ESMO Recommendations on Homologous Recombination Deficiency Testing to Predict PARP Inhibitor Benefit in Ovarian Cancer

Rowan E. Miller[1,2], Alexandra Leary[3], Clare Scott[4,5], Violetta Serra[6], Christopher J. Lord[7,8], David Bowtell[4,5], David Chang[9,10], Dale W. Garsed[4,5], Jos Jonkers[11], Jonathan A. Ledermann[12], Serena Nik-Zainal[13,14], Isabelle Ray-Coquard[15,16], Sohrab P. Shah[17], Xavier Matias-Guiu[18], Elizabeth M. Swisher[19] and Lucy R. Yates[20,21]*

[1]Department of Medical Oncology, University College London, UK

[2]Department of Medical Oncology St Bartholomew's Hospital, London, UK

[3]Department of Medicine and INSERM U981, Gustave Roussy Cancer Center, Université Paris-Saclay, Paris, France

[4]Peter MacCallum Cancer Centre, Melbourne, Australia

[5]The University of Melbourne, Melbourne, Australia.

[6]Experimental Therapeutics Group Vall d'Hebron Institute of Oncology, Spain

[7]The Breast Cancer Now Toby Robins Research Centre, The Institute of Cancer Research, London, UK

[8]CRUK Gene Function Laboratory, The Institute of Cancer Research, London, UK

[9] Glasgow Precision Oncology Laboratory, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow, UK

[10] West of Scotland Pancreatic Unit, Glasgow Royal Infirmary, Glasgow, UK

[11] Division of Molecular Pathology, Oncode Institute, Netherlands Cancer Institute, Amsterdam, The Netherlands

[12]UCL Cancer Institute, University College London, UK

[13] Department of Medical Genetics, School of Clinical Medicine, University of Cambridge, Cambridge, UK

[14]MRC Cancer Unit, School of Clinical Medicine, University of Cambridge, Cambridge, UK

[15]Centre Leon Berard, Lyon, France

[16]University Claude Bernard Groupe University of Lyon, France

[17]Computational Oncology, Dept of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, USA

[18]Departments of Pathology, Hospital U Arnau de Vilanova and Hospital U de Bellvitge, Universities of Lleida and Barcelona, Irblleida, Idibell, Ciberonc

[19]Department of Obstetrics and Gynecology, University of Washington, Seattle, WA, USA

[20]Wellcome Sanger Institute, Wellcome Genome Campus, Cambridge, UK

[21]Guy's Cancer Centre, Guys and St Thomas' NHS Foundation Trust, London, UK

*Corresponding author

Correspondence to: Dr Lucy R. Yates, ESMO Head Office – Scientific and Medical Division, Via Ginevra 4, Lugano CH-6900, Switzerland. Tel: 0041-91-973-1999; Fax: 0041-91-973-1902; E-mail: education@esmo.org

Abstract

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.

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 (1) define the term “HRD test”; (2) provide an overview of the biological rationale and the level of evidence supporting currently available HRD tests; (3) 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 are 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 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 world-wide 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 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) 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 cause 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 (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: (1) Define the term “HRD test” and recommend how an HRD test’s

clinical validity is currently best assessed in the context of HGSC (2) Provide an overview of the biological rationale and the level of evidence supporting currently available HRD tests, (3) 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). 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 1 and Supplementary Methods for details) [17]. A total of 343 relevant records were screened and 68 records were retained for critical evidence appraisal (Supplementary Table 2). 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 3) [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 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 80-89% 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 Gynaecological Malignancy Working Groups.

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, 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].

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 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 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 recognize that this may limit the future utility of these tests, particularly when considering other inhibitors of key targets involved in the DNA repair.

Glossary of Terms [EMBEDDED BOX]

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 *vs.* 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 cause platinum salt sensitivity. The term HRD is often used interchangeably with the term “BRCAness”, 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 DDR 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 onto 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.

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 summarized in Table 1 with a level of evidence (LOE) and EGAPP ranking provided for each test where relevant (see Supplementary Tables 4 – 10 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 randomized controlled trials that are summarized in Table 2. A comparison of the hazard ratios within the intention to treat and the mainly exploratory HRD test driven subgroup analyses is presented in Figure 3. For additional comments from the expert panel on methods of HRD testing see supplementary methods.

HRR Gene Level Tests

Germline mutations in BRCA Genes

Germline (inherited) *BRCA1* and *BRCA2* (*gBRCA)* 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 *gBRCA* 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 for 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, Table 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 form 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]. Whilst 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 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 PFS (HR = 0.23 vs 0.17, respectively). Within the NOVA trial, 47 (of 553) patients harboured a s*BRCA* mutation and derived a similar benefit from niraparib compared to 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 30% of HGSC [2]. Clinical studies have demonstrated that somatic mutations in non-*BRCA* HRR genes confer a PFS and OS advantage, similar to that seen with *BRCA* mutations in patients treated with platinum chemotherapy, when compared to patients who have neither a *BRCA* nor HRR mutation [4]. Due to the relative rarity of these mutations, data regarding the influence of non-*BRCA* HRR gene mutations on PARPi response is anecdotal.

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* [40]. 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) [40]. 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 performed on 12 patients with pre-treatment and post-progression biopsies [41]. 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.

Mutation variants of unknown significance (VUS) are particularly problematic 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 [42]. Indeed, *BRCA* mutation tests can have VUS rates of up to 5% in some laboratories despite the fact that these are incredibly well characterised genes [43].

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 [44-48]. 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, 46, 49-51] and positively associated with BRCA-associated genomic signatures [50, 52]. 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, 46, 48, 53, 54].

There is now evidence to suggest that existing studies were confounded by technical factors associated with the measurement of tumour DNA methylation [46-48]. It was only recently discovered, using a cohort HGSC patient derived xenograft models, that the zygosity of *BRCA1* methylation is a key determining factor for PARPi response [55]. Kondrashova *et al*, demonstrated that all copies of *BRCA1* must be methylated for PARPi response, and that losing methylation of a single *BRCA1* copy was sufficient to restore HRR DNA repair and cause platinum/PARPi resistance [55]. This finding was validated using BRCA1 samples from the ARIEL2 Part 1 trial, where “homozygous” BRCA1 methylation was carefully assigned using highly quantitative methylation-specific droplet digital PCR (to measure *BRCA1* methylation), as well as sample/tumour purity and *BRCA1* copy-number estimates [55]. 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

- *BRCA1/2* 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-*BRCA1/2* 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 characterized by a preponderance of short deletions (1 bp-100kbp), short tandem duplications (up to 10kbp) (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) [56], loss of heterozygosity (LOH) [57] or allelic imbalance extending to the telomere [58] (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 [59]. 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 TAI, LST and LOH (MyChoice HRD test , Myriad Genetics) or with an assessment of fraction of genomic sub-chromosomal LOH [60] (FoundationFocus CDxBRCA, Foundation Medicine) [61]. 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 [60]. The LOH test uses an NGS sequencing assay to determine the percentage of genomic LOH. A pre-defined cut-off of 14% or more defines LOH-high, based on the TGCA 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 (Table 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 randomized 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 3 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. 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 performed 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 [40]. 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 who 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 randomized controlled trials. 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 to GIS low subgroup (HR 0.5, 0.31-0.83 versus HR 0.68, 0.49-0.94) (Table 2, Figure 3). Unfortunately the VELIA study of veliparib versus placebo, 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 >=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 also reflect the lack of selection for platinum sensitivity or the utilization 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 a 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 [62, 63]. In HGSC, mutational signatures have been shown to correlate with clinical features such as survival and platinum response [63-67].

The most commonly cited approach for detecting point mutational signatures was developed by Alexandrov *et al.* [62]. 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 Factorization (NMF) method. Applying this approach to over 2,600 cancers has identified a total of 49distinct SBS mutational signatures to date [68]. 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 [69]. 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 [70].

As HRD causes different types of genomic alterations, an assay that utilizes as much genome-wide information as possible is likely to offer greater specificity and sensitivity. A BRCA deficiency detector termed HRDetect [70] 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 6 genomic features that are assigned different weightings as specified in brackets: 1) Indels – microhomology mediated deletions (2.398); 2) SBSs – Signature 3 (1.611) and Signature 8 (0.091); 3) SVs – rearrangement signature 3 (mainly short (<10kb) tandem duplications) (1.153) and rearrangement signature 5 (deletions of <100kb)(0.847); 4) 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% [60]. 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 [71, 72].

There is strong preclinical 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 [73]. 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
  (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 [74, 75]. 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 [76-78]. 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 onto 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 [79]. 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 Agency (FDA) for use in three clinical settings in the management of HGSCs: 1) as first-line maintenance therapy for platinum sensitive, advanced stage cancers 2) as second-line maintenance therapy in platinum sensitive, relapsed disease irrespective of *BRCA* mutation or other HRD test defined status and 3) as monotherapy treatment in *BRCA* mutant (olaparib/rucaparib) or HRD test positive (niraparib) HGSC beyond 2 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 [80]. 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 [81]. The Myriad myChoice assay was concurrently approved as a companion diagnostic for olaparib in this setting [81].

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 [82, 83]. 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 [82-86]. 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.

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 randomised patients between 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 were 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 HRD negative and unknown HRD status combined. In all three studies, HRP cohort was an exploratory end-point. 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) [82, 83, 85-87]. Recent data from the SOLO3 trial suggest that for PARPi naïve *gBRCA* patients with platinum resistant or partially sensitive ovarian cancer, olaparib is superior to nonplatinum chemotherapy with higher response rate and PFS [88]. 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 *BRCA1/2* mutation testing is routinely recommended to identify HGSC patients who should receive a PARPi. (Level of agreement = 100%; total agreement)
- In the 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 *BRCA1/2* 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 *BRCA1/2* 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 *BRCA1/2* 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 – genomic assays, by definition, provide information on mutations acquired in the past and do not necessarily provide information on the current HRD status. Specifically, genomic scars represent a historical record of HRD and will not reflect restoration of HRR as resistance to platinum or PARPi develops. The restoration of HRP and/or PARPi resistance may develop through diverse mechanisms and there are few assays that seek to identify these. However, we recognize that improvement in assessing HRD is required. The rate of inconclusive tests in PAOLA-1 (18%) and the observation that some patients with HGSC without detectable HRD may still benefit from bevacizumab and PARPi (e.g. highly platinum-sensitive) have prompted an ongoing European Network for Gynaecological Oncological Trial (ENGOT) initiative to evaluate several HRD tests, including RAD51 foci, on PAOLA-1 tumour samples. The expert panel members advised that better HRD biomarkers are needed and new HRD tests should address the problem of cancer evolution, provide a real-time read out of HRP/HRD and should ideally generate data in a format that permits on-going research (for example, whole genome sequence data rather than targeted data from a limited number of genes). The behaviour of new biomarkers may differ depending on the patient population (i.e. with HGSC that is platinum sensitive versus unknown) and the treatment schema (i.e, monotherapy versus combined with other therapies). Thus, the context of the trial will be important in developing and testing new biomarkers of PARPi benefit.

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, these assays have the potential to trace changes in subclone structure over a disease course. We should develop robust computational methods, locked down for clinical use, allowing academic centres to generate their own data for clinical decision making now, permitting future research and allowing local updating in response to advances in knowledge.

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)

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