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REVIEW

NEW PERSPECTIVES OF SELECTION TESTS FOR THE TREATMENT WITH PARP INHIBITORS OF OVARIAN CANCER PATIENTS: A MINI-REVIEW

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ABSTRACT: The introduction of PARP inhibitors has revolutionized the treatment landscape for ovarian cancer patients, particularly those with germline mutations in BRCA1/2 genes. Nevertheless, not all patients respond to these treatments in the same way, which shows the necessity of appropriate patient selection to determine which individuals are most likely to benefit. Innovative tests that are more reliable at predicting treatment outcomes have emerged as a result of recent developments in molecular diagnostics and biomarker identification. This review focuses on the most recent information on selection tests for PARP inhibitor therapy in patients with ovarian cancer and explores the importance of genomic scar, genetic alterations, and homologous recombination (HR) deficiency (HRD) scores.

HRD is a dynamic process that can be modified by chemotherapy; HRR may be restored by multiple mechanisms of resistance to PARPi or platinum agents. Differences between the test and the clinical response to PARPi can be attributed to these mechanisms of resistance, which genetic tests are unable to identify. The objective of this review is to describe different available methods to test homologous recombination functionality and to differences, weaknesses and straights of different tests. In addition, we want to describe a new academic functional assessment, RAD51 assay, which can detect the dynamicity of HR status, so it can help in the improvement of patient selection therapies.

By introducing these new selection tests into the clinical practice, the management of patients with ovarian cancer employing PARP inhibitors can be improved, leading to better treatment outcomes, reduced unnecessary toxicity, and an overall development in patient care.

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Impact statement: The article aims to present the available methodological differences for studying homologous recombination deficiencies, providing the pros and cons of each test. Additionally, we aim to present the advantages of the RAD51 academic assay and its utility in clinical practice.

Key words: *ovarian cancer; poly-ADP ribose inhibitors (PARPi); homologous recombination deficient (HRD); functional test.*

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INTRODUCTION

Ovarian cancer (OC) is the deadliest among gynecological malignancies and occupies the tenth place among all female cancers (3%) in Italy (1). Its high mortality is attributable to nonspecific, late symptomatology and the absence of validated screening strategies for early detection, except for women with germline mutations in BRCA1 and BRCA2 (BRCA1/2) genes that have a life-time risk of developing OC ranging from 30% to 70% (2, 3). Approximately 75-80% of patients have advanced disease at the time of diagnosis (FIGO stage III-IV); neoplasms limited to ovaries (FIGO stage I) or pelvis (FIGO II) are uncommon (10%) and they can be revealed during routine gynecologic checkups or, more commonly, incidentally during other surgical procedures (1).

The standard therapeutic approach consists of cytoreductive surgery followed by platinum-based chemotherapy; however, it is estimated that about 75%-80% of patients with OC will relapse in their lifetime with a five-year survival rate of about 47% (2). In recent years, the treatment of OC has deeply improved with the introduction of biological therapies, such as anti-angiogenic agents (bevacizumab) and PARP inhibitors (PARPi) (4). PARPi are a class of drugs that inhibit the enzyme poly (ADP-ribose) polymerase (PARP), which is crucial in the repair of DNA single-strand breaks (SSBs); by preventing the repair of SSBs, they lead to the accumulation of double-strand breaks (DSBs), which are lethal to the cells (4). Tumors harboring a deficiency in the homologous recombination repair (HRR) pathway (so called HRD tumors), such as tumors with mutations in BRCA1/2 genes, are sensitive to PARPi (4). The PARPi olaparib, niraparib, and rucaparib are currently approved for the treatment of High-Grade Serous OC (HGSOC) (4). Initially, these inhibitors were assessed and approved in the recurrent setting but following the groundbreaking results of trials such as SOLO1 (5), PRIMA (6) and ATHENA-MONO (7), they have been integrated into first-line therapy maintenance regimens. Olaparib was the first PARPi to be approved by the Food and Drug Administration (FDA) in 2014 thanks to the phase II Study 19 (8) and phase III SOLO2 (9) studies as second-line or later maintenance monotherapy in BRCA1/2-mutated patients in response to platinum-based chemotherapy. Moreover, olaparib was also approved by the FDA in 2018 in the first line setting based on the results of SOLO1 (phase III) (5) trial as maintenance treatment for platinum-responsive BRCA1/2-mutated HGSOC patients.

As stated, the efficacy of PARPi was initially restricted to platinum sensitive BRCA1/2-mutated OC. To improve the selection strategy, three methods to detect HRD, beyond BRCA1/2 mutations, have been tested in clinical trials (10): 1) alterations in HRR genes, such as gene panels revealing germline and somatic mutations, and epigenetic modifications in genes currently known to take part to the HRR pathway; 2) genomic scars and genomic signatures: cancer cells and cells with BRCA1/2 variants are characterized by genomic instability; they exhibit copy number modifications and numerous somatic changes in the genome, including single-nucleotide polymorphisms and structural aberrancies (structural variants, SVs) (10). The evaluation of these genomic characteristics allows the identification of tumors with a history of HRD, regardless of the underlying etiology. Two commercial tests related to genomic scars have been approved by the FDA and used to identify tumors with HRD: the 'myChoice HRD' kit by Myriad, which inquires the LOH (loss of heterozygosity), the TAI (telomeric allelic imbalance) and the LST (large-scale transitions) throughout the genome (11); the 'FoundationFocus™ CDx BRCA LOH', which is conceived to identify the occurrence of variants in BRCA1/2 and the amount of the genome impacted by LOH in tumor-derived DNA of patients with HGSOC (12). According to genomic assays, tumors can be classified in HRdeficient (HRD) and HR pro-

ficient (HRP); 3) functional assay of HRD (13): functional assay has the potential to provide a dynamic readout of actual, extant, HRR status. The most commonly used experimental system to assess HRR is to estimate the amount of nuclear RAD51, a downstream HR protein (a DNA recombinase) that enables high-fidelity double strand DNA repair by facilitating DNA strand invasion into the sister chromatid, a process supported by the BRCA1/PALB2/BRCA2 complex. Several approaches, both *in vivo* and *in vitro*, underpinned the highly sensitive and specific predictive value of the absence of nuclear RAD51 foci in response to PARPi therapy. According to the functional assay, tumors can be classified in HRD and HRP. **Table 1** resumes principal differences between these tests.

Genomic HRD was implemented in several clinical trials, including PAOLA1, PRIMA and ATHENA-MONO trials. The phase III PAOLA-1 (14) study evaluated olaparib in combination with bevacizumab

Table 1. *Different methodologies to evaluate* HRR *condition.*

	ANALYSES	TYPE OF TESTS	ADVANTAGES	LIMITATIONS
Genetic alterations	Mutations in HRR gene pathway Germline mutations Somatic mutations	NGS technology	Simultaneous analyses of 23 genes	Static test No data about pathway function
Genomic scars/ genomic signatures	LOH, TAI, LSTs	Myriad myChoice FoundationOne HRDetect	Predictive information Validated test	Static test
Functional dynamic assay	RAD51 Nuclear foci quantification	Immunofluorescence	Dynamic test Predictive test	Operator-dependent test No validation in clinical practice

NGS: Next Generation Sequencing; LOI: Loss of Heterozygosis; TAI: Telomeric Allelic Imbalance; LST: Large-scale transitions.

Table 2. *PARP inhibitors approval in clinical trials.*

	FIRST-LINE MONOTHERAPY MAINTENANCE			SECOND-LINE MONOTHERAPY MAINTENANCE		
	CLINICAL TRIAL	PATIENT SUBGROUP	APPROVAL GRANTED	CLINICAL TRIAL	PATIENT SUBGROUP	APPROVAL GRANTED
Olaparib	Phase III SOLO-1 trial	Platinum-responsive BRCA1/2 mutated HGSOC patients	FDA 2018 EMA 2019	Study 19 phase II and phase III SOLO-2 trials	BRCA1/2 mutated in response to platinum-based chemotherapy	FDA 2014 EMA 2018
Niraparib	Phase III PRIMA trial	HGSOC platinum-sensitive patients regardless the biomarker status	FDA 2020 EMA 2020	Phase III NOVA trial	BRCA1/2 mutated patient with recurrent, platinum-sensitive ovarian cancer	FDA 2017 EMA 2017
Rucaparib	Phase III ATHENA-MONO trial	Patients with advanced HGSOC regardless BRCA1/2 mutations	FDA 2023	Phase III ARIEL trial	BRCA1/2 mutated patient with high-grade, recurrent, platinum-sensitive ovarian cancer	FDA 2018 EMA 2018

PARP: Poli-ADP-Ribose Polymerase; HGSOC: High Grade Serous Ovarian Cancer; FDA: Food and Drug Administration; EMA: European Medicines Agency.

for the maintenance treatment of patients with advanced HGSOC who are in response to first-line platinum-based chemotherapy. It demonstrated that the efficacy of the combination was restricted to BRCA1/2-mutated and HRD+ tumors, leading to its FDA and the European Medicines Agency (EMA) approval in clinical practice. As a result of the PRIMA (6) trial niraparib was authorized by FDA in 2020 and in the same year was approved by EMA for HGSOC platinum-sensitive patients regardless the biomarker status. Thanks to ATHENA-MONO study (7), rucaparib was approved by FDA and EMA in 2023 for patients with advanced HGSOC, regardless of BRCA1/2 mutations (**Table 2**).

HRD testing is essential in the newly diagnosed setting, regardless of the PARPi employed, for identifying which patients may benefit most from PARPi maintenance therapy and to guide treatment choices. Newly diagnosed HGSOC patients with a BRCA1/2 mutation or those who tested positive for HRD in clinical trials benefit more from PARPi maintenance therapy.

The objective of this review is to prove that the identification and validation of these biomarkers, through RAD51 functional assay, are essential to improve the clinical use of PARPi, allowing for personalized treatment and improved outcomes for HGSOC patients.

HRD TESTS POTENTIALLY USEFUL FOR THE TREATMENT OF OVARIAN CANCER PATIENTS

HRD tests can be classified into genomic tests (NGS panels, genomic scars and gene signatures) and functional tests (*i.e.*, RAD51 assay) (**Figure 1**).

Genomic scars are currently used to assess HRD status use microarray technologies based on single nucleotide polymorphism (SNP) to detect the copy number variation (CNV). In three studies conducted in 2012 CNV was used to predict BRCA status through LST, LOH and TAI. Subsequent studies showed that the combination of these parameters was able to identify HRP and HRD tumors (10). Based on this, two tests are currently used in clinical practice: Myriad Genetics and FoundationFocus CDxBRCA Foundation Medicine. The latter determines the percentage of sub-chromosomic genomic LOH fraction through next generation sequencing (NGS), considering percentage higher than 16% as high LOH fraction. On the other side, Myriad test combines LOH, LST and TAI with BRCAmutated test thus revealing a genomic instability score (GIS) with a threshold of 42 (10).

Every mutational event leaves a 'genomic signature', which is defined by the type of DNA damage as a

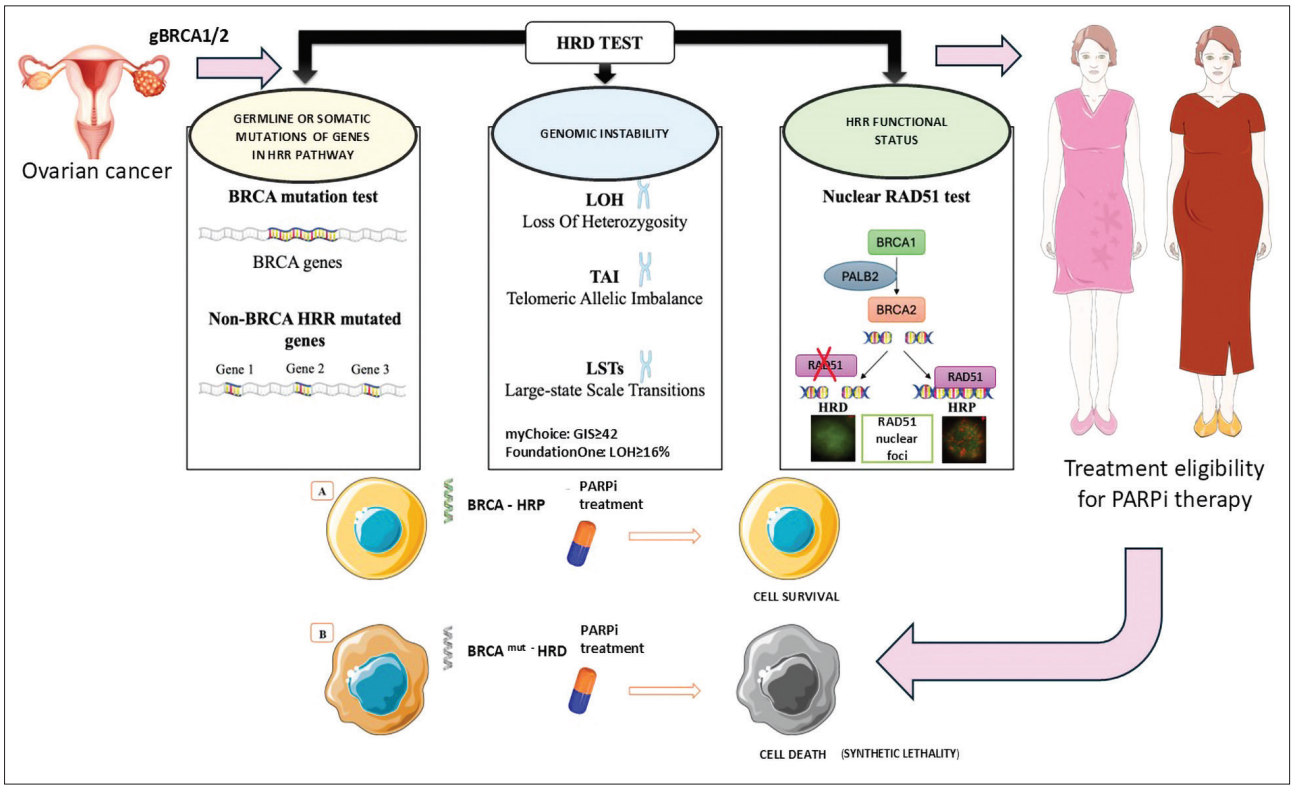

Figure 1. *Graphic summary of the HRD tests available. Abbreviations: gBRCA1/2: germinal BRCA1/2 mutation; HRR: Homologous Recombination Repair; LOI: Loss of Heterozygosis; TAI: Telomeric Allelic Impalance; LST: Large-Scale Transitions; HRP: Homologous Recombination Proficent; HRD: Homologous Recombination Deficent, PARPi: PARP inhibitor.*

result of internal/external agents and mechanisms of repair, and it could be due to the driver mutation, as well as some additional mutations which occur at different stages (15). These genomic signatures could be identified on the surgical specimen (15) and, in HGSOC, they are related to well-defined clinical characteristics, including long term outcomes and platinum response (10). Alexandrov *et al*. found a way to detect punctiform mutational signatures and, by analyzing 2400 tumors, they identified 49 SSB mutational signatures. Despite this, this is not a robust biomarker since lacks specificity, and it is currently difficult to determine a threshold (16). In addition, GIScar is a French academic test based on targeted DNA sequencing using a 127-gene panel in formalin-fixed paraffin embedded (FFPE) tumors determining a GIS (17). It has been shown that it reliably detects HRD positivity and predicts sensitivity to olaparib with bevacizumab maintenance therapy in patients with ovarian cancers with high analytical concordance with Myriad test. However, it was not superior to Myriad in predicting progression free survival (PFS) and it was validated using a retrospective cohort (17). In fact, the ideal test should be able to get as much information as possible on tumor genome and to ensure high sensitivity and specificity. With this goal, a new test has been developed, by considering the whole genome data: this is HRDetect. It exploits an algorithm based on all the 4 mutational classes (BRCA1/BRCA2 germline and somatic mutations), by measuring 6 genomic characteristics (10, 18). HRDetect was able to predict BRCA deficit with high sensitivity (98.7% in breast cancer, 86% in a breast cancer validation cohort of 80 patients, and up to 100% in ovarian cancer and in pancreatic cancer validation cohorts). Patients with monoallelic BRCA loss had a low HRDetect score (18). There is evidence on the possibility to predict clinical response to platinum in BC patients; on the contrary, the ability to predict PARPi response in HGSOC has not been proved yet. One limitation of these tests is sample conservation, since the majority of samples are FFPE, because DNA fragmentation and artefacts due to formalin fixation might impair the readout of these tests.

However, genomic scars and signatures reflect the HRD history of the lesion, but they do not reflect the current HRD status present at the time of core biopsy or tumor excision; thus, the HRR restoration is not detectable by genomic tests (10). All the HRD tests based on genomic scar evaluation can only indicate that a deficit of HRR occurred. However, genomic scarring would be detected also in tumors in which the function of HRR pathway has been restored, such as in tumors progressed following platinum-based systemic therapy or PARPi (19). Leibowitz *et al*. performed a genomic and transcriptomic profiling using NGS from Tempus xT, Tempus xO, Tempus xE, Tempus RS, and Tempus RS.v2 assays on 48,843 samples. Tempus HRD platform comprises two assays: HRD-DNA, which measures Genome-wide LOH (gwLOH) to distinguish BRCA-biallelic (HRD+) samples from HRR-WT (HRD-) samples for both breast and ovarian cancers to predict HRD status and HRD-RNA, a logistic regression model trained on whole-exome capture RNA sequencing data to identify a pan-tumor gene expression signature of HRD (20). This study suggested that an RNA-based measure of HRD may capture dynamic changes in HRD phenotype upon tumor evolution (20).

In fact, HRD is dynamic, is modified by chemotherapy, and HRR could be restored through different mechanisms of resistance to PARPi or to platinum agents. Genomic tests are not able to detect these mechanisms of resistance which are responsible for the discordance between the test response and the clinical response to PARPi (21). Hence the development of functional assays which could allow the dynamic detection of the current HRR status (22, 23). Currently, the most reliable functional test is the RAD51 assay, an immunofluorescence-based test to detect RAD51 nuclear foci in formalin-fixed paraffin (FFPE) samples (13, 24) which leads to the quantification of nuclear RAD51, whose function is explicated by the BRCA1/PALB2/BRCA2 complex (23). The pre-clinical and clinical utility of the assay has been tested in early triple-negative BC (TNBC), in HGSOC and in castration-resistant prostate cancer treated with the PARPi and/or platinum salts (23).

In the work by Pellegrino *et al*. (23) the RAD51 test accuracy was compared with other genomic tests. It showed higher accuracy than HRR gene mutations and genomic HRD analysis for predicting PARPi response (95% RAD51 score (≤10), 67% BRCA1/2, PALB2 mutations, and 71% Genomic HRD score (≥42)) and it captured dynamic changes in HRR status upon acquisition of PARPi resistance. In addition, the RAD51 test identifies early BC tumors that benefit from platinum salts and PARPi, as proved in GeparSixto and PETREMAC trials, respectively (25, 26). In biopsies of HGSOC patients (the CHIVA trial) (27), the RAD51 assay identified platinum sensitive tumors. However, in Capoluongo and Pellegrino *et*

al. (28), the predictive role to platinum salts of the RAD51 assay was not confirmed on surgery-derived HGSOC samples; indeed, the functional assay re-classified more than 20% of genomic HRD- tumors as HRP (77% *vs*. 58%).

In addition, RAD51 assay may be able to predict the pathogenicity of variant of unknown significance (VUS), as recently suggested by Casartelli and Tommasi *et al.* (29) and it should be investigated in a wider population to highlight the potential predictive role of this functional test.

To summarize, this assay is able to capture: 1) HRD in tumors with BRCA1/2 or PALB2 mutations, or with epigenetic silencing of an HRR gene; or 2) HRP in tumors with previous history of HRD that have restored HRR as a mechanism of resistance (23).

CLINICAL IMPACT OF HRD TESTS AND FUTURE PROSPECTIVES

Ovarian cancer treatment has improved significantly with the introduction of PARPi, especially for those with germline mutations in the BRCA1/2 gene. Because each patient responds differently, though, it is crucial to develop suitable predictive biomarkers in order to determine which patients may benefit the most from these treatments. In this setting, there is an increased need for new screening tests that can more precisely predict the effects of therapy. Identification of HRD tumor is fundamental to define the most effective maintenance treatment in HGSOC patients. As discussed earlier, HRD tumors are more sensitive to PARPi, so new methods have been assessed to improve the patient selection such as germline and somatic/epigenetic alteration in HRR genes, genomic scars and genomic signatures, and functional RAD51 assay.

The two main tests used in clinical practice are Myriad myChoice, which combines LOH, LST and TAI with BRCA1/2 mutational status, and FoundationFocus CDxBRCA Foundation Medicine that determines the sub-chromosomic genomic LOH fraction percentage through NGS (10, 30). According to PAOLA-1 study (14), administering bevacizumab with olaparib in advanced HGSOC patients as maintenance therapy, HRD+ patients most benefited from the treatment (with a score ≥ 42, tested by Myriad myChoice). By contrast in PRIMA (6) and ATHENA-MONO (7) trials, where niraparib and rucaparib, respectively, were compared with placebo, the HRD status correlates with the magnitude of benefit, but it is not capable to identify who do not benefit at all from PARPi. The updated results of the PRIMA trial did not show any statistically significant difference in terms of OS between treatment and placebo arm, confirming the lack of predictive role of genomic HRD (31). The hypothesis that there may be an HRP patients' subgroup who might benefit from PARPi, is supported by these data.

Myriad Genetics' MyChoice CDx assay was approved by FDA to determine HRD status. Recently, different providers have developed commercial assays that employ various methodologies to study the HRD status, assigning a negative or a positive GI value; HRD- cases were defined by negative BRCA1/2 mutational status and a negative GI score. An overall agreement rate of 97.6%, 90.7% and 90.4% was observed for TSO500, OCAPlus and SOPHIA when compared with Myriad, respectively (19). When patients were classified as HRD+/HRD- with TSO500, SOPHIA and OCAPlus, they observed a trend in favor of HRD+ patients for RR, PFS and OS similar to Myriad. In conclusion, they demonstrated a good agreement rate and similar correlations with outcome between different commercial assays and the reference Myriad test for HRD detection in HGSOC patients. Considering these results, their clinical implementation for BRCA1/2 and HRD testing is recommended to spread and reduce the costs of genomic HRD, as predictive biomarker of response to PAOLA1 combination.

To assess HRD status can be the identification of the genomic signature, a DNA damage determined by mechanism repair errors due to internal/external agents. It can be caused by driver mutations and other mutations that arise at different phases of ontogenetic process (15). The genomic signatures are detectable on the surgical sample and are associated with specific clinical features in HGSOC, such as survival and platinum response (10, 15). 49 SSB mutational signatures have been identified by Alexandrov *et al*. analysis over 2400 tumors. Nevertheless, because of its poor specificity and the difficulty in establishing a threshold, this signature is not very reliable (32). As seen before, in Leman *et al*. (17) GIScar French test was evaluated and compared to Myriad Genetics MyChoice test and it showed a lower proportion of inconclusive results (1% *vs*. 9%, respectively). However, further clinical validation is needed to confirm its predictive value.

The new developed test HRDetect (18) was able to identify BRCA1/2 mutations with high sensitivity and predict PARPi response in BC (33). Even if HR-

Detect had excellent sensitivity also in HGSOC, its predictive value to PARPi has not been proved yet, as mentioned in Davies *et al*. (18).

Genomic tests are not capable of recognizing the HRR restoration (23), possibly leading to a discordance between test responses and the clinical activity of PARPi, like in PRIMA and ATHENA trial.

To overcome this issue, the RAD51 functional and dynamic assay has been tested in preclinical and clinical samples (13, 24). Its abilities have been evaluated in TNBC, HGSOC and in castration-resistant prostate cancer treated with the PARPi Olaparib (25-27, 34). This technique has been proved to identify HRD in BRCA1/2- or PALB2-mutated tumors or epigenetic silencing in HRR genes, and HRP in tumors with previous history of HRD that have restored HRR as a mechanism of resistance. Nevertheless, this assay does not identify tumors whose sensitivity to PARPi lies on a DNA repair deficiency outside of the HRR core pathway; and it does not identify secondary PARPi-resistance due to mechanisms not involving restoration of HRR (23). Failure rates varied widely according to cancer and sample types (ranging from 5% in biopsies from BC to 30% in surgery samples of HGSOC). Capoluongo and Pellegrino *et al.* registered a certain discrepancy with genomic HRD score in HGSOC: RAD51 may identify this subgroup of HRD- tumors according to genomic scar that may respond to PARPi as classified HRD by the functional test. To verify this hypothesis, it will be crucial to prospectively test both genomic and functional assays on samples from BRCA1/2-WT patients enrolled in the MITO35 trial that have been treated with olaparib as first line maintenance treatment (35). In the future, RAD51 assay could be useful to better select treatment for patients in every phase of disease, thanks to its potential predictive impact. New clinical trials are necessary to amplify the knowledge about this test and to validate it in the clinical practice.

CONCLUSIONS

Several tests have been developed to select patients who are qualified for different treatments for OC therapy. Despite the aforementioned limitations, the new functional test of RAD51 is extremely useful in cases where the other tests are unable to identify the restoration of HRR. Further clinical validation is needed in order to confirm its predictive value to PARPi.

COMPLIANCE WITH ETHICAL STANDARDS

Funding and conflict of interests

MB has reported advisory board for GSK, MSD, EISAI, Astrazeneca, Roche and Abbvie; lectures fees from GSK, AstraZeneca, MSD, and has received a travel grant from Pharmamar outside the submitted work. GG reported personal fees from Mylan, AccMed, Polistudium, MIT and Collage SPA outside the submitted work.

Availability of data and materials

The data underlying this article are available within it.

Authors' contributions

BP: conceptualization; MC, CTom, CC, NC and BP: methodology; CT, MC, CTom, AI and BP: resources; CT, AI, CC, AS, DZ and BP: writing-original draft preparation; CT, CTom, AI and BP: writing-review and editing; CT and AI: visualization; OS, NC, GG, LM, MB, AM: supervision; CTom and BP: project administration. All Authors have read and agreed to the published version of the manuscript.

Ethical approval

Human studies and subjects

N/A.

Animal studies

N/A.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real.

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