Despite significant advances in surgical and medical management, epithelial ovarian cancer (EOC) remains the fifth most common cause of cancer death and the most lethal of all gynecologic malignancies in the USA. Given that EOC is a genetically and biologically heterogeneous disease, a personalized approach to management based on recognition of different EOC subtypes with distinct genotypic and phenotypic characteristics may be an effective strategy to improve outcomes in this disease. EOC is characterized by frequent genetic and epigenetic alterations in gene members of the homologous recombination DNA-repair pathway, most commonly in the BRCA1 and BRCA2 genes. Germline BRCA1 and BRCA2 mutations have been identified in approximately 15% of all EOCs while an additional 30–35% of tumors harbor other genetic or epigenetic alterations in the homologous recombination pathway. In this review, we summarize the phenotypic characteristics of BRCA1/2-associated tumors and their clinical implications, both in terms of routine patient management as well as clinical trial design.

Keywords: BRCA1/2-associated tumors • chemotherapy resistance • DNA repair • homologous recombination • ovarian cancer • PARP inhibitors • personalized therapy • survival
alterations in the HR pathway (including the alterations in BRCA1 and BRCA2 genes [9]). Identification of EOCs with BRCA1/2 mutations or other molecular alterations of the HR pathway is of increased clinical importance because of the advent of poly-ADP ribose polymerase inhibitors (PARPi), a novel class of anticancer agents that exhibit synthetic lethal effects when applied to cells with defective HR [14–17]. However, it is currently unclear whether these tumors should be treated differently compared with the remaining EOCs, and BRCA status is not currently used in the ongoing management of EOC patients and not routinely incorporated as a stratification factor in Phase III clinical trials of this disease. In this review we will summarize the clinical relevance and implications of BRCA status in EOC, both in terms of routine patient management as well as clinical trial design.

Phenotype of BRCA1/2 mutated EOCs: clinical implications

The clinical characteristics of patients with BRCA1/2-mutated tumors are summarized in Table 1 and presented in detail below.

- Association with hereditary breast/ovarian cancer syndrome

Hereditary breast/ovarian cancer (HBOC) syndrome is associated with germline mutations in BRCA1/2 genes [18] and is characterized by a familial clustering of breast and ovarian cancers [18]. It accounts for 10–15% of all EOCs [19,20], although its frequency is much higher among Ashkenazi Jewish women with EOC (29–41%) [21]. The National Comprehensive Cancer Network guidelines for breast and ovarian genetic risk assessment currently recommend referral for genetic testing for HBOC syndrome for every woman diagnosed with EOC, fallopian tube or primary peritoneal serous cancer [22,23]. However, although both BRCA1 and BRCA2 germline mutations cause HBOC syndrome, there are important differences between BRCA1- and BRCA2-associated hereditary cancer syndromes. BRCA1-mutation carriers are associated with higher lifetime risk of ovarian cancer compared with BRCA2 carriers (36–60% vs 16–27%, respectively) and tend to develop ovarian cancer approximately 8 years earlier on average than BRCA2 carriers (54 vs 62 years) [11,24–26]. Similarly, BRCA1-mutation carriers are associated with slightly higher lifetime risk of breast cancer compared with BRCA2 carriers (57 vs 49%, respectively) and tend to develop breast cancer approximately 4 years earlier on average than BRCA2 carriers (43 vs 47 years) [24]. Furthermore, risk-reducing salpingo-oophorectomy appears to confer different degrees of protection against gynecologic and breast cancers between BRCA1 and BRCA2 carriers, suggesting that future studies evaluating the efficacy of risk-reduction strategies in BRCA mutation carriers may need to stratify by the specific gene (BRCA1 vs BRCA2) mutated [27]. Finally, BRCA1 and BRCA2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BRCA1/2-associated EOCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hereditary breast–ovarian syndrome</td>
<td>BRCA1 carriers have higher lifetime frequency of EOC BXCA1 carriers develop EOC at an earlier age Risk of different cancers in BRCA1 vs -2 carriers</td>
</tr>
<tr>
<td>Pathology</td>
<td>Association with serous tumors Association with high-grade/undifferentiated tumors</td>
</tr>
<tr>
<td>Stage</td>
<td>Association with higher stage (stage III or IV) at presentation</td>
</tr>
<tr>
<td>Debulking status</td>
<td>No difference in the rates of optimal tumor debulking at primary surgery as compared with sporadic tumors Debulking status is independently associated with survival among patients with BRCA1/2-associated tumors</td>
</tr>
<tr>
<td>Patterns of recurrence</td>
<td>More likely to develop visceral metastases (parenchymal lung, liver, spleen, adrenal and brain metastases) This effect seems more prominent for BRCA1 tumors</td>
</tr>
<tr>
<td>Overall survival</td>
<td>Improved survival for BRCA1 vs sporadic (hazard ratio = 0.73) Improved survival for BRCA2 vs sporadic (hazard ratio = 0.49) Improved survival for BRCA2 vs BRCA1 (hazard ratio = 0.64)</td>
</tr>
<tr>
<td>Response to chemotherapy</td>
<td>Improved response to platinum and PARPi Improved response to other double strand DNA break-inducing agents, such as PLD BRCA2 are more responsive to platinum and have greater genomic instability than BRCA1-tumors BRCA1 loss may be associated with taxane resistance</td>
</tr>
</tbody>
</table>

EOC: Epithelial ovarian cancer; PARPi: PARP inhibitors; PLD: Pegylated liposomal doxorubicin.
mutation carriers have been reported to be associated with elevated risks for other tumors besides breast and ovarian cancer; that is, *BRCA1* carriers with gastric, pancreatic, prostate and uterine cancers and *BRCA2* with melanoma, gastric, pancreatic, prostate and biliary duct cancers [26–31]. The role of cancer screening for tumors other than breast or ovarian cancers in *BRCA1* and *BRCA2* carriers is currently unclear.

**Pathology of *BRCA1/2*-mutated tumors**

A higher proportion (as high as 22.6%) of high-grade (grade 2 or 3) papillary serous EOCs are associated with *BRCA1* or *BRCA2* mutations compared with endometrioid or clear cell histologies, while no or only exceedingly rare mutations have been identified in women with invasive mucinous ovarian tumors [11,12,32]. Furthermore, tumors in *BRCA1* and *BRCA2* carriers are more likely to be poorly differentiated or undifferentiated compared with non-carriers [33]. No differences in histology or grade have been identified between *BRCA1*- and *BRCA2*-associated tumors [32–34]. In one study, *BRCA1*/*2* mutations were identified in ten out of 119 endometrioid and four out of 63 clear-cell EOCs, but most of these cases (11 out of 14) were subsequently reclassified as high-grade serous or unclassified adenocarcinomas, suggesting that *BRCA1*/*2* mutations are almost exclusively associated with high-grade serous cancers [11]. Despite these findings, according to the National Comprehensive Cancer Network guidelines for breast and ovarian cancer genetic-risk assessment, *BRCA1/2* genetic testing should still be offered to all women with newly diagnosed EOC, regardless of histology [23].

In breast cancer, *BRCA1*-mutated tumors frequently exhibit a characteristic pathological phenotype (i.e., they commonly express basal, myoepithelial cell-type cytokeratins [CK5/6, CK14 and CK17], are commonly estrogen/progesterone receptor-/HER2-negative, are of higher mitotic count and show lymphocytic infiltration) [35], while *BRCA2*-mutated tumors lack a clear pathologic phenotype (although *BRCA2*-mutated tumors are more frequently estrogen receptor-/progesterone receptor-positive compared with *BRCA1*-mutated tumors) [32]. Conversely, in ovarian cancer, neither *BRCA1*- nor *BRCA2*-mutated tumors are associated with a distinct histopathological or immuno-histochemical phenotype that readily distinguishes them from sporadic cancers. However, in one small study from Memorial Sloan-Kettering Cancer Center (NY, USA) that included 43 high-grade serous EOCs from the TCGA project, *BRCA1*-mutated tumors were frequently associated with solid, pseudoendometrioid and transitional cell carcinoma-like morphology, higher mitotic indexes, more tumor-infiltrating lymphocytes and either geographic or comedonecrosis [36]. In the same study, *BRCA2*-associated tumors tended to show solid, pseudoendometrioid and transitional cell carcinoma-like morphology, but were relatively deficient in tumor-infiltrating lymphocytes and necrosis. Although larger studies are necessary to confirm these findings, these pathologic characteristics may raise the possibility of presence of a *BRCA* mutation in a patient who has otherwise not been offered genetic testing.

**Association with overall survival**

Four large studies have demonstrated that *BRCA1/2*-mutated ovarian cancers are associated with improved overall survival compared with their sporadic counterparts [9,26,33,37]. In three of these studies, *BRCA1* and *BRCA2* carriers were combined and compared together versus their sporadic counterparts [9,26,37]. The fourth and largest study included 1213 EOC patients with pathogenic germline mutations in *BRCA1* (*n* = 909) or *BRCA2* (*n* = 304) and 2666 non-carriers pooled from 26 international observational studies. The primary end point was 5-year overall mortality [33]. In that study, *BRCA1* and *BRCA2*-mutation carriers separately exhibited a statistically significantly improved survival compared with non-carriers (adjusted hazard ratio of 0.73 for *BRCA1* and 0.49 for *BRCA2* carriers vs non-carriers) and *BRCA2* carriers exhibited statistically significantly improved survival compared with *BRCA1* carriers (hazard ratio: 0.64). Interestingly, the survival advantage of *BRCA1* carriers over non-carriers differed depending on the location of the mutation; worse survival was observed as the mutation site moved from 5´ to 3´ end [33,38]. Two other studies also demonstrated a significant survival advantage for *BRCA2*-associated EOCs over *BRCA1*-associated and BRCA-negative EOCs, but a smaller not statistically significant advantage of *BRCA1*-associated EOCs over BRCA-negative tumors probably due to lack of power to detect such difference [34,39]. The survival advantage of *BRCA1*- and *BRCA2*-associated tumors relative to BRCA-negative tumors and the advantage of *BRCA2*-over *BRCA1*-associated tumors could be related to a more indolent natural history due to intrinsic biologic differences, or to differential response to therapy (as in the following section), or both. Finally, it is important to underscore that the duration of the survival advantage for the *BRCA1/2*-associated tumors over BRCA-negative tumors is unknown. In this regard, a recently published study that included 218 mutation carriers with EOC demonstrated a short-term survival advantage associated with the presence of *BRCA1/2* mutations, but that these patients did not have long-term survival benefit [40]. Longer follow up of the other studies discussed above is necessary to evaluate this possibility.
**Association with response to chemotherapy**

The standard-of-care first-line systemic therapy of EOC includes a combination of platinum and taxane chemotherapy [2,41]. Platinum analogs (carboplatin and cisplatin) induce intra- and inter-strand crosslinks and double strand breaks (DSB) in the DNA double helix backbone, which are normally repaired by the HR DNA-repair pathway [42]. Cells that are deficient in BRCA1 or BRCA2 function exhibit defective DNA repair via HR and are therefore particularly sensitive to platinum agents [43]. Furthermore, cells with defective HR use alternative mechanisms for the repair of DSB, such as the error-prone and mutagenic non-homologous end-joining (NHEJ) pathway that directly ligates the end of a DSB together and frequently causes deletions or mutations of DNA sequences around the DSB site [44]. For this reason, cells deficient in BRCA1 or BRCA2 function also show a high degree of genomic instability.

Patients with BRCA1/2-associated tumors exhibit higher response rates and prolonged disease-free survival after first-line platinum-based chemotherapy and increased response rates to subsequent lines of platinum-based chemotherapy compared with nonhereditary tumors [11,26,45–47]. Of note, enhanced responsiveness to platinum chemotherapy seems to be more prominent in BRCA2-associated tumors compared with BRCA1- and BRCA-negative tumors [34,46]. Specifically, in one study based on EOCs included in the TCGA project, BRCA2-associated tumors were associated with higher primary chemotherapy sensitivity rate, longer platinum-free duration and greater genomic instability compared with BRCA1- and BRCA wild-type tumors [34]. This is probably because, although both BRCA1 and BRCA2 genes are involved in DNA repair via HR, several studies suggest that they have distinct functions [48,49]. In this regard, the nature of the defect in DNA repair due to BRCA1 mutation may be different than that due to a BRCA2 mutation.

Importantly, the enhanced platinum sensitivity associated with BRCA-associated EOC tumors may challenge the traditional clinical definition of platinum resistance as relapse within 6 months after the last platinum dose in these patients. Specifically, in one study, patients with BRCA-associated tumors who were retreated with platinum within 6 months of the end of primary platinum therapy (i.e., classified as platinum resistant using the conventional clinical definition of platinum resistance) still exhibited high response rates to platinum therapy (i.e., eight out of ten patients [80%] showed a CA125 response defined as at least 50% reduction in CA125 maintained for at least 1 month) [11]. Although this phenomenon needs to be studied prospectively and in a randomized fashion in a larger number of patients, this study advocates for continuation of platinum therapy in patients with BRCA-associated tumors even if they are defined as platinum resistant using traditional criteria until clear tumor progression on platinum is observed.

The association between presence of BRCA1/2 mutations that cause defective DNA repair via HR and platinum sensitivity is further strengthened by the fact that development of platinum resistance in BRCA-associated tumors is frequently related to emergence of secondary BRCA1/2 mutations that restore BRCA1/2 function. Several studies have shown that in BRCA-associated tumors, restoration of BRCA1/2 function due to secondary BRCA1/2 mutations leads to restoration of DNA repair via HR and acquired platinum resistance [50–54]. In this case, the genetic reversion of the inherited BRCA1 or BRCA2 mutations provides a survival advantage to the cancer cells by protecting them from platinum chemotherapy. In one study, secondary BRCA1/2 mutations that restore BRCA function occurred in 12 out of 26 (46.2%) of platinum resistant relapsed BRCA-associated EOCs and cumulative exposure to chemotherapy may contribute to the development of these secondary genetic events [54].

BRCA-associated tumors have also been shown to exhibit enhanced sensitivity to non-platinum cytotoxic agents that induce double strand DNA breaks. For example, pegylated liposomal doxorubicin (PLD) is a topoisomerase II inhibitor that induces DNA double breaks and is US FDA-approved for the treatment of relapsed ovarian cancer [55,56]. Treatment of BRCA-associated EOC patients with PLD has been shown to result in longer time to treatment failure and improved overall survival compared with sporadic patients, independent of platinum sensitivity [57]. Similarly, other DSB-inducing agents such as PARPi (discussed below) exhibit high response rates in patients with BRCA-associated EOCs.

Unlike DSB-inducing agents such as platinum and PARPi, taxanes are mitotic spindle poisons that act by inhibiting microtubule depolymerization. Given that BRCA1 is also a regulator of the G2-M checkpoint and of the mitotic spindle assembly [58,59], several studies in breast and ovarian cancer have evaluated the association of BRCA1 status and taxane sensitivity [60–64]. Most of these studies suggest that intact BRCA1 is crucial for taxane cytotoxicity by directing cells towards apoptotic death after taxane treatment (i.e., exactly opposite from the association of BRCA1 with platinum response whereby intact BRCA1 is associated with platinum resistance) [60–63]. In this regard, inhibition of endogenous BRCA1 expression decreases sensitivity to taxanes in ovarian cancer cell lines and high BRCA1 levels in patients with sporadic ovarian cancer exhibit a nonsignificant trend towards improved survival after taxane-based chemotherapy [64]. Larger
prospective studies are necessary to confirm whether BRCA1 deficiency is indeed associated with taxane resistance and whether this is clinically significant for the medical treatment of EOC.

Association with other clinical characteristics (stage, debulking & patterns of recurrence)

Several studies have reported that BRCA1/2-associated tumors are associated with higher stage (stage III or IV) at presentation compared with their sporadic counterparts [11,35,65]. Conversely, no difference in the rates of optimal tumor debulking at primary surgery have been observed between BRCA1/2-associated and sporadic tumors after adjusting for differences in patient age [66]. However, debulking status is independently associated with survival among patients with BRCA1/2-associated tumors, and was the sole factor associated with survival in these patients in one study [11]. Of note, survival of BRCA1/2-associated tumors and suboptimally debulked disease is similar to survival of patients with BRCA-negative tumors and optimally debulked disease suggesting that the survival benefit of BRCA-associated tumors over their sporadic counterparts may be eliminated if these tumors are suboptimally debulked [11]. These data argue that presence of BRCA1/2 mutations does not obviate the need for optimal surgical debulking in these patients.

In terms of patterns of recurrence, two studies have demonstrated that BRCA1/2-associated tumors are more likely to develop visceral metastases (parenchymal lung, liver, spleen, adrenal and brain metastases) compared with the BRCA-negative tumors [11,67]. In the first study, the frequency of all visceral metastases was 58% among BRCA-associated tumors and 5% in matched sporadic controls, while the percentage of patients with visceral metastases as their first site of progression was 47% in BRCA-associated tumors and 5% in the sporadic controls [67]. This difference was particularly prominent in BRCA1-associated tumors, which also seemed to be associated with higher incidence of visceral metastases compared with BRCA2-associated tumors. Similarly, another larger study reported that patients with BRCA1/2 mutations were more likely to have developed visceral metastases within 2 months of first progression, but this difference decreased over time and the presence of visceral metastases did not affect survival among BRCA1/2-associated tumors [11]. However, although BRCA1/2 tumors are more likely to develop visceral metastases upon recurrence, there is no evidence that visceral metastases are more common in BRCA1/2-associated tumors at initial presentation. Specifically, while BRCA1/2-associated tumors present more commonly with advanced disease (stage III or IV) compared with their sporadic counterparts, no study has shown a prevalence of stage IV disease among BRCA1/2-associated tumors.

BRCAness phenotype in sporadic EOC

Patients with BRCA1/2-associated EOCs exhibit improved overall survival and high sensitivity to double strand DNA break-inducing agents due to an underlying defect in DNA repair via HR [68,69]. However, it is increasingly recognized that a subset of patients with sporadic EOCs also exhibit defective HR caused by mechanisms that are unrelated to germline BRCA1 or BRCA2 mutations [70]. These tumors may behave similarly to BRCA1/2-mutated EOCs and are commonly referred to as having a ‘BRCAness’ phenotype [70]. Identifying tumors with a BRCAness phenotype is of increased clinical importance not only due to the advent of PARPi (as discussed in the following section) but also because patients with this phenotype may need to be managed differently than the remaining patients.

Several molecular mechanisms may underlie defective HR in EOCs in the absence of germline BRCA1/2 mutations and are summarized in Table 2. These include genetic and epigenetic alterations involving members of the HR DNA-repair pathway, that is, somatic BRCA1/2 mutations, hypermethylation of BRCA1 or RAD51C, amplification or mutation of EMSY, focal deletion or mutation of PTEN, mutation of ATM or ATR, and mutation of...

---

**Table 2. Molecular alterations of homologous recombination pathway in epithelial ovarian cancer (based on The Cancer Genome Atlas dataset).**

<table>
<thead>
<tr>
<th>Molecular alteration</th>
<th>High-grade serous EOC (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Germline BRCA1/2 mutations</strong></td>
<td></td>
</tr>
<tr>
<td>BRCA1: 8.5</td>
<td></td>
</tr>
<tr>
<td>BRCA2: 6.3</td>
<td></td>
</tr>
<tr>
<td>Total: 14.7</td>
<td></td>
</tr>
<tr>
<td><strong>Somatic BRCA1/2 mutations</strong></td>
<td></td>
</tr>
<tr>
<td>BRCA1: 3.2</td>
<td></td>
</tr>
<tr>
<td>BRCA2: 2.9</td>
<td></td>
</tr>
<tr>
<td>Total: 6.1</td>
<td></td>
</tr>
<tr>
<td><strong>Epigenetic silencing of BRCA1</strong></td>
<td>10.8</td>
</tr>
<tr>
<td><strong>Amplification or mutation of EMSY</strong></td>
<td>7.9</td>
</tr>
<tr>
<td><strong>Homozogous deletion of PTEN</strong></td>
<td>6.7</td>
</tr>
<tr>
<td><strong>Mutations in Fanconi anemia genes</strong> (FANCA, FANCC, FANCD2, FANCE, FANCG, FANCI, FANCL, PALB2)</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Mutations in core HR RAD genes</strong> (RADS0, RADS1, RADS4L)</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Mutations in DNA damage response genes</strong> (ATM, ATR, CHEK2)</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>Epigenetic silencing of RAD51C</strong></td>
<td>2.5</td>
</tr>
</tbody>
</table>

†n = 316
EOC. Epithelial ovarian cancer; HR. Homologous recombination.
Data taken from [9].
Fanconi anemia genes [71–74]. In this regard, in the TCGA dataset, approximately 30% of high-grade serous EOCs harbored the aforementioned genetic or epigenetic alterations involving the HR pathway while approximately 20% harbored germline BRCA1 or BRCA2 mutations [9]. Interestingly, among the remaining 50% of high-grade serous EOCs in the TCGA dataset that did not harbor these alterations or germline BRCA1/2 mutations, several tumors were associated with enhanced sensitivity to platinum, suggesting that alternative mechanisms, that are yet to be identified, may underlie BRCAAness in EOC.

It is important to underscore that although the aforementioned molecular mechanisms of BRCAAness may cause defective HR and, thus, enhanced sensitivity to double strand DNA break-inducing agents, not all of them are necessarily associated with improved overall survival. For example, while sporadic EOCs with somatic BRCA1/2 mutations are associated with improved overall survival compared with BRCA-negative tumors [9,13], tumors with epigenetic silencing of BRCA1 through promoter methylation are not associated with improved survival (despite the fact that they are associated with platinum sensitivity) [9].

Given the heterogeneous molecular mechanisms that may underlie BRCAAness, it is challenging to prospectively identify sporadic patients with a BRCAAness phenotype. One approach would be to comprehensively profile each tumor for molecular abnormalities in HR pathway genes using next-generation sequencing [75]. Limitations of this approach include high cost and the fact that it is not certain that every molecular alteration in gene members of the HR pathway identified via this approach can result in sufficiently defective HR to induce sensitivity to platinum and PARPi. Alternative approaches include gene expression profiles of BRCAAness [76] or DNA repair [77], assessing loss of heterozygosity and copy number changes as a surrogate of genomic instability using single nucleotide polymorphism array data [78], assessing BRCA1 protein expression using immunohistochemistry [79], and assessing the wider tumor genome nucleotide sequences and mutational spectrums or ‘sequence scars’ that may be characteristic of defective DNA repair via HR [80]. These are promising assays but need to be independently and prospectively validated before they can be incorporated into routine clinical practice. Finally, functional biomarkers of BRCAAness have also been proposed whereby HR pathway is mechanistically evaluated by assessing RAD51 foci formation by immunofluorescence or by assessing other DNA repair complexes by immunohistochemistry [81,82]. The challenge with functional biomarkers of defective HR is that they require the tissue or the specimen to be exposed to some form of DNA damage (i.e., radiation or chemotherapy) before the molecular marker is assessed. Despite these challenges, identification of a reliable biomarker of BRCAAness that accurately predicts defective HR and responsiveness to platinum and PARP inhibitors is a high priority for ovarian cancer research.

Therapeutic opportunities for BRCA1/2-associated tumors

Concept of synthetic lethality

Two genes are synthetically lethal when loss-of-function in either of these two genes permits cell survival, while loss-of-function of both genes is incompatible with survival [83]. Synthetic lethality is an exciting anticancer strategy because targeted inhibition of one gene in the synthetic lethal pair leads to selective killing of cancer cells (cancer cells exhibit defects in the other gene of the synthetic lethal pair) while sparing normal cells (normal cells do not exhibit defects in the other gene of the synthetic lethal pair), thereby enabling wider therapeutic windows that can be achieved by conventional chemotherapy drugs. This strategy was applied to BRCA1/2-associated tumors and led to the development of a novel class of anticancer drugs – the PARPi. Specifically, it was shown that cells that exhibit defective HR due to BRCA1/2 mutations are exquisitely sensitive to inhibition of the PARP1 enzyme, which is a key component of the base excision DNA repair pathway that is responsible for repair of single-strand DNA breaks (SSB) [14,84]. Inhibition of PARPi enzyme leads to persistence of spontaneously occurring SSBs and subsequent formation of DSB (SSB stall and collapse replication forks and lead to DSB), which cannot be repaired by the defective HR pathway in BRCA1/2-mutated cells thereby resulting in cell death. Cells that exhibit normal DNA repair via the HR pathway are 1000-times less sensitive to PARPi compared with BRCA1/2-mutated cells, thus potentially providing a wide therapeutic window for these drugs. Importantly, PARPi have also been shown to stimulate the error-prone NHEJ DNA repair pathway (via phosphorylation of DNA-dependent protein kinase substrates) which leads to cytotoxicity in HR-deficient cells treated with PARPi. So, stimulation of the error-prone NHEJ pathway seems to play an important role in the synthetic lethality induced by PARPi in BRCA1/2-deficient tumors [85].

Clinical development of PARPi in BRCA1/2-associated EOC

Several PARPi including olaparib (AZD2281), rucaparib (AG014699), veliparib (ABT888), niraparib (MK4827) and iniparib (BSI201) have been evaluated in BRCA-associated EOC [86]. Iniparib is not considered a PARPi as it exhibits very low PARP inhibition in vitro, and its mechanism of action in vivo remains unclear [87]. Of these drugs, olaparib has been the most widely studied so far in BRCA-associated EOC (Table 3). In the initial proof-of-concept Phase I study of olaparib monotherapy in...
BRCA-associated refractory solid tumors, the maximum tolerated dose was established at 400 mg orally twice-daily using capsule formation and an impressive response rate was observed: nine of 19 BRCA-associated patients with breast, ovarian or prostate cancers exhibited a partial response according to Response Evaluation Criteria in Solid Tumors (RECIST; eight out of nine patients had EOC) and 63% of patients (12 out of 19) derived clinical benefit (tumor marker or radiologic response or stable disease of 4 or more months) from olaparib \(^{[15]}\). The expanded cohort of this study included 50 patients with BRCA1/2-associated EOC who received olaparib monotherapy at 200 mg p.o. daily and showed radiological or CA125 response in 40% of patients with a median duration of response of 28 weeks \(^{[88]}\). This study indicated an association between olaparib and platinum sensitivity, that is, the olaparib clinical benefit rate correlated with platinum sensitivity (23% in platinum refractory, 46% in platinum resistant and 69% in platinum-sensitive patients) probably due to the common mechanism of defective HR that confers sensitivity to both drugs.

Another Phase II international, multicenter study suggested a dose–response relationship of olaparib in BRCA1-associated EOC patients. Specifically, patients randomized to olaparib 400 mg by mouth (p.o.) twice-daily exhibited higher response rate and median progression-free survival (PFS) compared with patients randomized to olaparib 100 mg p.o. daily (33 vs 13% and 5.8 vs 1.9 months, respectively) \(^{[89]}\). Given that patients randomized in the lower dose cohort had poorer prognostic features it is still unclear whether there is a clinically meaningful dose–response relationship of olaparib in BRCA-associated EOC.

Subsequently, olaparib was compared with PLD in a Phase II, open-label study in patients with recurrent BRCA-associated EOC who progressed within 12 months of their most recent platinum regimen \(^{[90]}\). A total of 97 patients were randomized 1:1:1 between two olaparib doses (200 and 400 mg twice-daily) and PLD at its FDA-approved dose (50 mg/m\(^2\) intravenously every 28 days). The side-effect profile of olaparib reported in this and the aforementioned studies was generally mild and included nausea, vomiting, fatigue (which can sometimes be severe) and anemia. There were no statistically significant differences in median PFS, RECIST-assessed response rate and overall

<table>
<thead>
<tr>
<th>Table 3. Clinical Trials of PARP inhibitors as single agents in BRCA1/2-associated in epithelial ovarian cancer.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Agent</strong></td>
</tr>
<tr>
<td>Olaparib</td>
</tr>
<tr>
<td>Olaparib</td>
</tr>
<tr>
<td>Olaparib</td>
</tr>
<tr>
<td>Olaparib versus Doxil</td>
</tr>
<tr>
<td>Rucaparib</td>
</tr>
<tr>
<td>Niraparib</td>
</tr>
<tr>
<td>Veliparib</td>
</tr>
</tbody>
</table>

b.i.d.: Twice-daily; CR: Complete response; EOC: Epithelial ovarian cancer; iv.: Intravenously; ORR: Overall response rate; p.o.: By mouth; PFS: Progression-free survival; PR: Partial response; RECIST: Response Evaluation Criteria in Solid Tumors; SD: Stable disease.
survival, although the olaparib 400-mg arm exhibited a better CA125 response compared with the PLD arm, again suggesting a potential dose–response relationship of olaparib in BRCA-associated EOC. One explanation for the similar response between olaparib and PLD may be that patients with BRCA-associated EOC may also be more sensitive to PLD (as discussed above), which may have affected the power of the study to detect a smaller difference in PFS between olaparib and PLD [90,91]. In this regard, the PFS of 7.1 months observed in the PLD arm appears higher than the median PFS of PLD (4 months) observed in patients with unknown BRCA status. Finally, imbalances in platinum sensitivity and number of prior lines of therapy in favor of PLD may have underestimated an olaparib effect.

The clinical evaluation of other PARPi as single agents in BRCA-associated EOC is in a more preliminary stage (Table 3). Veliparib and niraparib (both oral agents) have been evaluated in Phase I studies as single agents while an oral formulation of rucaparib is currently in Phase II trials and the drug is administered every day in an effort to achieve a more prolonged PARP inhibition and possibly higher efficacy.

Finally, combinations of PARPi with conventional chemotherapy agents that induce DNA strand breaks such as temozolomide, platinum compounds and topoisomerase I or II inhibitors are also being evaluated in BRCA-associated EOCs [86]. The rationale behind these PARPi/chemotherapy combinations is that PARPi inhibit base excision repair, which is partly responsible for repair of the damage caused by these chemotherapy agents thus potentiating their action. Combinations of PARPi with antiangiogenic agents such as cediranib based on preclinical data of interaction between the VEGF pathway and PARP inhibition [92,93] and with PI3K inhibitors based on evidence of synergism between PI3K and PARP inhibition are also being evaluated [94].

■ PARPi in non-BRCA1/2-associated tumors

As discussed above, a subset of patients with sporadic EOCs also exhibit defective HR caused by mechanisms that are unrelated to germline BRCA1 or BRCA2 mutations [70]. These tumors, which are referred to as having a ‘BRCAness’ phenotype [70], may also be sensitive to PARPi because of their defective HR pathway, similar to their BRCA-associated counterparts. Two studies of single-agent olaparib have demonstrated significant activity of this PARPi in sporadic EOCs (Table 4) [95,96]. In a landmark Phase II study of 47 patients with high-grade serous/undifferentiated EOC and unknown or negative BRCA status, olaparib administered at 400 mg twice-daily was associated with a 24% objective response rate by RECIST, a 30% combined RECIST or CA125 response rate and a median PFS of 27 weeks [95]. This study included also 17 patients with BRCA-positive EOCs and the objective response rate to olaparib was 41%. As in the Fong et al. study [88], olaparib sensitivity was higher in platinum-sensitive compared with platinum-resistant tumors in both BRCA-positive and -negative cohorts. However, while responses were seen in a significant percentage of platinum-sensitive patients in both BRCA-positive and -negative tumors (60 and 50%, respectively) and in patients with platinum-resistant BRCA-positive tumors (33%), the response rate was very low in platinum-resistant BRCA-negative patients (one [4%] out of 26 patients).

Furthermore, a randomized, double-blind, placebo-controlled, Phase II study evaluated the role of maintenance olaparib treatment in 265 EOC patients, including those with non-BRCA-associated disease [96]. In an attempt to enrich for patients with tumors that may harbor defective HR (in the absence of any good biomarkers of BRCAiness), only patients with platinum-sensitive, high-grade serous EOC who had received two or more platinum-based regimens and had had a partial or complete response to their most recent platinum-based regimen were eligible. PFS, the primary end point of the study, was significantly longer in the olaparib versus placebo cohorts (8.4 vs 4.8 months, hazard ratio of progression or death was 0.35) but interim analysis showed no difference in overall survival. These two studies provide a strong rationale for use of PARPi in non-BRCA1/2-associated EOCs and studies of other PARPi in this patient population are currently underway.

■ Challenges for PARPi in EOC

Although PARPi have shown striking responses in BRCA-associated tumors, a substantial fraction of patients do not respond or develop resistance to these agents suggesting that de novo and acquired resistance to PARPi may be a significant clinical problem. Increased expression of p-glycoprotein efflux transporter mediating multidrug resistance has been shown to lead to acquired resistance to olaparib [97]. Furthermore, in BRCA-associated tumors, secondary BRCA1/2 mutations that restore BRCA1/2 function and lead to development of platinum resistance may also lead to PARPi resistance [50–54]. However, although restoration of defective HR is a common mechanism of resistance to both platinum and PARPi, it is important to underscore that the mechanisms of platinum and PARPi resistance are not completely overlapping. In this regard, patients who have developed resistance to PARPi may respond well to subsequent platinum therapy. One proposed mechanism for that is loss of 53BP1 in BRCA1-associated tumors, which has been shown to lead to PARPi resistance while preserving sensitivity to platinum and other interstrand crosslinking agents [98–100]. 53BP1 is involved in regulating the choice between NHEJ and HR-mediated repair of DNA DSB in favor of NHEJ, so loss of 53BP1 suppresses
Given that several studies suggest that BRCA2 drugs, but also in any clinical trial with a survival (PFS or OS) considered not only for clinical trials of PARPi or other DSB-inducing agents), an argument can also be made for stratification based on the specific mutation (i.e., BRCA1 versus BRCA2) in future clinical trial design. This is particularly relevant for trials of DSB-inducing agents such as PARPi or platinum because of the possible differential chemosensitivity of BRCA1- versus BRCA2-associated tumors and the different mechanisms of acquired resistance to chemotherapy that may develop in BRCA1 versus BRCA2 tumors. Furthermore, studies evaluating the efficacy of risk-reduction strategies in BRCA mutation carriers may need to stratify by the specific gene (BRCA1 versus BRCA2) mutated given that there may be different degrees of protection, as has been the case with risk reducing salpingo-oophorectomy [27].

It is important to underscore that for clinical trials of EOC in the relapsed/recurrent setting, stratification based on BRCA-status at the time of diagnosis may not be enough because of the potential bias that may arise due to the presence of secondary BRCA1/2 mutations that restore BRCA function at the time of relapse. Such mutations are quite prevalent and may occur in as many as 46.2% of recurrent platinum-resistant tumors [54]. In this regard, platinum-resistant BRCA1/2-associated tumors that harbor secondary BRCA1/2 mutations may have different chemosensitivity (i.e., be more resistant to PARPi and other DSB-inducing agents) than platinum-resistant BRCA1/2-associated tumors without secondary BRCA1/2 mutations. Therefore, it would be ideal if sequencing of patient tumors for the presence of secondary BRCA1/2 mutations is performed prior to their enrollment in clinical trials of PARPi or other DSB-inducing agents in the recurrent setting.

Another consideration, perhaps equally important for clinical trials in patients with BRCA1/2-associated EOC,
is the potential of higher sensitivity of these tumors to conventional chemotherapy drugs. As discussed previously, one explanation for the similar response between olaparib and PLD in the aforementioned randomized Phase II study may have been that patients with BRCA-associated EOC are more sensitive to PLD (90,91). This may have affected the power of the study to detect a smaller difference in PFS between olaparib and PLD. Therefore, future clinical trials that evaluate conventional chemotherapy agents in BRCA1/2-associated tumors should take into consideration the potential enhanced sensitivity of these tumors to conventional chemotherapy for power calculations and selection of appropriate end points.

Finally, given that 30–35% of EOCs exhibit a BRCAness phenotype associated with defective HR in the absence of germline BRCA1/2 mutations, it would be important to incorporate assays or biomarkers of BRCAness (discussed above) into clinical trials of PARPi or other DSB-inducing agents. Although these assays may not be ready at this point for use as a stratification factor in clinical trials of PARPi, they can still be incorporated as translational end points to aid the identification of a reliable biomarker of BRCAness, which is a high priority for ovarian cancer research.

Future perspective
EOC remains the most lethal gynecologic malignancy in the USA despite significant advances in its surgical and medical treatment. A personalized approach in management based on recognition of different EOC subtypes with distinct genotypic and phenotypic characteristics may be an effective strategy to improve outcomes in this disease. BRCA1/2-associated tumors exhibit defective DNA repair via HR and represent a distinct EOC subtype with unique clinical characteristics that have important implications for clinical management and clinical trial design. Importantly, certain sporadic EOCs exhibit defective HR due to mechanisms unrelated to BRCA1/2 germline mutations and possess similar characteristics with BRCA-associated tumors, a phenotype referred to as ‘BRCAness’. The striking activity of PARPi in BRCA-associated tumors and tumors associated with a BRCAness phenotype highlights the potential of synthetic lethality as anticancer strategy and exemplifies the paradigm of personalized medicine in EOC. However, several challenges remain, such as de novo or acquired PARPi resistance, identification of sporadic patients with a BRCAness phenotype and optimal incorporation of PARPi in our current armamentarium of drugs against this devastating disease.

Financial & competing interests disclosure
UA Matulonis is a consultant to Clovis and Tesaro. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. No writing assistance was utilized in the production of this manuscript.

Executive summary

<table>
<thead>
<tr>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ Approximately 50% of high-grade epithelial ovarian cancers (EOC) harbor genetic or epigenetic alterations in the homologous recombination pathway.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phenotype of BRCA1/2-mutated EOCs: clinical implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ Germline BRCA1 and BRCA2 mutations have been identified in approximately 15% of all EOCs and as high as 22.6% of high-grade serous EOCs.</td>
</tr>
<tr>
<td>■ BRCA1/2-associated tumors are frequently associated with serous histology, are of high grade and higher stage, and are associated with improved responsiveness to platinum and overall survival compared with their sporadic counterparts.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>‘BRCAness’ phenotype in sporadic EOC</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ The concept of ‘BRCAness’ refers to the phenomenon whereby a subset of sporadic EOCs may behave similarly to BRCA1/2-mutated EOCs.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Therapeutic opportunities for BRCA1/2-associated tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ PARP inhibitors (PARPi) exhibit synthetic lethal effects in tumors with a defective homologous recombination DNA repair pathway such as BRCA1/2-associated EOCs.</td>
</tr>
<tr>
<td>■ Clinical trials have demonstrated significant activity of PARPi in BRCA1/2-associated tumors and certain BRCA1/2 negative tumors, which correlates with platinum sensitivity.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Implications of BRCA status for clinical trial design</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ The unique phenotypic and genomic characteristics of BRCA1/2-associated tumors have important implications for their management as well as future clinical trial design in EOC.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Future perspective</th>
</tr>
</thead>
<tbody>
<tr>
<td>■ Overcoming de novo as well as acquired PARPi resistance, identification of sporadic patients with a BRCAness phenotype and optimal incorporation of PARPi in the current armamentarium of drugs are important priorities for ovarian cancer research.</td>
</tr>
</tbody>
</table>
References

Papers of special note have been highlighted as:

- of interest

10. Hallmark study showing the results of The Cancer Genome Atlas project for ovarian cancer.
14. Hennessy BT, Timms KM, Carey MS et al. Somatic mutations in BRCA1 and BRCA2 could expand the number of patients that benefit from poly (ADP ribose) polymerase inhibitors in ovarian cancer. J. Clin. Oncol. 28(22), 3570–3576 (2010).
16. First evidence of synthetic lethality between PARP inhibition and homologous recombination deficiency.
34. Largest study showing that BRCA1- and BRCA2-associated epithelial ovarian cancer (EOC) have improved survival compared with their sporadic counterparts.
Review: Clinical Trial Methodology

Konstantinopoulos & Matulonis


Secondary BRCA mutations are common and may underlie platinum resistance in ovarian cancer.


60 Lafarge S, Sylvain V, Ferrara M, Bignon YJ. Inhibition of BRCA1 leads to increased chemoresistance to microtubule-interfering agents, an effect that involves the JNK pathway. *Oncogene* 20(45), 6597–6606 (2001).


Very good review of Fanconi anaemia/BRCA pathway.


