Delivering Wide scale BRCA testing and PARP inhibitors in Ovarian Cancer

Authors: Angela George[1], Stan Kaye[2], Susana Banerjee[2]

Affiliation: [1]Gynaecology Unit and Cancer Genetics Unit, The Royal Marsden NHS Foundation Trust, Fulham Road, London, SW3 6JJ, United Kingdom
[2] Gynaecology Unit, The Royal Marsden NHS Foundation Trust, Fulham Road, London, SW3 6JJ, United Kingdom

Corresponding author:
Susana Banerjee MBBS MA MRCP PhD
Royal Marsden Hospital, 203 Fulham Road, SW3 6JJ London, United Kingdom
E-mail: susana.banerjee@rmh.nhs.uk
Phone: + 44 208 6613979 Fax: + 44 207 811 8103

Abstract

The treatment of ovarian cancer is rapidly changing in clinical practice following the success of PARP inhibitors in clinical trials. Olaparib is the first PARP inhibitor to have gained EMA and FDA approval in BRCA-mutation associated ovarian cancer. Germline *BRCA* mutation status is now established as a predictive biomarker of potential benefit from a PARP inhibitor and therefore knowledge of the BRCA status for an individual patient with ovarian cancer is undoubtedly essential in order to help guide treatment decisions. Up until recently, BRCA testing was only offered to women with a family or personal history of breast/ovarian cancer. It is now recognised that almost 20% of women with high grade serous ovarian cancer harbour a germline *BRCA* mutation and of these, over 40% may not have a significant family cancer history and therefore would not have routinely undergone BRCA testing. A strategy to implement BRCA testing more widely as routine care is necessary in order to deliver personalised therapy. In this review, we summarise key clinical trials of PARP inhibitors and discuss how to integrate these agents given the current treatment landscape in ovarian cancer. Germline BRCA testing models and other promising biomarkers of homologous deficiency will also be discussed.

Introduction

Epithelial ovarian cancer is now recognized as a heterogenous disease with each histological subtype (high grade serous, low grade serous, clear cell, endometrioid and mucinous) associated with distinct clinical behaviour characteristics and molecular pathway aberrations. However, regardless of this knowledge, ovarian cancer has been treated as one entity in clinical practice outside the context of recent clinical trials [1]. This has now changed as a result of the pivotal clinical trials of the PARP inhibitor, olaparib. In December 2014, Olaparib gained EMA approval for the use as monotherapy for maintenance treatment of patients with platinum-sensitive relapsed BRCA-mutated (germline or somatic) high-grade serous ovarian cancer who respond to platinum-based chemotherapy [2]. At the same time, the FDA approved the use of olaparib for a different indication: the treatment of patients with recurrent germline BRCA-

mutated advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy[3]. For the first time in the management of ovarian cancer, patients are now being selected in clinical practice for biomarker-directed therapy- based on the presence of a *BRCA* mutation. In this article, we summarise the key milestones in the development of PARP inhibitors and discuss the delivery of BRCA testing as a companion biomarker. In addition, challenges regarding how best to integrate PARP inhibitors in the treatment armamentarium available in ovarian cancer are discussed.

A summary of key clinical developments of PARP inhibitors

Poly(ADP)ribose polymerase (PARP) enzymes have been implicated in a number of cellular pathways including energy metabolism, gene transcription, cell death and epigenetic modification[4-8]. Of 17 PARP family members, PARP1, PARP2 and PARP3 have all been shown to have roles in DNA repair[9, 10]. A detailed discussion of the mechanism of DNA repair and PARP inhibitors has been reviewed previously and is beyond the scope of the aims of this article [10-12]. The mechanism of action of PARP inhibitors utilising synthetic lethality exploits the DNA repair function of PARP. Synthetic lethality describes the situation whereby two pathway defects acting individually have little effect, but when combined become lethal [13]. This has the potential for a therapeutic approach to be selectively lethal to the tumour cells, but not toxic to the normal cells thereby creating a substantial therapeutic window. A synthetic lethal interaction between *BRCA1* or *BRCA2* mutations and PARP was first reported in 2005 [14, 15]. Cell lines with a *BRCA1* or *BRCA2* mutation were shown to be highly sensitive to PARP inhibitors compared to heterozygous mutant or wild-type cells and led to the first phase I clinical trials of PARP inhibitors in germline *BRCA* mutation carriers. Base excision repair inhibition is the original and most widely described models proposed to explain the mechanism of PARP inhibitors [10, 12, 13, 16]. In brief, DNA single strand breaks (SSBs), normally repaired by base excision repair, persist in the presence of PARP inhibitors, leading to the accumulation of double strand breaks (DSBs). In a normal cell, DSBs are repaired by homologous recombination (HR). However, in an HR-deficient cell (eg. BRCA mutated) are unrepaired and lead to cell death. It is now well recognised that this model does not fully explain the synthetic lethal

action of PARP inhibitors [12]. Other models include PARP1 trapping; impaired BRCA1 recruitment and activation of non-homologous endjoining (NHEJ) [16-18]. A simplified description of the mechanisms of PARP inhibitors is shown in Figure 1.

The clinical proof of concept was first reported in the phase I trial of the PARP inhibitor, olaparib (AZD2281, AstraZeneca) which showed a response rate of 47% (9/19) in patients with a germline *BRCA* mutation [19]. Efficacy of olaparib was seen in heavily pre-treated patients and was associated with platinum-sensitivity: clinical benefit rate 69% in platinum-sensitive patients; 45% in platinum-resistant; and 23% in platinum-refractory disease[20]. Table 1 summarises the results of subsequent published, key II trials of olaparib and other PARP inhibitors in ovarian cancer[21-26].

A wider utility for the synthetic lethality approach was envisaged in view of the accumulating evidence indicating that up to 50% of high-grade serous ovarian cancers have homologous recombination defects (including *BRCA1/2* germline and somatic mutations; *BRCA1* methylation; *EMSY, PTEN, ATM, ATR, RAD51C,* Fanconia Anaemia gene alterations) which may confer sensitivity to PARP inhibition [16, 27-30]. This was explored as a maintenance strategy in a double-blind, placebo- controlled phase II study in which patients with platinum-sensitive, recurrent, high-grade serous ovarian cancer (who had achieved a response following their most recent platinum-based regimen) were randomised to receive either olaparib (400mg bd capsules) or placebo till progression irrespective of BRCA status (Study 19) [31]. The PFS was significantly prolonged with olaparib compared to the placebo arm (n=265, median 8.4 months vs. 4.8 months; HR 0.35, P<0.001). Following these striking results, the main question was whether the impact of olaparib may differ according to the BRCA mutation status. This information was made available for 96% of the participants and 51% of the overall study population was classed as having a BRCA mutation. A retrospective pre-planned analysis demonstrated an even greater improvement in PFS in patients with a BRCA mutation (germline and somatic) treated with olaparib compared with placebo (11·2 vs 4·3 months; HR 0.18; p<0·0001) [2]. The magnitude of the hazard ratio

is substantially greater than what has been seen in previous trials of relapsed, ovarian cancer. It is noteworthy that patients without a BRCA mutation also derived a significant benefit although the magnitude was less (7.4 vs 5.5 months; HR 0.54; p= 0.0075)[2]. This suggests that a proportion of patients without a *BRCA* mutation may also benefit from olaparib. Despite the improvement in PFS from olaparib maintenance therapy, the third updated survival analysis performed at 77% data maturity has not shown a statistically significant overall survival (OS) benefit thus far in the BRCA-mutated patients (median OS olaparib 34.9 months vs placebo 30.2 months; HR 0.62, p=0.02; criterion for statistical significance in view of multiple interim analysis p<0.0095) [32]. It is important to note that Study 19 was not designed to show a statistically significant difference in OS. Furthermore, 23% of the patients receiving the placebo switched to a PARP inhibitor after progression out of the context of the trial. An exploratory *post hoc* analysis that excluded all patients from sites where 1 or more placebo patients received a PARP inhibitor after progression was performed and suggests that post progression PARP inhibitor treatment had a confounding influence on the interim OS analysis in the BRCA-mutated group [33]. Based on the pivotal results of this phase II trial, the EMA approved the use of olaparib as a maintenance therapy for patients with platinum sensitive relapsed BRCA mutated (germ-line or somatic) high-grade serous ovarian cancer.

Olaparib gained FDA approval as a treatment for patients with germ-line BRCA-mutated ovarian cancer (as detected by the BRACAnalysis CDx (Myriad Genetics test) who have received three or more lines of chemotherapy based on the results of Study 42, a phase II single arm trial of olaparib (400mg bd capsules) in germline *BRCA* mutation carriers with advanced malignancy including ovarian, breast, pancreatic and prostate cancer [10]. In keeping with other studies, the response rate in the germline BRCA-mutated ovarian cancer cohort of 137 patients was 34% with a median duration of response of 7.9 months.

In addition to olaparib, there are several other PARP inhibitors undergoing clinical development that look very promising in ovarian cancer (Table 2).

Rucaparib, a potent oral PARP inhibitor has been granted breakthrough therapy designation by the FDA for the treatment of women with advanced BRCA-mutated ovarian cancer. ARIEL-2, a phase II open label study of rucaparib in patients with platinum-sensitive, relapsed, high-grade serous ovarian cancer, is the first clinical trial to prospectively demonstrate that an HR deficiency signature can identify BRCA wild-type patients with high-grade serous ovarian cancer most likely to respond to a PARP inhibitor. Using a next-generation sequencing-based HR deficiency (HRD) assay based on genomic loss of heterozygosity (LOH) levels, (Foundation Medicine) and algorithm to predict rucaparib sensitivity, three biomarker groups were defined; BRCA-mutant(^{mut}), BRCA wild-type (^{wt})/LOH^{high} and BRCA^{wt} /LOH ^{low}[34]. Using pre-specified cut-offs of LOH (≥14%), radiological response rates were 80%, 29% and 10% in BRCA^{mut}, BRCA^{wt}/LOH^{high} and BRCA^{wt}/LOH^{low} and the hazard ratio for PFS was 0.62 (95% CI 0.42, 0.90; P=0.01) in BRCA^{wt}/LOH^{high} vs BRCA^{wt}/LOH^{low} groups and 0.27 (95% CI 0.16-0.44; p<0.001) in BRCA^{mut} vs BRCA^{wt}/LOH^{low} groups [34] . Phase II/III trials of PARP inhibitors that are awaiting results are summarised in Table 3.

The clinical relevance of a BRCA mutation result

The rate of *BRCA1/2* mutations has historically been believed to be approximately 10% [35, 36]. However, the reported frequency of *BRCA1* and *BRCA2* mutations in women with ovarian cancer varies greatly by study-between 6-43% [37-41]. The highest rates have been reported in populations selected for factors which may enrich for *BRCA1/2* mutations such as a strong family history of breast and/or ovarian cancer, Ashkenazi Jewish ancestry younger age at diagnosis or serous histology and the lowest rates are seen in unselected series of ovarian cancer. This variation, as well as BRCA sequencing methods have made it difficult to establish the true frequency of *BRCA* mutations amongst ovarian cancer patients. Nevertheless, over recent years, several groups have consistently shown that the incidence in high grade serous ovarian cancer patients is generally greater than 15% [40, 42, 43]. For example, Alsop et al. found that the overall incidence of *BRCA* germline

mutations in 1001 patients with non-mucinous EOC was 14.1% and 17.1% in the high grade serous group.

The clinical relevance of a germline *BRCA1* or *BRCA2* mutation result in a woman with ovarian cancer extends beyond their increased risk of developing a further BRCA-associated malignancy and implications for family members who may also have inherited a mutation in the BRCA genes. Knowledge of the BRCA mutational status provides useful information regarding prognosis, the clinical behaviour of the disease, and influences clinical decision-making regarding treatment options. Several studies have demonstrated improved survival in patients with a mutation, with those carrying a *BRCA2* mutation noted to have the longest median survival, followed by the *BRCA1* carriers, then those without a mutation [44-48].

The highest rates of germline *BRCA1* and *BRCA2* mutations occur in patients with a histological diagnosis of the high grade serous subtype (8-18%) but mutations also occur in other subtypes including endometrioid, clear cell (5-15%) and rarely in carcinosarcomas [40, 42, 49, 50]. Visceral metastases (liver, lung splenic) are more common in ovarian cancer patients with a germline *BRCA1* or *BRCA2* mutation compared to non-BRCA patients. In a retrospective series, 74% of patients with a *BRCA* mutation developed visceral metastases compared with 16% in the control group [51].

It is now well established that germline *BRCA* mutation carriers are more likely to respond to platinum-based chemotherapy given as initial treatment and subsequent lines. In addition, these patients tend to retain platinum-sensitivity for longer (defined as disease progression > 6months since last platinum-containing regimen) [40, 52]. Higher response rates and survival following certain non-platinum chemotherapy agents such as PLD, trabectedin and cyclophosphamide have also been noted for germline *BRCA* carriers compared to non-carriers in recurrent disease [53, 54]. Clinicians are more likely to consider repeated rechallenges with platinum-containing therapy in patients with BRCA mutation-associated ovarian cancer. Moreover, given the promising

activity seen in early studies, these patients are directed into clinical trials of PARP inhibitors.

Somatic BRCA mutations have been identified in tumour samples of patients with ovarian cancer and although the reported rates vary, they are likely to be present in approximately 4-7% of cases [27, 75]. The full significance of somatic BRCA mutations is unfolding. It is not entirely clear whether the clinical implications, including the magnitude of benefit from PARP inhibitors for a patient with ovarian cancer harbouring either somatic BRCA1 or BRCA2 mutations are the same as if it were a mutation of germline origin. This is discussed further in the Challenges section. Nevertheless, as described earlier, the license for olaparib as maintenance therapy includes patients with a somatic BRCA mutation. Therefore in addition to germline BRCA testing, the optimisation of delivery of somatic BRCA testing in clinical practice is necessary.

There is a pressing need to be armed with the BRCA mutational status as patients now have the opportunity to receive a licensed PARP inhibitor as routine care. In view of this, BRCA1/2 testing should be incorporated into the routine investigations of patients with advanced EOC, as this will provide clinicians and patients with information that could impact on their clinical management.

Germline BRCA mutation Testing

BRCA1 and BRCA2 testing has traditionally been restricted to individuals meeting a prescribed threshold or set criteria which stipulate multiple familial cancers to be present to meet the requirements[55]. These limits, which vary across and within countries, were initially set due to the high cost and time taken to test for mutations. Multiple programmes and models have been developed to estimate the risk of an individual harbouring a germline BRCA mutation, all of which require family history details. Risk assessments such as the BOADICEA and BRCAPro computer programmes can be relatively cumbersome to use in every day practice[56-58]. Validated scoring systems such as the Manchester score are also popular because they can be utilised

during clinic consultations to determine if an individual meets BRCA testing thresholds[59].

Several studies have now demonstrated that selective testing based on family history misses a significant proportion of ovarian cancer patients with mutations. Moller et al [32] reported that 23% of patients presenting with ovarian cancer to the department were found to have a pathogenic *BRCA1* or *BRCA2* mutation. Of these, only one third qualified for testing based on family history. In an Australian series where 14% of the women with non-mucinous ovarian cancer were BRCA carriers, 44% reported no family history of breast or ovarian cancer [40]. Similar rates of mutations have been reported in women without a family history in other studies from Europe, Canada and USA [41, 60]. These findings led to several centres in Canada and the UK pioneering more widespread BRCA testing in ovarian cancer based on the histological subtype without the family/personal cancer history risk-based thresholds[60-62]. The NCCN has also produced guidelines recommending genetic risk evaluation for any woman diagnosed with ovarian, fallopian tube or primary peritoneal cancer[63]. This approach will detect many more women with mutations who would not have been tested using a selective approach. These women can then have the opportunity to benefit from tailored treatment choices and the potential of risk-reducing cancer strategies for themselves and family members.

The low rates of referral of ovarian cancer patients who are eligible for BRCA testing to Genetics departments has been an issue. This phenomenon includes specialist cancer centres [64, 65]. Overall, evidence suggests that only 20% of eligible women are referred for testing [64]. However, patients who are referred actually have a high uptake of consent, for BRCA testing suggesting that patient acceptance to be tested for the presence of a BRCA mutation is not the limiting factor [60]. Therefore, in addition to the eligibility criteria for BRCA testing, it appears that the general attitude in the medical field towards the testing for germline mutations needs to be addressed. For many years, the primary advantage for ovarian cancer patients to undergo genetic testing was the identification of at-risk family members who could choose risk-reducing interventions to modify their future risk of cancer. In order to move forward to

more patient-centred, personalised cancer treatment, BRCA testing must be considered an important part of the diagnostic process. Although slow to take off initially, there has been increasing international acceptance for the need of widespread BRCA testing for ovarian cancer patients and the delivery of this has gained significant momentum[63, 66-70].

The following example illustrates some of the practical issues in achieving this. In England, there is still variable access to testing for mutations in *BRCA1* or *BRCA2* in ovarian cancer patients. The Familial Breast Cancer Guidelines, revised by the National Institute for Clinical Excellence (NICE) in June 2013, recommends that all women with ovarian or breast cancer at a 10% risk of harbouring a mutation should be referred for BRCA testing [71]. However, it does not clarify which patients within these cancer types will meet the 10% threshold. As discussed earlier, multiple studies have reported BRCA mutation rates of 10% and greater for serous and endometroid ovarian cancer patients and therefore in theory, all women with these histological subtypes should meet NICE criteria for testing. However, it remains the domain of individual NHS Trusts to decide whether to and how to implement these guidelines as well as how to fund the inevitable increase in BRCA testing. The current criteria for ovarian cancer genetic testing still varies by region within England, despite recommendations in March 2015 from the UK Genetic Testing Network recommending that all women with ovarian cancer be offered testing [72]. As the cost of testing falls with the utilisation of Next Generation Sequencing, it is anticipated that BRCA testing will be more accessible to patients.

As new therapeutic targets are identified in a wide range of malignant and non-malignant conditions, demand for germline genetic testing is rapidly increasing. This will place increasing pressure on Genetics services worldwide, many of which are already facing difficulties to cope with the rise in referrals- waiting times can be up to ten months [72].

Germline BRCA mutation testing models

To address the increasing demand and provide rapid, expanded access to testing, several new models of genetic testing have been developed. The

following section describes the models currently in use in the UK, and are representative of approaches employed in other countries. These models all involve integration of oncology and genetics to different extents, with the aim of providing a streamlined approach for ovarian cancer patients to undergo germline BRCA testing.

The first model presented, developed within the Mainstream Cancer Genetics Programme and pioneered at the Royal Marsden Hospital, is now standard care for ovarian cancer patients treated at this institution[42, 61]. In brief, the oncogenetic pathway enables healthcare professionals working within the Gynaecology-unit (including nurses), who have successfully completed the mandatory online training, to initiate, counsel and consent patients for BRCA testing[73]. Women of any age with non-mucinous ovarian cancer, and those with ovarian cancer of any sub-type who also have a history of another cancer, are offered testing in their routine oncology clinic appointment by a member of the oncology team. Patients are offered BRCA testing at diagnosis or any time during their oncological journey. They are given an information sheet regarding the process and implications of BRCA testing. Results are returned directly to the patient in writing along with relevant information regarding the significance of the findings by the Genetics department. Patients found to carry a BRCA mutation also receive an appointment with a member of the Genetics team to discuss family risk of developing cancer, personal risk of developing a further malignancy, screening and prevention measures. Meanwhile, the oncology team discusses with the patient how the result may influence the management of the disease. This pathway means that geneticists have consultations with patients that are found to actually have a germline BRCA mutation and do not routinely see ovarian cancer patients who are BRCA negative (>80% of those tested), unless a patient or their oncologist request for this.

During the first 16 months of the model (July 2013-Nov 2014), 207 women with non-mucinous histology were tested and 16% were found to have a *BRCA1* or *BRCA2* mutation. More than 50% of patients with a *BRCA* mutation had no family history of breast/ovarian cancer or personal history of breast cancer that would meet the previous eligibility criteria for BRCA testing and therefore would

not have been offered the test. This model has proved acceptable to patients, geneticists and oncology clinicians and nurses. Furthermore, patients are receiving results within 3-4 weeks - a timeframe that allows for the result to influence the patient's treatment options [61]

In Scotland, all women with non-mucinous ovarian cancer are offered BRCA testing through oncology clinics. This was first established in Scotland in November 2012, when testing was initially offered to all women with high-grade serous ovarian cancer, regardless of family history. The consenting of patients is performed either by oncology clinicians or the Genetics team, depending on the region in Scotland. Of note, more complex cases are initially referred directly to the genetics team and all patients with a *BRCA* mutation are also referred. The Scottish Intercollegiate Guidelines Network (SIGN) were subsequently updated in 2013, recommending that all women with non-mucinous EOC are offered BRCA testing.

The Genetic Testing in Epithelial Ovarian Cancer (GTEOC) study assessed the feasibility and patient acceptabilty of BRCA testing. Over a two-year recruitment period (completed June 2015), women diagnosed with EOC within the prior 12 months, irrespective or age and family history were recruited though the existing genetics network in the East Anglia region. Patients were initially identified by the clinical team but, in contrast to the Royal Marsden oncogenetic pathway, counselling and consent were genetics-led. Patients returned questionnaires and consent forms by post. Results from the 232 women enrolled demonstrated a BRCA1/2 mutation yield of 12% in women <70 (17/146) and 1% in women over 70 (1/86) giving an overall rate of 8%. This figure is lower than other reported series and may reflect the patient population tested. The researchers conclude that population-based genetic testing is acceptable and less resource-intensive than the current standard practice in their region where all patients have a full assessment by the genetics team prior to testing [74-76].

Despite the fact that internationally, some centres have been offering patients BRCA testing based on a diagnosis of ovarian cancer for over 3 years,

widespread germline BRCA testing is still not accessible to a significant proportion of patients. However, an increasing number of centres are now implementing germline BRCA testing for women with ovarian cancer. The oncogenetic mainstreaming model (Royal Marsden/Institute of Cancer Research) is being taken up in cancer centres worldwide and is undergoing further evaluation in an international study (ENGAGE, NCT02406235).

Germline mutations beyond BRCA1 and BRCA2

The role of germline genetic mutations in ovarian cancer susceptibility genes other than *BRCA1* and *BRCA2,* such as *BRIP1, RAD51C* and *RAD51D* are becoming increasingly clinically relevant, in particular to identify patients with HRD who may benefit from PARP inhibitor strategies[43, 50, 77-81]. NGS technology enables the possibility of panel testing to be delivered in clinical practice. Panel testing means that rarer genes such as *BRIP1, RAD51C* and *RAD51D* can also be able to be tested in addition to *BRCA1* and *BRCA2*, giving more comprehensive genetic information which may guide patient care. For example, patients with a germline *RAD51C* mutation were shown to respond to rucaparib in the ARIEL-2 trial, while preclinical data have demonstrated sensitivity to PARP inhibitors in cell lines with *RAD51D* mutations [79, 82]

Challenges for PARP inhibitors in clinical practice

Several important issues remain to be addressed if ovarian cancer patients are to benefit from the full potential of PARP inhibitors.

1.**What are the challenges for the implementation of widespread BRCA testing and germline mutation testing beyond *BRCA*?**

There are a number of issues to address if the implementation of widespread BRCA testing using an oncogenetic pathway is to be successful. Firstly, collaboration with a genetics laboratory to provide testing in an appropriate timeframe to influence clinical practice and. robust processes for reporting and returning results to patients and clinicians are required. It is important that if oncologists are involved in returning and discussing results, any genetic variants must be clearly identifiable as either clinically actionable (pathogenic variants) or not (non-pathogenic variants). There must also be an option for patients who would benefit from further discussion with a geneticist before testing to be able to be referred promptly. In addition, support from the genetics team for the education of oncology team members is essential.

The eligibility criteria for germline BRCA testing of ovarian cancer patients varies between different countries and even within regions. Despite this, the recognition that women with high grade serous ovarian cancer regardless of family history warrant BRCA testing is now almost universal. Differences in criteria are mainly in relation to the inclusion of additional histological subtypes (eg. mucinous) and limiting the age at diagnosis.

A major hurdle to the success of any programme aimed at expanding access to genetic testing is appropriate funding. For example, in England, although there are national guidelines suggesting which patients should be offered testing, there is a lack of specific national funding for testing. This has also led to resistance for integrated programmes and disagreements over who should fund the testing – Genetics or Oncology departments.

To date, the economic modelling has demonstrated that offering genetic testing to all women with non-mucinous ovarian cancer is cost-effective [83]. In addition to the potential clinical management implications for an individual patient, the identification of individuals with *BRCA* mutations also allows cascade testing of at-risk family members, unaffected by cancer, to ascertain their own risk. In doing so, it offers a rare opportunity to prevent others from developing cancer themselves, by choosing risk-reducing strategies such as prophylactic surgery, appropriate screening or chemoprevention.

There is no consensus on regarding whether to check for germline and somatic BRCA mutations or in which order they should be performed. For many countries, germline testing is established in clinical practice. Some patients without a germline BRCA mutation may still benefit from somatic BRCA testing ie. to access a PARP inhibitor. Several centres are performing somatic testing first. Patients found to have a mutation in the tumour can then undergo germline testing, to establish the nature of the mutation. This approach limits the need for patients to undergo genetic counseling first and arguably, patients who do not have a BRCA mutation detected in the tumour will not require further germline BRCA testing. However, prior to taking up somatic testing as a screening tool to limit the number of patients requiring germline testing, several factors that are relevant for the implementation of widespread somatic BRCA mutation testing need to be considered: the quality of the extracted DNA for analysis; the interpretation of the sequencing data; the significance of tumour heterogeneity between and within biopsies of metastatic lesions; and the stability of a somatic BRCA mutation over time ie. Is the somatic BRCA mutation status of a sample taken at diagnosis relevant following progression after several lines of therapy? Preliminary results from the ARIEL-2 trial have shown that two patients were found to have a somatic BRCA mutation detected in the pretreatment biopsy but not in the archival (diagnosis) specimen and they derived durable responses to rucaparib[84]. This provides support for the consideration of pre-treatment biopsies before excluding PARP inhibitors for individual patients.

A practical issue for the delivery of germline panel testing of whole exome sequencing is that rare mutations, including protein-truncating mutations in many genes that have not been proven to have an association with ovarian cancer are likely to be found in patients. The difficulty lies in the interpretation of such results. Projects such as the 1000 Genome programme have shown that humans have more than 100 de-novo protein-truncating mutations, and that most of these mutations will not be disease-causing [74]. There is a concern that any protein-truncating mutation identified in a patient with cancer will be assigned as causative, unless clear guidelines for reporting are put in place. In addition, the analysis of the wealth of data generated by NGS also requires substantial bioinformatics support that can be beyond that available in most diagnostic laboratories currently.

2. **When should ovarian cancer patients be offered a PARP inhibitor?** The licensed indication for olaparib differs in Europe and the US. It is not known whether the optimal time to receive a PARP inhibitor for disease relapse is as a treatment or a maintenance strategy following response to platinum. Some of the following practical clinical scenarios are under investigation in clinical trials (table 2). In the relapsed treatment setting, after which line should patients receive a PARP inhibitor and should this be dependent on platinum-sensitivity? Durable responses to olaparib have been observed in germline BRCA mutated ovarian cancer patients who have received ≥3 lines of prior chemotherapy including patients with platinum-resistant disease [85, 86]. In the maintenance setting, should patients be receiving PARP inhibitor for first platinum-sensitive relapse or later? Should *BRCA* mutation carriers be treated with a maintenance PARP inhibitor in preference to bevacizumab for first platinum-sensitive relapse or vice versa? Alternatively, should patients be receiving maintenance PARP inhibitor therapy as first line therapy? The SOLO-1 trial (NCT01844986) which has completed patient recruitment will be the first trial to help address the role of first line maintenance in patients with a BRCA mutation.

Factors to consider are the potential long term toxicities of PARP inhibitors in a population with a good prognosis who may have been cured without PARP inhibitor therapy. Olaparib is generally well-tolerated with common toxicities

including gastro-intestinal toxicity (nausea and vomiting), fatigue and anaemia. These toxicities are generally low-grade, and usually improve over time. However, there is a risk, albeit low, (1-2%) of acute myeloid leukemia (AML) and myelodysplasia (MDS). This may not be different from the inherent risk of second malignancy in patients with BRCA mutations, but further careful long-term follow-up is necessary. Another factor to consider in the maintenance setting is the convenience of therapy for patients. In contrast to bevacizumab which is an intravenous maintenance therapy licensed in ovarian cancer, PARP inhibitors are oral. The current licensed formulation of olaparib is capsules and requires patients to take eight capsules twice daily. To improve compliance, a tablet formulation has been introduced and further randomised trials to confirm its efficacy are currently in progress [30].

Another important issue is whether following disease progression on a PARP inhibitor, the efficacy of other anti-cancer therapies is diminished. This information could influence decision-making regarding the point in a patient's disease course that they are used. Evidence so far, suggests that patients who receive olaparib as a treatment for disease progression and then develop olaparib resistance, retain the potential to respond to subsequent chemotherapy, including platinum but further data on larger numbers of PARP inhibitor treated patients are needed [87]. In the pivotal maintenance study, exploratory analyses of the BRCA mutation group showed that the time to second subsequent therapy or death (TSST) which is an approximation to the PFS2 (time to progression after subsequent treatment), was significantly longer in the olaparib arm compared to placebo (23.8 months vs 15.2 months; HR 0.44;p=0.00013) [2]. This suggests that olaparib may not be significantly detrimental to the effects of subsequent therapy. Ongoing phase III trials of olaparib (SOLO1 and SOLO2) and other PARP inhibitors (ARIEL-3, NOVA) will help address this important question.

2. Which patients should be treated with a PARP inhibitor?

The presence of a pathogenic *BRCA1* or *BRCA2* mutation is currently the best predictive biomarker of PARP inhibitor activity. Patients with a germline *BRCA1* or *BRCA2* mutation should be considered for a PARP inhibitor at some point in

their disease course. A major challenge is the identification of additional patients that are likely to also benefit from PARP inhibitors. Patients with a somatic BRCA mutation have also been shown to derive benefit and olaparib maintenance therapy is licensed for this group of patients [2, 82]. In study 19, fewer patients with somatic BRCA mutations were reported to have progression events in the olaparib arm (3/8, 38%) than the placebo group (6/10, 60%). The low number of patients with a somatic BRCA mutation in this study (n=18) means that it is not possible to undertake formal analyses in order to make definitive conclusions regarding the relative efficacy of olaparib in patients with a germline BRCA mutation compared to a somatic BRCA mutation. Of note, thus far, the response rates (combined RECIST and CA125) to rucaparib are similar for germline (n=20, 85%) and somatic (n=19, 84%) BRCA mutation groups [34]. Results from phase III studies (ARIEL-3 NCT01968213, NOVA NCT01847274) in which a larger number of patients with a somatic BRCA mutation are treated with a PARP inhibitor, will address this important point.

There is now clear evidence that another subgroup of patients those with HR-deficient cancers also derive benefit from PARP inhibitors. The question is how can these patients be identified? There are several HRD assays under development. The HRD signature developed within the ARIEL-2 described earlier (Foundation Medicine), appears promising. Using this signature, it was possible to identify a group of patients in which the response to rucaparib was higher compared to the biomarker-negative group although lower than the BRCA mutation group. There are also positive preclinical data on ovarian cancer models linking niraparib efficacy to the Myriad Genetics HRD assay, which is also based on genomic LOH. However, some have argued that targeted sequencing looking for defects in a range of DNA damage-repair genes would be a more accurate tool [88, 89]. Recently, a press release of the phase III NOVA trial results reported a significant improvement in PFS in HRD-positive (defined by myChoiceHRD® Myriad Genetics, Inc.) patients as well as the germline BRCA mutated group treated with maintenance niraparib compared to the placebo group (http://ir.tesarobio.com/releasedetail.cfm?ReleaseID=977524). The benefit of

PARP inhibitors is clearly expanding beyond germline BRCA-mutated ovarian cancer.

As well as defining which patient groups achieve a response or survival advantage, it is essential to work out which patients go on to derive durable, long-term benefits from PARP inhibitors. In Study 19, 13.2% of patients (18/136) received olaparib for more than 5 years [32].

3. How to overcome PARP inhibitor resistance and enhance the activity of PARP inhibitors?

Potential mechanisms of resistance to PARP inhibitors have been proposed and are reviewed in [10, 12]. These include enhanced PARP inhibitor cellular efflux, secondary BRCA reversion mutations, 'hypomorphic 'BRCA1 alleles, and reduced levels of 53BP1 [90-95]. The extent to which particular mechanisms play a role in an individual ovarian cancer patient developing resistance to a PARP inhibitor, remains to be established. It will be essential to understand better the mechanisms of resistance to PARP inhibitors so that the optimum strategy of PARP inhibitor use can be defined. This may best be addressed by careful analysis of tumour samples from patients whose disease has progressed on PARP inhibitor therapy. Although PARP inhibitors and platinum may share common mechanisms of resistance, some patients who have responded to olaparib and then subsequently develop resistance have been reported to retain sensitivity to further platinum-based treatment [87]. It is not known whether rechallenge with the same or different PARP inhibitor, potentially in combination with other agents, could benefit patients whose disease has progressed on a PARP inhibitor. If so, similar to the platinum-free interval, the PARP inhibitor free interval may be relevant. In addition to PARP inhibitor combination with chemotherapy, other combination strategies that have entered clinical trials are PARP inhibitors with PI3K/AKT inhibitors or antiangiogenic agents. A phase II trial of the VEGFR inhibitor cediranib with olaparib in platinum-sensitive ovarian cancer demonstrated that combination led to a significant improvement in PFS, compared with those treated with olaparib alone (17.7 vs 9 months, HR 0.42, p=0.005) [96]. However, the toxicities of the combination at the doses used were concerning with 77% of

patients requiring a dose reduction. Further studies of this combination are underway including a randomized study in relapsed disease (NCT02446600) where for the first time, an olaparib combination will be directly compared to platinum-based chemotherapy. Preclinical studies have provided rationales for pther potential clinical strategies which include the combination of PARP inhibitors with other targeted agents, such as ATR, c-Met [97]., Hsp90, CDK1 and HDAC inhibitors and immunotherapy[93, 98, 99]. Table 3 summarises PARP inhibitor and novel agent combination clinical trials.

Conclusions

A decade after the first preclinical studies demonstrated the synthetic lethal relationship between BRCA deficient cells and PARP inhibition, the first PARP inhibitor has been licensed (olaparib) for ovarian cancer patients with a BRCA mutation. Several other potent PARP inhibitors are also in late phase clinical development. An important challenge will be how to make the most out of the different PARP inhibitors and incorporate results from phase III trials due to report shortly for ovarian cancer patients. Widespread BRCA testing is essential for the maximum number of ovarian cancer patients to benefit from PARP inhibitors. The key areas of research relate to patient selection, overcoming resistance and how best to integrate PARP inhibitors into the ovarian cancer treatment landscape. In an era where antiangiogenic agents are in common use and immunotherapy approaches hold promise, this last point will become increasingly complex.

Acknowledgements

The authors would like to thank the Royal Marsden and Institute of Cancer Research National Institute for Health Research (NIHR). Biomedical Research Centre for Cancer (BRC) and the Gynaecological Cancers Fund.

References

1. Banerjee S, Kaye SB. New strategies in the treatment of ovarian cancer: current clinical perspectives and future potential. Clin Cancer Res 2013; 19: 961-968.
2. Ledermann J, Harter P, Gourley C et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. Lancet Oncol 2014; 15: 852-861.
3. Kaufman B, Shapira-Frommer R, Schmutzler RK et al. Olaparib monotherapy in patients with advanced cancer and a germline BRCA1/2 mutation. J Clin Oncol 2015; 33: 244-250.
4. Gibson BA, Kraus WL. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nat Rev Mol Cell Biol 2012; 13: 411-424.
5. Krishnakumar R, Kraus WL. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. Mol Cell 2010; 39: 8-24.
6. Rouleau M, Patel A, Hendzel MJ et al. PARP inhibition: PARP1 and beyond. Nat Rev Cancer 2010; 10: 293-301.
7. Bai P, Canto C. The role of PARP-1 and PARP-2 enzymes in metabolic regulation and disease. Cell Metab 2012; 16: 290-295.
8. Luo X, Kraus WL. On PAR with PARP: cellular stress signaling through poly(ADP-ribose) and PARP-1. Genes Dev 2012; 26: 417-432.
9. Quenet D, El Ramy R, Schreiber V, Dantzer F. The role of poly(ADP-ribosyl)ation in epigenetic events. Int J Biochem Cell Biol 2009; 41: 60-65.
10. Scott CL, Swisher EM, Kaufmann SH. Poly (ADP-ribose) polymerase inhibitors: recent advances and future development. J Clin Oncol 2015; 33: 1397-1406.
11. O'Connor MJ. Targeting the DNA Damage Response in Cancer. Mol Cell 2015; 60: 547-560.
12. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. Cancer Discov 2015; 5: 1137-1154.
13. Ashworth A. A Synthetic Lethal Therapeutic Approach: Poly(ADP) Ribose Polymerase Inhibitors for the Treatment of Cancers Deficient in DNA Double-Strand Break Repair. Journal of Clinical Oncology 2008; 26: 3785-3790.
14. Farmer H, McCabe N, Lord CJ et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005; 434: 917-921.
15. Bryant HE, Schultz N, Thomas HD et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature 2005; 434: 913-917.
16. Banerjee S, Kaye SB, Ashworth A. Making the best of PARP inhibitors in ovarian cancer. Nat Rev Clin Oncol 2010; 7: 508-519.
17. Murai J, Huang SY, Das BB et al. Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors. Cancer Res 2012; 72: 5588-5599.
18. Patel AG, Sarkaria JN, Kaufmann SH. Nonhomologous end joining drives poly(ADP-ribose) polymerase (PARP) inhibitor lethality in homologous recombination-deficient cells. Proc Natl Acad Sci U S A 2011; 108: 3406-3411.
19. Fong PC, Boss DS, Yap TA et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 2009; 361: 123-134.

20. Fong PC, Yap TA, Boss DS et al. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol 2010; 28: 2512-2519.
21. Audeh MW, Carmichael J, Penson RT et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet 2010; 376: 245-251.
22. Gelmon KA, Tischkowitz M, Mackay H et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. Lancet Oncol 2011; 12: 852-861.
23. Oza AM, Cibula D, Benzaquen AO et al. Olaparib combined with chemotherapy for recurrent platinum-sensitive ovarian cancer: a randomised phase 2 trial. Lancet Oncol 2015; 16: 87-97.
24. Coleman RL, Sill MW, Bell-McGuinn K et al. A phase II evaluation of the potent, highly selective PARP inhibitor veliparib in the treatment of persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer in patients who carry a germline BRCA1 or BRCA2 mutation - An NRG Oncology/Gynecologic Oncology Group study. Gynecol Oncol 2015; 137: 386-391.
25. Kummar S, Oza AM, Fleming GF et al. Randomized Trial of Oral Cyclophosphamide and Veliparib in High-Grade Serous Ovarian, Primary Peritoneal, or Fallopian Tube Cancers, or BRCA-Mutant Ovarian Cancer. Clin Cancer Res 2015; 21: 1574-1582.
26. Kaye SB, Lubinski J, Matulonis U et al. Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer. J Clin Oncol 2012; 30: 372-379.
27. Hennessy BTJ, 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. Journal of Clinical Oncology 2010; 28: 3570-3576.
28. Press JZ, De Luca A, Boyd N et al. Ovarian carcinomas with genetic and epigenetic BRCA1 loss have distinct molecular abnormalities. BMC Cancer 2008; 8: 17.
29. Baldwin RL, Nemeth E, Tran H et al. BRCA1 promoter region hypermethylation in ovarian carcinoma: a population-based study. Cancer Res 2000; 60: 5329-5333.
30. Integrated genomic analyses of ovarian carcinoma. Nature 2011; 474: 609-615.
31. Ledermann J, Harter P, Gourley C et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. N Engl J Med 2012; 366: 1382-1392.
32. Ledermann JAH, P.; Gourley, C.; Friedlander, M.; Vergote, I.; Rustin, G.J.S.; Scott, C.L.; Meier, W.; Shapira-Frommer, R.; Safra, T.; Matei, D.E.; Fielding, A.; Spencer, S.; Rowe, P.; Lowe, E.S.; Matulonis, U.A. Overall survival (OS) in patients (pts) with platinum-sensitive relapsed serous ovarian cancer (PSR SOC) receiving olaparib maintenance monotherapy: An interim analysis. Journal of Clinical Oncology 2016; 34: Abstr 5501.

33. Matulonis UA, Harter P, Gourley C et al. Olaparib maintenance therapy in patients with platinum-sensitive, relapsed serous ovarian cancer and a BRCA mutation: Overall survival adjusted for postprogression poly(adenosine diphosphate ribose) polymerase inhibitor therapy. Cancer 2016; 122: 1844-1852.
34. Coleman RL, Swisher E, Oza A et al. Refinement of prespecified cutoff for genomic loss of heterozygosity (LOH) in ARIEL2 part 1: A phase II study of rucaparib in patients (pts) with high grade ovarian carcinoma (HGOC). Journal of Clinical Oncology 2016; 34: Abstr 5540.
35. Rubin SC, Blackwood MA, Bandera C et al. BRCA1, BRCA2, and hereditary nonpolyposis colorectal cancer gene mutations in an unselected ovarian cancer population: Relationship to family history and implications for genetic testing. American Journal of Obstetrics and Gynaecology 1998; 178: 670-677.
36. Risch HA, McLaughlin JR, Cole DE et al. Prevalence and penetrance of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet 2001; 68: 700-710.
37. Soegaard M, Kjaer SK, Cox M et al. BRCA1 and BRCA2 mutation prevalence and clinical characteristics of a population-based series of ovarian cancer cases from Denmark. Clin Cancer Res 2008; 14: 3761-3767.
38. Satagopan JM, Boyd J, Kauff ND et al. Ovarian cancer risk in Ashkenazi Jewish carriers of BRCA1 and BRCA2 mutations. Clin Cancer Res 2002; 8: 3776-3781.
39. Moslehi R, Chu W, Karlan B et al. BRCA1 and BRCA2 mutation analysis of 208 Ashkenazi Jewish women with ovarian cancer. Am J Hum Genet 2000; 66: 1259-1272.
40. Alsop K, Fereday S, Meldrum C et al. BRCA Mutation Frequency and Patterns of Treatment Response in BRCA Mutation-Positive Women With Ovarian Cancer: A Report From the Australian Ovarian Cancer Study Group. J Clin Oncol 2012.
41. Moller P, Hagen AI, Apold J et al. Genetic epidemiology of BRCA mutations--family history detects less than 50% of the mutation carriers. Eur J Cancer 2007; 43: 1713-1717.
42. George AS, F.; Cloke, V.; Riddell, D.; Gore, M.; Hanson, H.; Banerjee, S.; Rahman, N. Implementation of Routine BRCA Testing of Ovarian Cancer (OC) Patients at The Royal Marsden Hospital. Annals of Oncology 2014; 25: iv305–iv326 (abst881PD).
43. Pennington KP, Walsh T, Harrell MI et al. Germline and Somatic Mutations in Homologous Recombination Genes Predict Platinum Response and Survival in Ovarian, Fallopian Tube, and Peritoneal Carcinomas. Clin Cancer Res 2014.
44. Yang D, Khan S, Sun Y et al. Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA 2011; 306: 1557-1565.
45. Bolton KL, Chenevix-Trench G, Goh C et al. Association between BRCA1 and BRCA2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA 2012; 307: 382-390.
46. Liu J, Cristea MC, Frankel P et al. Clinical characteristics and outcomes of BRCA-associated ovarian cancer: genotype and survival. Cancer Genetics 2012; 205: 34-41.

47. Chetrit A, Hirsh-Yechezkel G, Ben-David Y et al. Effect of BRCA1/2 Mutations on Long-Term Survival of Patients With Invasive Ovarian Cancer: The National Israeli Study of Ovarian Cancer. Journal of Clinical Oncology 2008; 26: 20-25.
48. Candido-dos-Reis FJ, Song H, Goode EL et al. Germline mutation in BRCA1 or BRCA2 and ten-year survival for women diagnosed with epithelial ovarian cancer. Clin Cancer Res 2015; 21: 652-657.
49. Norquist BM, Pennington KP, Agnew KJ et al. Characteristics of women with ovarian carcinoma who have BRCA1 and BRCA2 mutations not identified by clinical testing. Gynecol Oncol 2013; 128: 483-487.
50. Norquist BM, Harrell MI, Brady MF et al. Inherited Mutations in Women With Ovarian Carcinoma. JAMA Oncol 2015; 1-9.
51. Gourley C, Michie CO, Roxburgh P et al. Increased Incidence of Visceral Metastases in Scottish Patients With BRCA1/2-Defective Ovarian Cancer: An Extension of the Ovarian BRCAness Phenotype. Journal of Clinical Oncology 2010; 28: 2505-2511.
52. Tan DSP, Rothermundt C, Thomas K et al. "BRCAness" Syndrome in Ovarian Cancer: A Case-Control Study Describing the Clinical Features and Outcome of Patients With Epithelial Ovarian Cancer Associated With BRCA1 and BRCA2 Mutations. Journal of Clinical Oncology 2008; 26: 5530-5536.
53. Adams SF, Marsh EB, Elmasri W et al. A high response rate to liposomal doxorubicin is seen among women with BRCA mutations treated for recurrent epithelial ovarian cancer. Gynecologic Oncology 2011; 123: 486-491.
54. Lorusso D, Ferrandina G, Pignata S. Phase II prospective study on trabectedin (T) in BRCA-mutated and BRCAness phenotype advanced ovarian cancer (AOC) patients (pts): the MITO 15 trial. Journal of Clinical Oncology 2014; 32: Abstract 5530.
55. Eccles DM, Balmana J, Clune J et al. Selecting Patients with Ovarian Cancer for Germline BRCA Mutation Testing: Findings from Guidelines and a Systematic Literature Review. Adv Ther 2016.
56. Antoniou AC, Hardy R, Walker L et al. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. J Med Genet 2008; 45: 425-431.
57. Fischer C, Kuchenbacker K, Engel C et al. Evaluating the performance of the breast cancer genetic risk models BOADICEA, IBIS, BRCAPRO and Claus for predicting BRCA1/2 mutation carrier probabilities: a study based on 7352 families from the German Hereditary Breast and Ovarian Cancer Consortium. J Med Genet 2013; 50: 360-367.
58. Lee AJ, Cunningham AP, Kuchenbaecker KB et al. BOADICEA breast cancer risk prediction model: updates to cancer incidences, tumour pathology and web interface. Br J Cancer 2014; 110: 535-545.
59. Evans DG, Eccles DM, Rahman N et al. A new scoring system for the chances of identifying a BRCA1/2 mutation outperforms existing models including BRCAPRO. J Med Genet 2004; 41: 474-480.
60. Zhang S, Royer R, Li S et al. Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. Gynecologic Oncology 2011; 121: 353-357.

61. George A, Riddell D, Seal S et al. Implementing rapid, robust, cost-effective, patient-centred, routine genetic testing in ovarian cancer patients. Sci Rep 2016; 6: 29506.
62. Demsky R, McCuaig J, Maganti M et al. Keeping it simple: Genetics referrals for all invasive serous ovarian cancers. Gynecol Oncol 2013.
63. Network NCC. Genetic/familial high-risk assessment: Breast and Ovarian. 2015.
64. Meyer LA, Anderson ME, Lacour RA et al. Evaluating women with ovarian cancer for BRCA1 and BRCA2 mutations: missed opportunities. Obstet Gynecol 2010; 115: 945-952.
65. Lanceley A, Eagle Z, Ogden G et al. Family history and women with ovarian cancer: is it asked and does it matter?: An observational study. Int J Gynecol Cancer 2012; 22: 254-259.
66. Balmana J, Diez O, Rubio I et al. BRCA in breast cancer: ESMO Clinical Practice Guidelines. Ann Oncol 2010; 21 Suppl 5: v20-22.
67. oncology SoG. SGO Clinical Practice Statement: Genetic Testing for Ovarian Cancer 2014.
68. Marth C, Hubalek M, Petru E et al. AGO Austria recommendations for genetic testing of patients with ovarian cancer. Wien Klin Wochenschr 2015; 127: 652-654.
69. Llort G, Chirivella I, Morales R et al. SEOM clinical guidelines in Hereditary Breast and ovarian cancer. Clin Transl Oncol 2015; 17: 956-961.
70. Foretova L, Machackova E, Palacova M et al. [Recommended Extension of Indication Criteria for Genetic Testing of BRCA1 and BRCA2 Mutations in Hereditary Breast and Ovarian Cancer Syndrome]. Klin Onkol 2016; 29 Suppl 1: S9-13.
71. Excellence) NNIfC. Familial Breast Cancer: Classification and care of people at risk of familial breast cancer and management of breast cancer and related risks in people with a family history of breast cancer. 2013.
72. George A. UK BRCA mutation testing in patients with ovarian cancer. Br J Cancer 2015; 113 Suppl 1: S17-21.
73. Percival N, George A, Gyertson J et al. The integration of BRCA testing into oncology clinics. Br J Nurs 2016; 25: 690-694.
74. Tischkowitz MD, J.; Thompson, E.; Sagoo, G.; Newcombe, B.; Barter, E.; Ridley, P.; Miller, S.; Thompson, F; Webb, H.; Hodgkin, C.; Tan, L T.; Daly, M.; Ayers, S.; Rufford, B.; Parkinson, C.; Earl, H.; Duncan, T.; Pharoah, P.; Abbs, S.; Hulbert-Williams, N.; Crawford, R.; Brenton, J.; Shipman, H. The Genetic Testing in Epithelial Ovarian Cancer (GTEOC) Study: Direct access to BRCA1/2 genetic testing in oncology. 2014.
75. Tischkowitz M. Working together in the Genomics Era – Lessons from the GTEOC study 2015; NCRI abstract book.
76. Plaskocinska I, Shipman H, Drummond J et al. New paradigms for BRCA1/BRCA2 testing in women with ovarian cancer: results of the Genetic Testing in Epithelial Ovarian Cancer (GTEOC) study. J Med Genet 2016.
77. Coulet F, Fajac A, Colas C et al. Germline RAD51C mutations in ovarian cancer susceptibility. Clin Genet 2013; 83: 332-336.
78. Rafnar T, Gudbjartsson DF, Sulem P et al. Mutations in BRIP1 confer high risk of ovarian cancer. Nat Genet 2011; 43: 1104-1107.

79. Loveday C, Turnbull C, Ramsay E et al. Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nature Genetics 2011; 43: 879-884.
80. Loveday C, Turnbull C, Ruark E et al. Germline RAD51C mutations confer susceptibility to ovarian cancer. Nat Genet 2012; 44: 475-476; author reply 476.
81. Ramus SJ, Antoniou AC, Kuchenbaecker KB et al. Ovarian cancer susceptibility alleles and risk of ovarian cancer in BRCA1 and BRCA2 mutation carriers. Human Mutation 2012; 33: 690-702.
82. Kristeleit R, Swisher E, Oza A et al. Final results of ARIEL2 (Part 1): A phase 2 trial to prospectively identify ovarian cancer (OC) responders to rucaparib using tumor genetic analysis. European Cancer Congress 2015; Abstract 2700.
83. Slade I, Hanson H, George A et al. A cost analysis of a cancer genetic service model in the UK. J Community Genet 2016.
84. McNeish I, Coleman RL, Oza A et al. Preliminary results of ARIEL2, a phase 2 open-label study to identify ovarian cancer patients likely to respond to rucaparib. Annals of Oncology 2014; 25: iv305-iv326.
85. Domchek SM, Aghajanian C, Shapira-Frommer R et al. Efficacy and safety of olaparib monotherapy in germline BRCA1/2 mutation carriers with advanced ovarian cancer and three or more lines of prior therapy. Gynecol Oncol 2016; 140: 199-203.
86. Matulonis UA, Penson RT, Domchek SM et al. Olaparib monotherapy in patients with advanced relapsed ovarian cancer and a germline BRCA1/2 mutation: a multistudy analysis of response rates and safety. Ann Oncol 2016; 27: 1013-1019.
87. Ang JE, Gourley C, Powell CB et al. Efficacy of chemotherapy in BRCA1/2 mutation carrier ovarian cancer in the setting of PARP inhibitor resistance: a multi-institutional study. Clin Cancer Res 2013; 19: 5485-5493.
88. Rebbeck TR, Couch FJ, Kant J et al. Genetic heterogeneity in hereditary breast cancer: role of BRCA1 and BRCA2. Am J Hum Genet 1996; 59: 547-553.
89. Peng G, Chun-Jen Lin C, Mo W et al. Genome-wide transcriptome profiling of homologous recombination DNA repair. Nat Commun 2014; 5: 3361.
90. Fojo T, Bates S. Mechanisms of resistance to PARP inhibitors--three and counting. Cancer Discov 2013; 3: 20-23.
91. Jaspers JE, Kersbergen A, Boon U et al. Loss of 53BP1 causes PARP inhibitor resistance in Brca1-mutated mouse mammary tumors. Cancer Discov 2013; 3: 68-81.
92. Ceccaldi R, O'Connor KW, Mouw KW et al. A unique subset of epithelial ovarian cancers with platinum sensitivity and PARP inhibitor resistance. Cancer Res 2015; 75: 628-634.
93. Johnson N, Johnson SF, Yao W et al. Stabilization of mutant BRCA1 protein confers PARP inhibitor and platinum resistance. Proc Natl Acad Sci U S A 2013; 110: 17041-17046.
94. Edwards SL, Brough R, Lord CJ et al. Resistance to therapy caused by intragenic deletion in BRCA2. Nature 2008; 451: 1111-1115.
95. Barber LJ, Sandhu S, Chen L et al. Secondary mutations in BRCA2 associated with clinical resistance to a PARP inhibitor. J Pathol 2013; 229: 422-429.

96. Liu JF, Barry WT, Birrer M et al. Combination cediranib and olaparib versus olaparib alone for women with recurrent platinum-sensitive ovarian cancer: a randomised phase 2 study. Lancet Oncol 2014; 15: 1207-1214.
97. Du Y, Yamaguchi H, Wei Y et al. Blocking c-Met-mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors. Nat Med 2016.
98. Johnson N, Li YC, Walton ZE et al. Compromised CDK1 activity sensitizes BRCA-proficient cancers to PARP inhibition. Nat Med 2011; 17: 875-882.
99. Yalon M, Tuval-Kochen L, Castel D et al. Overcoming Resistance of Cancer Cells to PARP-1 Inhibitors with Three Different Drug Combinations. PLoS One 2016; 11: e0155711.

Figure 1. Putative anticancer mechanisms of action of PARP inhibitors

a | The classic model: impaired base-excision repair. DNA damage can cause single-strand breaks (SSBs) that are normally efficiently repaired by BER processes. PARP1 is a key component of the BER machinery and PARP inhibition results in persistent SSBs. A replication fork might encounter a persistent SSB during DNA replication, which either causes the replication fork to collapse or results in a DNA double- strand break (DSB). These breaks are normally repaired by the homologous recombination DSB-repair pathway, which requires BRCA1 and BRCA2 function. However, in the absence of functional BRCA1 or BRCA2, for example, owing to loss-of-function mutation, the DNA cannot be repaired, or is repaired through alternative pathways that are highly error prone, resulting in gross genomic instability and cell death. b | PARP trapping. PARP inhibition leads to inactivation of PARP1 and inhibition of subsequent poly [ADP-ribose] (pADPr) synthesis. PARP1 remains bound to damaged DNA, thus inhibiting DNA repair. c | Suppression of nonhomologous end-joining (NHEJ). Active PARP1 suppresses the error-prone DNA-repair pathway. In the presence of PARP inhibitors, NHEJ is no longer suppressed and is active in HR-deficient cells, leading to chromosomal rearrangements, genomic instability and cell death. d | Impaired BRCA1 recruitment. PARP inhibition reduces recruitment of the BRCA1-associated RING domain protein 1 (BARD1)–BRCA1 complex to damaged DNA, which impairs DSB repair. This process is potentially important in the presence of a BRCA1 mutation, whereby the BRCA1–histone 2A, family member X, phosphorylated at serine 139 (γH2Ax) interaction is diminished. DNA-PKCs, DNA-dependent protein kinase, catalytic subunit; HR, homologous recombination; Ku70, X-ray repair cross–complementing protein 6; Ku80, X-ray repair cross-complementing protein 5.

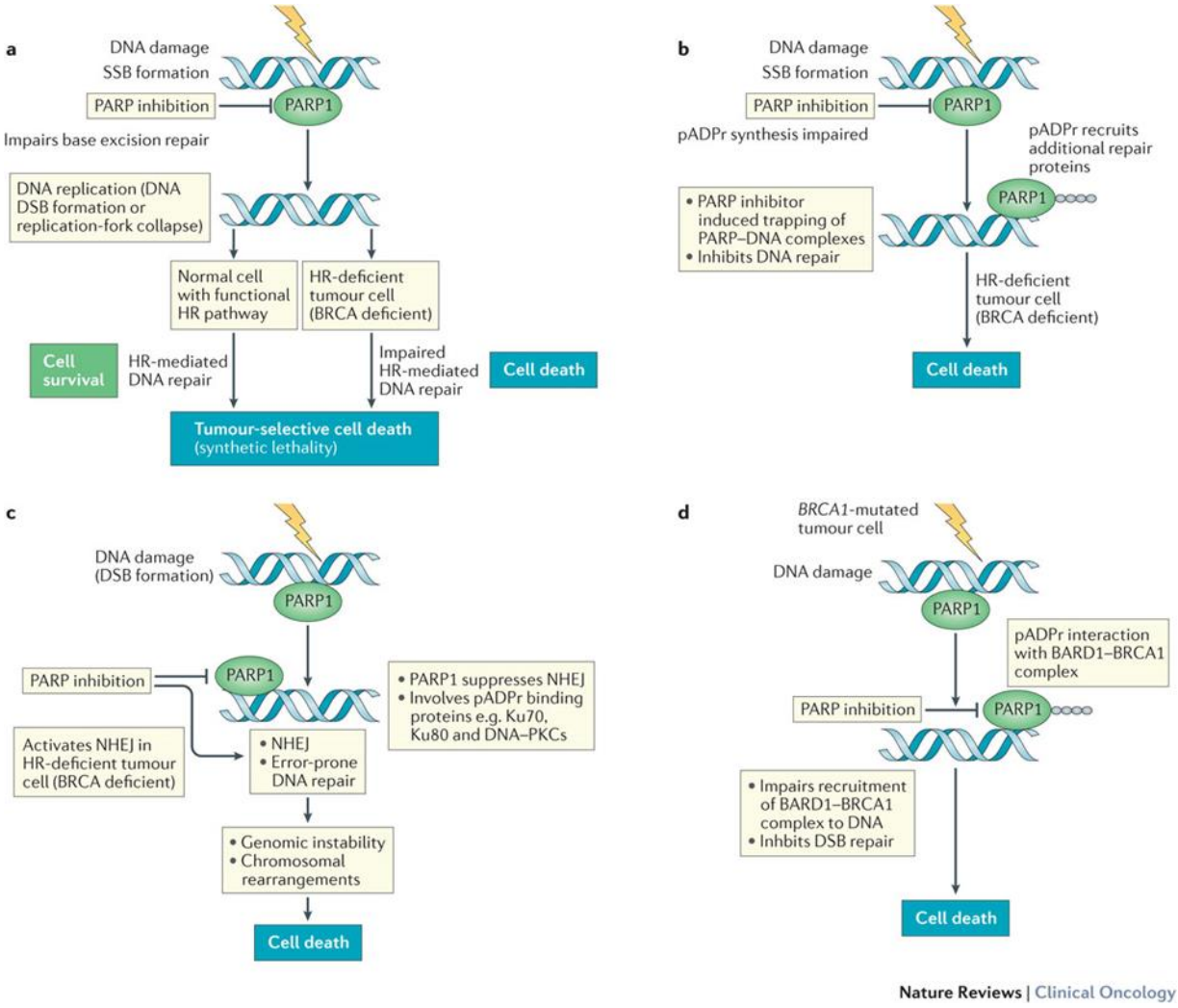

Table 1: Published results of key phase II/III studies of PARP inhibitors in patients with ovarian cancer

AUC, area under the curve; BD, twice daily; combo, combination; cyclo, cyclophosphamide; d, day; HR, hazard ratio; HRD, homologous recombination deficiency; n, number of patients; NR, not reported; OD, once daily; OR, odds ratio; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PLD, pegylated liposomal doxorubicin.

Study	Study population	Treatment	ORR	Mean PFS duration (months)	OS (months)
Audeh *et al.* (2010)[24] (ICEBERG 2)	Recurrent, germ line *BRCA*-mutated (*n* = 57)	Olaparib 400 mg BD (*n* = 33) vs olaparib 100 mg BD (*n* = 24)	33% vs 13%	5.8 vs 1.9	NR
Gelmon *et al.* (2011)[25]	Recurrent (*n* = 64)	Olaparib 400 mg BD	• *BRCA*-mutant: 41% • *BRCA* wild type: 24%	7.3 vs 6.3	NR
Kaufman *et al.* (2015)[38] (Study 42)	Recurrent, germ line *BRCA*-mutated; platinum-resistant, or unsuitable for further platinum therapy (*n* = 193)	Olaparib 400 mg BD	31%	7	16.6
Kaye *et al.* (2012)[29]	Recurrent germ line *BRCA*-mutated (*n* = 97)	Olaparib 200 mg BD vs olaparib 400 mg BD vs PLD 50 mg/m^2	25% vs 31% vs 18%, *P* = 0.31	6.5 vs 8.8 vs 7.1, *P* = 0.66	NR
Ledermann *et al.* (2012)[2], (2016)[34] (Study 19)	Platinum-sensitive, recurrent, high-grade, serous disease: unselected for *BRCA* status (*n* = 265)	Olaparib (maintenance therapy) 400 mg BD vs placebo	12% vs 4%, OR 3.36, *P* = 0.12	Overall: 8.4 vs 4.8, HR 0.35, *P* <0.001; *BRCA*-mutated group: 11.2 vs 4.3, HR 0.18, *P* <0.0001; *BRCA*-wild-type group: 7.4 vs 5.5, HR 0.54, *P* = 0.0075	Overall: 29.8 vs 27.8, HR 0.88, *P* = 0.44; *BRCA*-mutated group: 34.9 vs 31.9, HR 0.73, *P* = 0.19; *BRCA*-wild-type group: 24.5 vs 26.2, HR 0.99, *P* = 0.96
Oza *et al.* (2015)[26] (Study 41)	Platinum-sensitive, recurrent, high-grade, serous disease (*n* = 162)	Carboplatin AUC4 d1, paclitaxel 175 mg/m^2 d1, and olaparib 200 mg BD d1–10 every 21 d, then olaparib 400 mg BD maintenance vs carboplatin AUC6 d1 and paclitaxel 175 mg/m^2 every 21 d	64% vs 58%	12.2 vs 9.6, HR 0.51, *P* = 0.0012; *BRCA*-mutated group, HR 0.21, *P* = 0.0015	33.8 vs 37.6, HR 1.17, *P* = 0.44; *BRCA*-mutated group, not reached vs 39.2, HR 1.28, *P* = 0.69
Liu *et al.* (2014)[110]	Platinum-sensitive, recurrent, high-grade, serous/ endometrioid disease; unselected for *BRCA* status (*n* = 90)	Olaparib 200 mg BD and cediranib 30 mg OD vs olaparib 400 mg BD	79.6% vs 47.8%, OR 4.24, *P* = 0.002	17.7 vs 9.0, HR 0.42; *P* = 0.005; *BRCA*-mutated group: 19.4 vs 16.5 (HR 0.55; *P* = 0.16); *BRCA*-wild-type group: 16.5 vs 5.7 (HR 0.32; *P* = 0.008)	NR
Coleman *et al.* (2015)[27]	Germ line *BRCA*-mutant, recurrent disease (*n* = 50)	Veliparib 400 mg BD	Overall: 26%; platinum-sensitive: 35%; platinum-resistant: 20%	Overall: 8.2	NR
Kummar *et al.* (2015)[28]	Recurrent *BRCA*-mutated, or high-grade, serous disease (*n* = 75)	Veliparib 60 mg OD + cyclo 50 mg OD vs cyclo alone	Cyclo alone 7/36 (19.4%); combo 4/34 (11.8%)	Cyclo alone 2.3, combo 2.1	NR
Mirza *et al.* (2016)[40]	Platinum-sensitive, high-grade serous or known germ line *BRCA* mutation (*n* = 553)	Niraparib (maintenance therapy) 300mg OD vs placebo	NA	Germ line *BRCA*-mutated 21.0 vs 5.5, HR 0.27, *P* <0.001; non-germ-line *BRCA*-mutated 9.3 vs 3.9, HR 0.45, *P* <0.001; non-germ-line *BRCA*-mutated, HRD-positive 12.9 vs 3.8, HR 0.38, *P* <0.001; non-germ-line *BRCA*-negative, 6.9 vs 3.8. HR 0.58, *P* = 0.02	NR

Table 2: Phase III trials of PARP inhibitors in patients with ovarian cancer* with results pending

Data were accessed on the 18th of July 2016. HRD, homologous recombination deficiency. *Including patients with fallopian tube and/or primary peritoneal carcinomas. ‡Closed to recruitment, awaiting results. §Closed to recruitment, awaiting results (press release available indicating statistically significant improvement with olaparib)

Trial identifier	PARP inhibitor and context	Patient population
NCT01844986 (SOLO-1)[95‡]	Olaparib maintenance	*BRCA*-mutated following first-line platinum-based chemotherapy
NCT01874353 (SOLO-2)[97§]	Olaparib maintenance	Platinum sensitive, *BRCA*-mutated disease following a complete or partial response to platinum-based chemotherapy
NCT01968213 (ARIEL3)[98‡]	Rucaparib maintenance	Following platinum-based chemotherapy in patients with platinum-sensitive, high-grade serous or endometrioid disease
NCT02477644 (PAOLA-1)[114]	Olaparib maintenance (with bevacizumab)	Following first-line platinum-based chemotherapy with bevacizumab maintenance in patients with high-grade serous or endometrioid disease and germ line *BRCA* mutations
NCT02282020 (SOLO-3)[115]	Olaparib versus physician's choice of single-agent chemotherapy	Patients with platinum-sensitive, high-grade serous or endometrioid disease and germ line *BRCA* mutations
NCT02502266 (COCOS)[116]	Cediranib and olaparib or standard chemotherapy	Patients with platinum-resistant or refractory, high-grade serous or endometrioid, or known germ line *BRCA* mutations
NCT02446600 (Ref. 117)	Olaparib or cediranib and olaparib compared with standard platinum-based chemotherapy	Patients with platinum-sensitive, high-grade serous or endometrioid disease, or those with known germ line *BRCA* mutations
NCT02655016 (PRIMA)[118]	Niraparib maintenance	Patients with HRD-positive, advanced-stage ovarian cancer following response to first-line platinum-based chemotherapy
NCT02470585 (Ref. 119)	Veliparib maintenance	With carboplatin and paclitaxel and as continuation maintenance therapy in patients with newly diagnosed stage III or IV high-grade serous disease

Table 3: Ongoing clinical trials of PARP inhibitors combined with novel agents

Data were accessed on the 18th of July 2016. ATR, ataxia telangiectasia and Rad3-related protein; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed cell-death protein 1; PD-L1, programmed cell-death 1 ligand 1.

Trial identifier	Phase	PARP inhibitor	Combination novel agent(s)
NCT02723864 (Ref. 120)	I	Veliparib	VX-970 (ATR inhibitor) ± cisplatin
NCT01623349 (Ref. 121)	I	Olaparib	BKM120 or BYL719 (PI3K inhibitor)
NCT02338622 (Ref. 122)	I	Olaparib	AZD5363 (AKT inhibitor)
NCT02734004 (MEDIOLA)[123]	I	Olaparib	Durvalumab (anti-PD-L1 antibody)
NCT02681237 (Ref. 124)	II	Olaparib	Cediranib (VEGFR inhibitor)
NCT02576444 (OLAPCO)[125]	II	Olaparib	AZD5363 or AZD1775 (WEE1 inhibitor) or AZD2014 (mTORC1/2 inhibitor)
NCT02208375 (Ref. 126)	I	Olaparib	AZD2014 (mTORC1/2 inhibitor) or AZD5363 (WEE1 inhibitor)
NCT02484404 (Ref. 127)	I/II	Olaparib	Durvalumab (anti-PD-L1 antibody) and cediranib
NCT02657889 (Keynote-162)[128]	I/II	Niraparib	Pembrolizumab (anti-PD-1 antibody)
NCT02354131 (AVANOVA)[129]	I/II	Niraparib	Bevacizumab (anti-VEGF antibody)
NCT02571725 (Ref. 130)	I/II	Olaparib	Tremelimumab (anti-CTLA-4 antibody)
NCT01434316 (Ref. 131)	I	Veliparib	Dinaciclib (CDK inhibitor)