EDITORIAL

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Shallow whole genome re-sequencing to precisely predict benefit from PARP inhibitor

Advanced stage high-grade serous or endometroid ovarian cancer (HGOC) is the first cause of death from gynecological cancer. It is characterized by mutations of *TP53* in nearly all cases, leading to high genomic instability. About 50% of HGOC have defects of the homologous recombination (HR) pathway, with 30% being *BRCA*-deficient through inactivation of the *BRCA1/BRCA2* genes (either somatic or germline mutations or hypermethylation of the promotor of *BRCA1*). In the remaining 20% of cases, other members of the HR pathway are inactivated, such as *PALB2* or *RAD51C/RAD51D*.

BRCA-deficient cells are extremely sensitive to PARP inhibitors (PARPi) through synthetic lethality, representing the first and most successful example of applying this concept as a therapeutic approach in cancer. This success is best illustrated by the unprecedented survival benefit in *BRCA*-mutated HGOC when PARPi is given as frontline maintenance therapy after platinum [1]. Beyond *BRCA*-deficient tumors, several clinical trials have shown a prolonged progression-free survival (PFS) with PARPi in *BRCA* wild-type relapsing platinum-sensitive HGOC [2, 3]. Thus, extensive efforts have been put into identifying the genomic characteristics of *BRCA*-deficient tumors with the hypothesis that a genomic signature, historically named « BRCAness », could predict benefit from PARPi in *BRCA* wild-type tumors.

The HR pathway is an evolutionary conserved mechanism that coordinates error-free repair of DNA double-strand breaks. Since this high-fidelity DNA repair pathway is impaired in *BRCA*-deficient tumors, they rely on error-prone alternative pathways, accumulating characteristic genomic alterations that constitute the homologous recombination deficient (HRD) scars. These genomic scars can be measured quantitatively and qualitatively through next-generation sequencing (NGS) [4] or single nucleotide polymorphism (SNP) arrays [5]. The first HRD test to be approved by the regulatory authorities and is commercially available was the MyChoice CDx developed by Myriad Genetics Inc. It is a proprietary assay that combines *BRCA* mutations and three DNA-based measures of genomic instability by SNP arrays based on telomeric allelic imbalance, large-scale transitions and loss of heterozygosity. This commercial test was used to ascertain tumor HRD status in the 3 phase III clinical trials investigating PARPi as first-line maintenance treatment in HGOC. In the PAOLA-1, VELIA and PRIMA trials, women with HRD tumors had consistently longer PFS when treated with a PARPi than those with deemed HR-proficient tumors. However, this commercial test has several limitations. It is costly (around 4,500 €), requires at least 30% tumor content, and has a relatively high failure rate. When applied to the PAOLA-1 trial patients, up to 18% of tested tumors had an undetermined HRD status, preventing a significant proportion of women with advanced HGOC from having access to olaparib +bevacizumab as frontline maintenance therapy.

To address these limitations, the European HRD ENGOT initiative (EHEI) was launched in Paris in December 2019, shortly after the presentation of the results of VELIA, PAOLA-1 and PRIMA trials at the plenary session of ESMO annual meeting in 2019 and their concurrent publication. The EHEI is a unique European collaborative effort led by academic research groups aiming to refine HRD status determination. The ideal HRD test must be easy to implement, reliable, affordable, with a fast turnaround time, and use as little tumor material as possible. The EHEI made DNA extracted from 469 formalin-fixed paraffin embedded (FFPE) tumor samples of the PAOLA-1 trial [6] available to the research groups who joined the initiative. The results of several genomic HRD tests using NGS and/or SNP arrays and evaluated within EHEI have been recently published. They consistently reported a decrease in non-contributive results compared to MyChoice CDx while being able to predict PFS benefit of the olaparib +bevacizumab treatment [7–9]. The EHEI is still ongoing with other surrogates of HRD being tested, such as detection of RAD51 foci.

The report by Callens and colleagues published in this issue of *Oncogene*, is the result of an effort by the group that developed one of the original HRD scores, i.e. large-scale transitions [5], afterwards incorporated into the MyChoice CDx and several of the HRD tests listed above. Previously, HRD-related scale transitions were inferred from copy number alterations detected using an SNP array. In this study, the authors developed a new genomic scar assay from low coverage (shallow; 1-2X) whole genome sequencing (WGS), named *shallowHRD* [10]. The bioinformatics pipeline of *shallowHRD* was developed to estimate the number of large-scale genomic alterations, i.e. the number of copy number breaks between chromosomal segments measuring at least 10 Mb in size. Callens et al further adapted it to the clinical routine by addressing the specific noise associated with using FFPE samples and excluding tumors with *ERBB2* or *CCNE1* amplification, described as mutually exclusive with HRD phenotype. Compared to MyChoice Dx and in samples from the PAOLA-1 trial, the *ShallowHRDv2* substantially reduced the failure rate to 3% (compared to 11%), while having strong agreement (94%) for predicting HRD phenotype and benefit from olaparib+bevacizumab [11].

What makes *ShallowHRDv2* unique compared to other HRD tests is that it can be adaptable to any NGS platform and gene panel. Standard NGS performed in a clinical setting starts with a DNA fragmentation followed by capture and sequencing of the exons of targeted genes, resulting in over 99.9% of DNA not being analyzed. *ShallowHRDv2* strategy involves conducting WGS at shallow depth using the remaining DNA from standard NGS. Such approach saves time and resources related to DNA extraction from FFPE samples and reduces sequencing costs by approximately 50 times compared with standard diagnostic WGS. *ShallowHRDv2* workflow can then be used to interpret shallow WGS data, with the bioinformatics algorithm being freely available. For patients with

HGOC, somatic sequencing of a panel of 8-15 HR genes is routinely performed as a diagnostic tool to pre-select women for genetic counseling and/or for PARPi maintenance therapy in case of *BRCA* tumor mutations. Using *ShallowHRDv2*, the HRD score could reliably be obtained from re-sequencing the remaining NGS DNA library without additional genomic testing like SNP array. Such approach dramatically reduces the costs of HRD test, to as low as 100-200€ per test (plus bioinformatic analysis cost). Its application extends well beyond HGOC, to other cancers where HRD phenotype is frequent such as breast, endometrial and prostate.

This study illustrates well the importance of academic clinical/translational trials for innovation in cancer research. Among the six randomized phase III trials investigating PARPi as maintenance therapy in HGOC, PAOLA-1 was the only academic-led trial and made possible initiatives such as the unvaluable EHEI. As the sponsoring of randomized clinical trials shifted in the last two decades from academic collaborative groups to almost exclusively the pharmaceutical industry [12], it is important to advocate for access by the cancer research community to the databases and biobanks of registration trials to investigate predictive biomarkers and fullfill the promise of precision medicine for our patients.

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JLS and SILG wrote the report.

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ADDITIONAL INFORMATION