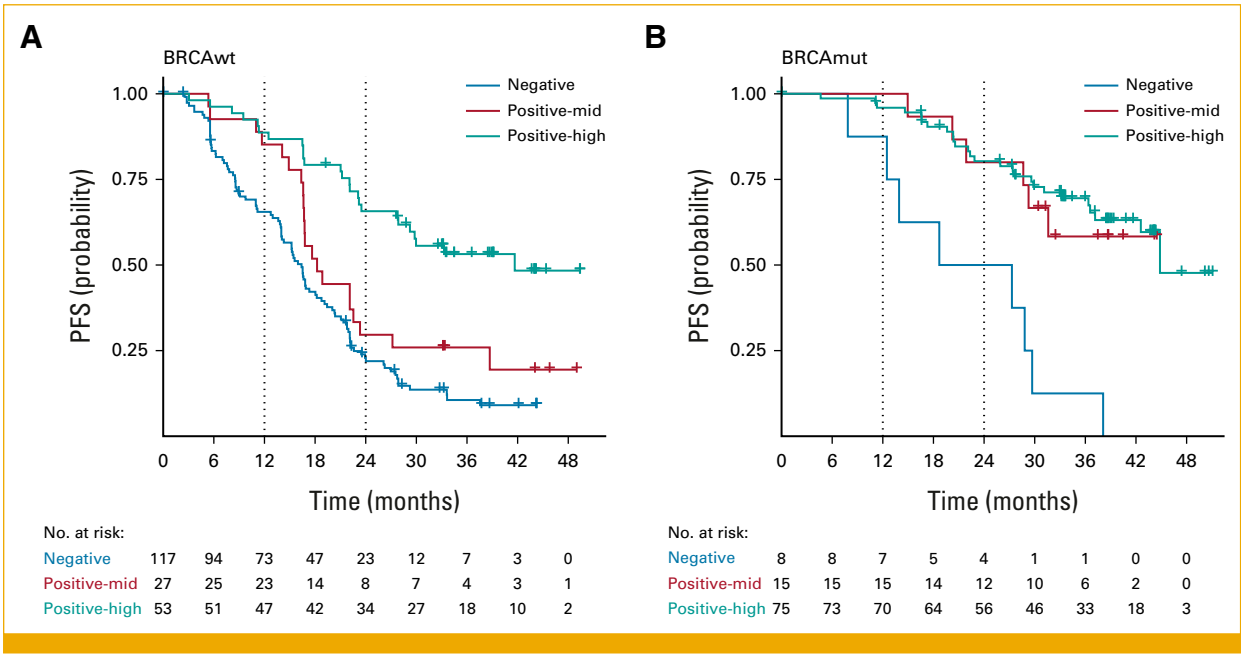

FIG 4. Kaplan-Meier plot of a three-way classification of the nLST score in the olaparib + bevacizumab arm: (A) BRCA wild-type subpopulation and (B) BRCA-mutated subpopulation. nLST, normalized LST.

We find that normalizing the LST using genomic doubling events is an efficient method to identify the genomic scars induced by the loss of HR and predicts response to olaparib therapy, in addition to bevacizumab, as well as the Myriad myChoice test in 469 samples from PAOLA-1 study, classifying more patients as HRD-positive than the Myriad test. However, the higher positivity rate also increases the risk for false positive, which is partly reflected in the hazard ratios. The set of BRCA wild-type patients that has an intermediate nLST score (between 15 and 20 nLSTs) represents an interesting subgroup. They displayed a better response to olaparib + bevacizumab than patients who are negative, but a worse long-term response than the patients with a high nLST score. One explanation was that this group represents a mixture of responders and nonresponders who were not efficiently separated by the test. However, these patients display an initial response to PARP inhibitors that is not sustained, suggesting both a biologic basis for this observation and the existence of partial responders despite a low number of patients in this group. Indeed, it raises the question of whether a 3-class system has a clinical utility, a suggestion supported by the existence of hypomorphic BRCA mutations[28] and the fact that different thresholds for the Myriad test have been used in clinical studies.[29,30] The inclusion of these patients in the HRD-positive category would have a negative effect on the global hazard ratio of the test, as observed in our case, but it still translates into a 12-month benefit for patients who receive treatment.

TABLE 2. 1-Year and 2-Year PFS in the BRCA Wild-Type Subpopulation With Respect to the Three-Way Classification Scheme With Olaparib + Bevacizumab Treatment

HRD Class	1-Year PFS, % (95%CI)	2-Year PFS, % (95%CI)
Negative	66 (57 to 75)	23 (16 to 32)
Positive-mid	85 (73 to 100)	30 (17 to 53)
Positive-high	89 (81 to 98)	66 (54 to 80)

Abbreviation: HRD, homologous recombination deficiency; PFS, progression-free survival.

The addition of the BRCA mutation status to the nLST score or the Myriad GIS score, as performed in the Myriad myChoice test, did not improve the performance of these tests. In ovarian cancer, more than 90% of the BRCA-mutated tumors show a genetic alteration of the second allele and mutations are thus homozygous. This leaves a small group of patients who have a BRCA mutation possibly without a HR deficiency. In the PAOLA-1 subcohort, we observed two cases with heterozygous mutations and low GIS and nLST scores that did not respond well to the olaparib treatment. The BRCA status, which is, in general, derived in parallel with the HRD score, is, however, beneficial in samples with a low tumor cell content as it eliminates some false negatives. It might also remove some false positives if a reversion mutation is detected. In our opinion, the BRCA status should be considered as a complement to HRD testing and the zygosity of the mutation should be taken into account in cases with a low HRD score.

We have developed and made available an academic assay for HRD detection that complements available assays and identifies a new group of patients with ovarian cancer who seem to respond to PARP inhibition but relapse earlier. We expect this assay to facilitate the availability of HRD testing and thus improve patient well-being.

AFFILIATIONS

[1]Hôpitaux Universitaires de Genève, Department of Clinical Pathology, Geneva, Switzerland
[2]Université de Genève, Geneva, Switzerland
[3]Gustave Roussy, Paris, France
[4]Charité—Universitätsmedizin Berlin (CVK), Berlin, Germany
[5]U.O.C. Oncologia Medica—Ospedale Senatore Antonio Perrino (Brindisi), Italy
[6]Medical Oncology Departament, Clinica Universidad de Navarra, Madrid, Spain
[7]Department for Gynaecology and Obstetrics, Institute for gynaecological oncology und senology—Karl Landsteiner, Vienna, Austria
[8]Saitama Medical University International Medical Center, Saitama, Japan
[9]University Hospitals Leuven and Leuven Cancer Institute, Leuven, Belgium
[10]Spedali Civili di Brescia, Brescia, Italy
[11]Turku University Hospital, Department of Obstetrics and Gynecology, Turku, Finland
[12]Centre Leon Bérard and University Claude Bernard Lyon I, Lyon, France
[13]ARCAGY-GINECO, Paris, France

CORRESPONDING AUTHOR

Yann Christinat, PhD, HUG, Service de Pathologie Clinique (CMU), Rue Michel-Servet 1, 1206 Geneva, Switzerland; e-mail: yann.christinat@hcuge.ch.

PREPRINT VERSION

Preprint version available on medRxiv: https://doi.org/10.1101/2022.08.22.22278669.

PRIOR PRESENTATION

Presented at the European Congress of Pathology, Basel, Switzerland, September 6, 2022; and ESGO, Berlin, Germany, October 27, 2022.

SUPPORT

This study was partially funded by AstraZeneca, Cambridge, UK, and Merck Sharp & Dohme LLC, a subsidiary of Merck & Co, Inc, Rahway, NJ.

AUTHOR CONTRIBUTIONS

Conception and design: Yann Christinat, Eric Pujade-Lauraine, Thomas A. McKee
Financial support: Thomas A. McKee
Provision of study materials or patients: Catherine Genestie, Jalid Sehouli, Saviero Cinieri, Antonio Gonzalez Martin, Ursula Denison, Keiichi Fujiwara, Ignace Vergote, Germana Tognon, Sakari Hietanen, Isabelle Ray-Coquard, Eric Pujade-Lauraine
Collection and assembly of data: Yann Christinat, Liza Ho, Sophie Clément, Jalid Sehouli, Saverio Cinieri, Antonio Gonzalez Martin, Ursula Denison, Keiichi Fujiwara, Ignace Vergote, Germana Tognon, Sakari Hietanen, Isabelle Ray-Coquard, Eric Pujade-Lauraine
Data analysis and interpretation: Yann Christinat, Liza Ho, Sophie Clément, Eric Pujade-Lauraine, Thomas A. McKee
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The following represents disclosure information provided by authors of this manuscript. All relationships are considered compensated unless otherwise noted. Relationships are self-held unless noted.
I = Immediate Family Member, Inst = My Institution. Relationships may not relate to the subject matter of this manuscript. For more information about ASCO's conflict of interest policy, please refer to www.asco.org/rwc or ascopubs.org/po/author-center.
Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians (Open Payments).

Yann Christinat
Research Funding: AstraZeneca, Cambridge, UK and Merck Sharp & Dohme LLC (Inst)

Jalid Sehouli
Honoraria: AstraZeneca, Eisai, Clovis Oncology, Olympus Medical Systems, Johnson & Johnson, PharmaMar, Pfizer, Teva, Tesaro, MSD Oncology, GlaxoSmithKline, Bayer
Consulting or Advisory Role: AstraZeneca, Clovis Oncology, PharmaMar, Merck, Pfizer, Tesaro, MSD Oncology, Lilly, Novocure, Johnson & Johnson, Roche, Ingress Health, Riemser, Sobi, GlaxoSmithKline, Novartis, Alkermes
Research Funding: AstraZeneca (Inst), Clovis Oncology (Inst), Merck (Inst), Bayer (Inst), PharmaMar (Inst), Pfizer (Inst), Tesaro (Inst), MSD Oncology (Inst), Roche (Inst)
Travel, Accommodations, Expenses: AstraZeneca, Clovis Oncology, PharmaMar, Roche Pharma AG, Tesaro, MSD Oncology, Olympus

Antonio Gonzalez Martin
Consulting or Advisory Role: Roche, Tesaro/GSK, Clovis Oncology, AstraZeneca, MSD, Genmab, Immunogen, Oncoinvent, Pfizer/EMD Serono, Amgen, Mersana, SOTIO, Sutro Biopharma, MacroGenics, Novartis, Alkermes, Hedera Dx, Novocure, Seagen, Takeda
Speakers' Bureau: Roche, AstraZeneca, Tesaro/GSK, PharmaMar, Clovis Oncology, MSD Oncology
Research Funding: Roche (Inst), Tesaro/GSK (Inst)
Travel, Accommodations, Expenses: Roche, AstraZeneca, PharmaMar, Tesaro/GSK, MSD Oncology

Keiichi Fujiwara
Honoraria: Zeria Pharmaceutical, Chugai Pharma, Eisai, Taiho Pharmaceutical, Daiichi Sankyo, Takeda, Genmab
Consulting or Advisory Role: MSD, Taiho Pharmaceutical, Eisai, Takeda, Genmab, NanoCarrier, Seagen
Research Funding: AstraZeneca (Inst), MSD (Inst), Regeneron (Inst), Genmab (Inst), Seagen (Inst)
Travel, Accommodations, Expenses: Genmab

Ignace Vergote
Consulting or Advisory Role: AstraZeneca, Elevar Therapeutics, Genmab, Immunogen, Jazz Pharmaceuticals, Mersana, MSD, Novocure, Sotio, Verastem, Zentalis, Roche, Agenus, Eisai, Novartis, Seagen, Akeso Biopharma, Bristol Myers Squibb, Deciphera, Exelixis, GlaxoSmithKline, Karyopharm Therapeutics, Oncoinvent, OncXerna Therapeutics, Regeneron, Sanofi
Research Funding: Roche (Inst), Amgen (Inst), Oncoinvent (Inst)
Travel, Accommodations, Expenses: Karyopharm Therapeutics, Genmab, Novocure

Germana Tognon
Consulting or Advisory Role: GlaxoSmithKline, MSD

Sakari Hietanen
Consulting or Advisory Role: GlaxoSmithKline, AstraZeneca, MSD
Speakers' Bureau: AstraZeneca, GlaxoSmithKline

Isabelle Ray-Coquard
Honoraria: Roche, PharmaMar, AstraZeneca, Clovis Oncology, Tesaro, MSD Oncology, Genmab, AbbVie, Pfizer, Bristol Myers Squibb, GlaxoSmithKline,

Deciphera, Mersana, Amgen, Advaxis, OxOnc, Seagen, MacroGenics, Agenus, Sutro Biopharma, Novartis, Daiichi Sankyo
Consulting or Advisory Role: Pfizer, AbbVie, Genmab, Roche, AstraZeneca, Tesaro, Clovis Oncology, PharmaMar, MSD Oncology, Bristol Myers Squibb, Deciphera, Mersana, GlaxoSmithKline, Agenus, MacroGenics, Seagen, BMS, Novartis, Novocure, OSE Pharma, Daichi, Sutro Biopharma, Eisai, Blueprint Medicines
Research Funding: MSD Oncology, BMS, Roche/Genentech (Inst)
Travel, Accommodations, Expenses: Roche, AstraZeneca, Tesaro, PharmaMar, GlaxoSmithKline, Clovis Oncology, Clovis Oncology, BMS, Advaxis
Uncompensated Relationships: Arcagy-Gineco, French National Cancer Institute (INCA), Italian Health Authorities, German Health Authorities, Belgium Health Authorities

Eric Pujade-Lauraine
Employment: Arcagy-Gineco
Honoraria: AstraZeneca, GlaxoSmithKline
Consulting or Advisory Role: AstraZeneca, Roche, Merck, Incyte, Agenus
Research Funding: AstraZeneca (Inst)
Other Relationship: Arcagy-Gineco

Thomas A. McKee
Honoraria: AstraZeneca, GlaxoSmithKline
Research Funding: AstraZeneca

No other potential conflicts of interest were reported.

ACKNOWLEDGMENT

We would like to thank AstraZeneca UK and Merck Sharp & Dohme for their financial support, ARCAGY for having put in place this wonderful HRD initiative and having invited us to participate in it, and the whole molecular pathology laboratory in Geneva for their quality work with the OncoScan assay.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, et al: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394-424, 2018
2. National Cancer Institute: Ovarian Cancer—Cancer Stat Facts. Surveillance, Epidemiology, and End Results Program. 2015. https://seer.cancer.gov/statfacts/html/ovary.html%0Ahttps://seer.cancer.gov/statfacts/html/mulmy.html
3. Foo T, George A, Banerjee S: PARP inhibitors in ovarian cancer: An overview of the practice-changing trials. Genes Chromosom Cancer 60:385-397, 2021
4. Farmer H, McCabe H, Lord CJ, et al: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434:917-921, 2005
5. Ray-Coquard I, Pautier P, Pignata S, et al: Olaparib plus bevacizumab as first-line maintenance in ovarian cancer. N Engl J Med 381:2416-2428, 2019
6. Pujade-Lauraine E, Brown J, Barnicle A, et al: Homologous recombination repair mutation gene panels (excluding BRCA) are not predictive of maintenance olaparib plus bevacizumab efficacy in the first-line PAOLA-1/ENGOT-ov25 trial. Gynecol Oncol 162:S26-S27, 2021
7. González-Martín A, Pothuri B, Vergote I, et al: Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med 381:2391-2402, 2019
8. Coleman RL, Fleming GF, Brady MF, et al: Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. N Engl J Med 381:2403-2415, 2019
9. Abkevich V, Timms KM, Hennessy BT, et al: Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. Br J Cancer 107:1776-1782, 2012
10. Birkbak NJ, Wang ZC, Kim J-Y, et al: Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. Cancer Discov 2:366-375, 2012
11. Popova T, Manié E, Rieunier G, et al: Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. Cancer Res 72:5454-5462, 2012
12. Macintyre G, Goranova TE, De Silva D, et al: Copy number signatures and mutational processes in ovarian carcinoma. Nat Genet 50:1262-1270, 2018
13. Castroviejo-Bermejo M, Cruz C, Llop-Guevara A, et al: A RAD 51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation. EMBO Mol Med 10:e9172, 2018
14. van Wijk LM, Kramer CJH, Vermeulen S, et al: The rad51-ffpe test; calibration of a functional homologous recombination deficiency test on diagnostic endometrial and ovarian tumor blocks. Cancers (Basel) 13:1-15, 2021
15. Bielski CM, Zehir A, Penson AV, et al: Genome doubling shapes the evolution and prognosis of advanced cancers. Nat Genet 50:1189-1195, 2018
16. Christinat Y: oconoscanR. https://github.com/yannchristinat/oncoscanR
17. TCGA_Pancancer. https://www.synapse.org/#!Synapse:syn1710464
18. Carter SL, Cibulskis K, Helman E, et al: Absolute quantification of somatic DNA alterations in human cancer. Nat Biotechnol 30:413-421, 2012
19. Bell D, Berchuck A, Birrer M, et al: Integrated genomic analyses of ovarian carcinoma. Nature 474:609-615, 2011
20. cBioPortal. https://www.cBioPortal.org
21. Pujade-Lauraine E, Christinat Y, D'incalci M, et al: 201 Homologous recombination deficiency testing in advanced ovarian cancer: Description of the ENGOT HRD European initiative. Int J Gynecol Cancer 31:A208, 2021
22. Andy Bunn MK: R: A language and environment for statistical computing. R Found Stat Comput 10:11-18, 2017
23. Sztupinszki Z, Diossy M, Krzystanek M, et al: Migrating the SNP array-based homologous recombination deficiency measures to next generation sequencing data of breast cancer. npj Breast Cancer 4:1-4, 2018
24. Melinda LT, Kirsten MT, Julia R, et al: Homologous recombination deficiency (hrd) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. Clin Cancer Res 22:3764-3773, 2016
25. Timms KM, Abkevich V, Hughes E, et al: Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. Breast Cancer Res 16:475, 2014
26. Tattersall A, Ryan N, Wiggans AJ, et al: Poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of ovarian cancer. Cochrane Database Syst Rev 2:CD007929, 2022
27. Hardenbol P, Banér J, Jain M, et al: Multiplexed genotyping with sequence-tagged molecular inversion probes. Nat Biotechnol 21:673-678, 2003
28. Krais JJ, Johnson N: BRCA1 mutations in cancer: Coordinating deficiencies in homologous recombination with tumorigenesis. Cancer Res 80:4601-4609, 2021
29. Banerjee SN, Lord CJ: First-line PARP inhibition in ovarian cancer—Standard of care for all? Nat Rev Clin Oncol 17:136-137, 2020
30. Swisher EM, Aghajanian C, O'Malley DM, et al: Impact of homologous recombination status and responses with veliparib combined with first-line chemotherapy in ovarian cancer in the Phase 3 VELIA/GOG-3005 study. Gynecol Oncol 164:245-253, 2022