Poly(ADP-Ribose) Polymerase (PARP) Inhibitors: Exploiting a Synthetic Lethal Strategy in the Clinic

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Abstract

Poly(ADP-ribose) polymerase (PARP) is an attractive antitumor target because of its vital role in DNA repair. The homologous recombination (HR) DNA repair pathway is critical for the repair of DNA double-strand breaks and HR deficiency leads to a dependency on error-prone DNA repair mechanisms, with consequent genomic instability and oncogenesis. Tumor-specific HR defects may be exploited through a synthetic lethal approach for the application of anticancer therapeutics, including PARP inhibitors. This theory proposes that targeting genetically defective tumor cells with a specific molecular therapy that inhibits its synthetic lethal gene partner should result in selective tumor cell killing. The demonstration of single-agent antitumor activity and the wide therapeutic index of PARP inhibitors in BRCA1 and BRCA2 mutation carriers with advanced cancers provides strong evidence for the clinical application of this approach. Emerging data also indicate that PARP inhibitors may be effective in sporadic cancers bearing HR defects, supporting a substantially wider role for PARP inhibitors. Drugs targeting this enzyme are now in pivotal clinical trials in patients with sporadic cancers. In this article, the evidence supporting this antitumor synthetic lethal strategy with PARP inhibitors is reviewed, evolving resistance mechanisms and potential molecular predictive biomarker assays are discussed, and the future development of these agents is envisioned. CA Cancer J Clin 2011;61:31–49. © 2011 American Cancer Society.

Introduction

The geneticist Theodosius Dobzhansky described the concept of synthetic lethality in 1946, to illustrate a phenomenon in which 2 non-lethal genetic mutations are innocuous when they occur individually, but which result in lethality to a cell in combination.1 This may involve genes in related pathways, or genes acting along a single pathway, in which loss of both genes, rather than just one, significantly affects pathway signaling or function. Although originally studied in highly genetically malleable organisms including Drosophila and Candida, this concept is now being exploited in cancer medicine.2

Many tumor cells have specific genetic lesions, which can be exploited by targeting synthetic lethal partner genes. In principle, this should result in selective tumor cell death, with a large therapeutic window (Fig. 1). Such strategies should favorably impact the equilibrium between tumor cell proliferation and cell death, and lead to disease regression, the patient’s symptomatic improvement, and potentially a survival advantage.3 They would also allow more “breathing room” or capacity to develop drug combinations. This is critically important because effective anticancer treatment is likely to involve combinations of multiple agents.

Preclinical screens for synthetic lethality are now being performed in different model organisms, including human cells, with the aim of identifying potential targets and strategies for the development of anticancer therapeutics.4,5 The first clinical trials exploiting this concept involved the study of poly(ADP-ribose) polymerase (PARP) inhibitors in BRCA1 and BRCA2 (BRCA1/2) mutation carriers with advanced solid tumors, which validated the clinical importance of this approach.6–9 This review will focus on the scientific rationale, preclinical...
work, and clinical evidence supporting the broad utility of this synthetic lethal approach with PARP inhibitors in both inherited and sporadic cancers. It will also detail the evolving molecular assays that may predict clinical benefit to these agents and the outstanding challenges that relate to the future development of PARP inhibitors, including the unravelling of underlying resistance mechanisms to such therapies.

Rationale for a Synthetic Lethal Strategy With PARP Inhibitors

DNA Repair

The mammalian genome is constantly exposed to both exogenous and endogenous stresses, resulting in multiple DNA damage events each day. A diverse range of DNA repair pathways are employed, depending on the types of lesions and repair required (Table 1) (Fig. 2). For example, homologous recombination (HR) and non-homologous end joining (NHEJ) pathways may be utilized for the repair of DNA double-strand breaks (DSBs), whereas the nucleotide excision repair, base excision repair (BER), or mismatch repair (MMR) pathways may be recruited instead to repair DNA single-strand breaks (SSBs). The HR system is highly conserved and error free, and is therefore the favored form of DNA DSB repair. Unlike HR however, the NHEJ pathway is error prone, and may lead to genomic instability. BER is a key pathway for the repair of DNA SSBs and encompasses the sensing of the DNA lesion, followed by recruitment of several other repair effectors through the action of the enzyme PARP.

PARP in DNA Repair

PARP consists of a family of 17 enzymes, although to date, PARP1 and PARP2 are the only ones known to be involved in DNA repair. The founding family member PARP1 is a ubiquitous nuclear enzyme that detects breaks in DNA and signals downstream through its enzymatic activity. In addition, PARP1 activation results in modification of hundreds of nuclear proteins, resulting in a massive reorganization of multiple cellular functions, notably that of BER, which is induced by active PARP1.

PARP2 was discovered after the observation of residual DNA-dependent PARP activity in embryonic fibroblasts obtained from PARP1-deficient mice. PARP2 is known to interact with several other repair effectors through the action of the enzyme PARP.
## TABLE 1. DNA Repair Pathways

| DNA REPAIR PATHWAY | LESIONS REPAIRED | KEY MOLECULES OF PATHWAY | GENETIC ABNORMALITY AS A CONSEQUENCE OF LOSS OF FUNCTION | INHERITED GENETIC LESIONS OF PATHWAY |
|-------------------|-----------------|--------------------------|----------------------------------------------------------|-------------------------------------|
| Single-strand break repair | | | | |
| Base excision repair<sup>11,12</sup> | Oxidized, alkylated, deaminated bases, or uracil | DNA glycosylase (MYH), AP lyase | APE1, AP lyase PARP1, XRCC1, Fen1, PCNA, Polβ, Polκ | Single-strand breaks leading to double-strand breaks if not repaired | BRCA1/2 mutations, Fanconi anemia (FANC genes), ataxia-telangiectasia (ATM) |
| Nucleotide excision repair<sup>13,14</sup> | Cyclobutane pyrimidine dimers, [6-4] photoproducts | RNA polymerase I and II, CSA and CSB | TFIIH, XPD, XPG, XPF-ERCC1 5'nuclease, RPA | C to T (and other) mutations | Xeroderma pigmentosum (XP gene mutations) |
| Mismatch repair<sup>11,15,16</sup> | Repair of G/T and A/C pairs | MutSα (MSH2, MSH6), exonuclease (EXO1) | MutLα (MLH1/PM2), Polb, PCNA, RPA, DNA ligase | Microsatellite instability | Lynch syndrome (hereditary nonpolyposis colorectal cancer) (MSH2 and MLH1) |
| Double-strand break repair | | | | |
| Homologous recombination<sup>17-19</sup> | Double-strand breaks during S- and G2 phases of cell cycle, regulated by CDK complex, comprised of gene conversion and single-strand annealing pathways | MRN complex, FANC proteins | ChiP, ATM, BRCA1/BARD1, MRN complex, RPA, PALB2, BRCA2, RAD51 | Failure of segregation of chromosomes at meiosis, inefficient DNA repair due to error-prone NHEJ, with translocations and telomere fusion | BRCA1/2 mutations, Fanconi anemia (FANC genes), ataxia-telangiectasia (ATM) |
| Non-homologous end joining<sup>20</sup> | Double-strand breaks in entire cell cycle, less precise than HR | Ku70/Ku80, DNA-PKcs | DNA-PKcs, Artemis | DNA polymerases | Repair by more error-prone MMEJ pathway | LIG4 syndrome (LIG4), XLF-SCID (XLF) |

APE1 indicates AP endonuclease 1; PARP1, poly(ADP-ribose) polymerase 1; Fen1, flap structure-specific endonuclease 1; PCNA, proliferating cell nuclear antigen; Polβ, DNA polymerase β; Polκ, DNA polymerase κ; CSA, Cockayne syndrome A; CSB, Cockayne syndrome B; TFIIH, transcription factor II H; XPD, xeroderma pigmentosum group D; XPG, xeroderma pigmentosum group G; RPA, replication protein A; MSH6, mutS homolog 6; MLH1, mutL homolog 1; CDK, cyclin-dependent kinase; FANC, Fanconi anemia; ChiP, ChIP-interacting protein; ATM, ataxia-telangiectasia mutated gene; PALB2, partner and localizer of BRCA2; NHEJ, nonhomologous end joining; HR, homologous recombination; DNA-PKcs, DNA-dependent protein kinase, catalytic subunit; MMEJ, microhomology-mediated end joining.
PARP1 and both are involved with XRCC1, DNA polymerase-β, and DNA ligase III, which are integral components of the BER and DNA SSB repair pathways. The functions of PARP2 do not completely overlap with PARP1; despite substantial PARP2-induced PARP activity in PARP1-knockout murine models with genotoxic stimulation, genomic instability is observed, suggesting that PARP2 is unable to fully compensate for the loss of PARP1.

**BRCA1 and BRCA2 in DNA Repair**

The BRCA1/2 genes encode proteins with many distinct functions in various cellular pathways, but share coordinating responsibilities in the response to DNA damage. BRCA1 plays a key role in the regulation of the cell cycle checkpoint and DNA repair signaling, including the recruitment of repair enzymes; BRCA2 directly translocates the DNA repair protein RAD51 to areas of DNA damage to facilitate repair. Individuals with heterozygous germline BRCA1 or BRCA2 aberrations have a cumulative lifetime risk of 84% and 60% to 80%, respectively, for breast cancer, whereas the chance of developing ovarian cancer is approximately 50% and 10% to 20%, respectively. These BRCA1/2 mutation carriers are also at an increased risk of developing other cancers, including cancers of the prostate and pancreas. Despite the improved understanding of these tumor suppressor genes, the clinical management of BRCA1/2-mutated cancers has remained generally similar to that of sporadic (ie, non-BRCA1/2 mutation–associated) malignancies. Emerging clinical data indicate that this is very likely to change.

**Tumor-Specific Cell Kill: Targeting BRCA Loss of Function With PARP Inhibitors**

When persistent DNA SSBs caused by PARP inhibition encounter DNA replication forks, they can result in stalling of the fork and may subsequently result in fork collapse or lead to DNA DSB formation. These DNA DSBs are normally efficiently repaired by the HR DNA repair pathway. A hypothesis has been proposed that because BRCA1/2-deficient (BRCA1/2-) tumor cells have a defective HR DNA repair pathway, when these cells are treated with PARP inhibitors, they are unable to effectively repair such DNA DSBs that accumulate from the generation of unrepaired DNA SSBs. These tumor cells resort to alternative error-prone DNA DSB repair mechanisms, resulting in chromatid instability, cell cycle arrest, and eventual apoptosis. In contrast, wild-type (BRCA1/2+) and BRCA1/2 heterozygous (BRCA1/2−) cells have an intact HR DNA repair pathway and therefore are able to repair such DSBs, thus maintaining chromosomal stability and cell viability. This model relies on the fact that tumor tissue in BRCA1/2 mutation carriers is HR deficient, providing a therapeutic window for selectively targeting such tumors (Figs. 1 and 2). Recent observations have provided further insights into the underlying mechanisms behind the specific targeting of BRCA1/2-deficient cells by PARP inhibitors. For example, Bryant et al showed
that PARP1 is also involved in HR repair at replication forks,\(^3^1\) possibly explaining the strong synthetic lethal relationship between PARP and BRCA1.\(^3^2\) It has also been suggested that PARP hyperactivity is not due to either increased DNA SSB repair or PARP induced at damaged replication forks,\(^3^3\) whereas deficiency of the NHEJ pathway factor p53-binding protein 1 (53BP1) was shown to abolish PARP inhibitor sensitivity in BRCA1-deficient cells.\(^3^4\) These new findings indicate that other factors may potentially contribute to the selective targeting of BRCA1/2-deficient cells by PARP inhibitors.

### Evidence Supporting a Synthetic Lethal Strategy With PARP Inhibitors in BRCA1/2-Mutated Cancers

#### Preclinical Evidence for PARP Inhibition in BRCA1/2-Mutated Cancers

Preclinical studies have been carried out to investigate the synthetic lethal relationship between PARP inhibitors and BRCA1/2-deficient cells. Farmer et al demonstrated that decreasing PARP1 expression with RNA interference resulted in reduced clonogenic survival of BRCA1/2-deficient embryonic stem cells compared with wild-type cells.\(^3^0\) Furthermore, clonogenic cell survival assays showed that BRCA1/2-deficient (BRCA\(^{-/-}\)) cell lines were much more sensitive to the potent PARP inhibitors KU0058684 and KU0058948, in contrast to heterozygous mutant (BRCA\(^{+/+}\)) or wild-type (BRCA\(^{+/+}\)) cells.\(^3^0\) Similarly, Bryant et al demonstrated that BRCA2-deficient V-C8 cells were profoundly sensitive to the PARP inhibitors AG14361 and NU1025, in contrast to the wild-type V79 cells.\(^3^5\)

These pivotal preclinical studies led us to design and conduct a first-in-human clinical trial of the PARP inhibitor olaparib in patients with advanced cancers, which was enriched with BRCA1/2 mutation carriers to evaluate this synthetic lethal strategy.\(^6\)

#### Proof-of-Concept Clinical Trials of a PARP Inhibitor in BRCA1/2-Deficient Carriers

Olaparib (AZD2281 [previously KU-0059436]; AstraZeneca) is a selective and potent PARP inhibitor with a half maximal inhibitory concentration (IC\(_{50}\)) in the nanomolar range for both PARP1 and PARP2 enzymes (Table 2).\(^6^9,3^6-4^9\) Although the phase 1 trial of olaparib initially enrolled patients with a range of advanced tumors, prospective enrichment of each dose escalation cohort with BRCA1/2 mutation carriers was carried out. After promising signals of antitumor activity, recruitment was then limited to patients with BRCA1/2 mutations during cohort expansion of this phase 1 trial.

Dose-limiting toxicities (DLTs) observed with olaparib included Common Terminology Criteria for Adverse Events (CTCAE) (version 3.0) grade 3 mood alteration, fatigue, and somnolence, as well as grade 4 thrombocytopenia, establishing the maximum tolerated dose (MTD) of olaparib at 400 mg twice daily. Olaparib-related toxicities resolved with drug discontinuation, with hematological toxicity being fully reversible on discontinuing the drug. The most common drug-related toxicities were grade 1 to 2 fatigue and gastrointestinal symptoms including nausea, with only a low incidence of drug-associated myelosuppression observed and no alopecia reported.

Overall, olaparib demonstrated a very acceptable side effect profile, especially when compared with conventional chemotherapies. Importantly, there were no obvious differences in toxicities observed between BRCA1/2 mutation carrier patients and the unselected patient population, supporting the predictions made from preclinical studies described above.\(^6,3^0,3^5\)

In this clinical trial, substantial and durable antitumor activity in patients with BRCA1/2-mutated cancers was observed, including those with ovarian, breast, and prostate cancers.\(^6\) No objective antitumor responses were noted in the unselected population. Of 50 patients with advanced BRCA1/2-mutated ovarian cancer treated in this trial (including those with primary peritoneal and fallopian tube carcinomas), there was an overall 46% clinical benefit rate, with 40% of patients achieving a complete response (CR) or partial response (PR) using Response Evaluation Criteria In Solid Tumors (RECIST) and/or Gynecologic Cancer InterGroup (GCIG) CA 125 tumor marker response, with a further 6% of patients experiencing prolonged RECIST disease stabilization lasting at least 4 months.\(^7\) The median duration of response was 28 weeks. Of note, a post hoc analysis demonstrated a significant association between the clinical benefit rate observed in these patients and the prior platinum-free interval across the platinum-sensitive, -resistant, and -refractory subgroups (69%, 45%, and 23%, respectively; \(P < .05\)).\(^7\)
| AGENT         | STUDY   | PHASE | NO. OF PATIENTS | PATIENT CHARACTERISTICS                                                                 | RR                                                                 | MEDIAN PFS, MONTHS | MEDIAN OS, MONTHS | SIGNIFICANT TOXICITIES                                                                 |
|--------------|---------|-------|----------------|-----------------------------------------------------------------------------------------|---------------------------------------------------------------------|-------------------|------------------|--------------------------------------------------------------------------------------------|
| Olaparib     | Fong 2009⁶ | 1     | 60             | Advanced solid tumors (enriched for BRCA1/2 mutations): 21 ovarian, 9 breast, 8 CRC, 4 melanoma, 4 sarcoma, 3 prostate | 9/60 patients, 9/19 with BRCA1/2 mutations (ovarian, breast, and prostate cancer) | NS                | NS               | 1 G3-4 anemia, 3 G3-4 lymphopenia, 2 G3-4 nausea, 1 G3-4 vomiting, 1 G3-4 fatigue, 1 G3-4 dizziness |
| Olaparib     | Fong 2010⁷ | 1     | 50             | Dose escalation and expansion phase cohort of above phase 1 trial in BRCA1/2 mutation carrier ovarian cancer | 20/50 (40%)                                                         | NS                | NS               | 4 (8%) G3-4 lymphopenia, 3 (6%) G3 nausea, 1 (2%) G3 neurocognitive change                  |
| Olaparib (400 mg bid or 100 mg bid) | Tutt 2010⁸ | 2     | 54             | BRCA1/2 mutation carrier breast cancer                                                      | 11/26 (42%) (400 mg bid) and 6/24 (25%) (100 mg bid)               | 5.7 mo (400 mg bid) and 3.7 mo (100 mg bid) | NS | 6 G3-4 fatigue, 5 G3-4 nausea, 3 G3-4 vomiting |
| Olaparib (400 mg bid or 100 mg bid) | Audeh 2010⁹ | 2     | 57             | BRCA1/2 mutation carrier ovarian cancer                                                      | 11/33 (33%) (400 mg bid) and 3/24 (13%) (100 mg bid)               | 5.8 mo (400 mg bid) and 1.9 mo (100 mg bid) | NS | 5 G3-4 nausea, 1 G3-4 fatigue, 4 G3-4 vomiting |
| Olaparib (400 mg bid) | Gelmon 2010¹⁰ | 2     | 90             | a) BRCA1/2 ovarian cancer (10)                                                            | 4/10                                                               | Breast cancer: 54 d; ovarian cancer: 219 d | NS | 7/90 G3-4 fatigue, 4/90 G3-4 dyspnea, 7/90 G3-4 anemia |
| Iniparib (BSI-201) | Kopetz 2008¹¹ | 1     | 23             | Advanced solid tumors                                                                    | 6 SD>2 mo                                                          | NS                | NS               | No MTD identified                                                                                     |
| MK-4287      | Sandhu 2010¹² | 1     | 59             | Advanced solid tumors; BRCA1/2 enriched 26 ovarian, 12 breast, 5 prostate                 | 9/59, including 5/19 evaluable ovarian cancer patients, 2/4 breast cancer patients | NS                | NS               | 7 G3-4 thrombocytopenia, 1 G3 neutropenia                                                                 |
| Veliparib (ABT-888) | Kummar 2009¹³ | 0     | 13             | Advanced solid tumors                                                                    | NS                                                                | NS                | NS               | Nil                                                                                                 |

**PARP INHIBITOR COMBINATION STUDIES**

| AG014699 + TMZ | Plummer 2006¹⁴ | 1     | 32             | Sarcoma, 3; melanoma, 15; CRC, 3; others, 8                                              | 1 CR, 2 PRs                                                        | NS                | NS               | 4 G3-4 neutropenia                                                                                      |
| AG014699 + TMZ | Plummer 2006¹⁵ | 2     | 46             | Melanoma                                                                                   | 7 PRs                                                             | NS                | NS               | 14/46 G4 thrombocytopenia; 19/46 G4 neutropenia; 1 toxic death due to febrile neutropenia; 4 hospitalized with myelosuppression; 18 patients required dose reduction of TMZ. |
| AGENT | STUDY | PHASE | NO. OF PATIENTS | PATIENT CHARACTERISTICS | RR | MEDIAN PFS, MONTHS | MEDIAN OS, MONTHS | SIGNIFICANT TOXICITIES |
|-------|-------|-------|----------------|-------------------------|----|-------------------|------------------|-----------------------|
| Carboplatin, gemcitabine ± iniparib (BSI-201) | O’Shaughnessy 201042 | 2 | 123 | Triple-negative breast cancer | 52.5% vs 32.3% (P = .023) | 5.9 mo vs 3.6 mo (P < .012) | 12.3 mo vs 7.7 mo (P = .014) | No significant differences in hematological/nonhematological toxicities between both arms. Most frequent G3-4 toxicities included neutropenia, thrombocytopenia, anemia, fatigue, leukopenia, and ALT increase. |
| Iniparib + TMZ | Blakely 201043 | 1 | 30 | Malignant glioma | NS | NS | NS | 1/30 LFT abnormality, 2/30 thrombosis, 2/30 lymphopenia |
| Iniparib + topotecan or gemcitabine or TMZ or carboplatin/paclitaxel | Mahany 200844 | 1b | 55 | NS | 1 CR, 5 PRs | NS | NS | No MTD identified |
| INO-1001 + TMZ | Bedikian 200945 | 1 | 12 | Melanoma | 1 PR | NS | NS | 3 G3-4 thrombocytopenia, 2 G3-4 neutropenia, 1 G3 LFT, 1 G3 VTE, 2 G3 fatigue, 2 G3 infection |
| Olaparib (200 mg bid) + wk paclitaxel | Dent 201046 | 2 | 19 | Triple/negative breast cancer | 7/19 | NS | NS | 1/19 G3 fatigue, 6/19 G3/4 neutropenia |
| Olaparib + cisplatin + gemcitabine | Giaccone 201047 | 1 | 23 | Advanced solid tumors including 8 NSCLC | 2/23 | NS | NS | 15/23 G3/4 neutropenia, requiring dose reduction of all drugs |
| Veliparib (ABT-888) + cyclophosphamide | Tan 201048 | 1 | 30 | 13 breast cancer, 8 ovarian cancer, 6 known BRCA | 1 PR | NS | NS | 6/30 with G3/4 neutropenia, 1/30 with G3 fatigue |
| Veliparib + TMZ | Isakoff 201049 | 2 | 41 | Metastatic breast cancer, 8 known BRCA | 7% overall 5/8 BRCA patients responded | 5.8 mo in BRCA patients vs 1.8 mo in non-BRCA patients | NS | 6/41 G3 thrombocytopenia, 9/41 G3 neutropenia |

PARP indicates poly(ADP-ribose) polymerase; RR, response rate; PFS, progression-free survival; OS, overall survival; CRC, colorectal cancers; NS, not stated; G, grade; bid, twice daily; ORR, objective response rate; MTD, maximum tolerated dose; TMZ, temozolomide; CR, complete response; PR, partial response; SD, stable disease; LFT, liver function tests; VTE, venous thromboembolism; NSCLC, non-small cell lung cancer; ALT, alanine aminotransferase; wk, weekly.
Two parallel open-label, multicenter, phase 2 studies of olaparib in germline BRCA1/2 mutation carriers with advanced breast (ICEBERG1) and ovarian (ICEBERG2) cancers have provided further support for this antitumor synthetic lethal approach (Table 2). \(^8\,\,^9\) Fifty-four patients with breast cancer in the ICEBERG1 study and 56 patients with ovarian cancer in the ICEBERG2 study were recruited sequentially to 2 non-randomized dose cohorts comparing olaparib at a dose of 100 mg twice daily (a pharmacodynamically active dose) and 400 mg twice daily (the previously established MTD). Overall, both phase 2 ICEBERG studies confirmed the tolerability of olaparib in BRCA1/2 mutation carriers, with mainly mild to moderate nausea, fatigue, and hematological events observed, which is in line with safety data from the phase 1 clinical trial.\(^6\,\,^9\)

The ICEBERG1 study reported an objective response rate (ORR) of 41% (11 of 27 patients) in BRCA1/2-mutated patients with breast cancer, who received olaparib at a dose of 400 mg twice daily, with one RECIST CR and 10 RECIST PRs reported, and a median progression-free survival (PFS) of 5.7 months (range, 4.6 months-7.4 months).\(^8\) This was in contrast to an ORR of 22% (6 of 27 patients) in patients with breast cancer who were treated with olaparib at a dose of 100 mg twice daily, with 6 RECIST PRs and a median PFS of 3.8 months (range, 1.9 months-5.5 months) reported.\(^9\)

In the ICEBERG2 study, BRCA1/2-mutated patients with ovarian cancer who received 400 mg twice daily of olaparib had a RECIST ORR of 33% (11 of 33 patients), compared with 13% (3 of 24 patients) for patients receiving olaparib at a dose of 100 mg twice daily.\(^9\) The median duration of response was 290 days (range, 126 days-506 days) for patients treated in the cohort receiving 400 mg twice daily, in contrast to 269 days (range, 169 days-288 days) for patients in the cohort receiving 100 mg twice daily.\(^9\)

Although both dose levels of olaparib demonstrated clinical activity in these 2 studies, the 400-mg dose of olaparib given twice daily appeared to be more efficacious with improved outcomes in this population of heavily pretreated BRCA1/2 patients with advanced cancer.\(^8\,\,^9\) This comparison should, however, be interpreted with caution because the allocation of patients to these doses was sequential and not randomized, and patients receiving olaparib at a dose of 100 mg twice daily had poorer prognostic features than those in the higher dose olaparib cohort. A randomized phase 2 trial comparing 400 mg twice daily of olaparib, 200 mg twice daily of olaparib, and pegylated liposomal doxorubicin in patients with BRCA1/2-mutated ovarian cancer has recently completed accrual (available at: www.clinicaltrials.gov; NCT00628251). These data are now in the public domain.

Overall, although the tumor response rates of 41% for patients with breast cancer and 33% for patients with ovarian cancer in both ICEBERG trials are impressive and provide proof-of-concept for this approach, these rates are still lower than those observed with other successful targeted therapies, including imatinib in gastrointestinal stromal tumors and gefitinib in lung cancer.\(^30\,\,^32\) This may reflect the more complex biology behind PARP inhibition and the urgent need for better predictive biomarkers. It is likely that these response rates will improve further through the use of robust assays of HR pathway integrity, rather than just BRCA1/2 genetic analyses. BRCA1/2 mutation status is thus better considered as an enrichment biomarker, rather than a predictive biomarker.\(^53\)

Although the ICEBERG2 BRCA1/2-mutated ovarian cancer study was not designed to compare differences in response between platinum-sensitive and platinum-resistant patient populations, responses to olaparib were observed in both cohorts, suggesting that resistance mechanisms to olaparib might only partly overlap with those for platinum chemotherapies. However, patient numbers were relatively small and thus the clinical implications of prior platinum-based chemotherapy for subsequent PARP inhibitor therapy require further study in a larger cohort of patients. Conversely, the sensitivity of BRCA1/2-mutated patients to various chemotherapies, especially platinum salts, after the development of resistance to PARP inhibitors needs to be examined. A recent small preliminary analysis in patients with BRCA1/2-mutated ovarian cancer has suggested that chemoresponsiveness, particularly with carboplatin and taxanes, is maintained in such patients after disease progression on olaparib.\(^54\)

Experience With Other PARP Inhibitors in BRCA1/2 Mutation Carriers

Another promising PARP inhibitor that has been studied in BRCA1/2 mutation carriers is MK-4827, a potent and oral PARP1/2 inhibitor with IC\(_{50}\) in the low nanomolar range (Table 2).\(^38\) At last
reporting, 59 patients with advanced solid tumors had been enrolled onto the phase 1 trial of MK-4827, with patient cohorts enriched with BRCA1/2 germline mutation carriers. Dose escalation involved the daily administration of MK-4827 from 30 mg to 400 mg. Pharmacodynamic analyses demonstrated target modulation from doses ≥80 mg with drug pharmacokinetics being dose proportional. MK-4827 was generally well tolerated. DLTs were observed in 4 patients and included grade 3 fatigue in a patient treated at a dose of 30 mg, reversible grade 3 pneumonitis in one patient treated at a dose of 60 mg, and 2 DLTs of reversible grade 4 thrombocytopenia in 2 of 6 patients treated at a dose of 400 mg daily. The MTD was established at a dose of 300 mg daily. Of 23 patients with germline BRCA1/2 mutations enrolled, 9 of 19 patients with ovarian cancer and 2 of 4 patients with breast cancer achieved a RECIST PR or had stable disease for ≥120 days. The study is currently recruiting patients with high-grade serous ovarian cancer and castration-resistant prostate cancer to explore the antitumor activity of MK-4827 in these sporadic tumors, which may potentially have an increased likelihood of acquired HR DNA repair defects.

Overall, these data clearly provide clinical validation for single-agent PARP inhibitor activity in patients with germline BRCA1/2 mutations. Multiple other trials of single-agent PARP inhibitors in patients with germline BRCA1/2-mutated cancers and sporadic malignancies, as well as clinical studies involving combination regimens, are currently ongoing, including AG014699 (Pfizer), veliparib (Abbott Laboratories), and iniparib (BiPar Sciences Inc). Table 2 summarizes the patient characteristics, toxicities, and efficacy results from reported clinical trials of PARP inhibitors.5-9,36-49 A critically important issue that requires continued vigilance remains the safety of chronic PARP inhibitor dosing, specifically with regard to the risk of drug-induced DNA damage and secondary tumorigenesis.

**Broadening the Therapeutic Scope for a Synthetic Lethal Strategy With PARP Inhibitors**

Beyond the immediate therapeutic application of PARP inhibitors in germline BRCA1/2-mutated cancers lies the broader prospect of treating a subgroup of sporadic tumors that display “BRCA
dness” and that carry abnormalities in other critical components of the HR DNA repair pathway, with potential synthetic lethal interactions with PARP inhibitors.55 BRCA
dness describes a phenomenon observed in certain sporadic cancers, which display clinical properties reminiscent of hereditary BRCA1/2-mutated cancers, but do not harbor inherited/germline BRCA1/2 mutations.56 Mechanisms responsible for this BRCA-like (BL) phenotype in sporadic tumors include the epigenetic silencing of BRCA and Fanconi anemia complementation group F (FANCF) genes through promoter methylation.56 BRCA1 gene inactivation via promoter methylation has been implicated in sporadic breast and ovarian cancers,57 whereas amplification of the EMSY gene, which encodes a BRCA2-interacting protein and suppresses BRCA2 transcription, has also been described.59

A recent study has suggested that germline and somatic BRCA1/2 mutations may occur in approximately 18% of ovarian cancers and in 23% of high-grade serous ovarian cancers, compared with previous reports that BRCA1/2 germline mutations only occur in 11% to 15% of unselected women with ovarian cancer.60-63 In this series, 9 of 21 (42.9%) BRCA1 and 2 of 7 (28.6%) BRCA2 mutations were demonstrated to be somatic.63 Loss of BRCA1/2 expression was also observed in 24 of 180 (13.3%) BRCA1/2 wild-type cancers, implicating other potential mechanisms (eg, methylation) for BRCA1/2 loss of expression. Overall, BRCA1/2 deficiency (defined as BRCA1/2 mutations or loss of expression) was present in 67 of 223 (30%) ovarian cancers and was significantly associated with improved PFS. These data suggest that there are a higher proportion of sporadic ovarian tumors, especially high-grade serous ovarian cancers, than previously estimated that display a BRCA
dness phenotype.

The first single-agent phase 2 trial of a PARP inhibitor to demonstrate antitumor activity of olaparib (at a dose of 400 mg twice daily) in high-grade sporadic serous ovarian cancer was recently presented by Gelmon et al at the 2010 American Society of Clinical Oncology Annual Meeting (Table 2).36 Of 63 women with ovarian cancer treated in the study, the objective RECIST response rate was 41.2% (7 of 17 patients) and 23.9% (11 of 46 patients), respectively, for patients with and without BRCA1/2 mutations. This study not only confirmed the tolerability and antitumor activity of olaparib in germline BRCA1/
2-mutated ovarian cancers as demonstrated in other phase 1/2 studies, but also showed the potential of PARP inhibitor therapy in sporadic high-grade serous ovarian cancer. Furthermore, in this trial, all patients had tumor biopsies at baseline as well as on day 57 and at the time of disease progression to assess olaparib activity; loss of heterozygosity; mutational changes; BRCA1/2 expression; and markers of response, including deep sequencing. These data will provide greater translational insights into the management of these groups of patients with PARP inhibitors in the near future. Apart from this study, a randomized placebo-controlled study of olaparib in patients with serous (sporadic) ovarian cancer at high risk of early recurrence is also ongoing (available at: www.clinicaltrials.gov; NCT00753545).64

PARP Inhibition in Triple-Negative Breast Cancer

An example of another important disease that may be treatable with a synthetic lethal strategy with PARP inhibitors is triple-negative (estrogen receptor-, progesterone receptor-, and human epidermal growth factor receptor 2 [HER2]-negative) breast cancer, which frequently shares clinical and pathological similarities with hereditary BRCA1-mutated breast cancers, suggesting a shared functional defect in a DNA repair pathway.56,65 A multicenter, open-label randomized phase 2 trial of gemcitabine and carboplatin, with or without the PARP inhibitor iniparib (BSI-201; BiPar Sciences), was recently reported (Table 2).42 This trial included 123 patients with metastatic triple-negative breast cancer who were randomized (1:1) to receive either gemcitabine and carboplatin, or gemcitabine with carboplatin and iniparib. Gemcitabine (1000 mg/m²) and carboplatin (area under the curve [AUC] of 2) were given on days 1 and 8, whereas iniparib (5.6 mg/kg intravenously, given biweekly) was administered on days 1, 4, 8, and 11 every 21 days.42 Crossover to the triple combination arm at the time of disease progression was permitted. There were highly significant improvements in efficacy parameters observed in patients receiving the iniparib triple combination, when compared with the control arm.42 The ORR increased from 32.3% (20 of 62 patients) in the chemotherapy alone arm to 52.5% (32 of 61 patients) in the triple combination arm \((P = .023)\). PFS also increased from 3.6 months to 5.9 months \((P = .012)\) and overall survival (OS) rose from 7.7 months to 12.3 months \((P = .014)\) in the triple combination arm. It is unclear whether these response rates are due to synthetic lethal interactions between triple-negative breast tumors and PARP inhibition, or whether iniparib is potentiating the chemotherapy by inhibiting repair of the chemotherapy-induced DNA damage. It should be noted that at last reporting, 30 of 62 patients randomized to the control arm crossed over to the iniparib triple combination arm at the time of disease progression.

The iniparib triple combination was well tolerated, with expected chemotherapy-related toxicities observed, but interestingly, no significant increases in hematological or nonhematological toxicities compared with the control arm for all parameters evaluated. This is in contrast with other combination studies involving PARP inhibitors, in which toxicities, especially hematological adverse events, have been observed (Table 2). This may potentially be due to the intermittent dosing schedule of the inhibitor used or, possibly, that iniparib is acting via independent mechanisms. Concerns have also been raised about the design of this study because the patients in the control arm were administered a suboptimal dose of carboplatin (AUC of 2 on days 1 and 8). This could have potentially led to a lower control arm ORR and PFS, in contrast to an ORR of approximately 40% and PFS of 6 months previously observed with other platinum-based regimens administered in this setting (eg, a carboplatin dose of AUC of 5 given every 3 weeks).66,67 Overall, however, this study supports the further evaluation of PARP inhibitors in triple-negative breast tumors in combination studies.

Nevertheless, it should be noted that in a different phase 2 study, no objective antitumor responses were observed in 15 patients with sporadic metastatic triple-negative breast cancer when treated with single-agent olaparib (400 mg bid) (Table 2).36 These patients had received a median of 3 (range, 1-6) prior chemotherapy regimens. The lack of responses may have been due to several factors, not least of which was the small number of patients evaluated and their extensive prior treatment, coupled with the fact that triple-negative breast cancer comprises a heterogeneous group of diseases.68 Olaparib (200 mg bid) was also administered in combination with paclitaxel (at a dose of 90 mg/m² weekly for 3 of 4
weeks) to patients with triple-negative breast cancer in a separate phase 1/2 study. An acceptable dose intensity of this regimen was not achieved because of toxicities (predominantly neutropenia) observed, resulting in dose delays. However, of 19 patients treated, 7 patients (37%) had confirmed PRs. Alternative doses and schedules are currently being pursued.

Other Potential Synthetic Lethal Strategies for PARP Inhibitors

Preclinical studies indicate that a defect in HR leads to BRCA-deficient cellular lethality with PARP inhibition. These studies also indicated that the loss of many other HR-related genes results in PARP inhibitor sensitivity. Since HR DNA repair is a complex process involving multiple components, loss of function of any critical cog in the repair machinery may potentially hinder DNA repair proficiency, including RAD51, RAD54, DSS1, replication protein A1 (RPA1), Nijmegen breakage syndrome 1 (NBS1), ataxia telangiectasia and Rad3-related (ATR), ataxia-telangiectasia mutated (ATM), Fanconi anemia complementation group D2 (FANCD2), Fanconi anemia complementation group A (FANCA), and Fanconi anemia complementation group C (FANCC) (Fig. 2). These may also confer selective sensitivity to PARP inhibition. Here, we examine some of the HR-related components that may be therapeutically targeted in HR-deficient cells.

The Fanconi Anemia Pathway

There are 13 Fanconi anemia (FA) genes, all of which encode proteins central to an integrated damage response network that recognizes and repairs DNA interstrand cross-links in cooperation with the HR DNA repair pathway (Fig. 2). Germline mutations in these genes confer chromosomal fragility and genomic instability and account for FA, a heterogeneous genetic disorder characterized by aplastic anemia, multiple congenital defects, and increased cancer susceptibility, in particular acute myelogenous leukemia, gynecologic cancers, and squamous cell carcinomas of the head and neck. Genetic and epigenetic disruptions of the FANC genes have been reported in a range of sporadic tumors. Somatic and inherited mutations in FANCC and Fanconi anemia complementation group G (FANCG) have been reported in pancreatic cancer, whereas epigenetic silencing of the FA-BRCA pathway by promoter methylation of the FANCF gene has been observed in several other sporadic cancers, including cervical, ovarian, and head and neck squamous cell carcinomas and non-small cell lung cancer. The genomic instability that results from the disruption of the FA-BRCA pathway may account for the underlying pathogenesis of these sporadic tumors, but importantly, may also select for sensitivity to DNA cross-linking agents, and forms the basis of the therapeutic rationale for targeting these tumors with PARP inhibitors.

Ataxia-Telangiectasia Mutated (ATM)

Ataxia-telangiectasia (A-T) is an autosomal recessive multisystem condition caused by mutations in the ATM gene located on chromosome 11 at the 11q22-q23 loci. Patients with A-T have an enhanced risk of developing lymphoid and epithelial malignancies, including breast cancer. Deletions and mutations of the ATM gene frequently characterize mantle cell lymphoma and have been found in 11% of B-cell chronic lymphocytic leukemia and in 46% of T-cell prolymphocytic leukemia. Furthermore, recent epidemiological studies have confirmed that heterozygous ATM mutations occur in 1% of the population and are associated with a 2-fold enhanced overall risk of breast cancer. ATM has multiple cellular functions, including a central role in DNA DSB damage signaling and response. The loss of function of ATM may thus impair HR repair proficiency and confer selective sensitivity to PARP inhibition. This has been demonstrated preclinically in ATM-deficient mantle cell lymphoma cell lines and xenografts, which were significantly more sensitive to olaparib when compared with ATM-proficient cell lines and xenografts.

Phosphatase and Tensin Homolog (PTEN)

The phosphatase and tensin homolog (PTEN) gene is one of the most commonly mutated or deleted tumor suppressor genes in cancer. In addition to its well-established role as a negative regulator of the phosphoinositide 3-kinase (PI3K)-AKT signaling cascade, PTEN has been shown to maintain chromosomal integrity through several mechanisms, including interactions with an integral centromeric binding and stabilizing protein CENP-C, as well as regulation of RAD51 transcription and modulation of cell cycle progression. In addition, PTEN
loss is associated with extensive centromeric breakage, diminished RAD51 expression, and defective DSB repair.\textsuperscript{83,86} Recent studies have provided mechanistic insights into the link between the loss of PTEN function and a compromised HR DNA repair pathway.\textsuperscript{87} Further preclinical studies have demonstrated a synthetic lethal relationship between PTEN-null tumors and PARP inhibition as a consequence of the impaired ability of these tumors to repair DSB via HR.\textsuperscript{86,88,89} Furthermore, the restoration of PTEN expression in certain PTEN-deficient cancer cell lines results in the return of PARP inhibitor resistance, supporting PTEN as a potential determinant of sensitivity to PARP inhibition. These data provide the rationale for the potential wider therapeutic application of PARP inhibitors in PTEN-deficient tumors.

If the loss of PTEN function associates with tumors harboring HR DNA repair defects and increased sensitivity to PARP inhibition, it may potentially lead to the use of PARP inhibitors in a wide range of cancers. For example, PTEN loss is common in prostate cancer and is associated with the presence of erythroblast transformation-specific gene rearrangements.\textsuperscript{90} In addition, from a series of 41 unselected, sporadic colorectal cancers (CRC), PTEN aberrations were found in approximately 20\% of cases, with allelic loss (in all or part of the PTEN gene) noted in a further 17\% of tumors.\textsuperscript{91} Importantly, in this study, all but one of the tumors with PTEN gene mutations were microsatellite stable, indicating that the PTEN mechanism of CRC tumorigenesis is distinct from that associated with MMR deficiency. PTEN is also mutated or deficient in 50\% to 80\% of endometrial cancers, whereas 20\% to 40\% have microsatellite instability (MSI).\textsuperscript{92} Further studies will be required to explore and substantiate the optimal criteria for patient selection and to discern the threshold level of PTEN loss that may predict responses to PARP inhibition.\textsuperscript{86}

Because PTEN is also a key negative regulator of the PI3K-AKT pathway,\textsuperscript{82} the concomitant targeting of this pathway with a PI3K or AKT inhibitor may potentiate the effects of PARP inhibition in BRCA-deficient tumors. A recent study assessed the combination of the PARP1 inhibitor AG14361 and PI3K inhibitor LY294002 in the BRCA1- and PTEN-mutated human breast cancer cell line MDA-MB-436. This strategy resulted in increased apoptosis and greater growth inhibition compared with AG14361 alone (\textit{P} < .01), supporting the utility of this potential strategy.\textsuperscript{93}

**MMR and PARP Inhibitor Sensitivity**

Apart from \textit{BRCA1} and \textit{BRCA2}, deficiencies in other mediators of DNA DSB repair pathways, such as the MRE11/RAD50/NBS1 (MRN) complex, have been shown to be synthetically lethal with PARP inhibition in preclinical models.\textsuperscript{55,94} Inactivating MRE11 mutations have been associated with MMR-deficient, MSI-positive CRC and endometrial cancers; laboratory studies have demonstrated frameshifts within the poly(T)11 microsatellite repeat sequence correlating with reductions in protein expression of MRE11 in up to 87\% of MSI-positive CRC.\textsuperscript{95} A recent preclinical study has shown that CRC cell lines with MRE11 mutation and loss of protein expression are highly sensitive to PARP inhibition with olaparib, suggesting that patients with MSI and MRE11-deficient CRC may benefit from treatment with PARP inhibitors.\textsuperscript{94} Germline mutations in MMR genes have also recently been shown to confer susceptibility to prostate cancer development.\textsuperscript{96}

**Predictive Biomarkers of Defective HR DNA Repair**

A critical challenge to broadening the therapeutic utility of PARP inhibitors is identifying the subgroup of patients with non-\textit{BRCA} mutation-related HR repair-deficient tumors. A recent study assessing the sensitivity of a panel of 95 cell lines to the PARP inhibitor olaparib showed that deleterious mutations in \textit{BRCA1}/\textit{2} genes were associated with only a small subset of highly sensitive cell lines, supporting the presence of other factors that determine olaparib responsiveness.\textsuperscript{97} Developing validated and readily applicable assays to delineate molecular determinants of response in these sporadic tumors with acquired HR defects is now critically important.

**Array-Based Assays**

Array-based strategies such as gene expression profiling\textsuperscript{98-101} or comparative genomic hybridization (CGH) have been evaluated to define distinct \textit{BRCA1}/\textit{2}-related profiles in ovarian and breast cancers.\textsuperscript{102,103} The hallmark of \textit{BRCA1}/\textit{2}-deficient tumors is an increased level of genomic aberrations by virtue of the inherent impairment in DNA repair. CGH arrays evaluate gross patterns of chromosomal gain and
deletions, and have been used to define distinct genetic signatures for sporadic and BRCA1/2-mutated breast tumors. A BRCA1 CGH molecular classifier has demonstrated some predictive accuracy in assigning tumors as either BRCA1 or sporadic on the basis of their genetic profile, although validation of this is still ongoing.\textsuperscript{103} In contrast, the BRCA2-mutated breast cancer CGH classifier has shown a very similar genomic profile to sporadic tumors and to date, has not been able to accurately discriminate BRCA2-mutated tumors from sporadic breast tumors.\textsuperscript{102} Overall, the objective molecular characterization of tumor material from patients with array CGH may generate a more accurate and precise estimation of an individual’s risk of carrying a BRCA-related mutation, in contrast to the currently utilized estimation of risk based on an individual’s family history.\textsuperscript{103}

One of the most promising array-based assays in epithelial ovarian cancer is a BRCA\textit{ness} gene expression profile with a reported 94% accuracy in discriminating platinum-sensitive from platinum-resistant disease.\textsuperscript{104} This gene profile is based on a 60-gene signature identified using genome-wide hierarchical clustering from a published microarray data set of 61 patients: 34 with BRCA1/2 germline mutations and 27 with sporadic ovarian cancer. The predictive accuracy of this gene profile was validated initially in 10 tumor biopsies from 6 patients with germline BRCA1/2 mutations and subsequently in 70 patients with sporadic ovarian cancer to ascertain the performance of the profile in defining platinum responsiveness, disease-free interval, and OS. This BRCA\textit{ness} mRNA expression profile accurately discriminated platinum sensitivity from platinum resistance in 8 of 10 biopsy specimens from patients with germline BRCA1/2 mutations. Of note, assignment of BL versus non-BL (NBL) profiles in these 8 cases also corresponded with the presence of either BRCA-mutated or BRCA-revertant (ie, reversion of the BRCA genotype with re-establishment of BRCA function) BRCA gene status. Furthermore, in 2 tumor biopsies, the BRCA\textit{ness} profile tracked the development of second BRCA mutations that restored BRCA function as the patient developed resistance to treatment.\textsuperscript{105,106} When used in the 70 sporadic cases of ovarian cancer, the BRCA\textit{ness} profile was able to segregate patients into those with a BL profile with a DFS of 34 months and an OS of 72 months, versus a group with an NBL profile with a DFS of 15 months and an OS of 41 months. The improved outcomes seen in patients with an identifiable BL phenotype is in keeping with the increased sensitivity to platinum chemotherapies, longer treatment-free intervals, and the better prognosis observed in patients with germline BRCA1/2-mutated ovarian cancer.\textsuperscript{107,108} This lends support for the validity of this BRCA\textit{ness} expression profile. Other strategies utilizing proteomics and expression array-based approaches to identify biomarker profiles of BRCA\textit{ness} are also currently being evaluated.\textsuperscript{109} There are of course multiple questions and challenges that need to be addressed with these assays, such as whether formalin-fixed paraffin-embedded material may be used in such analyses. It is clear that these biomarker assays require further validation and clinical qualification in prospective clinical trials before we can ascertain if they are the answer to our biomarker needs.

**RAD51 and γH2AX Foci Functional Assays**

Histone H2AX and RAD51 are key proteins involved in the DNA repair pathway.\textsuperscript{17,110} Histone H2AX is rapidly phosphorylated to γH2AX at the site of DNA DSBs and serves as a focus for the convergence of a host of other DNA repair proteins, including RAD51. γH2AX foci formation has therefore been utilized preclinically and in clinical samples as a measure of the extent of DNA damage.\textsuperscript{6,111} RAD51 is a key DNA repair protein that acts downstream of many of the proteins that make up the HR DNA repair pathway. RAD51 is recruited to sites of DNA damage, where it forms distinct nuclear foci in the setting of proficient HR repair when tumor cells are exposed to DNA-damaging agents. The ability of tumor cells to form RAD51 foci in the setting of exposure to DNA-damaging agents serves as a useful in vitro biomarker for assessing HR repair pathway integrity.\textsuperscript{30} The degree of RAD51 foci formation provides a functional readout of HR proficiency without a need to pinpoint the specific underlying component of the pathway that is disrupted.\textsuperscript{112} This is of particular value given the large number of genes, interactions, and complexities of this pathway.

In an endeavor to identify the subgroup of sporadic cancers with acquired functional HR defects that may potentially benefit from PARP inhibitors, several groups have focused on the development and clinical application of RAD51-based functional assays of HR. A prospective pilot study evaluating immunofluorescence-based RAD51 foci formation in 7 fresh breast tumor biopsies, which were irradiated ex
vivo, demonstrated for the first time the feasibility of subdividing tumors into RAD51 foci-positive and defective tumors based on their DNA damage response proficiency. A much larger prospective study examining similar identifiers of DNA damage response involved 60 patients with primary breast cancer receiving neoadjuvant epirubicin and cyclophosphamide. Quantification of 4 HR-related proteins, namely BRCA1, γH2AX, conjugated ubiquitin, and RAD51 foci, in paired tumor biopsies prior to and after the first cycle of treatment was undertaken as a strategy of assessing HR DNA repair competence as a predictive biomarker for chemosensitivity. The presence of BRCA1-positive baseline foci and the presence of either baseline or chemotherapy-induced RAD51 foci inversely correlated with response to chemotherapy. Conversely, proficient DNA repair indices based on all 4 markers correlated with drug resistance, supporting the hypothesis that DNA damage response competency predicts for reduced tumor responses to DNA damaging agents.

Mukhopadhyay et al have also developed a functional HR assay based on RAD51 foci formation by immunofluorescence, using primary cultures of epithelial ovarian cancer cells derived from ascites and pleural fluid obtained from patients undergoing debulking surgery. Twenty-five cultures were evaluated for γH2AX and RAD51 foci quantification, as well as cytotoxicity, after exposure to the PARP inhibitor AG014699. It was reported that 64% of the ovarian cancer cell cultures demonstrated HR deficiency, based on the lack of RAD51 foci formation, whereas 93% of these HR-deficient samples were sensitive to the cytotoxic effects of the PARP inhibitor AG014699. Of the 9 (36%) samples that resulted in an increase in RAD51 foci (therefore HR competent), none showed sensitivity to AG014699. These results will need to be validated in the setting of a clinical trial.

One of the drawbacks of the functional RAD51-based HR assays reported to date has been the failure to consider the cell cycle when quantifying the formation of RAD51 foci. This could potentially account for false-negative RAD51 scores if the cell is not in the S-phase of the cell cycle. Other issues relate to the need for a standardized and automated scoring system for broader and uniform application. The requirement for fresh tissue may also limit clinical application. These assays are however promising and with further validation could potentially be utilized as a patient enrichment biomarker in prospective clinical trials.

Circulating tumor cells (CTCs) have also been used to monitor γH2AX induction after treatment with DNA-damaging agents and offer a rapid method of assessing target modulation and anticancer effect in selected patients. Similar studies evaluating RAD51 foci formation and other DNA repair proteins in CTCs and tumor specimens with multicolor immunofluorescence are ongoing. Easy access to CTCs in many advanced tumors offers a unique platform with which to explore predictive biomarkers of response and to conduct longitudinal studies for interrogating the mechanistic basis of acquired resistance to PARP inhibitors. An ongoing debate with CTCs is whether their biological response to DNA damage truly represents the tumor as a whole. Preclinical and clinical validation studies involving the development of CTCs in this role are currently underway and will be essential to their future use as tumor surrogates.

CTC DNA and circulating tumor DNA in plasma can also be analyzed for the methylation status of the BRCA gene promoter using methylation-specific polymerase chain reaction with bisulfite-converted DNA. This can also be extracted directly from formalin-fixed paraffin-embedded tumor samples. Overall, identifying the patients who have acquired HR defects through a variety of epigenetic and genetic mechanisms remains a critically important challenge and a major focus of ongoing research.

Key Challenges in the Future Development of PARP Inhibitors

Understanding Mechanisms of PARP Inhibitor Sensitivity and Resistance

Understanding resistance to targeted therapies offers insights into the biology of tumors, the effects of treatments, and their molecular mechanisms of sensitivity and resistance (Fig. 3). Potentially, it also provides the opportunity to adapt strategies for current therapies (eg, changing schedules or doses) or to develop new therapies to circumvent resistance. Although the response rates to PARP inhibition in selected populations are encouraging, with evidence of durable responses noted in a significant proportion of patients, understanding resistance to PARP inhibition is crucial to improve outcomes from this therapy.

Studies in cancer cell lines have shown that secondary mutations in the BRCA2 gene contribute to
resistance to PARP inhibitors and cisplatin.\textsuperscript{105,106,123} Consistent with the cell line data, secondary mutations of \textit{BRCA1} and \textit{BRCA2} have been reported in cisplatin-resistant ovarian cancer samples from \textit{BRCA1}/2 mutation carriers, although it has not been reported whether secondary mutations of \textit{BRCA1}/2 occur in clinical samples of PARP inhibitor-resistant cancers.\textsuperscript{105,106,123,124} Secondary mutations may restore BRCA function and HR DNA repair in the face of PARP inhibition to maintain genomic integrity (Fig. 3B). For example, the CAPAN1 pancreatic cancer cell line has a deletion mutation in \textit{BRCA2}, resulting in sensitivity to treatment with both PARP inhibitors and platinum chemotherapy.\textsuperscript{125} Repeated exposure to the PARP inhibitor KU0058948 led to the development of 1000-fold resistance to PARP inhibition,\textsuperscript{105} associated with reconstitution of HR function, as evidenced by the formation of RAD51 foci after exposure to ionizing radiation. Sequencing of the \textit{BRCA2} gene in these resistant cell line variants revealed restoration of the reading frame for the gene, and regeneration of a functioning BRCA2 protein. Similar findings were made in samples from \textit{BRCA2} mutation carriers who became resistant to platinum-based chemotherapy.\textsuperscript{105} These findings are supported by data from other studies, which also reported restoration of \textit{BRCA1} or \textit{BRCA2} function as a result of a secondary mutation in the affected gene.\textsuperscript{106,123,124} These preliminary data now need confirmation in larger studies.

Although resistance to platinum compounds and PARP inhibitors may both be mediated by secondary mutations, which restore BRCA function, more complex molecular circuitry is likely to be involved (Fig. 3). For example, in \textit{BRCA1}/2 mutation carrier patients with ovarian cancer treated in the phase 1 clinical trial of olaparib, not all platinum-sensitive patients responded, whereas antitumor responses were still noted in platinum-resistant patients.\textsuperscript{7} Both of these facts suggest that other mechanisms of resistance to PARP inhibition are relevant. The P-glycoprotein efflux pump has been implicated in resistance to chemotherapy, and recent evidence also suggests a role for it in the development of resistance to PARP inhibitors (Fig. 3C).\textsuperscript{126} Other potential mechanisms for resistance may include compensatory upregulation of other proteins mediating DNA repair, or other canonical mechanisms of cancer progression, such as PI3K-AKT or Ras-Raf pathway protein abnormalities, as
well as mutations in PARP itself leading to altered drug-target interactions (Figs. 3D-3F).

Enhancing the Therapeutic Potential of PARP Inhibitors

Radiotherapy and DNA cross-linking agents such as platinum compounds confer their antitumor effects by inducing DNA damage, which, if left unrepaired, leads to tumor cell death. However, treatment resistance often occurs because of an intrinsic or acquired ability of tumor cells to repair the DNA damage incurred.

Because PARP inhibition hinders the ability of the cell to respond to DNA damage, many groups have sought to use PARP inhibitors to enhance the cell killing of existing cytotoxic chemotherapy. However, several issues still need to be addressed with this strategy. The optimal PARP inhibitor-chemotherapy drug combination remains to be established, with a wide range of combination studies currently ongoing (Table 2). Additional challenges of concurrent treatment relate to the establishment of the optimal dose and schedule of both the PARP inhibitor and cytotoxic agent to optimize efficacy while minimizing toxicity and considerations of whether to give low-dose cytotoxics with full-dose PARP inhibition or vice versa. Additive or synergistic cytotoxic effects generated through such combinations of treatment may potentially permit lower doses of chemotherapy to be utilized in conjunction with standard doses of PARP inhibitor to achieve similar benefits. Moreover, an intermittent rather than continuous schedule may be necessary for these combinations. A detailed account of the utility of PARP inhibitors in combination with other DNA-damaging agents is beyond the scope of this review, but the reader is directed to Table 2 and the following reviews, which discuss this important issue.

Moving away from the more traditional approach of combining a targeted agent with conventional cytotoxics or radiotherapy, the next step forward in the development of PARP inhibitor regimens is likely to involve rational combinations with targeted agents, with particular emphasis on blocking the HR pathway. Among the potential agents of interest for combination therapies are histone deacetylase inhibitors, the tyrosine kinase inhibitor imatinib, proteasome inhibitors, and cyclin-dependent kinase 2 inhibitors. These agents have been shown to reduce RAD51 foci formation and lead to HR repair perturbation with corresponding enhanced cytotoxic effects when combined with DNA-damaging agents or PARP inhibitors preclinically. The concern with the development of these combinations is the potential abrogation of regulatory pathways required for normal cellular functions and the potential subsequent narrowing of the therapeutic window.

PARP Inhibitors in Chemoprophylaxis

As discussed earlier, patients with BRCA1/2 germline mutations have a significantly increased lifetime risk of developing cancers, in particular breast, ovarian, pancreatic, and prostate cancers. Despite cancer prevention strategies including prophylactic risk-reducing surgery and chemoprevention, many BRCA1/2 mutation carriers will still develop BRCA mutation-related cancers. Mitigating cancer risks in these patients remains a priority and continues to be an unmet clinical need. PARP inhibitors have demonstrated the ability to selectively kill BRCA2-deficient cells in vivo while sparing normal tissue with heterozygous BRCA2 loss. These data support the study of PARP inhibitors in chemoprophylaxis studies to kill off clones of dysplastic cells with biallelic BRCA2 loss prior to the development of cancer. Nonetheless, there are still unresolved concerns about the long-term safety of PARP inhibition, in particular the effects of chronic inhibition of a DNA repair pathway leading to mutagenesis and the development of secondary malignancies, such as myelodysplasia or acute leukemia. Further chronic exposure safety data from the use of these agents are required before this approach is adopted. An alternative potential strategy to consider is repeated short courses of exposure to PARP inhibition.

Conclusions

The synthetic lethal targeting of DNA repair pathways, as exemplified by PARP inhibitors, in cancers bearing HR DNA repair defects is showing considerable potential for delivering selective tumor cell kill while sparing normal cells, and offers a scientifically rational and potentially broad clinical application in oncology. Several challenges related to the development of these inhibitors remain, including the identification of robust predictive biomarkers of HR deficiency in cancers. The dissection of the underlying mechanisms of PARP inhibitor
resistance and establishment of optimal drug combinations and strategies for chemoprophylaxis with these therapies remain high priorities. It is important to be aware that different PARP inhibitors may have varying potencies on individual members of the PARP superfamily and also affect other targets, resulting in distinct toxicity and efficacy profiles. In the future, it is envisioned that this tumor-specific synthetic lethal strategy with PARP inhibitors may potentially be utilized against cancers with similar molecular defects but diverse anatomical origins.118 Such a paradigm shift in drug discovery may crucially bring us closer to our ultimate goal of personalized medicine. ■

References

1. Dobzhansky T. Genetics of natural populations. XIII. Recombination and variability in populations of Drosophila pseudobscura. Genetics. 1946;31:269-290.

2. Kaelin WG Jr. The concept of synthetic lethality in the context of anticancer therapy. Nat Rev Cancer. 2005;5:689-698.

3. de Bono JS, Tolcher AW, Rowinsky EK. The future of cytotoxic therapy: selective cytotoxicity based on biology is the key. Breast Cancer Res. 2003;5:154-159.

4. Ooi SL, Pan X, Peyser BD, et al. Global synthetic-lethality analysis and yeast functional profiling. Trends Genet. 2006;22:56-63.

5. Iorns E, Lord CJ, Turner N, et al. Utilizing RNA interference to enhance cancer drug discovery. Nat Rev Drug Discov. 2007;6:556-568.

6. Feng PC, Boss DS, Yap TA, et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med. 2009;361:123-134.

7. Feng PC, Yap TA, Boss DS, et al. Poly-(ADP-ribose) polymerase inhibition: frequent durable responses in BRCA carriers ovarian cancer correlating with platinum-free interval. J Clin Oncol. 2010;28:2512-2519.

8. Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet. 2010;376:235-244.

9. Audeh MW, Carnmichael J, Penston RT, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer: a proof-of-concept trial. Lancet. 2010;376:245-251.

10. Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature. 2001;411:366-374.

11. Kinsella TJ. Coordination of DNA mismatch repair and base excision repair: a genetic and molecular basis of heterogeneity. Nat Rev Genet. 2009;10:756-768.

12. Cleaver JE, Lam ET, Revet I. Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. Nat Rev Genet. 2009;10:756-768.

13. Cleaver JE. Cancer in xeroderma pigmentosum and related disorders of DNA repair. Nat Rev Cancer. 2005;5:564-573.

14. Shah SN, Hile SE, Eckert KA. Defective mismatch repair, microsatellite mutation bias, and variability in clinical mismatch repair phenotypes. Cancer Res. 2010;70:431-435.

15. Jirinyc J. The multifaceted mismatch repair system. Nat Rev Mol Cell Biol. 2006;7:335-346.

16. Gudmundsdottir K, Ashworth A. The roles of BRCA1 and BRCA2 and associated proteins in the maintenance of genomic stability. Oncogene. 2005;25:5584-5594.

17. O’Donovan PJ, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis. 2010;31:961-967.

18. D’Andrea AD. Susceptibility pathways in Fanconi’s anemia and breast cancer. N Engl J Med. 2010;362:1909-1919.

19. Lieber MR. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu Rev Biochem. 2010;79:181-211.

20. Ashworth A. A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. J Clin Oncol. 2008;26:3785-3790.

21. Ame JC, Spenhauer C, de Munza G. The PARP superfamily. Bioessays. 2004;26:882-893.

22. Shieh WM, Ame JC, Wilson MV, et al. Poly(ADP-ribose) polymerase null mouse cells synthesize ADP-ribose polymers. J Biol Chem. 1998;273:30869-30874.

23. Ame JC, Rolli V, Schreiber V, et al. PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase. J Biol Chem. 1999;274:17860-17868.

24. Trucco C, Rolli V, Oliver FJ, et al. A dual approach in the study of poly(ADP-ribose) polymerase: in vitro random mutagenesis and generation of deficient mice. Mol Cell Biochem. 1999;193:53-60.

25. Venkitaraman AR. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell. 2002;108:171-182.

26. Lord CJ, Ashworth A. RAD51, BRCA2 and DNA repair: a partial resolution. Nat Struct Mol Biol. 2007;14:461-462.

27. Wooster R, Weber BL. Breast and ovarian cancer. N Engl J Med. 2003;348:2339-2347.

28. Haber JE. DNA recombination: the replication connection. Trends Biochem Sci. 1999;24:21-27.

29. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005;434:917-921.

30. Bryant HE, Petermann E, Schultz N, et al. PARP is activated at stalled forks to mediate Mre11-dependent replication restart and recombination. EMBO J. 2009;28:2601-2615.

31. Hellday T. Homologous recombination in cancer development, treatment and development of drug resistance. Carcinogenesis. 2010;31:955-960.

32. Gottipati P,Vischioni B, Schultz N, et al. Poly(ADP-ribose) polymerase is hyperactivated in homologous recombination-defective cells. Cancer Res. 2010;70:5389-5398.

33. Bunting SF, Callen E, Wong N, et al. S3BP1 inhibits homologous recombination in Brca1-deficient cells by blocking resection of DNA breaks. Cell. 2010;141:243-254.

34. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005;434:913-917.

35. Gelmon KA, Hirte HW, Robidoux A, et al. Can we define tumors that will respond to PARP inhibitors? A phase II correlative study of olaparib in advanced serous ovarian cancer and triple-negative breast cancer [abstract]. J Clin Oncol. 2010;28(15 suppl): Abstract 3002.

36. Kopez S, Mita MM, Mok I, et al. First in human phase I study of BSI-201, a small molecule inhibitor of poly ADP-ribose polymerase (PARP) in subjects with advanced solid tumours [abstract]. J Clin Oncol. 2008; 26(suppl): Abstract 3577.

37. Sandhu SK, Wenham RM, Wilding G, et al. First-in-human trial of a poly(ADP-ribose) polymerase (PARP) inhibitor MK-4827 in advanced cancer patients (pts) with anti-tumor activity in BRCA-deficient and sporadic ovarian cancers [abstract]. J Clin Oncol. 2010;28(15 suppl): Abstract 3001.

38. Kummar S, Kinders R, Gutierrez ME, et al. Phase 0 clinical trial of the poly (ADP-ribose) polymerase inhibitor ABT-888 in patients with advanced malignancies. J Clin Oncol. 2009;27:2705-2711.

39. Plummer R, Jones C, Middleton M, et al. 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.

40. Plummer R, Lorigan P, Evans J, et al. First and final report of a phase II study of the poly (ADP-ribose) polymerase (PARP) inhibitor, AG014699, in combination with temozolomide in patients with metastatic melanoma (MM) [abstract]. J Clin Oncol. 2006;24(18 suppl): Abstract 3013.

41. O’Shaughnessy J, Osborne C, Pippen J, et al. Final efficacy and safety results of a phase II study of the PARP inhibitor iniparib (BSI-201) in combination with gemcitabine/carboplatin in metastatic triple negative breast cancer [abstract]. In: Proceedings of the 35th European Society of Medical Oncology; October 8-12, 2010; Milan, Italy. Abstract LBA11.
43. Blakely JO, Ye X, Grossman SA, et al. Poly (ADP-ribose) polymerase-1 (PARP1) inhibitor BSI-201 in combination with temozolomide (TMZ) in malignant glioma [abstract]. J Clin Oncol. 2010;28(15 suppl): Abstract 1212.

44. Mahany JJ, Lewis N, Heath EI, et al. A phase IB study evaluating BSI-201 in combination with chemotherapy in subjects with advanced solid tumors [abstract]. J Clin Oncol. 2008;26(suppl): Abstract 3579.

45. Bediakian AY, Padapadosus NE, Kim KB, et al. A phase IB trial of intravenous INO-1001 plus oral temozolomide in subjects with unresectable stage-III or IV meta-

46. Dent RA, Lindeman GI, Clemons M, et al. Safety and efficacy of the oral PARP inhibitor olaparib (AZD2281) in combination with paclitaxel for the first- or second-line treatment of patients with metastatic triple-negative breast cancer: results from the safety cohort of a phase I/II multicenter trial [abstract]. J Clin Oncol. 2010;28(15 suppl): Abstract 1019.

47. Giacone G, Rajan A, Kelly RJ, et al. A phase I combination study of olaparib (AZD2281, KU-0059436) and cisplatin (C) plus gemcitabine (G) in adults with solid tumors [abstract]. J Clin Oncol. 2010;28 (15 suppl): Abstract 3027.

48. Tan AR, Gibbon D, Stein MN, et al. Preliminary results of a phase I trial of ABT-888, a poly(ADP-ribos) polymerase (PARP) inhibitor, in combination with cyclophosphamide [abstract]. J Clin Oncol. 2010;28(15 suppl): Abstract 3000.

49. Isakoff SJ, Overmoyer B, Tung NM, et al. A phase II trial of the PARP inhibitor veliparib (ABT-888) and temozolomide for metastatic breast cancer [abstract]. J Clin Oncol. 2010;28(15 suppl): Abstract 1019.

50. Chan SL, Mok T. PARP inhibition in BRCA-mutated breast and ovarian cancers. Lancet. 2010;375:211-213.

51. Darr TJ, jegers von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med. 2002;347:472-480.

52. Mok TS, Wu YL, Thongprasert S, et al. Poly(ADP-ribose) polymerase (PARP) inhibitors in platinum-resistant ovarian cancer: a phase III randomized placebo-controlled trial. J Clin Oncol. 2009;27:565-571.

53. Yap TA, Sandhu SK, Workman P, et al. Envisioning the future of early anticancer drug development. Nat Rev Cancer. 2010;10:514-523.

54. Ang J, Yap TA, Fong PC, et al. Preliminary experience with the use of chemotherapeutic (CT) following treatment with olaparib, a poly(ADP-ribose) polymerase (PARP) inhibitor, in patients with BRCA1/2-deficient ovarian cancer (BDOC) [abstract]. J Clin Oncol. 2010;28(15 suppl): Abstract 5041.

55. McCabe N, Turner NC, Lord CJ, et al. Deficiency in the repair of DNA damage by homologous recombination and sensitivity to poly(ADP-ribose) polymerase inhibitors. Cancer Res. 2006;66:8109-8115.

56. Turner N, Tutt A, Ashworth A. Hallmarks of ‘BRCA’ness’ in sporadic cancers. Nat Rev Cancer. 2004;4:814-819.

57. Esteller M, Silva JM, Dominguez G, et al. Poly(ADP-ribose) polymerase inactivation and BRCA1 inactivation in sporadic breast and ovarian tumors. J Natl Cancer Inst. 2000;92:564-569.

58. Baldwin RL, Nemeth E, Tran H, et al. BRCA1 promoter region hypermethylation in ovarian carcinoma: a population-based study. Cancer Res. 2000;60:5329-5333.

59. Hughes-Davies L, Huntsman D, Ruas M, et al. EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer. Cell. 2003;115:523-535.

60. Risch HA, McLaughlin JR, Cole DE, et al. Population BRCA1 and BRCA2 mutation frequencies and cancer penetrances: a kin-

61. Risch HA, McLaughlin JR, Cole DE, et al. Prevalence of germline BRCA1 and BRCA2 mutations in a population series of 649 women with ovarian cancer. Am J Hum Genet. 2001;68:700-710.

62. Pal T, Permutt-Wey J, Betts JA, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005;104:2807-2816.

63. Hennessy BT, Timms KM, Carey MS, et al. Somatic mutations in BRCA1 and BRCA2 could explain the number of patients that benefit from poly (ADP-ribose) polymerase inhibitors in ovarian cancer. J Clin Oncol. 2010;28:3570-3576.

64. Carden CP, Yap TA, Kaye SB. PARP inhhi-

65. Turner NC, Reis-Filho JS, Basal-like breast cancer and the BRCA1 phenotype. Oncogene. 2006;25:5846-5853.

66. Sirohi B, Arnedos M, Popat S, et al. Platinum-based chemotherapy in triple-negative breast cancer. Ann Oncol. 2008;19: 1847-1852.

67. Uhm JE, Park YH, Yi SY, et al. Treatment outcomes and clinicopathologic characteristics of triple-negative breast cancer patients who received platinum-containing chemothera-

68. Reis-Filho JS, Tutt AN. Triple negative tumours: a critical review. Histopathology. 2008;52:108-118.

69. Auerbach AD, Allen RG. Leukemia and preleukemia in Fanconi anemia patients. A review of the literature and report of the International Fanconi Anemia Registry. Cancer Genet Cytogenet. 1991;51:1-12.

70. Alter BP. Fanconi’s anemia and malignancies. Am J Hematol. 1996;53:99-110.

71. van der Heijden MS, Yeo CJ, Hruban RH, et al. Disruption of the Fanconi anemia-FANCF: disruption of Fanconi Anemia-Related Complementation Group C. J Clin Oncol. 2003;21:2305-2311.

72. Yap TA, Garrett MD, Walton MI, et al. Pathogenicity of the P82R frameshift mutation in FANCA. J Clin Oncol. 2003;21:2943-2949.

73. Taniguchi T, Tischkowitz M, Ameziane N, et al. Disruption of the Fanconi anemia-BRCA pathway in isocapane-sensitive ovarian tumors. Nat Genet. 2003;32:565-570.

74. Neri P, Morazzoni P, Scaltriti M, et al. Neoplastic transformation of mouse embryonic fibroblasts by a truncated BRCA1 mutant. Nature Genet. 2003;34:221-224.

75. Xu Y, Saccone F, Tafliger S, et al. Somatic inactivating mutations of the BRCA1 gene in sporadic breast cancer. Nat Genet. 1997;17:96-99.
olaparib in homologous recombination deficient (HRD) MRE11 mutant microsatellite instable (MSI) colorectal cancer [abstract]. In: 20th Annual AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; November 15-19, 2009; Boston, MA. Abstract A114.

95. Miquel C, Jacob S, Grandjouan S, et al. Frequent alteration of DNA damage signalling and repair pathways in human colorectal cancers with microsatellite instability. Oncogene. 2007;26:5919-5926.

96. Grindecad EM, Moller P, Eeles R, et al. Germline mutations in mismatch repair genes associated with prostate cancer. Cancer Epidemiol Biomarkers Prev. 2009;18:2460-2467.

97. Dry JR, Lau A, Knights C, et al. Identifying pre-clinical predictive biomarkers for the PARP inhibitor olaparib [abstract]. In: Proceedings of the 101st Annual Meeting of the American Association for Cancer Research; April 17-21, 2010; Washington, DC. Abstract 3497.

98. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A. 2003;100:8418-8423.

99. Jazaeri AA, Yee CJ, Sotiriou C, Brantley lan BY, et al. Gene expression profile of BRCA1 and BRCA2-linked, and sporadic ovarian cancers. J Natl Cancer Inst. 2002;94:990-1000.

100. Hedenfalk I, Duggan D, Chen Y, et al. Gene-expression profiles in hereditary breast cancer. N Engl J Med. 2001;344:519-526.

101. Hedenfalk IA. Gene expression profiling of hereditary and sporadic ovarian cancers reveals unique BRCA1 and BRCA2 signatures. J Natl Cancer Inst. 2002;94:960-961.

102. van Beers EH, van Beers EH, Tielen IH, et al. Gene expression profiles of BRCA1-linked, BRCA2-linked, and sporadic ovarian cancers. J Natl Cancer Inst. 2003;95:228-237.

103. Joosse SA, van Beers EH, Luu CM, et al. Utility of DNA repair protein focci for the detection of putative BRCA1 pathway defects in breast carcinomas. Mol Cancer Res. 2009;7:1304-1309.

104. Akasaka H, Koizumi H, Koihe A, et al. Prediction of breast cancer sensitivity to neoadjuvant chemotherapy based on status of DNA damage repair proteins. Breast Cancer Res. 2010;12:R17.

105. Mukhopadhyay A, Elattar A, Cerbinskaite A, et al. Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. Clin Cancer Res. 2010;16:2344-2351.

106. Wang LH, Pfister TD, Patchen RE, et al. Monitoring drug-induced gammaH2AX as a pharmacodynamic biomarker in individual circulating tumor cells. Cancer Res. 2010;70:1073-1084.

107. Carden CP, Sarker D, Postel-Vinay S, et al. Can molecular biomarker-based patient selection in Phase I trials accelerate anti-cancer drug development? Drug Discov Today. 2009;15:88-97.

108. Yap TA, Sandhu SK, Workman P, et al. Envisioning the future of early anticancer drug development. Nat Rev Cancer. 2010;10:514-523.

109. Herman JG, Graff JR, Myohanen S, et al. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A. 1996;93:8821-8826.

110. Press JZ, De Luca A, Boyd N, et al. Monitoring drug-induced gamma-H2AX expression in tumors exposed to cisplatin and fractionated irradiation. Clin Cancer Res. 2009;15:3344-3353.

111. Kennedy RD, D’Andrea AD. DNA repair pathways in clinical practice: lessons from pediatric cancer susceptibility syndromes. J Clin Oncol. 2006;24:3799-3808.

112. Williams H, Taghian AG, Luo CM, et al. Identification of new PARP inhibitors in breast cancer. Mol Cancer. 2009;7:1304-1309.

113. Plummer ER, Calvert H. Targeting poly(ADP-ribose) polymerase: a two-armed strategy for cancer therapy. Clin Cancer Res. 2007;13:6252-6256.

114. Helleyda T, Petermann E, Lundin C, et al. DNA repair pathways as targets for cancer therapy. Nat Rev Cancer. 2008;8:193-204.

115. Sandhu SK, Yap TA, de Bono JS. Poly(ADP-ribose) polymerase inhibitors in cancer treatment: a clinical perspective. Eur J Cancer. 2010;46:9-20.

116. Ferraris DV. Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. J Med Chem. 2010;53:4561-4584.

117. Jacquetmont C, Taniguchi T. Proteasome function is required for DNA damage response and foci formation. J Biol Chem. 2008;283:23465-23473.

118. Deans AJ, Khanna KK, McNees CJ, et al. Cyclin-dependent kinase 2 functions in normal DNA repair and is a therapeutic target in BRCA1-deficient cancers. Cancer Res. 2008;68:8219-8226.

119. Adimooolam S, Sirisawad M, Chen J, et al. HDAC inhibitor PCI-24781 increases RAD51 expression and inhibits homologous recombination. Proc Natl Acad Sci U S A. 2007;104:19482-19487.

120. Choudhury A, Zhao H, Jalali F, et al. Targeting homologous recombination using imatinib results in enhanced tumor cell chemosensitivity and radiosensitivity. Mol Cancer Ther. 2009;8:203-213.

121. Hay T, Jenkins H, Sansom OJ, et al. Efficient deletion of normal Brca2-deficient intestinal epithelium by poly(ADP-ribose) polymerase inhibition models potential prophylactic therapy. Cancer Res. 2005;65:10145-10148.

122. Tong WM, Yang YG, Cao WH, et al. Poly(ADP-ribose) polymerase-1 plays a role in suppressing mammary tumourigenesis in mice. Oncogene. 2007;26:3857-3867.

123. Rottenberg S, Jaspers JE, Kerstenberg A, et al. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci U S A. 2008;105:17079-17084.

124. Roden J, Iniesta MD, Papadopoulos K. Development of PARP inhibitors in oncology. Expert Opin Investig Drugs. 2009;18:31-43.

125. Plummer ER, Calvert H. Targeting poly(ADP-ribose) polymerase: a two-armed strategy for cancer therapy. Clin Cancer Res. 2007;13:6252-6256.

126. Bellaloya T, Petermann E, Lundin C, et al. DNA repair pathways as targets for cancer therapy. Nat Rev Cancer. 2008;8:193-204.

127. Sandhu SK, Yap TA, de Bono JS. Poly(ADP-ribose) polymerase inhibitors in cancer treatment: a clinical perspective. Eur J Cancer. 2010;46:9-20.

128. Ferraris DV. Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. J Med Chem. 2010;53:4561-4584.

129. Jacquetmont C, Taniguchi T. Proteasome function is required for DNA damage response and foci formation. J Biol Chem. 2008;283:23465-23473.

130. Deans AJ, Khanna KK, McNees CJ, et al. Cyclin-dependent kinase 2 functions in normal DNA repair and is a therapeutic target in BRCA1-deficient cancers. Cancer Res. 2008;68:8219-8226.

131. Ferraris DV. Evolution of poly(ADP-ribose) polymerase-1 (PARP-1) inhibitors. From concept to clinic. J Med Chem. 2010;53:4561-4584.

132. Deans AJ, Khanna KK, McNees CJ, et al. Cyclin-dependent kinase 2 functions in normal DNA repair and is a therapeutic target in BRCA1-deficient cancers. Cancer Res. 2008;68:8219-8226.

133. Adimooolam S, Sirisawad M, Chen J, et al. HDAC inhibitor PCI-24781 increases RAD51 expression and inhibits homologous recombination. Proc Natl Acad Sci U S A. 2007;104:19482-19487.

134. Choudhury A, Zhao H, Jalali F, et al. Targeting homologous recombination using imatinib results in enhanced tumor cell chemosensitivity and radiosensitivity. Mol Cancer Ther. 2009;8:203-213.

135. Hay T, Jenkins H, Sansom OJ, et al. Efficient deletion of normal Brca2-deficient intestinal epithelium by poly(ADP-ribose) polymerase inhibition models potential prophylactic therapy. Cancer Res. 2005;65:10145-10148.

136. Tong WM, Yang YG, Cao WH, et al. Poly(ADP-ribose) polymerase-1 plays a role in suppressing mammary tumourigenesis in mice. Oncogene. 2007;26:3857-3867.

137. Rottenberg S, Jaspers JE, Kerstenberg A, et al. High sensitivity of BRCA1-deficient mammary tumors to the PARP inhibitor AZD2281 alone and in combination with platinum drugs. Proc Natl Acad Sci U S A. 2008;105:17079-17084.

138. Roden J, Iniesta MD, Papadopoulos K. Development of PARP inhibitors in oncology. Expert Opin Investig Drugs. 2009;18:31-43.