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 G2 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.
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. Treszczamsky 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 (HNPPCs) and other HNPPC 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 toxic potency 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 G2 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 iododeoxuryridine (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 mono-therapy 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 ‘BRCaless’

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
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

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**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.
core complex ubiquitin ligase and is required for FANCD2-1 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
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 \( \text{BRCA1} \) or \( \text{BRCA2} \) 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 \( \text{BRCA} \) carriers had a response according to Response Evaluation Criteria in Solid Tumors (RECIST). No patients without known \( \text{BRCA} \) mutations experienced objective anti-tumor responses.

BSI-201, a small-molecule inhibitor of PARP, has also been tested in a phase 1 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].

### 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.

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].
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. 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/m² [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/m² 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 chemo naive patients with metastatic melanoma.

Early results from a phase II trial examining AG014699 and TMZ in patients with chemo naive 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

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| 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.
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 down-regulated [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 opportunities.

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; 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.

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