Review: Clinical Trial Methodology

CLINICAL INVESTIGATION

BRCA status in epithelial ovarian cancer: implications for management and future clinical trial design

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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

Epithelial ovarian cancer (EOC) remains the fifth commonest cause of cancer death in women and the most lethal gynecologic malignancy in the USA [1]. Although important advances in surgical and chemotherapeutic strategies over the last three decades have significantly improved the median survival of EOC patients, the plateau of the survival curve has not changed appreciably [1–5]. Given that EOC is a genetically and biologically heterogeneous disease, identification of specific molecular abnormalities that can be targeted in each individual ovarian cancer on the basis of predictive biomarkers may be an effective strategy to improve outcome in this disease [6–8]. In this regard, a plethora of molecular studies, and most recently The Cancer Genome Atlas (TCGA) project, have evaluated mRNA and miRNA expression, promoter methylation, DNA copy number and whole exome DNA sequence information on clinically annotated EOC samples in an effort to identify novel genomic and epigenomic aberrations that may affect outcome or constitute therapeutic targets in this disease [9,10]. These studies have consistently shown that EOC is characterized by frequent genetic and epigenetic alterations in gene members of the homologous recombination (HR) DNA-repair pathway, most commonly in the *BRCA1* and *BRCA2* genes. Specifically, germline *BRCA1* and *BRCA2* mutations have been identified in approximately 15% of all EOCs [11,12] and as many as 22.6% of high-grade serous EOCs [9,11,12], while somatic *BRCA1* and *BRCA2* mutations have been identified in as many as 6–7% of EOCs [9,13]. Overall, approximately 50% of high-grade EOCs have been shown to harbor genetic or epigenetic

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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 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 1. Clinical characteristics of *BRCA1/2*-associated tumors.

Characteristic	*BRCA1/2*-associated EOCs
Hereditary breast–ovarian syndrome	*BRCA1* carriers have higher lifetime frequency of EOC *BRCA1* carriers develop EOC at an earlier age Risk of different cancers in *BRCA-1* vs *-2* carriers
Pathology	Association with serous tumors Association with high-grade/undifferentiated tumors
Stage	Association with higher stage (stage III or IV) at presentation
Debulking status	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
Patterns of recurrence	More likely to develop visceral metastases (parenchymal lung, liver, spleen, adrenal and brain metastases) This effect seems more prominent for *BRCA1* tumors
Overall survival	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)
Response to chemotherapy	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

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 [28–31]. 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 immunohistochemical 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 [11,33,65]. 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).

Molecular alteration	High-grade serous EOC (%)†
Germline *BRCA1/2* mutations	*BRCA1*: 8.5 *BRCA2*: 6.3 Total: 14.7
Somatic *BRCA1/2* mutations	*BRCA1*: 3.2 *BRCA2*: 2.9 Total: 6.1
Epigenetic silencing of *BRCA1*	10.8
Amplification or mutation of *EMSY*	7.9
Homozygous deletion of *PTEN*	6.7
Mutations in Fanconi anemia genes (*FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCG*, *FANCI*, *FANCL*, *PALB2*)	5.1
Mutations in core HR RAD genes (*RAD50*, *RAD51*, *RAD54L*)	1.6
Mutations in DNA damage response genes (*ATM*, *ATR*, *CHEK2*)	2.2
Epigenetic silencing of *RAD51C*	2.5

†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 BRCAness in EOC.

It is important to underscore that although the aforementioned molecular mechanisms of BRCAness 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 BRCAness, it is challenging to prospectively identify sporadic patients with a BRCAness 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 BRCAness [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 BRCAness 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 BRCAness 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 PARP1 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

Table 3. Clinical Trials of PARP inhibitors as single agents in *BRCA1/2*-associated in epithelial ovarian cancer.

Agent	Design	Patients/dose	Results	Ref.
Olaparib	Initial proof-of-concept Phase I	15 BRCA-associated EOCs (oral dose escalation)	Eight out of 15 (53%) had objective response per RECIST criteria and one had stable disease for 6 months	[15]
Olaparib	Expanded cohort of above Phase I	50 BRCA-associated EOCs (200 mg p.o. b.i.d.)	40% ORR and/or CA125 (>50% decline) Clinical benefit in platinum-sensitive, -resistant and -refractory subgroups (69, 46 and 23%, respectively)	[88]
Olaparib	Phase II	33 BRCA-EOCs (400 mg p.o. b.i.d.) 24 BRCA-EOCs (100 mg p.o. b.i.d.)	ORR: 33% in 400 mg b.i.d. and 13% in 100 mg b.i.d. Median PFS 5.8 (400 mg) versus 1.9 months (100 mg)	[89]
Olaparib versus Doxil	Randomized Phase II	97 BRCA-associated EOCs 32: Olaparib 400 mg p.o. b.i.d. 32: Olaparib 200 mg p.o. b.i.d. 33: Doxil 50 mg iv. every 28 days	ORR: 25 (200 mg), 31 (400 mg) and 18% (Doxil) PFS: 6.5 (200 mg), 8.8 (400 mg) and 7.1 months (Doxil) GCIG CA125 response: 34 (200 mg), 56 (400 mg) and 33% (Doxil)	[90]
Rucaparib	Phase I	41 BRCA-associated breast and ovarian tumors (iv. on days 1–5 of a 21-day cycle; dose escalation)	RECIST clinical benefit rate (CR + PR + SD ≥ 4 months) = 32% A more frequent dosing schedule of oral AG-014699 is planned in this patient population	[102]
Niraparib	Phase I	11 BRCA-associated tumors (p.o. dose escalation)	RECIST PR in two patients with BRCA-associated EOCs SD ≥ 4 months in two patients with BRCA-associated EOCs	[103]
Veliparib	Phase I	38 BRCA-associated tumors (20 BRCA-associated EOCs) (p.o. dose escalation)	Two RECIST PR (one breast and one EOC) SD ≥ 4 months in ten patients	[104]

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.

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 twice-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 *BRCA1/2*-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. twice-daily (33 vs 12.5% 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

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 BRCAness), 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

Table 4. Clinical trials of PARP inhibitors as single agents in epithelial ovarian cancer patients with sporadic or unknown *BRCA1/2* status.

Agent	Design	Patients/dose	Results	Ref.
Olaparib	Phase II, open-label, multicenter, non-randomized study	17 BRCA-associated EOCs 46 sporadic EOCs (400 mg b.i.d.)	BRCA-associated EOCs ORR: 41% (platinum sensitive: 60%; platinum resistant: 33%) Sporadic-EOC ORR: 24% (platinum sensitive: 50%, platinum resistant: 4%)	[95]
Olaparib maintenance versus placebo	Randomized, double-blind, placebo-controlled, Phase II	265 high-grade EOC patients with unknown BRCA-status who had received two or more platinum-based regimens and experienced a PR or CR to their most recent platinum-based regimen 136: Olaparib 400 mg b.i.d. 129: Placebo	PFS significantly longer in olaparib (8.4 vs 4.8 months; $p < 0.001$; hazard ratio for progression or death: 0.35) Adverse events with an incidence that was at least 10% higher in the olaparib group than in the placebo group, were nausea, fatigue, vomiting and anemia A CR (vs PR) to the final platinum-based therapy was associated with longer PFS, regardless of study group Interim OS analysis no difference between two arms	[96]

b.i.d.: Twice-daily; CR: Complete response; EOC: Epithelial ovarian cancer; ORR: Overall response rate; OS: Overall survival; PFS: Progression-free survival; PR: Partial response.

NHEJ and may rescue *BRCA1* defective cells from PARPi cytotoxicity [98]. Similarly, as discussed above, patients who have developed platinum resistance may respond well to subsequent PARPi therapy, particularly if they have BRCA-associated tumors. Elucidation of the mechanisms of PARPi resistance and how these relate to resistance to platinum and other chemotherapeutics, may aid the development of novel therapies to overcome PARPi resistance and also optimization of the sequence that PARPi are incorporated in the clinical management of both BRCA-associated and sporadic EOC tumors (i.e., before or after platinum, first-line or advanced setting, maintenance or not). Finally, as discussed in detail previously, apart from BRCA-associated patients who respond well to PARPi, identification of sporadic patients with a BRCAness phenotype that are more likely to respond to PARPi is a major challenge for the clinical development of these agents.

Implications of *BRCA*-status for clinical trial design

BRCA1/2-associated EOCs exhibit improved survival and enhanced sensitivity to DSB-inducing agents compared with their sporadic counterparts. These phenotypic characteristics have important implications for clinical trial design in EOC. If BRCA-status is not incorporated in clinical trials as a stratification factor or is not accounted for by preplanned statistical analysis then there is a significant risk for bias that may be introduced by unequal numbers of *BRCA1/2*-associated patients included in different study cohorts [38,101]. BRCA-status should be considered not only for clinical trials of PARPi or other drugs, but also in any clinical trial with a survival (PFS or overall survival) or chemosensitivity end point. Furthermore, given that several studies suggest that *BRCA2*-associated tumors exhibit distinct phenotypic characteristics compared with *BRCA1*-associated tumors (better survival and chemosensitivity), an argument can also be made for stratification based on the specific mutation (i.e., *BRCA1* vs *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* vs *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.

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Executive summary

Background

- Approximately 50% of high-grade epithelial ovarian cancers (EOC) harbor genetic or epigenetic alterations in the homologous recombination pathway.

Phenotype of *BRCA1/2*-mutated EOCs: clinical implications

- 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.
- *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.

'BRCAness' phenotype in sporadic EOC

- The concept of 'BRCAness' refers to the phenomenon whereby a subset of sporadic EOCs may behave similarly to *BRCA1/2*-mutated EOCs.

Therapeutic opportunities for *BRCA1/2*-associated tumors

- PARP inhibitors (PARPi) exhibit synthetic lethal effects in tumors with a defective homologous recombination DNA repair pathway such as *BRCA1/2*-associated EOCs.
- Clinical trials have demonstrated significant activity of PARPis in *BRCA1/2*-associated tumors and certain *BRCA1/2* negative tumors, which correlates with platinum sensitivity.

Implications of *BRCA* status for clinical trial design

- 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.

Future perspective

- Overcoming *de novo* as well as acquired PARPi resistance, identification of sporadic patients with a BRCAness phenotype and optimal incorporation of PARPis in the current armamentarium of drugs are important priorities for ovarian cancer research.

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