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### Title

Role and clinical application of next-generation sequencing (NGS) for ovarian cancer.

### Permalink

<https://escholarship.org/uc/item/5w639875>

### Journal

Journal of gynecologic oncology, 28(4)

### ISSN

2005-0380

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### Publication Date

2017-07-01

### DOI

10.3802/jgo.2017.28.e51

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Peer reviewed

## Editorial



# Role and clinical application of next-generation sequencing (NGS) for ovarian cancer

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## OPEN ACCESS

Received: May 11, 2017

Accepted: May 15, 2017

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### Conflict of Interest

No potential conflict of interest relevant to this article was reported.

► See the article “Germline and somatic mutations in homologous recombination genes among Chinese ovarian cancer patients detected using next-generation sequencing” in volume 28, e39.

The inability to repair double stranded DNA breaks, or homologous recombination deficiency (HRD), is the first clinically actionable molecular aberration to be identified in gynecologic cancers. Germline mutations in *BRCA1* and *BRCA2* were the first biomarkers of this phenotype to be identified. Further investigation of these mutations revealed that they are key biomarkers for prevention in affected blood relatives, improved prognosis, response to platinum-based therapy, and, of more recent importance, maximum benefit from poly (adenosine diphosphate [ADP]-ribose) polymerase (PARP) inhibitor (PARPi) treatment [1]. Mutations in additional genes including *RAD51C*, *RAD51D*, *BRIP1*, *ATM*, *CHEK2*, *BARD1*, *MRE11*, *NBS1*, *PALB2*, and *NBN* are now also implicated in this pathway and are rationale predictors of PARPi sensitivity [2-4], as loss of their protein products impair the homologous recombination (HR) pathway's task to maintain genomic stability [5]. The Cancer Genome Atlas (TCGA) project in the USA reported up to alterations in the HR pathway in 51% of high grade serous ovarian cancers, with 16% of tumors harboring germline *BRCA1* or *BRCA2* mutations, 9% with somatic mutations, and 11% with epigenetic silencing of *BRCA1* by way of methylation [6].

Germline *BRCA* mutation (*gBRCAmut*) has been established as a strong predictor of response rate, progression-free survival (PFS), and overall survival (OS) with PARPi. Olaparib (Lynparza™; AstraZeneca, Wilmington, DE, USA) first gained approval in Europe for maintenance following complete response to third line or greater platinum-based chemotherapy [7] and in the USA for 4th treatment or greater [8], both indications limited to *gBRCAmut* carriers. However, somatic mutations were also postulated to predict PARPi sensitivity [9], and in fact, rucaparib broadened the indications for PARPi when it was approved in the USA for 3rd line or greater treatment in women with either germline or somatic *BRCA* mutations [10]. Somatic mutations in this study, rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1), were assessed by loss of heterozygosity (LOH). PFS was significantly longer in the *gBRCAmut* (hazard ratio [HR]=0.27; 95% confidence interval [CI]=0.16–0.44;  $p<0.001$ ) and LOH high (HR=0.62; 95% CI=0.42–0.90;  $p=0.011$ ) subgroups compared with the LOH low subgroup. This study also led to the

approval of FoundationFocus™ CDx<sub>BRCA</sub> test (Foundation Medicine Inc., Cambridge, MA, USA), a tissue-based next-generation sequencing (NGS) tool to detect alteration in *BRCA1* and *BRCA2* genes in formalin-fixed paraffin-embedded (FFPE) ovarian tumor tissues as a companion diagnostic for rucaparib. Simplified sample collection using archived specimen 1 mm<sup>3</sup> FFPE ovarian cancer tumor tissue specimens with >20% of malignant origin, 14-day turnaround time, and multiple cancer gene evaluations at a time is strengths of NGS. Exploratory analyses in ARIEL2 documented significant tumor responses in women with mutations in non-*BRCA* HR genes *ATM*, *NBN*, *RAD51C*, or *RAD51D* plus those with methylation of *BRCA1* or *RAD51C* [11], further supporting their classification as actionable mechanisms of HRD and their inclusion in comprehensive HRD assessment.

Given these advances, and the likelihood that an additional 30% of serous and an unknown proportion of non-serous histologies harbor non-*gBRCAm*-associated HRD, it is now imperative to discover what other genomic alterations might predict PARPi-sensitive biology. In addition, it will be important to know if these alterations vary by ethnicity.

In this issue of *Journal of Gynecologic Oncology*, Zhao et al. [12] report the results of their NGS analysis of 50 matched serum and samples collected from women undergoing ovarian cancer surgery in China. These specimens were tested for germline and somatic mutations in a broad panel of HRD genes including *BRCA1/2*, Fanconi anemia pathway (*FANCA* and *PALB2*), plus *ATM*, *ATR*, *CHEK1*, *CHEK2*, *BARD1*, *BRIP1*, *FAM175A*, *MRE11A*, and *NBN*. Mutations were classified as deleterious or not based on the publicly available ClinVar database (National Library of Medicine, Bethesda, MD, USA). The prevalence of germline and somatic mutations in this sample was 18 (36%) and 5 (10%), respectively. Interestingly, *BRCA2* was the most common germline mutation and *RAD50* was the most frequent somatic mutation. Additional germline mutations included *BRCA1* (8%), *ATR* (6%), *RAD52* (4%), *RAD54B* (4%), *CHEK2* (2%), and *RAD50* (2%) and other somatic mutations were found in *RAD50* (4%), *RAD54B* (2%), *BRCA2* (2%), and *BRCA1* (2%). This is in contrast to Western women where in 360 women from a single institution, deleterious *BRCA1* (13.4%), *BRCA2* (5.2%), *RAD51D* (1.1%), *BRIP1* (1.1%), *RAD51C* (0.8%), *CHEK2* (0.8%), *PALB2* (0.5%), *FAM175A* (0.5%), *BARD1* (0.5%), *NBN* (0.3%), and *CHEK1* (0.3%) mutations were identified in the germline and *BRCA1* (5.2%), *BRCA2* (1.6%), *ATM* (0.8%), *CHEK2* (0.8%), *BRIP1* (0.5%), *MRE11A* (0.3%), and *RAD51C* (0.3%) mutations were identified in tumors. In a second analysis of *BRCA1* and *BRCA2* only mutations were particularly common (23%) in high-grade serous cancers. In 28 patients with available germline DNA, 9 (42.9%) of 21 and 2 (28.6%) of 7 *BRCA1* and *BRCA2* mutations were demonstrated to be somatic, respectively.

The current study is consistent with prior reports in that germline mutations are more common than somatic ones. However, at least in this sample, the higher incidence of *BRCA2* mutation (18%) than *BRCA1* mutation (10%) in Chinese women is quite different pattern compared to that in Western women who exhibit a higher incidence of *BRCA1* (18.6%) than *BRCA2* (6.8%) [3,12]. This pattern in Chinese women is consistent with the previous results in Asian women from the Japanese women with ovarian, fallopian tubal, or peritoneal cancer (5.3% *BRCA1* and 7.4% *BRCA2*) [13]. In Korean women with these cancers, *BRCA1* mutations are more frequently identified than *BRCA2* mutations [14-16]. Other HRD genes remain lower in prevalence but are consistently identified across studies in which they are probed. Diversity in mutational prevalence according to races or regions has been shown in founder germline mutations, and is important to explore in larger populations with comparable methodologies. These differences could further advance our understanding of ovarian carcinogenesis and treatment approach.

Current Korean Society of Gynecologic Oncology (KSGO) recommendations are to perform genetic testing for germline mutation of *BRCA1* and *BRCA2* in all women with a pathologically-confirmed ovarian, tubal, or peritoneal cancer [17]. NGS, as performed here, has become the standard sequencing strategy for most laboratory genetic testing due to higher throughput at a lower cost. The limitations of NGS include difficulty detecting certain mutation types (i.e., genomic rearrangements), generation of large amounts of data that require formal bioinformatics analysis, and need for investment in time and funding to validate a new system. Moreover, multigene testing creates complexity in the interpretation of variants of uncertain clinical significance and clinical significance of non-*BRCA* gene mutations [7,11]. Finally, public databases, such as ClinVar (National Library of Medicine) employed in this study, have been criticized for being incomplete when compared to a proprietary, commercial database, accounting for 27% of discrepancies in variant classification across the 2 databases [18]. Database sharing across multiple institutions and/or centralization of testing would be long-term solutions to this problem.

Meanwhile, tissue-based NGS is easy and do not needs an additional invasive procedure compared to blood-based germline test. And more comprehensive genetic test from non-*BRCA1/2* HR gene and somatic mutation could be achieved. Given the importance of HRD in ovarian cancer, we must continue in our efforts to understand the genetic background and share the individualized treatment outcome based on NGS test in the management of ovarian cancer. Future directions of HRD assessment include non-genomic tissue-based testing that is more phenotypic in nature such as LOH, telomeric allelic imbalance, and large-scale state transitions [19]. This is commercially available as myChoice® HRD test (Myriad Genetics, Inc., Salt Lake City, UT, USA) and is in development as a companion diagnostic to niraparib, the newest of the PARPi's to be approved in the US for maintenance in non-selected women with a complete or partial response to platinum-based chemotherapy [20].

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