PARP inhibitor combination therapy

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Abstract

In 2014, olaparib (Lynparza) became the first PARP (Poly(ADP-ribose) polymerase) inhibitor to be approved for the treatment of cancer. When used as single agents, PARP inhibitors can selectively target tumour cells with *BRCA1* or *BRCA2* tumour suppressor gene mutations through synthetic lethality. However, PARP inhibition shows considerable promise when used together with other therapeutic agents. Here, we summarise both the pre-clinical and clinical evidence for the utility of such combinations and discuss the future prospects and challenges for PARP inhibitor combinatorial therapies.

Introduction

The PARP (Poly(ADP-ribose) polymerase) family of enzymes utilise beta nicotinamide adenine dinucleotide (β-NAD^+) to covalently add Poly(ADP-ribose) (PAR) chains onto target proteins a process termed PARylation (De Vos et al. 2012). This form of post-translational modification has the ability to alter the function of target proteins and has been found to be involved in a diverse set of cellular processes including chromatin modification, transcription regulation, the control of cell division, Wnt signalling and the maintenance of telomeres (Gibson and Kraus 2012). The best-studied PARP enzyme is PARP1, which has a well-established role in the repair of damaged DNA (reviewed in (De Vos et al. 2012)). As part of this role, PARP1 is involved in the repair of single stranded DNA breaks (Woodhouse et al. 2008), but has also been implicated in the repair of other DNA lesions (Krishnakumar and Kraus 2010). PARP1 binds to damaged DNA via a series of Zinc finger domains, and then PARylates a series of DNA repair effector proteins as part of the repair process, releasing nicotinamide as a by product (Krishnakumar and Kraus 2010). Once the role of PARP1 in this repair process is complete, PARP1 autoPARylation causes the release of the protein from DNA (De Vos et al. 2012).

The role of PARP1 and the related enzyme PARP2, in DNA repair, prompted the development of potent small molecule PARP1/2 inhibitors (PARPi) (reviewed in (Zaremba and Curtin 2007)). The original proposed use of these inhibitors was as chemo- or radiosensitizing agents. Indeed, as early as the 1980s, a toolbox PARP superfamily inhibitor, 3-aminobenzamide (3AB), was shown to enhance the cytotoxic effect of the DNA methylating agent, dimethyl sulphate (Purnell and Whish 1980). Classical structure activity relationship-based drug discovery efforts led to the discovery of the first set of clinical PARPi to enter clinical trials; rucaparib (AG014699, PF-01367338/ Pfizer), veliparib (ABT-888/Abbott Pharmaceuticals), olaparib (AZD2281, KuDOS/AstraZeneca, now marketed as Lynparza), and niraparib (MK-4827, Merck/Tesaro) (recently reviewed in (Lord et al. 2015)). In general, these

inhibitors tend to have PARP1 IC_{50} (the drug concentration needed to kill 50% of a cell population) values in the nanomolar range, but more recently, a second generation of more potent PARPi with picomolar PARP1 IC_{50} values, such as talazoparib (BMN 673, Biomarin/Medivation) have been developed (Shen et al. 2013). Each of these small molecule inhibitors impairs the catalytic activity of PARP1 by interacting with the β-NAD^+ binding catalytic domain. However, there are distinct differences in other aspects of their function. For example, recent work has suggested that the cytotoxicity caused by PARPi is in part caused by PARP1 being "trapped" on DNA by PARPi (Murai et al. 2012), a likely consequence of impairing PARP1 autoPARylation. It seems that whereas drugs such as talazoparib and olaparib are effective PARP1 trapping agents, veliparib has considerably less effect in this regard (Murai et al. 2012; Murai et al. 2014b).

Although clinical PARPi were developed with a chemo- or radiosensitisation role in mind, their utility as single agents has superseded this effort. PARP1/2 inhibitors can selectively target tumour cells with defects in either the *BRCA1* or *BRCA2* tumour suppressor genes that normally maintain the integrity of the genome by mediating a DNA repair process, known as homologous recombination (HR) (recently reviewed in (Lord et al. 2015)). This "synthetic lethal" effect of PARPi is likely caused by PARPi causing a persistent DNA lesion that is normally repaired by HR; in the absence of *BRCA* gene function and HR, tumour cells seem unable to effectively repair these DNA lesions and die, whilst normal (non-tumour) cells are unaffected (Bryant et al. 2005; Farmer et al. 2005). The effectiveness of PARPi in being able to selectively target *BRCA* mutant tumour cells in pre-clinical model systems (tumour cell lines and animal models) was reflected in clinical trials where significant and sustained anti-tumour responses were observed in heavily pre-treated breast or ovarian cancer patients with germ-line *BRCA1* or *BRCA2* mutations (Lord et al. 2015). Additionally, efficacy was observed when olaparib was used in a Phase 2 clinical trial as a maintenance therapy in high-grade serous ovarian cancer (HGSOC) following carboplatin first-line therapy. For example, when compared to patients receiving placebo after carboplatin, HGSOC patients

who received olaparib maintenance therapy exhibited a marked improvement in progression free survival (PFS) (8.4 months compared to 4.8 months in the placebo arm). HGSOC is a disease with a relatively high frequency of BRCA mutations and in this same clinical trial, patients harbouring *BRCA1* or *BRCA2* gene mutations showed the most profound improvement in PFS (11.2 months) (Ledermann et al. 2012; Ledermann et al. 2014). On the basis of these trial results, olaparib was approved for use by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2014, specifically as a maintenance monotherapy in patients with deleterious or suspected deleterious germline *BRCA* mutated, advanced ovarian cancer, who have been treated with three or more prior lines of chemotherapy (2014; Simon 2014).

Although PARPi can elicit profound and sustained anti-tumour responses in *BRCA*-mutant patients, in some cases PARPi resistance emerges (Lord and Ashworth 2013). In *BRCA* mutant patients, clinical PARPi resistance has been associated with the presence of additional mutations in the either the *BRCA1* or *BRCA2* genes. These “secondary” mutations, which have been observed in tumour DNA recovered from patients with profound PARPi resistance (Barber et al. 2013), restore the open reading frame (ORF) of either the *BRCA1* or *2* gene and cause the formation of a functional protein product that can restore HR and repair the DNA lesion caused by PARPi (Edwards et al. 2008; Sakai et al. 2009). Additional mechanisms of acquired resistance to PARPi have been proposed and include up-regulation of P-glycoprotein (PGP) pumps (which transport small molecules such as PARPi across the plasma membrane), additional loss of function in either DNA repair proteins, 53BP1 and REV7, or stabilization of the BRCT domain of mutant BRCA1 by the heat-shock protein 90 (HSP90) (Lord and Ashworth 2013).

Further investigation of PARPi utility outwith a *BRCA* context has also been explored. Several sporadic tumours have been shown to exhibit a BRCAness phenotype, where they possess molecular and histopathological characteristics similar to BRCA-deficient disease (Turner et al. 2004). For example, the presence of oncogenic fusions such as *EWS-FLI1* (caused by a

chromosomal translocation) in Ewing sarcoma (EWS) and subsequently derived tumour cell lines and xenografts exhibit sensitivity to PARPi (Brenner et al. 2012; Garnett et al. 2012). However, a Phase 2 clinical trial evaluating efficacy of a single-agent PARPi therapeutic approach exhibited no partial or complete response in 12 Ewing sarcoma patients (NCT01583543) (Choy et al. 2014). Defects in the *ATM* DNA repair tumour suppressor gene have also been associated with PARPi sensitivity (McCabe et al. 2006; Williamson et al. 2010); in contrast to EWS, clinical responses to *ATM* mutant prostate cancers have recently been described (Mateo et al. 2015).

Before we summarize the current understanding of PARPi combinatorial effects, it is perhaps useful to consider why combination therapy might be useful in contrast to single agent treatment. Combinations of therapeutic agents that have fundamentally different mechanisms of action and varying normal tissue toxicity have great potential for improving survival outcomes for cancer patients. In some instances, some agents fail to have any anti-tumour effects when used as single agents, but can elicit effects when combined with secondary agents. Although the use of single agent PARPi has received considerable attention as of late, a notable amount of data evaluating the potential of PARPi in combination has accrued over the last decade. Specifically, strategies to optimize the use of PARPi as chemopotentiators, as well as to circumvent the development of resistance have been, and remain, under investigation.

Combinations of PARPi with Cytotoxic Chemotherapy

Together with radiotherapy and surgery, cytotoxic chemotherapy remains one of the core elements of most cancer treatments. However, chemotherapy responses are treatments limited by either *de novo* or acquired resistance and therefore combination therapies that improve chemotherapy responses are eagerly sought. Many chemotherapies work by causing DNA damage and/or exploiting DNA repair defects that exist in tumour cells and therefore additional drugs, such as PARP inhibitors, which also impair DNA repair processes are often assessed for their ability to enhance chemotherapy

responses. As mentioned earlier, the discovery and pre-clinical optimization of small molecule PARPi was originally driven by the potential of these agents to enhance the effects of commonly used cytotoxic chemotherapies such as temozolomide (TMZ), illustrating the potential for exploiting combinations between PARPi and these commonly used drugs.

Temozolomide

TMZ is a cytotoxic chemotherapy used in the treatment of anaplastic astrocytomas and glioblastoma multiforme (GBM). TMZ mediates its cytotoxic effect by attaching alkyl (C_nH_{2n+1}) groups to nitrogen atoms in guanine bases in DNA (Newlands et al. 1997). As early as 1996, preclinical data established that 3-aminobenzamide (3-AB), a toolbox PARPi, enhanced the cytotoxic effect of this alkylating agent (Boulton et al. 1999; Wedge et al. 1996), observations which have subsequently been replicated using more specific and potent clinical PARPi (Gill et al. 2015). The synergistic interaction between PARPi and TMZ appears to be, at least in part, dependent upon the ability of PARPi to trap PARP on DNA (Gill et al. 2015; Murai et al. 2014a; Wedge et al. 1996). For example olaparib, which has a greater ability to trap PARP1 on DNA than veliparib, also enhances the cytotoxic effect of TMZ to a greater extent than veliparib (Murai et al. 2014a). The PARPi/TMZ synergistic effect is also seemingly not reliant upon defective HR (Gill et al. 2015) rather it appears to be related to the role of PARP1 in the repair of abasic sites in DNA, i.e. short “gaps” in the double helix that lack a base. Abasic sites, an intermediary state formed during repair of alkylated DNA, are processed by the APE1 enzyme (apurinic/apyrimidinic endonuclease 1), which generates 1-nucleotide gaps flanked by 3′-OH and 5′-deoxyribose phosphate (5′-dRP) groups (Hazra et al. 2007). PARP1 preferentially binds DNA lesions with 5′-dRP ends, as opposed to 5′-phosphate ends, perhaps explaining why TMZ induces such an enhanced level of PARP DNA binding (Cistulli et al. 2004) and why more DNA-PARP complexes occur in the presence of TMZ, enhancing cytotoxic effects of efficient PARP trapping PARPi.

In preclinical models, PARPi/TMZ combinations have been shown to be effective in targeting models of DNA mismatch repair-defective leukemia, EWS, and GBM, including models that are normally resistant to single-agent TMZ (Balvers et al. 2015; Bowman et al. 1998; Daniel et al. 2009; Norris et al. 2014; M. A. Smith et al. 2015; Tentori et al. 1999). In some cases a PARPi/TMZ combination has been seen to enhance the apoptotic rate of a population of cells by decreasing expression levels of MCL-1, a key anti-apoptotic mediator, and by increasing levels of pro-apoptotic factors such as BAX and BAK (Engert et al. 2015). In addition, high-dose PARPi treatment in concert with low-dose of TMZ is able to potentiate TMZ efficacy; dose-limiting toxicity is often a feature of TMZ treatment and therefore a combinatorial approach that uses a low dose of TMZ with an elevated dose of a PARP inhibitor might reduce toxicity while maintaining efficacy (M. A. Smith et al. 2015).

Based on the early pre-clinical work identifying the PARPi/TMZ inhibitor synergy, the first human trial using a clinical PARPi assessed whether a therapeutic window could be achieved with a PARPi/TMZ combination (Plummer et al. 2008). In this Phase 1 trial, a dose-escalation study was performed in patients with advanced malignancy and a combination dose regime of 12 mg/m^2 of rucaparib plus 200 mg/m^2 TMZ was established as tolerable (Plummer et al. 2008). Building on these results, a number of subsequent clinical trials have been performed to attempt to identify anti-tumour efficacy using PARPi/TMZ combinations but also to identify and/or validate biomarkers that might predict which patients might receive the most benefit from this approach. However, to date, results from these trials have been mixed. For example, a randomized Phase 1/2 study in recurrent, TMZ-resistant glioblastoma showed that combining veliparib with TMZ, though well-tolerated, had no additional benefit compared to TMZ, as assessed by PFS or overall survival (OS) (Robins et al. 2015). Similarly, Middleton *et al*. reported results from a double blind, Phase 2 study, in which patients with stage III or IV metastatic melanoma were treated with a combination of veliparib and TMZ (Middleton et al. 2015). In this trial, median PFS improved from 2 months in the placebo arm to 3.7 and 3.6 months for either 20 mg or 40 mg doses of the

PARPi, respectively (NCT00804908) (Middleton et al. 2015). However, these improvements in PFS failed to translate into enhanced OS (Middleton et al. 2015). However, a possible explanation to the lack of OS benefit could be that veliparib is a relatively poor PARP1 trapping inhibitor, PARPi with greater trapping potential such as olaparib or talazoparib might be more suitable to combine with TMZ (Murai et al. 2014a). These trials are currently underway (NCT02049593). Furthermore, a biomarker to define patients where a PARPi/TMZ combination might achieve most benefit has yet to be identified. To address this, Gupta *et al.* recently used a panel of GBM patient-derived xenograft (PDX) models, and found that tumours with hypermethylation of the *MGMT* promoter responded better to a veliparib/TMZ combination than PDX models without this methylation biomarker (Gupta et al. 2015). Based on these findings, patients with *MGMT* promoter hypermethylated GBM are currently being recruited into a Phase 2/3 study evaluating the efficacy of this combination (NCT02152982) (Gupta et al. 2015).

Platinum salts

Platinum-based chemotherapy induces both intra- and interstrand crosslinks in DNA through covalent interaction with nucleophilic N-7 sites of purine residues (Siddik 2003; Sikov 2015). These crosslinks inhibit DNA synthesis by preventing RNA polymerase II from past the site of the DNA adduct progressing. This may result in collapse of replication forks, and subsequently, the formation of DNA double stand breaks (DSBs) or single strand breaks (SSBs) (W. H. Ang et al. 2010). An intact nucleotide excision repair (NER) pathway is required for the correction of platinum-induced crosslinks; cells lacking functional NER show exquisite sensitivity to platinum agents (Husain et al. 1985; Zamble et al. 1996).

Extensive preclinical data combining platinum-based chemotherapies, such as cisplatin, carboplatin, or oxaliplatin, with PARPi has demonstrated that PARPi can enhance anti-tumour efficacy independent of PARP catalytic inhibition (Murai et al. 2014a; Nguewa et al. 2006). Early *in vivo* studies

showed that a cisplatin-resistant rat ovarian cancer cell line model responded to nicotinamide-induced PARP inhibition (G. Chen and Zeller 1993). Subsequently, a considerable amount of data has shown PARP inhibition can potentiate the chemotherapeutic effect of platinum agents and increase DNA damage in tumour cells including in a *BRCA1/2* deficient setting (Donawho et al. 2007; Miknyoczki et al. 2003; Rottenberg et al. 2008; Xu et al. 2015). Importantly, Donawho *et al*. showed that a PARPi/platinum combination resulted into a sustained and statistically significant regression of tumour growth in breast cancer xenografts (Donawho et al. 2007).

Despite this promising data, a recent study by Murai *et al.* has suggested that the extent of synergy achieved with PARPi/platinum combinations is minimal compared to other combinations, particularly the combination of PARPi with TMZ (Murai et al. 2014a). Miknyoczik *et al*. found that PARPi did not alter the quantity of cisplatin-induced DNA adducts, but rather the repair of such adducts was delayed (Miknyoczki et al. 2003; Plooy et al. 1984), supporting the observation that PARPi do not potentiate platinum sensitivity, but rather the combinatorial effects observed are additive (Murai et al. 2014a). Additionally, a retrospective analysis on a cohort of PARPi treated, *BRCA* mutant ovarian cancer patients showed that some patients who become resistant to PARPi therapy maintain the potential to benefit from subsequent chemotherapeutic treatment, including platinum agents (J. E. Ang et al. 2013). Taken together, this data suggests a PARPi/platinum combination may be acting outside of the PARP1/*BRCA* synthetic lethal interaction.

Several clinical trials have assessed whether a therapeutic window could be achieved with PARPi/platinum combinations. Balmana *et al*. reported tolerability results from a Phase 1 olaparib/cisplatin combination study in breast and ovarian cancer patients. A maximum tolerated dose (MTD) was reached with intermittent olaparib at 50 mg twice a day (*b.i.d.*) combined with cisplatin 60 mg/m^2 (NCT00782574) (Balmana et al. 2014). Results from a Phase 1/1b study in *BRCA* mutated breast and ovarian cancer identified 400 mg *b.i.d.* olaparib in combination with AUC5 carboplatin (once every 21-days)

as a tolerable dose (NCT01445418) (Lee et al. 2014). Another Phase 1 study evaluated the tolerability of a triple combination with PARPi given alongside both cisplatin and the cytotoxic chemotherapy gemcitabine (GEM) in patients with advanced solid tumours (NCT00678132) (Rajan et al. 2012). The identified MTD was 100 mg/m^2 olaparib every 12 hours and 60 mg/m^2 cisplatin on day followed by 500 mg/m^2 GEM on days 1 and 8. However, even while using suboptimal doses of both cisplatin and GEM, the addition of olaparib to the combination resulted in severe myelosuppresion, which may limit the therapeutic effectiveness of this combination moving forward (Rajan et al. 2012).

A Phase 2, randomized trial in recurrent platinum-sensitive ovarian cancer reported olaparib in combination with another chemotherapy paclitaxel (PXL) and carboplatin followed by monotherapy olaparib improved PFS from 12.2 months from 9.6 months in the PXL/carboplatin treated arm (NCT01081951) (Oza et al. 2015). The most common AE observed in this trial was neutropenia, reported in 43% of patients (Oza et al. 2015). Van der Noll *et al.* reported patients initially treated with combination olaparib plus carboplatin or PXL followed by maintenance mono-olaparib therapy exhibited a prolonged favorable response (NCT00516724) (van der Noll et al. 2015). Efforts to identify a patient subset and/or predictive biomarker would significantly advance this area; such studies are currently underway.

Taxanes

Taxanes, for example paclitaxel (PXL) and docetaxol (DOC), are a class of anticancer drugs that induce mitotic arrest by polymerizing tubulin and inhibiting the disassembly of microtubules (Horwitz et al. 1986). Since its development in the 1960s, PXL has been approved for use in the treatment of breast, ovarian, non-small cell lung carcinoma (NSCLC), and sarcomas (Barbuti and Chen 2015). PXL is also currently under evaluation for activity in several other cancer types (Barbuti and Chen 2015). For example, in advance-stage gastric cancer, response rates from PXL monotherapy range

from 15 to 21% and PXL therapy extends OS by an average of eight months (Sakamoto et al. 2009). As a result, PXL has become widely used as a second-line therapy in gastric cancers (Hironaka et al. 2013).

In preclinical studies, synthetic lethality between deficiency of the DNA repair sensor, ATM (ataxia telangiectasia mutated), and PARPi was identified and subsequently confirmed in ATM defective gastric tumour cell lines (Kubota et al. 2014; McCabe et al. 2006). As discussed above, PLX is widely used in gastric cancer and because roughly 60% of gastric cancers exhibit some form of ATM defect, a PARPi/PXL combination was proposed as a therapeutic approach in this patient population (Haince et al. 2007; Kang et al. 2008). In a randomized, double blind Phase 2 trial in recurrent or metastatic gastric cancer an olaparib/PXL combination elicited an average OS of 13.1 months compared to 8.3 months in a cohort of patients who received only PXL (NCT01063517) (Bang et al. 2015). Additionally, when patients were dichotomized by tumour expression of ATM (assessed by tumour biopsy immunohistochemistry), those with reduced ATM expression exhibited an OS benefit compared to those with higher ATM expression. As a result of this promising data, an olaparib/PXL combination is now been evaluated in a Phase 3 study (NCT01924533).

Extensive preclinical data have characterized two main mechanisms of taxol resistance: (i) modulation of tubulin which impairs the effectiveness of taxol binding and (ii) upregulation of drug efflux pumps (Cabral et al. 1983; Horwitz et al. 1986; Monzo et al. 1999; Roy and Horwitz 1985). Upregulation of PGP drug efflux pumps has been observed as an *in vitro* resistance mechanism to PARPi (Henneman et al. 2015). As a potentially shared mechanism of resistance in a PARPi/taxane combination, future studies should include serial evaluation of modulation of drug efflux pump levels and other potential mechanisms of resistance.

Gemcitabine

Gemcitabine (GEM), a deoxycytidine analog, prevents DNA synthesis when incorporated into DNA by preventing chain elongation during DNA replication (Gandhi et al. 1996; P. Huang et al. 1991). Cells exposed to GEM exhibit increases in DNA replication stress, stalled replication forks, and dependency on cell cycle checkpoints (Karnitz et al. 2005). GEM has been used both as single agent, and more recently in combination with radiation for the treatment of advanced pancreatic cancer (Loehrer et al. 2011).

Resistance to single agent GEM limits the overall response rate in pancreatic cancer to 20%, and therefore, drug combination strategies that might extend the therapeutic potential of GEM (El-Rayes and Philip 2003; Schniewind et al. 2004). Preclinical studies showed a combination of PARPi/GEM caused a more sustained retardation of cell proliferation, enhanced the retention of DNA damage and ultimately increased apoptosis in pancreatic and triple-negative breast cancer cell lines (Hastak et al. 2010; Jacob et al. 2007).

Evaluation of tolerability and therapeutic potential of a PARPi/GEM combination was evaluated in a Phase 1 dose escalation / dose expansion clinical trial. Patients with advanced pancreatic solid tumours were treated with either GEM alone or a combination of olaparib/GEM (NCT00515866) (Bendell et al. 2015). During the dose escalation phase, tolerability of continuous olaparib (100 mg *b.i.d.*) alongside 800 mg/m^2 GEM had 2/3 patients experiencing DLT with grade 3 or higher AEs. Consequently, intermittent dosing of olaparib (days 1-14) 100 mg *b.i.d.* was evaluated with a reduced dose of 600 mg/m^2 GEM. This combination, deemed the MTD, showed good tolerability. However, in the dose expansion phase of this trial, 26/47 (55%) of patients experienced considerable haematological toxicity, suggesting a PARPi/GEM may be limited clinically.

Topoisomerase inhibitors/poisons

Topoisomerases are responsible for catalysing the formation of DNA strand breaks, which are required to alleviate the torsional structure of DNA prior to

naturally occurring processes such as DNA replication (Champoux 2001). Topoisomerase enzymes are classified as type 1 topoisomerases (Top1), which cut the DNA phosphodiester backbone one strand at a time, generating single strand DNA breaks (SSBs) and type 2 topoisomerases (Top2), which excise both strands of the DNA backbone simultaneously, generating double strand DNA breaks (DSBs).

Topoisomerase 1 poisons

Derivatives of the Top1 poison camptothecin (CPT), topotecan, irinotecan and belotecan, are used clinically; each of which covalently fix (poison) Top1 onto DNA and generate unrepaired SSBs (Hsiang et al. 1985). Dose limiting toxicities still hinder the wide-scale application of Top1 poisons but these still remain therapeutically viable options for several malignancies, including ovarian, gastric, colorectal and esophageal cancers as well as glioblastomas and sarcomas (Pommier 2013).

PARP1 is critical for the repair of Top1 cleavage sites in DNA (L. M. Smith et al. 2005) and utilization of PARP inhibitors block the repair of topoisomerase poison-induced SSBs (Kummar et al. 2011). Specifically, PARP1 is required for the recruitment of TDP1 (tyrosyl-DNA phosphodiesterase 1), a key enzyme for Top1-induced DNA lesion repair, to the damage site (Das et al. 2014). This mechanistically supports observations of synergy between SN-38, the active metabolite of irinotecan, and olaparib (Tahara et al. 2014). Moreover, in contrast to TMZ, where PARP1 trapping appears to be critical for the synergy with PARPi, the synergy between Top1 poisons and PARP inhibitors is more reliant upon catalytic inhibition of PARP1 and less influenced by the trapping ability of PARPi. For example, the poor PARP-1 trapper but effective PARP1 catalytic inhibitor veliparib effectively synergizes with irinotecan and camptothecin but not TMZ (Murai et al. 2014a). Therefore, discrimination between PARPi on their ability to catalytically block PARP1 function is necessary for future evaluation of potential efficacy of a PARPi/Top1 inhibitor combination.

In initial Phase 1 clinical trials evaluating the tolerability of a PARPi/Top1 combination, the combination of PARPi and topotecan was stopped following the negative results showed patients experienced increased AE with subtheraputic doses of the cytotoxic chemotherapy (Sanz et al. 2009). Specifically, when combined with 10 mg veliparib, topotecan was only tolerated at a dose of 0.6 mg/m^2 due to hematological toxicities, significantly compromising the effectiveness of topotecan (Kummar et al. 2011). However, this trial showed clinical mechanistic interaction between a PARPi (veliparib) and a Top1 inhibitor retards repair of Top1-mediated DNA damage, suggesting additional clinical evaluation may be warranted if better tolerability can be achieved (Kummar et al. 2011).

Topoisomerase 2 inhibitors / poisons

Topoisomerase 2 (Top2) enzymes generate staggered double strand breaks to aid the removal of supercoils in the DNA structure, a prerequisite for processes such as transcription and replication, and utilize ATP to reseal the nick (Champoux 2001; Lodish H 2000). Small molecules that target Top2 can be classified as those that impair catalytic (ATP) turnover (Top2 inhibitors such as bisdioxopiperazines, novobiocin, and the anthracycline, aclarubicin) and Top2 poisons (which include the majority of clinically active agents including etoposide and doxorubicin) that fix Top2 on DNA increasing the frequency of Top2-DNA complexes (Nitiss 2009). Catalytic Top2 inhibitors are believed to cause cell death by eliminating essential Top2 enzymatic activity (Nitiss 2009). While Top2 poison-generated Top2-DNA complexes result in DSBs independent of DNA replication(Nitiss 2009).

Evaluation of a PARPi/etoposide combination in a Phase 1 dose escalation study demonstrated a triple combination of 100 mg dose of veliparib with cisplatin (75 mg/m^2 on day 1) plus etoposide (100 mg/m^2 on days 1-3) in a 21-day cycle is well tolerated in treatment-naive small cell lung cancer (SCLC) (NCT01642251) (Owonikoko et al. 2015). Out of the nine patients enrolled in the study, minimal AE were observed (Owonikoko et al. 2015).

Doxorubicin (DOX; also known as Adriamycin) is an anthracycline chemotherapy that intercalates into DNA (Gewirtz 1999; Hortobagyi 1997). In doing so, DOX impairs the activity of the Top2 enzyme (Gewirtz 1999). Pegylated liposomal DOX (PLD) is an approved treatment in patients with epithelial ovarian carcinoma (EOC) that presented with either *de novo* resistance or recurrent disease following first-line, platinum-based chemotherapy (Gordon et al. 2001). A retrospective analysis of over 14 randomized controlled trials showed overall PLD was better tolerated than paclitaxel in combination with carboplatin (the previous standard therapy) and showed some efficacy in platinum-sensitive relapsed EOC (Lawrie et al. 2013). Because the best responses to single agent PARPi was observed in platinum-sensitive disease, evaluating the potential of PLD combined with a PARPi was a logical next step (Fong et al. 2009).

To compare the efficacy of monotherapy of olaparib or PLD, 97 patients with advanced ovarian carcinoma who progressed within 12 months of platinum-based chemotherapeutic treatment were randomized into three study arms: (i) 200 mg *b.i.d.* of olaparib, (ii) 400 mg *b.i.d.* of olaparib, or (iii) intravenous infusions of PLD 50 mg/m^2 in a 28-day cycle (NCT00628251) (Kaye et al. 2012). As a monotherapy, olaparib failed to reach the primary objective of improving PFS, partly due to a better PFS observed in the PLD arm than expected. PFS were seen in 8/32 in the 200 mg, 10/31 in the 400 mg, and 6/33 patients in the PLD arms. No notable difference in OS was observed while twice as many grade ≥ 3 toxicities were seen in the PLD arm. Though reported as a negative study, this trial showed consistent response and decreased toxicity with the use of single agent olaparib in *BRCA* mutation ovarian cancer patients. Moreover, 32% of patients who crossed over to the olaparib 400 mg twice daily arm from the PLD arm continued on PARPi treatment suggesting there may be continued benefit of PARPi therapy for patients with *BRCA1/2* mutated disease after progression on PLD (Kaye et al. 2012).

To assess the efficacy of a PARPi/PLD combination, a Phase 1 dose escalation study was initiated in patients with advanced solid tumours

(NCT00819221) (Del Conte et al. 2014). This study reported 400 mg *b.i.d*. olaparib in combination with 40 mg/m^2 PLD was generally well tolerated with either continuous or intermittent scheduling (Del Conte et al. 2014). Of the 44 patients treated, 2 exhibited high-grade (≥ 3) dose-limiting toxicities that included thrombocytopenia and stomatitis. Additionally, results showed some antitumor activity in ovarian carcinoma patients with 33% of patients responding and 13/14 of the responders had ovarian cancer (Del Conte et al. 2014). In another Phase 1 study, Landrum *et al*. reported the MTD of veliparib in combination with both PLD and carboplatin to be 80 mg orally b.i.d. in women with recurrent, platinum-sensitive ovarian cancer (Landrum et al. 2015). Further evaluation of potential efficacy of a PARPi/PLD combination is warranted.

PARPi in combination with radiation

The therapeutic effect of high dose ionizing radiation (IR) arises from DNA damage to which some tumours are particularly sensitive (Mo et al. 2015). IR exposure results in the rapid activation and recruitment of PARP1 to damaged DNA (Satoh et al. 1993). Furthermore, PARP-1-null cell lines and mice exhibit exquisite sensitization to IR (de Murcia et al. 1997; Schreiber et al. 1995) and clinical PARPi induce radiosensitization in pre-clinical model systems (Chalmers et al. 2004; Jacobson et al. 1985), including *in vivo* tumour cell xenograft models. In this latter system, PARPi/IR combinations can elicit tumour inhibition with minimal effects on proliferating normal tissue, suggesting an actionable therapeutic window (Albert et al. 2007; Gani et al. 2015).

Clinical studies using PARPi/IR combinations have attempted to exploit these effects by enhancing the efficacy of IR without compounding normal tissue toxicity. For example, in a Phase 1, multicenter study, 22 patients with peritoneal carcinomatosis were treated with 60 cGy (on days 1 and 5 for 4 weeks) of fractionated whole abdominal radiation alongside incremental escalations of veliparib (NCT01264432) (Reiss et al. 2015). This treatment regimen was well-tolerated and showed some disease stabilization (Reiss et

al. 2015). Mehta *et al*. have also reported results from a Phase 1, dose escalation study that identified the MTD for veliparib in combination with whole brain radiation therapy (WBRT) (NCT00649207) (Mehta et al. 2015). Here, 81 patients with brain metastases secondary to primary breast or NSCLC were treated with WBRT (30.0 or 37.5 Gy in 10 or 15 fractions) with oral *b.i.d.* dosing of veliparib (10 – 300 mg). The combination was well also well tolerated, and though uncontrolled, preliminary results suggest the combination arm exhibited increased efficacy with median survival time (MST) increasing from the nomogram-model-predicted MST of 4.2 months to 8.85 months. In another Phase 1 trial, patients with rectal carcinoma were treated with veliparib in combination with capecitabine and radiation (NCT01589419) (Michael Michael 2015). Again, this combination showed good tolerability and complete responses were observed in 28% of patients. Further evaluation of PARPi/IR combination treatments is currently underway in multiple Phase 2 clinical trials in patients with NSCLC (NCT02412371, NCT01386385).

At present, it remains to be determined whether a concurrent PARPi/IR combination versus neoadjuvant PARPi therapy followed by IR will be more efficacious in a clinical setting. Additionally, efforts to identify a biomarker for response to a PARPi/radiotherapy combination have so far remained fruitless, but this would clearly facilitate the application of this combination.

PARPi in combination with targeted agents

Although PARP inhibitor combinations with chemotherapies and IR have received the most attention, a growing body of investigation has assessed the potential for combination therapy involving agents targeted against molecular targets in cancer.

EGFR inhibitors

EGFR, a member of the erbB family, is comprised of an extracellular ligand-binding domain, a transmembrane portion, and intracellular tyrosine kinase regulatory domains (Siegelin and Borczuk 2014). EGFR-activating mutations,

including amplification of the *EGFR* gene, as well as point mutations within the kinase-coding domain of *EGFR*, have been detected in several tumour types, including lung, colorectal, ovarian, breast, and glioblastoma (Siegelin and Borczuk 2014). Targeting EGFR has been achieved clinically using either small molecule tyrosine kinase inhibitors (EGFR TKIs such as erlotinib and genfitinib) or monoclonal antibodies (mAbs such as cetuximab) which target epitopes in the extracellular domain of EGFR (Cohen et al. 2004; J. R. Johnson et al. 2005; Pirker et al. 2009).

Erlotinib when combined with olaparib can elicit apoptosis in an EGFR-overexpressing, ovarian cancer cell line model with wild-type *BRCA* function (Sui et al. 2015). Furthermore some *EGFR* mutations have been proposed to elicit Fanconi anemia-like phenotypes, reducing cells to a HR deficient state similar to that generated by *BRCA* gene mutation i.e. BRCAness (Pfaffle et al. 2013), potentially explaining the rationale for a PARPi/EGFRi combinatorial effect.

IGF-1R inhibitors

Insulin-like growth factor type 1 receptor **(**IGF-1R), is activated by the ligands insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-2 (IGF-2), and has been implicated in the pathogenesis of several cancer types, including breast and colorectal cancers (H. X. Chen and Sharon 2013). Similar to EGFRi, IGF-1R inhibitors (IGF-1Ri) are either monoclonal antibodies against IGF-1R / IGF-1R ligands (IGF-1 and IGF-2) or TKIs, such as OSI-906 and BMS-754807 (H. X. Chen and Sharon 2013).

In preclinical models, *BRCA1*-mutated ovarian and breast tumour cells with impaired HR function have been linked to hyper activation of IGF-1R, resulting in an increased sensitivity to IGF-1R inhibition, suggesting BRCA-deficient cells may be hyper-vulnerable to dual inhibition of PARP and IGF-1R (Amin et al. 2015). Additionally, in HR proficient cells, IGF-1Ri exposure was found to reduce RAD51 mRNA transcript and protein levels and enhance sensitivity of these cells to olaparib (Amin et al. 2015). Taken together, this data suggests

cells may be reliant on IGF-1R for functional HR, and therefore, IGF-1R inhibition may potentiate PARPi sensitivity by generating BRCAness.

VEGFR inhibitors

Combinations of vascular endothelial growth factor receptor inhibitors (VEGFRi) with several chemotherapies have already been licensed for treatment in metastatic colorectal cancer, renal cancer, and advanced non-squamous non-small cell lung cancer (Escudier et al. 2010; Hurwitz 2004). These approvals include the use of bevacizumab, a VEGFRi which sequesters VEGF-dependent signalling by competitively inhibiting the binding of its ligand, and cediranib, an oral ATP-competitive VEGFR1/2/3 tyrosine kinase inhibitor (Tewari 2015). Central to the proposed mechanism of action of these VEGFRi is their role in inhibiting tumour angiogenesis and current clinical studies are also evaluating bevacizumab plus cediranib combinations, as these inhibitors have non-overlapping mechanisms of actions targeting the extracellular and intracellular domains of VEGFR, respectively (Hong et al. 2014). Extensive preclinical data suggests angiogenesis and VEGFR orchestrates a particularly pivotal role in the development of ovarian cancers, specifically vascular endothelial growth factor A (VEGF-A) overexpression and its role in vascular permeability and the development of ascites in epithelial ovarian cancers (EOCs) (Byrne et al. 2003). Accordingly, significant efforts have been made to evaluate efficacy of VEGFRi within EOC. Specifically, patients with recurrent, platinum-resistant EOC exhibited a response rate of 18% and 19% for bevacizumab and cediranib, respectively (Cannistra et al. 2007; Matulonis et al. 2009).

Extensive preclinical data suggests induction of a hypoxic state, as is sometimes caused by restricting angiogenesis, can generate BRCAness, specifically by decreasing BRCA1 and RAD51 expression (Bindra et al. 2005). For example, anti-angiogenesis therapies, including bevacizumab and cediranib induce a higher incidence of hypoxia, a reduction in HR, and subsequent sensitivity to PARP inhibition (Chan and Bristow 2010; Hegan et al. 2010). Additionally, inhibition of VEGFR-3 results in down-regulation of

BRCA1 and BRCA2 expression and can restore chemosensitivity in ovarian cancer models (Lim et al. 2014).

Several trials have assessed whether these observations are clinically actionable using PARPi/VEGFRi combinations. The safety and toxicity of an olaparib/bevacizumab combination was assessed in a Phase 1 study and showed no dose-limiting toxicities (Dean et al. 2012). Results from a randomized, Phase 2 study in recurrent platinum-sensitive, ovarian cancer showed that olaparib in combination with cediranib improved PFS from 9.0 months (monotherapy of olaparib) to 17.7 months (olaparib plus cediranib NCT01116648) (Liu et al. 2014). Eight patients (6 with *BRCA* gene mutant disease) had complete responses in the olaparib/cediranib combination arm compared to two in the olaparib monotherapy arm (Liu et al. 2014). Consistent with the preclinical data, this study supports the hypothesis that the combination of PARPi with anti-angiogenic agents may function synergistically and may serve as a viable treatment option for patients with platinum-sensitive, ovarian disease.

HDAC inhibitors

Histone deacetylases are critical for functional HR and modulate DSB repair pathways (Ha et al. 2014; Kachhap et al. 2010). Preclinical data has shown in PTEN-functional, triple negative breast cancer (TNBC) the combination of olaparib and suberoylanilie hydroxamic acid (SAHA), an HDAC inhibitor, synergistically increased tumour cell death via activation of apoptosis and increased accumulation of DNA damage (Min et al. 2015). Additionally, HDAC inhibitors combined with olaparib in pancreatic cancer cell lines exhibited downregulation of HR-related protein expression, specifically RAD51 and BRCA1, and accumulation of DNA damage, as measured by γH2AX foci (Chao and Goodman 2014). This data suggests treating cells with HDAC inhibitors may prime cells for PARPi sensitivity, allowing an accumulation of DNA damage to increase the amount of unrepaired DNA lesions past an apoptotic initiation threshold. It has yet to be determined whether this

combination will have a therapeutic window in a clinical setting or what a predictive biomarker of combinatorial sensitivity might be.

PI3K/mTOR inhibitors

Phosphoinositide 3-kinase (PI3K) signalling is fundamental to a variety of cellular processes, including double strand break repair (DSBR) (Kao et al. 2007; Kumar et al. 2010). *PIK3CA* (which encodes the PI3K catalytic isoform p110α) and *PTEN* (phosphatase and tensin homolog) are two of the most frequently mutated oncogene and tumour suppressor genes, respectively, in human cancer. Over a dozen PI3K inhibitors (PI3Ki) are currently in clinical development, and in 2014, Gilead's first-in-class PI3Ki, idelalisib, received FDA approval for the treatment of chronic lymphocytic leukaemia (CLL) and non-Hodgkin's lymphoma (Fruman and Rommel 2014). However, despite this approval, trial results have been disappointing; substantial and sustained responses to single agent PI3Ki therapy remain limited for this class of inhibitors (Fruman and Rommel 2014).

Recent data has implicated PI3K-signaling with the detection of DNA breaks, as well as the maintenance of BRCA1 and BRCA2 expression (Fruman and Rommel 2014). Therefore, abrogation of PI3K signalling has been proposed as an approach to debilitate HR and therefore potentiate PARPi therapy, potentially independent of *BRCA* gene mutation status. Juvekar *et al.* demonstrated that PARPi and PI3K inhibitors (PI3Ki) synergize in both *BRCA*-deficient and *BRCA*-proficient preclinical models (Juvekar et al. 2012). Specifically, the PARPi/PI3Ki combination enhanced tumour cell death by reducing the localization of another key DNA repair protein, 53BP1, and by decreasing proliferation in *BRCA*-deficient models (Juvekar et al. 2012; Yi et al. 2015). Additionally, in *BRCA*-proficient TNBC cells, blocking PI3K signalling results in retardation of HR, sensitization to PARPi, and increased radiation-induced cell death (Ibrahim et al. 2012; Jang et al. 2015). The inhibition of the related mTOR signalling cascade, has also been assessed in combination with PARPi. Mo *et al.* demonstrated that in *BRCA*-proficient TNBC tumour cell models, a combination of everlimous with talazoparib

synergistically interacted to increase overall efficacy of either agent alone (Mo et al. 2015). Mo *et al.* also found that mTOR modulates SUV39H1, a H3K9 histone methyltransferase involved in DSB repair. Subsequently, mTOR inhibition results in a decrease of SUV39H1 and a decrease in DSBR (Ayrapetov et al. 2014).

On the basis of these pre-clinical studies, Phase 1 clinical trials assessing the tolerability of PARPi/PI3Ki combinations using olaparib with buparlisib (BKM120), BYL719, AZD5363, or AZD2014 are currently underway (NCT01623349, NCT02338622, NCT02576444). At present, preliminary findings suggest in women with HGSOV or TNBC, olaparib/buparlisib (BKM120) combination is well tolerated, with some evidence of anti-tumour effects in patients with germline mutations in BRCA genes (NCT01623349) (Matulonis et al. 2015).

HSP90 inhibitors

HSP90 is an ATP-dependent chaperone protein responsible for the modulation, stabilization, and activation of more than 200 proteins (Neckers 2007; Whitesell and Lindquist 2005). Preclinical data for PARPi/HSP90 inhibitor (HSP90i) combinations demonstrated that 17-AAG, an HSP90i, combined with olaparib can increase olaparib sensitivity of HR-proficient epithelial ovarian cancer cell lines (Choi et al. 2014). Specifically, 17-AAG decreases HR in the presence of DNA damage, placing the cells in a HR-deficient-like state (Choi et al. 2014). In addition, Johnson *et al.* found that BRCA1 function is dependent upon HSP90, with the proper folding of BRCA1 and its subsequent activity being HSP90 reliant (N. Johnson et al. 2013). Moreover, when treated with the HSP90i 7-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), PARPi-resistant cells exhibited a decrease in the quantity of mutant BRCA1 protein, suggesting HSP90 is involved in folding and stabilizing mutant BRCA1, as well as functional BRCA1 protein. No changes in BRCA1 protein levels were observed in *BRCA*-WT cells suggesting HSP90 is only instrumental in the folding of mutant BRCA1 protein. Taken together, this data suggests a PARPi/HSP90i

combination could be an effective treatment strategy to potentiate PARPi therapy in an HR-proficient setting, including in *BRCA* mutant cancers that gain secondary reversion mutations that might somewhat alter the normal folding of the protein. This combination is currently being evaluated for tolerability in a Phase 1 trial (NCT02627430).

CHK1/2 inhibitors

Cells harboring mutations in the tumor suppressor protein TP53, which lack the G_1 checkpoint, leave them heavily reliant on checkpoint kinases, such as CHK1 and CHK2, that tightly regulate G_2/M progression. The majority of *BRCA*-mutant cancers harbor co-occurring mutations in TP53. It has been suggested that combining CHK inhibitors (CHKi) with chemotherapeutic agents result in a lethal accumulation of DNA damage alongside unregulated cell cycle progression (Morgan et al. 2010). Booth *et al*. showed combining any one of four different PARPi (olaparib, veliparib, rucaparib, and NU-1025) with the CHKi, AZD7762, LY2603618, or UCN-01, increased the overall DNA damage burden and caused cell death in *BRCA*-proficient breast carcinoma cell lines (Booth et al. 2013). Booth and colleagues also found that tumour beta cells (which secrete insulin) pre-treated with a PARPi/CHKi combination were hypersensitive to radiotherapy resulting in increased γH2AX levels (Booth et al. 2013). Currently, this combination has not as yet been assessed in clinical trials.

Trastuzumab

Overexpression or amplification of the ERBB2 tyrosine kinase receptor, HER2, is found in around 20% of breast cancers (Vu and Claret 2012). Trastuzumab, a monoclonal antibody targeting the extracellular domain IV of HER2, has been shown to promote HER2 degradation, antibody-dependent cytotoxicity, and suppression of MAPK/PI3K signalling in HER2-positive breast tumour cells (Vu and Claret 2012). Recent preclinical data evaluating the efficacy of PARPi in combination with trastuzumab within HER2-positive

disease showed breast cancer cell lines treated with a combination of either olaparib or rucaparib with trastuzumab notable decreases in cell proliferation and increases in DNA damage accumulation (Garcia-Parra et al. 2014). Further preclinical evaluation is needed to elucidate the potential of a PARPi/trastuzumab combination.

Anti-endocrine agents

Endocrine therapy that impairs signalling cascades mediated by estrogenic or androgenic hormones are central to the treatment of hormone-receptor positive cancers. In estrogen receptor positive breast cancer, inhibitors of estrogen receptor function, such as tamoxifen, and/or aromatase inhibitors that block estrogen synthesis are ubiquitously used. In prostate cancer, androgen receptor modulators, such as enzalutamide, and cytochrome 17 (CYP17) inhibitors that block androgen synthesis, such as abiraterone, are approved therapies.

Most patients with metastatic prostate cancer patients progress to advanced, castration-resistant disease (mCRPC) within two years following androgen deprivation therapy (Lorente and De Bono 2014). Both abiraterone and enzalutamide have small but significant benefit in terms of OS for patients with mCRPC (Fizazi et al. 2012) (Lorente and De Bono 2014).

Recent genomic studies uncovered more than 19% of mCRPC exhibit at least one mutation in DNA-repair genes, including both *BRCA1* and *BRCA2* (Robinson et al. 2015). Mateo *et al.* reported in TO-PARP, a multipart Phase 2 trial, single agent olaparib showed anti-tumour activity in unselected mCRPC (NCT01682772) (Mateo et al. 2015). Of the 49 patients treated, 16/49 had confirmed mutations in at least one DNA-damage repair gene, with 14/16 (88%) responding to olaparib (Mateo et al. 2015). Additionally, preclinical data has also shown inhibition of AR results in the downregulation of DNA damage repair proteins and subsequent DNA damage repair (Karanika et al. 2015).

Together, these observations have provided a foundation for clinical evaluation of PARPi/anti-androgen combinations in attempt to concurrently inhibit AR, generate PARPi-induced genomic instability, and trigger PARPi synthetic lethality. Evaluation of a PARPi/anti-androgen combination is currently clinical trial evaluation (NCT01576172).

PARPi combined with immunotherapeutics

Therapies that harness the host immune system have been revolutionary in the treatment of a subset of cancers (D. S. Chen and Mellman 2013). Currently most success has been achieved with immune checkpoint inhibitors such as anti-CTLA4 and anti-PD1/PDL-1. Response to these agents appears to correlate with the mutagenic burden of the tumour; presumably these mutations produce a larger number of neo-antigens, which can be recognised by the immune system (Rizvi et al. 2015). It is possible, therefore, that tumours with deficient BRCA1, BRCA2 and other HR proteins may be particularly sensitive to such approaches given the potentially high mutagenic burden in tumours with BRCAness. Moreover, the PARPi, BMN 673, has been shown to have immuno-modulatory effects Brca1-deficient ovarian tumour cell line grown *in vivo* in the peritoneum (J. Huang et al. 2015). In particular the percentage of cytotoxic CD8 T cells, B cells, and NK cells was increased in the peritoneal cavity of tumour-bearing mice. Taken together these observations have suggested the therapeutic efficacy of immune checkpoint blockade in combination with PARPi may have potential.

To test this, Higuchi *et al.* investigated the efficacy of various immune checkpoint blockade agents in combination with PARPi (Higuchi et al. 2015). The model utilised was an *in vivo* tumour model of mouse Brca1-deficient ovarian cancer in an immune-competent host. They showed that a CTLA-4 antibody, but not PD-1/PDL-1 blockade, synergized therapeutically with veliparib. This resulted in long-term survival in the majority of animals. The mechanism remains to be fully described but survival benefit was dependent on T cells and associated with elevated IFN-gamma production in the tumour microenvironment.

A number of trials investing PARPi/immunotherapy combinations are now being planned or are underway (for example, NCT02571725).

PARP inhibitor combinations – challenges and opportunities

It is clear from the data described above that there is already considerable evidence that combination therapies that involving PARPi could be of considerable utility in a wide variety of cancers. Along with others, we believe the re-categorization of PARPi based on their dual molecular mechanism of action (PARP inhibitor via catalytic inhibition or PARP poison via PARP trapping) is imperative to correctly identifying appropriate combinatorial approaches (Fojo and Bates 2013; Murai et al. 2012; Murai et al. 2014a). However, before the full potential of such combinations might be realised, we believe that there are a number of key issues require resolution. These are summarised below:

(i) Foremost amongst the challenges facing the effective use of PARPi combinations is the issue of predictive biomarkers. Significant advances have been made in the discovery and clinical validation of predictive biomarkers of single agent PARPi responses, but far less is understood about what predicts a favourable response to PARPi combinations. In the absence of such biomarkers, the clinical development of combinatorial approaches might be problematic. In principle, identifying and validating such combinatorial biomarkers could be achieved in much the same way as the identification of biomarkers of single agent activity. The efforts to assess *MGMT* promoter methylation as a predictive biomarker of PARPi/TMZ combination responses (Gupta et al. 2015) provide one such example, but we believe much more activity in this area is required.

(ii) How might the impact of resistance to single agent PARPi in *BRCA* gene mutant patients be minimised by the use of combinatorial therapy? At present it is not at all clear whether combinations of PARPi with secondary agents might delay or prevent the

emergence of PARPi resistance in these patients, providing a more durable response. Equally the sequencing and scheduling of agents requires attention.

(iii) The delivery of PARPi combinations has been limited by the observation of dose limiting toxicities. In preclinical studies, some PARPi combinatorial synergies, notably PARPi/TMZ, have been achieved by using high-dose PARPi combined with relatively low doses of chemotherapy. A key question is: should more consideration be given to using similar “high PARPi/low chemo” approaches to achieve anti-tumour efficacy with well-tolerated regimens? This is starting to be exemplified in on-going studies, including NCT02049593.

(iv) The majority of PARPi combinations identified to date involve DNA damaging chemotherapies, some of which might have overlapping mechanisms of actions (MoA) to PARPi and might therefore be limited by shared mechanisms of resistance. Whilst this area of research has been fruitful (i.e. PARPi/platinum combinations), perhaps more focus be given to systematically assessing PARPi combinations that involve drugs with clearly different MoA and which target distinct characteristics of tumour cells. These combinations might not elicit synergistic effects *per se* (i.e. supra-additive drug combination effects on tumour cells) but might deliver more durable responses as they will likely be less limited by the emergence of common drug resistance mechanisms.

(v) How might an understanding of the biology of PARPi responses be better used to design PARPi combination therapies? Already, there is an understanding of how PARPi cause tumour cell death by inducing an excessive level of genomic instability. Can this phenotype be exploited by combining PARPi with agents that enhance cell death programmes that are instigated by elevated levels of genomic instability such as the apoptotic or necroptotic machinery (Yuan et al. 2013)?

(vi) How might PARPi be combined with immunotherapeutics both in the context of wild-type *BRCA1/2* or with BRCAness where there may be a higher mutagenic load?

Now that the first PARPi has been licenced as a single agent, it seems appropriate to consider once again, how these agents might be used in combination. As we have described in this review, the work that has been carried out in the last few decades has shown that there is strong potential for PARPi combinatorial approaches as cancer treatments, if the considerable challenges can be overcome.

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Conflict of interest statement

CJL and AA are named inventors on patents describing the use of PARP inhibitors and stand to gain from their development as part of the ICRs “Rewards to Investors” Scheme.

Figure 1. Proposed mechanism for PARPi combinations therapy

Several agents are currently undergoing both preclinical and clinical evaluation in combination with PARPi. Combinatorial PARPi therapeutic rationale can be classified into three broad mechanisms: 1) increased accumulation of DNA damage and subsequent dependence on PARP-mediated DNA damage repair, 2) increased levels of trapped PARP-DNA complexes, and 3) induction of BRCAness phenotype to elicit PARPi/BRCAness synthetic lethality.

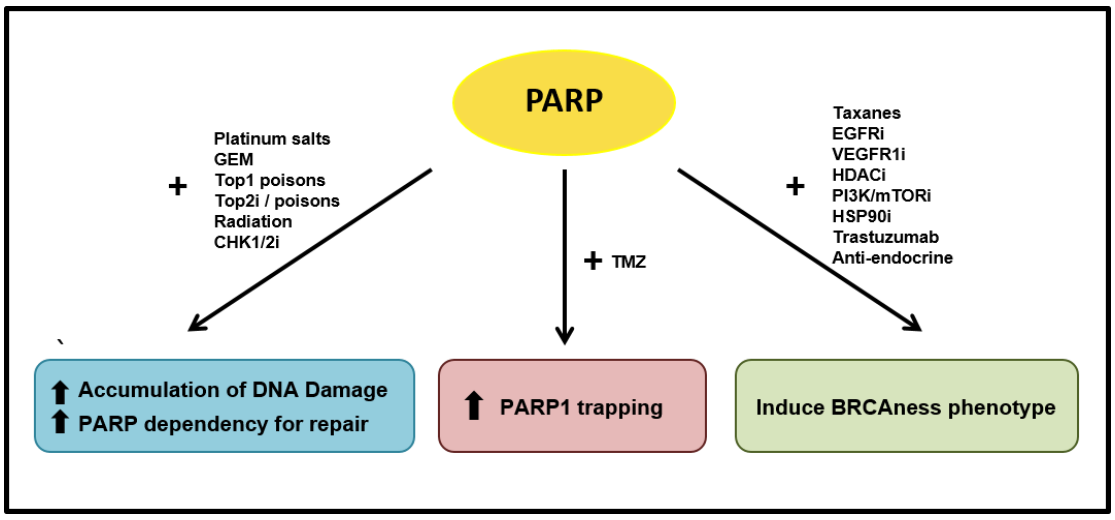
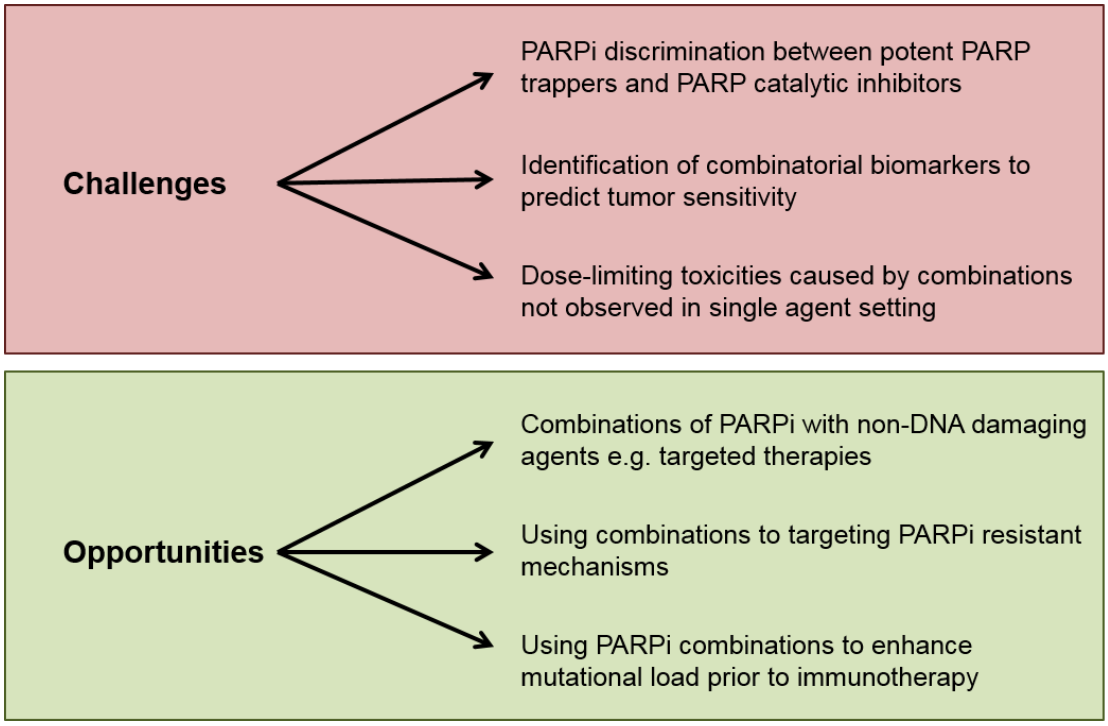


Figure 2. Challenges and opportunities for PARPi combinations

Though PARPi therapy holds enormous potential as a targeted therapy, challenges for optimising its use within a combination context remain. Principally, these challenges are reclassification of PARPi agents to distinguish between PARPi trappers and PARP catalytic inhibitors, identification of predictive biomarkers for each combinatorial approach, and avoidance of creating conditions for new DLTs. That being said, if these challenges are met, enormous opportunities remain for PARPi combinations to generate a profound and sustained anti-tumour response within the clinical setting.



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