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## PARP Inhibition: “Targeted” Therapy for Triple Negative Breast Cancer

Carey K. Anders<sup>1</sup>, Eric P. Winer<sup>2</sup>, James M. Ford<sup>3</sup>, Rebecca Dent<sup>4</sup>, Daniel P. Silver<sup>2</sup>, George W. Sledge<sup>5</sup>, and Lisa A. Carey<sup>1</sup>

<sup>1</sup>University of North Carolina, Chapel Hill, NC

<sup>2</sup>Dana Farber Cancer Institute, Boston, MA

<sup>3</sup>Stanford University, San Francisco, CA

<sup>4</sup>Sunnybrook Odette Cancer Center, Toronto, Canada

<sup>5</sup>Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN

### Abstract

In contrast to endocrine-sensitive and HER2-positive breast cancer, novel agents capable of treating advanced triple negative breast cancer (TNBC) are lacking. PARP (Poly-(adenosine diphosphate [ADP]-ribose) polymerase) inhibitors are emerging as one of the most promising ‘targeted’ therapeutics to treat TNBC, with the intended ‘target’ being DNA repair. PARP’s 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 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 TNBC. The focus of this review will include 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, conclude with current challenges and future directions for the development of PARP inhibitors in the treatment of breast cancer.

### Introduction

Inhibition of PARP (Poly-(adenosine diphosphate [ADP]-ribose) polymerase) is emerging as one of the most exciting and promising ‘targeted’ therapeutic strategies to treat advanced triple negative breast cancer – the intended ‘target,’ being DNA repair. Diagnosed in an estimated 180,000 women worldwide, triple negative breast cancer (TNBC) is an aggressive subset of breast cancer that lacks expression of the estrogen and progesterone receptors (ER and PR) and the 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 triple negative breast cancer 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 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)(10,11). Similarly, while there was a progression-free survival benefit of 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) (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(13,14). 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, progression free (PFS) and overall survival (OS) when adding PARP inhibition to DNA-damaging chemotherapeutics with minimal additional toxicity(15,16). We will review the preclinical rationale for evaluating PARP inhibitors in 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, while exogenous forms include chemical mutagens, cytotoxic agents and both ultra-violet and ionizing radiation(17). 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 (DSB). DNA double strand breaks are highly toxic to cells and two main pathways contribute to their inherent repair – error-prone non-homologous end joining (NHEJ) and error-free homologous recombination (HR, Figure 1a)(17-19). HR is dependent on functional *BRCA 1* and *2* pathways and *BRCA* maintains genome stability, at least in part, by regulating HR according to the type of DNA damage(20-23). 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(17,24,25). 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, Poly-(adenosine diphosphate [ADP]-ribose) polymerase (PARP).

PARP's are a family of enzymes involved in cellular processes such as genomic stability, DNA repair, cell cycle progression, and apoptosis(26,27). PARP-1, a nuclear, zinc-finger, deoxyribonucleic acid (DNA)-binding protein, localizes to DNA strand breaks as part of the base excision repair (BER) process(28). 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 (Figure 1b) – a hypothesis that has borne out in both preclinical models and the clinical trial arena.

It well-recognized that *BRCA*-deficient, basal-like (as defined by microarray) and triple negative (as defined by immunohistochemistry [IHC]) 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)(4,7,9,15,29, 30). Several mechanisms have been proposed to explain these similarities based on presumed *BRCA* pathway and subsequent HR dysfunction in sporadic basal-like and TNBC. These mechanisms may include (1) over-expression and copy number gain of *ID4*, a negative regulator of *BRCA1*(31,32), (2) decreased expression of *BRCA1* messenger RNA(32), (3) *BRCA1* gene promoter methylation(33) and (4) copy number aberrations affecting genes within a ‘*BRCA* DNA Damage Response’ pathway(32). 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 “*BRCAness*” of sporadic TNBC(34). Thus, the “*BRCAness*” 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 (15,35-37).

### Preclinical Efficacy of PARP Inhibition in *BRCA*-deficient and TNBC/Basal-like Breast Cancer Models

Two landmark preclinical studies report (1) feasibility of targeting DNA repair defects inherent to *BRCA* mutant cell lines as a therapeutic strategy and (2) the acute sensitivity of *BRCA*-2 deficient embryonic stem cells to PARP inhibition due to deficiencies in HR(38,39). Farmer et al. conducted a series of experiments illustrating a reduction in clonogenic survival of *BRCA-1* 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 *BRCA-1* and *BRCA-2* deficient embryonic stem cells compared to heterozygous mutant and wild-type cells. An *in vivo* *BRCA-2* deficient murine model supported these findings(39). Parallel studies conducted by Bryant et al. also demonstrated 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( $\gamma$ )-H2AX foci, a marker of DNA double strand breaks, and *BRCA2* deficient tumors in a xenograft model were susceptible to PARP inhibition(38). Taken together, results from these studies suggested a new, mechanism-based approach for the treatment of patients with *BRCA-1* 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 homologous recombination.

As discussed above, *BRCA*-mutated, basal-like and TNBC share a number of clinico-pathologic characteristics (Table 1). *BRCA-1* and *BRCA-2* mutation carriers, however, comprise a minority of breast cancer cases(24,25). The “*BRCAness*” of TNBC, which is classified as basal-like by cDNA microarray over 80% of the time(9,40), led investigators to evaluate the sensitivity of PARP inhibition in breast cancer cell lines of varying subtypes (i.e. luminal, basal, etc.) to not only determine subtype-specific sensitivity, but 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 base excision repair (BER)(41). 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 *BRCA-1* mutated breast cancers share defects in maintaining genomic stability via aberrant regulation of ODD. When a variety of breast cancer cell lines of a varying subtypes, including luminal, basal-like and *BRCA-1* mutated breast cancers were tested for sensitivity to H<sub>2</sub>O<sub>2</sub>, (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. (41).

A second set of parallel experiments concluded that the relative inefficiency of basal-like and *BRCA-1* mutated breast cancer cell lines to repair ODD was a result of defective BER(41). Briefly, a BER assay involving BER-dependent expression of a GFP [green fluorescent protein] reporter gene showed a relative decrease in GFP expression 24 hours following DNA damage among basal-like and *BRCA-1* mutated breast cancer cell lines when compared to luminal breast cancer cell lines. Moreover, transfection of the *BRCA-1* mutated cell line SUM149 with wild-type *BRCA1* resulted in a 4-fold increase in BER, while 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(42). 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.

## 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 CNS), and inherently poor prognosis(13,14). Based on elegant preclinical rationale, PARP inhibitors are being tested in *BRCA*-deficient and TNBC and illustrate clinical efficacy. Although there are several PARP inhibitors in early phase development (Table 2), the lion's share 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(35). In addition to standard dose-escalation and pharmacokinetic/pharmacodynamic 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. There was no obvious increase in adverse effects among *BRCA*-mutation carriers. Objective anti-tumor activity was reported only in mutation carriers (all heavily pre-treated for breast, ovarian or prostate cancer).

A second phase II, multi-center, single-arm, sequential cohort design study sought to determine the efficacy/tolerability of the olaparib among patients with *BRCA1/BRCA2*-deficient, advanced breast cancer(43). Fifty-four patients were enrolled in one of two dose-based cohorts (cohort 1 = 27 treated with 400mg orally twice daily; cohort 2 = 27 treated with 100mg orally twice daily). The primary objective was objective tumor response (complete response [CR] plus partial response [PR]). Secondary objectives included progression-free survival (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. Over 50% of patients had TNBC and median number of prior therapies was 3. Overall response rates were 41% and 22% among patients in cohort 1 and 2, respectively. PFS was 5.7 (4.6 – 7.4) months in cohort 1 and 3.8 (1.9 – 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 20 study is a phase II study enrolling 4 cohorts of 91 patients with advanced breast or ovarian cancer: A) Ovarian, *BRCA* negative/unknown, B) TNBC, *BRCA* negative/unknown, C) Ovarian, *BRCA*-mutated and D) Breast, *BRCA*-mutated, ER - or +. Patients received olaparib 400 mg orally twice daily. Assessments at eight weeks include imaging, tumor re-biopsy, 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(44).

Canadian Study 11, a randomized, double-blind, multi-center study assessing efficacy of olaparib in combination with paclitaxel in the 1<sup>st</sup> or 2<sup>nd</sup> line treatment of metastatic TNBC, yielded notable toxicity patterns(45). In the phase I cohort, 8 of 9 patients required paclitaxel dose modifications and 4 of 9 required olaparib dose modifications due to grade 2 – 4 neutropenia prompting the use of G-CSF rescue (granulocyte colony-stimulating factor) in cohort 2. Overall response in cohorts 1 and 2 were 33% (3/9) and 40% (4/10), 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 or not 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(46,47). A phase I study of BSI-201 in 23 heavily-pretreated patients with advanced solid tumors report tolerability at doses ranging from 0.5 mg/kg – 8.0 mg/kg. A maximum tolerated dose (MTD) 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 (PBMC's) was suppressed by > 50% at the fifth dose level (2.8 mg/kg) and best response of stable disease for > 2 months was reported in 6 of 23 patients(46). 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/paclitaxel). Among 66 treated patients

(24 breast cancer patients), 2 serious adverse events were possibly related to BSI-201 and all patients tolerated doses up to 5.6mg/kg. The addition of BSI-201 did not potentiate expected toxicities of individual cytotoxics. Fifty-three of 66 reported some clinical benefit with partial response observed in 2 advanced breast cancer patients(47).

Both the shared clinico-pathologic features between *BRCA*-deficient and sporadic TNBC and preclinical evidence illustrating synergy for BSI-201 and gemcitabine/carboplatin provided the rationale to investigate the efficacy of BSI-201 with chemotherapy in advanced TNBC (15,16). One-hundred and twenty women with metastatic TNBC were enrolled in a multi-center, open-label, randomized, clinical trial in the United States. Enrolled patients who had received 0–2 prior lines of chemotherapy in the metastatic setting were randomized to receive gemcitabine (1000mg mg/m<sup>2</sup>, IV, days 1, 8) plus carboplatin (AUC 2, IV, days, 1, 8) with or without BSI-201 (5.6mg/kg, IV, days 1, 4, 8, 11) every 21 days. Clinical benefit rate (CR + PR + SD [stable disease]) at 6 months and safety of combination therapy was the primary objective of this trial. Secondary objectives included overall response rates, PFS and overall survival (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%, prior bevacizumab. Clinical benefit rates were superior among patients who received BSI-201 in combination with chemotherapy compared to 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 [HR] = 0.342,  $p < 0.0001$  and 9.2 versus 5.7 months; HR = 0.348,  $p = 0.0005$ , respectively)(15). An updated analysis continues to confirm an OS advantage among patients who received BSI-201 in combination with chemotherapy compared to chemotherapy alone (12.2 versus 7.7 months,  $p = 0.005$ ; Figure 2)(36). The updated safety analysis indicates 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/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 one 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 multi-drug resistance (MDR 1,2) efflux pumps(48) and reversion of *BRCA* mutation with restoration of *BRCA* function(49). A series of several studies support the proposed mechanisms of PARP resistance.

Long-term treatment of a *BRCA-1* 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 co-administration of tariquidar, a P-glycoprotein inhibitor(48). 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(49). Finally, based on the knowledge that 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, 4 of 6 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(50). 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 TNBC patient population) most likely to respond to the PARP inhibition, (2) discovering and validating candidate biomarkers to predict responders (i.e.  $\gamma$  H2AX, RAD51 [as a marker of intact HR], germline DNA studies, etc.), (3) determining the optimal chemotherapy back-bone to combine with PARP inhibitors, (4) defining the most effective schedule of administration (ie. 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 is particularly important as these drugs move to the adjuvant setting since 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 (51). The role of PARP inhibitors in non-TNBC phenotypes is also of interest since 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 pre-clinical, translational, and clinical investigators will 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.

### Statement of Translational Relevance

The treatment of triple negative breast cancer with 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, such that strides at the bedside continue – the essence of translational medicine.

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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 is a report 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.

Members attending this symposium include: Marianne Alciati, PhD; Carey K. Anders, MD; Allison Axenrod; Sunil Badve, MD; Judith Balmaña, MD; Powel Brown, MD, PhD; Lisa A. Carey, MD; Jennifer De Los Santos, MD; Rebecca Dent, MD; Hayley Dinerman; James Ford, MD; Andres Forero, MD; William D. Foulkes, MB, PhD; Liz Frank; Sharon Fredman; Shridhar Ganesan, MD, PhD; Judy E. Garber, MD, MPH; Montserrat Garcia-Closas, MD, Dr.P.H.; Vincent L. Giranda, MD, PhD; Cindy Geoghegan; Lyndsay N. Harris, MD; Gabriel N. Hortobagyi, MD, FACP; Clifford A. Hudis, MD; Steven Jay Isakoff, MD, PhD; Gilbert Jirau-Lucca, MS; Helen Krontriras, MD; Gabriella L. Mariani, MD; Olufunmilayo I. Olopade, MD, FACP; Charles M. Perou, PhD; Peggy L. Porter, MD; Lajos Pusztai, MD, PhD; Andrea Richardson, MD, PhD; Hope Rugo, MD; Daniel Silver, MD, PhD; George W. Sledge, Jr. MD; Sandra M. Swain, MD; Melinda L. Telli, MD; Elizabeth Thompson; Andrew Tutt, MRCP, FRCR, PhD; Eric P. Winer, MD; Antonio C. Wolff, MD; Jo Anne Zujewski, MD.

## References

- Swain, S. Triple-Negative Breast Cancer. Metastatic Risk and Role of Platinum Agents 2008 ASCO Clinical Science Symposium 2008; June 3, 2008;
- Carey L, Perou C, Livasy C, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA* 2006;295:2492–502. [PubMed: 16757721]
- Dent R, Trudeau M, Pritchard K, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007;13:4429–34. [PubMed: 17671126]
- Livasy C, Karaca G, Nanda R, et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Mod Pathol* 2006;19:264–71. [PubMed: 16341146]
- Millikan RC, Newman B, Tse CK, et al. Epidemiology of basal-like breast cancer. *Breast Cancer Research and Treatment* 2008;109:123–39. [PubMed: 17578664]
- Nielsen T, Hsu F, Jensen K, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;10:5367–74. [PubMed: 15328174]
- Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747–52. [PubMed: 10963602]
- Smid M, Wang Y, Zhang Y, et al. Subtypes of breast cancer show preferential site of relapse. *Cancer Research* 2008;68:3108–14. [PubMed: 18451135]
- 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;100:8418–23. [PubMed: 12829800]
- Miller K, Wang M, Gralow J, et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *New England Journal of Medicine* 2007;357:2666–76. [PubMed: 18160686]
- Burstein, HJ. Therapeutic Options for Treatment Refractory Breast Cancer. Discussion 2010 Metastatic Breast Cancer Oral Abstract Presentation 2010; June 8, 2010;
- Thomas ES, Gomez HL, Li RK, et al. Ixabepilone Plus Capecitabine for Metastatic Breast Cancer Progressing After Anthracycline and Taxane Treatment. *Journal of Clinical Oncology* 2007;25:5210–7. [PubMed: 17968020]
- Dent RTM, Pritchard KI, Hanna WM, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007;13:4429–34. [PubMed: 17671126]
- Lin N, Claus E, Sohl J, et al. Sites of Distant Recurrence and Clinical Outcomes in Patients With Metastatic Triple-negative Breast Cancer High Incidence of Central Nervous System Metastases. *Cancer* 2008;113:2638–45. [PubMed: 18833576]



15. O'Shaughnessy J, Osborne C, Pippen J, et al. Efficacy of BSI-201, a poly (ADP-ribose) polymerase-1 (PARP1) inhibitor, in combination with gemcitabine/carboplatin (G/C) in patients with triple-negative breast cancer (TNBC): Results of a randomized phase II trial. *J Clin Oncol* 2009;27 Abstract 3.
16. O'Shaughnessy, J.; Yoffe, M.; Osborne, C., et al. Triple negative breast cancer: a phase 2, multi-center, open-label, randomized trial of gemcitabine/carboplatin (G/C), with or without BSI-201, a PARP inhibitor. *San Antonio Breast Cancer Symposium*; 2008. Abstract 2120
17. Ford, J. DNA Damage Response Pathways and Cancer. In: Abeloff, MD., editor. *Clinical Oncology*. 3rd. Churchill Livingstone; 2004.
18. Hartman A, Ford J. BRCA1 induces DNA damage recognition factors and enhances nucleotide excision repair. *Nature Genetics* 2002;32:180–4. [PubMed: 12195423]
19. Zhang J, Powell S. The role of the BRCA1 tumor suppressor in DNA double-strand break repair. *Mol Cancer Res* 2005;3:531–9. [PubMed: 16254187]
20. Moynahan ME, Pierce AJ, Jasin M. BRCA2 is required for homology-directed repair of chromosomal breaks. *Molecular Cell* 2001;7:263–72. [PubMed: 11239455]
21. Cousineau I, Belmaaza A. BRCA1 haploinsufficiency, but not heterozygosity for a BRCA1-truncating mutation, deregulates homologous recombination. *Cell Cycle* 2007;6:962–71. [PubMed: 17404506]
22. Powell SN, Kachnic LA. Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. *Oncogene* 2003;22:5784–91. [PubMed: 12947386]
23. Snouwaert JN, Gowen LC, Latour AM, et al. BRCA1 deficient embryonic stem cells display a decreased homologous recombination frequency and an increased frequency of non-homologous recombination that is corrected by expression of a *brca1* transgene. *Oncogene* 1999;18:7900–7. [PubMed: 10630642]
24. Narod S. Modifiers of risk of hereditary breast and ovarian cancer. *Nat Rev Cancer* 2002;2:113–23. [PubMed: 12635174]
25. Narod S, Foulkes W. BRCA1 and BRCA2: 1994 and beyond. *Nat Rev Cancer* 2004;4:665–76. [PubMed: 15343273]
26. Tentori L, Graziani G. Chemopotentiality by PARP inhibitors in cancer therapy. *Pharmacol Res* 2005;52:25–33. [PubMed: 15911331]
27. Tentori L, Porarena I, Graziani G. Potential Clinical Applications of Poly (ADP-ribose) Polymerase Inhibitors. *Pharmacological Research* 2002;45:73–85. [PubMed: 11846617]
28. Plummer E. Inhibition of poly(ADP-ribose) polymerase in cancer. *Current Opinion in Pharmacology* 2006;6
29. Cleator S, Heller W, Coombes R. Triple-negative breast cancer: therapeutic options. *Lancet Oncol* 2007;3:235–44. [PubMed: 17329194]
30. Sorlie T, Perou C, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A* 2001;98:10869–74. [PubMed: 11553815]
31. Turner N, Reis-Filho J, Russell A, et al. BRCA1 dysfunction in sporadic basal-like breast cancer. *Oncogene* 2007;26:2126–32. [PubMed: 17016441]
32. Natrajan R, Weigelt B, Mackay A, et al. An integrative genomic and transcriptomic analysis reveals molecular pathways and networks regulated by copy number aberrations in basal-like, HER2 and luminal cancers. *Breast Cancer Research and Treatment* 2009;121:575–89. [PubMed: 19688261]
33. Grushko T, Nwachukwu C, Caroenthammaraksa S, et al. Evaluation of BRCA1 inactivation by promoter methylation as a marker of triple-negative and basal-like breast cancer. *Journal of Clinical Oncology* 2010;28:7S. Abstract 10510.
34. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. *Nat Rev Cancer* 2004;4:814–9. [PubMed: 15510162]
35. Fong P, Boss D, Yap T, Tutt A, Wu P, Mergui-Roelvink M. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. *New England Journal of Medicine* 2009;361:123–34. [PubMed: 19553641]

36. O'Shaughnessy J, Osborne C, Pippen J, et al. Updated results of a randomized phase II study demonstrating efficacy and safety of BSI-201, a PARP inhibitor, in combination with gemcitabine/carboplatin in metastatic triple-negative breast cancer. *Cancer Research* 2009;69 Abstract 3122.
37. Tutt A, Robson M, Garber JE, et al. Phase II trial of the oral PARP inhibitor olaparib in BRCA-deficient advanced breast cancer. *Journal of Clinical Oncology* 2009;27 Abstract CRA501.
38. Bryant H, Schultz N, Thomas H, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature* 2005;434:913–7. [PubMed: 15829966]
39. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* 2005;434:917–21. [PubMed: 15829967]
40. Silver D, Richardson A, Eklund A, et al. Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. *Journal of Clinical Oncology* 2010;28:1145–53. [PubMed: 20100965]
41. Alli E, Sharma V, Sunderesakumar P, Ford J. Defective Repair of Oxidative DNA Damage in Triple Negative Breast Cancer Confers Sensitivity to Inhibition of Poly(ADP-Ribose) Polymerase. *Cancer Research* 2009;69:3589–96. [PubMed: 19351835]
42. Hastak K, Alli E, Ford J. Synergistic chemosensitivity of triple-negative breast cancer cell lines to a PARP inhibitor, cisplatin and gemcitabine. *Proc AACR* 2009;50 Abstract 5642.
43. Tutt A, Robson M, Garber JE, et al. Phase II trial of the oral PARP inhibitor olaparib in BRCA-deficient advanced breast cancer. *J Clin Oncol* 2009;27:CRA501.
44. Gelmon K, Hirte H, Robidoux A, et al. Can we define tumors that respond to PARP inhibitors? A phase II correlative study of olaparib in advanced serous ovarian cancer and triple negative breast cancer. *Journal of Clinical Oncology* 2010;28 Abstract 3002.
45. Dent R, Lindeman G, 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. *Journal of Clinical Oncology* 2010;28:1018.
46. Kopetz 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 tumors. *J Clin Oncol* 2008;26 Abstract 3577.
47. Mahany J, Lewis N, Heath E, et al. A phase IB study evaluating BSI-201 in combination with chemotherapy in subjects with advanced solid tumors. *J Clin Oncology* 2008;26 Abstract 3579.
48. Rottenberga S, Jaspersa JE, Kersbergena 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 USA* 2008;105:17079–84. [PubMed: 18971340]
49. Edwards S, Brough R, Lord C, et al. Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* 2008;451:1111–6. [PubMed: 18264088]
50. Swisher E, Sakai W, Karlan B, Wurz K, Urban N, Toshiyasu T. Secondary BRCA1 Mutations in BRCA1-mutated Ovarian Carcinomas with Platinum Resistance. *Cancer Research* 2008;68:2581–6. [PubMed: 18413725]
51. Rodriguez, A.; Rimawi, M.; Wu, M., et al. A BRCA1-Like, 25-gene Assay Predicts for Anthracycline-chemosensitivity in Sporadic Triple-Negative Breast Cancer. 2009 San Antonio Breast Cancer Symposium; 2009. Abstract 110
52. Comen E, Robson M. Inhibition of Poly(ADP)-Ribose Polymerase as a Therapeutic Strategy for Breast Cancer. *Oncology* 2010;24:55–68. [PubMed: 20187322]

Figure 1a

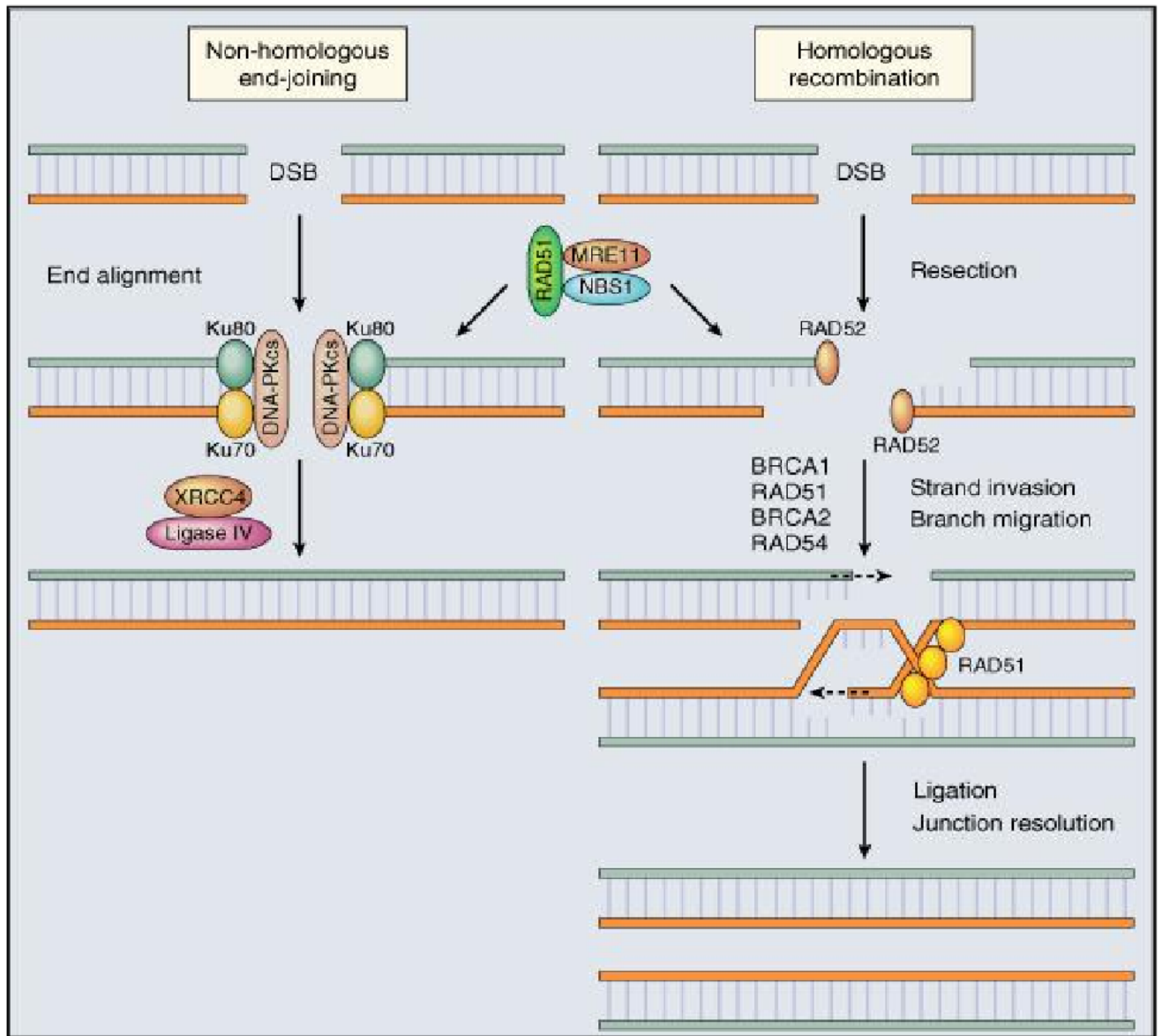
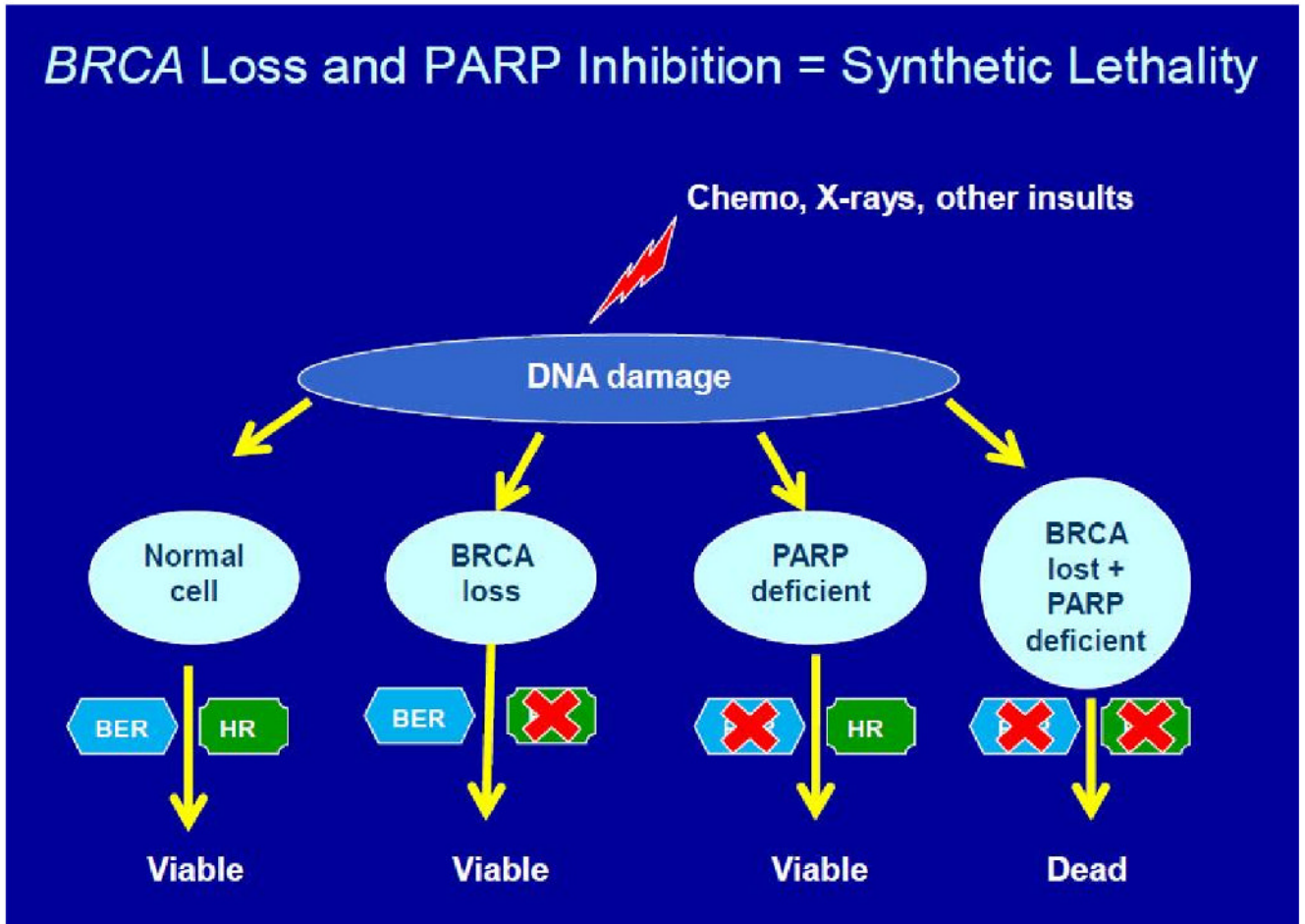


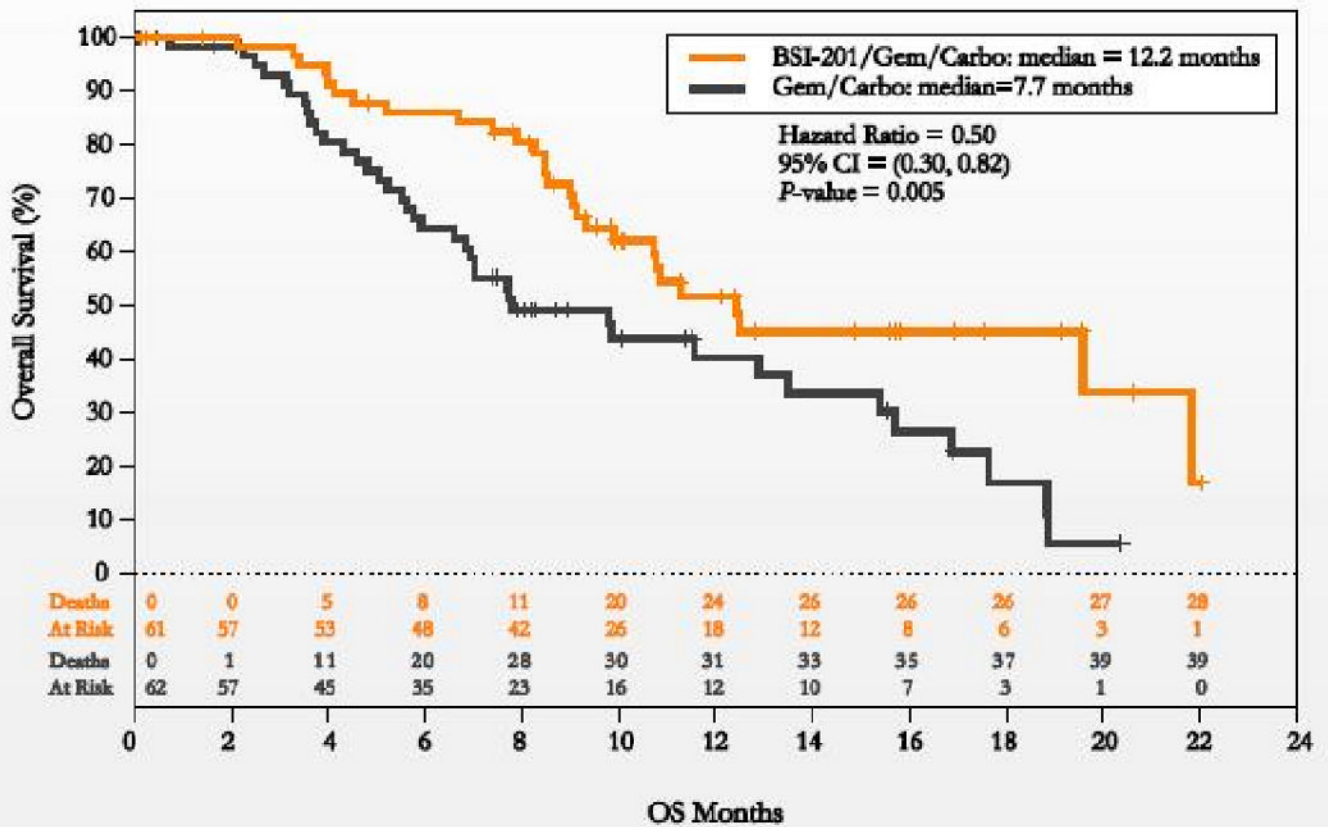
Figure 1b

**Figure 1.**

**Figure 1a:** Two main pathways which contribute to repair of DNA double strand breaks: non-homologous end joining (NHEJ) and homologous recombination (HR). This figure was published in Abeloff's Clinical Oncology, 4th Edition. JM Ford and MB Kastan, Chapter 10: DNA damage response pathways and cancer. p. 149. (Reprinted with permission, Copyright 2008, Churchill Livingstone, an Imprint of Elsevier) (5).

**Figure 1b:** '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 (Reprinted with permission, from EA Comen and M Robson: ONCOLOGY 24(1):55-68, 2010) (52).

**Overall Survival, ITT Analysis**  
**Kaplan Meier Plot, Hazard Ratio, and Log-Rank Test**



**Figure 2.** Kaplan Meier curves illustrating an overall survival 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 to chemotherapy alone (12.2 versus 7.2 months,  $p = 0.005$ )(36).

**Table 1**

Shared clinic-pathologic features between *BRCA-1* deficient and triple negative/Basal-like breast cancer. Figure adapted from 2009 ASCO Plenary Session(15).

Clinico-pathologic Characteristics	Hereditary <i>BRCA1</i> Breast Cancer	Triple Negative/Basal-like Breast Cancer
ER/PR/HER2 status	Negative	Negative
TP53 status	Mutant	Mutant (Up to 80%)
<i>BRCA1</i> status	Mutational Inactivation	Diminished Expression (?)
Gene-expression patterns	Basal-like	Basal-like
Tumor Histology	Poorly-differentiated (high grade)	Poorly-differentiated (high grade)
Chemosensitivity to DNA-damaging chemotherapy	Highly sensitive	Likely sensitive
Genome-wide Aneuploidy	Yes	Yes

**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 1 and 2
Veliparib/(ABT-888)	Abbott	Oral	Phase 1 and 2
BS1-201	BiPar/SanofiAventis	IV	Phase 2 and 3
AG014699	Pfizer	IV	Phase 1 and 2
MK482	Merck	Oral	Phase 1
INO-1001	Inotek	IV	Phase 1
CEP9272	Cephalon	Oral	Phase 1