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REVIEW

BIOMARKERS OF HOMOLOGOUS RECOMBINATION DEFICIENCY IN THE ERA OF PARP INHIBITORS

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ABSTRACT

Homologous Recombination Deficiency (HRD) was initially described in cancers with germline mutations of *BRCA1* and *BRCA2* and thereafter in both sporadic and hereditary cancers carrying muta-tions or epigenetic inactivation of other genes in-volved in HR. Since cancers harbouring HRD are particularly susceptible to PARP inhibitors (PARPi), identifying methods to detect HRD that can accu-rately predict clinical sensitivity to PARPi beyond *BRCA1/2* mutations has been challenging. In this review, we describe the HRD biomarkers identified up to now, pointing out strengths and weakness-es of each associated assay.

Multigene panel test-ing, genomic scar assays and the most recent func-tional assays developed in the last ten years are associated with several drawbacks, mainly due to the possible restoration of HR proficiency and tu-mor heterogeneity. The use of functional assays on samples obtained from liquid biopsy could overcome these issues, providing a dynamic readout of HRD status and helping in clinical decision-making especially in the recurrent setting. Composite HRD scores involving two or more biomarkers would be probably required to define "HRDness" and to predict response to PARPi alone or in combination regimens.

KEY WORDS

Omologous recombination deficiency; PARP inhibitors; genomic scar; BRCA1; BRCA2.

IMPACT STATEMENT

The current available biomarkers to infer the presence of HRD, including multigene panel testing, genomic scar and functional assays, are inadequate predictors of response to PARPi.

INTRODUCTION

Homologous recombination (HR) is a fundamental pathway that allows error-free repair of double-stranded DNA breaks (DSBs). HR operates during S and G2 phase of the cell cycle when a homologous sister chromatid is available as template and relies on many proteins including BRCA1 and BRCA2, MNR complex (MRE11/RAD50/NBS), RAD51, ATM, ATR, PALB2, BRIP1, and BARD1 (1). HR deficiency (HRD) induces activation of the more error-prone template-independent non-homologous end-joining (NEJH) pathway, which results in the accumulation of additional mutations and chromosomal instability (2).

HRD was initially described in cancers with germline mutations of *BRCA1* and *BRCA2* (*BRCA1/2*) (3). However, germline or somatic mutations or epigenetic inactivation of other genes involved in HR can lead to HRD in both sporadic and hereditary cancers, broadly termed BRCAness (4, 5). Cells whit HRD are particularly susceptible to the DNA damage induced by DSBs and crosslinks generating agents like platinum compounds (6, 7). Moreover, cells with mutant *BRCA1/2* are exquisitely sensitive to poly-(ADP-ribose) polymerase (PARP) enzyme PARP inhibitors (PARPi) (8, 9). The PARP1 subunit binds single-stranded DNA breaks (SSBs) and then organizes their repair by synthesising PAR chains on target proteins (the so-called PARylation) (10). Inhibition of PARP1 promotes SSBs, which, if unrepaired, consequently lead to DSBs by collapsing of the stalled replication fork during DNA replication (11). PARPi act mainly in a double way: by inhibition of the catalytic activity of PARP1, which results in synthetic lethality in cells with impaired HR, and by trapping PARP1 at sites of DNA damage (12, 13).

Other mechanisms of HR impairment beyond *BRCA1/2* mutations can similarly confer PARPi sensitivity; however, identifying methods to detect HRD that can accurately predict clinical sensitivity to PARPi has been challenging (14-16). *BRCA1/2* mutations and/or HRD status have been evaluated in clinical trials with PARPi (16-18). Multiple genomic biomarkers have been evaluated to presume the presence of HRD; although promising, these biomarkers are inadequate predictors of response to PARPi, with clinical benefits observed both with and without HRD as measured by current clinical assays (19). In this review, we aim to describe the HRD biomarkers identified up to know, pointing out strengths and weaknesses of each associated assay, and key challenges in the clinical use of HRD testing. An overview of HRD assays and their biological principles are summarized in **figure 1**.

GERMLINE AND SOMATIC MUTATIONS IN HR-RELATED GENES

Testing for germline and somatic mutations in *BRCA1/2* and other HR-related genes may be used to infer the presence of HRD. Germline *BRCA1/2* (*gBRCA*) mutations are present in 13-15% of epithelial non-mucinous ovarian cancer (OC) patients and an additional 5-7% of OC harbour somatic *BRCA1/2* (*sBRCA*) mutation that have arisen during cancer development or progression (20, 21). *BRCA1/2* mutant cells show clear evidence of HRD in vitro (22, 23). The main randomised clinical trials indicate that *gBRCA* mutations remain the best clinical biomarker for response to PARPi while limited data are available on *sBRCA* mutations alone, although the clinical outcomes for patients with *sBRCA* mutations were similar to those with *gBRCA* mutations (24-33). Retrospective analysis from Study 19 identified bi-allelic inactivation in > 80% of cases of *sBRCA* mutation and mutations were predominantly clonal, suggesting that *sBRCA* mutations arise early in tumorigenesis (34).

In vitro studies showed that beyond *BRCA1/2*, mutations in other HR-related genes can also confer an HRD phenotype and increased sensitivity

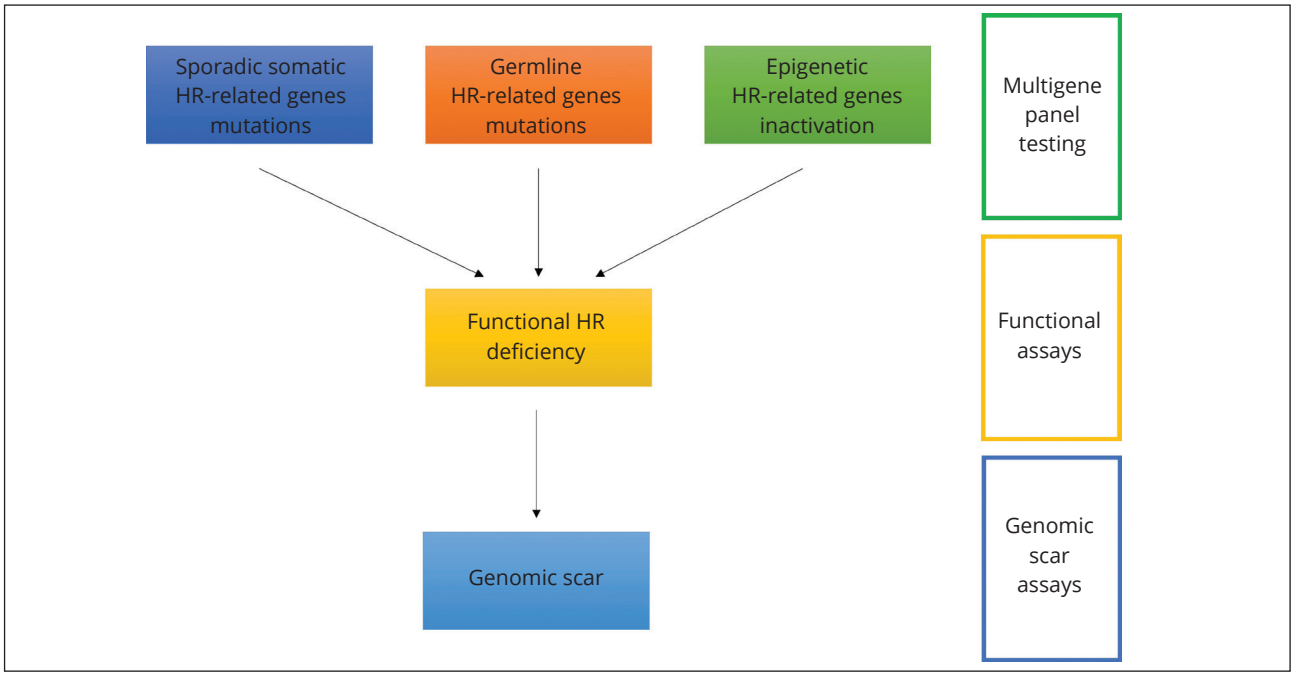

Figure 1. *An overview of HRD assays and their biological principles. Sporadic somatic or germline mutations (that can be detected with multigene panel testing) as well as epigenetic inactivation of HR-related genes induce a functional deficiency of HR recombination that can be revealed by functional assays. HRD and consequent defective DNA repair induces chromosomal aberrations, called genomic scars, detectable by specific assays.*

to platinum and/or PARPi (35). Cancer-associated mutations in *PALB2*, *BARD1*, *BRIP1*, *RAD51B*, *RAD51C*, *RAD51D*, *ATM*, *FAAP20*, *CHEK2*, *FAN1*, *FANCE*, *FANCM*, and *POLQ* (20,36,37), are potential biomarkers of HRD in cancer but how much these genes impact on PARPi response *in vivo* is still being defined due to the relative rarity of non-*BRCA* HR-related genes mutations (19). For example, mutations or methylation of *RAD51C* were identified in OC patients with clinical PARPi responses (38), and patients harbouring *RAD51C/D* mutations had long-term responses with rucaparib (39). *ATM* pathogenic variants are associated with olaparib response in OC and prostate cancer (40, 41).

Germline genetic testing is recommended for all women with OC, ideally with genetic counselling (18, 42). The blood-based assay Myriad Genetics BRACAnalysis CDx platform (Myriad Genetics; Salt Lake City, UT) has been FDA approved to identify OC patients with suspected pathogenic *gBRCA* variants eligible for treatment with olaparib (43). The phase III studies of PARPi in OC (Study 19 (33) and the NOVA trial (24)) and breast cancer (BC) (OlympiAD (25)) used BRACAnalysis to establish *gBRCA* mutation status (**table I**). Multigene germline panels, which extend the analysis to other genes as-

TRIAL	GBRCA TEST	SBRCA TEST	HRD TEST
SOLO1 (30)	Myriad BRCAnalysis	FoundationFocus BRCA	NA
PRIMA (29)	Local testing	Myriad myChoice HRD	Myriad myChoice HRD
PAOLA-1 (32)	NA	Myriad myChoice HRD	Myriad myChoice HRD
VELIA (27)	Myriad BRCAnalysis	Myriad myChoice HRD	Myriad myChoice HRD
Study 19 (33)	Myriad BRCAnalysis or local testing	Foundation medicine NGS	NA
SOLO2 (31)	Myriad BRCAnalysis	NA	NA
NOVA (24)	Myriad BRCAnalysis	Myriad myChoice HRD	Myriad myChoice HRD
ARIEL2 (37)	BRCA-HR Sequencing	FoundationFocus BRCA	FoundationFocus BRCA LOH
ARIEL3 (28)	Myriad BRCAnalysis	Foundation medicine NGS	FoundationFocus BRCA LOH

Table I. *HRD biomarkers and relative assays used in clinical trials of PARPi in ovarian cancer.*

sociated with increased cancer risk such as *BRIP1*, *RAD51C/D*, and *PALB2*, include both commercial and academic laboratory tests (44). There is currently no approved diagnostic assay for HRD based on germline mutations of other HR-related genes. Using germline mutations in HR genes to classify tumors as HRD has several disadvantages. In fact, is not always clear if a mutation truly disrupts gene function or is benign: the American College of Medical Genetics and Genomics provides guidelines for variants interpretation but in case of variants of uncertain significance (VUS) the genotype-phenotype correlation remains unclear (45). Somatic reversion mutations in *BRCA1/2* could restore HR function and confer platinum and PARPi resistance even with germline mutation (46).

The tissue based FoundationFocus CDx BRCA assay (Foundation medicine; Cambridge, MA) detects both *gBRCA* and *sBRCA* mutations in the tumor and is FDA approved as a companion diagnostic to rucaparib based on the ARIEL trials (28, 38) (**table I**). Multigene panels detecting somatic mutations in other genes than *BRCA1/2* may add additional information although mutations in non-*BRCA* HR-related genes are not currently part of an FDA-approved test to assess PARPi eligibility in OC (19). The limits of somatic testing include not only the difficult interpretations of VUS and the possibility of reversion mutations as for germline testing, but also the impossibility to analyse the intratumoral heterogeneity in a single tumor specimen.

PLATINUM SENSITIVITY

Platinum sensitivity *in vitro* is a feature of HRD, and *BRCA1/2* mutant OC and BC have increased platinum sensitivity (7, 47). As platinum is a key component of first line chemotherapy in OC, prior platinum sensitivity has been considered a surrogate clinical marker for prediction of PARPi efficacy (48). For example, in the phase III NOVA trial, Niraparib conferred a benefit in all subsets of platinum-sensitive OC, also in non-*BRCA* mutated patients (24). However, PARPi sensitivity does not completely overlap with platinum sensitivity in all cases (**figure 2**) (26). Considering cancers with defects in nucleotide excision repair, the response to platinum therapy does not confer a concurrent PARPi sensitivity (49). On the other hand, there is also a fraction of platinum-resistant patients who maintain PARPi sensitivity (50).

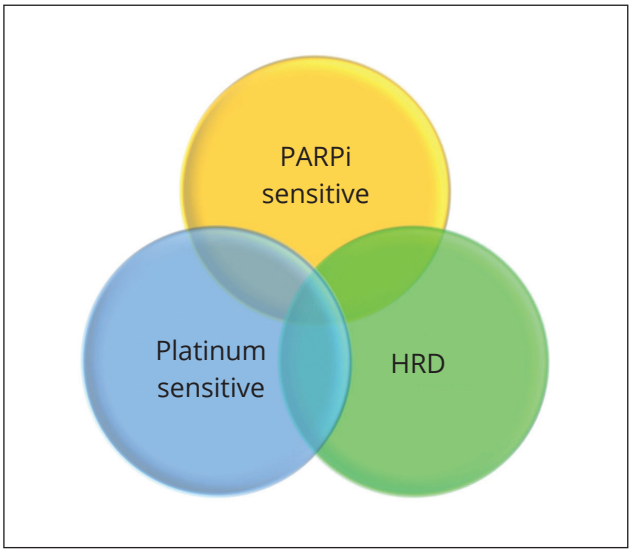

Figure 2. *HRD, platinum and PARPi sensitivity. Tumors with evidence of HRD, determined by the current available tests, are more likely to respond to platinum compounds and PARPi. However, PARPi sensitivity does not completely overlap with platinum sensitivite in all cases.*

GENOMIC SCAR ASSAYS

The loss of HR function and consequent defective DNA repair induces chromosomal aberrations, irrespectively of which component of the pathway was lost. "Genomic scars" of HRD consist of specific patterns of mutations and structural chromosomal aberrations, including rearrangements, insertions, and deletions in the genome (51). Current genomic scar assays are based on a combination of different genomic profiling techniques including array-based comparative genomic hybridization (aCGH), single nucleotide polymorphism (SNP) genotyping, and next generation sequencing (NGS).

aCGH of structural chromosomal rearrangements

The aCGH assay detects genomic copy number variation (CNV) in tumors (52). An aCGH genomic profiles analysis of primary BC identified four subgroups, two of which were enriched for *BRCA1/2* deficiency (53). However, only two-thirds of *BRCA1*-like tumors harbour either *BRCA1* mutation or promoter methylation. A *BRCA1*-like aCGH signature predicted favourable response to platinum, suggesting that this signature identifies a wider spectrum of HRD tumors (54). The *BRCA1*-like and *BRCA2*-like profiles were later combined to create a *BRCA*-like aCGH score that was evaluated ret-

rospectively in a BC clinical trial, where *BRCA*-like aCGH patients showed a statistically significant benefit from high-dose platinum-based therapy (55). Up to now, aCGH assays have not been evaluated in the context of PARPi.

SNP-based "genomic-scar" assays

In 2012, three studies reported SNP-based CNV assays to assess up to three types of genomic scarring patterns (56-58). Loss of heterozygosity (LOH) is the absence of one of two gene alleles at a heterozygous site or uniparental disomy due to inaccurate repair of sister chromatids during the S/G2 phase of cell cycle. A study in OC detected that a "HRD-LOH" score defined by the number of LOH regions of more than 15 Mb and shorter than the whole chromosome was associated with *BRCA1/2* deficiency (56). Large-scale transitions (LST) are chromosomal breaks between adjacent genomic regions longer than 10 Mb (after exclusion of region shorter than 3 Mb). Break points may be caused by chromosomal inversions, deletions, duplications, translocations, or other rearrangements. *BRCA1/2* and *RAD51C* deficient BC show higher LST than sporadic cancers (57, 59). Telomeric allelic imbalance (TAI) considers subchromosomal regions displaying allelic imbalance extended to one of the telomeres but not crossing the centromere longer than 11 Mb. TAI is consequence of aberrant chromosomal end fusion due to inappropriate end-joining during mitosis. TCGA data show elevated TAI in *gBRCA* mutated OC and higher levels of TAI correlates significantly with response to neoadjuvant platinum-based chemotherapy in triple-negative BC (58). HRD-LOH, LST, and TAI are correlated each other (60) and are associated with *BRCA1/2* deficiency independently and when combined into a single score (56-61).

Several combination HRD score have been described (62), with most data for the three-factor combination scar assay by Myriad Genetics (60). The Myriad myChoice test joins a combined HRD score called "Genomic Instability Score" (GIS) with mutation and rearrangement analysis of *BRCA1/2*. GIS consists of the unweighted sum of LOH, LST, and TAI which produces a continuous score between 0 and 100. A threshold for GIS was decided on a pooled set of BC and OC in which HRD was defined as biallelic *BRCA1/2* loss of function. A score of 42 corresponded to the 5th percentile of the set of known *BRCA*-mutant tumors, therefore a score of ≥ 42 was established to denote HRD and a score of < 42 was considered HR-proficient (63). Several PARPi clinical trials have incorporated the myChoice test (**table I**), with a score of ≥ 42 considered HR deficient in most trials (24, 29, 32). This assay was FDA-approved as a companion diagnostic for niraparib in relapsed OC and for olaparib with bevacizumab in newly diagnosed patients following front-line therapy (19).

The FoundationFocus™ CDx BRCA LOH assay was applied in clinical trials of rucaparib (28, 38) (**table I**) and it has been approved as a complementary diagnostic to determine tumor HRD status. In this assay, a percent genomic LOH is calculated based on the fraction of genome regions with LOH. The optimal cut-off from analysis of OC (56) was 14% genomic LOH, which was prospectively validated in the ARIEL2 study, where progression-free survival was longer in the LOH-high subgroup compared with LOH-low (38). In a subsequent phase III study of rucaparib ARIEL3, the cut-off was adjusted to 16% genomic LOH as the threshold to identify HRD tumors (28).

The combined HRD score and the percent genomic LOH only partial correlated in predicting HRD status (64). Both HRD tests have several drawbacks, since they estimate the likelihood of HRD in the tumor based on evidence of genomic scarring. However, genomic alterations induced by HRD are permanent, even if functional capacity of HR is restored, for example in case of reversions in *BRCA1/2*, hence HRD testing via one of these assays on archival tumor may not represent the current HRD status of the cancer cells. Furthermore, HRD test results may not perfectly predict PARPi response due to PARPi resistance mechanisms which overcome HRD. Finally, HRD tests can have false positives or negatives due to technical factors, empiric threshold to classify HRD patients not accurate for all and heterogeneity in HRD between biopsy site and other disease sites (19).

NGS-based mutational signatures

Cancer types carry distinct mutational signatures which reveal the impact of different mutational processes including aging, UV light, and DNA damage repair and replication defects. A set of mutational signatures were detected from whole-exome sequencing of human tumors using NGS and computational technologies (65, 66). One of these, "signature 3" is enriched in cancers with *BRCA1/2* mutations and other mechanisms of HRD and has

been shown to exist in several cancers, including BC, OC, pancreatic, prostate, and gastric. It has been proposed as a biomarker for HRD (67, 68). A computational tool called Signature Multivariate Analysis (SigMA) can identify the presence of signature 3 on targeted gene panel data and does not require whole-exome sequence data. However, the sensitivity for identification of signature 3 is only 74% (67).

HRDetect (69) was developed using whole genome sequence data from *BRCA* mutant and wild-type (control) BC samples. The algorithm uses information from all four aCGH genomic profiles and incorporates a weighted score of microhomology mediated deletions, base substitutions/rearrangements signatures and the HRD score (as used in Myriad myChoice HRD). Using a probabilistic cut-off of 70%, HRDetect predicted *BRCA* deficiency with a sensitivity of 98.7% in BC and reaching 100% in OC and pancreatic cancer validation cohorts. There is some evidence that the HRDetect score can predict clinical outcome and response to platinum therapy in BC but its ability to predict PARPi benefit has not yet been established (70, 71).

There is strong pre-clinical evidence that mutation-based assays that use information from multiple mutation types could outperform existing scar assays (for example the GIS had a sensitivity of 60% (63)). A major limitation, however, is the need of fresh frozen material while most trial samples are formalin fixed paraffin embedded (FFPE). A second limitation is that mutation-based assays remain genomic scar assays, so they by definition reflect the historical presence of HRD and do not provide information about current HR status that can be restored through different mechanisms as above mentioned.

FUNCTIONAL ASSAYS

Functional assays have the potential to provide a dynamic indicator of the actual HR status, giving the challenge of measuring a single downstream event that would reflect proficiency of multiple upstream components of HR (16). The most used experimental system in this setting has been quantification of RAD51 nuclear foci. RAD51 is a DNA recombinase which act as a downstream HR protein facilitating DNA strand invasion into the sister chromatid and consequent faithful DSBs repair. Reduced DNA damaged-induced nuclear RAD51 foci has been associated with *BRCA1/2* deficiency as well as PARPi responses, both in OC and BC laboratory models and in small cohorts of patient samples (72-74).

One of the most frequently used RAD51-based functional HRD tests that has been validated on different tumor and specimen types is the REcombination CAPacity (originally termed Repair CAPacity) or RECAP test (75-78). However, this test relies on the use of fresh tumor tissue and requires *ex vivo* induction of DNA damage; so, a RAD51 score on FFPE tumor tissue has been developed (79-81). The RAD51 score is dependent on the combination of two parameters: the percentage of geminin-positive GMN+ cells (an S/G2 phase cell proliferation marker (82)) with RAD51 foci and the number of RAD51 foci per nucleus. In BC samples, an RAD51 score threshold of 10% GMN+ cells with RAD51 foci and a cut-off of five foci/nucleus showed the best correlation with PARPi response in *gBRCA1* patient-derived xenografts and *gBRCA1/2* patient samples (79). This outcome was confirmed by a second study that identified all *BRCA1/2*-deficient BC tumors as HRD (80). An RAD51 score threshold of 15% GMN+ cells with RAD51 foci in combination with a RAD51 foci number cut-off of two foci/nucleus yielded the highest sensitivity, identifying 90% of *BRCA*-deficient and 87% of RECAP-HRD cases on endometrial and OC specimens (81). More recently, Pellegrino *et al.* (83) using a panel of patient-derived tumor xenografts models from BC, OC and pancreatic cancer demonstrated that a RAD51 score ≤ 10% predict PARPi response more accurately than HR-related gene mutations and genomic scar analysis. This work furnished a preclinical *in vivo* validation of the RAD51-immunofluorescence test for dynamic identification of tumors with HRD, differentiating PARPi-sensitive tumors from those that become PARPi-resistant after restoration of functional HR.

Drawbacks of RAD51 foci as a surrogate of HRD include the impossibility to identify defects in HR downstream of RAD51 loading on to DNA and technical aspects, such as the possibility of non-informative results (due to insufficient number of proliferating tumour cells) (82). Retrospective analyses of larger clinical cohorts are also needed to clinically validate the RAD51 score thresholds above mentioned and prospective trials selecting patients according to their RAD51 score are also awaited.

FUTURE PERSPECTIVES

Although promising, the current available biomarkers (multigene panel testing, genomic scar and functional assays) are inadequate predictors of response to PARPi, with clinical benefits observed both with and without HRD (84). Moreover, the current HRD assays do not provide a dynamic readout and are only valid for the time point at which the cancer tissue sample is obtained, usually at diagnosis, and do not consider tumor heterogeneity. Considering cancer's capacity to continuously evolve and to develop therapy resistance, functional assays are expected to be able to detect acquired resistance to PARPi due to HR restoration in HRD tumors. The so-called liquid biopsy that sample circulating tumor cells or circulating tumor DNA may overcome these issues. Hence, analysing feasibility of functional assays on multiple and serial samples obtained from liquid biopsy could majorly impact in clinical decision-making in the recurrent setting.

Several mechanisms of resistance to PARPi have been suggested, with only reversions in *BRCA1/2* clinically proved; however, other mechanism different from HR restoration have been described in preclinical models (85). Moreover, several ongoing clinical trials are investigating the combination of PARPi (especially Olaparib) with inhibitors of the replication stress, particularly ATR inhibitors (86), in order to elicit additive or synergistic effects and possibly overcome PARPi resistance. Given the complexity of the HR pathway and its interaction with cell cycle regulation, response to stress replication, and other DNA damage repair pathways it is unlike that one single biomarker will suffice: in all likelihood, composite HRD scores involving two or more biomarkers would be required to define "HRDness" and to predict response to PARPi alone or in combination regimens.

CONCLUSIONS

The need of predictive markers of response to PARPi is raising alongside with the increasing use of PARPi in clinical practice and the emerging of resistance to these agents. However, the current available biomarkers to infer the presence of HRD, including multigene panel testing, genomic scar and functional assays, are not able to accurately predict clinical sensitivity to PARPi. In the next future, the implementation of composite HRD scores involving multiple biomarkers identified on tumor samples from liquid biopsy will be challenging.

ETHICS

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Conflict of interests

CP declares no conflicts of interests. LC declares to receive honoraria from AstraZeneca, Pfizer, Novartis, MSD, Gilead Sciences.

Availability of data and materials

The data underlying this article can be shared just before a reasonable request to the corresponding author.

Authors' contribution

All the authors contributed equally to conception, data collection, analysis and writing of this paper.

Ethical approval

N/A.

Consent to participate

N/A.

REFERENCES

1. Sun Y, McCorvie TJ, Yates LA, Zhang X. Structural basis of homologous recombination. Cell Mol Life Sci 2020;77(1):3-18.
2. Ceccaldi R, Rondinelli B, D'Andrea AD. Repair Pathway Choices and Consequences at the Double-Strand Break. Trends Cell Biol 2016;26(1):52-64.
3. Venkitaraman AR. Linking the cellular functions of BRCA genes to cancer pathogenesis and treatment. Annu Rev Pathol 2009;4:461-87.
4. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 2004;4(10):814-9.
5. Lord CJ, Ashworth A. BRCAness revisited. Nat Rev Cancer 2016;16(2):110-20.
6. Deans AJ, West SC. DNA interstrand crosslink repair and cancer. Nat Rev Cancer 2011;11(7):467-80.

7. Tan DS, Rothermundt C, Thomas K, et al. "BRCA-ness" syndrome in ovarian cancer: A case-control study describing the clinical features and outcome of patients with epithelial ovarian cancer associated with BRCA1 and BRCA2 mutations. J Clin Oncol 2008;26(34):5530-336.
8. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434(7035):917-21.
9. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005;434(7035):913-7.
10. Mortusewicz O, Amé JC, Schreiber V, Leonhardt H. Feedback-regulated poly(ADP-ribosyl)ation by PARP-1 is required for rapid response to DNA damage in living cells. Nucleic Acids Res 2007;35(22):7665-75.
11. Liao H, Ji F, Helleday T, Ying S. Mechanisms for stalled replication fork stabilization: new targets for synthetic lethality strategies in cancer treatments. EMBO Rep 2018;19(9):e46263.
12. Lord CJ, Ashworth A. PARP inhibitors: Synthetic lethality in the clinic. Science 2017;17;355(6330):1152-8.
13. Murai J, Huang SY, Das BB, et al. Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. Cancer Res 2012;72(21):5588-99.
14. Stover EH, Konstantinopoulos PA, Matulonis UA, Swisher EM. Biomarkers of Response and Resistance to DNA Repair Targeted Therapies. Clin Cancer Res 2016;22(23):5651-60.
15. Nesic K, Wakefield M, Kondrashova O, et al. Targeting DNA repair: the genome as a potential biomarker. J Pathol 2018;244(5):586-97.
16. Hoppe MM, Sundar R, Tan DSP, Jeyasekharan AD. Biomarkers for Homologous Recombination Deficiency in Cancer. J Natl Cancer Inst 2018;110(7):704-13.
17. Liu JF, Konstantinopoulos PA, Matulonis UA. PARP inhibitors in ovarian cancer: current status and future promise. Gynecol Oncol 2014;133(2):362-9.
18. Konstantinopoulos PA, Norquist B, Lacchetti C, et al. Germline and Somatic Tumor Testing in Epithelial Ovarian Cancer: ASCO Guideline. J Clin Oncol 2020;38(11):1222-45.
19. Stover EH, Fuh K, Konstantinopoulos PA, et al. Clinical assays for assessment of homologous recombination DNA repair deficiency. Gynecol Oncol 2020;159(3):887-98.
20. Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. Nature 2011;474(7353):609-15.
21. Kanchi KL, Johnson KJ, Lu C, et al. Integrated analysis of germline and somatic variants in ovarian cancer. Nat Commun 2014;5:3156.
22. Patel KJ, Yu VP, Lee H, et al. Involvement of BRCA2 in DNA repair. Mol Cell 1998;1(3):347-57.
23. Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. Mol Cell 2001;7(2):263-72.
24. Mirza MR, Monk BJ, Herrstedt J, et al. Niraparib maintenance therapy in platinum-sensitive, recurrent ovarian cancer. N Engl J Med 2016;375(22):2154-64.
25. Robson M, Im SA, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 2017;377(6):523-33.
26. Gelmon KA, Tischkowitz M, Mackay H, et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple negative breast cancer: A phase 2, multicentre, open-label, non-randomised study. Lancet Oncol 2011;12(9):852-61.
27. Coleman RL, Fleming GF, Brady MF, et al. Veliparib with first-line chemotherapy and as maintenance therapy in ovarian cancer. N Engl J Med 2019;381:2403-15.
28. Coleman RL, Oza AM, Lorusso D, et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017;390:1949-61.
29. Gonzalez-Martin A, Pothuri B, Vergote I, et al. Niraparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med 2019;381:2391-402.
30. Moore K, Colombo N, Scambia G, et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. N Engl J Med 2018;379:2495-505.
31. Pujade-Lauraine E, Ledermann JA, Selle F, et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18:1274-84.
32. Ray-Coquard I, Pautier P, Pignata S, et al. Olaparib plus bevacizumab as first line maintenance in ovarian cancer. N Engl J Med 2019;381:2416-28.
33. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer:

a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014;15:852-61.
34. Dougherty BA, Lai Z, Hodgson DR, et al. Biological and clinical evidence for somatic mutations in BRCA1 and BRCA2 as predictive markers for olaparib response in high-grade serous ovarian cancers in the maintenance setting. Oncotarget 2017;8:43653-61.
35. McCabe N, Turner NC, Lord CJ, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition. Cancer Res 2006;66(16):8109-15.
36. Riaz N, Blecua P, Lim RS, et al. Pan-cancer analysis of bi-allelic alterations in homologous recombination DNA repair genes. Nat Commun 2017;8(1):857.
37. Toh M, Ngeow J. Homologous Recombination Deficiency: Cancer Predispositions and Treatment Implications. Oncologist 2021;26(9):e1526-e1537.
38. Swisher EM, Lin KK, Oza AM, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol 2017;18(1):75-87.
39. Swisher EM, Kristeleit RS, Oza AM, et al. Characterization of patients with long-term responses to rucaparib treatment in recurrent ovarian cancer. Gynecol Oncol 2021;163(3):490-97.
40. de Bono J, Mateo J, Fizazi K, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med 2020;382(22):2091-102.
41. Mateo J, Porta N, Bianchini D, et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol 2020;21(1):162-74.
42. Miller RE, Leary A, Scott CL, et al. ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer. Ann Oncol 2020;31(12):1606-22.
43. Gunderson CC, Moore KN. BRACAnalysis CDx as a companion diagnostic tool for Lynparza. Expert Rev Mol Diagn 2015;15(9):1111-6.
44. Pennington KP, Walsh T, Harrell MI, et al. Germline and somatic mutations in homologous recombination genes predict platinum response and survival in ovarian, fallopian tube, and peritoneal carcinomas. Clin Cancer Res 2014;20(3):764-75.
45. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405-24.
46. Tobalina L, Armenia J, Irving E, et al. A meta-analysis of reversion mutations in BRCA genes identifies signatures of DNA end-joining repair mechanisms driving therapy resistance. Ann Oncol 2021;32(1):103-12.
47. Tutt A, Ellis P, Kilburn L, et al. Abstract S3-01: The TNT trial: A randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or BRCA1/2 breast cancer (CRUK/07/012). Cancer Res 2015;75(9 Supplement):S3-01-S3-01.
48. Fong PC, Yap TA, Boss DS, et al. Poly(ADP)-ribose polymerase inhibition: Frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol 2010;28(15):2512-9.
49. Ceccaldi R, O'Connor KW, Mouw KW, et al. A unique subset of epithelial ovarian cancers with platinum sensitivity and PARP inhibitor resistance. Cancer Res 2015;75(4):628-34.
50. Kaufman B, Shapira-Frommer R, Schmutzler RK, et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol 2015;33(3):244-50.
51. Lord CJ, Ashworth A. The DNA damage response and cancer therapy. Nature 2012;481(7381):287-94.
52. Theisen A. Microarray-based comparative genomic hybridization. Nat Educ 2008;1(1):45.
53. Stefansson OA, Jonasson JG, Johannsson OT, et al. Genomic profiling of breast tumours in relation to BRCA abnormalities and phenotypes. Breast Cancer Res 2009;11(4):R47.
54. Vollebergh MA, Lips EH, Nederlof PM, et al. An aCGH classifier derived from BRCA1 mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann Oncol 2011;22(7):1561-70.
55. Vollebergh MA, Lips EH, Nederlof PM, et al. Genomic patterns resembling BRCA1- and BRCA2 mutated breast cancers predict benefit of intensified carboplatin-based chemotherapy. Breast Cancer Res 2014;16:R47.
56. Abkevich V, Timms KM, Hennessy BT, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair de-

fects in epithelial ovarian cancer. Br J Cancer 2012;107(10):1776-82.
57. Popova T, Manie E, Rieunier G, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. Cancer Res 2012;72(21):5454-62.
58. Birkbak NJ, Wang ZC, Kim JY, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents. Cancer Discov 2012;2(4):366-75.
59. Manié E, Popova T, Battistella A, et al. Genomic hallmarks of homologous recombination deficiency in invasive breast carcinomas. Int J Cancer 2016;138(4):891-900.
60. 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 2014;16(6):475.
61. Marquard AM, Eklund AC, Joshi T, et al. Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. Biomark Res 2015;3:9.
62. Isakoff SJ, Mayer EL, He L, et al. TBCRC009: A multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. J Clin Oncol 2015;33(17):1902-9.
63. Telli ML, Timms KM, Reid J, et al. Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. Clin Cancer Res 2016;22(15):3764-73.
64. Timms KM, Mills GB, Perry M, Gutin A, Lanchbury J, Brown R. Comparison of genomic instability test scores used for predicting PARP activity in ovarian cancer. J. Clin Oncol 2020;38(15):1586.
65. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500(7463):415-21.
66. Funnell T, Zhang AW, Grewal D, et al. Integrated structural variation and point mutation signatures in cancer genomes using correlated topic models. PLoS Comput Biol 2019;15(2):e1006799.
67. Gulhan DC, Lee JJ, Melloni GEM, et al. Detecting the mutational signature of homologous recombination deficiency in clinical samples. Nat Genet 2019;51(5):912-9.
68. Polak P, Kim J, Braunstein LZ, et al. A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. Nat Genet 2017;49(10):1476-86.
69. Davies H, Glodzik D, Morganella S, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med 2017;23(4):517-25.
70. Zhao EY, Shen Y, Pleasance E, et al. Homologous Recombination Deficiency and Platinum-Based Therapy Outcomes in Advanced Breast Cancer. Clin Cancer Res 2017;23(24):7521-30.
71. Staaf J, Glodzik D, Bosch A, et al. Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. Nat Med 2019;25(10):1526-33.
72. Mukhopadhyay A, Plummer ER, Elattar A, et al. Clinicopathological features of homologous recombination-deficient epithelial ovarian cancers: sensitivity to PARP inhibitors, platinum, and survival. Cancer Res 2012;72(22):5675-82.
73. Hill SJ, Decker B, Roberts EA, et al. Prediction of DNA Repair Inhibitor Response in Short-Term Patient-Derived Ovarian Cancer Organoids. Cancer Discov 2018;8(11):1404-21.
74. Naipal KA, Verkaik NS, Ameziane N, et al. Functional ex vivo assay to select homologous recombination-deficient breast tumors for PARP inhibitor treatment. Clin Cancer Res 2014;20(18):4816-26.
75. Meijer TG, Verkaik NS, Sieuwerts AM, et al. Functional ex vivo assay reveals homologous recombination deficiency in breast cancer beyond BRCA gene defects. Clin Cancer Res 2018;24:6277-87.
76. de Jonge MM, Auguste A, van Wijk LM, et al. Frequent homologous recombination deficiency in high-grade endometrial carcinomas. Clin Cancer Res 2019;25:1087-97.
77. van Wijk LM, Vermeulen S, Meijers M, et al. The RECAP test rapidly and reliably identifies homologous recombination-deficient ovarian carcinomas. Cancers (Basel) 2020;12:2805.
78. Cruz C, Castroviejo-Bermejo M, Gutiérrez-Enríquez S, et al. RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. Ann Oncol 2018;29(5):1203-10.
79. Castroviejo-Bermejo M, Cruz C, Llop-Guevara A, et al. A RAD51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation. EMBO Mol Med 2018;10(12):e9172.

80. 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) 2021;13:2994.
81. Wright WD, Shah SS, Heyer WD. Homologous recombination and the repair of DNA double-strand breaks. J Biol Chem 2018;293:10524-35.
82. van Wijk LM, Nilas AB, Vrieling H, Vreeswijk MPG. RAD51 as a functional biomarker for homologous recombination deficiency in cancer: a promising addition to the HRD toolbox? Expert Rev Mol Diagn 2022;22(2):185-199.
83. Pellegrino B, Herencia-Ropero A, Llop-Guevara A, et al. Preclinical In Vivo Validation of the RAD51 Test for Identification of Homologous Recombination-Deficient Tumors and Patient Stratification. Cancer Res 2022;82(8):1646-57.
84. Gonzalez D, Stenzinger A. Homologous recombination repair deficiency (HRD): From biology to clinical exploitation. Genes Chromosomes Cancer 2021;60(5):299-302.
85. Giudice E, Gentile M, Salutari V, et al. PARP Inhibitors Resistance: Mechanisms and Perspectives. Cancers (Basel) 2022;14(6):1420.
86. Forment JV, O'Connor MJ. Targeting the replication stress response in cancer. Pharmacol Ther 2018;188:155-67.