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Review

Clinical Cancer Research

Poly(ADP-Ribose) Polymerase Inhibition: "Targeted" Therapy for Triple-Negative Breast Cancer

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Abstract

In contrast to endocrine-sensitive and human epidermal growth factor receptor 2 (HER2)–positive breast cancer, novel agents capable of treating advanced triple-negative breast cancer (TNBC) are lacking. Poly (ADP-ribose) polymerase (PARP) inhibitors are emerging as one of the most promising "targeted" therapeutics to treat TNBC, with the intended "target" being DNA repair. PARPs are a family of enzymes involved in multiple cellular processes, including DNA repair. TNBC shares multiple clinico-pathologic features with *BRCA*-mutated breast cancers, which harbor dysfunctional DNA repair mechanisms. Investigators hypothesized that PARP inhibition, in conjunction with the loss of DNA repair via *BRCA*-dependent mechanisms, would result in synthetic lethality and augmented cell death. This hypothesis has borne out in both preclinical models and in clinical trials testing PARP inhibitors in both *BRCA*-deficient and triple-negative breast cancer. The focus of this review includes an overview of the preclinical rationale for evaluating PARP inhibitors in TNBC, the presumed mechanism of action of this novel therapeutic class, promising results from several influential clinical trials of PARP inhibition in advanced breast cancer (both TNBC and *BRCA* deficient), proposed mechanisms of acquired resistance to PARP inhibitors, and, finally, concludes with current challenges and future directions for the development of PARP inhibitors in the treatment of breast cancer. *Clin Cancer Res; 16(19); 4702–10. ©2010 AACR.*

Inhibition of poly(ADP-ribose) polymerase (PARP) is emerging as one of the most exciting and promising "targeted" therapeutic strategies to treat advanced triple-negative breast cancer (TNBC), the intended "target," being DNA repair. Diagnosed in an estimated 180,000 women worldwide, TNBC is an aggressive subset of breast cancer that lacks expression of the estrogen and progesterone receptors (ER and PR) and the human epidermal growth factor receptor 2 (HER2) protein (1). TNBC, classified as basal like by gene expression 80% of the time, is characterized by distinct risk factors: aggressive and early patterns of metastases, unique molecular characteristics, association with *BRCA1* mutations, a relative lack of targeted therapeutics, and poor prognosis (2–9).

In contrast to endocrine-sensitive and HER2-positive breast cancer, novel agents capable of treating advanced TNBC are, at present, lacking. Currently available therapies are limited to cytotoxic chemotherapy with or without the addition of the anti-angiogenic agent bevacizumab (Avastin, Genentech). Despite improvements in progression-free survival (PFS) when combining bevacizumab with paclitaxel among patients with HER2-negative advanced breast cancer (11.8 versus 5.9 months), absolute improvements for the triple-negative subset were more limited (9 versus 5 months; refs. 10, 11). Similarly, although a PFS benefit was seen in adding ixabepilone (Ixempra, Bristol Meyer Squibb), a newer generation microtubule stabilizing agent, to capecitabine chemotherapy among the triple-negative subset (4.1 versus 2.1 months), the benefit was still only modest, in part based on poor baseline outcomes among women with advanced breast cancer regardless of subtype (5.8 versus 4.2 months; refs. 11, 12).

Given the poor prognosis and high rate of visceral metastases (including central nervous system recurrence) associated with TNBC, investigators have been actively searching for innovative therapeutic strategies to effectively treat this aggressive disease (3, 13). Building on the observation that TNBC shares several clinical and pathologic characteristics with *BRCA*-deficient breast cancers known to harbor deficient DNA repair mechanisms, PARP inhibitors have been tested in early phase clinical trials among patients with advanced TNBC. Preliminary results of phase II trials are encouraging and report improvements in response rates, PFS, and overall survival (OS) when adding PARP inhibition to DNA-damaging chemotherapeutics with minimal additional toxicity (14, 15). We review the preclinical rationale for evaluating PARP inhibitors in

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Translational Relevance

The treatment of triple-negative breast cancer with poly(ADP-ribose) polymerase (PARP) inhibitors is a perfect example of translational medicine. Close to a decade ago, investigators hypothesized that preventing DNA repair via PARP inhibition, in conjunction with the loss of DNA repair via *BRCA*-dependent mechanisms, would result in synthetic lethality and augmented cell death. This hypothesis has borne out in preclinical studies evaluating the effect of PARP inhibition on *BRCA*-deficient cell lines and *BRCA*-deficient preclinical tumor models. Profound efficacy observed in the preclinical arena was readily transferrable to the clinical setting. Recognizing the clinico-pathologic similarities between *BRCA*-deficient and triple-negative breast tumors (often termed "*BRCA*-ness"), PARP inhibitors have been tested in both patient populations, and illustrate apparent clinical efficacy. Future studies to define PARP inhibitor mechanisms of resistance and response biomarkers to define optimal patient populations will involve returning back to the bench, so that strides at the bedside continue, which is the essence of translational medicine.

triple-negative (and *BRCA*-deficient) advanced breast cancer, the presumed mechanism of action of this novel therapeutic class, promising results from several influential clinical trials of PARP inhibition in advanced breast cancer (both TNBC and *BRCA* deficient), proposed mechanisms of acquired resistance to PARP inhibitors, and, finally, conclude with current challenges and future directions for the development of PARP inhibitors in the treatment of breast cancer.

DNA Repair as a Therapeutic "Target" and Proposed Mechanism of PARP Inhibitors

DNA damage is an ongoing process resulting from both endogenous and exogenous (environmental) assaults to the human genome. Endogenous forms for DNA damage arise from spontaneous base changes, replication errors, and oxygen-free radicals, whereas exogenous forms include chemical mutagens, cytotoxic agents, and both UV and ionizing radiation (16). The genome is armed with multiple DNA repair mechanisms, including but not limited to mismatch repair (MMR), base excision repair (BER), nucleotide excision repair (NER), and double-strand break repair (DSR). DNA double-strand breaks are highly toxic to cells, and two main pathways contribute to their inherent repair: error-prone nonhomologous end-joining (NHEJ) and error-free homologous recombination (HR; Fig. 1A; refs. 16–18). HR is dependent on functional *BRCA1* and *-2* pathways, and *BRCA* maintains genome stability, at least in part, by regulating HR according to the type of DNA damage (19–22). As follows, germline mutations in either the *BRCA1* or *BRCA2* genes are associated with a high risk of developing a number of cancers, including breast, ovarian, and prostate cancer (16, 23, 24). When the *BRCA*-associated DNA repair pathway, namely HR, is lost or dysfunctional, repair shifts toward alternate DNA repair mechanisms dependent on a unique class of enzymes, PARPs.

PARPs are a family of enzymes involved in cellular processes, such as genomic stability, DNA repair, cell cycle progression, and apoptosis (25, 26). PARP-1, a nuclear, zinc-finger, DNA binding protein, localizes to DNA strand breaks as part of the BER process (27). Cell death from targeting two genes, which, alone, do not result in cell death, is termed "synthetic lethality." Investigators hypothesized PARP inhibition, in conjunction with the loss of DNA repair via *BRCA*-dependent mechanisms, would result in synthetic lethality and augmented cell death (Fig. 1B), a hypothesis that has borne out in both preclinical models and the clinical trial arena.

It is well recognized that *BRCA*-deficient, basal-like (as defined by microarray), and triple-negative (as defined by immunohistochemistry) breast cancers share clinical and pathologic similarities, including high rates of p53 mutation, aneuploidy, high pathological grade, and relative sensitivity to DNA-damaging chemotherapeutics (Table 1; refs. 4, 7, 9, 14, 28, 29). Several mechanisms have been proposed to explain these similarities on the basis of presumed *BRCA* pathway and subsequent HR dysfunction in sporadic basal-like and TNBC. These mechanisms may include: (1) overexpression and copy number gain of *ID4*, a negative regulator of *BRCA1* (30, 31); (2) decreased expression of *BRCA1* messenger RNA (31); (3) *BRCA1* gene-promoter methylation (32); and (4) copy number aberrations affecting genes within a "*BRCA* DNA-damage response" pathway (31). Each of these observations suggests a role for defective HR DNA repair among sporadic TNBC and basal-like breast tumors providing the basis for the term "*BRCA*-ness" of sporadic TNBC (33). Thus, the "*BRCA*-ness" of sporadic TNBC has provided the rationale to test PARP inhibitors, not only in advanced *BRCA*-mutated tumors, but also sporadic TNBC. Results from early phase clinical trials evaluating the efficacy of PARP inhibitors in *BRCA*-mutated and TNBC, although preliminary, are quite promising (15, 34–36).

Preclinical Efficacy of PARP Inhibition in BRCA-Deficient and Triple-Negative and/or Basal-Like Breast Cancer Models

Two landmark preclinical studies report (1) the feasibility of targeting DNA-repair defects inherent to *BRCA* mutant cell lines as a therapeutic strategy and (2) the acute sensitivity of *BRCA2*-deficient embryonic stem cells to PARP inhibition due to deficiencies in HR (37, 38). Farmer and colleagues conducted a series of experiments

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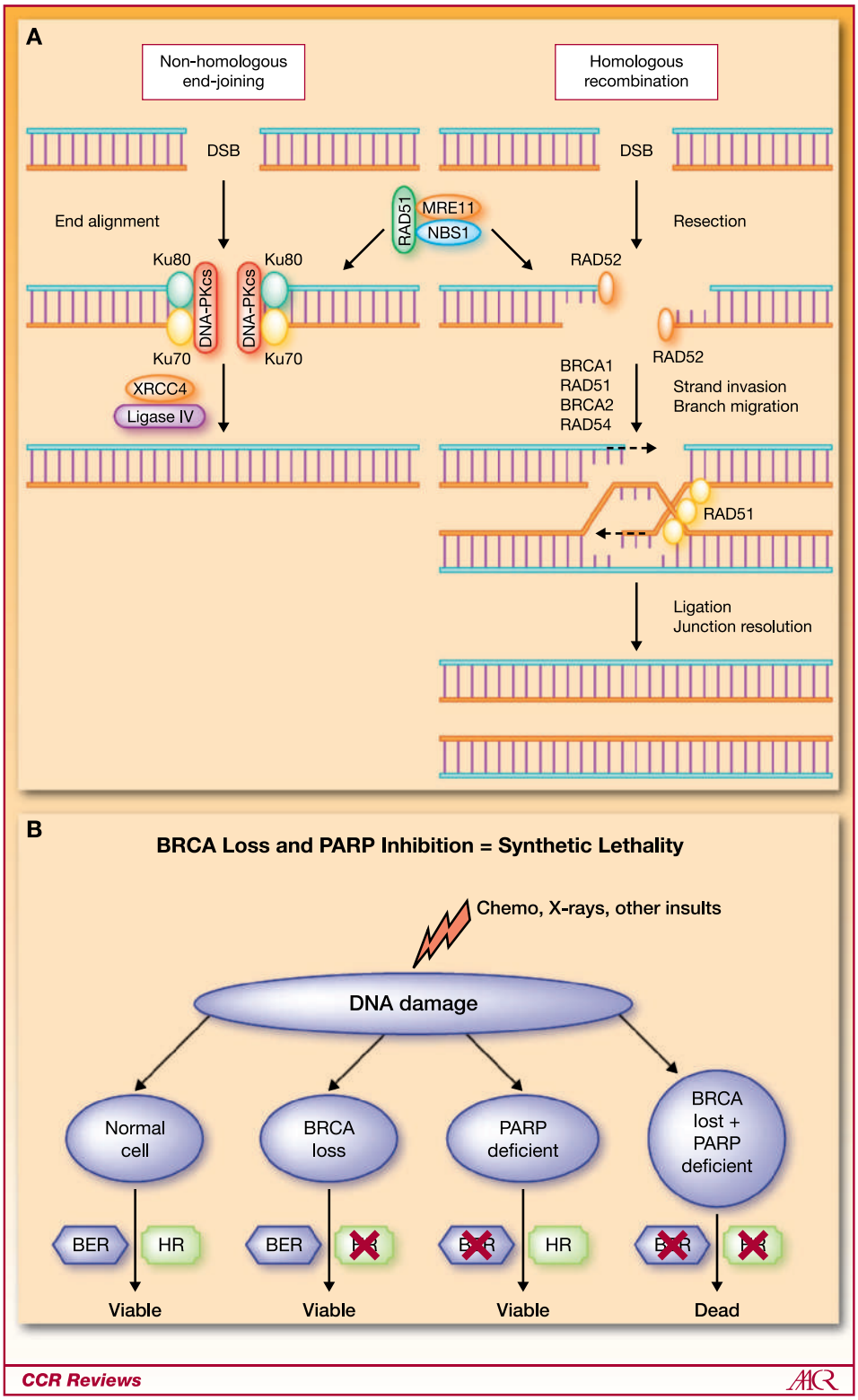

Fig. 1. A, two main pathways that contribute to repair of DNA double-strand breaks: NHEJ and HR. This figure was published in Ford JM and Kastan MB, Chapter 10, DNA damage response pathways and cancer, p. 149. In: Abeloff MD, editor. Clinical oncology, 4th ed. Reproduced with permission, copyright 2008, from Churchill Livingstone, an Imprint of Elsevier; ref. 4. B, "Synthetic lethality" and subsequent cell death due to loss of parallel DNA repair pathways. In the presence of one (or both) functional BRCA- or PARP-dependent DNA repair pathways, cells survive. In the absence of both, cell death ensues. Reproduced with permission from Comen et al. (51).

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Table 1. Shared clinico-pathologic features between *BRCA1*-deficient and triple-negative and/or basal-like breast cancer

Clinico-pathologic characteristics	Hereditary *BRCA1* breast cancer	Triple-negative and/or basal-like breast cancer
ER/PR/HER2 status	Negative	Negative
TP53 status	Mutant	Mutant (up to 80%)
BRCA1 status	Mutational inactivation	Diminished expression (?)
Gene expression patterns	Basal-like	Basal-like
Tumor histology	Poorly differentiated (high grade)	Poorly differentiated (high grade)
Chemosensivity to DNA-damaging chemotherapy	Highly sensitive	Likely sensitive
Genome-wide aneuploidy	Yes	Yes

NOTE: Adapted from 2009 ASCO Plenary Session (14).

illustrating a reduction in clonogenic survival of *BRCA1*- and *BRCA2*-deficient cell lines when transfected with a plasmid expressing a short-interfering RNA (siRNA) targeting *PARP1*. Chemical inhibition of PARP1 was more potent in *BRCA1*- and *BRCA2*-deficient embryonic stem cells compared with heterozygous mutant and wild-type cells. An *in vivo BRCA2*-deficient murine model supported these findings (38). Parallel studies conducted by Bryant and colleagues also showed that cell lines deficient in HR (via defective *XRCC2*, *XRCC3*, and *BRCA2*) were highly sensitive to PARP inhibition. Treatment of *BRCA2*-deficient cells lines with PARP inhibition induced gamma(γ)-H2AX foci, a marker of DNA double-strand breaks, and *BRCA2*-deficient tumors in a xenograft model were susceptible to PARP inhibition (37). Taken together, results from these studies suggested a new, mechanism-based approach for the treatment of patients with *BRCA1*- and *BRCA2*-deficient tumors. Importantly, models of sensitivity to PARP inhibition suggest that the key ingredient is deficiency in HR, indicating that this approach may be more widely applicable in the treatment of sporadic cancers sharing functional *BRCA* loss or other impairments of homologus recombination.

As discussed above, *BRCA*-mutated, basal-like, and TNBC share a number of clinico-pathologic characteristics (Table 1). *BRCA1* and *BRCA2* mutation carriers, however, compose a minority of breast cancer cases (23, 24). The "*BRCA-ness*" of TNBC, which is classified as basal-like by cDNA microarray more than 80% of the time (9, 39), led investigators to evaluate the sensitivity of PARP inhibition in breast cancer cell lines of varying subtypes (i.e., luminal, basal, etc.), not only to determine subtype-specific sensitivity, but also to further elucidate the mechanism of cellular cytotoxicity.

A series of informative preclinical studies evaluating DNA damage–response pathways in breast cancer cell lines of different subtypes report selective response of basal-like breast cancer cell lines to PARP inhibition, which may, in addition to defective HR, be due to inefficient BER (40). Oxidative DNA damage (ODD) constitutes a large majority of endogenous DNA damage in human cells. DNA damage from reactive oxygen species usually occurs as a single-base alteration repaired via BER. Investigators hypothesized that basal-like and *BRCA1*-mutated breast cancers share defects in maintaining genomic stability via aberrant regulation of ODD. When a variety of breast cancer cell lines of varying subtypes, including luminal, basal-like, and *BRCA1*-mutated breast cancers, were tested for sensitivity to H_20_2 (as an indicator of response to ODD), basal-like and *BRCA1*-mutated breast cancer cell lines were most sensitive, thus, least effective in the repair of oxidative damage (40).

A second set of parallel experiments concluded that the relative inefficiency of basal-like and *BRCA1*-mutated breast cancer cell lines to repair ODD was a result of defective BER (40). Briefly, a BER assay involving BER-dependent expression of a green fluorescent protein (GFP) reporter gene showed a relative decrease in GFP expression 24 hours following DNA damage among basal-like and *BRCA1*-mutated breast cancer cell lines, when compared with luminal breast cancer cell lines. Moreover, transfection of the *BRCA1*-mutated cell line SUM149 with wild-type *BRCA1* resulted in a four-fold increase in BER, whereas small hairpin RNA (shRNA) knock-down of *BRCA1* resulted in decreased BER. Inefficient BER repair was correlated with sensitivity to PARP inhibition *in vitro*. In addition, using this same panel of cell lines, it was shown that the basal-like cells were selectively sensitive to platinum and gemcitabine, and that these drugs exhibited synergy when combined with a PARP inhibitor in basal-like, but not luminal, breast cancer cell lines (41). This series of informative studies continues to shed light on the complicated mechanism of action inherent to PARP inhibitors and provides a plausible mechanism for the selective efficiency of PARP inhibitors in *BRCA*-mutated and basal-like cell lines, an observation that has borne out in clinical studies.

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PARP Inhibitors: Overview of Current Clinical Data

In parallel to strides in the preclinical arena, pharmacologic inhibition of PARP has translated into advances in the clinical management of patients with TNBC and *BRCA*-deficient advanced breast cancer. Historically, the treatment of advanced TNBC has been fraught with unique challenges. Specifically, in contrast to endocrine-sensitive and HER2-enriched breast cancer, TNBC is characterized by a relative lack of "drug-able" targets, rapid recurrence in visceral organs (including the central nervous system), and inherently poor prognosis (3, 13). On the basis of elegant preclinical rationale, PARP inhibitors are being tested in *BRCA*-deficient and triple-negative breast cancer and illustrate clinical efficacy. Although several PARP inhibitors are in early phase development (Table 2), the majority of clinical experience has been with olaparib (AZD2281, AstraZenca/KuDOS) and BSI-201 (BiPAR Sciences/Sanofi Aventis).

Olaparib

The efficacy of olaparib, an oral PARP inhibitor, among patients with *BRCA*-mutated advanced solid tumors was initially reported in the phase I setting (34). In addition to standard dose escalation and pharmacokinetic and/or pharmacodyamic analyses, toxicity and efficacy were reported among 60 patients with advanced tumors, a population enriched for *BRCA* mutation carriers (n = 22). Pharmacokinetic data indicated rapid absorption and elimination; pharmacodynamic studies confirmed PARP inhibition in surrogate samples (peripheral-blood mononuclear cells and plucked eyebrow-hair follicles) and tumor tissue. No obvious increase in adverse effects was seen among *BRCA* mutation carriers. Objective antitumor activity was reported only in mutation carriers (all heavily pretreated for breast, ovarian, or prostate cancer).

A second phase II, multicenter, single-arm, sequential cohort design study sought to determine the efficacy and/or tolerability of olaparib among patients with *BRCA1*- and/or *BRCA2*-deficient, advanced breast cancer (42). Fifty-four patients were enrolled in one of two dose-based cohorts (cohort 1, 27 patients treated with 400 mg orally twice daily; cohort 2, 27 patients treated with 100 mg orally twice daily). The primary objective was objective tumor response [complete response (CR) plus partial response (PR)]. Secondary objectives included PFS, safety, and tolerability. At the time of progression, patients in cohort 2 were permitted to cross-over to cohort 1. The majority of patients in both cohorts harbored *BRCA1* mutations, 67% and 56%, respectively; a smaller proportion had *BRCA2* mutations, 33% and 41%, respectively. More than 50% of patients had TNBC, and the median number of prior therapies was three. Overall response rates were 41% and 22% among patients in cohort 1 and 2, respectively. PFS was 5.7 (4.6 to 7.4) months in cohort 1 and 3.8 (1.9 to 5.5) months in cohort 2. The most commonly reported grade 3 adverse events were fatigue, nausea, and vomiting. Treatment discontinuation due to adverse events was uncommon.

Several Canadian studies are ongoing to define efficacy, mechanisms of resistance, and patient population most likely to respond to olaparib. Canadian study 20 is a phase II study enrolling four cohorts of 91 patients with advanced breast or ovarian cancer: (1) ovarian, *BRCA* negative, and/or unknown; (2) TNBC, *BRCA* negative, and/or unknown; (3) ovarian, *BRCA*-mutated; and (4) breast, *BRCA*-mutated, ER–, or ER+. Patients received olaparib 400 mg orally twice daily. Assessments at 8 weeks include imaging, tumor rebiopsy, blood collection, and response assessment. Although responses were seen in arms A, C, and D, arm B closed early as no responses were seen in 15 enrolled patients with sporadic TNBC. This trial is one of the first to report response assessment for PARP inhibitors as a single agent to treat sporadic TNBC, and, with limited and unimpressive results, further supports the use of combination PARP inhibitor plus chemotherapy to treat patients with sporadic TNBC (43).

Canadian study 11, a randomized, double-blind, multicenter study assessing efficacy of olaparib in combination

Table 2. A summary of representative PARP inhibitors in clinical development by route of administration and current clinical status

Agent	Company	Route of administration	Clinical status
Olaparib (AZD2281)	AstraZeneca/KuDOS	Oral	Phase I and II
Veliparib (ABT-888)	Abbott	Oral	Phase I and II
BS1-201	BiPar/SanofiAventis	IV	Phase II and III
AG014699	Pfizer	IV	Phase I and II
MK482	Merck	Oral	Phase I
INO-1001	Inotek	IV	Phase I
CEP9272	Cephalon	Oral	Phase I

Abbreviation: IV, intravenous.

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with paclitaxel in the first or second line treatment of metastatic TNBC, yielded notable toxicity patterns (44). In the phase I cohort, 8 of 9 patients required paclitaxel dose modifications, and 4 of 9 required olaparib dose modifications because of grade 2 to 4 neutropenia, prompting the use of granulocyte colony–stimulating factor rescue in cohort 2. Overall response in cohorts 1 and 2 were 33% (3 out of 9) and 40% (4 out of 10), respectively. Ongoing trials with olaparib and DNA-damaging chemotherapeutics (i.e., phase II, olaparib with carboplatin in advanced *BRCA*-deficient and TNBC, and phase I-II, olaparib with cisplatin in the neoadjuvant treatment of TNBC) will shed light on this toxicity. Moreover, these trials will further define whether dose-limiting neutropenia is specific to olaparib in combination with paclitaxel or is more generalizable across the broader class of DNA-damaging chemotherapeutics.

BSI-201

Initial safety, tolerability, and efficacy of the intravenous PARP inhibitor BSI-201 was reported in a series of phase I studies as a single agent and in combination with several DNA-damaging chemotherapeutics, respectively (45, 46). A phase I study of BSI-201 in 23 heavily pretreated patients with advanced solid tumors reported tolerability at doses ranging from 0.5 mg/kg to 8.0 mg/kg. A maximum tolerated dose was not reached, with the most common side effects being fatigue (56%), nausea (47%), vomiting (39%), and constipation (21%). PARP activity as measured in peripheral blood mononuclear cells was suppressed by >50% at the fifth dose level (2.8 mg/kg), and best response of stable disease (SD) for >2 months was reported in 6 of 23 patients (45). A second phase Ib study reported safety and efficacy of BSI-201 in combination with several DNA-damaging chemotherapeutic agents (i.e., gemcitabine, topotecan, temozolomide, or carboplatin and/or paclitaxel). Among 66 treated patients (24 breast cancer patients), two serious adverse events were possibly related to BSI-201, and all patients tolerated doses up to 5.6 mg/kg. The addition of BSI-201 did not potentiate expected toxicities of individual cytotoxics. Fifty-three of 66 reported some clinical benefit with PR observed in two advanced breast cancer patients (46).

Both the shared clinico-pathologic features between *BRCA*-deficient and sporadic triple-negative breast cancer and preclinical evidence illustrating synergy for BSI-201 and gemcitabine and/or carboplatin provided the rationale to investigate the efficacy of BSI-201 with chemotherapy in advanced TNBC (14, 15). One-hundred and twenty women with metastatic TNBC were enrolled in a multicenter, open-label, randomized, clinical trial in the United States. Enrolled patients who had received 0 to 2 prior lines of chemotherapy in the metastatic setting were randomized to receive gemcitabine (1,000 mg mg/m^2 intravenously, days 1 and 8) plus carboplatin [area under the curve (AUC) 2, intravenously, days 1 and 8] with or without BSI-201 (5.6 mg/kg, intravenously, days 1, 4, 8, and 11) every 21 days. Clinical benefit rate (CR + PR + SD) at 6 months and safety of combination therapy were the primary objective of this trial. Secondary objectives included overall response rates, PFS, and OS.

Patients' baseline characteristics were well balanced between treatments arms. Notably, >50% of patients in both arms had received prior taxane- and anthracycline-based chemotherapy, and 10% received prior bevacizumab. Clinical benefit rates were superior among patients who received BSI-201 in combination with chemotherapy compared with those who received chemotherapy alone (62% versus 21%, respectively, $P = 0.0002$). In addition, PFS and OS was superior among patients who received BSI-201 (6.9 versus 3.3 months, hazard ratio = 0.342, $P < 0.0001$; and 9.2 versus 5.7 months, hazard ratio = 0.348, $P = 0.0005$, respectively; ref. 14). An updated analysis continues to confirm an OS advantage among patients who received BSI-201 in combination with chemotherapy compared with chemotherapy alone (12.2 versus 7.7 months, $P = 0.005$; Fig. 2; ref. 35). The updated safety analysis indicates that the addition of BSI-201 does not potentiate side effects of chemotherapy. Preliminary efficacy results prompted the design of an ongoing multicenter, randomized, phase III trial to confirm efficacy of BSI-201 in combination with gemcitabine and/or carboplatin chemotherapy, results of which are eagerly awaited.

Proposed Mechanisms for Resistance to PARP Inhibition

PARP inhibitors have illustrated clinical efficacy in historically challenging, often treatment-refractory malignancies, however, disease control rarely exceeds 1 year. Although not entirely understood, resistance to PARP inhibitors is hypothesized to result from one of several mechanisms including, but not limited to, up-regulation of the multidrug resistance (MDR 1,2) efflux pumps (47), and reversion of *BRCA* mutation with restoration of *BRCA* function (48). A series of several studies support the proposed mechanisms of PARP resistance.

Long-term treatment of a *BRCA1*-deficient genetically engineered mouse model (GEMM) treated with the PARP inhibitor AZD2281 caused up-regulation of the *Abcb1a/b* genes encoding for the P-glycoprotein efflux pumps, and occasionally up-regulation of the drug target *PARP1*. Moreover, resistance in all cases was reversed by coadministration of tariquidar, a P-glycoprotein inhibitor (47). A separate series of experiments employed a PARP inhibitor–resistant clone from a human pancreatic cell line homozygous for the *BRCA2* protein–truncating c.6174delT frameshift mutation. Resistant cell lines were found to express new *BRCA2* isoforms as a result of intragenic deletion spanning the c.6174delT mutation, resulting in restoration of the open reading frame, creating a novel BRCA2 allele functional for homologous recombination and causing decreased sensitivity to PARP inhibition (48). Finally, on the basis of the knowledge that

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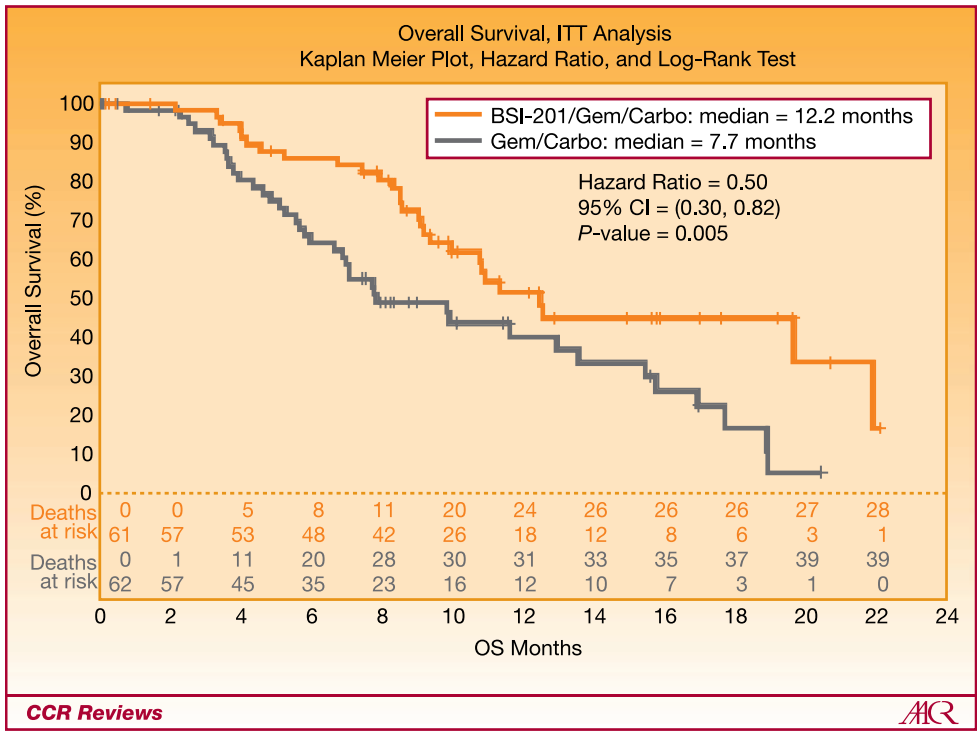

Fig. 2. Kaplan Meier curves illustrating an OS advantage for patients with triple-negative metastatic breast cancer treated with the PARP inhibitor BSI-201 (BiPAR Sciences/Sanofi Aventis) plus carboplatin-gemcitabine chemotherapy compared with chemotherapy alone (12.2 versus 7.2 months, $P = 0.005$). Adapted from O'Shaughnessy J, et al., San Antonio Breast Cancer Symposium, 2009, poster # 3122. Final results of a randomized phase II study demonstrating efficacy and safety of BSI-201, a poly (ADP-ribose) polymerase (PARP) inhibitor, in combination with gemcitabine/carboplatin (G/C) in metastatic triple negative breast cancer (TNBC).

PARP inhibitors and platinum chemotherapeutics have overlapping targets, platinum resistance may inform mechanisms of PARP inhibitor resistance. Among a series of recurrent *BRCA1*-mutated ovarian cancers, four of six recurrent, platinum-resistant tumors had developed secondary genetic changes in *BRCA1* that restored the open reading frame of the *BRCA1* protein, thus conferring diminished platinum sensitivity (49). Although complex, rational strategies to circumvent the two flavors of PARP inhibitor resistance, up-regulation of MDR efflux pumps and reversion mutations, may exist. Such strategies will be challenging to translate clinically and underscore the need for tissue collection from the numerous early phase clinical trials evaluating PARP inhibition in both *BRCA*-mutated and non-*BRCA*–mutated advanced malignancies to study mechanisms of resistance.

Conclusions, Challenges, and Future Directions

Results from elegant preclinical studies and promising clinical trials continue to highlight PARP inhibitors as one of the most promising "targeted therapies" for aggressive TNBC. As we as a medical community move forward, we are challenged with several tasks including: (1) more precisely defining the patient population (even within the TNBC patient population) most likely to respond to the PARP inhibition; (2) discovering and validating candidate biomarkers to predict responders [i.e., (γ) H2AX, RAD51 (as a marker of intact HR), germline DNA studies, etc.]; (3) determining the optimal chemotherapy backbone to combine with PARP inhibitors; (4) defining the most effective schedule of administration (i.e., continuous versus intermittent dosing with chemotherapy); and finally, (5) moving PARP inhibitors into "niche" settings (i.e., brain metastases arising from TNBC). In this era of personalized medicine, investigators are actively working to further define TNBC patients most likely to respond to PARP inhibitors. This direction is particularly important as these drugs move to the adjuvant setting, because long-term toxicities to normal tissues as a result of prolonged suppression of DNA repair have yet to be defined. As an example, an as-of-yet unvalidated, but intriguing, 25-gene assay was developed to identify *BRCA*-like sporadic TNBC; an approach such as this may help us select patients most likely to respond to this class of drugs (50). The role of PARP inhibitors in non-TNBC phenotypes is also of interest because the mechanism of PARP inhibition may serve to sensitize endocrine sensitive and/or HER2-positive breast tumors to DNA-damaging chemotherapy. Each of the aforementioned challenges are areas deserving of further study and are the subject of ongoing clinical investigation. Although challenging at first glance, thoughtful planning and coordinated efforts between collaborating preclinical, translational, and clinical investigators will

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continue to move the development of PARP inhibitors forward and hold the potential to improve the lives of the hundreds and thousands of women diagnosed with breast cancer worldwide each year.

Disclosure of Potential Conflicts of Interest

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On December 9, 2009, the Triple Negative Breast Cancer Foundation and Susan G. Komen for the Cure convened a meeting of clinicians, investigators, and advocates to review the state of current clinical and translational research on TNBC, specifically as it relates to novel therapies and the direction of future research opportunities in this subset of breast cancer. This report is derived from that meeting. We would like to acknowledge and sincerely thank the Triple Negative Breast Cancer Foundation and the Susan G. Komen for the Cure for their support of this symposium.

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