PARP Inhibitors in Breast Cancer

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Abstract: The therapeutic implications of DNA damage in cancer therapy have long been appreciated and form the basis of many successful cytotoxic chemotherapy and radiotherapy treatment strategies. A novel class of DNA repair defect targeted therapeutics that inhibit poly (ADP-Ribose) polymerase (PARP) are being rapidly developed in breast cancer based on exciting preliminary clinical activity as single agents in BRCA mutation–associated breast cancer and in combination with chemotherapy in triple-negative breast cancer. Though there is widespread enthusiasm to move these drugs forward quickly, much remains to be understood about the optimal use of the novel agents. Here we review the clinical development of PARP inhibitors in breast cancer and highlight clinical trials in progress. We also provide commentary on a series of outstanding questions in the field, the answers to which will be critical for the successful development of PARP inhibitor–based strategies in early- and late-stage breast cancer.

Introduction

PARP and the DNA Damage Response

The cellular response to DNA damage is highly regulated and may result in survival of a normal cell, cell death, or mutagenesis depending on the magnitude of the insult, efficiency of repair, and the cellular context.[1] The human genome is protected from various endogenous and exogenous DNA damaging insults by a complex system of DNA repair machinery designed to respond to and repair a wide spectrum of DNA damage. Double-strand DNA breaks are highly toxic lesions, and 2 major pathways—nonhomologous end joining and homologous recombination—contribute to the repair of these lesions. The excision repair pathways, including base excision repair (BER), nucleotide excision repair (NER), and mismatch repair, employ a "cut and patch" mechanism to excise the damaged or incorrect DNA sequence and fill the resulting gap using the complementary DNA strand as a template.[1]

As a member of a large family of poly (ADP-ribose) polymerases (PARP), PARP1 is an abundant nuclear enzyme that is activated by and recruited to sites of DNA base damage.[2] The enzyme itself is composed of 3 functional domains, including a DNA binding domain, an automodification domain, and a catalytic domain.[3] Following DNA damage, PARP1 is recruited and binds to the damaged DNA with a subsequent increase in catalytic activity that results in the formation of poly(ADP-ribose) polymers using the substrate NAD+ that are transferred to acceptor proteins and to PARP1 itself. Formation of these poly(ADP-ribose) polymers are important for

recruitment of the BER machinery to the site of the DNA damage and relaxation of the chromatin structure to facilitate repair. Early work that contributed to the elucidation of this mechanism implicated a potential role of inhibitors of PARP in the treatment of cancer after it was observed that 3-aminobenzamide (3AB) could enhance the cytotoxicity of alkylating agents.[2]

Strategies Employing PARP Inhibitors in the Treatment of Cancer

Two primary therapeutic strategies employing PARP inhibitors in the treatment of cancer are currently under investigation; the first is the use of PARP inhibitors as sensitizers to DNA damaging chemotherapy or radiation, while the second approach exploits specific genetic characteristics of certain cancers that leave them vulnerable to DNA damage via the mechanism of "chemical synthetic lethality"[4] (Figure 1). Since many effective cytotoxic chemotherapies and ionizing radiotherapy exert their anti-tumor effect through production of DNA damage, it was hypothesized that interference with cellular DNA repair using PARP inhibitors may mitigate repair of this injury, resulting in decreased treatment resistance. Indeed, following the observation that inhibitors of PARP enhance the cytotoxicity of DNA methylating agents, potentiation of ionizing radiation effect via inhibition of PARP was demonstrated.[5] In the 1990s, more potent PARP inhibitors were developed and evaluated preclinically for their potential as chemotherapy or radiation sensitizers. In 2005, however, a pair of pivotal papers suggested a novel application of PARP inhibitors in the treatment of human cancers. These studies demonstrated that the use of inhibitors of PARP in cells deficient in BRCA1 and BRCA2 function resulted in selective cytotoxicity, compared to cells that are wild-type or heterozygous for BRCA1 or BRCA2.[6-7] This concept of "chemical synthetic lethality" of PARP inhibitors in the treatment of BRCA1 and BRCA2 mutation-associated cancer generated tremendous enthusiasm and fueled the rapid development of clinical investigation in this area. Based on these 2 principles, a number of PARP inhibitors are currently in clinical development for the treatment of cancer (Table 1). In genetics, a synthetic lethal interaction describes the scenario where mutation in either of 2 genes alone has no phenotypic effect, but the combination of both mutations results in death of the cell.[8] Tumors arising in patients with a germline BRCA mutation are associated with reduced DNA repair capacity due to the loss of BRCA function and are hypothesized to rely more heavily on alternate compensatory DNA repair processes for survival. The BRCA1 and BRCA2 proteins are best known for their important role in homologous recombination, though BRCA1 has been implicated as having additional roles in NER and BER.[9-10] PARP plays a central role in

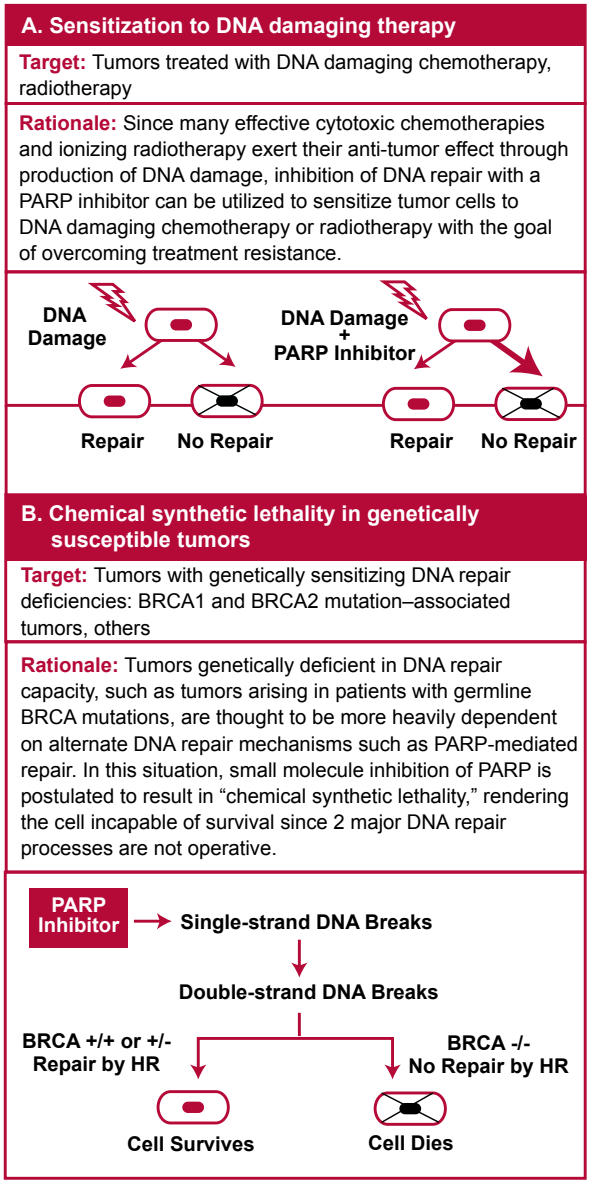

Figure 1. Two distinct strategies for targeting poly (ADP-ribose) polymerase in the treatment of cancer.

HR=homologous recombination.

BER. It is required for the repair of oxidative damage associated with breaks in single-strand DNA. If PARP is inhibited, repair-associated breaks result in replication fork–mediated double-strand break formation, which requires BRCA1- and BRCA2-associated recombination to resolve.[11] Thus, in tumors lacking intact BRCA function due to loss of a second allele, chemical inhibition of PARP with a small molecule inhibitor is postulated to be the "second hit" that renders the cell incapable of survival and mimics genetic absence of PARP. However, in the host's somatic tissues that are heterozygous for a

Table 1. PARP Inhibitors in Clinical Development for the Treatment of Cancer

Drug Name	Manufacturer	Route of Administration	Tumors Targeted in Ongoing Clinical Trials	Phase
Veliparib ABT-888	Abbott	Oral	Breast, ovarian, primary peritoneal, fallopian tube, colon, prostate, melanoma, HCC, hematologic malignancies/CLL, lymphoma, advanced solid tumors	I, II
AG014699 PF01367338	Pfizer	Intravenous	Triple-negative breast, BRCA-associated breast, BRCA-associated ovarian, advanced solid tumors	I, II
Olaparib AZD2281	AstraZeneca	Oral	Triple-negative breast, BRCA-associated breast, BRCA-associated ovarian, ovarian, gastric, colon, pancreas, melanoma, advanced solid tumors	I, II
Iniparib BSI-201 SAR240550	BiPar Sciences/ Sanofi-Aventis	Intravenous	Triple-negative breast,* BRCA-associated breast, BRCA-associated pancreas, BRCA-associated ovarian, ovarian, primary peritoneal fallopian tube, uterine carcinosarcoma, glioblastoma multiforme, NSCLC, squamous cell lung*	I, II, III*
CEP8983	Cephalon	Oral	Advanced solid tumors	I
MK-4827	Merck	Oral	Advanced solid tumors,prostate, ovarian, primary peritoneal, fallopian tube	I

CLL=chronic lymphocytic leukemia; HCC=hepatocellular carcinoma; NSCLC=non–small cell lung cancer

BRCA mutation and that maintain normal BRCA protein function, PARP inhibitors are thought to have little or no effect.

PARP Inhibitors in Breast Cancer

BRCA1 and BRCA2 Mutation-associated Breast Cancer

The breast and ovarian cancer syndromes caused by germline mutations in the BRCA1 and BRCA2 genes account for a minority of breast cancers overall (approximately 5%), though it was this population that provided clinical "proof of principle" of chemical synthetic lethality with PARP inhibitors. To date, the majority of clinical investigation in BRCA mutation–associated cancer has been with the oral PARP inhibitor olaparib (AstraZeneca), and few data exist for this drug outside of this population. Fong and colleagues reported a phase I dose escalation study of olaparib in patients with advanced refractory solid tumors.[12] A total of 60 patients were enrolled, including 22 patients with known BRCA1 or BRCA2 mutations. Objective responses were seen in this study, though only in patients with BRCA mutation–associated ovarian, breast, or prostate cancer. Few patients had breast cancer, however, including 6 with no BRCA mutation and 3 with documented BRCA2 mutations. No responses were observed in patients lacking known BRCA mutations. At the 2009 American Society of Clinical Oncology (ASCO) Annual Meeting, Tutt and colleagues presented the first phase II results of olaparib for the treatment of women with BRCA1 and BRCA2 mutation–associated stage IIIB–IV breast cancer that had progressed on at least 1 prior systemic chemotherapy regimen.[13] Two sequential cohorts of 27 patients were enrolled at dose levels of 100 mg twice daily orally and 400 mg twice daily orally every 28 days. Patients had received a median of 3 prior chemotherapy regimens for advanced breast cancer, and 64% and 50% of patients had the triple-negative subtype of breast cancer in the 100 mg and 400 mg cohorts, respectively. The objective response rate was 41% in the 400 mg cohort and 22% in the 100 mg cohort. One complete response was observed with the higher dose. The responses seen in both triple-negative (estrogen receptor [ER]-negative, progesterone receptor [PR]-negative and human epidermal growth factor [HER] 2/neu nonoverexpressing) and nontriple-negative (but BRCA-mutation carrier) patients were an important observation and suggest that the selection of patients based on shared DNA repair defects due to BRCA mutations supersedes phenotypic subtype. To date, the activity of olaparib in non-BRCA–mutant triple-negative breast cancer remains unclear. Gelmon and colleagues recently reported results of a phase II study of single-agent olaparib 400 mg orally twice daily.[14] In a cohort of fifteen BRCA-negative, triple-negative breast cancer patients with advanced disease, no objective responses were observed.

Following these successes, many companies are examining the efficacy of their PARP inhibitors in patients with BRCA mutation-associated breast cancer. The majority of these studies are recruiting patients with advanced BRCA mutation-associated breast cancer, though BSI-201 (BiPar Sciences/Sanofi-Aventis) is being investigated in the neoadjuvant setting in combination with gemcitabine

(Gemzar, Eli Lilly) and carboplatin for patients with stage I–IIIA BRCA1 and BRCA2 mutation–associated breast cancer, regardless of breast cancer subtype. Pfizer has also recently launched a randomized phase II study of cisplatin, with or without PF-01367338, in early-stage BRCA1- or BRCA2-positive patients with residual disease after neoadjuvant chemotherapy. BRCA1 and BRCA2 carriers with triple-negative or ER and/or PR-positive, HER2-negative breast cancer subtypes are eligible for this study. Another exciting area of PARP inhibitor development relates to their potential use as chemoprevention agents in patients with high breast cancer risk. Studies examining this hypothesis in BRCA mutation carriers are currently in development.

Triple-negative Breast Cancer

Sporadic triple-negative breast cancers share many pathologic and molecular features with breast cancers caused by hereditary BRCA1 mutations, including basal-like gene expression, high histologic grade, frequent p53 mutations, cytogenetic abnormalities, increased genomic instability, and EGFR overexpression.[15-17] Based on these similarities and the role of BRCA1 in multiple DNA repair pathways, the hypothesis emerged that sporadic triple-negative breast tumors may possess similar DNA repair deficiencies and exhibit similar chemosensitivities as BRCA1 mutation-associated breast tumors. Interestingly, basal-like breast cancer cell lines, like BRCA1-deficient cancer cell lines, are more sensitive to oxidative DNA damage compared to luminal breast tumor cells or normal breast epithelial cells, and are deficient in BER.[10] Similar to BRCA1-deficient cells, basal-like tumor cells also demonstrate increased sensitivity to PARP inhibition, cisplatin, and gemcitabine,[10,18] and PARP1 has been observed to be overexpressed in triple-negative breast cancer.[19] These observations provided the rationale to investigate DNA damaging chemotherapies and PARP inhibitors in triple-negative breast cancer patients.

At ASCO 2009, O'Shaughnessy and colleagues reported the first clinical results exploring the role of PARP inhibition in the treatment of sporadic triple-negative advanced breast cancer.[20] In this randomized phase II trial, women with advanced breast cancer were treated with intravenous gemcitabine 1,000 mg/m^2 and intravenous carboplatin area under the concentration of 2 on days 1 and 8, with or without the PARP inhibitor BSI-201 dosed at 5.6 mg/kg intravenously on days 1, 4, 8, and 11. A total of 123 women treated with up to 2 prior chemotherapy regimens for metastatic disease were enrolled. This study demonstrated improvements in the clinical benefit rate (21% vs 62%; *P*=.0002), overall response rate (16% vs 48%; *P*=.002), median progression-free survival (3.3 vs 6.9 months; hazard ratio [HR], 0.342; *P*<.0001) and median overall survival (5.7 vs 9.2 months; HR, 0.348; *P*=.0005) among patients who received BSI-201. Prolongation of survival is rarely observed in clinical trials of women with advanced breast cancer and, as such, these results were received with great enthusiasm in the oncology community. The overall survival data were updated at the 2009 San Antonio Breast Cancer Symposium, and they continued to show a significant survival advantage among women treated with BSI-201 in an intention-to-treat analysis (7.7 vs 12.2 months; HR, 0.50; *P*=.005).[21] Of particular note, approximately 40% of women treated on the control arm crossed over to receive BSI-201 at the time of disease progression.

In July 2009, a randomized phase III registration study of gemcitabine and carboplatin, with or without BSI-201, was launched to examine the safety and efficacy of this combination in a larger patient population (n=420). Accrual was extremely rapid, with the study closing to accrual in February 2010. A subsequent study examining this combination in advanced triple-negative breast cancer has recently been launched in Europe and is testing gemcitabine and carboplatin with 2 different doses and schedules of BSI-201: 11.2 mg/kg intravenously on days 1 and 8 versus 5.6 mg/kg intravenously on days 1, 4, 8, and 11. Other ongoing PARP inhibitor studies in triple-negative breast cancer include a phase II study of neoadjuvant gemcitabine, carboplatin, and BSI-201 in women with stage I–IIIA triple-negative breast cancer and a phase I/II study of cisplatin, with or without olaparib, as neoadjuvant therapy in women with early-stage, triple-negative breast cancer. Pfizer has recently launched a randomized phase II study of cisplatin with or without PF-01367338 in early-stage triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy. ABT-888 (Veliparib, Abbott) is being investigated in combination with weekly paclitaxel and every 3 weekly carboplatin followed by doxorubicin and cyclophosphamide in the neoadjuvant treatment of early breast cancer as part of the multicenter I-SPY2 TRIAL (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging and Molecular Analysis).

Data with single-agent BSI-201 in breast cancer, in particular triple-negative breast cancer, are limited. Likewise, data with single-agent olaparib in sporadic triple-negative breast cancer are also limited. Though PARP inhibitors were investigated in triple-negative disease based on the hypothesis that this breast cancer subtype is "BRCA-like," it is unknown at present whether the activity seen in the BSI-201 study was a result of a synthetic lethal interaction between a genetic defect characteristic of this subtype and the PARP inhibitor, a result of enhanced chemosensitivity of gemcitabine and carboplatin in combination with BSI-201, or possibly a combination of both. It is quite possible that the mechanism may vary by patient, and identification of a means to distinguish these patients and their responses is a high priority of research.

Future Direction and Unanswered Questions

Despite the exciting preliminary results of PARP inhibitors in BRCA mutation–associated and triple-negative breast cancer and the widespread enthusiasm to move these drugs forward quickly, it is important to recognize that this field is still in its infancy and there is much that remains to be understood about the optimal use of these novel agents.

Mechanism of Action

As alluded to above, the mechanism by which the PARP inhibitor BSI-201 improves outcome in patients with triple-negative breast cancer treated with gemcitabine and carboplatin is unknown. Successful and logical development of this drug and its application to other clinical settings will be highly dependent on understanding this mechanism and identifying predictors of response. Data with single-agent PARP inhibitor therapy in patients with sporadic triple-negative breast cancer are surprisingly limited and, thus, it is unclear if PARP inhibitors possess single-agent activity in this breast cancer subtype, as was seen in BRCA mutation–associated breast cancers. To date, the concept of chemical synthetic lethality of PARP inhibitors in BRCA mutation–associated tumors is explained by the loss of homologous recombination (genetic loss) and loss of BER (chemical inhibition of PARP). The current hypothesis is that when BRCA-mediated homologous recombination is lost, the tumor cell is more dependent on PARP-mediated repair, which is presumably normal. Preclinical work from our group suggests that BRCA1 has important functions in DNA repair beyond homologous recombination and that BRCA1 mutant and triple-negative breast tumors share deficiencies in BER.[10] Whether the clinical activity of PARP inhibitors relates to this DNA repair defect requires further investigation. If there is truly a defect in BER in triple-negative and BRCA1 mutation-associated breast cancers, these tumor cells may already be compromised for PARP-mediated repair, and this may contribute to PARP inhibitor sensitivity. In vitro, PARP inhibitors do show selective single-agent cytotoxicity to basal-like, but not luminal, breast cancer cell lines. Furthermore, we have demonstrated that PARP inhibition is synergistic with both platinum and gemcitabine in basal-like, but not luminal, breast cancer cell lines,[18] supporting the idea that PARP inhibitors are not functioning as nonspecific DNA damage sensitizers in basal-like breast tumors, but require certain underlying genetic defects for activity. Complicating matters further is the fact that other PARPs exist besides PARP1, and all known PARP inhibitors have some effect on at least the PARP2 enzyme. Therefore, there may be "off-target" effects that at present are poorly understood both in terms of efficacy and toxicity.

Optimal Dosing Strategies and Long-term Safety

Another area where much work is needed is in determining optimal treatment frequency, dosing, and combination with cytotoxic chemotherapy. In the BSI-201 phase II study in triple-negative breast cancer, BSI-201 was given as a bolus dose every 4 days for 4 treatments in combination with low-dose weekly carboplatin and gemcitabine. This dosing schedule and combination was clearly efficacious, though it is unclear if the same results could be achieved with less frequent BSI-201 dosing or whether results may have been better if higher-dose platinum once per cycle was used as an alternative. The kinetics of dosing oral PARP inhibitors such as olaparib and ABT-888 are different, and continuous PARP inhibition may have a different efficacy and toxicity profile compared to intermittent therapy. Preclinical studies examining the scheduling of PARP inhibitors with DNA damaging agents are very limited. Of particular interest is long-term safety of these agents, as they are being developed rapidly in potentially curable patient populations. Secondary malignancies and other long-term toxicities, particularly neurocognitive toxicities,[12] need to be carefully assessed, particularly in patients treated with continuous PARP inhibition.

Biomarkers of Response

The key for successful PARP inhibitor development lies in biomarker discovery and validation. At present, no predictive biomarkers of response for PARP inhibitor therapy in triple-negative breast cancer exist. For that matter, no validated biomarker of prognosis in triple-negative breast cancer currently exists. Therefore, a major area of importance is to better define biologic markers predictive of tumor sensitivity to PARP inhibitors, either alone or in combination with DNA damaging agents. Outside of BRCA mutations, and more recently, PTEN mutations,[22] there are no validated biomarkers for this purpose. Our increasing understanding of the mechanism of action of PARP inhibitors suggests that markers indicative of underlying DNA repair defects should be most informative, though whether these are restricted to proteins involved in homologous recombination, or include members of additional DNA repair pathways, also remains unknown.

To date, within breast cancer, the triple-negative subtype has served as the most widely used phenotype for selecting patients for PARP inhibitor treatment in clinical trials. Although the immunohistochemistry approaches commonly used in most histopathology laboratories to define breast cancer are relatively simple, they are associated only with an underlying DNA repair defect in cell line studies, and clinical correlates remain lacking.

PARP1 levels themselves have been suggested as a potential biomarker for response, and in one study they were reported to be elevated in triple-negative breast

cancers,[19] but this remains to be validated. In a recent study, von Minckwitz and associates observed a relationship between cytoplasmic PARP expression by immunohistochemistry in 638 breast tumors of various subtypes and response to standard neoadjuvant chemotherapy.[23] Methods for detection of PARP activity through measurement of poly(ADP-ribose) in tumor biopsy samples are feasible, but they are not yet well established, and further investigation in this area is of importance.

DNA damage response pathway genes have been suggested as obvious biomarker candidates. For example, DNA damage-inducible histone γ-H2AX phosphorylation and RAD51 foci formation both have been widely used in tissue culture and mouse xenograft studies to correlate with DNA strand breaks.[24-25] However, as biomarkers for the DNA damage response in human tumors, these are less practical in the clinic, as they require induction by a DNA-damaging agent prior to assaying. Thus, a biopsy within hours of drug treatment or ex vivo irradiation of biopsied tissues would be required.[26] One recent report of core needle biopsies obtained prior to and within 24 hours after neoadjuvant treatment with epirubicin and cyclophosphamide in breast cancer patients showed induction of γ-H2AX and RAD51 foci in most cases, but also found an inverse correlation between pretreatment foci levels and chemoresponsiveness.[27]

From a mechanistic point of view, the most robust biomarker in this setting would be a functional assay for actual tumor DNA repair activity, regardless of the underlying genetic or molecular determinants. Indeed, attempts at this approach have been made by our group and others, using the Comet assay for single- or double-strand DNA breaks and an assay for host-cell reactivation of a viral green fluorescence protein reporter gene containing transcription-blocking oxidative DNA damage that measures BER activity.[10] However, while conceptually attractive, these assays are unlikely to contribute to large clinical trials or clinical practice given the need for fresh tumor samples.

Thus, there is a great need for more standard biomarkers for predicting sensitivity to PARP inhibitors and/or DNA repair defects in untreated tumor samples, for breast cancer and other potential malignant targets. Hopefully, investigational genomic analyses of tissues obtained in ongoing clinical trials will identify gene expression signatures or underlying DNA alterations that serve as biomarkers for response and emphasize the need for appropriate tissue acquisition in these trials.

Though tissue collection for biomarker discovery would be ideally incorporated into all studies of PARP inhibitors in breast cancer, a number of barriers exist, including, most importantly, cost and feasibility. Though prospectively collected tumor tissues offer the promise of improved understanding of mechanisms of response and resistance to novel therapies, requirement for complex tumor tissue collection must be balanced against the practical realities of the system in which we operate, and expeditious accrual to important studies cannot be jeopardized. Though many studies in the advanced breast cancer setting aim to collect archived tissue for biomarker analyses, the use of tissue collected from the primary breast tumor diagnosed years earlier may simply not be reflective of the disease at the time of recurrence and has the potential to bias results. A biopsy of the recurrent tumor in these situations would be most helpful, though patient acceptance of invasive biopsy procedures and cost considerations often result in low rates of research tissue acquisition, thereby reducing the power of the correlative question being asked.

Ideally, biomarker questions would be asked in the setting of randomized clinical trials among newly diagnosed patients whose tumors at the time of treatment can be biopsied with ease from the breast. Since pathologic complete response (pCR) is an important surrogate endpoint for breast cancer survival, the neoadjuvant strategy is particularly well suited to the evaluation of novel agents and combinations. Importantly, the neoadjuvant approach allows assessment of a new regimen's activity in a relatively small number of patients over a short time period. Moreover, collection of tumor tissue before and after neoadjuvant treatment is standard and provides a unique opportunity for high-impact correlative translational science. Given limited resources and numerous practical barriers, allocation of biomarker discovery resources to neoadjuvant studies of PARP inhibitors is most likely to accelerate the rational clinical development of this promising group of drugs.

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