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SPECIAL ARTICLE

ESMO recommendations on predictive biomarker testing for homologous recombination deficiency and PARP inhibitor benefit in ovarian cancer

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Background: Homologous recombination repair deficiency (HRD) is a frequent feature of high-grade serous ovarian, fallopian tube and peritoneal carcinoma (HGSC) and is associated with sensitivity to PARP inhibitor (PARPi) therapy. HRD testing provides an opportunity to optimise PARPi use in HGSC but methodologies are diverse and clinical application remains controversial.

Materials and methods: To define best practice for HRD testing in HGSC the ESMO Translational Research and Precision Medicine Working Group launched a collaborative project that incorporated a systematic review approach. The main aims were to (i) define the term 'HRD test'; (ii) provide an overview of the biological rationale and the level of evidence supporting currently available HRD tests; (iii) provide recommendations on the clinical utility of HRD tests in clinical management of HGSC.

Results: A broad range of repair genes, genomic scars, mutational signatures and functional assays are associated with a history of HRD. Currently, the clinical validity of HRD tests in ovarian cancer is best assessed, not in terms of biological HRD status *per se*, but in terms of PARPi benefit. Clinical trials evidence supports the use of *BRCA* mutation testing and two commercially available assays that also incorporate genomic instability for identifying subgroups of HGSCs that derive different magnitudes of benefit from PARPi therapy, albeit with some variation by clinical scenario. These tests can be used to inform treatment selection and scheduling but their use is limited by a failure to consistently identify a subgroup of patients who derive no benefit from PARPis in most studies. Existing tests lack negative predictive value and inadequately address the complex and dynamic nature of the HRD phenotype.

Conclusions: Currently available HRD tests are useful for predicting likely magnitude of benefit from PARPis but better biomarkers are urgently needed to better identify current homologous recombination proficiency status and stratify HGSC management.

Key words: homologous recombination deficiency (HRD), poly-ADP ribose inhibitors (PARPi), *BRCA*, genomic scar assays

INTRODUCTION

Every year, almost 250 000 women worldwide are diagnosed with high-grade serous carcinoma of the ovary, fallopian tube or peritoneum (HGSC). Following standard treatment approaches of cytoreductive surgery and platinum and taxane based chemotherapy the average 5-year survival rate is approximately 30%.[1] Around half of HGSCs

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exhibit defects within the homologous recombination DNA repair pathway and are therefore reliant on more error prone means of DNA repair such as non-homologous end joining.[2,3] HGSC with homologous recombination repair deficiency (HRD) (see Box 1 for Glossary of terms) exhibit a distinct clinical phenotype including a superior response to platinum salt chemotherapies and sensitivity to poly-ADP ribose inhibitors (PARPi).[4,5] The introduction of PARPis has transformed the management of HGSC in both relapsed and first-line treatment settings.[6-13] Developing methods to reliably determine the HRD status of a HGSC is of critical importance to optimise clinical benefit from these drugs.

The best characterised causes of HRD in HGSC are germline or somatic mutations in the *BRCA1* and *BRCA2* genes (*BRCA*) that encode the breast cancer type 1 and type 2 susceptibility proteins and are detected in 12%-15% and 5%-7% of cases, respectively.[2,14] However, there is now clear evidence that HRD can arise through germline and somatic mutations or methylation of a wider set of homologous recombination repair (HRR) related genes, or other as yet undefined mechanisms.[3] Furthermore, a range of mechanisms such as reversion mutations in the *BRCA* genes can reinstate homologous recombination proficiency (HRP) revealing that HRD status is both a complex and dynamic phenotype.[15,16] A wide range of assays, referred to as 'HRD tests', have been developed to try to better define which cancers, beyond *BRCA* mutant, are most likely to have HRD. These HRD tests fall into three main categories: (i) HRR pathway related genes that identify specific causes of HRD, (ii) genomic 'scars' or mutational signatures that measure the patterns of somatic mutations that accumulate in HRD cancers irrespective of the underlying defect and (iii) functional assays that have the potential to provide a real-time read out of HRD or HRP (Figure 1).

The European Society for Medical Oncology (ESMO) Translational Research and Precision Medicine Working Group identified that there is currently uncertainty within the oncology community surrounding the different methods for HRD testing in HGSC. To address this, a collaborative project was launched with a number of clinicians and scientists with expertise in the fields of PARPi clinical trials, cancer genomics and DNA repair. The group defined three main aims for the project: (i) Define the term 'HRD test' and recommend how an HRD test's clinical validity is currently

Box 1. Glossary of Terms

Homologous recombination repair (HRR). A form of DNA recombination often used to repair DNA double strand breaks (DSBs). HRR predominantly acts in S and G_2 phases of the cell cycle and is a conservative process, restoring the original DNA sequence at the site of damage. During HRR, part of the DNA sequence around the DSB is removed (resection), revealing regions of single stranded DNA (ssDNA). The DNA recombinase RAD51 binds ssDNA and invades the DNA sequence on a homologous sister chromatid, using this as a template for the synthesis of new DNA at the DSB site. Crucial proteins involved in mediating HRR include those encoded by *BRCA1*, *BRCA2*, *RAD51*, *RAD51C*, *RAD51D* and *PALB2*.

Homologous recombination deficiency (HRD). A defect in DNA repair by hampered HRR. In cancers, this is often caused by loss of function mutations in *BRCA1*, *BRCA2*, *RAD51C*, *RAD51D* or *PALB2*, promoter hypermethylation of the *BRCA1* gene promoter (leading to reduced expression of BRCA1) or a series of as yet to be defined causes. HRD can be defined in multiple ways, for example, by the use of experimental assays that measure the conservative versus non-conservative repair of DSBs, mutational signatures that are the result of HRD or the inability of cells to relocalise the DNA recombinase RAD51 to sites of DNA damage. HRD is also characterised by the cellular sensitivity to PARP inhibitors, topoisomerase inhibitors or platinum salts although other causes of sensitivity to these agents also exist, including defects in nucleotide excision repair (NER) which causes platinum salt sensitivity. The term 'HRD' is often used interchangeably with the term '*BRCA*ness', although this latter term describes a broader concept that describes cancers that share molecular, histological, clinical and phenotypic features of germline *BRCA* mutant cancers (gBRCAm phenocopies), including, but not exclusive to, HRD, sensitivity to PARP inhibitors, topoisomerase inhibitors and platinum salts.

HRD cancer. Cancers that exhibit HRD. HRD is enriched in cancers of the ovary, prostate, pancreas and breast, where defects in *BRCA1*, *BRCA2*, *RAD51*, *RAD51C*, *RAD51D* or *PALB2* are most prevalent.

Homologous recombination proficiency (HRP). The scenario where cells/tumour cells are able to effectively repair DNA damage by HRR. Often associated with primary or acquired resistance to PARP inhibitors, topoisomerase inhibitors or platinum salts.

Poly-ADP ribose inhibitors (PARPi). PARPi are small molecule inhibitors of the PARP family of proteins, which play critical roles in DNA repair through multiple DNA damage response pathways, with HRD cells showing a greater reliance on PARP activity to maintain cell survival. The finding that single-agent PARP inhibition selectively killed BRCA-deficient cells was a key discovery in exploiting synthetic lethal approaches in oncology. PARP inhibitors trap PARP1 protein on to DNA at sites of single-strand DNA breaks. When this trapped PARP1 is encountered by the DNA replication machinery it leads to stalling of the replication fork, collapse and the generation of a double strand break, which cannot be repaired in cells with HRD such as *BRCA*-mutated cells.

best assessed in the context of HGSC, (ii) provide an overview of the biological rationale and the level of evidence supporting currently available HRD tests, and (iii) provide recommendations on the clinical utility of HRD tests in clinical management of HGSC.

MATERIALS AND METHODS

The expert panel was comprised of oncologists, a geneticist, pathologist and basic scientists operating in Europe, USA and Australia (see supplementary Methods, available at https://doi.org/10.1016/j.annonc.2020.08.2102). All panel members offered expertise in two or more areas relevant to the topic including but not limited to ovarian cancer management, DNA repair, cancer genomics, mutational signatures, cancer evolution, functional genomics, clinical trials, biomarker development and PARPi development and biology. To formally capture a balanced representation of experts' opinions on current HRD test usage, challenges and future opportunities, we employed a questionnaire-based approach that supplemented regular discussions.

A systematic review-based approach, adhering to the PRISMA statement pre-set-up protocol, was used as the starting point for identifying studies that combined HRD testing methodologies with PARPi or platinum chemotherapies (supplementary Table S1 and supplementary Methods, available at https://doi.org/10.1016/j.annonc.2020.08.2102).[17] A total of 343 relevant records were screened and 68 records were retained for critical evidence appraisal (supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2020.08.2102). For each HRD biomarker test shortlisted, studies were categorised by panel members using the level of evidence (LOE) approach and for genomics-based tests using the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) ranking where appropriate evidence was available (supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2020.08.2102).[18,19] The EGAPP approach aims to determine whether there is direct evidence that using the test leads to clinically meaningful improvement in outcomes or is useful in medical or personal decision-making.[18,19] To this end, the agreed definition for assessing clinical validity of an HRD test is 'accuracy of prediction of PARP inhibitor benefit' (see supplementary Methods, available at https://doi.org/10.1016/j.annonc.2020.08.2102, for further details).

Final consensus statements were generated in agreement by all panel members in light of the evidence review. The final degree of consensus was obtained by the mean percentage of agree responses to each statement from the 16 expert panel members (values range from 0%: total disagreement to 100%: total agreement), was judged as inconsistent if $<60\%$, low in the range 60%-69%, moderate in the range 70%-79%, strong from 80% to 89% and very strong if $>90\%$. The manuscript and consensus statements were reviewed by the wider ESMO Translational Research and Precision Medicine Working Group and the Faculty in Gynaecological Cancers.

RESULTS

Pathological considerations

Concordance between histopathological and molecular features is essential in cancer, particularly when assessing somatic alterations in tissues. The recommendations in this article relate to HGSCs of the ovary, fallopian tube and the peritoneum that share morphological and molecular features. Pathological diagnosis is straightforward in most cases but can be more challenging in the subgroup with solid, pseudo-endometrioid or transitional (SET) features, that like other HGSCs frequently exhibit HRD.[20,21] These tumours were historically classified as endometrioid or transitional cell carcinomas, which explains the occurrence of HRD in some older series of endometrioid carcinomas or mixed endometrioid—serous carcinomas.[22,23] The two most recent WHO classifications (2014 and 2020)[24,25] clearly state that these tumours are variants of HGSC, and provide information for distinguishing between HGSC and high-grade endometrioid carcinomas. A panel of antibodies (including WT-1, TP53, NAPSIN A and oestrogen and progesterone receptor) is helpful for confirming diagnosis.[26]

The pathologist is responsible for controlling the pre-analytical conditions of tumour tissue samples and is therefore critical to the success of the range of HRD tests discussed below. Inappropriate tissue handling (delayed fixation and over-fixation) may modify the quality of the sample, impacting on molecular test results. For molecular tissue-based HRD tests, representative tumour area selection and assessment of the percentage of malignant cells, necrosis and inflammatory component is of fundamental importance. Typically, a minimum of 30% tumour component is recommended to guarantee the detection of a variant through molecular techniques. For some cancers with HRD this can be difficult to achieve due to abundant inflammatory cell infiltrates.[27,28] It is recommended that molecular laboratories and pathology departments maintain quality standards within both pre-analytical and analytical steps by adhering to national or international standards, such as ISO 15189 or equivalent.

Consensus recommendation

Pathological evaluation of the tumour tissue specimens used for assessment of somatic molecular alterations is essential. It is recommended that a pathologist with experience in gynaecological pathology should be a member of the team and responsible for confirming diagnosis, assessing sample adequacy, selection of tumour area, and quantification of tumour cells, inflammatory cells and necrosis. An integrated pathology-molecular report is highly recommended.

(Level of agreement = 100%; total agreement)

Defining the HRD test

While the ideal method for detecting HRD would measure HRR capacity directly, HRD functional tests are some way off routine clinical use. The HRD tests that are used in the clinic

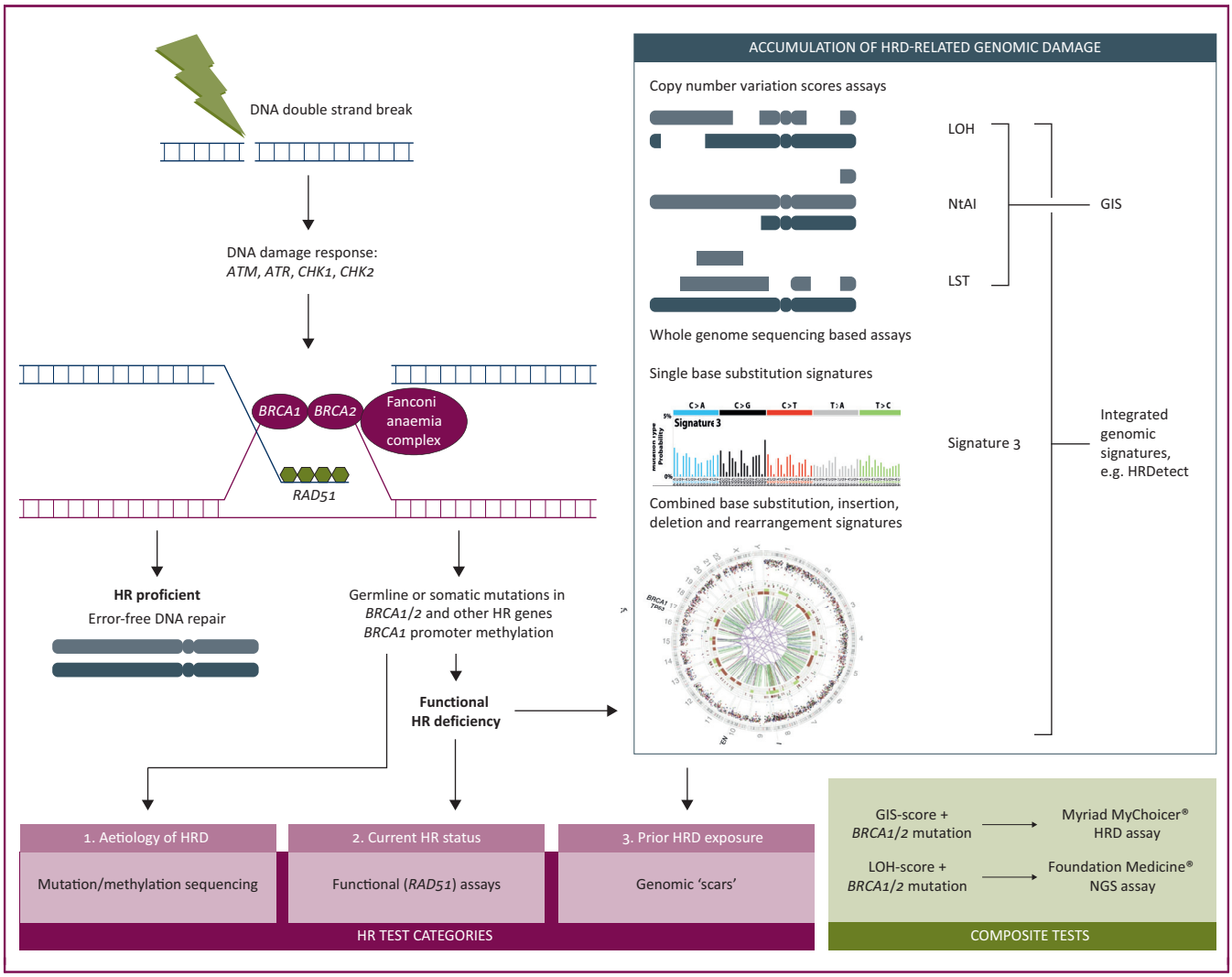

Figure 1. Methods for detecting homologous recombination repair deficiency (HRD).
HR, homologous recombination. Individual assays [HRDetect, loss of heterozygosity (LOH), NtAI (number of subchromosomal regions with allelic imbalance extending to the telomere), large scale transitions (LST) and genomic instability scores (GIS)] are described in the text. The two commercially available assays that combine *BRCA* mutation and GISs are described in the green box.

or have been tested within published randomised clinical trials to date measure a genotype (gene mutation/methylation or genomic scar) that correlates with an HRD phenotype and deficient HRR but not HRR itself. The majority of HRD tests currently under investigation are being developed to identify patients who benefit from PARPi and therefore will only indirectly identify cancers with HRD (Figure 2A). As discussed below, currently an HRD test result is most likely to have clinical utility in the context of PARPi treatment stratification and therefore PARPi benefit is the preferred outcome against which HRD test performance should be measured (Figure 2B). This underlies the decision to focus this recommendation article on the methods of HRD testing to guide PARPi therapy rather than their ability to detect HRD *per se*. It is important, however, to recognise that this may limit the future utility of these tests, particularly when considering other inhibitors of key targets involved in the DNA repair.

Methods for detecting HRD in HGSC

The systematic review confirmed that the currently available HRD testing methods fall into three main categories: HRR gene level tests, genomic scars and signatures and functional assays (Figure 1). The critical evidence review for individual tests is summarised in Table 1 with an LOE and EGAPP ranking provided for each test where relevant (see supplementary Tables S4-S10, available at https://doi.org/10.1016/j.annonc.2020.08.2102, for details for each test category). The main evidence supporting (or refuting) the clinical validity and clinical utility of these tests is derived from eight pivotal randomised controlled trials that are summarised in Table 2. A comparison of the hazard ratios within the intention to treat and the mainly exploratory HRD test driven subgroup analyses are presented in Figure 3. For additional comments from the expert panel on methods of HRD testing see supplementary Methods, available at https://doi.org/10.1016/j.annonc.2020.08.2102.

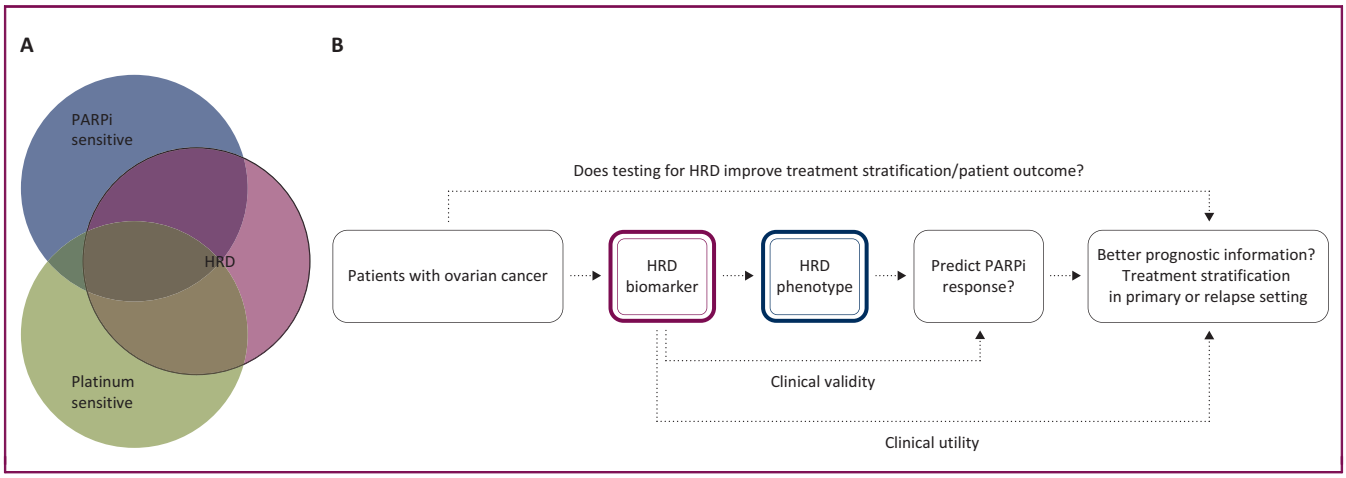

Figure 2. Rationale for using homologous recombination deficiency (HRD) tests to establish PARP inhibitor (PARPi) benefit in ovarian cancer.
(A) Tumours with evidence of HRD, determined using currently available tests, are more likely to respond to platinum salt chemotherapy and PARPis but factors such as resistance mechanisms mean overlap is incomplete. (B) Schema for assessing clinical validity and clinical utility of HRD biomarkers.

HRR gene level tests

Germline mutations in *BRCA* genes. Germline (inherited) *BRCA1* and *BRCA2* (g*BRCA*) mutations are implicated in the development of 13%-15% of HGSC.[2,14] Functional BRCA1 and BRCA2 proteins are crucial to the repair of double-stranded DNA breaks by HRR.[29] Cancers that arise in individuals with a deleterious g*BRCA* mutation frequently harbour a somatic loss of function aberration in the corresponding wild-type BRCA allele and therefore have defective HRR. The development of PARP inhibitors as treatment of HGSC was prompted by observations that *BRCA* mutations greatly increased the *in-vitro* sensitivity of cancer cells to PARP inhibition.[30,31]

Across the main randomised clinical trials in both first-line and relapse maintenance settings, whether as monotherapy or as combination therapy, a common theme is observed—*BRCA* mutation status consistently identifies the subgroup of patients who derive the greatest benefit from PARPi treatment in platinum-sensitive disease (LOE 1, Tables 1 and 2, Figure 3).[6-8,10-13,32] Despite some differences in trial design, patient characteristics and the treatment setting, the hazard ratio (HR) for PARPi maintenance therapy benefit in patients with a *BRCA* mutation is remarkably similar between all the above trials suggesting a robustness of the biomarker as a positive predictor of response (Figure 3). However, the negative predictive value (NPV) of *BRCA* mutation status is universally poor in the setting of platinum-sensitive relapsed HGSC, with *BRCA* wild-type (*BRCA*wt) subgroups also deriving a significant, although numerically smaller benefit from PARPi (Table 2, Figure 3).[7,10,12] Similarly, in the first-line setting PARPi treatment benefit extended to patients without *BRCA* mutations, which probably reflects the fact that platinum sensitivity is itself a powerful biomarker of HRD (Figure 2A).[6,8,13]

Somatic *BRCA* mutations. An additional 5%-7% of HGSC harbour somatic *BRCA* (s*BRCA*) mutations that have arisen during cancer development or progression.[2] While many studies utilised tumour *BRCA* (t*BRCA*) status (incorporating both g*BRCA* and s*BRCA*) as a biomarker to determine PARPi benefit,[7,13,32] data on s*BRCA* mutations alone is more limited. Retrospective analysis from Study 19 identified s*BRCA* mutation in 10% of patients.[33] There was bi-allelic

Table 1. Summary of critical evidence review of homologous recombination deficiency (HRD) tests

HRD test	Test LOE	Clinical validity	Clinical utility 1st line maintenance	Clinical utility platinum sensitive relapsed disease
Germline *BRCA* mutations	I	Good	Good	Good
Tumour *BRCA* mutations	I	Good	Good	Good
Somatic *BRCA* mutations	I/II	Good/fair	Good	Good
Non-*BRCA* mutations HRR genes	II	Marginal	No evidence	Marginal
HR genomic scar assays:				
GIS	I	Good	Good	Good
LOH	II	Good	No evidence	Good

For each HRD test, where relevant, the level of evidence (LOE) as per Simon criteria[18] (supplementary Table 3, available at https://doi.org/10.1016/j.annonc.2020.08.2102) and evaluation of genomic applications in practice and prevention (EGAPP) ranking[17] is provided. For EGAPP ranking, clinical validity is defined as 'accuracy of prediction of PARP inhibitor sensitivity' and clinical utility describes the 'accuracy of prediction of PARP inhibitor benefit' in the first-line and platinum-sensitive relapsed maintenance settings. Clinical utility is reported as good, fair, or marginal, where marginal reflects the fact that the studies may not have been poor in general but may not have been designed to address the specific question. LOE and EGAPP ranking is designed to evaluate genomic (not functional) tests and is only provided for HRD tests for which there is sufficient clinical evidence to evaluate. Tumour *BRCA* incorporates both germline (inherited) *BRCA* and somatic (acquired) *BRCA* mutations.
GIS, genomic instability score; LOH, loss of heterozygosity score.

Table 2. Pivotal randomised controlled trials of PARP inhibitor (PARPi) maintenance therapy in HGSC (relates to Figure 3)

Study details	Drugs (patients)	Platinum sensitivity eligibility criteria	*BRCA* status	Randomisation stratification criteria	Biomarkers used (test details)	Analysis subgroup	HR (95% CI)	PFS in months
Maintenance therapy in platinum sensitive relapse (≥2 previous lines of platinum-based chemotherapy)								
ARIEL3 (NCT01968213) Coleman et al. *Lancet*, 2017	Rucaparib 600 mg bd ($n = 375$) versus placebo ($n = 189$)	Stringent:		Mutations in *BRCA* or HRR related genes	Foundation Medicine T5 NGS assay:	ITT (all patients)	0.37 (0.3-0.45)	10.8 versus 5.4; $P < 0.001$
		PR/CR on serology or radiology		PFS following penultimate chemotherapy	(1) g/sBRCA mutations	HRD (g/s*BRCA* mutation)	0.23 (0.16-0.34)	16.6 versus 5.4; $P < 0.001$
		CA125 normalisation		Best response to most recent chemotherapy	(2) LOH (genomic scar)	HRD (g/s*BRCA* mutation or LOH-high)	0.32 (0.24-0.42)	13.6 versus 5.4; $P < 0.001$
					(3) Mutations in 28 HRR genes	HRD (LOH-high & *BRCA*wt)	0.44 (0.29-0.66)	9.7 versus 5.4; $P < 0.001$
						HRP (LOH-low & *BRCA*wt)	0.58 (0.4-0.8)	6.7 versus 5.4; $P = 0.0049$
NOVA (NCT01847274) Mirza et al. *NEJM*, 2016	Niraparib 300 mg od ($n = 372$) versus placebo ($n = 181$)	Stringent:		PFS following penultimate chemotherapy	BRACAnalysis test (Myriad Genetics):	HRD (g*BRCA* mutation)	0.27 (0.17-0.41)	21 versus 5.5; $P < 0.001$
		Radiological PR/CR		Bevacizumab use with last/ penultimate chemo	g*BRCA* mutations	HRD (GIS-high & g*BRCA*wt)	0.38 (0.24-0.59)	12.9 versus 3.8; $P < 0.001$
		Low disease burden (<2 cm size)		Best response to most recent platinum chemo	myChoice® HRD (Myriad Genetics):	HRP (g*BRCA*wt)	0.45 (0.34-0.61)	9.3 versus 3.9; $P < 0.001$
		Sustained normalisation/ 90% decrease in CA125			GIS-score (genomic scar)	HRP (GIS-low & g*BRCA*wt)	0.58 (0.36-0.92)	6.9 versus 3.8, $P = 0.02$
SOLO2 (NCT01874353) Pujade Lauraine, *Lancet Oncol*, 2017	Olaparib 300 mg bd tablets ($n = 196$), placebo ($n = 99$)	Moderate:	Germline *BRCA* mutation	Best response to most recent platinum chemo	BRACAnalysis test (Myriad Genetics):	ITT (g*BRCA* mutation)	0.33 (0.24-0.44)	19.1 versus 5.5; $P < 0.001$
		PR/CR or no detectable disease		Platinum free interval	g*BRCA* mutations			
		CA125 not rising						
Study19 (NCT00753545) Ledermann et al. *Lancet Oncol*, 2014	Olaparib 400 mg bd capsules ($n = 136$), placebo ($n = 129$)	Moderate:		PFS following penultimate chemotherapy	Foundation medicine:	ITT (all patients)	0.35 (0.25-0.49)	10.8 versus 5.4; $P < 0.001$
		PR/CR		Best response to most recent platinum chemo	t*BRCA* mutations	HRD (*BRCA* mutation)	0.18 (0.1-0.31)	11.2 versus 4.3; $P < 0.001$
				Ethnic descent		HRP (*BRCA*wt)	0.54 (0.34-0.85)	7.4 versus 5.5; $P = 0.0075$
Monotherapy maintenance in advanced platinum sensitive cancers—first-line setting								
PAOLA-1 (NCT02477644) Ray-Coquard et al. *NEJM*, 2019	Olaparib 300 mg ($n = 537$) bd plus bevacizumab (15 mg/ kg d1, q3w) versus placebo ($n = 269$) plus bevacizumab	Stringent:		Best response to most recent chemotherapy	myChoice® HRD Plus assay (Myriad Genetics):	ITT (all patients)	0.59 (0.49-0.72)	22.1 versus 16.6; $P < 0.001$
		CR/PR/no disease after chemotherapy		Tumour *BRCA* mutation status (mutant versus wild-type)	(1) GIS-score ≥42 (genomic scar)	HRD (t*BRCA* mutation)	0.31 (0.2-0.47)	37.2 versus 21.7
					(2) t*BRCA* mutations	HRD (GIS-high or t*BRCA* mutation)	0.33 (0.25-0.45)	37.2 versus 17.7
		No evidence of PD (CA125/imaging/ physical exam) during first line chemo/before randomisation				HRD (GIS-high & t*BRCA*wt)	0.43 (0.28-0.66)	28.1 versus 16.6
						HRP (t*BRCA*wt)	0.71 (0.58-0.88)	18.9 versus 16
						HRP (GIS-low /unknown)	0.92 (0.72-1.17)	16.9 versus 16

Continued

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Table 2. Continued

Study details	Drugs (patients)	Platinum sensitivity eligibility criteria	*BRCA* status	Randomisation stratification criteria	Biomarkers used (test details)	Analysis subgroup	HR (95% CI)	PFS in months
PRIMA (NCT02655016) Gonzalez-Martin, *NEJM*, 2019	Niraparib 300 mg ($n = 487$) versus placebo ($n = 246$)	Stringent:		Best response to most recent platinum chemo	myChoice® HRD Plus assay (Myriad Genetics):	ITT (all patients)	0.62 (0.5-0.76)	13.8 versus 8.2; $P < 0.001$
		CR/PR after chemotherapy		Receipt of neoadjuvant chemotherapy	(1) GIS-score ≥42 (genomic scar)	HRD (t*BRCA* mutation)	0.4 (0.27-0.62)	22.1 versus 10.9
		Stage III patients must have residual disease after surgery		Tumour HRD status (HRD versus HRP or unknown status)	(2) t*BRCA* mutations	HRDpos (GIS-high or t*BRCA* mutation)	0.43 (0.31-0.59)	21.9 versus 10.4; $P < 0.001$
						HRD (GIS-high & t*BRCA*wt)	0.5 (0.31-0.83)	19.6 versus 8.2
						HRP (GIS-low and t*BRCA*wt)	0.68 (0.49-0.94)	8.1 versus 5.4
VELIA (NCT0247058) Coleman et al, *NEJM*, 2019	carboplatin/taxane + maintenance placebo ($n = 375$), carboplatin/ taxane and maintenance veliparib ($n = 383$) carboplatin/ taxane with veliparib and maintenance veliparib ($n = 382$)	None specified		Germline *BRCA* status & disease stage	myChoice® HRD Plus assay (Myriad Genetics):	ITT (all patients)	0.68 (0.56-0.83)	23.5 versus 17.3; $P < 0.001$
				Paclitaxel schedule & geographic region	(1) GIS-score ≥ 33 (genomic scar)	HRD (t*BRCA* mutation)	0.44 (0.28-0.68)	34.7 versus 22; $P < 0.001$
						HRD (GIS-high or t*BRCA* mutation)	0.57 (0.43-0.76)	31.9 versus 20.5; $P < 0.001$
						HRP (*BRCA*wt)	0.8 (0.64-1.00)	NA
				Surgical timing & residual disease post-surgery	(2) t*BRCA* mutations	HRP (GIS-low and t*BRCA*wt)	0.81 (0.6-1.09)	NA
SOLO1 (NCT01844986) Moore et al. *NEJM*, 2018	Olaparib 300 mg bd tablets ($n = 260$), placebo ($n = 131$)	Stringent: CR/PR/no disease after chemotherapy No radiological evidence of PD or rising CA125 after chemo	Deleterious/ suspected deleterious *BRCA* mutation	Best response to most recent platinum chemo	Myriad or BGI: g*BRCA* mutations	ITT (g*BRCA* mutation)	0.3 (0.23-0.41)	NR versus 13.8; $P < 0.001$

Benefit from PARPi versus placebo is displayed as progression-free survival (PFS) and hazard ratios (HR) with 95% confidence intervals (CI). These HRs are presented in the same order in the forest plot in Figure 3. Primary endpoint analyses are denoted by purple highlighting. Heterogeneity between trials includes but is not limited to eligibility criteria and stratification criteria, some of which are presented. Additional exploratory analyses of homologous recombination deficiency (HRD) and homologous recombination proficiency (HRP) subgroups that were predefined (dark grey) or carried out post-hoc (white) are presented.

BGI, Beijing Genomics Institute; *BRCA*wt, Absence of *BRCA1* or *BRCA2* mutation; CR, complete response; HRR, homologous recombination deficiency; ITT, Intention to treat; NGS, next-generation sequencing; PD, progressive disease; PR, partial response.

BRCA mutation, mutation in *BRCA1* or *BRCA2* gene (nature of mutation denoted by prefix: s, somatic, t, tumour and g, germline).

Genomic scar tests include the genomic instability score (GIS) and loss of heterozygosity score (LOH).

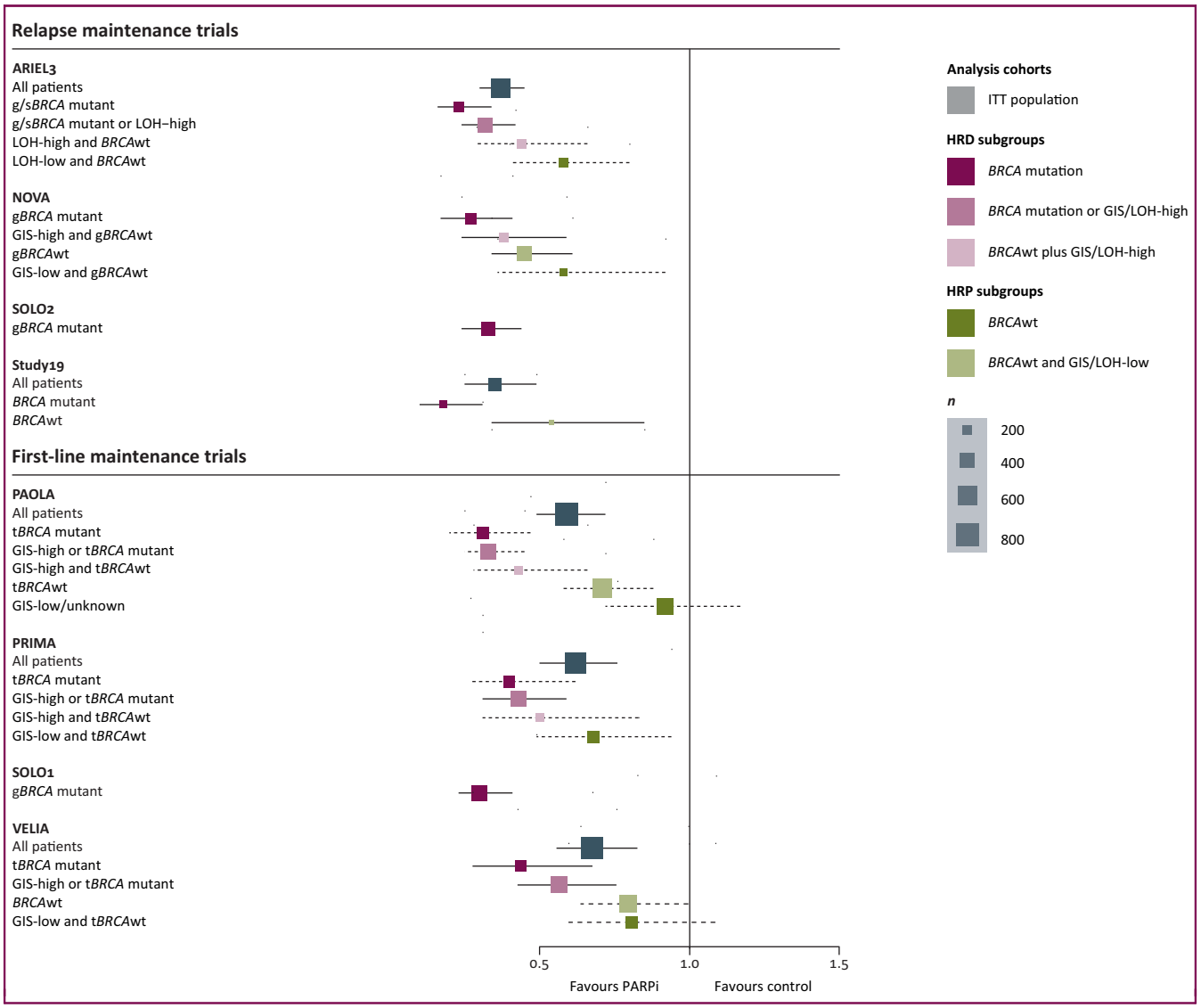

Figure 3. Trends for incremental benefit from PARP inhibitor (PARPi) across homologous recombination deficiency (HRD) defined subgroups.
A forest plot displays the hazard ratios and 95% confidence intervals for PARPi benefit as reported in the key phase II/III clinical trials of high grade ovarian cancer, detailed in the same order as Table 2. The box size indicates the number of patients (*n*). Solid and dashed error bars indicate primary and exploratory analyses, respectively. Similar trends are seen across trials with incremental PARPi benefit across HRD and homologous recombination proficient (HRP) subgroups. The greatest benefit is observed in the *BRCA* mutation cohort (s, t and g prefix = somatic, tumour and germline, respectively) (dark purple), followed by those with high genomic instability scores (GIS), loss of heterozygosity (LOH) scores or a *BRCA* mutation (medium purple) (these are equivalent to the Myriad Genetics and Foundation medicine commercial assay 'HRD positive' subgroups), the *BRCA*wt group with GIS/LOH-high (light purple) and finally the HRP *BRCA*wt (dark green) and the *BRCA*wt/GIS/LOH-low score (light green) subgroups. Caution is advised in comparing absolute results between trials due to important differences in trial design (some of which are described in Table 2). Results are included where they were presented in the original publications.

inactivation in >80% of cases and mutations were predominantly clonal, suggesting that s*BRCA* mutations arise early in tumourigenesis. The clinical outcomes for patients with s*BRCA* mutations were similar to those with g*BRCA* mutations in terms of progression-free survival (PFS, HR 0.23 versus 0.17, respectively). Within the NOVA trial, 47 (of 553) patients harboured an s*BRCA* mutation and derived a similar benefit from niraparib compared with placebo (PFS increase 11 to 20.9 months, HR 0.27) as the *gBRCA* population (PFS 5.5. to 21.0 months, HR 0.27).[10] Similarly, for rucaparib, data are available for monotherapy treatment in patients with platinum-sensitive advanced disease; for 19 patients with s*BRCA* mutation the response rate was 74% which was similar to those with g*BRCA* mutations (85%) and PFS was also similar.[34] Finally, within the VELIA first-line study, a similar benefit was observed for g*BRCA* (HR 0.5, 0.30-0.82) and s*BRCA* (HR 0.35, 0.14-0.87) with veliparib versus placebo treatment.[6]

Non-*BRCA* HRR gene mutations. Germline or homozygous somatic mutations in other members of the Fanconi anaemia family, such as *RAD51C*, *RAD51D* and *BRIP1*, increase susceptibility to HGSC[35-37] and pre-clinical studies have established that deficiencies in these genes and possibly other HRR-associated genes, such as *ATM*, *CHEK1*, *CHEK2* and *CDK12* also confer sensitivity to DNA repair inhibition.[5,35,38,39] The Cancer Genome Atlas (TCGA) identified mutations related to the HRR pathway in approximately

https://doi.org/10.1016/j.annonc.2020.08.2102

30% of HGSC.[2] Clinical studies have demonstrated that somatic mutations in non-*BRCA* HRR genes confer a PFS and overall survival advantage, similar to that seen with *BRCA* mutations in patients treated with platinum chemotherapy, when compared with patients who have neither a *BRCA* nor HRR mutation.[4] However, due to the relative rarity of non-*BRCA* HRR mutations, these studies grouped all HRR genes together while other data on individual HRR genes is anecdotal which makes it difficult to interpret the relevance of any individual HRR gene at present. Indeed, emerging evidence reveals that mutations in different HRR genes, such as *ATM* and *BRCA*, can be associated with distinct sensitivities to PARPi[40] and a one-size-fits-all approach when using HRR mutations to predict PARPi response should be avoided.

In a retrospective analysis from Study 19, tumour tissue testing identified that 21 HGSCs without *BRCA* mutations had mutations in other genes implicated in DNA repair including *BRIP1* (*BRCA1* interacting protein C-terminal helicase 1) that co-operates with BRCA1 to perform DNA repair. Other DNA repair genes altered in more than one patient included *CDK12, RAD54L* and *RAD51B*.[41] The cohort of HGSC that lacked a *BRCA* mutation but carried a mutation in other HRR genes derived a similar benefit to those with a *BRCA* mutation (HR 0.21 and HR 0.18, respectively) and this was of a greater magnitude to that observed in the cohort that lacked mutations in either *BRCA* or the wider set of HRR genes (HR 0.71).[41] Caution is required in interpreting the data from this retrospective analysis, as the numbers of patients with defects in any one gene (other than *BRCA1* or *BRCA2*) are small and a similarly sized study could generate a different set of recurrently altered HRR genes. Within the ARIEL2 (NCT01891344) rucaparib monotherapy study, exploratory analysis was carried out on 12 patients with pre-treatment and post-progression biopsies.[42] Two patients had a mutation in a non-*BRCA* HRR gene (*RAD51C* and *RAD51D*) with both patients deriving clinical benefit to rucaparib treatment. Interestingly, both post-progression biopsy samples contained reversion mutations that were predicted to restore the respective gene functions, which was confirmed *in vitro*.

Variants of uncertain significance

Not all mutations falling within the *BRCA* or other HRR genes alter protein function or contribute towards cancer development. A major challenge for *BRCA* and wider HRR gene testing is determining the clinical relevance of variants of uncertain significance (VUS) that are typically rarer missense mutations and also include intronic or exonic mutations that may alter RNA splicing.[43-45] The biological relevance of variants that lead to partial or 'leaky' splicing defects, particularly in *BRCA2*, that reduce full length transcript production are particularly hard to interpret.[46] The problem of VUS is even more pronounced for wider gene panel tests where the functional and clinical consequences of most individual genomic loci are not well characterised and individual mutations are not highly recurrent. Furthermore, somatic VUS may be more numerous and diverse than germline variants as they may arise in the context of an elevated mutation rate and/or genomic instability that is characteristic of many cancers.[47]

A recent international questionnaire-based study revealed surprising variation between individual laboratories worldwide, in the rate of *BRCA* VUS detection/reporting (3%-50%) and in the approaches used for detection, reporting and clinical management of patients with a VUS.[48] This reflects the challenges in VUS annotation and is the subject of an existing ESMO publication.[49] Given the difficulty of predicting the functional relevance of an individual point mutation or structural variant within a given gene footprint, corroborating evidence of HRD from a genomic mutation/scar test and/or a functional assay, as discussed below, would ideally be acquired.

HR gene promoter methylation

Although the impact of deleterious *BRCA* gene mutations on PARPi and platinum responses in HGSC is established, the clinical relevance of HRR gene promoter methylation is more difficult to interpret.[50-54] There is biological evidence that *BRCA1* and *RAD51C* gene promoter methylation can result in HRD. Promoter methylation results in reduced expression of these key HRR genes, and in cancers it is generally mutually exclusive with *BRCA* mutation[2,52,55-57] and positively associated with BRCA-deficiency associated genomic signatures.[56,58] However, clinical studies that included screening for HRR gene methylation provide conflicting evidence and its accuracy and reliability as a biomarker for predicting PARPi (or platinum) responses in HGSC patients cannot currently be established.[2,34,52,54,59,60]

There is now evidence to suggest that existing studies were confounded by technical factors associated with the measurement of tumour DNA methylation.[52-54] It was only recently discovered, using cohort HGSC patient-derived xenograft models, that the zygosity of *BRCA1* methylation is a key determining factor for PARPi response.[61] Kondrashova et al. demonstrated that all copies of *BRCA1* must be methylated for PARPi response and that losing methylation of a single *BRCA*1 copy was sufficient to restore HRR DNA repair and cause platinum/PARPi resistance.[61] This finding was validated using *BRCA1* samples from the ARIEL2 Part 1 trial, where 'homozygous' *BRCA*1 methylation was carefully assigned using highly quantitative methylation-specific droplet digital polymerase chain reaction (PCR) (to measure *BRCA1* methylation), as well as sample/tumour purity and *BRCA1* copy number estimates.[61] Although the same principles of methylation zygosity may apply to *RAD51C* methylated cases, this remains to be confirmed. Thus, in future, great caution should be taken in assigning methylation status to these HRR genes, with quantitative methylation assays, sample purity and gene copy number all being critical for accurate HRD assessment and predicting platinum/PARPi responses.

Consensus statements on HRR gene tests

- *BRCA* mutation tests [germline (LOE I), tumour (incorporating germline and somatic) (LOE I) and somatic (LOE I/II)] exhibit good clinical validity by consistently identifying the subgroup of ovarian cancer patients who derive the greatest magnitude of benefit from PARPi therapy.

(Level of agreement = 100%; total agreement)

- There is currently an insufficient quantity of evidence to determine the clinical validity of individual or panels of non-*BRCA* HRR genes for predicting a PARPi response and further prospectively collected data is required (LOE II).

(Level of agreement = 100%; total agreement)

- There is currently insufficient evidence to determine the clinical validity of *BRCA1* or *RAD51C* promoter methylation to predict PARPi benefit, partly due to concerns regarding the analytic validity of previous studies.

(Level of agreement = 100%; total agreement)

GENOMIC SIGNATURES AND SCARS

Cancers and cell lines with *BRCA* mutations exhibit genomic instability, manifesting in abnormal copy number profiles and thousands of somatic mutations genome-wide that include both single base substitutions (SBS) and structural variants (SVs) that are characterised by a preponderance of short deletions (1 bp to 100 kbp) and short tandem duplications (up to 10 kbp) (Figure 1). Measuring some or all of these genomic features provide ways of identifying cancers with a history of HRD, irrespective of the underlying aetiology.

Copy number based 'scar' assays

Most HRD genomic assays in current use were developed using SNP-based microarray technologies and measure somatic copy number variation (CNV). In 2012 three studies reported SNP-based CNV assays that predicted *BRCA* status through the quantification of large scale transitions (LST),[62] loss of heterozygosity (LOH)[63] or number of subchromosomal regions with allelic imbalance extending to the telomere[64] NtAI (Figure 1). Subsequent studies suggested that combining the information derived from two or more of these assays further enhanced the ability to distinguish between HRR competent and deficient cancers.[65] The most common genomic scar assays reported to date are two commercially available tests that combine tumour *BRCA* mutation testing with a genomic instability score derived from the unweighted sum of NtAI, LST and LOH (myChoice HRD test, Myriad Genetics) or with an assessment of fraction of genomic sub-chromosomal LOH[66] (FoundationFocus CDxBRCA, Foundation Medicine)[67] (Figure 1). The Myriad genomic instability score (GIS) uses a dichotomous threshold, determined within a training cohort of 497 breast and 461 ovarian cancers, including 268 *BRCA* mutant or promoter methylated tumours to classify cancers as GIS-high or GIS-low.[66] The LOH test uses a next generation sequencing assay to determine the percentage of genomic LOH. A predefined cut-off of 14% or more defines LOH-high, based on the TCGA data.[2] As discussed below, both GIS and LOH tests were developed with predefined thresholds but these were not adopted in all studies. The biomarker potential of LOH-high versus LOH-low and the MyChoice assay have been investigated in high quality (LOE I) prospective clinical trials of PARPis in the first-line and/or relapse settings (Tables 1 and 2).[6-8,10,12,13,34]

Only the monotherapy ARIEL2 trial was designed to evaluate genomic scarring within the *BRCA*wt population. All of the maintenance studies in both the primary and recurrent setting completed to date which have included genomic instability as molecular assay used a nested approach for the primary outcomes in which the HRD population included *BRCA*-mutated HGSC (Table 2). Therefore, evaluating the utility of LOH or GIS to predict benefit from PARPi in the *BRCA*wt populations were preplanned secondary analyses that were not adequately powered to allow definitive analyses in any of the large randomised controlled trials. The strongest evidence for LOH status as a marker of PARPi response is derived from the ARIEL studies of rucaparib. The ARIEL2 (part 1) phase II monotherapy study classified patients into three predefined subgroups according to HRD status: *BRCA* mutant; *BRCA*wt/LOH-high and HRP (*BRCA*wt/LOH-low).[34] Amongst patients with *BRCA*wt cancers, PFS was superior in the LOH-high compared with the LOH-low subgroup (0.62, 0.42-0.90, $P = 0.011$). Because ARIEL2 is a monotherapy study without a control arm, it is possible that LOH status functioned as a prognostic not predictive marker. In the phase III ARIEL3 study of rucaparib versus placebo as maintenance therapy in relapsed disease, the primary endpoint of PFS was further explored within prespecified HRD categories including *BRCA*wt/LOH-high and *BRCA*wt with LOH-low (HRP), but these analyses were limited by lack of LOH status as a stratification factor and inadequate power for such secondary comparisons (Table 2). The threshold for determining LOH status (16%) also differed to that determined in the original studies (14%).[34] Treatment benefit (PFS) was greatest in *BRCA* mutant (HR 0.23, 0.16-0.39), followed by HRD positive (*BRCA* mutant or LOH-high; HR 0.32, 0.24-0.42), BRCAwt/LOH-high (HR 0.44, 0.29-0.66) and finally the HRP (BRCAwt and LOH-low) cohort (HR 0.58, 0.4-0.8)[7] (Figure 3).

The NOVA study of niraparib versus placebo included two parallel cohorts: g*BRCA* mutant and *BRCA*wt. A hierarchical analysis was carried out within the BRCAwt group for the GIS-high and then all g*BRCA*wt subgroups. GIS was not a stratification factor.[10] Findings echoed those of ARIEL3, including an intermediate benefit in the BRCAwt/GIS-high and failure to identify an HRP group who do not benefit (Table 2). A retrospective analysis of Study 19, combined with GIS testing further confirmed that GIS did moderately separate the *BRCA*wt population into higher and lower benefit groups but does not adequately define an HRP group who derive no benefit from a PARPi.[41] In the relapse platinum-sensitive setting the LOH score and GIS score therefore demonstrate good clinical validity in their ability to define a *BRCA*wt subgroup that derive a greater benefit from PARPi. However, the clinical utility of these tests, at least in the platinum-sensitive setting, as discussed in the

next section, is limited by the fact that neither test can consistently identify a *BRCA*wt subgroup that derives no benefit from PARPi (Table 1, Figure 2A). There have been no side-by-side comparisons of these tests within clinical trials to draw a direct comparison of performance.

GIS is the only genomic scar assay that has been tested to date in first-line randomised controlled trials (Table 2). The PRIMA study compared niraparib to placebo and stratified treatment according to HRD status (combined t*BRCA* status and GIS score) in patients with documented platinum-responsive disease after primary treatment.[8] Like ARIEL3 and NOVA, analyses of GIS within the *BRCA*wt population was a preplanned exploratory analysis in PRIMA. Possibly reflecting the stringent platinum responsiveness inclusion criteria (including at least 90% reduction in serum CA125), the results were similar to those seen in the relapse setting with benefit observed in all *BRCA*wt HGSC irrespective of GIS, although the magnitude of benefit was higher in the GIS-high compared with GIS-low subgroup (HR 0.5, 0.31-0.83 versus HR 0.68, 0.49-0.94) (Table 2, Figure 3). In contrast to PRIMA, in the VELIA study, veliparib or placebo was given concurrently with chemotherapy as well as maintenance therapy. However, this study was not designed or powered to detect a difference within the *BRCA*wt population so we do not know how to interpret the fact that the GIS-high subgroup (defined in this study as a score $\geq$33) appears to derive almost identical benefit to the overall *BRCA*wt cohort (HR 0.81, 0.6-1.09 and HR 0.8, 0.64-1.0, respectively), which could reflect the lack of selection for platinum sensitivity or the utilisation of PARPi in combination with chemotherapy before maintenance.[6] The PAOLA-1 study investigated the benefit of adding olaparib to bevacizumab maintenance therapy.[13] Amongst *BRCA*wt HGSC, PARPi benefit was restricted to those with a high GIS (HR 0.43, 0.28-0.66 versus HR 0.92, 0.72-1.17 with low GIS) indicating that in some patient populations the GIS has the potential to identify an HRP population who do not derive benefit from PARPi, when given in combination with bevacizumab.

Mutational signatures

Whole genome sequencing of a typical cancer will reveal thousands of somatic mutations. The pattern of mutations reflects historical endogenous and exogenous mutational processes that have operated in the cell. Each mutational process may contain components of DNA damage, repair and replication and can generate a characteristic mutational signature that can be detected using computational methodologies.[68,69] In HGSC, mutational signatures have been shown to correlate with clinical features such as survival and platinum response.[69-73]

The most commonly cited approach for detecting point mutational signatures was developed by Alexandrov et al.[68] Every SBS in the genome is first assigned to one of 96 possibilities determined by the base change (C>A, C>G, C>T, T>A, T>C, T>G) and the immediate 5′ and 3′ base. Mutational signatures are then extracted using a non-negative matrix factorisation (NMF) method. Applying this approach to over 2600 cancers has identified a total of 49 distinct SBS mutational signatures to date.[47] SBS Signature 3 is associated with *BRCA* mutation and *BRCA1* promoter methylation in breast, ovarian, pancreatic and stomach cancers. It has been proposed as a biomarker for HRD.[74] However, in isolation SBS Signature 3 is unlikely to provide a sufficiently robust clinical biomarker for guiding PARPi therapy in HGSC. Firstly, it probably lacks specificity (the vast majority of HGSCs have some contribution from Signature 3). Secondly, ascertaining appropriate thresholds will be difficult as the relatively indistinct nature of the signature makes it particularly sensitive to a reduction in the number of mutations that occurs in low tumour cellularity or when swamped by other competing mutational signatures.[75]

As HRD causes different types of genomic alterations, an assay that utilises as much genome-wide information as possible is likely to offer greater specificity and sensitivity. A *BRCA* deficiency detector termed HRDetect[75] was developed using whole genome sequence data from *BRCA* mutant and wild-type (control) breast cancer samples. The algorithm uses information from all four mutation classes and measures six genomic features that are assigned different weightings as specified in brackets: (i) Indels, microhomology mediated deletions (2.398); (ii) SBSs, Signature 3 (1.611) and Signature 8 (0.091); (iii) SVs, rearrangement Signature 3 [mainly short (<10 kb) tandem duplications] (1.153) and rearrangement Signature 5 (deletions of <100 kb)(0.847); (iv) CNV, the HRD score (as used in Myriad myChoice HRD) (0.667). Using a probabilistic cut-off of 70%, HRDetect predicted *BRCA* deficiency with a sensitivity of 98.7% in 560 breast cancers (including the training cohort), 86% in a validation breast cancer cohort (n = 80) and approaching 100% in ovarian cancer (n = 73) and pancreatic cancer (n = 96) validation cohorts. Cases with monoallelic *BRCA* loss had low HRDetect scores. The HRDetect assay significantly outperformed existing genomic scar measures such as the GIS that had a sensitivity of 60%.[66] In breast cancer, there is some evidence that the HRDetect score can predict clinical outcome and response to platinum therapy (AUC 0.89, P = 0.006) but its ability to predict PARPi benefit in HGSC has not yet been established.[76,77]

There is strong pre-clinical evidence that mutation-based assays that use information from multiple mutation types could outperform existing scar assays. A major limitation, however, is the reliance on fresh frozen material while most trial samples are formalin fixed paraffin embedded (FFPE). While FFPE-related artefacts can be managed with relative ease in targeted sequencing experiments, in whole genome data, although some solutions have been developed, these artefacts remain challenging.[78] A second limitation of all genomic scar or signature assays is that they by definition reflect the historical existence of HRD and do not provide information about current HRP status that can be reinstated through different mechanisms.

Consensus statement on the use of genomic scar tests of HRD

- HRD tests that incorporate scores of allelic imbalance (GIS or LOH) identify a subgroup of *BRCA* wild-type, platinum-

sensitive cancers that derive a greater magnitude of benefit from PARPi therapy in some settings (LOE I).
(Level of agreement = 100%; total agreement)

- There is currently insufficient evidence to ascertain the clinical validity of whole genome sequencing based mutational signatures for predicting PARPi benefit in HGSC.

(Level of agreement = 100%; total agreement)

- Pre-clinical evidence suggests that whole genome sequencing based mutational signature tests may compare favourably to existing genomic scar assays in terms of identifying cancers with HRD—their clinical validity in terms of PARPi benefit should be ascertained in archived clinical trial specimens and/or prospective clinical trial specimens.

(Level of agreement = 100%; total agreement)

Functional assays

Functional assays have the potential to provide a dynamic readout of actual, extant, HRR status. The most commonly-used experimental system to estimate HRR has been 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. Reduced DNA damaged-induced nuclear RAD51 foci has been associated with *BRCA1* or *BRCA2* gene defects as well as PARPi responses, both in ovarian and breast cancer laboratory models and in small cohorts of patient samples, including *ex-vivo* cultures derived from ascites or from solid HGSC.[79,80] Further evidence exists in breast cancer where low RAD51 foci (induced by DNA-damaging chemotherapy) are associated with patient treatment responses to neoadjuvant chemotherapy or to PARPi.[81-83] Two limitations of measuring reduced RAD51 as a surrogate of HRD are (i) the RAD51 assay will not identify defects in HR downstream of RAD51 loading on to DNA and (ii) when used experimentally, the RAD51 signal is normally elicited by exogenous DNA damage, limiting the clinical applicability of the approach. However, the ability to estimate nuclear RAD51 levels in the absence of exogenous damage as an estimate of HRD has now been demonstrated in treatment naive, archival FFPE tumour specimens, suggesting that clinical application of this assay might be possible.[84] Retrospective analyses of larger clinical cohorts are also needed to demonstrate the clinical validity of the RAD51 assay. Prospective trials selecting patients according to their RAD51 score are also awaited.

Consensus statement on the use of functional assays of HRD

- There is currently insufficient evidence to ascertain the clinical validity of functional assays in predicting response to PARPi therapies, but these pre-clinical assays provide promise for ascertaining real-time estimates of HRD and their development should be a priority. The potential for using functional assays alongside HRR gene tests and genomic tests should be investigated.

(Level of agreement = 100%; total agreement)

CLINICAL UTILITY OF AVAILABLE HRD TESTS

PARPis are licensed by European Medicines Agency (EMA) and/or the US Food and Drug Administration (FDA) for use in three clinical settings in the management of HGSCs: (i) as first-line maintenance therapy for platinum-sensitive, advanced stage cancers, (ii) as second-line maintenance therapy in platinum-sensitive, relapsed disease irrespective of *BRCA* mutation or other HRD test defined status and (iii) as monotherapy treatment in *BRCA* mutant (olaparib/rucaparib) or HRD test positive (niraparib) HGSC beyond two prior lines of therapy. There is some variation in specific license details as summarised in Table 3. Notably, EMA but not FDA regulations limit PARPi use to high-grade cancers while FDA approvals depend on the use of FDA approved companion diagnostics for HRD status testing including for *BRCA* mutations. Clinical trials evidence has informed recent approvals by the FDA for first-line maintenance therapy, with EMA approvals awaited (Table 3). Based on the PRIMA trial data, in April 2020 the FDA approved the use of niraparib for 'all comers' based on positive data in the intention to treat populations.[85] Following PAOLA-1 trial data the FDA extended approval for olaparib beyond *BRCA* mutation to those with *BRCA*wt/GIS-positive HGSC but only when given in combination with bevacizumab.[86] The Myriad myChoice assay was concurrently approved as a companion diagnostic for olaparib in this setting.[86]

Maintenance therapy in platinum-sensitive relapse

In the platinum-sensitive relapsed setting initial approvals for PARPi maintenance were limited to olaparib for use in ovarian cancers with *BRCA* mutations.[87,88] Subsequent data identified benefit in all subgroups and supported an extended scope for PARP inhibitor use. This is reflected in approvals by the FDA and EMA for niraparib, rucaparib and olaparib as maintenance therapy for all patients with platinum-sensitive relapsed ovarian cancer, irrespective of *BRCA* or HRD status.[87-91] However, despite regulatory approval for 'all comers', as discussed in relation to individual tests above, there is an incremental reduction in benefit observed from the *BRCA* mutant to HRD to HRP populations as defined by GIS/LOH score assays in maintenance monotherapy. The clinical utility of HRD tests (*BRCA* mutation and genomic 'HRD' scars) in these settings therefore results from the magnitude of PARPi benefit. The expert panel commented that in the relapsed setting this can be helpful for deciding whether to initiate chemotherapy and bevacizumab or chemotherapy alone with the intention of using a PARPi if there is a partial or complete response. Furthermore, it identifies the group of patients predicted to derive the least benefit from PARPi maintenance and where clinical trials may be more appropriate.

Table 3. Regulatory approvals of PARP inhibitor use in gynaecological malignancy

	First-line monotherapy maintenance		Second-line monotherapy maintenance		Monotherapy beyond second-line	
Approval criteria	Advanced epithelial OC, FTC or PPC Complete or partial response to platinum-based chemotherapy		Recurrent epithelial OC, FTC or PPC Complete or partial response to platinum-based chemotherapy No mutation/HRD test restrictions			
	Approval granted	**Additional restrictions**	**Approval granted**	**Additional restrictions**	**Approval granted**	**Additional restrictions**
Olaparib	FDA (2018)	Suspected or known deleterious *BRCA* mutation detected using FDA-approved companion diagnostic[a]	FDA (2017)		FDA (2014)	OC only Germline *BRCA*-mutated only ≥3 prior lines of chemotherapy FDA-approved companion diagnostic[a]
	EMA (2019)	High-grade cancers only Germline or somatic *BRCA*-mutation	EMA (2014)	High-grade cancers only		
Rucaparib			FDA (2018)		FDA (2016)	Epithelial OC, FTC or PPC Germline or somatic BRCA-mutated ≥2 prior lines of chemotherapy FDA-approved companion diagnostic[a]
			EMA (2019)	High-grade cancers only	EMA (2018)	High-grade epithelial OC, FTC or PPC ≥2 prior lines platinum therapy Platinum sensitive relapsed cancers only Unable to tolerate further platinum therapy Germline or somatic *BRCA* mutation
Niraparib	FDA (2020)	No mutation/HRD test restrictions	FDA (2017)		FDA (2019)	Epithelial OC, FTC, or PPC Progressed >6 months after last platinum HRDpositive status—deleterious or suspected deleterious *BRCA* mutation, or genomic instability FDA-approved companion diagnostic[a]
			EMA (2017)	High-grade cancers only		

EMA, European Medicines Agency; FDA, US Food and Drug Administration; FTC, fallopian tube cancer; HRD, homologous recombination deficiency; OC, ovarian cancer; PPC, primary peritoneal cancer.

[a] See https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools for details of FDA approved companion diagnostics.

Maintenance therapy after response to first-line chemotherapy

The use of olaparib maintenance following first-line chemotherapy in patients with advanced *BRCA*-mutated HGSC significantly improves PFS.[11] Within the recent studies exploring the role of first-line PARPi maintenance in 'all comers', *BRCA*wt but HRD positive (i.e. high GIS on Myriad myChoice HRD) cancers constituted 20%-30% of HGSC. In two of these trials, preplanned, but exploratory analyses of this subgroup demonstrated a clinically meaningful increase in median PFS (of greater than 10 months in each study) from first-line PARP inhibition, although the magnitude of benefit was less than that observed for patients with *BRCA* mutant HGSC[6,8,13] (Table 2).

The non-HRD (GIS-low and *BRCA*wt) subgroup constituted up to 50% of all HGSC in these trials.[6,8,13] A more difficult question to answer is whether existing HRD tests can consistently identify a group of patients who do not derive sufficient benefit to justify PARPi therapy in this setting. No trial was powered to determine whether the HRP population by itself derived benefit from maintenance PARPi in either the recurrent or primary setting but all showed benefit across the intention to treat populations.

The expert panel commented that translating these data into clinical practice is somewhat challenging due to fundamental differences in study design and patient inclusion. The PAOLA-1 study randomly assigned patients to olaparib with bevacizumab or bevacizumab with placebo maintenance therapy (Table 2). Academic research on PAOLA-1 samples should help to unpick which patients benefit from PARPi plus bevacizumab but unfortunately it did not include a PARPi only maintenance arm, so this question will remain unanswered. The expert panel advises that caution is required when evaluating these biomarkers as none of these trials was prospectively designed to evaluate the HRD test in all subgroups, including the HRP population. Indeed, PAOLA-1 was stratified for *BRCA* mutant versus *BRCA*wt, while *BRCA*wt/HRD positive was an exploratory analysis. PRIMA was stratified for HRD positive versus HRP and unknown HRD status combined. In all three studies, HRP cohort was an exploratory endpoint. The Myriad myChoice assay was the only one used in these studies.

Monotherapy treatment with PARPi

There are limited opportunities to use a PARPi as a single-agent treatment in both Europe and the USA and each indication requires either a *BRCA* mutation or HRD positive cancer (Myriad myChoice) (Table 3).[87,88,90-92] Recent data from the SOLO3 trial suggest that for PARPi naive *gBRCA* patients with platinum-resistant or partially sensitive ovarian cancer, olaparib is superior to nonplatinum chemotherapy with higher response rate and PFS.[93] However, as PARPi maintenance therapy is now routinely available for all patients with platinum-sensitive relapsed disease and for all *BRCA* mutant patients in the first-line setting the opportunities for monotherapy use are increasingly limited.

Consensus recommendations on the clinical utility of HRD tests

- In the first-line maintenance setting, germline and somatic *BRCA* mutation testing is routinely recommended to identify HGSC patients who should receive a PARPi.

(Level of agreement = 100%; total agreement)

- In the first-line maintenance setting, it is reasonable to use a validated scar based HRD test to establish the magnitude of benefit conferred by PARPi use in *BRCA* wild-type HGSC.

(Level of agreement = 100%; total agreement)

- In the first-line maintenance setting, it is reasonable to use a validated scar based HRD test to identify the subgroup of *BRCA* wild-type patients who are least likely to benefit from PARPi therapy.

(Level of agreement = 100%; total agreement)

- In the platinum-sensitive relapse maintenance setting, it is reasonable to use *BRCA* mutation testing and validated scar based HRD tests to predict the likely magnitude of PARPi benefit for consideration of risks and benefits of maintenance therapy.

(Level of agreement = 100%; total agreement)

FUTURE PERSPECTIVES: DEVELOPING THE OPTIMAL HRD BIOMARKER

Cancer's capacity to continuously evolve and change is a common challenge in the era of precision medicine. The HRD assays currently available in clinical practice do not provide a dynamic readout and are only valid for the time point at which the sample is obtained. In reality, the tested sample is usually archival, typically obtained at diagnosis or surgical debulking. If an HRD test is to be used to guide treatment at relapse or in the maintenance setting it ideally should be carried out on a sample obtained at that point in time. Successful strategies would therefore need to tackle the associated problems of minimal residual disease and inter-tumour heterogeneity (at any point in time there may be multiple cancer subclones present). So-called liquid biopsies that sample circulating tumour cells, circulating tumour DNA or ascitic fluid may offer hope for addressing these problems. There are some promising data from prostate cancer that demonstrated the utility of ctDNA for identifying *BRCA* mutations and reversion mutations and their correlation to prostate specific antigen level[94] but clearly more nuanced HRD testing methodologies have not been explored in this way.

A second challenge is that all of the gene based and genomic assays, by definition, provide information on mutations acquired in the past. The footprints from mutational processes active early in tumourigenesis may not reflect contemporaneous activity of DNA repair mechanisms. Genomic scars will be detected within relapsed tumours even if they have developed treatment resistance. Importantly, at present, none of the DNA sequencing approaches assess the presence of the known mechanism of clinical resistance, namely HR gene reversion. It seems logical that the known reversion events should be included in genomic assays and further research is needed to elucidate the full range of mechanisms of clinical resistance. Another route to tackling this problem might be measuring changes in subclone specific mutational signatures across serial samples. It remains that, based on the biology of the disease, we cannot rely on any of the existing tests to accurately predict whether HRD is extant at the time of treatment or not. It is highly likely that some form of functional assay will be required for this, probably in combination with other HRD tests.

An additional consideration for current HRD assays is the need to combine them with other predictive biomarkers as PARPi therapy evolves to include combination therapy with other agents such anti-angiogenesis, checkpoint inhibitors and other DNA repair inhibitors (reviewed in Pilie et al.[95]). Further validation using adequately powered clinical trials either prospectively or retrospectively will be required for each combination as these develop.

Consensus recommendation

- An optimised HRD biomarker needs to be developed to address the problem of cancer evolution, provide a real-time read out of HRP and should ideally generate data in a format that permits on-going research. (Level of agreement = 100%; total agreement)

CONCLUSION

We predict that the development of composite biomarkers will improve treatment stratification and these should be a priority for translational research. Indeed, the likely impact of platinum sensitivity (itself a strong biomarker of HRD) on the heterogeneity of HRD-related outcomes in the clinical trials discussed above indicates that we need to develop systematic ways to integrate this clinical information with HRD test results. Real-time composite markers may include a combination of a platinum sensitivity, genomic scar/mutational signature test and a functional assay to provide both robust historical evidence of HRD and to estimate

current HRR capacity. Alternatively, (or within this strategy) comprehensive genomic assays, based on high quality whole genome sequencing data, could be developed to provide simultaneous read outs of HRR gene mutations, mutational signatures and reversion mutations. Furthermore, if combined with multi-sampling strategies to obtain contemporaneous and representative tumour tissue samples, these assays have the potential to trace changes in subclone structure over a disease course. Given the significant rate of HRD in other gynaecological histologies and other cancers including those of the breast, prostate and pancreas, investing in the development of optimised biomarker strategies could have far reaching implications.

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