Breast Cancer RESEARCH

REVIEW

Emergence of rationally designed therapeutic strategies for breast cancer targeting DNA repair mechanisms

Bryan P Rowe and Peter M Glazer*

Abstract

Accumulating evidence suggests that many cancers, including BRCA1- and BRCA2-associated breast cancers, are deficient in DNA repair processes. Both hereditary and sporadic breast cancers have been found to have significant downregulation of repair factors. This has provided opportunities to exploit DNA repair deficiencies, whether acquired or inherited. Here, we review efforts to exploit DNA repair deficiencies in tumors, with a focus on breast cancer. A variety of agents, including PARP (poly[ADP-ribose] polymerase) inhibitors, are currently under investigation in clinical trials and available results will be reviewed.

Introduction: DNA repair and cancer

Mammalian cells exist under constant genotoxic stress from both endogenous and exogenous sources. Replication errors, chemical decay of bases, and reactive oxygen species generated during metabolism all contribute to DNA damage from within the cell while UV light, ionizing radiation (IR), and chemical exposures assault the cell's DNA from outside [1]. To mitigate damage to DNA, a number of mechanisms have evolved to repair a variety of lesions.

Several processes repair single-stranded DNA damage by using the undamaged strand as a template. Base excision repair (BER) uses DNA glycosylases to recognize and remove non-bulky damaged bases [2]. BER has been reviewed in detail previously [3]. Nucleotide excision repair (NER) removes bulky distortion in the DNA helix and is crucial for the processing of UV-induced damage and chemical adducts [4]. The mismatch repair system (MMR) removes base-base mismatches and small insertion or deletion mismatches that can occur during replication [5].

Double-strand breaks (DSBs) are repaired by either non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ is more prone to deletions and other alterations since the fragmented ends are processed and re-ligated with no available template to ensure accuracy. HR is essentially an error-proof mechanism that occurs during the S or G_2 phases of the cell cycle, when the sister chromatid can provide a template for accurate repair [1]. HR is also involved in repairing lesions that disrupt the replication fork. A more complete review of DSB repair is available elsewhere [6].

Translesion synthesis (TLS) is a DNA tolerance process that allows DNA replication to bypass certain lesions (for example, thymine dimers and abasic sites) by substituting specialized translesion polymerases that function in the presence of damaged nucleotides. TLS is involved in the removal of interstrand crosslinks (ICLs) [7].

All of the above processes are crucial for a cell's ability to maintain genomic fidelity. Disruptions in these pathways cause a predisposition to accumulate DNA damage and, subsequently, mutations. Mutations in tumor-suppressor genes, oncogenes, and other genes involved in cell survival and growth can lead to the development of cancer. Furthermore, there is a growing body of evidence that tumors accumulate mutations in DNA repair proteins as they progress, becoming increasingly malignant [8]. In addition to playing a central role in the development of cancer, DNA repair mechanisms greatly affect the response to cytotoxic treatments, including radiation and chemotherapy, which target cellular DNA.

Not surprisingly, there is intense interest in DNA repair pathways in the field of oncology. As the molecular and genetic details of DNA repair pathways and their regulation have become increasingly characterized, new opportunities for therapeutic intervention have emerged. For a variety of reasons, the treatment of breast cancer plays a central role in these new areas of development.

*Correspondence: peter.glazer@yale.edu
Department of Therapeutic Radiology, Yale University School of Medicine, P.O. Box 208040, New Haven, CT 06520-8040, USA

BRCA1, BRCA2, and homologous recombination

In the early 1990s, *BRCA1* and *BRCA2* were identified as the tumor-suppressor genes responsible for a significant proportion of hereditary breast cancers. For women who are carriers, the estimated risks of developing breast cancer and ovarian cancer by age 70 are 40% to 66% and 13% to 46%, respectively [9]. Carriers also have an elevated risk of prostate, pancreatic, and other cancers. BRCA2 serves as a co-factor for Rad51, facilitating nuclear filament formation and stimulating Rad51-mediated recombination reactions required for HR [10-12]. The molecular functions of BRCA1 are somewhat less well characterized but it appears that BRCA1 is required for efficient HR, acts in the DNA damage-signaling cascade, is involved in chromatin remodeling, and is involved in the activation of the Fanconi anemia (FA) pathway [13-17].

The discovery that BRCA1 and BRCA2 are involved in HR explains, at least partly, the genomic instability and predisposition to cancer that are seen in *BRCA* carriers. Approximately 5% to 10% of breast cancers result from loss of heterozygosity at the *BRCA* locus in *BRCA* mutation carriers. As a result, the tumor cells are most deficient in HR and are therefore potentially vulnerable to therapeutic strategies that target this weakness.

Characteristics of homologous recombination-deficient cells

It is well established that cells deficient in HR are particularly sensitive to DNA crosslinking agents, including the platinum-based drugs cisplatin and carboplatin as well as mitomycin C, a natural anti-tumor antibiotic. Cells deficient in BRCA1, BRCA2, XRCC2, and XRCC3 – all important components in HR – display this increased sensitivity to ICLs [18-20].

ICLs prevent DNA unwinding by covalently linking the two DNA strands to each other, thereby disrupting replication and transcription. These lesions are extremely toxic to cells and not easily repaired. It appears that the combined action of several DNA repair pathways – NER, TLS, and HR – in conjunction with the FA pathway is required to repair an ICL and that removal of the lesion occurs almost exclusively during DNA replication [21].

This cellular sensitivity of HR-deficient cells to crosslinking agents suggests that these drugs may be particularly effective in BRCA-associated tumors. Several studies have shown that patients with BRCA-associated ovarian cancer have a better prognosis than their sporadic counterparts. In a case series of 71 patients with advanced ovarian cancer, including 34 patients with *BRCA* mutations, Cass and colleagues [22] found that the patients with *BRCA* mutations had a significantly better response to platinum-based chemotherapy. The authors hypothesized that this increased sensitivity to cisplatin was the primary reason for the observed improvement in overall survival (OS) [22]. An ongoing phase II trial of BRCA-associated breast cancer patients ('the BRCA trial') aims to discover whether carboplatin is a safer and more effective chemotherapy than docetaxel [19].

BRCA-deficient cells have also shown hypersensitivity to etoposide, a topoisomerase II inhibitor. Etoposide binds to topoisomerase II and forms a stable drug-enzyme-DNA complex, thereby inhibiting the final re-ligation step required for replication and eventually resulting in a DSB. Treszezamsky and colleagues [23] showed that both BRCA1- and BRCA2-deficient human breast cancer cell lines showed an increased sensitivity to etoposide compared with their BRCA-complemented counterparts.

Fanconi anemia pathway

FA is a rare x-linked and recessive genetic disorder characterized by chromosomal instability, which leads to a wide variety of clinical findings, including bone marrow failure, skeletal anomalies and other birth defects, and early onset of leukemias and solid tumors. One cellular hallmark of FA is hypersensitivity to crosslinking agents, including mitomycin C and diepoxybutane [7]. In fact, quantification of chromosomal abnormalities induced by these agents is used for clinical diagnosis of FA.

Thirteen FA genes (designated *FANCA* [Fanconi anemia complementation group A] through *FANCN*), each with a protein product that plays a role in DNA repair, have been identified. Most of the FA proteins are involved in the formation of a core complex with ubiquitin ligase activity which monoubiquitinates FANCD2 and FANCI in response to DNA lesions during replication. The FA family members appear to be key regulators of DNA repair, thereby helping to maintain genetic stability. One primary function of the FA pathway appears to be in coordinating several repair pathways – NER, TLS, and HR – to remove ICLs, thus explaining the sensitivity of Fanconi cells to crosslinking agents. FA proteins also interact with several important proteins, including ataxia-telangiectasia mutated (ATM), ATM and Rad3 related (ATR), and meiotic recombination 11 (MRE11), which are responsible for genetic instability syndromes [24-26]. Furthermore, FA proteins are involved in suppression of sister chromatid exchanges, regulation of cell cycle checkpoints, and cytokinesis [7].

Though discovered independently, BRCA2 and FANCD1 have been shown to be the same protein. This discovery clarified some of the previously noted similarities and interactions between the BRCA proteins and the FA family of proteins, including the shared hypersensitivity to mitomycin C and the finding that targeted inactivation of the BRCA2 protein in mice produced an FA-like phenotype [27]. Although mechanistic details have yet to be worked out, there is accumulating evidence that the BRCA and FA DNA repair pathways are intimately related.

Because of the role of the FA pathway in repairing ICLs, the status of the FA pathway is an important determinant of sensitivity to cisplatin and other cross-linking agents. In fact, reactivation of the FA pathway appears to be a mechanism by which tumors acquire resistance to cisplatin [28]. Conversely, it has been shown that disruption of the FA pathway leads to increased cisplatin sensitivity in tumor cell lines. This has been accomplished by using a gene therapy approach [29] or by inhibiting the monoubiquitination of FANCD2 by a small-molecule inhibitor such as curcumin [30].

Mismatch repair system-deficient tumors

Genetic defects in the MMR pathway are well known to cause microsatellite instability and predispose patients to hereditary non-polyposis colorectal cancers (HNPCCs) and other HNPCC spectrum tumors, including endometrial, gastric, and ovarian cancer. There are some early data suggesting that epigenetic silencing of the MMR genes may contribute to the development of sporadic breast cancers. A substantial proportion of sporadic breast cancers (24% to 46%) contain hypermethylated promoters at *hMLH1* and *hMSH2* and this may be associated with more advanced breast cancers and reduced OS [31-34].

In contrast to other DNA repair systems (for example, HR and BER), a functional MMR pathway actually enhances the cytotoxicity of a variety of chemotherapeutic agents. Following administration of chemotherapeutic agents such as temozolomide (TMZ) or 6-thioguanine (6-TG), MMR-proficient cells repeatedly and unsuccessfully attempt to process chemically induced mispairs. This futile cycling of the MMR pathway is believed to signal a G_2 checkpoint arrest and apoptosis. Damage induced by IR is also recognized by MMR, resulting in MMR-mediated cytotoxicity, which is most pronounced at low dose rates [35,36]. Thus, MMR-deficient cells can be resistant to both chemotherapy and radiotherapy.

Currently, the use of iododeoxyuridine (IUdR) and other radiosensitizing agents that preferentially accumulate in MMR-deficient cells is being investigated as a way to selectively target these therapy-resistant cells. In an attempt to maximize the therapeutic ratio, computational models based on extensive experimental data are being used to predict the optimal dose of IUdR and timing of IR [5]. In addition, knowledge of resistance mechanisms to specific chemotherapeutic agents should help guide drug selection.

PARP inhibition, base excision repair, and synthetic lethality

Poly(ADP-ribose) polymerase 1 (PARP1) is the most well-characterized member of the PARP superfamily. An abundant nuclear protein, PARP1 is involved in a wide variety of cellular processes ranging from inflammation to apoptosis and, importantly, BER. PARP1 contains zinc-finger motifs that allow it to detect and bind to sites of single-stranded DNA damage. Using NAD^+ as a substrate, PARP1 catalyzes the addition of ADP-ribose polymer sidechains to itself, DNA ligase III, DNA polymerase-β, XRCC1, and other repair components, thereby recruiting and regulating the effectors of BER [37,38]. The presence of PARP1 has been shown to be required for efficient functioning of BER [39,40]. A variety of molecules, most of which mimic the nicotinamide moiety of NAD^+, have been developed to inhibit the action of PARP1, thereby inhibiting efficient BER [41]. These agents have shown promising potential both as monotherapy for patients with HR-deficient tumors and in potentiating effects of traditional cytotoxic agents, including chemotherapy and radiotherapy.

In 2005, two groups published the finding that BRCA-deficient cells are sensitive to agents that inhibit PARP1 [42,43]. This discovery generated intense interest, in part because of the potentially large therapeutic window that exists in a situation in which synthetic lethality is present. Synthetic lethality occurs when two lesions that are individually non-lethal become lethal when combined (Figure 1). In this particular situation, the HR-deficient BRCA mutant cells become highly dependent on other DNA repair pathways, including BER, that help prevent development of DSBs in order to compensate for their inability to repair DSB in an error-free manner. When PARP1 and therefore BER are inhibited, the unrepaired single-strand breaks (SSBs) eventually cause the collapse of the replication fork and become DSBs, overwhelming the cell's repair machinery and leading to cell death. The non-tumor cells are better able to tolerate the PARP inhibition because their HR machinery is intact.

Synthetic lethality represents a new strategy for the development of anti-cancer drugs. Traditional chemotherapeutic agents are relatively non-selective, often targeting rapidly dividing cells, which include both tumor and some normal cells. Using a synthetic lethality approach, screening programs can be designed to identify target genes that, when mutated or inhibited, lead to the death of cancer cells that already carry additional alterations in different genes [44,45]. Normal cells should be spared since it is the combination of a drug-induced alteration with a cancer-related alteration that is lethal.

DNA repair defects, epigenetic inactivation, and the concept of 'BRCAness'

Although germline mutations in *BRCA1* or *BRCA2* account for 5% to 10% of breast cancers, these loci are rarely mutated in sporadic tumors. Nonetheless, there are gene expression profiles as well as clinical and pathological phenotypes of some sporadic tumors that closely resemble those of BRCA-associated tumors. Using gene expression microarray analysis, sporadic tumors can be

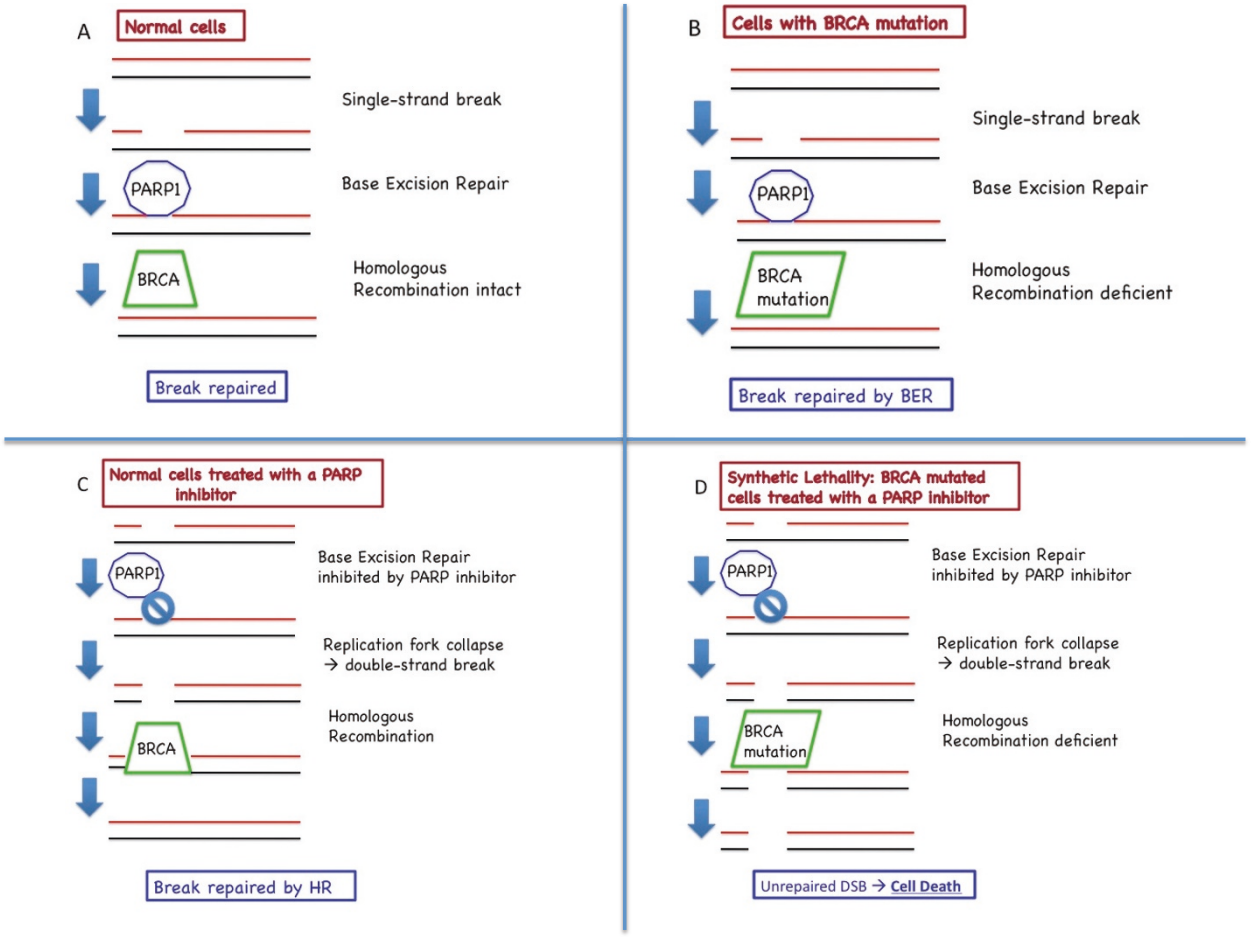

Figure 1. Example of synthetic lethality: PARP inhibition in cells deficient in homologous recombination (HR). (a) In normal cells, repair pathways, including base excision repair (BER) and HR, are intact. Single-strand breaks (SSBs) are readily repaired by BER, with the participation of PARP1, and double-strand breaks (DSBs) are reliably repaired by HR, with the participation of BRCA1 and BRCA2. **(b)** Cells with mutations in *BRCA1* or *BRCA2* are deficient in HR. Other repair pathways, including the BER pathway, are able to minimize the number of lesions that become DSBs. **(c)** Normal cells treated with PARP inhibitors may become deficient in BER. Therefore, more SSBs go unrepaired. During replication, the replication fork may stall, resulting in the conversion of SSBs into DSBs. HR can repair these DSBs in an error-free manner. **(d)** When PARP inhibitors are delivered to cells deficient in HR (for example, *BRCA*-mutated cells), synthetic lethality can result. Inhibition of BER by PARP inhibitors results in the conversion of unrepaired SSBs into DSBs. These DSBs cannot be repaired by HR in an error-free manner and can therefore result in cell death. PARP, poly(ADP-ribose) polymerase.

divided into five main groups. One, known as basal-like tumors, expresses high levels of myoepithelial cytokeratins found in the outer basal layer of cells in a normal breast duct. These tumors share a similar gene expression profile with BRCA1-associated tumors, suggesting a common etiology. Furthermore, both groups tend to be estrogen receptor-negative and human epidermal growth factor receptor 2/neu (HER2)-negative, have a higher mitotic count, show lymphocytic infiltration, and appear to have a 'pushing margin' pattern of invasion at the tumor edge [46].

BRCA1 promoter methylation

While *BRCA1* and *BRCA2* are infrequently mutated in sporadic tumors, there is increasing evidence for epigenetic mechanisms that result in silencing of DNA repair genes. The most well-characterized epigenetic mechanism is that of *BRCA1* promoter hypermethylation leading to undetectable *BRCA1* expression. Gene promoters frequently contain CpG dinucleotide islands, which, under normal conditions, are unmethylated. Methylation of these cytosine residues leads to silencing of transcription. Abnormal methylation of the *BRCA1* promoter is found in 11% to 14% of sporadic breast tumors [46].

FANCF promoter methylation

Another potentially important mechanism of epigenetic inactivation of repair pathways is methylation of the *FANCF* promoter. FANCF is a member of the Fanconi

core complex ubiquitin ligase and is required for FANCD2-I ubiquitination. FA patients harboring homozygous mutations to *FANCF* display extreme sensitivity to DNA crosslinking agents. It appears that *FANCF* methylation is a frequent mechanism by which sporadic tumors inactivate the BRCA/FA pathways. *FANCF* methylation is found in approximately 17% of sporadic breast cancers and has also been detected in ovarian, non-small cell lung cancer (NSCLC) and cervical cancer [46]. High sensitivity to cisplatin has been found in two ovarian cancer cell lines lacking expression of FANCF due to *FANCF* promoter methylation [28].

EMSY amplification

While hypermethylation of the *BRCA2* promoter region does not appear to contribute to the development of sporadic breast cancers, there is evidence that *BRCA2* transcription can be silenced by amplification of the *EMSY* gene. *EMSY* is located on 11q13 and has been found to be amplified in 13% of sporadic breast cancers. The *EMSY* protein product binds to *BRCA2* at exon 3, causing silencing of *BRCA2* transcription [46]. Recent data suggest that *EMSY* amplification may be associated with reduced OS [47].

'BRCAness'

The sensitivity of BRCA-deficient cells to PARP inhibitors is likely due to the underlying defect in HR. This was illustrated by McCabe and colleagues [48], who showed that cells deficient in a variety of proteins involved in HR – including RAD51, RAD54, DSS1, RPA1, NBS, ATR, ATM, CHK1, CHK2, FANCD2, FANCA, and FANCC – displayed sensitivity to PARP inhibition. Thus, cancer cells with alterations in these and other proteins might also be included in the group of tumors displaying properties of 'BRCAness'.

The clinical significance of 'BRCAness' lies in the idea that, taken together, a substantial proportion of sporadic breast cancers may harbor defects in repair pathways. Like BRCA-associated tumors, these 'BRCAness' tumors might be susceptible to synthetic lethality approaches involving PARP inhibitors or other inhibitors of BER. Alternatively, these tumors might be better treated with crosslinking chemotherapeutic agents rather than standard taxanes.

A number of clinical trials that aim to address these issues are under way. Various PARP inhibitors are currently being tested alone or in combination with chemotherapeutic agents in the treatment of triple-negative, BRCA-deficient, and metastatic breast cancers. Chemotherapeutic agents being tested include carboplatin and cisplatin, topotecan, gemcitabine, doxil, TMZ, and paclitaxel. The results of these many clinical trials will help to clarify the therapeutic potential of these strategies.

Screening approaches

Given the mechanistic heterogeneity of the different breast cancers harboring defects in DNA repair, novel screening approaches could help in determining which patients may benefit from PARP inhibition and similar therapies. Recently, Willers and colleagues [49] reported on a pilot study of an *ex vivo* biomarker assay for several DNA repair protein foci (BRCA1, FANCD2, and RAD51) with the goal of identifying the BRCA1-deficient phenotype, regardless of the underlying mechanism leading to the HR deficiency. Core biopsies from seven previously untreated breast cancers were treated with 8 gray (Gy) of x-irradiation with corresponding untreated controls from the same tumor. After incubation, sectioning, and staining of the breast biopsy specimens, RAD51, FANCD2, and BRCA1 foci were successfully detected. Four of the seven tumors displayed a BRCA1 defect with corresponding impairment of FANCD2 and RAD51 foci as well [49]. Of interest, three of the four tumors with a BRCA1 defect were triple-negative, lending support to the idea of 'BRCAness' [50]. Screening biopsy tissue for potential therapeutic response is a compelling idea that may play an important role in selection of therapies.

Targeting DNA damage signaling and checkpoints

A significant amount of work has gone into targeting the DNA damage-sensing pathways and cell cycle checkpoints. The phosphatidylinositol-3-kinase-related kinases (PIKKs), including ATM, ATR, and DNA-dependent protein kinase (DNA-PK), have emerged as promising targets for small-molecule inhibitors. This topic is beyond the scope of this article but has been reviewed in detail elsewhere [50,51].

Early clinical development of PARP inhibitors

PARP inhibitors as monotherapy

Several phase I and II trials using PARP inhibitors for patients with breast, ovarian, and a variety of other malignancies are currently under way (Table 1). Fong and colleagues [52] recently published results from a phase I trial of olaparib – a potent, orally active PARP inhibitor – administered as monotherapy. Sixty patients with advanced solid tumors, 22 of whom were carriers of a *BRCA1* or *BRCA2* mutation, were enrolled and treated. Dose escalation was performed using a modified accelerated-titration design. Once the maximum tolerated dose was determined, a cohort of only BRCA carriers was enrolled [52].

Olaparib was found to be absorbed rapidly with a peak plasma concentration between 1 and 3 hours after administration. Terminal-elimination half-life was 5 to 7 hours, which led the investigators to choose a twice-daily dosing scheme. PARP inhibition was confirmed in peripheral blood mononuclear cells (PBMCs) and by

Table 1. PARP inhibitors currently in clinical trials

Agent	Route	Phase of development	Comments
ABT-888	Oral	Phase 2	Being tested in combination with TMZ for patients with metastatic breast cancer and metastatic melanoma
AG014699	Intravenous	Phase 2	Being tested in locally advanced or metastatic BRCA-associated breast or ovarian cancer
AZD2281 (olaparib)	Oral	Phase 2	Being tested in multiple phase 2 trials for BRCA-associated advanced breast cancer and ovarian cancer
BSI-201	Intravenous	Phase 2	Being tested in neoadjuvant setting in combination with gemcitabine plus carboplatin for patients with triple-negative breast cancer
CEP-9722	Subcutaneous	Phase 1	Being tested as a single agent and in combination with TMZ in patients with advanced solid tumors
INO-1001	Intravenous	Phase 1B	Recently completed phase 1B trial in combination with TMZ for patients with stage III or IV melanoma
MK4827	Oral	Phase 1	Being tested in phase 1 for patients with advanced solid tumors

PARP, poly(ADP-ribose) polymerase; TMZ, temozolomide.

immunoblotting of cell extracts from paired tumor biopsy specimens collected before initiation of olaparib and after 8 days of treatment.

Overall, olaparib was well tolerated and resulted in less toxicity than standard chemotherapeutic agents. Three of sixty patients experienced toxicity of grade 3 or higher, including grade 3 mood alteration and fatigue, grade 4 thrombocytopenia, and grade 3 somnolence. Otherwise, adverse events (AEs) were largely grade 1 or 2, gastrointestinal (GI)-related (28% nausea, 18% vomiting, and 12% dysgeusia) or general disorders (28% fatigue and 12% anorexia).

Although this was a phase I trial, some clinical response data were reported. Twelve of the nineteen evaluable patients with a *BRCA1* or *BRCA2* mutation and ovarian, breast, or prostate cancer had a clinical benefit, with radiologic or tumor-marker responses or disease stabilization of at least 4 months. Nine *BRCA* carriers had a response according to Response Evaluation Criteria in Solid Tumors (RECIST). No patients without known *BRCA* mutations experienced objective anti-tumor responses.

BSI-201, a small-molecule inhibitor of PARP, has also been tested in a phase I dose-escalation trial as monotherapy for patients with refractory, advanced solid tumors. PARP inhibition was confirmed in PBMCs. All doses were well tolerated, and no maximum tolerated dose was identified. Again, the most common observed AEs were GI-related (39% of AEs) or general disorders (21% of AEs). Six of the twenty-three subjects, all of whom had been heavily treated previously, achieved stable disease for 2 months or more [53].

PARP inhibitors in combination with cytotoxic agents

By inhibiting BER, PARP inhibitors have the potential to enhance the lethality of cytotoxic agents, especially in tumor cells that already have defects in DNA repair pathways. Several chemotherapeutic agents, in combination with PARP inhibition, have shown promising preclinical results (Table 2).

Preclinical

Temozolomide

The mechanism of action of the methylating agent, TMZ, makes it a particularly attractive agent to use in combination with PARP inhibition. Although the predominant methylation products of TMZ are N7-methylguanine and N3-methyladenine, these lesions are repaired very efficiently by BER and so do not normally contribute to cytotoxicity. By inhibiting BER, PARP inhibitors have the potential to increase the number of cytotoxic lesions generated. In addition, TMZ resistance frequently develops due to efficient repair of toxic O6-methylguanine adducts or due to defects in the MMR, which, when functional, contributes to TMZ cell killing. Indeed, the PARP inhibitor, AG14361, has been shown to restore sensitivity to TMZ in mismatch repair-deficient human colon and ovarian cancer cells [54]. Another PARP inhibitor, INO-1001, restored sensitivity to TMZ in xenografts of glioblastoma multiforme (GBM) tumor cells deficient in mismatch repair [55].

Several preclinical studies have shown promising synergy between TMZ and PARP inhibition in a variety of human cancer cell lines and murine xenograft models. Using an SW620 colorectal cell murine xenograft model, Calabrese and colleagues [56] showed that, when added to TMZ, AG14361 increased cytotoxicity fourfold to fivefold in LoVo colorectal cancer cell lines. Furthermore, using an SW620 colorectal cell murine xenograft model, a 100% complete remission rate was achieved when AG14361 was added to TMZ [56]. ABT-888 has shown potentiation of TMZ in HCT116 colorectal and other cancer cells [57]. CEP-6800, a novel inhibitor of both PARP1 and PARP2, in combination with TMZ showed 100% tumor regression in U251MG human glioblastoma xenografts in nude mice [58].

Table 2. Preclinical testing of PARP inhibitors and other inhibitors of base excision repair

Agent	Mechanism	Cancer cell lines/tumor models	Agents potentiated	References
ABT-888	PARP inhibition	Breast, lung, ovarian, colon, melanoma, glioma	TMZ, cisplatin, carboplatin, irinotecan, cyclophosphamide, IR	[57,62,63]
AG14361	PARP inhibition	Lung, colorectal	TMZ, topotecan, irinotecan, IR	[56]
CEP-6800	PARP inhibition	Colon, GBM, NSCLC	TMZ, irinotecan, cisplatin	[58]
CEP-8983	PARP inhibition	GBM, colon, rhabdomyosarcoma, neuroblastoma	TMZ, camptothecin, irinotecan	[85]
INO-1001	PARP inhibition	Breast, GBM, sarcoma	TMZ, doxorubicin, IR	[55,64]
Lithocholic acid	DNA pol-β inhibition	BRCA2-deficient Chinese hamster ovary cells	TMZ	[59]
Methoxyamine	AP site binding	Colon	TMZ, BCNU	[68]

AP, apurininc/pyrimidinic; BCNU, 1,3-bis(chloroethyl)-1-nitrosourea; GBM, glioblastoma multiforme; IR, ionizing radiation; NSCLC, nucleotide excision repair; PARP, poly(ADP-ribose) polymerase; TMZ, temozolomide.

Alternative base excision repair targets

Recent work in our laboratory indicates that alternative means of BER inhibition similarly potentiate the effects of TMZ. We investigated the effects of lithocholic acid, an inhibitor of the key BER enzyme DNA polymerase β, in combination with TMZ. The two agents displayed synergism when given together in BRCA2-complemented cell lines. Furthermore, when the two agents were co-administered in BRCA2-deficient cells, the degree of synergism was increased [59]. The mechanism of potentiation appears to be similar to that seen with PARP inhibition, namely, persistent single-stranded DNA breaks incompletely repaired by BER being converted into DSB during replication, thereby leading to cell death.

Topoisomerase inhibitors

The combination of PARP inhibitors with the topoisomerase I inhibitors has also been explored. Early work showed that camptothecin cytotoxicity was potentiated by PARP inhibition [60]. Further work by Delaney and colleagues [61] showed that topotecan cytotoxicity was enhanced in a variety of human cancer cell lines, but this effect did not hold true for etoposide, a topoisomerase II inhibitor.

Ionizing radiation

IR induces cell killing primarily through the induction of DSBs. Several preclinical trials have shown that PARP inhibition can enhance the lethality of IR. Calabrese and colleagues [56] administered AG14361 30 minutes prior to 2 Gy of x-irradiation to mice with colorectal cancer xenografts and found that the addition of AG14361 increased anti-tumor activity by approximately twofold. ABT-888 has been shown to potentiate fractionated radiotherapy in preclinical lung cancer and colon cancer murine models [62,63]. Brock and colleagues [64] treated a murine sarcoma cell line with a single fraction of radiation with and without INO-1001 and found that these cells were radiosensitized by PARP inhibition with an enhancement ratio of 1.7.

To our knowledge, no clinical trials that combine IR with PARP inhibition are currently under way. The key clinical question that remains to be answered is to what extent PARP inhibition will differentially increase lethality to tumor cells over normal cells, thereby resulting in an improved therapeutic ratio.

Clinical trials

The therapeutic strategy of PARP inhibition in combination with chemotherapy is currently being investigated in several clinical trials, some of which have been completed. Plummer and colleagues [65] performed a phase I study investigating AG014699, a tricyclic indole administered intravenously, with TMZ in patients with advanced solid tumors. In the first phase of the trial, AG014699 was dose-escalated to establish the PARP inhibitory dose (PID) in peripheral blood lymphocytes (PBLs) with no dose-limiting toxicity observed. In the second phase, a cohort of metastatic melanoma patients received AG014699 at the previously established PID while the TMZ dose was escalated up to 200 mg/m^2 [65].

The combination of AG014699 and TMZ was well tolerated, with no observed toxicity attributable to AG014699 alone. Minimal myelosuppression was observed using the PID of AG014699 and 200 mg/m^2 TMZ. The dose-toxicity curve appeared to be steep, with myelosuppression observed when either the AG014699 dose or the TMZ dose was increased. Clinical benefit was observed in several patients with one documented complete response and one partial response in two chemonaive patients with metastatic melanoma.

Early results from a phase II trial examining AG014699 and TMZ in patients with chemonaive metastatic melanoma have also been reported. More myelosuppression was observed compared with the phase I trial, with 12% of patients experiencing grade 4 thrombocytopenia and 15% experiencing grade 4 neutropenia. One patient died from febrile neutropenia after one cycle, and 12 patients required dose reduction of TMZ. Encouraging activity was seen as several patients achieved partial responses or

prolonged disease stabilization, although it was too early to evaluate most of the patients [66].

BSI-201 has been tested in combination with topotecan, gemcitabine, TMZ, and carboplatin/paclitaxel in a phase IB trial involving patients with advanced solid tumors. BSI-201 was well tolerated in all combinations and at all doses tested. No serious AEs were attributed to the study drug. One patient with ovarian cancer obtained a complete response at 6 months, and several other patients with a variety of primary tumors achieved partial responses [53]. Given these encouraging results, BSI-201 is being tested in several phase II clinical trials, including as part of a neoadjuvant regimen with gemcitabine and carboplatin for triple-negative breast cancer.

Several other inhibitors of BER are being tested in combination with TMZ in phase I trials. INO-1001, a highly potent PARP inhibitor, was recently tested with TMZ in a phase IB trial for patients with unresectable stage III/IV melanoma. Dose-limiting toxicities of the combination were myelosuppression and hepatic toxicity, manifest by elevated transaminases that returned to normal upon withdrawal of the medication. The median time to progression was 2.2 months, and of the 12 evaluable patients, one had a partial response and four had stable disease [67]. Methoxyamine is a small molecule that inhibits BER by binding directly to apurinic/apyrimidinic sites and preventing their processing by APE-1 [68]. Methoxyamine and TMZ are currently being tested in combination in a phase I trial for patients with advanced solid tumors.

Mechanisms of resistance

Hypoxia

Hypoxic cells are known to be more resistant to radiotherapy and chemotherapy than normoxic cells are [69]. Hypoxic cell populations within tumors are believed to be a significant reason for radiotherapy failures, and, indeed, the clinical targeting of hypoxic cell populations is associated with improved locoregional control and OS [70]. Not only does hypoxia mediate resistance to therapy, it promotes genetic instability and aggressive mutagenesis, in part by impairing DNA repair pathways in tumor cells.

Acute hypoxia

Hypoxia appears to decrease radiation damage by multiple mechanisms. The classic 'oxygen fixation hypothesis' holds that DNA lesions produced by x-rays in the presence of oxygen cannot be chemically restored and are therefore more lethal to cells [71]. Recent data support the idea that, under acutely hypoxic conditions, the checkpoint kinases ATM and ATR are activated and limit DNA damage through cell cycle arrest [72]. The coordinated cellular response to hypoxic stress in conjunction with the damage-potentiating role of oxygen following IR may largely explain the classic finding of hypoxic radioresistance. Interestingly, it has been shown that the PARP inhibitor ABT-888 can radiosensitize acutely hypoxic human prostate and NSCLC cell lines to a level similar to that of oxic radiosensitivity [73]. The mechanism for this radiosensitization may be related to transcriptional downregulation of HR by PARP inhibition [74].

Chronic hypoxia

Following the initial, acute DNA damage response, it appears that a chronic hypoxic response develops whereby important genes in the MMR and HR pathways – including MLH1, MSH2, BRCA1, and Rad51 – are downregulated [75-79]. Chan and colleagues [80] recently found that chronically hypoxic cells display increased sensitivity to crosslinking agents cisplatin and mitomycin C. Given that increased sensitivity to crosslinking agents is a hallmark of HR-deficient cells, these findings support the idea that radioresistance during chronic hypoxia is decreased compared with acute hypoxia due to downregulation of repair pathways. MicroRNAs – small, non-protein-coding RNAs that bind to and regulate mRNAs – also appear to be important participants in the regulation of DNA repair in response to chronic hypoxia [81]. As the details of microRNA regulatory mechanisms emerge, they may reveal therapeutic opportunities to be exploited.

Secondary mutations

Recent discoveries are shedding light on how *BRCA*-mutated cancer cells acquire resistance to therapies. While ovarian cancers with a mutation in *BRCA1* or *BRCA2* are generally sensitive to cisplatin or carboplatin, these cancers eventually become resistant. Sakai and colleagues [82] recently showed that secondary intragenic mutations in *BRCA2* that restore the wild-type *BRCA2* reading frame can mediate resistance to cisplatin. Similarly, Edwards and colleagues [83] showed that intragenic deletions causing restoration of the open reading frame in *BRCA2* mutant cells can also result in resistance to PARP inhibition. The same mechanism has been implicated in the development of platinum resistance in *BRCA1*-mutated ovarian carcinomas [84]. Ironically, the HR deficiency that is being targeted therapeutically also increases the likelihood of additional mutations, some of which will restore the open reading frame and thereby restore BRCA function.

Conclusions

DNA repair pathways play a central role in cancer, both in the development of cancer and in the response to therapies. The elucidation of the molecular mechanisms

of DNA repair and the discovery that tumors are frequently repair-deficient provide a therapeutic opportunity to selectively target this weakness, especially in breast cancers. In BRCA-associated breast cancer, the inhibition of BER with agents such as the PARP inhibitors may provide an effective synthetic lethality approach resulting in tumor cell death with minimal toxicity to normal tissues. Furthermore, a substantial proportion of sporadic breast cancers, including the therapeutically challenging basal-like subset, may have similar repair pathway deficiencies that make them susceptible to these agents. Inhibiting DNA repair may also enhance the effectiveness of cytotoxic therapies such as chemotherapy and radiation therapy, although it remains to be seen to what extent this increased cytotoxicity will differentially affect tumor cells in patients. Knowledge of the mechanisms of DNA damage and repair may help to guide selection of chemotherapeutic agents and also may help elucidate mechanisms of resistance. The role of hypoxia in the regulation of DNA repair is still under investigation and may offer additional therapeutic targets.

Abbreviations

AE, adverse event; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia mutated and Rad3 related; BER, base excision repair; DSB, double-strand break; FA, Fanconi anemia; FANC, Fanconi anemia complementation; GI, gastrointestinal; Gy, gray; HR, homologous recombination; ICL, interstrand crosslink; IR, ionizing radiation; IUdR, iododeoxyuridine; MMR, mismatch repair system; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NSCLC, non-small cell lung cancer; OS, overall survival; PARP, poly(ADP-ribose) polymerase; PBMC, peripheral blood mononuclear cell; PID, poly(ADP-ribose) polymerase inhibitory dose; TLS, translesion synthesis; TMZ, temozolomide.

Competing interests

The authors declare that they have no competing interests.

Published: 30 April 2010

References

1. Hall E, Giaccia A: Repair of radiation damage and the dose-rate effect. In *Radiobiology for the Radiologist*. 6th edition. Edited by McAllister L. Philadelphia: Lippincott Williams & Wilkins; 2006:67-90.
2. Chan KK, Zhang QM, Dianov GL: **Base excision repair fidelity in normal and cancer cells.** *Mutagenesis* 2006, **21:**173-178.
3. Robertson AB, Klungland A, Rognes T, Leiros I: **DNA repair in mammalian cells: base excision repair: the long and short of it.** *Cell Mol Life Sci* 2009, **66:**981-993.
4. Hanawalt PC: **Subpathways of nucleotide excision repair and their regulation.** *Oncogene* 2002, **21:**8949-8956.
5. Kinsella TJ: **Coordination of DNA mismatch repair and base excision repair processing of chemotherapy and radiation damage for targeting resistant cancers.** *Clin Cancer Res* 2009, **15:**1853-1859.
6. Pardo B, Gomez-Gonzalez B, Aguilera A: **DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship.** *Cell Mol Life Sci* 2009, **66:**1039-1056.
7. Moldovan GL, D'Andrea AD: **How the Fanconi anemia pathway guards the genome.** *Annu Rev Genet* 2009, **43:**223-249.
8. Roschke AV, Glebov OK, Lababidi S, Gehlhaus KS, Weinstein JN, Kirsch IR: **Chromosomal instability is associated with higher expression of genes implicated in epithelial-mesenchymal transition, cancer invasiveness, and metastasis and with lower expression of genes involved in cell cycle checkpoints, DNA repair, and chromatin maintenance.** *Neoplasia* 2008, **11:**1222-1230.
9. Chen S, Parmigiani G: **Meta-Analysis of *BRCA1* and *BRCA2* Penetrance.** *J Clin Oncol* 2007, **25:**1329-1333.
10. Jasin M: **Homologous repair of DNA damage and tumorigenesis: the BRCA connection.** *Oncogene* 2002, **21:**8981-8993.
11. Chen JJ, Silver D, Cantor S, Livingston DM, Scully R: **BRCA1, BRCA2, and Rad51 operate in a common DNA damage response pathway.** *Cancer Res* 1999, **59 (7 Suppl):**1752s-1756s.
12. Yang H, Li Q, Fan J, Holloman WK, Pavletich NP: **The BRCA2 homologue Brh2 nucleates RAD51 filament formation at a dsDNA-ssDNA junction.** *Nature* 2005, **433:**653-657.
13. Boulton SJ: **Cellular functions of the BRCA tumour-suppressor proteins.** *Biochem Soc Trans* 2006, **34:**633-645.
14. Moynahan ME, Chiu JW, Koller BH, Jasin M: **Brca1 controls homology-directed DNA repair.** *Mol Cell* 1999, **4:**511-518.
15. Scully R, Livingston DM: **In search of the tumour-suppressor functions of BRCA1 and BRCA2.** *Nature* 2000, **408:**429-432.
16. Kastan MB, Bartek J: **Cell-cycle checkpoints and cancer.** *Nature* 2004, **432:**316-323.
17. Ganesan S, Silver DP, Greenberg RA, Avni D, Drapkin R, Miron A, Mok SC, Randrianarison V, Brodie S, Salstrom J, Rasmussen TP, Klimke A, Marrese C, Marahrens Y, Deng CX, Feunteun J, Livingston DM: **BRCA1 supports XIST RNA concentration on the inactive X chromosome.** *Cell* 2002, **111:**393-405.
18. Bhattacharyya A, Ear U, Koller B, Weichselbaum R, Bishop D: **The breast cancer susceptibility gene *BRCA1* is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin.** *J Biol Chem* 2000, **275:**23899-23903.
19. Tutt A, Lord C, McCabe N, Farmer H, Turner N, Martin N, Jackson S, Smith G, Ashworth A: **Exploiting the DNA repair defect in BRCA mutant cells in the design of new therapeutic strategies for cancer.** *Cold Spring Harb Symp Quant Biol* 2005, **70:**139-148.
20. Liu N, Lamerdin J, Tebbs R, Schild D, Tucker J, Shen M, Brookman K, Siciliano M, Walter C, Fan W, Narayana L, Zhou Z, Adamson A, Sorensen K, Chen D, Jones N, Thompson L: **XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages.** *Mol Cell* 1998, **1:**783-793.
21. Akkari YM, Bateman RL, Reifsteck CA, Olson SB, Grompe M: **DNA replication is required To elicit cellular responses to psoralen-induced DNA interstrand cross-links.** *Mol Cell Biol* 2000, **20:**8283-8289.
22. Cass I, Baldwin R, Varkey T, Moslehl R, Narod S, Karlan B: **Improved survival in women with *BRCA*-associated ovarian carcinoma.** *Cancer* 2003, **97:**2187-2195.
23. Treszezamsky A, Kachnic L, Feng Z, Zhang J, Tokadjian C, Powell S: **BRCA1- and BRCA2-deficient cells are sensitive to etoposide-induced DNA double-strand breaks via topoisomerase II.** *Cancer Res* 2007, **67:**7078-7081.
24. Taniguchi T, Garcia-Higuera I, Xu B, Andreassen PR, Gregory RC, Kim ST, Lane WS, Kastan MB, D'Andrea AD: **Convergence of the Fanconi anemia and ataxia telangiectasia signaling pathways.** *Cell* 2002, **109:**459-472.
25. Andreassen PR, D'Andrea AD, Taniguchi T: **ATR couples FANCD2 monoubiquitination to the DNA-damage response.** *Genes Dev* 2004, **18:**1958-1963.
26. Pichierri P, Averbeck D, Rosselli F: **DNA cross-link-dependent RAD50/MRE11/NBS1 subnuclear assembly requires the Fanconi anemia C protein.** *Hum Mol Genet* 2002, **11:**2531-2546.
27. Garcia-Higuera I, Taniguchi T, Ganesan S, Meyn MS, Timmers C, Hejna J, Grompe M, D'Andrea AD: **Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway.** *Mol Cell* 2001, **7:**249-262.
28. Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje H, Mok SC, D'Andrea AD: **Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors.** *Nat Med* 2003, **9:**568-574.
29. Ferrer M, de Winter JP, Mastenbroek DC, Curiel DT, Gerritsen WR, Giaccone G, Kruyt FA: **Chemosensitizing tumor cells by targeting the Fanconi anemia pathway with an adenovirus overexpressing dominant-negative FANCA.** *Cancer Gene Ther* 2004, **11:**539-546.
30. Chirnomas D, Taniguchi T, de la Vega M, Vaidya AP, Vasserman M, Hartman AR, Kennedy R, Foster R, Mahoney J, Seiden MV, D'Andrea AD: **Chemosensitization to cisplatin by inhibitors of the Fanconi anemia/BRCA pathway.** *Mol Cancer Ther* 2006, **5:**952-961.
31. Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D, Sinnett D: **Frequent loss of heterozygosity at the DNA mismatch-repair loci hMLH1 and hMSH3 in sporadic breast cancer.** *Br J Cancer* 1999, **79:**1012-1017.
32. Naqvi RA, Hussain A, Deo SS, Kukreti H, Chauhan M, Sarin R, Saxena A, Asim

M, Shukla NK, Husain SA, Pasha ST, Basir SF: **Hypermethylation analysis of mismatch repair genes (hmlh1 and hmsh2) in locally advanced breast cancers in Indian women.** *Hum Pathol* 2008, **39:**672-680.
33. Karray-Chouayekh S, Trifa F, Khabir A, Boujelbane N, Sellami-Boudawara T, Daoud J, Frikha M, Gargouri A, Mokdad-Gargouri R: **Clinical significance of epigenetic inactivation of hMLH1 and BRCA1 in Tunisian patients with invasive breast carcinoma.** *J Biomed Biotechnol* 2009, **2009:**369129.
34. Murata H, Khattar NH, Gu L, Li GM: **Roles of mismatch repair proteins hMSH2 and hMLH1 in the development of sporadic breast cancer.** *Cancer Lett* 2005, **223:**143-150.
35. Zeng M, Narayanan L, Xu XS, Prolla TA, Liskay RM, Glazer PM: **Ionizing radiation-induced apoptosis via separate Pms2- and p53-dependent pathways.** *Cancer Res* 2000, **60:**4889-4893.
36. Fritzell JA, Narayanan L, Baker SM, Bronner CE, Andrew SE, Prolla TA, Bradley A, Jirik FR, Liskay RM, Glazer PM: **Role of DNA mismatch repair in the cytotoxicity of ionizing radiation.** *Cancer Res* 1997, **57:**5143-5147.
37. Jagtap P, Szabó C: **Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors.** *Nat Rev Drug Discov* 2005, **4:**421-440.
38. Huber A, Bai P, de Murcia JM, de Murcia G: **PARP-1, PARP-2 and ATM in the DNA damage response: functional synergy in mouse development.** *DNA Repair* 2004, **3:**1103-1108.
39. de Murcia JM, Niedergang C, Trucco C, Ricoul M, Dutrillaux B, Mark M, Oliver FJ, Masson M, Dierich A, LeMeur M, Walztinger C, Chambon P, de Murcia G: **Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells.** *Proc Natl Acad Sci U S A* 1997, **94:**7303-7307.
40. Schreiber V, Amé JC, Dollé P, Schultz I, Rinaldi B, Fraulob V, Ménissier-de Murcia J, de Murcia G: **Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1.** *J Biol Chem* 2002, 277:23028-**230**36.
41. Ratnam K, Low JA: **Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology.** *Clin Cancer Res* 2007, **13:**1383-1388.
42. Farmer H, McCabe N, Lord C, Tutt A, Johnson D, Richardson T, Santarosa M, Dillon K, Hickson I, Knights C, Martin N, Jackson S, Smith G, Ashworth A: **Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy.** *Nature* 2005, **434:**917-921.
43. Bryant H, Schultz N, Thomas H, Parker K, Flower D, Lopez E, Kyle S, Meuth M, Curtin N, Helleday T: **Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase.** *Nature* 2005, **434:**913-917.
44. Kaelin WG Jr.: **The concept of synthetic lethality in the context of anticancer therapy.** *Nat Rev Cancer* 2005, **5:**689-698.
45. Iglehart JD, Silver DP: **Synthetic lethality- a new direction in cancer-drug development.** *N Engl J Med* 2009, **361:**189-191.
46. Turner N, Tutt A, Ashworth A: **Hallmarks of 'BRCAness' in sporadic cancers.** *Nat Rev Cancer* 2004, **4:**814-819.
47. Kirkegaard T, Nielsen KV, Jensen LB, Campbell FM, Müller S, Tovey SM, Brown S, Cooke TG, Bartlett JM: **Genetic alterations of CCND1 and EMSY in breast cancers.** *Histopathology* 2008, **52:**698-705.
48. McCabe N, Turner NC, Lord CJ, Kluzek K, Bialkowska A, Swift S, Giavara S, O'Connor MJ, Tutt AN, Zdzienicka MZ, Smith GC, Ashworth A: **Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibition.** *Cancer Res* 2006, **66:**8109-8115.
49. Willers H, Taghian AG, Luo CM, Treszezamsky A, Sgroi DC, Powell SN: **Utility of DNA repair protein foci for the detection of putative BRCA1 pathway defects in breast cancer biopsies.** *Mol Cancer Res* 2009, **8:**1304-1309.
50. Powell SN, Bindra RS: **Targeting the DNA damage response for cancer therapy.** *DNA Repair (Amst)* 2009, **8:**1153-1165.
51. Choudhury A, Cuddihy A, Bristow R: **Radiation and new molecular agents part I: targeting ATM-ATR checkpoints, DNA repair, and the proteasome.** *Semin Radiat Oncol* 2006, **16:**51-58.
52. Fong P, Boss D, Yap T, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor M, Ashworth A, Carmichael J, Kaye S, Schellens J, de Bono J: **Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers.** *N Engl J Med* 2009, **361:**123-134.
53. Kopetz S, Mita M, Mok I, Sankhala K, Moseley J, Sherman B, Bradley C, Tolcher A: **First in human phase I study of BSI-201, a small molecule inhibitor of poly ADP-ribose polymerase (PARP) in subjects with advanced solid tumors [abstract].** *J Clin Oncol* 2008, **26:**s3577.
54. Curtin N, Wang L, Yiakouvaki A, Kyle S, Arris C, Canan-Koch S, Webber S, Durkacz B, Calvert H, Hostomsky Z, Newell D: **Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells.** *Clin Cancer Res* 2004, **10:**881-889.
55. Cheng C, Johnson S, Keir S, Quinn J, Ali-Osman F, Szabo C, Li H, Salzman A, Dolan M, Modrich P, Bigner D, Friedman H: **Poly(ADP-ribose) polymerase-1 inhibition reverses temozolomide resistance in a DNA mismatch repair-deficient malignant glioma xenograft.** *Mol Cancer Ther* 2005, **4:**1364-1368.
56. Calabrese CR, Almassy R, Barton S, Batey MA, Calvert AH, Canan-Koch S, Durkacz BW, Hostomsky Z, Kumpf RA, Kyle S, Li J, Maegley K, Newell DR, Notarianni E, Stratford IJ, Skalitzky D, Thomas HD, Wang LZ, Webber SE, Williams KJ, Curtin NJ: **Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361.** *J Natl Cancer Inst* 2004, **96:**56-67.
57. Liu X, Shi Y, Guan R, Donawho C, Luo Y, Palma J, Zhu G, Johnson EF, Rodriguez LE, Ghoreishi-Haack N, Jarvis K, Hradil VP, Colon-Lopez M, Cox BF, Klinghofer V, Penning T, Rosenberg SH, Frost D, Giranda VL, Luo Y: **Potentiation of temozolomide cytotoxicity by poly(ADP)ribose polymerase inhibitor ABT-888 requires a conversion of single-stranded DNA damages to double-stranded DNA breaks.** *Mol Cancer Res* 2008, **6:**1621-1629.
58. Miknyoczki SJ, Jones-Bolin S, Pritchard S, Hunter K, Zhao H, Wan W, Ator M, Bihovsky R, Hudkins R, Chatterjee S, Klein-Szanto A, Dionne C, Ruggeri B: **Chemopotentiation of temozolomide, irinotecan, and cisplatin activity by CEP-6800, a poly(ADP-ribose) polymerase inhibitor.** *Mol Cancer Ther* 2003, **2:**371-382.
59. Stachelek GC, Dalal S, Donigan KA, Campisi Hegan D, Sweasy JB, Glazer PM: **Potentiation of temozolomide cytotoxicity by inhibition of DNA polymerase beta is accentuated by BRCA2 mutation.** *Cancer Res* 2010, **70:**409-417.
60. Bowman KJ, Newell DR, Calvert AH, Curtin NJ: **Differential effects of the poly (ADP-ribose) polymerase (PARP) inhibitor NU1025 on topoisomerase I and II inhibitor cytotoxicity in L1210 cells *in vitro*.** *Br J Cancer* 2001, **84:**106-112.
61. Delaney CA, Wang LZ, Kyle S, White AW, Calvert AH, Curtin NJ, Durkacz BW, Hostomsky Z, Newell DR: **Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly(adenosine diphosphoribose) polymerase inhibitors in a panel of human tumor cell lines.** *Clin Cancer Res* 2000, **6:**2860-2867.
62. Albert JM, Cao C, Kim KW, Willey CD, Geng L, Xiao D, Wang H, Sandler A, Johnson DH, Colevas AD, Low J, Rothenberg ML, Lu B: **Inhibition of poly(ADP-ribose) polymerase enhances cell death and improves tumor growth delay in irradiated lung cancer models.** *Clin Cancer Res* 2007, **13:**3033-3042.
63. Donawho CK, Luo Y, Luo Y, Penning TD, Bauch JL, Bouska JJ, Bontcheva-Diaz VD, Cox BF, DeWeese TL, Dillehay LE, Ferguson DC, Ghoreishi-Haack NS, Grimm DR, Guan R, Han EK, Holley-Shanks RR, Hristov B, Idler KB, Jarvis K, Johnson EF, Kleinberg LR, Klinghofer V, Lasko LM, Liu X, Marsh KC, McGonigal TP, Meulbroek JA, Olson AM, Palma JP, Rodriguez LE, *et al.*: **ABT-888, an orally active poly(ADP-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models.** *Clin Cancer Res* 2007, **13:**2728-2737.
64. Brock WA, Milas L, Bergh S, Lo R, Szabó C, Mason KA: **Radiosensitization of human and rodent cell lines by INO-1001, a novel inhibitor of poly(ADP-ribose) polymerase.** *Cancer Lett* 2004, **205:**155-160.
65. Plummer R, Jones C, Middleton M, Wilson R, Evans J, Olsen A, Curtin N, Boddy A, McHugh P, Newell D, Harris A, Johnson P, Steinfeldt H, Dewji R, Wang D, Robson L, Calvert H: **Phase I study of the poly(ADP-ribose) polymerase inhibitor, AG014699, in combination with temozolomide in patients with advanced solid tumors.** *Clin Cancer Res* 2008, **14:**7917-7923.
66. Plummer R, Lorigan P, Evans J, Steven N, Middleton M, Wilson R, Snow K, Dewji R, Calvert H: **First and final report of a phase II study of the poly(ADP-ribose) polymerase (PARP) inhibitor, AG014699, in combination with temozolomide (TMZ) in patients with metastatic malignant melanoma (MM) [abstract].** *J Clin Oncol* 2006, **24:**s8013.
67. Bedikian AY, Papadopoulos NE, Kim KB, Hwu WJ, Homsi J, Glass MR, Cain S, Rudewicz P, Vernillet L, Hwu P: **A phase IB trial of intravenous INO-1001 plus oral temozolomide in subjects with unresectable stage-III or IV melanoma.** *Cancer Invest* 2009, **27:**756-763.
68. Yan L, Bulgar A, Miao Y, Mahajan V, Donze JR, Gerson SL, Liu L: **Combined treatment with temozolomide and methoxyamine: blocking apurinic/pyrimidinic site repair coupled with targeting topoisomerase IIα.** *Clin Cancer Res* 2007, **13:**1532-1539.
69. Shannon AM, Bouchier-Hayes DJ, Condron CM, Toomey D: **Tumour hypoxia, chemotherapeutic resistance and hypoxia-related therapies.** *Cancer Treat Rev* 2003, **29:**297-307.
70. Overgaard J: **Hypoxic radiosensitization: adored and ignored.** *J Clin Oncol*

2007, **25:**4066-4074.
71. Ewing D: **The oxygen fixation hypothesis: a reevaluation.** *Am J Clin Oncol* 1998, **21:**355-361.
72. Bindra RS, Crosby ME, Glazer PM: **Regulation of DNA repair in hypoxic cells.** *Cancer Metastasis Rev* 2007, **26:**249-260.
73. Liu SK, Coackley C, Krause M, Jalali F, Chan N, Bristow RG: **A novel poly(ADP-ribose) polymerase inhibitor, ABT-888, radiosensitizes malignant human cell lines under hypoxia.** *Radiother Oncol* 2008, **88:**258-268.
74. Hegan DC, Lu Y, Stachelek GC, Crosby ME, Bindra RS, Glazer PM: **Inhibition of poly(ADP-ribose) polymerase down-regulates BRCA1 and RAD51 in a pathway mediated by E2F4 and p130.** *Proc Natl Acad Sci* 2010, **107:**2201-2206.
75. Mihaylova VT, Bindra RS, Yuan J, Campisi D, Narayanan L, Jensen R, Giordano F, Johnson RS, Rockwell S, Glazer PM: **Decreased expression of the DNA mismatch repair gene Mlh1 under hypoxic stress in mammalian cells.** *Mol Cell Biol* 2003, **23:**3265-3273.
76. Shahrzad S, Quayle L, Stone C, Plumb C, Shirasawa S, Rak JW, Coomber BL: **Ischemia-induced K-ras mutations in human colorectal cancer cells: role of microenvironmental regulation of MSH2 expression.** *Cancer Res* 2005, **65:**8134-8141.
77. Koshiji M, To KK, Hammer S, Kumamoto K, Harris AL, Modrich P, Huang LE: **HIF-1alpha induces genetic instability by transcriptionally downregulating MutSalpha expression.** *Mol Cell* 2005, **17:**793-803.
78. Bindra RS, Schaffer PJ, Meng A, Woo J, Måseide K, Roth ME, Lizardi P, Hedley DW, Bristow RG, Glazer PM: **Down-regulation of Rad51 and decreased homologous recombination in hypoxic cancer cells.** *Mol Cell Biol* 2004, **24:**8504-8518.
79. Bindra RS, Gibson SL, Meng A, Westermark U, Jasin M, Pierce AJ, Bristow RG, Classon MK, Glazer PM: **Hypoxia-induced down-regulation of BRCA1 expression by E2Fs.** *Cancer Res* 2005, **65:**11597-11604.
80. Chan N, Koritzinsky M, Zhao H, Bindra R, Glazer PM, Powell S, Belmaaza A, Wouters B, Bristow RG: **Chronic hypoxia decreases synthesis of homologous recombination proteins to offset chemoresistance and radioresistance.** *Cancer Res* 2008, **68:**605-614.
81. Crosby ME, Kulshreshtha R, Ivan M, Glazer PM: **MicroRNA regulation of DNA repair gene expression in hypoxic stress.** *Cancer Res* 2009, **69:**1221-1229.
82. Sakai W, Swisher EM, Karlan BY, Agarwal MK, Higgins J, Friedman C, Villegas E, Jacquemont C, Farrugia DJ, Couch FJ, Urban N, Taniguchi T: **Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers.** *Nature* 2008, **451:**1116-1120.
83. Edwards SL, Brough R, Lord CJ, Natrajan R, Vatcheva R, Levine DA, Boyd J, Reis-Filho JS, Ashworth A: **Resistance to therapy caused by intragenic deletion in *BRCA2*.** *Nature* 2008, **451:**1111-1116.
84. Swisher EM, Sakai W, Karlan BY, Wurz K, Urban N, Taniguchi T: **Secondary *BRCA1* mutations in *BRCA1*-mutated ovarian carcinomas with platinum resistance.** *Cancer Res* 2008, **68:**2581-2586.
85. Miknyoczki SJ, Chang H, Grobelny J, Pritchard S, Worrell C, McGann N, Ator M, Hursten J, Deibold J, Hudkins R, Zulli A, Parchment R, Ruggeri B: **The selective poly(ADP-ribose) polymerase-1(2) inhibitor, CEP-8983, increases the sensitivity of chemoresistant tumor cells to temozolomide and irinotecan but does not potentiate myelotoxicity.** *Mol Cancer Ther* 2007, **6:**2290-2302.

doi:10.1186/bcr2566
Cite this article as: Rowe BP, Glazer PM: Emergence of rationally designed therapeutic strategies for breast cancer targeting DNA repair mechanisms. *Breast Cancer Research* 2010, **12:**203.