MINIREVIEW

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# The development of PARP inhibitors in ovarian cancer: from bench to bedside

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The nuclear enzyme poly (ADP-ribose) polymerase (PARP) represents an important novel target in the treatment of ovarian cancer. This article charts over 50 years of research from the discovery of the first PARP enzyme in 1963, to the approval and licensing in 2015 of the first PARP inhibitor, olaparib (Lynparza), in the treatment of *BRCA*-mutated ovarian cancer.

Ovarian cancer is the fifth most common cancer in women in developed countries, accounting for 140 000 deaths per year worldwide (World Health Organization, 2008; Siegel *et al*, 2012). The majority of women present with advanced-stage (3 or 4) disease, where 5-year survival rates are poor at around 27% (Siegel *et al*, 2012). Despite initial high responses to platinum-based chemotherapy and cytoreductive surgery, more than 70% of these patients will relapse with limited subsequent treatment options (Hanker *et al*, 2012). There is a pressing need for improved treatments that can extend survival, delay disease progression and maintain quality of life for patients with ovarian cancer.

A better understanding of cancer is leading to the identification of distinct cancer molecular sub-types, new anticancer targets, and more individualised patient treatment approaches. The development of poly(ADP-ribose) polymerase (PARP) inhibitors for the treatment of *BRCA*-mutated (*BRCA*m) ovarian cancer is an example of this approach in action. This review summarises the research behind this development; charting the discovery of the first PARP enzyme (Chambon *et al*, 1963) and the development of PARP inhibitors as a class; highlighting why cancers defective in DNA repair could be selectively sensitive to these agents, and why the approval of the PARP inhibitor olaparib (Lynparza) has changed the management of *BRCA*m ovarian cancer.

## DNA DAMAGE RESPONSE, REPAIR PATHWAYS AND *BRCA*

The accurate and efficient repair of DNA damage is essential for cells to function and maintain genomic stability (Hoeijmakers, 2001). In humans, acquired or inherited defects in DNA damage response and repair pathways can result in an increased lifetime risk of cancer (Hoeijmakers, 2009). DNA double-strand breaks (DSBs) are regarded as the most lethal of the DNA insults and, if left unrepaired, result in genomic instability, carcinogenesis and ultimately cell death (Hoeijmakers, 2001). DNA DSBs can arise as a result of direct damage to both strands of DNA from exogenous agents, such as ionising radiation or chemotherapy (Helleday *et al*, 2008), or as part of normal cell physiology, for example, to permit genetic recombination during meiosis (Neale and Keeney, 2006) and the rearrangements needed for the development of immunoglobulin genes during V(D)J (variable, diversity and joining) recombination (Leavy, 2010).

The two primary DSB repair pathways in humans are non-homologous end joining (NHEJ) and homologous recombination repair (HRR). These two pathways operate independently but do share some common proteins (Figure 1). The pathway that is used to repair the DNA damage depends principally on the origin of the DSB and the stage in the cell cycle in which the DSB occurs (Takata *et al*, 1998). The preferred pathway is HRR, as it is an error-free pathway; however, it is dependent on the availability of sister chromatids and can only take place in late S and the $G_2$ phases of the cell cycle (O'Driscoll and Jeggo, 2006). A significant number of DSBs can also arise during DNA replication when a replication fork encounters an unrepaired, single-strand break (SSB); the HRR pathway and the nuclear enzyme PARP-1 have a vital role in repairing these DSBs (Bryant *et al*, 2009; Helleday *et al*, 2007). Homologous recombination repair involves a variety of proteins, including BRCA1 and BRCA2. BRCA1 has a role in signalling of the DNA DSB damage response and subsequent repair via HRR, but also in transcriptional regulation and cell-cycle checkpoint control; whereas BRCA2 has a more direct repair role in HRR through its regulation of Rad51 (Gudmundsdottir and Ashworth, 2006). It is proposed that the BRCA2–Rad51 complex

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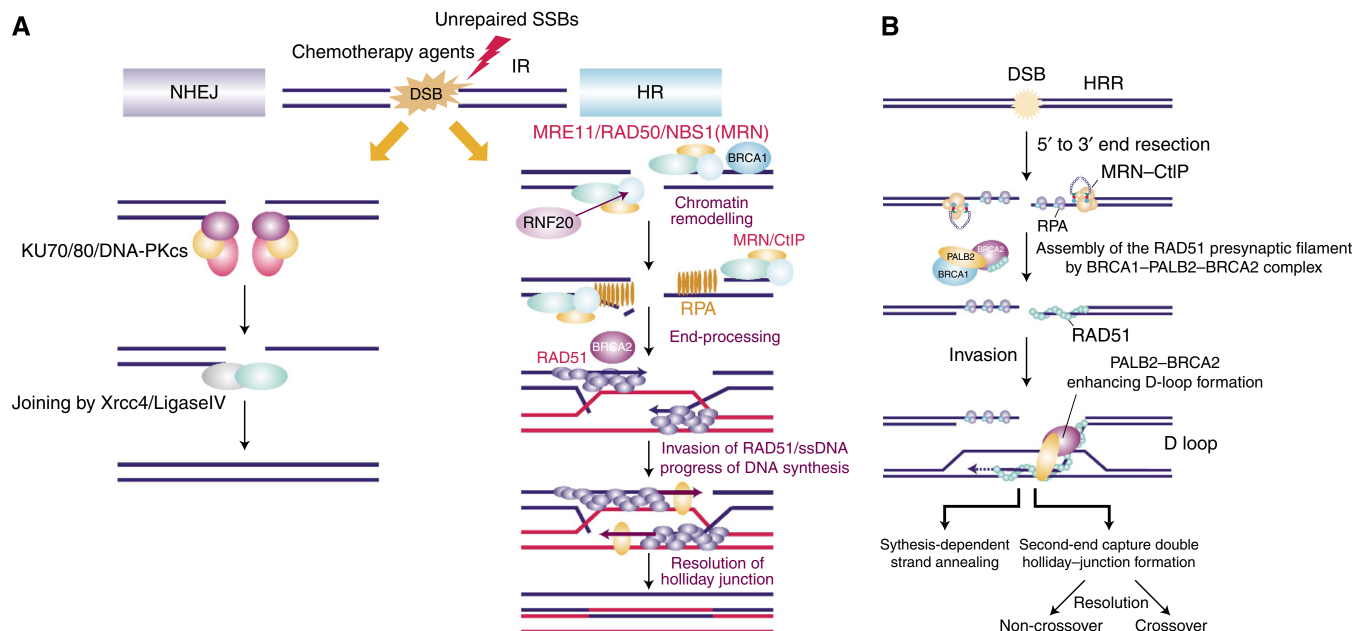


**Figure 1. Mechanisms of DNA double-strand break (DSB) repair.** Double-stranded breaks in DNA are typically repaired through one of two pathways: (**A**) non-homologous end joining (NHEJ); (**A, B**) homologous recombination (HR). Proteins involved in NHEJ include KU70/80, DNA-PKcs, XRCC4 and DNA ligase IV. Proteins involved in HR include MRE11, RAD50 and NBS1 (which form the MRN complex); CtIP; RNF20; RPA; RAD51; PALB2; BRCA1 and BRCA2. Abbreviations: HRR, homologous recombination repair; IR, ionising radiation; SSB, single-strand break; ssDNA, single-stranded DNA. Note: (**A**) Reproduced with permission from Pioneer Bioscience Publishing Company (© Saito *et al*, 2013). (**B**) Reprinted with permission from Nature America, Inc. (© Buisson *et al*, 2010).

binds to the exposed DNA, and this binding then enables the loading of Rad51 onto the break and the formation of the presynaptic filament (Yang *et al*, 2002). Given the functions of BRCA1 and 2, it would be logical to hypothesise that deficiencies within either gene will result in defective HRR and subsequent loss of efficient and effective DNA DSB repair.

## *BRCA* MUTATIONS AND OVARIAN CANCER

The *BRCA1* gene was identified in 1990 by Mary King's group working at Berkeley, CA, USA. The name BRCA was originally chosen to stand for Berkeley California, but was later changed to represent breast cancer susceptibility (Hall *et al*, 1990). The gene was subsequently cloned in 1994 by Myriad Genetics (Miki *et al*, 1994). Around the same time, the *BRCA2* gene was discovered by Stratton and Wooster working at the Institute of Cancer Research, London, UK (Wooster *et al*, 1994). The identification of these genes represented a significant breakthrough in the management of breast and ovarian cancer families, enabling the introduction of risk assessment, genetic counselling and *BRCA* mutational analysis. Subsequently, over 2000 distinct mutations and sequence variations in the *BRCA* genes have been identified (Audeh, 2014), with *BRCA1* mutations more common, occurring approximately twice as frequently as *BRCA2* (Chen and Parmigiani, 2007).

Women who inherit a deleterious *BRCA1* or *BRCA2* mutation have up to a 40% and 20% lifetime risk, respectively, of developing ovarian cancer, and higher risks of developing breast cancer (Chen and Parmigiani, 2007). The prevalence of germline (g) *BRCA* mutations in ovarian cancer has historically been estimated to be around 10–15% (Risch *et al*, 2001). However, recent reports suggest that this may be a gross underestimate, especially in women with high-grade serous ovarian cancer (HGSOC) (Risch *et al*, 2006; Cancer Genome Atlas Research Network, 2011; Alsop *et al*, 2012). In addition, in one series where 17% of patients with HGSOC were found to carry a *BRCA* mutation, almost half (44%) of these women had no family history of cancer (Alsop *et al*, 2012). Such data support the use of *BRCA* mutation testing in all patients with HGSOC, regardless of family history. This expansion in *BRCA* testing will require changes to the traditional genetic service pathways in which patients are screened and referred based on family history, moving to a more streamlined oncology-based genetic testing service.

Over the past two decades the main focus in the treatment of women identified as *BRCA* mutation carriers has been ovarian and breast cancer prevention through prophylactic surgery, and early cancer detection through screening (Domchek *et al*, 2006). However, surveillance and surgery will not prevent all carriers developing cancer and many already have cancer at the time their mutation status is diagnosed. The current management of *BRCA*m-associated ovarian cancer is not different to the treatment of the non-*BRCA* stage-matched cases. However, recent data suggest that these *BRCA*m cancers should be treated as a distinct disease entity and that *BRCA* mutation status has a major influence on ovarian cancer patient outcomes. *In vitro* studies have demonstrated that *BRCA1*- and *2*-deficient cells are more sensitive than their wild-type controls to platinum analogues and less sensitive to anti-microtubule agents, such as the taxanes (Bhattacharyya *et al*, 2000; Tassone *et al*, 2003; Tan *et al*, 2008). Data from 26 observational clinical studies of 3879 women with ovarian cancer reported that those with *BRCA*m cancers have a better outcome following cytoreductive surgery and platinum-based chemotherapy than their non-*BRCA*m counterparts, with prolonged progression-free and greater 5-year overall survival (Bolton *et al*, 2012). A recent meta-analysis of 14 ovarian cancer studies has confirmed this, showing that *BRCA* status in ovarian cancer is an independent predictor of outcome (Zhong *et al*, 2015). In the relapsed setting, *BRCA*m carriers have also been shown to respond better to both platinum- and non-platinum-containing regimens (Alsop *et al*, 2012).

Knowing the *BRCA* mutation status of a patient with ovarian cancer is important in terms of managing individual risk and identifying other family members at risk. In addition, a patient's *BRCA1* and *2* mutation status can now inform the physician and patient regarding treatment outcomes, and, with the development of PARP inhibitors, offers patients the potential for personalised anticancer treatment.

## POLY (ADP-RIBOSE) POLYMERASE AND THE DEVELOPMENT OF PARP INHIBITORS

The discovery of the first PARP was made over 50 years ago when researchers in Paul Mandel's laboratory observed the synthesis of a new polyadenylic acid after adding nicotinamide mononucleotide to rat liver extracts (Chambon *et al*, 1963). By 1980 it was known that this nuclear enzyme, PARP-1, was activated by DNA damage and played a pivotal role in the repair of DNA SSBs via the base-excision repair/single-strand break repair (BER/SSBR) pathway (Figure 2) (Benjamin and Gill, 1980). Seminal work by Sydney Shall's group subsequently demonstrated that PARP-1 was not only involved in the repair of SSBs, but inhibiting it could enhance the cytotoxic effects of methylating agents in leukaemic mice cells (Durkacz *et al*, 1980), suggesting that PARP inhibitors could act as chemosensitisers. There are now 17 members of the PARP nuclear superfamily and it is PARP-1 and 2 that are involved in DNA repair (Rouleau *et al*, 2010).

The first inhibitor of PARP, 3-aminobenzamide (3-AB), was identified over 30 years ago following the observation that nicotinamide and 5-methylnicotinamide competed with NAD+ as a PARP substrate (Purnell and Whish, 1980). Poly (ADP-ribose) polymerase inhibitor development pipelines initially investigated the potential for PARP inhibition to act as potentiators of chemotherapy and radiotherapy (Ferraris, 2010). More recently, they have pursued their therapeutic application as single agents, selectively killing cells with defects in DNA repair pathways, such as those with *BRCA1/2* mutations. There are currently four PARP inhibitors in Phase III development for ovarian cancer (Table 1). The most developed in the class is olaparib, a potent, oral inhibitor of PARP-1 and 2 that induces lethality in tumours with HRD, such as *BRCA1/2* mutations (Evers *et al*, 2008; Rottenberg *et al*, 2008). Olaparib is associated with significant clinical benefit in high-grade ovarian cancers with germline and/or somatic mutations within the *BRCA1/2* genes (Fong *et al*, 2009; Audeh *et al*, 2010; Tutt *et al*, 2010; Gelmon *et al*, 2011; Ledermann *et al*, 2014; Kaufman *et al*, 2015; Oza *et al*, 2015). This topic is reviewed within this Supplement (Ledermann, 2015). Why single-agent PARP inhibitors are active in *BRCA*m cancers is explained below through the concept of 'synthetic lethality'.

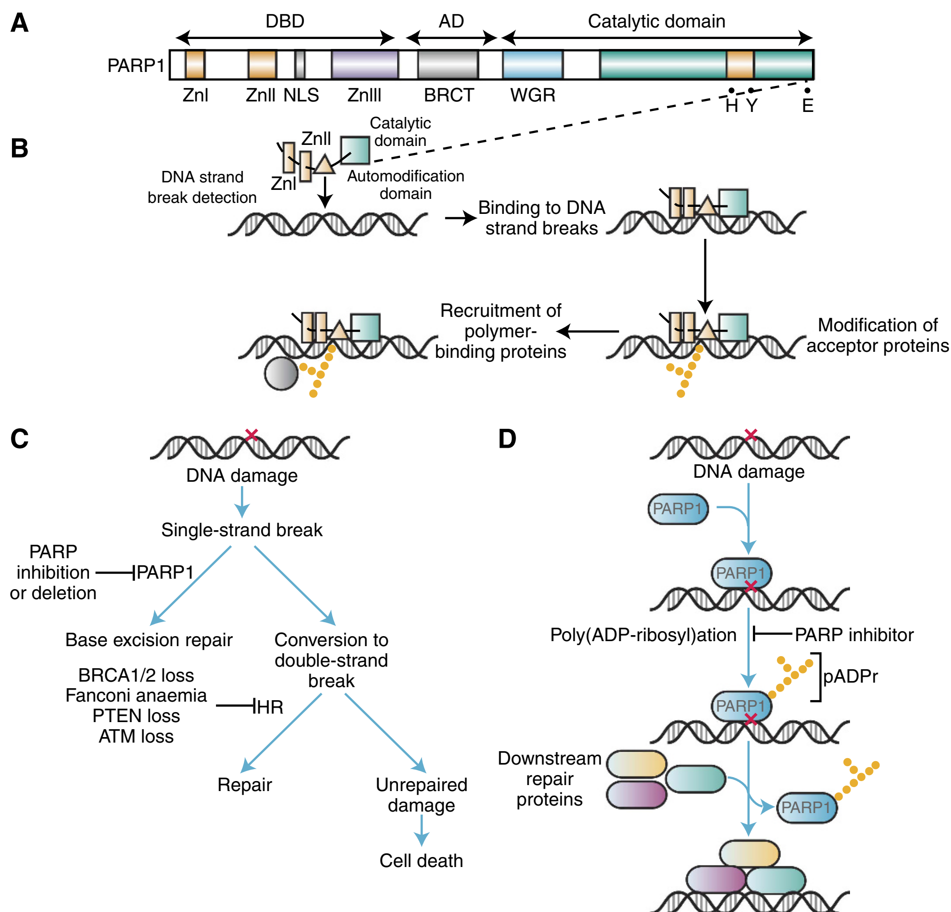


Figure 2. Base-excision repair/single-strand break pathway. (**A**) Structure of PARP1. (**B**) Activation of PARP1 in response to DNA damage. After binding to damaged DNA, the enzymatic activity of PARP1 increases following a conformational change to the active site. PARP1 synthesises poly(ADP) ribose chains that alter protein function and recruit additional proteins. (**C**) Role of PARP1 in base excision repair. (**D**) Model showing recruitment of DNA repair proteins following DNA damage. Abbreviations: AD, automodification domain; BRCT, *BRCA1* C-terminal domain; DBD, DNA-binding domain; HR, homologous recombination; NLS, nuclear localisation signal; WGR, tyrptophan–glycine–arginine-rich domain; Zn, zinc finger. Note: Reproduced with permission from the American Society of Clinical Oncology (© Scott *et al*, 2015).

**Table 1. PARP inhibitors in Phase III clinical trial development for ovarian cancer, 2015**

| Agent | Company | $IC_{50}$ | Ongoing clinical trials | Patient population | Indication |
|---|---|---|---|---|---|
| Olaparib (AZD2281) | AstraZeneca | 5 nM (PARP1)<br>1 nM (PARP2)<br>(Menear *et al*, 2008) | SOLO1 (NCT01844986) | *BRCA*-mutated, advanced (FIGO Stage III–IV), high-grade serous/endometrioid; response (CR or PR) to initial platinum-based chemotherapy | First line |
| | | | SOLO2 (NCT01874353) | *BRCA*-mutated, high-grade serous/endometrioid; response (CR or PR) following ⩾2 lines of platinum-based chemotherapy | Relapsed |
| | | | SOLO3 (NCT02282020) | Germline *BRCA*-mutated, platinum-sensitive relapsed, high-grade serous/endometrioid | Relapsed |
| | | | SOLOiST (NCT02392676) | Platinum-sensitive relapsed, high-grade epithelial; deficient DNA damage repair (must not be caused by a germline *BRCA* mutation) | Relapsed |
| Niraparib (MK4827) | Merck (licensed to Tesaro) | 3.8 nM (PARP1)<br>2.1 nM (PARP2)<br>(Jones *et al*, 2009) | NOVA (NCT01847274) | *BRCA*-mutated or high-grade serous; sensitive to penultimate platinum-based regimen; response (CR or PR) to current platinum-based chemotherapy | Relapsed |
| Rucaparib (AG014699) | Clovis Oncology | 1.4 nM (Ki; PARP1)<br>0.5 nM (Ki; PARP2)<br>(Thomas *et al*, 2007) | ARIEL3 (NCT01968213) | High-grade serous/endometrioid; sensitive to penultimate platinum-based regimen; response (CR or PR) to current platinum-based chemotherapy | Relapsed |
| Talazoparib (BMN-673) | Medivation | 0.58 nM (PARP1)<br>(Shen *et al*, 2013) | None | — | — |
| Veliparib (ABT-888) | AbbVie and BMS | 5.2 nM (PARP1)<br>2.9 nM (PARP2)<br>(Donawho *et al*, 2007) | NCT02470585 | Advanced (FIGO Stage III or IV), high-grade serous | First line |

Abbreviations: CR = complete response; FIGO = Féderation Internationale de Gynécologie et d'Obstétrique; $IC_{50}$ = the concentration of a drug required for 50% inhibition; PR = partial response.

## POLY (ADP-RIBOSE) POLYMERASE INHIBITORS AS SINGLE AGENTS IN *BRCA*m CANCERS—THE CONCEPT OF SYNTHETIC LETHALITY

In 2005, two articles published in *Nature* reported that cells deficient in *BRCA1* and *2* were 100- to 1000-fold more sensitive to PARP inhibitors than *BRCA1/2* heterozygote or wild-type cell lines (Bryant *et al*, 2005; Farmer *et al*, 2005). Bryant *et al* used the PARP inhibitors NU1025 and AG14361, both forerunners to rucaparib (Clovis Oncology, Boulder, CO, USA). In mice xenografts, three out of five V-C8 tumours responded to a 5-day dosing of AG14361, with one mouse showing complete remission and no sign of tumour at autopsy. In addition, the articles reported an induction in γH2AX foci formation (representing DNA DSBs) and Rad51 foci formation (indicating functional HR repair) in the XRCC1-deficient EM9 (Chinese hamster ovary) cell lines. In the V-C8 cells, an increase in γH2AX foci formation, but not Rad51, was observed following exposure to NU1025.

In the *Nature* sister article, Farmer *et al* (2005) demonstrated the sensitivity of both *BRCA1*- and *BRCA2*-deficient cell lines to the specific inhibition of PARP-1 by two small-molecule inhibitors KU0058684 and KU0058948, forerunners to olaparib. They demonstrated that 24-h exposure to the PARP inhibitor resulted in permanent G2/M cell-cycle arrest or apoptosis. They also reported a three-fold increase in sensitivity over the DNA-damaging agent cisplatin for *BRCA1/2*-deficient cells. Both research groups independently concluded that *BRCA*-deficient cells were selectively sensitive to PARP inhibition by a mechanism of 'synthetic lethality'.

'Synthetic lethality' is the concept by which cancer cells are selectively sensitive to the inactivation of two genes or pathways when inactivation of either gene or pathway alone is non-lethal (Kaelin, 2005). This proposed mechanism of synthetic lethality of PARP inhibitors in *BRCA*-deficient cells is outlined in Figure 3. Poly (ADP-ribose) polymerase inhibition leads to the accumulation of DNA SSBs that result in unrepaired stalled replication forks and ultimately DSBs. These DNA DSBs are normally repaired by the HRR pathway (Hoeijmakers, 2001). In HRR-defective cells, that is, those with *BRCA1/2* mutations, these DSBs are left unrepaired or are repaired in an error-prone way by alternative non-homologous end-joining DNA repair; both outcomes can result in genomic instability and ultimately cell death. Whereas, in cells with functional HRR, that is, those with heterozygous mutations or wild-type *BRCA*, DSBs will be accurately and efficiently repaired, and inhibiting PARP will not result in cell death. Clinical trials are now confirming these preclinical data demonstrating that, as a class, PARP inhibitors are active in *BRCA*m cancers.

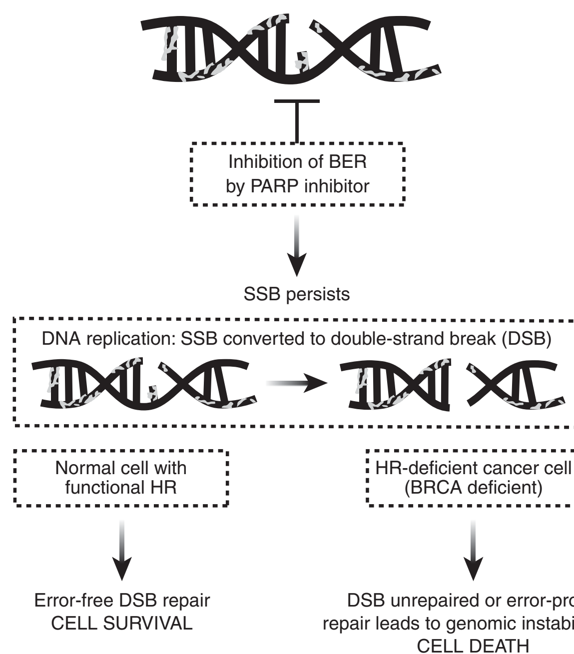


Figure 3. Synthetic lethality of PARP inhibitors in *BRCA*-deficient cells.

## FUTURE DIRECTIONS FOR PARP INHIBITORS

The majority of ovarian cancers are not attributed to hereditary germline mutations in the *BRCA1* and *2* genes (Venkitaraman, 2002), so a key question is whether single-agent PARP inhibitors can be used to treat patients within the larger ovarian cancer population. It is known that HRD is not exclusive to germline *BRCA*m cancers, for example; molecular analysis of HGSOC as part of The Cancer Genome Atlas revealed that approximately 50% were shown to harbour HRD (Cancer Genome Atlas Research Network, 2011). This HRD included somatic *BRCA* mutations (6–8%) and epigenetic silencing in non-*BRCA* genes, such as *ATM* and *RAD51*. In addition, by using a functional assay of HRR, Mukhopadhyay *et al* (2010) demonstrated that 50% of primary cultures generated from ascites in unselected HGSOC patients had HRD and were sensitive to PARP inhibitors. Developing a diagnostic signature of HRD in cancers is the focus of the ongoing rucaparib studies (www.clinicaltrials.org). Preliminary results from the rucaparib ARIEL 2 study (NCT 01891344) indicate efficacy in patients who have *BRCA*m ovarian cancer, but also in those who are *BRCA* wild-type with high tumour genomic loss of heterozygosity (McNeish *et al*, 2014). The study hopes to develop a companion diagnostic to use within the ongoing Phase III trial (ARIEL 3; NCT01968213) of rucaparib in platinum-sensitive ovarian, fallopian tube or primary peritoneal high-grade cancer patients.

Another therapeutic approach is to induce HRD in otherwise HRR-competent cancers by altering the tumour microenvironment through hypoxia, or to combine PARP inhibitors with agents that can downregulate HRR, such as vascular endothelial growth factor (VEGF) inhibitors. This concept, known as 'contextual' synthetic lethality, could further broaden the application of this class of drugs and is the rationale behind many ongoing clinical trials. Preliminary data from a Phase II trial combining olaparib with the potent, oral VEGF tyrosine kinase inhibitor, cediranib, was shown to significantly improve progression-free survival over olaparib alone (9.0 months *vs* 17.7 months) (Liu *et al*, 2014); a confirmatory study is awaited.

Based on a wealth of preclinical data showing that PARP inhibitors potentiate the effects of DNA-damaging chemotherapy agents, such as the platinums, temozolomide and topoisomerase inhibitors (Delaney *et al*, 2000; Calabrese *et al*, 2004; Donawho *et al*, 2007), the original therapeutic intention of these agents was as chemopotentiators. Furthermore, inhibition of PARP has been shown to augment the antitumour activity of other agents that impair HRR, such as the DNA-synthesis inhibitor, gemcitabine (Virag and Szabo, 2002; Jacob *et al*, 2007). However, early clinical trials investigating multiple chemotherapy and PARP inhibitor combinations have reported enhanced myelosuppression as the main dose-limiting toxicity, and this may limit the future use of PARP inhibitors with chemotherapy (Chen, 2011).

Radiotherapy induces DNA damage by multiple mechanisms including base damage and single- and double-strand DNA breaks; damage that is dependent on PARP activity for its repair. Numerous *in vitro* and *in vivo* studies (Powell *et al*, 2010) using different classes of PARP inhibitors have reported enhancement of the cytotoxicity of radiation in a number of tumour types, including colorectal cancers (Calabrese *et al*, 2004) and gliomas (Dungey *et al*, 2009; Russo *et al*, 2009). More recently, work by Anthony Chalmers' group has shown that this radio-potentiation is enhanced in rapidly proliferating cells and cells defective in DNA DSB repair compared with normal tissue (Loser *et al*, 2010). These data support a role for combining radiotherapy and PARP inhibitors in patients with cancer, and clinical trials are finally underway (www.clinicaltrials.gov) with results eagerly awaited.

## SUMMARY

Poly (ADP-ribose) polymerase inhibitors are an exciting new development in the treatment of cancer, with clinical trials of single agents showing significant benefits in patients with *BRCA*m ovarian cancer. The mechanism underlying this benefit is the HRD of *BRCA*m cancers. Historically, germline *BRCA1/2* mutations were thought to be associated with approximately 10% of all ovarian cancers, but this is now known to be an underestimate. In addition, HRD is reported to be present in approximately 50% of all HGSOC cases. This suggests that the use of PARP inhibitors may have a much broader role in the treatment of ovarian cancer and the development of a validated HRD signature would facilitate this.

Finally, the recent licensing of olaparib in *BRCA*m ovarian cancer brings together over 50 years of research and is the first targeted treatment option for this patient population, taking another step further towards personalised medicine in ovarian cancer.

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## CONFLICT OF INTEREST

Dr Yvette Drew has previously received honoraria as a clinical advisory board member for AstraZeneca and for Clovis Oncology.

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